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SYMPOSIA AND ORAL SESSIONS

Late-Breaking Original Research

LB1 Composition, nitrogen fraction, and amino acid profile of mare's milk produced in the mountains and highlands. A. T. Mazhitova* and A. A. Kulmyrzaev, *Kyrgyz-Turkish Manas University*, *Bishkek, Kyrgyzstan.*

The study was carried out to determine the effect of 2 ecological regions and lactation period on chemical ($P < 0.01$) and amino acid ($P < 0.01$) composition and nitrogen fraction ($P < 0.01$) of mares grazing on pastures at 1700 m (vegetation is dominated by shrubs and sub-shrubs) and 2200 m (vegetation is dominated by grasses, forbs, sub-shrubs) above sea level. The animals were kept under extensive pasture conditions and received no additional feed supplements. Milk samples were collected monthly from May to July and from May to August from mares grazing at 1700 m (Mountains) and 2200 m (Highlands), respectively. Total solids (11.39–10.47% and 11.56–10.86%), milk fat (1.80–1.33% and 1.83–1.60%) and ash (0.48–0.26% and 0.55–0.30%) content of milk for the Mountain and Highland regions respectively were decreased to the end of lactation period. The average percentages of casein to whey protein ratio were 52:47 and 50:42 for milk obtained from Mountain and Highland pastures. The highest content of essential amino acids were obtained in May milk for both regions (1251 and 1284 mg/100 g of milk) and the lowest content were in July (852 mg/100 g of milk) and August milk (880 mg/100 g of milk), but the percentages of essential amino acids in protein of mare's milk were the highest in June (49%) and July milk (49%) produced at 1700 m and 2200 m respectively. The results of the study have shown that the changes in chemical composition, nitrogen fraction, and amino acid profile of the mare's milk during lactation period are influenced by geographical location of pastures.

Key Words: mare's milk, amino acid composition, pasture

LB2 Colorimetric detection of volatile organic compounds for shelf-life monitoring of milk. M. Ziyaina*, *Washington State University*, *Pullman, WA, USA.* Colorimetric nanosensors for monitoring food quality and shelf life provide an exciting development with obvious economic benefits. In this study, a colorimetric sensor based on silicon dioxide (SiO_2) nanoparticles and Schiff's reagent to detect volatile organic compounds (VOCs) generated by the growth of spoilage bacteria in pasteurized whole milk stored at 7, 13, 15, and 19°C was developed. Volatile organic compounds formed from microbial growth were detected using solid-phase microextraction (SPME) and gas chromatography. Volatile organic compounds levels were correlated with microbial growth (aerobic plate counts) and color change throughout the shelf-life period. Color changes due to VOCs generated by spoilage bacteria were detected at all storage temperatures except 7°C. Nanosensor response correlated well with microbial growth in milk. Milk spoilage occurred at 32, 60, and 84 h at 19, 15, and 13°C, respectively and could be detected colorimetrically. Color change, recorded as total color difference (ΔE) for the nanosensor, correlated well with aerobic plate counts (5.0–7.0 $\log_{10}$ cfu/mL) in milk ($R^2 = 0.81–0.96$). This colorimetric sensor could be incorporated into food packaging to predict remaining shelf life, benefitting consumers as well as manufacturers and retailers and reducing food loss.

Key Words: nanoparticles, sensor, Schiff reagent

LB3 Selective dry cow therapy on US dairy farms: Impact on udder health and productivity. S. Rowe*[1], S. Godden[1], E. Royster[1], J. Timmerman[1], D. Nydam[2], A. Vasquez[2], P. Gorden[3], and A. Lago[4], [1]*University of Minnesota*, *Saint Paul, MN, USA,* [2]*Cornell University*, *Ithaca, NY, USA,* [3]*Iowa State University*, *Ames, IA, USA,* [4]*DairyExperts*, *Tulare, CA, USA.*

Culture- and algorithm-guided selective dry cow therapy (SDCT) programs were each compared with blanket dry cow therapy (BDCT) in a clinical trial for the following outcomes: antibiotic (ABX) use at dry-off; risk of new and cured infections (IMI) during the dry period; 120-d clinical mastitis (CM) and culling risks; milk yield and somatic cell count (SCC) in the first 120 d in milk. Seven herds were recruited from 4 study sites (CA, IA, MN, and NY). Cows (n = 1,275) were randomly allocated to BDCT, Culture-SDCT, and Algorithm-SDCT. All quarters of the Blanket group were treated with intramammary ABX. Quarters of Culture cows received ABX if any growth was observed after milk culture using the MN Easy 4Cast plate. Algorithm cows received ABX in all quarters if they met any of the following criteria: ≥ 2 cases of CM during lactation, CM during the 14 d before dry-off, or any test day SCC >200,000 cells/ml during lactation. All quarters were treated with an internal teat sealant. Risk differences (RD), Hazard ratios (HR) and adjusted means were estimated using marginal standardization, Cox proportional hazards and linear mixed models, respectively. Quarter-level ABX use was reduced by 55% in each SDCT group. IMI cure risk was similar in Blanket (89.8%), Culture (90.0%, RD = +0.2%, 95%CI: −4.4, 4.7%) and Algorithm (90.4%, RD = +0.6%, 95%CI: −3.9, 5.2%) quarters. New IMI risk was similar in Blanket (15.1%), Culture (15.3%, RD = +0.2%, 95%CI: −2.5, 2.9%) and Algorithm (14.9%, RD = −0.2%, 95%CI: −2.9, 2.5%) quarters. CM incidence was similar for Blanket (14.5%), Culture (12.2%, HR = 0.82, 95%CI: 0.6–1.2), and Algorithm (12.2%, HR = 0.82, 95%CI: 0.6, 1.1) cows. Risk of culling was similar for Blanket (10.8%), Culture (9.8%, HR = 0.89, 95%CI: 0.6, 1.3) and Algorithm (10.6%, HR = 0.98, 95%CI: 0.7, 1.4) cows. Adjusted geometric mean SCC was similar for Blanket (55, 95%CI: 47, 65), Culture (57, 95% CI: 49, 68), and Algorithm (59, 95%CI: 50, 69) cows. Adjusted average daily milk yield (kg/day) was: Blanket (48.6, 95%CI: 46.2, 51.1), Culture (48.6, 95%CI: 46.2, 51.1), and Algorithm (47.8, 95% CI: 45.3, 50.2). SDCT successfully reduced ABX use by 55%, without causing negative effects on health and productivity.

Key Words: selective dry cow therapy, on-farm culture, algorithm

LB4 Ileal digestibility of an enzyme-treated soybean meal for milk replacer in preweaned dairy calves. I. Ansia*[1], H. H. Stein[1], C. Brøkner[2], D. A. Vermeire[3], and J. K. Drackley[1], [1]*University of Illinois*, *Urbana, IL,* [2]*Hamlet Protein A/S*, *Horsens, Denmark,* [3]*Nouriche Nutrition*, *Lake St. Louis, MO.*

Our objective was to measure and compare apparent (AID), standard (SID), and true ileal digestibility (TID) of protein and AA in milk replacers (MR) containing all milk proteins (CON) or an enzyme-treated soybean meal-based (ESBM) protein. A T-cannula was placed in the ileum of 9 Holstein calves at 15 d of age. After 2 wk post-surgery, calves were randomly assigned to a 3 × 3 replicated Latin square with 5-d periods. Calves were fed 2× daily at a rate of 2% (DM) of BW, adjusted weekly. No starter was offered to minimize rumen development. Digesta samples were collected continuously during 12 h on d 4 and 5 of each period. Basal endogenous losses of AA (AA_{endo}) and CP (CP_{endo}) were estimated by feeding a nitrogen-free MR to each calf during 1 period. Total (basal + specific) AA_{endo} and CP_{endo} were estimated by multivariate regression of the χ^2 distances between digesta and reference protein AA profiles. Ileal digesta pH with the ESBM (7.27) diet was lower ($P = 0.01$) than with CON (7.51). According to the piecewise nonlinear model of pH fluctuation, digesta pH during ESBM decreased slower after feeding and reached its nadir later than with the CON diet. Diet did not affect ($P = 0.45$) ADG, but calves on the ESBM diet showed a bigger increment of withers height (0.89 vs 1.61 cm) and lower mean fecal scores (2.81 vs 1.71). Basal ΣAA_{endo} and CP_{endo} were 13.9 and 22.4 g/kg of DMI, respectively. Total ΣAA_{endo} (24.6 vs 32.1 g/kg DMI) and CP_{endo} (28.2 vs 37.1 g/kg DMI) were higher ($P < 0.05$) with ESBM than with CON. Accordingly, AID and SID of most AA, CP, and ΣAA were lower or tended to be lower with ESBM. However, TID did not differ between diets for CP and all AA except Ala and Ile; TID for Arg tended

($P = 0.07$) to be greater with ESBM. According to the estimation model, the differences of protein endogenous losses were caused by an increase of gut bacterial protein rather than by host protein. In fact, we found that flows of digesta DNA were greater ($P < 0.01$) with ESBM (3.2 vs 4.7 ng/kg DMI), but the mucin flow (estimated using glucosamine concentration as marker) did not differ (2.1 vs 1.8 g/kg DMI). Adjusting digestibilities of AA in MR by endogenous losses is crucial when comparing alternative proteins to milk proteins.

Key Words: amino acid, ileal digestibility, calf

LB5 Transcription effect of exogenous galectin-8 and LPS-treated bovine neutrophils using RNA-sequencing. E. Eluka-Okoludoh, B. Mulakala, K. Ekwemalor, S. Harrison, and M. Worku*, *North Carolina Agricultural and Technical State University*, *Greensboro, NC, USA.*

Neutrophils are cells of the innate immune system that have the ability to respond to stimuli. Galectin-8, a part of the family of galectins, has been shown to modulate the innate and adaptive immune system. The objective of this study was to analyze the effect of exogenous galectin-8 on global transcription in bovine neutrophils. Whole blood was collected from the jugular vein of clinically healthy Holstein-Friesian cows from the North Carolina A&T State University Dairy Unit (n = 5). Neutrophils were isolated by differential centrifugation and hypotonic lysis and TC20 was used to measure viability. Neutrophils were then treated (1×10^6 cells/mL viable) with rGal-8 (2 μg), LPS (10 μg), rGal-8 + LPS, or maintained in PBS at 37°C for 1 h, 5% CO_2. Total RNA was extracted using Trizol, RNA integrity (RIN) was determined using Agilent Bioanalyzer, and RNA with RIN of >7 was used. The RNA sequences were generated using an Illumina HiSeq 4000 sequencer. Short RNA fragment reads were in FASTQ format and aligned to the cow reference genome (bosTau8), using Spliced Transcripts Alignment to a Reference (STAR) software. Mapped reads were counted with HTSeq. Genes were normalized against the control, PBS. Differentially expressed genes were analyzed with DESeq2. Pathway analysis was conducted using Ingenuity Pathway Analysis (IPA) software and Database for Annotation, Visualization and Integrated Discovery (DAVID). Our results show that 14,023 genes were expressed. Transcriptome profiling identified differentially expressed transcripts with the treatment of galectin-8 (2037), LPS (477), and Gal-8+LPS (1065) ($P < 0.05$). Galectin-8 targeted 78 pathways, including MAPK signaling pathway; Gal8+LPS targeted 63 pathways including TNF signaling pathway; and LPS targeted 41 pathways including toll-like receptor signaling pathway using DAVID. Integrin Signaling pathway was one of the top canonical pathways targeted by galectin-8 using IPA. These results suggest galectin-8 has broad interactions with several molecules modulating cytokines, chemokines, and innate and adaptive immune genes.

Key Words: cow, galectin, RNA sequencing

LB6 Synergistic associations of bacteria and archaea in DNA and cDNA components of rumen samples collected using stomach tube and cannula methods in dairy cows. D. W. Pitta*[1], C. F. A. Lage[2,5], J. S. Bender[1], N. Indugu[1], M. L. Hennessy[1], V. K. Shabtai[1], B. Vecchiarelli[1], A. Fernandez[6], A. Spitzer[7], S. E. Raisanen[2], A. Melgar[2], K. Nedelkov[3], X. Chen[4], J. Oh[2], and A. N. Hristov[2], [1]*University of Pennsylvania*, *Kennett Square, PA, USA,* [2]*The Pennsylvania State University*, *University Park, PA, USA,* [3]*Universidade Federal de Minas Gerais*, *Belo Horizonte, Brazil,* [4]*College of Pastoral Agriculture Science and Technology*, *Lanzhou University, China,* [5]*Faculty of Veterinary Medicine, Trakia University*, *Stara Zagora, Bulgaria,* [6]*Eberly College of Arts and Sciences, West Virginia University*, *Morgantown, WV, USA,* [7]*School of Veterinary Medicine, Cornell University*, *Ithaca, NY, USA.*

The rumen microbial ecosystem is comprised of bacteria, protozoa, fungi and archaea that work synergistically to facilitate feed digestion. However, the synergistic interactions between different microbes is seldom investigated. Here we investigated rumen bacteria and archaea associations of 6-cannulated Holstein cows that were adapted to a basal total mixed ration. Rumen samples were collected via stomach tube (ST) and rumen cannula (RC) at 0, 2, 4, 6, 8 and 12 h post feeding and filtered to collect solid rumen contents. These samples were extracted for genomic DNA (total) and RNA (cDNA; metabolically active), PCR amplified, sequenced and analyzed using QIIME pipeline for DNA and cDNA-based bacteria and archaea diversity. Based on PERMANOVA analysis for bacteria and archaea communities differed ($P \leq 0.05$) between DNA and cDNA but showed no differences ($P \geq 0.05$) between ST and RC. At the individual taxa level, ANCOM analysis showed that lineages of *Actinobacteria* and *Bacteroidetes* were lower ($P \leq 0.05$) and those of *Proteobacteria* and *Fibrobacter* bacterial phyla were higher in the cDNA compared with DNA bacteria across ST and RC samples. Particularly, the abundance of *Ruminococcus* and *Succinivibrionaceae* were more than doubled in the cDNA compared with DNA bacteria. Similarly, for archaea, *Methanobrevibacter* was lower ($P \leq 0.05$) and *VadinCA11* ($P \leq 0.05$) was higher in cDNA compared with DNA archaea in ST and RC samples. These results indicate that the cDNA approach is more discriminatory than the DNA approach for microbial diversity analysis in ST and RC methods. Correlation analysis of cDNA bacteria with molar proportions of volatile fatty acids in both ST and RC revealed strong positive correlations ($P \leq 0.05$) between *Ruminococcus* and acetate, *Succinivibrionaceae, Prevotella* and *Bulledia* and propionate and *Clostridium* and butyrate. Co-occurrence analysis revealed that co-associations existed between *Ruminococcus* and *Methanobrevibacter*, and *Succinivibrionaceae* and *Methanosphaera*. This is the first study demonstrating the synergistic associations of bacteria-archaea cohorts in the rumen that are individual specific but differed between individual cows.

Key Words: bacteria-archaea cohorts, proxy, ANCOM

LB7 Effects of increasing propionate concentration on short-term metabolism in liver explants from dairy cows in the postpartum period. K. M. Kennedy* and M. S. Allen, *Michigan State University*, *East Lansing, MI, USA.*

Our objective was to determine the temporal effects of increasing concentrations of propionate on hepatic metabolism of dairy cows in the postpartum (PP) period. Liver of 6 dairy cows (3 primiparous: 9.00 ± 1.00 (mean ± SD) d PP and 3 multiparous: 4.67 ± 1.15 d PP) was biopsied and used in a block-design liver explant experiment. Treatments consisted of 3 concentrations of $^{13}C_3$-sodium propionate at 1, 2 or 4 mM. Explants were incubated in 2 mL of supplemented medium 199 at 38°C and sampled at 0.5, 15 and 60 min. Explants were analyzed for [M + n] citrate, isocitrate, succinate, fumarate, malate, pyruvate, lactate, glutamate, and glucose by GC-MS and for acetyl CoA, propionyl CoA, succinyl CoA, and methylmalonyl CoA by LC-MS/MS. Data were analyzed with mixed models and repeated measures. Increased concentrations of $^{13}C_3$-propionate increased total ^{13}C% enrichment of propionyl CoA, succinyl CoA, succinate, fumarate, malate, and citrate over time ($P \leq 0.01$). Treatment did not affect total ^{13}C% enrichment of hepatic glucose (0.89 vs. 0.89 vs. 1.00%; $P = 0.79$) or acetyl CoA (4.23 vs. 4.48 vs. 4.87%; $P = 0.79$) but total ^{13}C% enrichment increased over time for both ($P < 0.001$). Total ^{13}C% enrichment of pyruvate (2.18 vs. 3.36 vs. 4.18%; $P < 0.001$) and [M+2] pyruvate (1.39 vs. 2.15 vs. 2.54%; $P < 0.001$) increased over time with increased concentrations of propionate. Increases in ^{13}C% enrichment of [M+4] citrate (2.36 vs. 3.30 vs. 4.00%; $P < 0.001$) and [M+5] citrate (1.28 vs. 1.81 vs. 2.30%; $P < 0.001$) indicate propionate conversion to acetyl CoA and subsequent entry of acetyl CoA into the TCA cycle. Because these cows were in a lipolytic state, the conversion of pyruvate to acetyl CoA is inhibited. However, the relative partitioning of pyruvate to oxaloacetate or acetyl CoA is dependent upon enzyme activities. This research indicates that at least some propionate can be converted to acetyl CoA and oxidized in the TCA cycle. Metabolic reactions occur very rapidly and understanding short-term metabolism may improve feeding strategies for dairy cows in the PP period.

Key Words: propionate metabolism, liver, dairy cows

LB8 ***Moringa oleifera* polyphenols modulate galectin expression in LPS-induced bovine peripheral blood mononuclear cells.** S. Adjei-Fremah[1], K. Ekwemalor[1], E. Asiamah[2], and M. Worku*[1], [1]*North Carolina A&T State University*, *Greensboro, NC, USA,* [2]*University of Arkansas at Pine Bluff*, *Pine Bluff, AR, USA.*

Galectins are carbohydrate-binding proteins that function to regulate immune and inflammatory response. The expression of galectin genes *LGALS*1, *LGALS*3, and *LGALS*9 has been associated with innate and adaptive immunity. Bioactive plant-derived polyphenols enhance immunological health in animals. Studies have shown that bioactive plant compounds are able to bind and regulate galectins in inflammatory diseases. Polyphenols derived from *Moringa oleifera* have antioxidant and anti-inflammatory properties but their effect on galectin expression in the bovine peripheral blood mononuclear cells (PBMC) has not been studied. The objective of this study was to investigate the effects of *Moringa oleifera* polyphenol extract (MOPE) on galectin gene transcription and translation in LPS-challenged bovine PBMC *ex vivo.* Bovine PBMC was isolated from blood collected from lactating Holstein cows (n = 10; age = 3.73 ± 0.35 yr, milk yield = 25.71 ± 3.11 kg/cow per day) using Ficoll technique. LPS-challenged PBMC (1.0 µg/mL *Escherichia coli* O111:B4), was incubated with MOPE (10 µg/mL) for 3 h. Quantitative real-time PCR (qRT-PCR) was used to evaluate the mRNA levels of bovine galectin 1 (*LGALS1*), galectin 3 (*LGALS3*), and galectin 9 (*LGALS9*), using *TBP*, *ACTB*, and *RPLP0* as internal controls. Secretion of GAL-1, GAL-3, and GAL-9 in culture supernatant was measured using ELISA. Data were analyzed by GLM procedure (SAS 9.4). Results from the qRT-PCR showed decreased mRNA of *LGALS1* (fold change, FC = −2.01), *LGALS3* (FC = −3.84) and *LGALS9* (FC = −3.98) after treatment with MOPE. Treatment with MOPE decreased mean concentration of GAL-1 (41.71 vs. 172.76 ng/mL; $P < 0.01$), GAL-3 (3.30 vs. 9.95 ng/mL; $P < 0.01$) and GAL-9 (854.05 vs. 2336.76 pg/mL; $P < 0.01$). Overall, results from this study showed that polyphenol from *Moringa* decreased GAL-1, GAL-3 and GAL-9 mRNA and protein expression in bovine PBMC. Our finding suggest the use of polyphenols extract from *Moringa* enriched feed supplements may have immunomodulatory properties for bovine health, and aid in the design of galectin-based strategy to counteract LPS-induced inflammation and morbidities.

Key Words: bovine PBMC, galectin, *Moringa*

NANP Nutrition Models Workshop

1 Welcoming remarks. J. McNamara*, *McNamara Research in Agriculture Firm, Pullman, WA.*

The animal sciences are made up of a wide range of processes from single gene inheritance through cellular, organ, whole-animal, herd, farm, system, and economic and social processes. Research over the last 100 years has given us tremendously detailed insight and massive data sets on all aspects of food animal production. Nutrition and feeding, including all the interactions with genetics, the environment and reproduction are central to supplying health and efficient animals for a healthy human population. Although no one or one research group can simultaneously teach or do research in all these sections, our charge to improve the security, safety and efficiency of the animal food product system dictates that several efforts integrate 2 or even many of these systems toward a common end goal. Many government, NGO and private companies and organizations now are involved in and in fact require complex approaches to solving the complex issues of food production. Various research and educational approaches are valid and useful, but they all make use of sound quantitative biological and statistical approaches, including various types of mathematical and statistical modeling techniques. The National Animal Nutrition Program and the National Research Support Program exist to support and encourage excellence in education and research in Animal Sciences Nutrition and all aspects of the food system that are integrated with that (we don't feed animals in a separate system, the inputs into the animals and outputs from them are an integral part of the system as a whole. The workshop today encompasses a variety of useful concepts and practices that will allow the student or scientist at any stage of their career to more fully understand and use a variety of biological, mathematical, and statistical approaches to systems biology research and education. The topics, practices, and information will be useful for anyone starting a career in the animal sciences or wishing to integrate their existing teaching and research into a relevant systems approach to providing a wholesome food supply.

Key Words: systems biology, nutrition, modelling

2 Tutorial on R software. T. Hackmann*, *University of California, Davis, Davis, CA.*

R statistical software will be used throughout the NANP Nutrition Models Workshop. The objective of this tutorial is to familiarize workshop attendees with use of this software. Specifically, this tutorial will walk attendees through basic data manipulation, visualization, and other functions. Assistance will be available for attendees who have difficulty installing the software on their laptops.

Key Words: R, tutorial, modeling

3 Estimation of parameter values: Lecture and exercises. M. D. Hanigan*[1] and V. L. Daley[1,2], [1]*Virginia Tech, Blacksburg, VA,* [2]*National Animal Nutrition Program, University of Kentucky, Lexington, KY.*

Application of nutritional knowledge generally requires expression in mathematical form. It is insufficient to conclude that animals should be fed more of a nutrient; the recommendation must include an estimate of how much more. Thus, models that accurately and precisely represent animal responses to varying nutrient supply are a critical product of nutrition science. They also allow quantitative hypothesis testing which guides the scientific process. Hence, the construction of models and the derivation of parameter estimates for those models are a critical component of nutrition science. For this learning exercise, it is assumed the participant is proficient in the use of R and the use of linear and nonlinear regression functions, and has participated in the National Animal Nutrition Program Level 1 Workshop, which includes a module on building a portion of this model, or has gained that expertise through self-study. Participants will be given a data set and a model containing 4 components that interact. These are (1) a pool of insoluble N in the rumen, (2) a pool of soluble N in the rumen, (3) a pool of ruminal microbes that are consuming soluble N, and (4) digestion and absorption of N from the small intestine. The model will be fitted to observed ruminal and fecal N outflow data to derive parameter estimates for the conversion of insoluble N to soluble N and the fractional use of the soluble N in support of microbial growth, and digestion of N in the intestines. The exercise demonstrates the use of an optimizer to fit model parameters to observed data.

Key Words: mathematical model, parameter estimation, instruction

4 Cross validation and bootstrapping: Part I (lecture). J. A. D. R. N. Appuhamy*[1] and L. E. Moraes[2], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Department of Animal Science, The Ohio State University, Columbus, OH.*

Cross-validation (CV) and bootstrapping are resampling methods that refit a model to samples drawn from the data. CV helps in choosing a "best" model associated with the lowest prediction error rate, whereas bootstrapping allows determining the uncertainty of parameter estimates. One may be tempted to use whole data set to develop and evaluate a model simultaneously. This approach however has issues such as overfitting and thus selects models that would potentially fail on an independent data set. These limitations can be overcome successfully with CV. Traditionally, CV is applied by splitting the data into 2 sets training, test that are used for model development and evaluation, respectively. This method called Hold-out is not recommended particularly for small data sets as the error rate would depend exclusively on the split and be misleading for a different split. Data splitting methods such as K-fold and Leave-one-out are recommended to overcome those limitations. K-fold CV involves dividing the data into K number of samples and holding out one as the test set to determine the error rate. In leave-one-out CV, only one observation is held out at a time as the test set. In both cases, the true error rate for models with continuous responses is generally estimated as the average of the separate error estimates. Bootstrapping is a powerful statistical tool involving resampling with replacement and commonly used to quantify standard error or the confidence interval of statistical estimates. Consequently, bootstrapping allows for determining bias, standard errors, and confidence intervals of statistical estimates. Traditionally, the uncertainty of model parameters are estimated by deriving the sampling distribution based on assumptions about distribution of the population. In contrast, bootstrapping allow estimating the uncertainty without explicitly deriving the sampling distribution that way although it is important to keep in mind that the bootstrap depends on the bootstrap principle "Sampling with replacement behaves on the original sample the way the original sample behaves on a population."

This lesson will cover the principles and implementations of CV and bootstrapping for models frequently used in animal nutrition.

Key Words: model evaluation, prediction error, resampling

5 Cross validation and bootstrapping: Part II (exercises). J. A. D. R. N. Appuhamy*[1] and L. E. Moraes[2], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Department of Animal Science, The Ohio State University, Columbus, OH.*

Here we demonstrate a few applications of cross validation and bootstrapping in evaluating the predictive ability and determining uncertainty of the parameter estimates of a linear regression model using R, a freely available and widely used statistical programming language. The packages such as "design," "DAAG," "caret," and "boot" are capable of performing cross validation of linear models in R. The "boot" package particularly provides extensive facilities for bootstrapping and thus estimating the standard error or confidence interval of a single statistic (e.g., mean), or a vector (e.g., regression coefficients). A data set including a given number of enteric methane emission (CH_4) measurements, and corresponding dry matter intake (DMI) and dietary fat content is used. A simple linear regression model to predict CH_4 is first developed including DMI and evaluated separately using Hold-up, K-fold, and Leave-one-out cross validation methods. The outputs are discussed and the methods are compared related to the variability of MSPE, and computational cost. The K-fold cross validation is performed with traditional K = 10 (90% of data for training and 10% for test), and compared with lower (K = 5) and higher (K = 20) number of folds. One of the issues with K-fold cross validation is that it often has a high variability, if performed multiple times on the same data. The replicated K-fold cross-validation method addresses this issue by performing the whole process several times averaging over replications. Therefore, we perform replicated K-fold cross validation and compare the MSPE with previous values. We then use our simple prediction model to demonstrate an application of nonparametric bootstrapping to estimate bias, standard error, and 95% confidence interval of the parameter estimate. Histograms and normal quantile-comparison plots for the bootstrap replications are obtained and discussed. If time permits, the bootstrapping will be repeated with a multiple regression model including both DMI and dietary fat content. The data and all the R scripts will be available in advance for download.

Key Words: confidence interval, K-fold cross validation, standard error

6 Automated model selection: Part I (lecture). V. L. Daley*[1], T. J. Hackmann[2], and M. D. Hanigan[3], [1]*National Animal Nutrition Program (NANP), University of Kentucky, Lexington, KY,* [2]*University of California, Davis, CA,* [3]*Virginia Tech, Blacksburg, VA.*

Automated model selection (AMS) is a procedure to select the best model from a set of candidate models (multi-model inference). This approach can be very useful when the investigator is dealing with a large number of predictor variables to explain a subject of interest (dependent variable). The objective of this lecture is to present the concepts of AMS and illustrate how this procedure can be used in the development of empirical models in Animal Science. The attendees should have some experience in data analysis and empirical models. At the beginning, the hypothesis, objectives, and potential variables associated with the subject of study will be discussed. Then, key concepts in development of a meta-analytic data set and examples from the NANP website (https://animalnutrition.org) will be presented. Data should be assessed for biological coherence and outliers need to be removed. For AMS, a mixed model is fitted using all predictor variables that potentially affect the dependent variable (global model), then a set of sub-models are derived from the fixed terms of the global model. The parameters of those models are estimated by the maximum likelihood method. The AMS approach can use one or more information criterion, but the Akaike's information criterion corrected for small sample size (AICc) is often adopted. All candidate models are ranked based on the lowest AICc to obtain model weights, then the best set of candidate models are selected. The variance inflation factor (VIF) is used to evaluate the correlation between predictors in the model, and biological coherency of the best models are evaluated. The final stage compares the best candidate models selected (often <10 models) using the root mean square error (RMSE) and the concordance correlation coefficient (CCC). AMS is useful for the development of prediction models when a large set of potential predictor variables are available.

Key Words: multi-model inference, empirical models, review

7 Automated model selection: Part II (exercises). V. L. Daley*[1], T. J. Hackmann[2], and M. D. Hanigan[3], [1]*National Animal Nutrition Program (NANP), University of Kentucky, Lexington, KY,* [2]*University of California, Davis, CA,* [3]*Virginia Tech, Blacksburg, VA.*

Automated model selection (AMS) can be applied in different research areas for the selection of the best fitting models. The objective of this exercise is to apply the AMS approach as a tool for the selection of prediction models. A hypothetical example will be used to help the audience better understand and apply AMS. This exercise will use RStudio program, which can be freely downloaded from the internet. As an example, the investigator will develop empirical models to predict the dry matter intake of lactating dairy cows using AMS and parallel computation in R (MuMIn). A meta-analytical data set from the National Animal Nutrition Program (NANP, https://animalnutrition.org) is available for this exercise. First, data quality and range will be checked to identify and remove outliers, and data will be visualized (ggplot2). A global mixed model (lme4) will be fitted to the data using all potential predictor variables from the data set. A set of candidate models will be generated using combinations of the fixed terms of the global model. Then, Akaike's information criterion corrected for small sample size (AICc) is calculated and used to rank the models. The candidate models are collected in a data set named "all models" for future use. The models with the lowest AICc values are collected in another data set named "best models." The estimated parameters of best candidate models are automatically collected. Only candidate models with a variance inflation factor (VIF) less than 10 are kept in the "best models" data set. Evaluation of the biological coherence and ANOVA to compare the models are performed. The best candidate models will also be evaluated using the root mean squared error of prediction (RMSE) and concordance correlation coefficient (CCC). The best 3 models are automatically collected in a table (sjPlot). During the exercise, additional instructors will be available to help the participants. The attendees will be able to undertake AMS to select the best models and apply those models in research.

Key Words: multi-model inference, mixed models, Akaike information criterion

8 Molly and other dynamic models: Part I (lecture). H. A. Rossow*, *University of California, Davis, Davis, CA.*

Mathematical models are tools to examine existing theories, find gaps in knowledge and explain phenomena such as nutrient digestion and metabolism. Simulated data from a model can be used to examine model behavior, as in sensitivity analysis, and compared with experimental data to determine if the model makes biological ‘sense’. The objective of this session is to explore how concepts or theories of nutrient digestion, metabolism, and lactation physiology are translated into mechanistic mathematical equations and combined into a whole animal model using the Molly model as an example. Molly is a mechanistic model of a dairy cow composed of a digestive element and an animal element. The digestive element converts chemical composition of the diet to volatile fatty acids, microbial growth and absorbed nutrients using physical attributes of the diet such as proportions of large and small particles and water passage. The animal element converts products from the digestive element into tissues (protein), waste products, heat production or secreted products (e.g., milk, milk fat). Three processes that have been translated into equations in the Molly model will be examined in this session: (1) Passage of digesta through the rumen described in Baldwin et al. (1977; Agric. Syst. 2:255–288); (2) milk production by the mammary gland described in Neal and Thornley (1983; (J. Agric. Sci. Camb. 101:389–400); and (3) growth (protein synthesis) described in Oltjen et al. (1986; J. Anim. Sci. 62:86–97). In the lecture, representations of these processes will be examined using conceptual diagrams and differential equations. Then a full lactation simulation will be used to demonstrate how equations of passage, protein accretion, and milk synthesis fit into the model.

Key Words: computer simulation model, dairy cow, metabolism

9 Molly and other dynamic models: Part II (exercises). H. Rossow*, *University of California, Davis, Davis, CA.*

Mechanistic models representing physiological processes can be connected to represent whole animal systems. When the models use Michealis-Menton type kinetic equations, examples of ways to connect physiological processes are (1) including central pools (blood pool) of nutrients available for physiological processes, (2) using differential equations that represent nutrient input and output relationships for nutrient pools, and (3) assuming a physiologic process is the same across all tissues, i.e., protein synthesis. Based on the 3 example processes discussed in lecture, (1) passage of digesta through the rumen described in Baldwin et al. (1977; Agric. Syst. 2:255–288); (2) milk production by the mammary gland described in Neal and Thornley (1983; J. Agric. Sci. Camb. 101:389–400); and (3) growth (protein synthesis) described in Oltjen et al. (1986; J. Anim. Sci. 62:86–97), participants will conduct a simulation exercise that uses these modeled processes in Molly to predict lactation performance. In the exercise, participants will observe effects of altering milk production processes on production of the dairy cow to understand how metabolic processes can be represented by mathematical equations to provide a conceptual framework that improves our understanding of animal biology.

Key Words: computer simulation model, dairy cow, metabolism

34th ADSA Discover Conference Mini Symposium

10 Mini-symposium on Discover 34—Re-examining amino acid and energy interactions in the dairy cow. G. A. Broderick*[1], J. A. Metcalf*[2], J. L. Firkins[3], and L. R. Miller[4], [1]*Broderick Nutrition & Research LLC, Madison, WI,* [2]*Trouw Nutrition Agresearch, Guelph, ON, Canada,* [3]*The Ohio State University, Columbus, OH,* [4]*ADSA Discover Conference Series, Centreville, MD.*

The 34th ADSA Discover Conference (DC34), "Reexamining Amino Acid and Energy Interactions in the Dairy Cow," was held May 29 to June 1, 2018. The meeting addressed the following themes: (1) pre-absorptive interactions: where protein and energy first interact; (2) post-absorptive interactions: where cows get stuff to make milk; (3) changes in energy and AA interactions over the lactation cycle; (4) translating dynamic elements of nutrient metabolism into feeding systems; and (5) research gaps and urgent needs for the dairy industry. At the meeting wrap-up, conference organizers summarized the challenges and recommendations from the 3-d conference to help focus ongoing and future research to improve the efficiency of amino acid utilization in lactating dairy cows. This mini-symposium summarizes the findings presented at DC34 and discusses strategies to implement future research related to amino acid and energy interactions. All members, whether or not they attended DC34, are encouraged to participate. The ADSA Discover conferences address important contemporary issues in food animal agriculture and are organized to provide unique opportunities for professional interactions that facilitate development and use of frontier science for the benefit of the global food industries and society. The conferences offer an environment that fosters creativity, emphasizes interaction and open discussion, and focuses on thrusts that will synergize the development and application of science. This mini-symposium is an example of conference follow-up by the Discover program to further enhance conference discussions, recommendations, and networking.

Key Words: amino acid nutrition, energy nutrition, nutrient interactions

David M. Barbano Recognition Symposium

11 Introductory remarks on a biographical presentation of our honoree, Professor David Barbano. R. Jimenez-Flores*, *The Ohio State University, Columbus, OH.*

David Barbano is a professor in the Department of Food Science at Cornell University. Dave received his BS in biology/food science in 1970 at Cornell University and his MS/PhD in food science from Cornell (MS in 1973 and PhD in 1976). He joined the Department of Food Science as an assistant professor in 1980. In 1988, he became the director of the Northeast Dairy Foods Research Center. He is a member of ADSA, IFT, IDFA, AOACI, IAMFES, IDF, and NYS Assoiation of Milk and Food Sanitarians. Dave is past president of the ADSA, and a fellow of ADSA and the Association of Official Analytical Chemists. Dave received the Harvey Wiley award of AOAC in 2010. Dave is on numerous International Dairy Federation committees for milk analysis. The National Cheese Institute (NCI) named him the 2018 NCI Laureate. The award recognizes individuals who have made significant long-term contributions to the development and growth of the cheese industry. The speakers in this special symposium are distinguished former students of Dr. Barbano; each exemplifies a different scientific and technological area of his numerous contributions to dairy science.

12 Milk composition testing: From dairy farmer payment to dairy processor efficiency to dairy farm and animal diagnostics. L. Metzger*, *South Dakota State University, Brookings, SD.*

A cornerstone of Dr. Barbano's research program is the development and application of analysis methods for milk and dairy products. A major portion of this work has focused on rapid milk component testing using mid infrared analysis. Mid infrared milk analysis is the critical technology that allowed a transition from producer payment based on milk weight and fat to payment based on multiple components (fat, protein, and other solids). The successful application of mid infrared milk analysis for producer payment as well as dairy herd improvement testing would not have been possible without the detailed and systematically research conducted by Dr. Barbano. The central theme of this research was that mid infrared milk analyzers needed to be accurately calibrated to provide useful information. This research involved numerous facets over a 30-year period including: ensuring accuracy of primary methods, identification of factors influencing mid infrared performance, and development of calibrations standards. The financial implications of this work are enormous and ensure that both producers and processors are fairly compensated for the composition of the milk they buy/sell. Subsequently, Dr Barbano facilitated the application of mid infrared analysis to improve processor efficiency during the manufacture of cheese and concentrated milk products. Finally, in the last several years, Dr. Barbano discovered that some of the factors which influence calibration of mid infrared milk analyzer could be utilized to diagnose rumen health and productivity of dairy cattle. This information has led to a new area of research and has returned him full circle back to the farm.

Key Words: mid infrared milk analyzers, milk composition, analysis methods

13 The Mozzarella/*pasta filata* years: From pizza cheese to traditional Sicilian Ragusano. J. Yun[1] and P. Kindstedt*[2], [1]*Parmlat Canada, Toronto, ON, Canada,* [2]*University of Vermont, Burlington, VT.*

In the decades that followed the end of World War II, Mozzarella cheesemaking in the United States grew steadily as the pizza restaurant established an ever-expanding footprint across America and beyond. By the 1980s, Mozzarella cheesemaking had attained unprecedented scales of production, yet even as new cheese plants were coming on line across the country and production capacities were reaching dizzying levels, the scientific and technological knowledge base needed to standardize production schedules, control product quality, and maximize cheese yields and efficiency lagged far behind the industry needs. It was within this historical context that David Barbano turned his systematic and meticulous research program toward the needs of the Mozzarella cheese industry during the 1980s. By the early 1990s, Barbano was leading a team of graduate students, post-docs, technical staff, and collaborators in a systematic evaluation of every step in the Mozzarella cheesemaking process. The end product of these studies was nothing less than the transformation of what had been (to an astonishing degree) a poorly understood "black box" process into a precisely controlled make procedure that lent itself to precision tailoring of cheese functionality, tight control over manufacturing schedules and efficiency, and maximization of cheese yields. Barbano's international collaborators also included research scientists from Italy, where Mozzarella originated. Working with them, Barbano led a systematic evaluation of the scientific and technological aspects of Ragusano cheese, a very traditional PDO *pasta filata* cheese from Sicily. In the process, Barbano's team demonstrated a new approach to traditional artisanal practices that merged both the art and science of cheesemaking, in effect combining the best of both worlds, toward the goal of sustaining traditional cheesemakers and the working landscapes that they support. Throughout studies, Barbano's research led to innovations in cheesemaking technology (such as improved salting methods, preacidification treatments and strategies to improve low-fat Mozzarella functionality), and new analytical methods to evaluate product functionality, that have revolutionized the Mozzarella industry worldwide.

Key Words: Mozzarella, cheese, p*asta filata*

14 The milk filtration revolution: Pioneering "milk refining." B. Nelson*, *Daisy Brand, Dallas, TX.*

David Barbano's extensive milk separation experience, through both cheesemaking and filtration, contributed to the development of a system that directs components to uses that maximize milk's value: "milk refining." During the early years of cross-flow filtration, the dairy industry used reverse osmosis and ultrafiltration (UF) to reduce transportation costs and improve plant throughput. Additionally, capturing proteins from whey was increasing in popularity. Microfiltration (MF) was key to the milk-refining concept because serum proteins no longer went through the cheesemaking process to be separated from casein. To realize the conceivable benefits of filtration, the dairy industry needed to understand changes to product characteristics, how to efficiently operate equipment, economic benefits, changes to analytical measurements, and other dimensions of this burgeoning unit operation. Barbano and his team delivered. They quantified milk component recoveries and cheese yield and determined aging characteristics of cheeses made from retentates. One example of the practical nuggets found throughout Barbano's research is the change from a pressure-regulating valve with a high pressure drop to a small-diameter pipe that gradually reduces pressure. This pragmatic solution alleviated the destruction of fat globules causing high levels of free fatty acids in cheese made from whole milk retentate.

Additionally, his group found that the coagulant used for cheese making, whey bleaching agents, and adsorbed proteins change UF flux. In the past 20 years, Barbano's contribution to the body of knowledge of MF cannot be overstated. His research group has determined approaches to increase the efficiency of serum protein removal, determined critical and limiting protein levels and fluxes, compared ceramic and polymeric membrane materials, elucidated foulants, compared channel geometries and diameters, altered analytical methods for accurate measurements of filtration products, and pushed the boundaries of operating conditions. Along the way he used filtration to improve calibration samples for milk testing and showed how to produce products with extended shelf life and diverse attributes. A revolution indeed!

Key Words: filtration

ADSA GSD Symposium: Grant Writing

15 Grantsmanship: Top ten tools for success in grant writing. J. Williams*, *USDA National Institute of Food and Agriculture, Washington, DC.*

The purpose of the symposium is to improve the number of applications to, and the success rate of, proposals from new investigators for USDA's National Institute of Food and Agriculture. I will provide a top 10 list of tools to assist stakeholders in developing and strengthening their grant-writing skills and knowledge. This list of tools will enhance an applicant's ability to manage and understand proposal development and provide a map to assist new investigators with navigating the proposal submission process. As an institute, it is imperative that we make a concerted effort to reach out to the graduate student community, as they are our rising new investigators. Making this community more comfortable with USDA's funding portfolio and system will increase the likelihood of success and ensure the success of our next generation of agriculture researchers and educators.

Key Words: grantsmanship, USDA, NIFA

ADSA Graduate Student Dairy Foods Poster Competition

M1 The role of sweeteners and protein source on high-protein low-carbohydrate bar shelf life. H. Keefer*, S. Nishku, and M. A. Drake, *North Carolina State University, Raleigh NC.*

A desirable protein bar has a favorable flavor and texture to consumers and a shelf life of at least 6 mo. There are several available protein sources and sweeteners, so it is important to investigate implications on bar sensory properties and shelf stability. A previous study evaluated the impact of protein source and sweetener on bar flavors and sweetness profiles. The objective of this study was to characterize the impact of sweetener and protein source on the texture and shelf life of high protein low carbohydrate bars. The protein bars (15 formulas, replicated twice) consisted of a protein source (whey, milk, or pea) and a sweetener (sucrose, fructose, monk fruit, stevia, or sucralose) with fiber and vegetable shortening. Bars (15 g protein per serving) were placed in moisture barrier pouches and stored 35 d at 35C for accelerated shelf life testing. Instrumental hardness (g) and color measurements (L a b*) were taken and descriptive analysis (DA) of texture was conducted at d 1, 7, 14, 21, 28, and 35. Linear mixed model analyses were conducted for each time point with sugar and protein source and their interaction as fixed effects. Protein source and sweetener had a significant interaction for instrumental and sensory properties ($P < 0.05$). Bars with whey protein and fructose were harder than other bars until d 21. Whey protein bars, regardless of sweetener, were denser at all time points than pea or milk protein bars. Bars made with pea protein were the driest, least cohesive, and had the fastest rate of breakdown across shelf life ($P < 0.05$). Whey protein bars had the greatest change in color compared with pea or milk protein, but all protein bar formulas visibly darkened and increased in yellow/red color over time ($P < 0.05$). Both sweetener and protein source affect protein bar characteristics and should be chosen carefully. These results are important to understand and predict how sugar reduction and/or sweetener replacement affects the sensory and textural properties of protein bars with different protein types.

Key Words: protein bar, texture, hardening

M2 Developing a blood glucose meter-based method for the rapid measurement of lactose in dairy ingredients. C. Wagner*[1,2], J. Amamcharla[1], A. Rao[2], and L. Metzger[3], [1]*Kansas State University, Manhattan, KS,* [2]*Agropur Ingredients, La Crosse, WI,* [3]*South Dakota State University, Brookings, SD.*

Commonly used lactose assays (enzymatic absorbance [EZ] and HPLC) for dairy ingredients are relatively expensive and time-consuming. A blood glucose meter (BGM)-based method has been used successfully as a rapid lactose assay in milk. The BGM method involves diluting the sample to an appropriate level with water or buffer, adding lactase enzyme, incubating at 40°C for ~15 min, and measuring the resulting glucose content with the BGM. A standard curve developed between the known lactose concentration and BGM reading is used to quantify lactose in unknown samples. The objective of this study was to evaluate the BGM-based lactose analysis method in whey- and skim milk-derived (WD and SMD, respectively) ingredients and was done in 4 phases. In phase 1, the effect of pH and lactose concentration on the BGM reading was investigated using a factorial design with 2 factors (pH: 6.03–6.89 and lactose: 0.2% or 0.4%) and found that BGM readings are significantly ($P < 0.05$) affected by pH at both lactose levels. In phase 2, the effect of total solids and ingredient type on the BGM reading was investigated using a factorial design with 2 factors (ingredient type: WD or SMD and total solids: 0–8%). It was observed that the BGM reading was significantly ($P < 0.05$) affected by both ingredient type and total solids. Phase 3 involved developing a linear relationship between the BGM reading and an EZ reference method to ascertain the accuracy of the proposed BGM method. Different ingredient type (WD or SMD) and total solids (0.5–27%) model solutions were measured using the BGM and EZ methods. The percent bias (BGM method – EZ method) was found to be between 2.2 and 6.8%. In phase 4, 15 samples procured from commercial sources were evaluated using the BGM method and EZ method as the reference method. High lactose (>47%) and low lactose (<13%) samples yielded absolute biases between −5.3 and 1.4% and 0.3 and 0.8%, respectively. Overall, the BGM method is a promising tool for rapid, low-cost analysis of lactose in both high-lactose and low-lactose dairy ingredients.

Key Words: lactose analysis, biosensor, protein ingredients

M3 Characterization of milk and soy phospholipid liposomes and their effects on inflammation using an adipocyte model. E. Kosmerl*, D. Rocha-Mendoza, I. García-Cano, O. Ziouzenkova, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

Milk phospholipids (PLs) are valuable dairy components and appear to impart human health benefits, including improved cognitive function in infants and adults. Furthermore, PLs have been shown to lower LDL cholesterol and improve intestinal barrier function. The commercial food industry uses other dietary sources of PLs, such as soy lecithin. However, it remains unclear whether dissimilar composition of PLs from different dietary sources convey the same benefits. The hypothesis of this work was that milk and soy PLs (MPLs and SPLs, respectively) will produce different physiological responses in cell culture. Our objective was to develop a cell-based method to compare the potential health benefits of milk and soy PLs to ultimately focus on inflammation. To improve stability of PLs in cell culture media, liposome structures made from milk and soy PLs (MPL and SPL, respectively) were prepared, optimized, and fully characterized. Large and stable unilamellar vesicles (LUVs) were attained with particle sizes of 232.1 ± 7.5 and 221.8 ± 10.0 nm in diameter and zeta potentials of −16.41 ± 2.49 and −28.01 ± 2.81 mV for MPL- and SPL-LUVs, respectively. Subsequently, 3T3-L1 adipocytes were treated with 0.05, 0.25, 0.5, and 1.5 mg/mL MPL- or SPL-LUVs and analyzed for changes in cell viability and cytotoxicity. The optimized conditions showed that the non-toxic, physiological range for cell culture was between 0.05 and 0.5 mg/mL. There also was a significant difference ($P < 0.05$) between cell viability of MPL- and SPL-LUVs treated cells at 0.25 and 0.5 mg/mL, suggesting that MPL-LUVs may have greater bioavailability compared with SPL-LUVs. These findings lead to our prediction that MPL-LUVs will have a greater anti-inflammatory effect than SPL-LUVs because of differences in PL composition, specifically sphingomyelin. To test this, adipocytes will be stimulated with bacterial endotoxins and characterized for NF-kB-mediated inflammation after treatment with LUVs. We propose that PL compositional differences may be the basis for differences in physiological response.

Key Words: phospholipids, liposomes, inflammation

M4 Forward osmosis concentration of skim and whole milk at different temperatures: effect on flux and milk powder quality. A. Beldie* and C. I. Moraru, *Cornell University, Ithaca, NY.*

Milk concentration is typically achieved either by thermal evaporation or reverse osmosis (RO). Thermal evaporation leads to cooked flavor and color changes, and it is energy intensive. Additionally, thermal evaporators are prone to fouling and biofilm formation. On the other hand, RO is affected by fouling, which limits the achievable concentration level. The main objective of this work was to evaluate forward osmosis (FO) as an alternative method for concentrating milk, at different temperatures. Another objective was to assess the quality of milk powders obtained by spray drying the FO concentrates. Pasteurized skim and whole milk (Cornell Dairy, Ithaca, NY) was concentrated at 4°C, 15°C and 25°C, in triplicate, using a laboratory scale FO unit (Ederna, France), equipped with a polymeric membrane. Concentrates were dried using a lab-scale spray drier (Armfield, UK). Data were analyzed statistically by one-way ANOVA. For skim milk, water flux at the beginning of the process (15–20 min) was 2.21 ± 1.64 L/(m^2h) at 25°C, 2.98 ± 0.62 L/(m^2h) at 15°C, and 2.52 ± 0.39 L/(m^2h) at 4°C. These values were not statistically different ($P > 0.05$). Comparable initial fluxes were obtained for whole milk: 2.29 ± 0.94 L/(m^2h) at 25°C, 2.67 ± 0.26 L/(m^2h) at 15°C and 2.59 ± 0.23 L/(m^2h) at 4°C. Flux decreased with time under all processing conditions. Flux drop was less pronounced at higher temperatures for both skim and whole milk: for whole milk, the flux after 8h reached 52% of the initial flux at 25°C, 30% at 15°C and 24% at 4°C. Higher temperatures also allowed faster FO concentration. For skim milk, 40°Bx was reached after 7h at 25°C, after 8.5h at 15°C and after ~10h at 4°C. Whole milk concentration was slower, with 30°Bx achieved after ~7h at 25°C, 8h at 15°C and ~9h at 4°C. No differences in reconstitution behavior were observed for skim powders obtained from FO concentrates vs thermal concentrates. Slight differences in color were observed, but they were not significant ($P > 0.1$). These results demonstrate that FO is a viable method for nonthermal concentration of milk, able to achieve concentration factors higher than RO, comparable to thermal evaporation.

Key Words: forward osmosis, milk concentration, nonthermal processing

M5 Utilization of acid whey as a fermentation aid to developing an enriched feed ingredient.

A. Mayta-Apaza*, I. García-Cano, D. Rocha-Mendoza, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

The processing of strained yogurts, and fresh cheeses, such as ricotta or cottage, generates large amounts of waste streams known as acid whey (AW) that require significant capital investments for proper disposal. Similarly, the fishery industry produces about 45% of the whole fish as waste at the end of its production chain. To valorize these by-products, this study is based on the hypothesis that fermentation of the mixture of AW and fish waste (FW) would break down complex nutrients, increasing its nutritional value for aquaculture. The objective of this study was to optimize fermentation parameters using lactic acid bacteria (LAB) and a mixture of AW and FW. The first step was screening of the LAB in the OSU "Parker Chair" LAB Collection to identify the strain with the highest proteolytic activity. Then, 3 fermentation systems (FS) were inoculated with *Lactobacillus rhamnosus* and set up as follows: 1) FS1: 36% FW, 12% fish muscle, 48% AW, and 4% molasses; 2) FS2: 38% FW, 12% fish muscle, and 50% AW; 3) FS3: 48% FW, 48% AW, and 4% molasses. The fermentation lasted 14 d at room temperature, and samples were collected every 48 h. The soluble protein concentration and peptide concentration of the samples were evaluated with colorimetric assays (Bradford and cadmium-ninhydrin), SDS-PAGE, pH, and microbial count of LAB was monitored simultaneously. The results showed a significant increase ($P < 0.05$) of peptides over time in the 3 fermentation systems. However, FS2 had 3.6 times more peptides than that of day zero's, and the values of Bradford and SDS-PAGE images were consistent with the results presented in the peptide assay. The pH dropped significantly ($P < 0.05$) from 5.88 to 4.09 for FS1 and FS3 on the first 6 d due to the content of molasses and lactic acid production. Conversely, FS2 had a significant increase from 5.96 to 7.07. The LAB count fluctuated within each fermentation system and over the 14 d. In conclusion, this study presents an alternative to the current handling of acid whey and fish waste, exploiting their residual nutrients for delivery of higher quality protein to animal diets with minimal treatment.

Key Words: acid whey, lactic acid bacteria, protein hydrolysis

M6 A natural antimicrobial from *Bacillus subtilis,* a predominant constituent of membrane biofilms.

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Current cleaning and sanitation protocols may be ineffective in cleaning separation membranes and result in the formation of resilient multispecies biofilms. These old biofilms may show bacterial predominance on prolonged use of the membranes. In our previous study, we isolated organisms such as *Bacillus subtilis*, *Bacillus licheniformis*, *Exiguobacterium aurantiacum*, and *Acinetobacter radioresistens* from an 18-mo-old reverse osmosis membrane. Competitive exclusion studies revealed the predominance of *B. subtilis* within the membrane biofilm microflora. This study investigates the antimicrobial activity of *B. subtilis* as a cause of its predominance. The culture was incubated in tryptic soy broth (TSB) at 37°C, and microfiltered to prepare cell-free extracts (CFEs) at 8-, 10-, 12-, 14-, 16- and 18-h intervals. The CFEs were freeze-dried and re-suspended in minimum quantities of HPLC grade water to create concentrated solutions. The antimicrobial activities of CFEs were tested using agar-well assay against the biofilm constitutive microflora and some common food pathogens. The experiments were conducted in triplicates and means were compared for significant differences using a general linear mixed model procedure of SAS. The results indicated the highest antimicrobial activity of 12 h CFE of *B. subtilis* against other *Exiguobacterium* sp., *E. auranticum*, *A. radioresistens*, *Listeria monocytogenes*, and *Bacillus cereus*, with average inhibition zone sizes of 16.5, 16.25, 20.6, 18.0, and 13.8 mm, respectively. On treating with proteinase K, the CFE completely lost its antimicrobial activity, establishing it to be a proteinaceous compound. The amino acid profiling revealed the total crude protein in CFE to be 51% (wt/wt) having its major constituent to be glutamic acid (13% wt/wt). The CFE was thermally stable on exposure to the common temperature used for sanitizer applications (23.8°C for 5 and 10 min). Based on this study, the proteinaceous antimicrobial compound produced by *B. subtilis* may result in its predominance.

Key Words: biofilms, antimicrobial activity, predominance

M7 Manufacturing low-spore-count skim milk powders by combining optimized raw milk holding conditions and hydrodynamic cavitation.

N. Awasti*, P. Chaudhary, and S. Anand, *Department of Dairy and Food Science, South Dakota State University, Brookings, SD.*

The presence of high numbers of *Bacillus* spores in skim milk powder limits its application for creating UHT products. The dairy industry has been emphasizing on developing strategies for reducing sporeformers and spores in skim milk powder. Previous studies conducted in our lab showed that it is possible to produce lower spore count skim milk powder, either by optimizing raw milk holding conditions or by using hydrodynamic cavitation as a process intervention. It was hypothesized that by combining the 2 processes it may be possible to further lower the sporeformers and spore counts in the final product. Pilot-scale challenge studies were conducted by spiking raw skim milk with approx. 4.0 log cfu/mL sporeformers and 2.0 log cfu/mL spores of *Bacillus licheniformis*. The inoculated raw skim milk was divided in to 3 parts and exposed to 3 treatment (T1, T2 and control) for keeping the spore and sporeformer populations low in raw milk, before manufacturing skim milk powder. Powder prepared using treatment 1 (T1) includes raw milk holding at 4°C for 24 h, treatment 2 (T2) includes holding at 4°C for 24 h combined with 2 pass hydrodynamic cavitation, whereas, control includes holding raw spiked skim milk at 10°C for 4 h and then at 7°C for up to 72 h (PMO based conditions). Spiked raw milk samples from all 3 treatments were HTST pasteurized (73°C for 15 s), evaporated, and dried (outlet and inlet temperature of 200 and 95°C) to obtain skim milk powders. Spore and sporeformer counts of samples from 3 treatments were statistically compared after initial treatments (T1, T2, and control), pasteurization, evaporation, and drying steps. Final spore and sporeformer counts from powder prepared using T1 (0.58 ± 0.04, 1.82 ± 0.05 log cfu/g), T2 (0.33 ± 0.27, 1.49 ± 0.07 log cfu/g) and control (2.74 ± 0.03 and 1.03 ± 0.06 log cfu/g) were significantly different ($P < 0.05$) with respect to each other. Our results demonstrate that combining cavitation with optimized raw milk holding conditions (treatment T2) produces skim milk powder with least sporeformers and spore counts.

Key Words: cavitation, spore, powder

M8 Effect of lactic acid and galactose on the physical, chemical, and thermal properties of deproteinized whey powders. P. Thorakkattu* and J. Amamcharla, *Kansas State University, Manhattan, KS.*

Deproteinized whey (DPW) is obtained as a permeate fraction during ultrafiltration of whey and generally contains a minimum of 76% (db) lactose. It is further processed into a non-hygroscopic and free-flowing DPW powder by crystallization and spray drying. The DPW may undergo changes such as caking and Maillard browning during storage. The aim of this study was to investigate the effect of different levels of lactic acid (LA) and galactose (GA) on the physical, chemical, and thermal properties of DPW powders. Two lots of concentrated permeate with total solids of ~77% were procured from a commercial manufacturer. The total solids were adjusted to 45% TS and was divided into 9 subsamples with treatment combinations of high, medium, low levels of lactic acid by adjusting the pH to 5.0 by adding 0.2% lactic acid, pH 5.55 with no addition and pH 6.10 by adding 2% NaOH and galactose (0, 0.15, and 0.30%) as per the experimental design. The DPW powders were spray dried in a lab-scale spray dryer with inlet temperature 180°C and the outlet air temperature 83–88°C. The water activity (a_w) and L* values of the powders immediately after spray drying were not significantly different ($P > 0.05$) and was in the range 0.074 to 0.098 and 91.89 to 92.81, respectively. It was observed that the pH of the powders decreased with increase in lactic acid contents. The pH of low LA and high LA powder were found to be 6.60 and 5.78, respectively. The titratable acidity also increased with increasing lactic acid content and ranged from 0.03 to 0.08% LA. The hygroscopicity values were not significantly different ($P > 0.05$) for the powders and were determined to be in the range 7.8 to 11.3% and the caking tendency of the powders ranged from 97.73 to 98.55%. The thermal properties of the stored DPW powders were determined with differential scanning calorimetry and a dehydration endothermic peak at around 142°C was observed which represented loss of crystalline water. Overall, the DPW powders did not show any significant difference for all the treatment combinations immediately after production.

Key Words: deproteinized whey powder, lactic acid, galactose

ADSA Graduate Student (MS) Production Poster Competition

M9 Experimental heat stress alters the fecal microbiome of lactating dairy cows. M. Witzke*, R. Rodrigues, E. Shangraw, A. Ericsson, T. McFadden, and P. Adkins, *University of Missouri, Columbia, MO.*

The objective of this study was to evaluate the effects of heat stress on the fecal microbiome of lactating dairy cows. We hypothesized there would be an increase in the richness and diversity of the bacterial communities in the feces of heat stressed cows. Six Holstein cows were housed in tie stalls in an environmental chamber. Cows averaged 175 ± 7 d in milk, 1.5 ± 0.5 parities and 36.3 ± 3.7 kg/d of milk and were fed and milked twice-daily. Cows were allowed 5-d acclimation to the chambers (d −5 to 0; temperature humidity index (THI)~65) and were then subjected to constant heat stress for 16d (d0 to 16; THI~76), followed by a 9d recovery period (d 16 to 24; THI~66). Feed and water were available ad libitum. Fecal samples were collected per rectum on d −1, 0, 6, 13, 16, 20 and 24. Samples were immediately frozen at −20°C. Fecal DNA was extracted using PowerFecal kits (Qiagen), the V4 hypervariable region of the 16S rRNA gene was sequenced using the Illumina MiSeq platform, and operational taxonomic units (OTUs) were assigned to the SILVA database using BLAST based on a 97% nucleotide identity to evaluate richness and composition of fecal bacterial populations. Number of OTUs, and diversity assessed by the Shannon Index (Bonferroni *t*-test), increased approximately 20% and 5%, respectively, during heat stress compared with d −1 ($P < 0.05$, ANOVA). Diversity returned to d −1 levels by d 20 whereas the total number of OTUs was not fully restored until d 24. Principal component analysis (Bray-Curtis distances) revealed that community composition was similar during acclimation but diverged during heat stress and into the recovery period ($P < 0.001$, PERMANOVA). Results revealed individual OTUs displaying markedly different patterns of abundance across the experiment ($P < 0.05$). For example, compared with the acclimation period, Bacteroides species abundance increased 112% by d 16 of heat stress, then increased an additional 15% by d 24. We conclude that heat stress altered the fecal microbiome of lactating dairy cows affecting both diversity and abundance of individual OTUs. Altered fecal microbiome may impact gut health or environmental pathogen loads.

Key Words: bacteria, hyperthermia, metagenomics

M10 Rectal temperature, respiration rate, and heart rate of slick-hair and wild-type lactating Holstein cows under heat stress. C. G. Ríos-Solís*, N. R. Cid-Hernández, E. Ruiz-Cortés, E. Valencia, J. E. Curbelo-Rodríguez, and G. Ortiz-Colón, *University of Puerto Rico, Mayagüez, PR.*

Dairy cattle in the tropics are exposed to chronic heat stress, which have an effect on the cow's ability to maintain body temperature at normal levels. The objective of this experiment was to evaluate rectal temperatures (RT), heart rates (HR), and respiration rates (RR) in lactating Holstein cows with 2 hair genotypes, slick-hair (SL) and wild-type (WT). An allelic discrimination assay was used to determine the presence or absence of the SL gene mutation. Slick-hair (n = 8; BW 595.1 kg ± 5.9; DIM 135.4 ± 28.5; milk yield 20.5 kg ± 0.9) and WT (n = 8; BW 563.1 kg ± 4.9; DIM 145 ± 28.4; milk yield 19.5 kg ± 0.9) Holstein cows were used in this experiment that lasted 60 d. Animals were fed ad libitum a TMR based on Brachiaria spp. forage, soybean meal, steam flaked corn grain, brewers' grains, and minerals. The average ambient temperature during the study was 25.7°C ± 0.03, while the average ambient humidity was 78.5% ± 0.24, resulting in an average temperature-humidity index (THI) of 75.8 ± 0.03. Weekly RT, RR, and HR were determined at 0600 and 1800h. A digital thermometer was used to determine RT. A stethoscope was used to determine HR, while RR was determined by means of visual observation of the flank for 1 min. Weekly averages of RT, RR, and HR were analyzed using Proc Mixed of SAS (SAS University Edition, 2018) using a model that included genotype (SL and WT), week and their interactions as fixed effects. For each parameter, week was used as a repeated measurement, with cow as the subject. There was no interaction between genotype and week in terms of RT, RR, and HR ($P \geq 0.29$). While SL had an average RT of 38.4°C ± 0.08; WT showed a higher RT of 38.7°C ± 0.08 ($P < 0.0001$). Likewise, while SL had an average RR of 43 ± 1.08 respirations per minute, WT had a higher average RR of 59 ± 1.18 respirations per minute ($P < 0.0001$). Moreover, whereas SL had an average HR of 70 ± 0.13; WT showed a higher HR of 73 ± 1.14 ($P < 0.05$). Overall, under the conditions of this experiment, SL and WT dairy cows seem to experience and cope with heat stress in a dissimilar manner. Slick-hair cows seem to be better adapted to conditions of heat stress.

Key Words: slick hair, heat stress, heart rate

M11 Evaluating the impact of stage of life on anti-Müllerian hormone in dairy cattle. K. Alward*[1], W. Graves[1], R. Palomares[2], A. Nelson[1], and J. Bohlen[1], [1]*Animal and Dairy Science, University of Georgia, Athens, GA,* [2]*College of Veterinary Medicine, University of Georgia, Athens, GA.*

Anti-Müllerian hormone (AMH) is produced by granulosa cells, found in early, antral follicles on the ovary. Higher circulating AMH concentrations are indicative of a larger number of viable follicles present and published data shows that heifers with high AMH have longer productive lives than low AMH counterparts. The objective of this study was to examine whether stage of life at sampling affects AMH level and to describe changes in AMH level from virgin heifers through calving and early lactation. Virgin Holstein heifers (n = 111) meeting minimum weight and height requirements were enrolled pre-breeding at 13–15 mo of age. Upon enrollment, blood was collected and analyzed for AMH (Ansh Labs, Webster, TX) and transrectal ultrasonography was performed to record antral follicle count (AFC). In addition, presence of corpora lutea (CL) were noted for cyclicity status and any reproductive tract anomalies were recorded. Heifers were inseminated upon standing estrus with all service data recorded. After calving, blood was collected at 5–20 d fresh and at 45–60 d in milk (DIM) to analyze for AMH concentration. Transrectal ultrasonography was also performed at 45–60 DIM and the same parameters were recorded as previously described. Following sampling, heifers were split based on AMH concentration to form a HIGH AMH group (>275 pg/mL) and LOW AMH group (<275 pg/mL) for analysis. AMH was consistent across stages of life with animals maintaining their categorization as HIGH or LOW throughout all 3 sampling times ($P < 0.0001$). As heifers, the high AMH categorization correlated with fewer services per conception ($P < 0.0001$). AMH and AFC were positively correlated when evaluated as heifers and at 45–60 DIM in the lactating herd ($P < 0.0001$). AMH was in the highest concentration within the heifer group ($P < 0.0001$) while the 45–60 DIM sample was higher than fresh ($P < 0.0001$). This data indicates that calving may temporarily depress AMH concentrations; however, these animals recover and maintain their AMH categorization.

Key Words: anti-Müllerian hormone, stress, fertility

M12 Effects of timing of artificial insemination and use of semen extenders on fertility of dairy heifers subjected to timed artificial insemination. C. C. Figueiredo*[1], D. Z. Bisinotto[1], R. C. Chebel[1], R. Le Boucher[2], S. Camugli[2], E. Schimitt[2], C. Arnoult[3], W. W. Thatcher[1], and R. S. Bisinotto[1], [1]*University of Florida, Gainesville, FL,* [2]*IMV-Technologies, L'Aigle, France,* [3]*University of Grenoble Alpes, Grenoble, France.*

Approximately 25% of dairy heifers subjected to timed AI are observed in estrus 24 h before prescheduled AI, creating an asynchrony between ovulation and insemination. Objectives were to evaluate the effects of timing of AI and use of 2 semen extenders (SE) on pregnancy per AI (P/AI) and pregnancy loss. Holstein heifers ($\geq$13 mo of age, body weight $\geq$350 kg, wither height $\geq$122 cm) received GnRH and an intravaginal insert containing progesterone on study d −8. Inserts were removed 5 d later and heifers received injections of $PGF_{2\alpha}$ on d −3 and −2, followed by GnRH on d 0. Heifers were assigned randomly to receive timed AI with untreated semen on d 0 (72 h after first $PGF_{2\alpha}$; positive control; 72-CON; n = 103), timed AI with untreated semen on d −1 (48 h after first $PGF_{2\alpha}$; negative control; 48-CON; n = 100), timed AI on d −1 with SE1-treated semen (48-SE1; n = 98), timed AI on d −1 with SE2-treated semen (48-SE2; n = 102). A total of 4 bulls were used. Sample size was sufficient to detect a 20-percentage point difference among treatments (55 vs. 35%; $\alpha = 0.05$; $\beta = 0.20$). Heifers were fitted with automated estrus detection monitors. Pregnancy was diagnosed by transrectal ultrasonography 29 and 54 d after AI. Data were analyzed by logistic regression. Statistical models included fixed effects of treatment and enrollment week. Orthogonal contrasts were used to assess the effects of day of AI (72-CON vs. 48-CON+48-SE1+48-SE2), use of semen extenders (48-CON vs. 48-SE1+48-SE2), and extender type (48-SE1 vs. 48-SE2). Onset of estrus between 24 and 48 h after first $PGF_{2\alpha}$ was detected in 66.6% of heifers. Pregnancy per AI on d 29 (72-CON = 60.1, 48-CON = 35.4, 48-SE1 = 39.8, 48-SE2 = 32.2%) and 54 (72-CON = 57.1, 48-CON = 31.7, 48-SE1 = 36.0, 48-SE2 = 29.7%) was greater ($P < 0.001$) for heifers inseminated on d 0 compared with d −1; however, no effect semen extender or extender type was observed. Treatment did not affect pregnancy loss (72-CON = 3.1, 48-CON = 7.9, 48-SE1 = 7.3, 48-SE2 = 5.6%). Hastening AI by 24 h decreased likelihood of pregnancy, which was not improved by the use of semen extenders.

Key Words: semen extender, fertility, sperm lifespan

M13 Using 30-h in vitro NDF digestibility of feedstuffs in ration formulation: evaluation of predictions for milk and methane production in lactating dairy cows. K. C. Krogstad*[1], D. L. Morris[1], P. J. Kononoff[1], and K. J. Herrick[2], [1]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE,* [2]*POET Nutrition, Sioux Falls, SD.*

In vitro NDF digestibility (NDFd) is often used to determine forage quality, and its increased availability has spurred interest for use in ration formulation procedures. The objectives of this study were to compare the predictions of metabolizable energy (ME) allowable milk and methane production when default NDFd values or lab-determined 30-h NDFd were used in the Cornell Net Carbohydrate and Protein System (CNCPS; v 6.5). We hypothesized that the use of 30-h NDFd would improve ME-allowable milk and methane predictions from CNCPS. The 30-h NDFd was measured on forages and byproducts included in diets fed to lactating dairy cows. Predictions of carbon dioxide production and DMI were also evaluated. Treatment means (n = 32) originating from 8 energy balance studies were assembled to evaluate the prediction of ME allowable milk and methane production when either a default value or observed 30 h NDFd was entered into the feed library. Of the 32 means, 26 were made on Jersey cows and 6 were made on Holstein cows. Our data set averaged 18.8 ± 2.26 kg of DMI, 25.8 ± 6.00 kg of milk, and 15.1 ± 2.65 L of methane/kg of milk. Headbox-style indirect calorimeters were used to measure methane and carbon dioxide production in all studies. Comparison statistics were generated using R (v 3.5.2) and predictions were evaluated based upon the root mean square prediction error (RMSPE). A concordance correlation coefficient (CCC) was used to evaluate the agreement between the predicted and observed values. Carbon dioxide and the mean of the upper and lower bounds of predicted DMI resulted in CCC of 0.48 and 0.75 and RMSPE of 8.8% and 20.5% of the mean, respectively. The use of lab-determined NDFd resulted in a decreased CCC (0.87 to 0.82) and an increased RMPSE (10.7% to 12.5% of the mean) for ME allowable milk but an increased CCC (0.55 to 0.62) and reduced RMSPE (18.4% to 15.7% of the mean) for methane production. These results suggest that determination of NDFd may not be advantageous in predicting milk yield but may improve prediction of methane production.

Key Words: neutral detergent fiber, digestibility, Cornell Net Carbohydrate and Protein System (CNCPS)

M14 Ruminal degradation and intestinal digestibility of hydrolyzed feather meal with and without blood. K. Buse*, D. Morris, and P. Kononoff, *University of Nebraska-Lincoln, Lincoln, NE.*

Hydrolyzed feather meal (HFM) is a readily available, high protein feedstuff that can be used as a cost-effective dairy feedstuff. Because the production process may vary, the chemical composition of HFM may also vary. Additionally, some processes may incorporate blood into the final product. The objective of this study was to evaluate the ruminal and intestinal digestibility of HFM originating from processes that differ in their inclusion of blood. Ten samples of HFM, 5 without blood (FM) and 5 with blood (FMB), were collected from 10 different production plants across the United States. Two multiparous lactating Holstein cows fitted with rumen and proximal duodenal cannulas were used to quantify rumen undegradable protein (RUP), and RUP digestibility (dRUP) by employing the mobile bag technique. Approximately 1.5 g of each was weighed into 10 N-free nylon bags with a mean pore size of 50 μm and a dimension of 5 × 10 cm and incubated in the rumen for 16 h. A subset of rumen bags were then used to determine RUP. The remaining bags were placed in a pepsin-HCl bath for 3 h and then inserted in the duodenal cannula of each cow. Bags were recovered in the feces and used to quantify dRUP. Data were analyzed as a complete randomized design to test the effect of blood inclusion on RUP and dRUP of HFM. The CP content was similar ($P = 0.57$) between FMB and FM averaging 94.5 ± 0.90%. The RUP content of FMB tended ($P = 0.13$) to be greater than FM (81.6 vs. 74.1 ± 3.19%). The dRUP was not different ($P = 0.77$) averaging 61.1 ± 2.36% across treatments. There was also no difference detected ($P = 0.40$) between FMB and FM in total-tract DM ($P = 0.40$) and CP ($P = 0.52$) digestibility averaging 74.2 ± 3.34 and 69.4 ± 4.07%. Results of this study suggest that although there are modest differences in chemical composition in hydrolyzed feather meal associated with the inclusion of blood, very little differences are observed in either ruminal or intestinal digestion of protein.

Key Words: intestinal digestibility, rumen degradation, rumen undegraded protein

M15 Effect of feeding switchgrass hay to dairy cows during the dry period.

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Switchgrass (*Panicum virgatum*) is a native tall grass species. Hay from this forage is high in fiber and low in potassium, making it a promising feed for dry cows. Our objective was to evaluate the effect of feeding switchgrass hay or straw in a single dry period ration. Holstein cows (n = 36) were randomly assigned to one of 2 "controlled energy" diets at dry-off. Forage in the TMR consisted of an approximately 50:50 ratio of corn silage and either switchgrass hay (SGR) or wheat straw (CON). Cows were fed twice daily during the dry period, and refusals were weighed once daily. After calving, cows entered the lactating herd, and daily milk yield was collected up to 21 DIM. Ketones were measured with a cow-side test at 7, 10, 14, and 21 DIM, and BCS was recorded once weekly from the beginning of the dry period through the first month of lactation. Data were analyzed using PROC MIXED in SAS, with the random effect of cow and the fixed effects of calving month, diet, parity, days relative to calving (DRC), and the interactions of diet with parity and DRC. There was a significant effect of parity and DRC on DMI, BCS (prepartum and postpartum), and milk yield ($P \leq 0.05$). Ketones were affected by parity ($P = 0.01$) but only tended to differ due to DRC ($P = 0.07$). Prepartum DMI was not affected by diet (CON = 14.8 ± 0.86 kg/d, SGR = 15.4 ± 0.70 kg/d; $P = 0.20$), nor was daily milk yield (CON = 31.2 ± 1.62 kg/d, SGR = 32.9 ± 1.31 kg/d; $P = 0.31$). Postpartum ketone levels also were not affected by diet (CON = 1.2 ± 0.14 mmol/L, SGR = 0.8 ± 0.12 mmol/L; $P = 0.20$). There was no overall effect of diet on BCS prepartum (CON = 3.45 ± 0.070, SGR = 3.39 ± 0.056; $P = 0.59$), although postpartum BCS differed due to dry period diet (CON = 3.24 ± 0.070, SGR = 3.01 ± 0.057; $P = 0.02$). Interactions between diet and DRC or parity were not significant ($P \geq 0.11$), except for DMI, where DRC × diet was significant ($P < 0.01$). Although cows who received SGR in the dry period mobilized more body fat in the month following parturition than CON, cows on both treatments maintained a healthy BCS, and overall there were no apparent negative effects of feeding switchgrass in the dry period.

Key Words: dry period, body condition score, switchgrass hay.

M16 Effect of weaning and supplemental butyrate on nutrient transporter expression in Holstein calves.

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This study examined the effect of the weaning transition and supplemental sodium butyrate—a primary stimulator of rumen development—on rumen fermentation and volatile fatty acid transporter (VFA) abundance. Holstein bull calves (n = 36; age = 10.7 ± 4.1d) were assigned to 1 of 4 treatment groups: 2 pre-weaning groups, animals fed either milk only (PRE-M) or milk, calf starter, and hay (PRE-S); and 2 post-weaning groups: animals fed milk, calf starter, and hay either without supplementation (POST-S) or with 1% wt/wt supplemental butyrate during the weaning transition (POST-B). Milk was provided at 1200 g/d; starter, water, and hay were provided ad libitum. Weaning transition occurred in POST-S and POST-B by reducing milk replacer to 800 g/d in wk 7 and 400 g/d in wk 8, 0 g/d at wk 9 and harvest at wk 10. Rumen pH was measured continuously for 7 d before harvest. At harvest, rumen fluid was analyzed for VFA and rumen tissue was analyzed for VFA transporters. Data were analyzed in SAS with fixed effect of treatment and, where appropriate, repeated effect of week. Between PRE-M and PRE-S, total VFA concentrations increased (11.8 ± 5.8 vs. 35.6 ± 5.6 mM, $P < 0.01$), mean rumen pH was unaffected (6.16 ± 0.83 vs. 7.44 ± 0.79, $P = 0.28$), and MCT1 expression was unaffected (6.70 ± 135 vs. 8.39 ± 143 × 10^5 A.U., respectively, $P = 0.30$). Between PRE-S and POST-S, total VFA concentrations increased (35.6 ± 5.6 vs. 154.3 ± 15.0 mM, $P < 0.01$), but mean rumen pH was unaffected (7.44 ± 0.79 vs. 6.39 ± 0.19, respectively; $P = 0.48$), as was MCT1 expression (8.39 ± 1.43 vs. 7.28 ± 1.35 A.U., respectively; $P = 0.58$). Between POST-S and POST-B, total VFA concentrations were unaffected (154 ± 15 vs. 131 ± 16 mM, $P = 0.23$), and mean rumen pH decreased (6.39 ± 0.19 vs. 5.83 ± 0.18, $P = 0.05$), while MCT1 expression was unaffected (7.28 ± 1.35 vs. 8.17 ± 1.59 × 10^5 A.U., respectively; $P = 0.61$). Expression of MCT1 was unaffected by changes in calf starter intake or rumen pH and is not correlated with average daily gain during the weaning transition. These data suggest improvements in nutrient transport may be driven by other transporters or mechanisms.

M17 Supplementation of serotonin or fluoxetine impacts bioenergetics in dairy calves.

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Serotonin (5-HT) is a monoamine that regulates energy balance through the modulation of insulin and lipid metabolism. Here, we hypothesize that manipulating 5-HT pathway by administering Fluoxetine (FLX, a 5-HT reuptake inhibitor) or 5-hydroxytryptophan (5-HTP, a 5-HT precursor), would improve energy metabolism in pre-weaned dairy calves. Bull Holstein calves (21 ± 2 d) were fed milk replacer (8 L/d) with saline (CON, 8 mL/d n = 8), FLX (40 mg/d, n = 8) or 5-HTP (90 mg/d, n = 8) for 10 consecutive d in a complete randomized block design. Blood samples were collected before (d-1), daily during treatment administration (d1 to 10) and during a 14-d withdrawal period (at d2, 3, 4, 7, 14) to measure insulin and NEFA concentrations. Calves were euthanized after the 10-d treatment or after the 14-d withdrawal period to harvest pancreas and adipose tissue. Gene expression of 5-HT receptors (*5-HTR*), 5-HT transporter (*SERT*) and tryptophan hydroxylase (*TPH1*) was measured by real-time PCR. Data were analyzed by period using 1- and 2-way ANOVAs in R. Insulin had a treatment by day interaction ($P < 0.01$), where 5-HTP group had higher circulating concentrations compared with the CON, particularly on d 5, 6, 7 and 9 of treatment; but had only a day effect during withdrawal period ($P < 0.001$). Circulating NEFA concentrations were not different during treatment or withdrawal period ($P > 0.10$). After treatment period, adipose gene expression of *5-HT1A* tended to be downregulated in the 5-HTP group ($P < 0.10$), but after withdrawal period *5-HT1F* was upregulated by 5-HTP and FLX ($P < 0.01$) when compared with CON. After treatment period, there was a tendency for downregulation of pancreatic gene expression of *SERT* ($P < 0.08$) and *5-HT1F* ($P < 0.10$), in FLX and 5-HTP group, respectively, when compared with CON. After withdrawal, serotonin pancreatic receptors were not differentially expressed ($P > 0.11$). Manipulating serotonin bioavailability of dairy calves increases circulating insulin and modifies the expression of serotonin receptors in the pancreas and adipose tissue.

Key Words: adipose, pancreas, serotonin

M18 Comparison of IgG absorption in calves fed a commercial colostrum replacer or supplement maternal colostrum.

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Successful passive transfer of antibodies in neonatal calves can be achieved by feeding maternal colostrum (MC) or colostrum replacer (CR). An alternative could be a supplemented low-quality maternal colostrum (CS). The objective of this study was to determine if a commercial colostrum product (Premolac PLUS Bovine IgG, Zinpro Corporation, Eden Prairie, MN) fed to replace or supplement MC could lead to adequate IgG levels and apparent efficiency of absorption (AEA) 24 h after birth in neonatal dairy calves. Holstein calves (n = 20/treatment; TRT) were separated from their dam after birth and randomly assigned to 1 of 2 levels of CR (110 g or 150 g of IgG), low-quality colostrum (41 g IgG/L) supplemented with CR (154 g IgG fed) or MC (106 g IgG/L; 401 g IgG fed; positive control) within 1.5 h of birth. Colostrum was obtained from first (MC TRT) or second and third milking (CS TRT) of cows from The Pennsylvania State University dairy. Then, it was pooled in 2 batches and analyzed for total IgG concentration using radial immunodiffusion. Blood samples were taken before feeding colostrum and 24 h after birth and analyzed for serum total protein, total IgG, hematocrit, and Brix. In the statistical model, data were analyzed as a completely randomized design. Hematocrit was tested as a covariate but not used in the final model. Reported means are followed by their standard errors. Feeding 150 g of IgG in CR led to higher 24 h serum IgG values than feeding 110 g of IgG (16.90 ± 1.09 and 12.79 ± 1.08 mg/mL, respectively; $P < 0.01$). MC had higher 24 h IgG values than CS (27.04 ± 1.07 and 22.33 ± 1.08 mg/mL, respectively; $P < 0.01$). Serum IgG levels were statistically different between CR and MC ($P < 0.01$), but both had average values above 10 mg/mL IgG. Calves fed CS had greater AEA than calves fed MC (54.58 ± 2.39 and 24.38 ± 2.36%, respectively; $P < 0.01$). Among calves fed CR with 110 g or 150 g of IgG, AEA was not different ($P = 0.68$), but was greater than previous reports (40.47 and 40.09%, respectively). Total protein and Brix had a strong correlation (r = 0.98, $P < 0.01$). These results indicate that CR can be an alternative to MC or a supplement to colostrum with low IgG.

Key Words: calf, colostrum, immunoglobulin G

M19 Alfalfa establishment: Evaluating the effects of underseeding sudangrass and ryegrass on forage yields and chemical composition under drought conditions.

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This study aimed at evaluating the effects of underseeding alfalfa with different annual companion crops on forage yield, botanical composition, chemical composition, alfalfa establishment and alfalfa persistence. Treatments included alfalfa (13 kg/ha) seeded in solo (control) or with an annual companion forage such as sudangrass (15 or 20 kg/ha), ryegrass (20 kg/ha) or oat (90 kg/ha) which were assigned to a randomized complete block design. Experimental plots (4 replicates/treatment) were seeded twice (in May and June). All plots were harvested (3 and 2 times in May and June, respectively) at the budding stage of alfalfa. For each plot, harvested forage mix was weighed for total yield determination (DM basis). The harvested mix was then manually separated and weighed by forage type. Forage subsamples were chemically analyzed for NDF, ADF and CP. Alfalfa establishment and winter survival were determined by measuring alfalfa stem density in the fall of the seeding year and after one winter (spring) in the following year. Data were analyzed according to seeding dates using the GLM procedure of SAS. Overall, total forage yields were highest ($P < 0.05$) with sudangrass (3.6 T/ha) and lowest ($P < 0.05$) with control (2.4 T/ha) or ryegrass (2.1 T/ha). Alfalfa yield was markedly reduced ($P < 0.05$) with oat when compared with other companion crops. For June established plots, yield of companion crop was higher with sudangrass (average 1.6T/ha) and oat (1.2T/ha) than ryegrass (0.2 T/ha). However, sudangrass and oat produced forages with lower ($P < 0.001$) CP but higher ($P < 0.001$) NDF and ADF levels than control and ryegrass. Calculated relative feed values of forages were highest ($P < 0.001$) with control and ryegrass treatments. Sudangrass yielded more CP (1.4×) and NDF (1.9×) than control per hectare of cultivated land. Companion crops had no detrimental effects on alfalfa establishment and persistence. In conclusion, underseeding alfalfa with sudangrass may improve both forage and NDF yields for better feeding of dairy cows.

Key Words: alfalfa, companion crops, dairy cow

ADSA Graduate Student (PhD) Production Poster Competition

M20 Effect of duration of exposure to diets differing in DCAD on calcium metabolism after a parathyroid hormone challenge in dairy cows. A. Vieira-Neto*, I. M. R. Leao, J. G. Prim, R. Zimpel, K. V. de Almeida, M. M. Nehme, J. Bollatti, A. C. M. Silva, A. Revilla-Ruiz, C. D. Nelson, and J. E. P. Santos, *University of Florida, Gainesville, FL.*

Objectives of this experiment were to determine the length of exposure to an acidogenic diet (ACD) to elicit an increased response to parathyroid (PTH)-induced changes in blood Ca in prepartum cows. The hypothesis was that cows have increased PTH responsiveness within 3 d of feeding an ACD. Ten parous Holstein cows at 242 ± 7 d of gestation were blocked by lactation (1 or > 1) and pretreatment DMI and, within block, they were assigned randomly to an alkalogenic (ALKD; DCAD = +209 mEq/kg DM; n = 5) or an ACD (DCAD = −168 mEq/kg DM; n = 5) on experiment d 0. Water and DMI were measured and blood sampled daily. Urine was sampled every 3 h for 36 h and then daily. The PTH challenges were performed on d 3, 8, and 13. Cows received 0.05 mg PTH/kg BW i.v. every 20 min for 9 h to mimic the pulsatile release of endogenous PTH. Jugular blood was sampled at 0 h, and hourly thereafter until 10 h, and at 12, 18, 24, 36, and 48 h relative to the challenge. Blood acid-base measures and concentrations of ionized Ca (iCa) were evaluated. Results were available for the first challenge on d 3 and data were analyzed by ANOVA with mixed models with SAS. Cows fed ACD had lower ($P < 0.01$) blood pH (7.382 vs. 7.429 ± 0.005), base excess (−2.4 vs. 4.3 ± 0.5 m*M*), and bicarbonate (22.8 vs. 28.5 ± 0.4 m*M*) within 24 h of the experiment compared with cows fed ALKD. Urine pH decreased ($P < 0.01$) by 15 h of feeding ACD (7.32 vs. 8.18 ± 0.17), and differences increased by 24 h (6.46 vs. 8.10 ± 0.17). Blood iCa increased ($P < 0.01$) in ACD compared with ALKD by d3 (1.28 vs. 1.22 ± 0.01 m*M*). During the PTH challenge on d3, cows fed ACD had a higher ($P < 0.01$) concentration of blood iCa than cows fed ALKD (1.42 vs. 1.33 ± 0.01 m*M*). Nevertheless, the increment in iCa in the first 36 h after the challenge, relative to baseline at 0 h, did not differ between treatments (ACD = 0.16 vs. ALKD = 0.15 ± 0.01 mM). Diet-induced metabolic acidosis occurred within 24 h of treatment; however, an increase in blood iCa concentration was observed after 3 d of metabolic acidosis. Blood iCa response to a PTH challenge did not differ between treatments on experiment d 3.

Key Words: parathyroid hormone, dietary cation-anion difference (DCAD), calcium

M21 Nitrogen metabolism in lactating dairy cows supplemented with *N*-acetyl-L-methionine as a source of rumen-protected methionine. F. X. Amaro*[1], E. J. C. Duvalsaint[1], D. Kim[1], R. Restelatto[1], P. Carvalho[1], A. Oyebade[1], Y. Jiang[1], A. P. Cervantes[1], K. G. Arriola[1], L. F. Ferraretto[1], A. T. Adesogan[1], J.-S. Eun[2], J. S. Park[2], S. H. Lee[2], D. Vyas[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Institute of Integrated Technology, CJ CheilJedang, Suwon, South Korea.*

The objective was to determine if supplementing a methionine (Met) derivative, *N*-acetyl-L-Met (NALM; CJ CheilJedang, Seoul, South Korea) would affect nitrogen (N) metabolism and improve N efficiency in lactating dairy cows. Sixty multiparous Holstein dairy cows in early lactation (27 ± 4.3 DIM) were assigned to 4 treatments in a randomized complete block design. Cows were blocked by actual milk yield and calving date. Treatments were: (1) Control (no NALM); (2) 15 g/d NALM (15NALM); (3) 30 g/d NALM (30NALM); and (4) 45 g/d NALM (45NALM). The NALM product contained 78% Met with 99.5% purity and based on bioavailability values provided from manufacturer, adding 15, 30, and 45 g/d as top-dress onto corresponding experimental diets provided 8, 16, and 24 g/d of metabolizable Met, respectively. Diets were formulated to meet nutritional requirements of lactating dairy cows producing 42 kg/d and to under supply metabolizable Met (−8 g/d; Control) or provide adequate (15NALM), or excess (+8 g/d with 30 NALM; +16 g/d with 45 NALM) metabolizable Met. Samples (feces, urine, blood, rumen fluid, and milk) were collected during the covariate period (2 wk), and at wk 4, 8, 16, and 24 of the treatment period. Data were analyzed using GLIMMIX procedure of SAS using covariates for N intake, fecal-N, urine-N, blood urea nitrogen (BUN), milk urea nitrogen (MUN), ammonia-N (NH_3-N) and N efficiency. Contrast statements were included to test linear and quadratic effects of NALM along with Control versus all NALM treatments. Intake of N ($P = 0.40$) and N loss in feces ($P = 0.79$), and urine ($P = 0.50$) and ruminal NH_3-N concentration ($P = 0.57$) were not affected by NALM supplementation, compared with Control. The efficiency of N utilization, measured as conversion of intake N into milk protein, was quadratically ($P = 0.03$) improved with NALM. No effect was observed on MUN concentration; however, BUN levels were linearly ($P < 0.01$) and quadratically ($P < 0.01$) reduced with NALM supplementation. In summary, NALM supplementation improved efficiency of N utilization and decreased BUN concentration.

Key Words: dairy cow, *N*-acetyl-L-methionine (NALM), nitrogen metabolism

M22 Feeding rumen-protected choline to prepartum Holstein cows in negative energy balance increases circulating lipoprotein phosphatidylcholine and triglyceride levels while preventing hepatic triglyceride accrual. W. A. Myers*[1], M. G. Zenobi[2], B. A. Barton[3], C. R. Staples[2], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Florida, Gainesville, FL,* [3]*Balchem Corp., New Hampton, NY.*

Rumen-protected choline (RPC) supplementation may increase hepatic phosphatidylcholine (PC) synthesis to promote triglyceride (TG) secretion within very-low density proteins. To assess whether RPC enhances lipoprotein PC and TG levels in dairy cows, 41 pregnant, nonlactating, multiparous Holstein cows were fed a RPC (ReaShure, Balchem Corp., New Hampton, NY) that provided 0 (control), 6.5, 12.9, 19.4, and 25.8 g/d of choline ions, respectively. Diets were fed to exceed nutrient requirements for 5 d, then cows were restricted to consume ~31% of their net energy requirements for 9 d. Preprandial plasma and liver were collected on d 9 of feed restriction. Plasma TG-rich and low-density lipoprotein (LDL) fractions were isolated using liquid chromatography. Lipoprotein fraction total TG, cholesterol and phospholipids were quantified. Lipoprotein fractions and liver were processed for lipidomics. Statistical analyses were done using the Mixed procedure of SAS. Birth weight of the calves and number of days prepartum at enrollment were covariates. Normalized omic data were natural log-transformed. A significant linear increase ($P < 0.01$) in TG-rich lipoprotein total TG levels was observed with choline ion supplementation. Likewise, RPC linearly increased TG levels within LDL fractions ($P = 0.02$). Total LDL fraction phospholipids tended to be modified by treatment (quadratic, $P = 0.09$). The majority of PC within the TG-rich lipoprotein fraction increased linearly with increasing RPC (40 PC out of 45 PC detected;

e.g., PC 38:5; $P < 0.01$). A similar linear outcome was observed for select TG-rich lipoprotein TG (168 TG out of 317 TG detected; $P < 0.05$). In LDL, RPC increased majority of PC detected (control vs. RPC [all levels], $P < 0.05$); however, TG was not overtly modified. In liver, RPC increased a limited number of PC (<15% detected; control vs. RPC, $P < 0.05$). Hepatic total TG was lowered by RPC (17.5 vs. 13.6% of tissue DM; control vs. RPC, $P < 0.05$). We conclude that RPC increased lipoprotein PC and TG concentrations, and reduced hepatic TG deposition in dairy cows.

Key Words: choline, liver, phosphatidylcholine

M23 Amino acid composition of cattle tissue and milk, and various feeds used in ruminant diets using multiple hydrolysis times.

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Nutrition models have improved in terms of predicting AA requirements and their supply for diet formulation. However, data have demonstrated the essential AA (EAA) content of feeds or animal products are not adequately described using 24h hydrolysis time. Thus, the objective was to evaluate the EAA content of milk, tissues, and feeds after multiple hydrolysis times, from 2 h to 168 h, and determine a correction factor for each AA. Twenty-six feeds were chosen, as well as 6 tissue samples representing whole body composition of Holstein heifers from serial harvest studies. The milk sample was pooled from 4 bulk tank samples over 3 d from the Cornell University Dairy. Feed samples were analyzed for all EAA by HPLC following acid hydrolysis at 110°C in a block heater for 2, 4, 6, 12, 18, 21, 24, 30, 48, 72, 120 and 168h. Performic acid preoxidation was conducted for the sulfur AA and barium hydroxide was used for Trp hydrolysis. Tissues and milk were analyzed for 21, 72 and 168h using the same methods. A least-squares nonlinear regression was used to determine the true AA content of the samples and significance declared at $P < 0.05$. The EAA of feeds, milk, and tissues continued to increase after 24h and overall, had the greatest EAA concentration after 168h hydrolysis ($P < 0.05$). The branched chain AA (BCAA) for most feeds, and all milk and tissue samples increased from 24h to 168h hydrolysis ($P < 0.05$). Lys increased in concentration up to 168h for most feeds ($P < 0.05$) but decreased in milk after 72 h and tissue after 21 h ($P < 0.002$). Two ratios, A_0/24h and Max/24h, were determined and compared among sources to be used as correction factors for the 24h hydrolysis. The A_0 is the AA concentration at 0h of hydrolysis and Max being the maximal concentration of AA. The ratios for BCAA vary among sources of EAA and different ratios should be determined for each source to have an accurate correction factor. In summary, a single time point hydrolysis is not an accurate representation of the EAA concentration of a substrate and correction factors could be used to determine the actual EAA content by substrate.

Key Words: amino acid, hydrolysis, correction factor

M24 Importance of including body weight loss in assessing cow responses to dietary protein reduction.

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Our goal was to determine the importance of including body weight (BW) loss in assessing the response of cows to low protein diets. 169 Holstein cows in mid-lactation (92 primiparous) and 69 in late lactation (42 primiparous) were fed diets high or low in protein in crossover designs with 2 28-d treatment periods. Mid-lactation diets were 18% or 14% CP, and late-lactation diets were 16% or 12% CP. All diets had adequate RDP with expeller soybean meal added for high protein diets. Cows were milked 2×/d; intake and milk yield were recorded daily. Milk composition was measured 2 d/wk, and BW was measured 3d/wk. Historical prices of corn and soybean meal, milk class and component prices, and cull cow values were used to set financial parameters. Captured energy, captured protein, and gross income from milk production and BW change were calculated for each cow in each diet. Protein responses within each lactation stage were analyzed with the MIXED Procedure (SAS 9.4), including fixed effects of experiment, diet, parity, and period nested within experiment, and random effect of cow nested within experiment and parity. In mid lactation, reducing protein from 18 to 14% saved \$0.73 per cow in daily feed cost but resulted in daily losses of: 1) 2.9 Mcal milk energy and 1.8 Mcal body energy, 2) 0.14 kg milk protein and 0.03 kg body protein, 3) \$1.40 milk income and \$0.29 body salvage value (all $P < 0.05$), so that 38% of the total energy loss, 18% of total protein loss, 17% of gross income loss, and 24% of income over feed cost (IOFC) loss were due to BW loss. In late lactation, reducing protein from 16 to 12% saved \$0.60 in daily feed cost but resulted in daily losses of: 1) 2.3 Mcal milk energy and 2.2 Mcal body energy, 2) 0.11 kg milk protein and 0.05 kg body protein, 3) \$1.07 milk income and \$0.38 body salvage value (all $P < 0.05$), so that 50% of total energy loss, 31% of total protein loss, 26% of gross income loss, and 45% of IOFC loss were due to BW loss. In addition, the importance of BW loss was greater for primiparous than multiparous cows. In conclusion, BW loss was an important part of the response to low protein, and we suggest that it should not be neglected when assessing responses to dietary protein.

Key Words: protein reduction, milk response, body weight loss

M25 The effects of citral as a therapeutic treatment for *Escherichia coli* mastitis in lactating dairy cattle.

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Citral (OO), a component of orange oils, has bacteriostatic and bactericidal properties against isolated mastitis pathogen *Escherichia coli* P4 at relatively low concentrations that do not interfere with the cow's cellular immune response in vitro. The objective of this study was to evaluate the effectiveness of OO as an intramammary therapy for experimentally induced *E. coli* mastitis. One rear quarter from 18 healthy, multiparous mid-lactation dairy cows was inoculated with ~800 cfu of *E. coli* P4. Infections were established in all inoculated quarters. One of 3 intramammary treatments were administered into the infected quarter at 24 h post-infection for 4 consecutive days (6 cows/treatment). Treatments were 1% vol/vol OO twice daily, ceftiofur hydrochloride once daily, and sterile phosphate buffer solution twice daily. Foremilk from the infected quarter was aseptically sampled twice daily before milking (0700 and 1600 h) and coccygeal blood was sampled once daily through d 7 post inoculation and weekly through d 35 post inoculation. Health examinations were performed before blood sampling, and milk production and feed intake were recorded daily. Results were analyzed by ANOVA using the MIXED procedure of SAS 9.4 with treatment, time and treatment by time interaction declared as fixed effects and cow declared as a random effect. Milk *E. coli* cfu/mL was log10 transformed and not affected by treatment or treatment across time ($P > 0.05$). Treatments did not affect peak bacteria concentrations in milk ($P > 0.05$). Somatic cell count ($P = 0.03$) differed across time by treatment as OO lowered somatic cell count on d 2.5 and 3.5 following inoculation as compared with the antibiotic and control treatments. Somatic cell score, clinical score, health parameters, total daily milk production and daily dry matter intake were not affected by treatment or treatment across time ($P > 0.05$). In summary, OO did not alter production parameters or health of *E. coli* mastitis-induced cows; however, the anti-inflammatory effects of OO may be useful to reduce the severity of the infection.

Key Words: mastitis, *Escherichia coli*, alternative therapy

M26 Starch content of the close-up dry cow diet can affect insulin sensitivity of newborn dairy calves early in life.

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The objective of this study was to determine the effect of starch content in the close-up diet on insulin sensitivity of female calves early in life. Thirty-eight female Holstein heifer calves were born to dams fed either a high (26% starch; HI; n = 20) or moderate (14% starch; MOD; n = 18) starch close-up diet commencing at 28 d before expected calving date. Following birth, calves were removed from the dam within 2 h, and fed 3 2-L meals of colostrum within the first 24 h of life. Calves were housed individually and offered 10 L/d of milk replacer (26% CP, 18% fat mixed to 130 g/L) fed through a Calf Rail feeding system. There was no difference in birth body weight of calves between HI or MOD. A glucose tolerance test (GTT) was performed a minimum of 6 h after their last colostrum, or milk meal on d 2, d 10 ± 2 and d 20 ± 2. The GTT involved an intravenous infusion of glucose at a dose of 180 mg/kg BW via a jugular catheter, with sequential blood sampling for 90 min after the infusion, and samples were analyzed for plasma glucose and insulin concentrations. Data were analyzed using the FIT model of JMP and included the fixed effects of dam treatment, parity and their interaction. There was no difference in basal concentrations of glucose before the GTT at d 2, 10 or 20, and no difference in basal insulin at d 2 or 10, however, at d 20, HI calves had increased basal concentrations of insulin as compared with MOD (3.04 vs. 1.92 ng/mL; $P = 0.05$). On d 2, HI calves had greater maximum insulin concentrations (11.1 vs. 6.55 ng/mL; $P = 0.02$) and greater area under the curve for insulin (17.2 vs. 10.8 ng/mL × min; $P = 0.03$) following the glucose infusion, but during the GTT, with no difference in glucose response. On d 10 HI calves had reduced insulin sensitivity (8.51 vs. 14.3 mg/min × ng/mL; $P = 0.03$) and tended to have reduced glucose clearance rates, and on d 20, HI calves tended to have higher maximum insulin concentration following the glucose infusion as compared with MOD. These findings suggest that feeding a HI close-up diet may reduce insulin sensitivity of female offspring early in life.

Key Words: close-up diet, insulin sensitivity, newborn calf

M27 Withdrawn

M28 α_{S1}-Casein (*CSN1S1*) suppresses β-casein expression via JAK2/STAT5a signaling pathway in goat mammary epithelial cells.

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α_{S1}-Casein, encoded by the *CSN1S1* gene, is one of the main milk proteins, which can provide amino acids and other nutrients for the bodies, nevertheless, it can also lead to human allergy. However, the effects of *CSN1S1* on other milk proteins in goat mammary epithelial cells (GMEC) remain unknown. To explore the regulatory mechanism of *CSN1S1* on major milk proteins, *CSN1S1* overexpression adenovirus and small interference RNA targeting *CSN1S1* were designed and transfected into GMEC, respectively. GMEC were incubated with treatments for 48 h, and then total RNA and protein of cells were isolated. The mRNA levels and protein levels of caseins (*CSN1S1*, *CSN1S2*, *CSN2*, *CSN3*), major whey proteins (*LALBA*, *BLG*), proteins of Janus kinase 2/ signal transducer and activator of transcription 5a (JAK2/STAT5a) signaling pathway (*JAK2*, *STAT5a*, *SOCS3*, *ELF5*, *PRLR*) were detected by RT-qPCR and Western blotting, and analyzed with Student's *t*-tests. Results showed that overexpressing *CSN1S1* decreased the β-casein mRNA and protein abundance, as expected, *CSN1S1* silencing resulted in a significant increase in β-casein ($P < 0.05$). However, *CSN1S1* did not alter the expression of *CSN1S2*, *CSN3*, *LALBA*, *BLG* ($P > 0.05$). Previous studies have shown that JAK2/STAT5a signaling promotes β-casein transcription and synthesis in ruminants, which implied the possibly involvement of JAK2/STAT5a signaling pathway in *CSN1S1* regulating β-casein metabolism. We observed that overexpression of *CSN1S1* induced reductions of JAK2 and STAT5a mRNA level, as well as STAT5a phosphorylation level ($P < 0.05$). Similarly, knockdown of *CSN1S1* upregulated the mRNA level of JAK2 and STAT5a, also increased phosphorylation abundance of STAT5a ($P < 0.05$). In conclusion, our findings indicated the regulatory role of *CSN1S1* on β-casein through JAK2/STAT5a signaling pathway in goat mammary epithelial cells.

Key Words: α_{S1}-casein, β-casein, JAK2/STAT5a signaling pathway

M29 Effects of feeding moderate- or high-starch close-up diet to cows on response of newborn calves to intravenous injection of glucagon-like peptide 1.

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The objective of this study was to evaluate the effects of feeding moderate- or high-starch close-up diet to close-up cows on response of newborn calves to intravenously (i.v.) injected glucagon-like peptide 1 (GLP-1). Holstein heifer calves (n = 37) born to cows fed a moderate- (M, 14% starch; n = 17) or high-starch (H, 26% starch; n = 20) diet during the last 28 d of gestation were assigned to one of 2 treatment groups, which were i.v. injected with saline as control (MC and HC, n = 9 and 10, respectively) or GLP-1 solution (MG and HG, n = 8 and 10, respectively) immediately after milk replacer (MR) feeding finished (within 5 s after MR feeding finished) at 2, 10, and 20 d after birth. Blood samples were collected at −10, 0, 10, 20, 30, 40, 50, 60, 90, and 120 min relative to treatment injection and plasma glucose, insulin, and GLP-1 concentrations were measured. Data were analyzed by ANOVA using fit model procedure of JMP® 14 pro (SAS Institute Inc., Cary, NC). Plasma GLP-1 concentration increased rapidly after GLP-1 injection and was higher for the calves injected with GLP-1 than those injected with saline ($P < 0.01$) at 2 (1.97 vs 1.02 ng/mL for G and C, respectively), 10 (2.10 vs 0.67 ng/mL for G and C, respectively) and 20 d after birth (1.79 vs 0.38 ng/mL for G and C, respectively), but no difference was observed between MG and HG at all sampling days. Both in M and H calves, the rise in postprandial plasma glucose concentration was suppressed ($P < 0.01$) by direct glucose-lowering action of i.v. injected GLP-1 at 10 (137 vs 150 mg/dL for G and C, respectively) and 20 d after birth (147 vs 158 mg/dL for G and C, respectively), and similar tendency was observed at 2 d after birth (133 vs 144 mg/dL for G and C, respectively; $P = 0.09$): this direct glucose-lowering action by GLP-1 was greater ($P = 0.02$) for H than for M calves at 20 d after birth (141 vs 152 mg/dL for HG and MG, respectively). These results indicate that feeding a high-starch diet to cows in close-up period enhances glucose-lowering

action by GLP-1 after feeding depending on age of calves, which can affect glucose status in newborn calves.

Key Words: heifer calf, glucagon-like peptide 1, prepartum diet

M30 Effects of recent and ancient inbreeding on performance of Dutch Holstein Friesian dairy cattle.

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Inbreeding decreases animal performance (inbreeding depression), but not all inbreeding is expected to be equally harmful. Inbreeding on recent ancestors is expected to be more harmful than inbreeding on more ancient ancestors, because of purging. Purging is the removal of deleterious recessive alleles over time by selection. We investigated inbreeding depression in Dutch Holstein Friesian cattle, expecting to find stronger effects of recent inbreeding compared with ancient inbreeding. The effect of inbreeding on yield, fertility and udder health traits was determined with linear mixed models using 38,792 first-parity cows. Pedigree data were used to compute traditional inbreeding (F_{PED}) and 75k genotype data were used to identify regions of homozygosity (ROH) and compute ROH-based inbreeding (F_{ROH}). Inbreeding depression was apparent, e.g., a 1% increase in F_{ROH} was associated with a decrease in 305-d milk yield of 36.3 kg (SE = 2.4), an increase in calving interval of 0.48 d (SE = 0.15) and an increase in mean somatic cell score in d 150 to 400 of 0.86 units (SE = 0.28). Distinguishing recent from ancient inbreeding gave mixed results. For example, only very long ROHs (indicating more recent inbreeding) significantly increased calving interval, whereas both long and short ROHs decreased protein yield. Across all traits, standard errors were larger for inbreeding that was more ancient. When F_{PED} was split into new and ancestral components, based on whether alleles were identical by descent for the first time or not, there was clear evidence of purging. For example, a 1% increase in new inbreeding was associated with a 2.2 kg (SE = 0.4) decrease in 305-d protein yield, compared with a 0.9 kg (SE = 0.8) increase for ancestral inbreeding. The mixed results we obtained may be partly due to difficulties in determining ancient inbreeding. Distant ancestors are less well registered, and short ROHs may be less reliable than long ROHs. Furthermore, selection history is complex and purging may have acted on some, but not on all alleles. Results suggest that, despite the presence of purging, both recent and ancient inbreeding contribute to inbreeding depression and should be considered in management strategies.

Key Words: inbreeding depression, purging, dairy cattle

ADSA-SAD Undergraduate Original Research Poster Competition

M31 Effect of air and probiotics on in vitro fermentation. R. Y. Rha*, S. L. Ratiff, J. S. Scott, and R. A. Kohn, *University of Maryland, College Park, MD.*

Many fiber-digesting microorganisms are strict anaerobes so fiber digestion could be decreased when ruminants swallow air during feed consumption. The objective of this study was to evaluate the effect of adding air on rumen fermentation, and to determine whether adding aerobic probiotics can ameliorate the effects of air. Twelve treatments were analyzed in a 4 × 3 factorial design with 4 levels of air treatments and 3 probiotic treatments. Air treatments included: no air added to chemically reduced medium, and 0, 25 mL or 50 mL air added to 40 mL unreduced medium with 10 mL rumen inoculum at the start of fermentation in 125-mL flasks. Probiotic treatments were: no additive (control), Dairyman's Edge (DE), and live yeast. Timothy hay (0.5 g) and corn grain (0.5 g) were incubated at 39°C for 24 h with 5 replicates. Results were analyzed by the model: $Y = \mu + A + P + A\times P + S + E$, where Y is the response variable, and A is a fixed effect of air or reducing agent, P is the effect of yeast or probiotic additive, S is sequence, and E is error. Significant differences were accepted at $P < 0.05$, and tendencies at $P < 0.1$. Air decreased NDF digestibility from 51.8% for reduced medium to 46.1, 44.2 and 41.3% with 0, 25, and 50 mL air respectively. Air treatment tended to decrease pH from 6.41 for reduced medium to 6.25 for other treatments, but DE increased pH to 6.60 compared with 6.32 for control or yeast. There were no main effects of treatments on gas production, but there was an interaction of air treatment with additive. For yeast treatment, gas production decreased from 228 mL for reduced treatment, to 221, 170, and 108 mL for treatments with 0, 25 or 50 mL of air, while gas production for other treatments was not affected by air addition. Acetate production tended to decrease ($P < 0.1$) with air addition, and decreased for DE and yeast treatments. Air decreased butyrate production. Acetate to propionate ratio decreased with air or probiotic, but air did not further decrease the acetate to propionate ratio in the presence of probiotics. The addition of air to in vitro fermentation decreased NDF digestibility, and decreased expected acetate production. Use of probiotics did not ameliorate the decrease in fiber digestion due to presence of air.

Key Words: in vitro fermentation, fiber digestion, live yeast

M32 Fecal nutrient content of slick-hair and wild-type dairy cows under heat stress. C. G. Ríos-Solís, N. L. Rosa-Padilla*, and G. Ortiz-Colón, *University of Puerto Rico, Mayagüez, PR.*

Dairy cattle exposed to high ambient temperatures and humidity suffer heat stress, which may result in lower DM digestibility. Our objective was to evaluate the content of neutral detergent fiber (aNDFom), nitrogen (N), phosphorus (P), potassium (K), lignin and starch in the feces of slick-hair (SL) and wild-type (WT) lactating dairy cows under heat stress. SL (n = 8) and WT (n = 8) lactating cows with an average of 140 DIM, were in a feeding trial for 60 d during April and May of 2018 in a commercial dairy farm in southwestern Puerto Rico (18.027435, −67.094955). Average temperature-humidity index (THI) during the feeding trial was 75.8 ± 0.03. Animals were fed a totally mixed ration (TMR) based on *Brachiaria* spp. forage, soybean meal, steam flaked corn grain, brewer's grains, and minerals. The DMI was recorded daily with Calan Feeding System and no differences in DMI in were documented between the genotypes ($P = 0.72$). For the fecal analysis TMR samples were collected from a.m. feedings on d 58 and 59. Fecal samples were collected from each cow intra-rectum at 0900 h and 1700 h on d 59 and 60. Fecal and TMR samples were analyzed by wet chemistry. On average the TMR contained 15% CP, 1.39 Mcal NE_L/kg, 18.7% starch, 4.8% lignin, 0.32% P, 1.17% K, and 36.7% aNDFom. All fecal components were similar between genotypes. Average fecal aNDFom % in SL cows was 42.7 ± 1.1 while WT cows average was 40.9 ± 1.1 ($P = 0.235$). Average fecal lignin % in SL cows was 4.29 ± 0.2, and 4.11 ± 0.2 in WT ($P = 0.591$). Average fecal K % was 0.723 ± 0.045 while WT cows average was 0.718 ± 0.042 ($P = 0.93$). Average fecal N was 2.12 ± 0.04 while WT cows average was 2.13 ± 0.036 ($P = 0.8262$). When evaluating P fecal concentration, SL cows had an average P of 0.66 ± 0.032 while WT cows average 0.597 ± 0.022 ($P = 0.098$). Average fecal starch % in SL cows was 9.09 ± 1.05, and 8.67 ± 0.98 in WT ($P = 0.776$). In this experiment, where SL and WT dairy cows were exposed to heat stress, we found no differences in their fecal content of aNDFom, N, P, K, lignin and starch.

Key Words: slick hair, heat stress, fecal starch

M33 Effects of feeding 25-hydroxyvitamin D_3 versus vitamin D_3 to dairy calves: Effects on responses to endotoxin challenge. S. M. Buoniconti*[1], L. P. Blakely[1], M. Reese[1], M. F. Kweth[1], T. L. Williams[1], M. Zenobi[1], C. R. Staples[1], P. Celi[2], C. Cortinhas[2], and C. D. Nelson[1], [1]*University of Florida, Gainesville, FL,* [2]*DSM Nutritional Products, Columbia, MD.*

Objectives were to test the effects of feeding vitamin D_3 compared with 25-hydroxyvitamin D_3 on physiological and immune responses of dairy calves to an intravenous endotoxin challenge. Forty-five Holstein bull calves (<7 d of age) were blocked into weekly cohorts and randomly assigned to one of 5 daily supplements; control (CON, 0.25 mg of vitamin D_3/kg BW), and 2 levels (1.5 or 3.0 μg/kg BW) of vitamin D3 (VitD), or 25-hydroxyvitamin D_3 (25D) added to the CON treatment resulting in the following treatment groups: CON, Vit1.5, VitD3.0, 25D1.5, 25D3.0. Calves were fed milk replacer until weaning at 56 d of age and had ad libitum access to water and a starter grain throughout the experiment. Treatments were added to milk replacer once daily until weaning, then to starter grain thereafter. On d 91 of the experiment, calves were challenged with lipopolysaccharide (0.1 μg/kg BW) via intravenous injection and physical and plasma measures were evaluated in response to the challenge. Data were analyzed with mixed model that included fixed effects treatment and time and random effects of calf within block. Average rectal temperatures from 1 to 72 h after challenge were 39.1, 39.1, 38.8, 38.9 and 39.1 ± 0.1°C, for CON Vit1.5, Vit3.0, 25D1.5 and 25D3.0 calves, respectively. A similar pattern was observed for reactive oxygen metabolites in serum and the interaction between source and dose was significant ($P < 0.01$) for rectal temperatures and reactive oxygen metabolites in blood such that responses were greater in VitD1.5 and 25D3.0 calves compared with VitD3.0 and 25D1.5 calves. Concentrations of Ca, NEFA, glucose and haptoglobin in serum changed over time but were not affected by treatment; however, serum P was decreased ($P < 0.01$) in CON calves (2.3 ± 0.1 mM) compared with VitD and 25D treated calves (2.6, 2.5, 2.4 and 2.5 ± 0.1 mM). Leukocyte counts and expression CD11b, CD14, CD62L proteins did not differ between treatments. Feeding VitD at 3.0 μg/kg BW and 25D at 1.5 μg/kg BW resulted in better tolerance of calves to an endotoxin challenge compared with 1.5 μg/kg BW VitD or 3.0 μg/kg BW 25D.

Key Words: 25-hydroxyvitamin D_3, calf, immunity

M34 Colony stimulating factors modulate uterine caruncle immune cell phenotype in dairy cows. M. Smith*, J. Laguna, R. Nelli, and G. A. Contreras, *Michigan State University, East Lansing, MI.*

Uterine diseases affect 25% of dairy cows in the US and often develop from retention of the placenta (RP). One of the major causes of RP is an impairment of the inflammatory responses at the uterine-placenta junction. The dysregulation of certain components of the immune system, including macrophages and neutrophils, appears to trigger RP. Immunomodulatory cytokines such as granulocyte colony stimulating factor (G-CSF), granulocyte macrophage CSF (GM-CSF), and macrophage CSF (M-CSF) alter phagocytic cells function and increase their circulating numbers in dairy cows. However, their effect on the inflammatory responses that trigger placental expulsion is currently unknown. Transcriptional studies were performed to determine the effects of G-CSF, GM-CSF, and M-CSF on the gene expression of markers of immune cell phenotype and function at the uterine-placenta junction. Caruncles were collected transvaginally from singleton-bearing multiparous cows (n = 7) at 1–2 h after calving. Caruncle apexes were dissected and then exposed to 50 and 500 ng/gram of caruncle of recombinant bovine CSFs: G-CSF (Elanco Animal Health), GM-CSF and M-CSF (Kingfisher Biotech), and a vehicle control (CON) for 3 h at 37°C. Gene expression data were analyzed using lognormal distributions and pairwise comparisons. Independently of the dose, caruncles exposed to all CSFs increased the expression of *TNFA*, *IL8*, and *IL10* compared with CON ($P < 0.05$). G-CSF and M-CSF, at both doses, enhanced the expression of *CCL24,* a neutrophil chemoattractant, *MMP9*, extracellular matrix metalloproteinase 9, compared with those exposed to GM-CSF, and CON ($P < 0.05$). G-CSF tended to increase the transcription of STAT3, a key regulator of the proteolytic activity, compared with GM-CSF and CON ($P = 0.06$). In contrast to the pro-inflammatory transcriptional patterns exerted by G-CSF, GM-CSF at both doses decreased the expression of the mature macrophage markers *CD68* and *CD14* compared with CON and the other CSF. Collectively, these findings suggest that G-CSF may promote inflammatory responses at the uterine caruncle immediately after calving by enhancing chemotaxis and by triggering proteolytic activity.

Key Words: granulocyte colony stimulating factor (G-CSF), retained placenta

M35 Behavioral response of dairy cows after subcutaneous insertion of real-time temperature-detecting biosensor: A pilot study. K. Sheng*, K. Reuscher, H. Chung, C. Choi, Y. Kim, S. Brounts, and J. Van Os, *University of Wisconsin-Madison, Madison, WI.*

Heat stress in dairy cows negatively affects production and animal welfare. To improve early detection of heat stress, a real-time body-temperature detecting microchip was developed for subcutaneous insertion at the base of the ear of dairy cows. Although previous research has validated the accuracy of microchip temperature detection, little is known about how well cows tolerate this device. Our objective was to evaluate dairy cows' behavioral reactions toward the microchip as potential indicators of negative effects on animal welfare. We predicted that, if the cows responded negatively to the microchip, there would be an increase in the frequency of the focal behaviors after the implant. This pilot study enrolled lactating Holstein cows (n = 3). The cows were video recorded for 90 – 100 min/d starting at 1330 h for 5 d:2 d before and 3 d after the microchip (2 mm × 12 mm) insertion. A single trained observer (blind to both the ear with the implant and the day relative to insertion) recorded the frequency of ear flicks, head rubs, and head shakes. The frequency of each behavior (in bouts/min) were calculated and averaged among the days before and after the microchip insertion for each cow, and differences between the implanted and control ears were calculated within the pre- and post-implant periods. Differences between the pre- and post-implant periods were evaluated using a Wilcoxon signed-rank test. No difference was detected between pre- and post-implantation for ear flicks (control ear before: 0.04 ± 0.01 bouts/min, after: 0.03 ± 0.02 bouts/min; implanted ear before: 0.03 ± 0.01 bouts/min, after: 0.23 ± 0.38 bouts/min; differences between ears: $P = 0.18$), head rubs (before: 0.01 ± 0.01 bouts/min, after: 0.02 ± 0.01 bouts/min; $P = 0.79$), and head shakes (before: 0.01 ± 0.01 bouts/min, after: 0.04 ± 0.03 bouts/min; $P = 0.42$). Our results suggest that, in this pilot study, the subcutaneous insertion of the biosensor had little observable adverse impact on the welfare of the dairy cows. Future research is planned with a larger sample size and longer behavioral observation periods to further evaluate how well cows tolerate the biosensor.

Key Words: heat stress, body temperature, biosensor

M36 Disrupting circadian rhythms during the dry period decreases blood glucose but significantly increases milk production. G. Wernert*[1], A. Suarez-Trujillo[1], M. Grott[1], J. Johnson[2], T. Steckler[1], J. Townsend[3], K. Plaut[1], J. Boerman[1], and T. Casey[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*USDA-ARS, West Lafayette, IN,* [3]*Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN.*

The circadian timing system (CTS) functions to maintain homeostasis and we hypothesized that disrupting the CTS during the prepartum dry period would affect metabolic homeostasis and decrease milk yield in the subsequent lactation. Our objectives were to determine the effect of disrupting the CTS on: 1) Blood glucose, β-hydroxy butyrate (BHBA) and nonesterified fatty acids (NEFA) in the pre and postpartum, and 2) milk production to 60 d in milk (DIM). Five weeks before expected calving (BEC) multiparous cows (n = 32) were moved to a tie-stall barn and divided into 2 treatments: control (n = 16; 16 h light: 8 h dark) or phase-shifted (PS, n = 16), a chronic jet lag paradigm which shifted the light-dark phase 6 h every 3 d until parturition. All cows were exposed to control lighting after calving. Blood samples were taken at least biweekly at 0600 in the pre- and postpartum to measure glucose, BHBA and NEFA, and every 4 h over 48 h at 3 time points: d 23 BEC, d 9 BEC, and 5 d postpartum (PP). NEFA was measured using colorimetric assay; blood glucose and BHBA were measured with Centrivet monitor. Core body temperature was recorded every 30 min vaginally using ibutton data loggers at 23 BEC, 9 BEC, and 5 PP. PS ($P < 0.05$) shifted phase and attenuated circadian rhythms of core body temperature. Daily feed intake was not different between control (30.5 ± 0.6 kg) and PS (29.8 ± 0.6 kg) cows. Blood glucose was ($P < 0.05$) decreased in PS cows pre and postpartum, however there was no effect of treatment on BHBA or NEFA concentration. Cosine fit analysis found control cows exhibited circadian rhythms of plasma NEFA only at 5PP ($R^2 = 0.69$; $P = 0.002$); whereas PS cows lacked circadian rhythms throughout study. Milk yield and fat corrected milk yield were significantly increased ($P < 0.05$) through 60 DIM in PS (42 ± 0.92 and 43.5 ± 1.04 kg/d) versus control (39.3 ± 0.92 and 40.3 ± 1.04 kg/d) cows. Thus, lack of difference in BHBA, NEFA and feed intake along with decreased blood glucose, supports that disrupting circadian rhythms in late parturition increases milk production efficiency.

M37 Co-dispersion of chia plant protein and casein micelles in milk. S. Tieu*, A. Mayta-Apaza, J. Ortega-Anaya, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

Food proteins are the basis for imparting functionality in many foods. Not all proteins are able to form associations with other proteins, but the casein micelles present properties that make them a unique system for protein blending. This has the potential to provide novel functionalities in industrial food applications. To capitalize on this, our study investigated processing methods to stabilize the interactions of casein micelles in milk with chia protein. Most plant proteins are not soluble in water, so a co-dispersion of casein micelles with these globular proteins was sought via homogenization. First, the temperature of the skim milk was lowered, and sodium citrate was added to allow the casein micelles to dissociate enough as to interact with the chia protein. The soluble chia protein was extracted from microfine powder of the seed. The fiber, starch material, and pericarp were precipitated and separated. The supernatant containing the dispersed chia protein was added to the skim milk. This was homogenized under cold condition (4°C) and relatively higher pressure (350 bar) to increase the stability of the colloidal dispersion by applying shear. The particle size, zeta potential, and total protein were analyzed before and after homogenization. The zeta potential increased significantly after homogenization from a mean value of 33.98 to 45.83 mV. This indicates a stable co-dispersion of the chia protein and casein micelles. Overall, dissociating casein micelles and homogenizing at colder temperatures and higher pressures may effectively co-disperse and stabilize chia plant protein and casein micelles for use in a wide range of dairy applications.

Key Words: casein, chia, protein interaction

M38 Rumen-protected methionine supplementation during early lactation and associations with plasma amino acid, metabolite concentrations and a novel metabolite health index.

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Multiparous dairy cattle in the Northeastern US were enrolled in a prospective cohort study with the objective of quantifying circulating AA and plasma metabolites during the transition period and creating a Metabolite Health Index (MHI). Farms were enrolled as either experiment (EXP; n = 7 farms): postpartum (POST) rumen-protected Met supplementation of > 11.25 g/d (Smartamine M; Adisseo, Antony, France) or control (CON; n = 5 farms): POST rumen-protected Met supplementation of ≤ 11.25 g/d. Prepartum (265 to 280 DCC; n = 168) and POST (5 to 17 DIM; n = 179) cows were sampled within each farm. All cows sampled were considered eligible for milk analysis whereas only POST cows were eligible for the metabolite analysis. Data were analyzed in SAS with PROC MIXED and repeated measures analysis was conducted for measures with multiple time points. Multivariable models were created using PROC MIXED to predict wk 4 milk (WK4M) and energy-corrected milk (ECM) at first test day, with MHI as the main risk factor of interest. Plasma urea concentrations were lower for EXP than CON cows (8.83 vs. 10.61 mg/dL; $P < 0.01$). Yield of ECM was higher in EXP through the 4th test day (49.2 vs. 45.4 kg/d; $P < 0.01$). There was a treatment × parity interaction for WK4M such that there was no difference between treatments for 2nd lactation cows ($P = 0.31$) whereas ≥ 3rd lactation EXP cows had greater WK4M than CON ≥ 3rd lactation cows (51.9 vs. 44.0 kg; $P = 0.01$). A MHI (range: −10.50 to 6.57) was created based on postpartum plasma bilirubin, cholesterol, and albumin concentrations sampled between 5 and 17 DIM. The EXP cows had a greater index than CON cows (0.25 vs. −0.42; $P = 0.02$) indicating better postpartum health status. A 1-unit increase in MHI resulted in a +0.86 kg WK4M response ($P < 0.01$). Similarly, a 1-unit increase in MHI resulted in a +1.32 kg ECM response ($P < 0.01$). Supplementation of Met was shown to improve MHI, ECM, and WK4M for ≥ 3rd lactation cows. A greater MHI was associated with greater WK4M and ECM, indicating healthy and more productive cows.

Key Words: methionine, transition cow, metabolic health

M39 The effects of regrouping dairy cattle in pairs or individually on fecal cortisol metabolite concentrations.

P. L. Knickerbocker* and J. M. Huzzey, *California Polytechnic State University, San Luis Obispo, CA.*

Regrouping occurs when a dairy cow is moved from one pen to another. We predict this is a stressful time for a cow; however, no research has quantified a physiological stress response. Further, no work has attempted to determine if the anticipated physiological stress response is mitigated when cows are moved with a familiar partner. We predict that cows moved individually may lack the social support needed to buffer the negative effects of regrouping. The objective of this study was to measure fecal cortisol metabolite concentrations during the days following regrouping, among cows moved individually or as a pair. Holstein and Jersey cows were divided equally into 2 treatments: 1) Individual Regrouping 3 d after calving; n = 17, and 2) Paired Regrouping 3 d after calving; n = 17). Treatment groups were balanced by parity and breed. After calving, all cows stayed in a postpartum pen for 3 d that was adjacent to the maternity pen. The afternoon of d 3, cows in the individual treatment were regrouped alone while the focal cow in the paired treatment was moved with a familiar partner (i.e., both spent 3 d in the postpartum pen together). Fresh fecal samples were taken during the afternoon feeding, for 5 d, starting on the day of regrouping. Samples were analyzed for fecal cortisol metabolite (11,17-dioxyandrostane; 11,17-DOA) concentration. Data were analyzed using a mixed model in SAS. Primiparous cows had higher 11,17-DOA than multiparous cows after regrouping (1.9 ± 0.2 vs. 1.1 ± 0.2 ng/mL, respectively; $P < 0.001$) and Holsteins tended to have higher 11,17-DOA relative to Jerseys (1.7 ± 0.2 vs. 1.3 ± 0.1 ng/mL, respectively; $P = 0.08$). Overall, cows regrouped as individuals had higher 11,17-DOA concentrations compared with paired cows (1.8 ± 0.2 vs. 1.2 ± 0.2 ng/mL, respectively; $P = 0.01$). These differences were greatest during the 24 h after regrouping ($P < 0.01$); by d 3 after regrouping there were no detectable differences in 11,17-DOA concentration ($P = 0.90$) between treatments. Moving cows in pairs may help reduce the physiological stress response observed within 24-h after regrouping.

Key Words: regrouping, dairy cattle, cortisol

M40 Acute behavioral effects of regrouping postpartum dairy cattle in pairs or individually.

K. A. Mazer* and J. M. Huzzey, *California Polytechnic State University, San Luis Obispo, CA.*

Regrouping involves moving cows from one pen to another and is associated with decreased feeding time and increased competition toward the regrouped cow. Pen changes occur frequently around calving, a time when cows are most vulnerable to health problems. Regrouping cows along with a familiar conspecific may make social integration easier; however, this hypothesis has yet to be tested among postpartum cattle. The objective of this study was to describe acute changes in behavior in the first hour following regrouping among cows moved with a partner vs. individually. Holstein and Jersey cows were divided into 2 treatments: 1) Individual Regrouping 3 d after calving; n = 16, and 2) Paired Regrouping 3 d after calving; n = 16. After calving, cows were held in a postpartum pen, adjacent to the maternity pen, for 3 d. On the afternoon of d 3, cows in the individual treatment were regrouped into the main lactation pen alone while the focal cow in the paired treatment was moved with a familiar partner (i.e., both spent 3 d in postpartum pen together). Time spent feeding, standing/lying in stalls, and at various locations in the pen were quantified over 1 h using 30 s time sampling. Data collection began immediately following the focal cow's entrance into the lactation pen. Grooming time and frequency of competitive displacements (from feed or lying stall) were quantified using continuous live-observation. Differences in behavior between the individual and paired treatments were analyzed using a mixed model in SAS. In the first hour after regrouping there were no treatment differences in grooming behavior, or time spent in the outdoor yard, lying stalls, back alley, feed alley, and water alleys of the pen ($P \geq 0.16$). Cows regrouped individually were displaced from the feed bunk more often (1.3 ± 0.3 vs 0.4 ± 0.3 times/60 min, respectively; $P = 0.04$) than cows regrouped as a pair. Regrouping cows with a familiar partner may help mitigate changes in behavior associated with this stressful relocation.

Key Words: postpartum, regrouping, behavior

M41 The effects of polymorphisms in growth hormone receptor, insulin-like growth factor-1, and tumor necrosis factor α genes on hepatic gene expression in postpartum dairy cattle.

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The objective of this study was to determine the effect of SNP in growth hormone receptor (GHR), IGF-I, and tumor necrosis factor α (TNFa) on hepatic gene expression. Multiparous Holstein cows (n = 40) were part of a separate study evaluating effects of diet on postpartum parameters. Cows were fed a common prepartum ration and assigned randomly at calving to one of 2 postpartum diets differing in fiber content. High fiber (35.3% NDF, 12.2% $uNDF_{240}$, 23.2% peNDF; n = 27) and low fiber (32.8% NDF, 9.5% $uNDF_{240}$, 21.6% peNDF; n = 29) treatment diets were formulated for equivalent metabolizable protein and starch. DNA from blood of each cow was genotyped for all SNPs by PCR-restriction fragment length (PCR-RFLP) methods. Liver biopsies (n = 40) were obtained on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum, liver tissue was immediately frozen in liquid nitrogen. Liver samples were analyzed via RT-qPCR to evaluate mRNA expression of GHR, IGF-I, TNFa, and tumor necrosis factor receptor super family 1a (TNFRSF1). Data were analyzed by repeated measures ANOVA with the random effect of cow within treatment and fixed effects of dietary treatment, genotype for all genes, time, parity group, and all interactions. There were no main effect differences ($P > 0.15$) of dietary treatment on hepatic gene expression of IGF-I, GHR, TNFa, or TNFRSF1. Cows with GHR SNP AB tended ($P < 0.15$) to have higher hepatic IGF-I gene expression compared with cows with GHR SNP AA or BB, while cows with TNFa SNP AB tended ($P < 0.15$) to have lower hepatic IGF-I gene expression compared with those with TNFa SNP BB. Hepatic gene expression was also influenced by GHR SNP such that genotype AB cows tended ($P < 0.15$) to have higher hepatic GHR gene expression than cows with GHR SNP AA or BB. There were no effects ($P > 0.15$) of genotype on hepatic TNFa or TNFRSF1 gene expression; however both differed ($P < 0.01$, $P = 0.12$ respectively) over time, with higher expression at d 14 compared with d 7. Genotype of GHR and TNFa, but not IGF-I, influenced hepatic gene expression in postpartum dairy cattle.

Key Words: transition cow, polymorphism, gene expression

Animal Health 1

M42 Transient versus persistent subclinical hypocalcemia: Association of calcium status with early lactation disease and production in Holstein cows. J. A. A. McArt*[1] and R. C. Neves[2], [1]*Cornell University, Ithaca, NY,* [2]*Texas Tech University, Lubbock, TX.*

Our objectives were to evaluate the association of subclinical hypocalcemia (SCH) duration with the risk of early lactation negative events and milk production. Data from a cohort of 407 Holstein cows in 2 herds in NY were used. Primiparous (P) and multiparous (M) cows were classified into 1 of 4 groups based on postpartum plasma total Ca (tCa): normocalcemic (NC; $[\mathrm{tCa}]_{\mathrm{P}} > 2.15$ mmol/L at 1 and 2 DIM, n = 67; $[\mathrm{tCa}]_{\mathrm{M}} > 1.77$ at 1 DIM, > 2.20 mmol/L at 4 DIM, n = 109); transient SCH (tSCH; $[\mathrm{tCa}]_{\mathrm{P}} \leq 2.15$ at 1 DIM, > 2.15 mmol/L at 2 DIM, n = 25; $[\mathrm{tCa}]_{\mathrm{M}} \leq 1.77$ at 1 DIM, > 2.20 mmol/L at 4 DIM, n = 50); persistent SCH (pSCH; $[\mathrm{tCa}]_{\mathrm{P}} \leq 2.15$ mmol/L at 1 and 2 DIM, n = 33; $[\mathrm{tCa}]_{\mathrm{M}} \leq 1.77$ at 1 DIM, ≤ 2.20 mmol/L at 4 DIM, n = 34); or delayed SCH (dSCH; $[\mathrm{tCa}]_{\mathrm{P}} > 2.15$ at 1 DIM, ≤ 2.15 mmol/L at 2 DIM, n = 19; $[\mathrm{tCa}]_{\mathrm{M}} > 1.77$ at 1 DIM, ≤ 2.20 mmol/L at 4 DIM, n = 70). Evaluated outcomes were development of a negative event (NEG; hyperketonemia at 3, 5, 7, and/or 10 DIM, metritis, or displaced abomasum or herd removal within 60 DIM) and average milk yield per d across the first 10 wk of lactation. Multivariable Poisson models were used to analyze the NEG outcome and generalized linear mixed models for milk yield. Both P and M cows with tSCH were no more likely to have a NEG event than NC cows (risk $\mathrm{ratio}_{\mathrm{P}}$ (RR) = 1.3, 95% CI = 0.5 to 3.2; $\mathrm{RR}_{\mathrm{M}} = 1.4$, 95% CI = 1.0 to 2.1). However, P and M pSCH cows were 4.1 (95% CI = 2.1 to 7.9) and 1.8 (95% CI = 1.2 to 2.7), and dSCH cows 3.2 (95% CI = 1.5 to 7.0) and 1.8 (95% CI = 1.4 to 2.6) times more likely to have a NEG event than NC cows, respectively. Both P and M cows with tSCH produced more milk per d than NC, pSCH, or dSCH cows across the first 10 wk of lactation. Primiparous cows averaged 28.5 ± 0.7, 31.9 ± 1.1, 29.7 ± 0.9, and 28.7 ± 1.2 kg per d for NC, tSCH, pSCH, and dSCH cows, respectively, and M cows averaged 44.6 ± 0.7, 49.1 ± 1.1, 46.4 ± 1.3, and 41.4 ± 0.9 kg per d for NC, tSCH, pSCH, and dSCH, respectively. Our results suggest that cows with tSCH adapt better to early lactation, develop fewer NEG events, and produce more milk than NC, pSCH, or dSCH cows. Primiparous or M cows with pSCH or dSCH are at an increased risk for early lactation NEG events and reduced milk production.

Key Words: calcium, subclinical hypocalcemia

M43 Effect of postpartum milking strategy on plasma calcium concentration and risk of subclinical hypocalcemia in dairy cows. A. Valldecabres*[1], R. Lopes[1], A. Lago[2], C. Blanc[3], and N. Silva-del-Río[1], [1]*Veterinary Medicine Teaching and Research Center, University of California Davis, Tulare, CA,* [2]*DairyExperts Inc., Tulare, CA,* [3]*GTV Dairies, Tipton, CA.*

The aim of the present study was to evaluate the effect of different postpartum milking strategies on plasma Ca concentration in multiparous dairy cows. A total of 83 Jersey and Jersey × Holstein crossbreed cows of 2nd to 8th parity, were enrolled in the study before 1st postpartum milking. Milking strategies implemented during the first 2 d postpartum were: once-a-day milking (1M; cows were milked every 24 h; n = 24), twice-a-day milking (2M; cows were milked every 12 h; n = 21), delayed milking (DM; cows were not milked for the first 2 postpartum milkings and were milked every 12 h afterward; n = 19), and restricted milking (RM; cows were milked 3 L every 12 h; n = 19). Blood samples for total plasma Ca analysis were collected from the coccygeal vessels into heparinized vacuum tubes starting before 1st postpartum milking, every 4 h up to 48 h and at 72 h postpartum. Plasma Ca concentration changes during the study period and risk of subclinical hypocalcemia (SCH; Ca ≤ 2.12 mmol/L) at 48 and 72 h postpartum were evaluated using MIXED and GENMOD procedures of SAS, respectively. Prevalence of SCH before 1st postpartum milking was 48%. There were effects of treatment ($P = 0.03$), parity ($P = 0.003$), time ($P < 0.001$), initial calcemic status ($P < 0.001$; normocalcemic or subclinically hypocalcemic) and time by initial calcemic status ($P < 0.001$) on plasma Ca concentration. Overall, lower plasma Ca concentration was observed for 2M cows (2.04 mmol/l) compared with DM (2.17 mmol/L; $P = 0.04$) and RM cows (2.17 mmol/L; $P = 0.04$), but no differences were observed with 1M (2.12 mmol/L). At 48 h postpartum the risk of SCH was lower for 1M (Risk ratio; RR = 0.27; $P < 0.001$), DM (RR = 0.55; $P = 0.02$) and RM cows (RR = 0.41; $P < 0.001$) than for 2M cows. At 72 h postpartum the risk of SCH was lower for 1M (RR = 0.26; $P < 0.001$) and DM cows (RR = 0.41; $P < 0.001$) than for 2M cows. Our results suggest that postpartum plasma Ca concentration and risk of SCH may be influenced by postpartum milking strategies, warranting its study as a prophylactic strategy for hypocalcemia. Project funded by USDA-NIFA (1013457 CFAH).

Key Words: dairy cow, hypocalcemia, transition cow

M44 The effect of prepartum negative dietary cation-anion difference and serum calcium concentration on blood neutrophil function in the transition period. R. Couto Serrenho*, E. I. Morrison, O. Bogado Pascottini, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

Our objectives were to assess the effects of a negative dietary cation-anion difference (DCAD) on phagocytosis (P) and oxidative burst (OB) function of circulating neutrophils and to determine the associations of serum ionized (iCa) and total calcium concentrations (tCa) with P and OB. We hypothesized that multiparous cows fed a negative DCAD prepartum would have greater serum ionized (iCa) and total calcium concentrations (tCa), and thus improved P and OB. From 3 wk before expected parturition until calving, 38 healthy multiparous cows from 3 farms were randomly assigned to negative DCAD (NEG; −100 meEq/kg DM; n = 21) or a control diet (CON; 12 meEq/kg DM; n = 17). Urine pH was measured weekly and in NEG was 6.3 ± 0.8 with 76% of 21 samples <7 and 62% < 6.5. Each farm was on 1 treatment or the other. P, OB, iCa, and tCa were measured at −7, 1, and 4 DIM. Mean fluorescence intensity for P (MFIP) and OB (MFIOB), and the shift of percentage of active cells for P (PP) and OB (POB) were measured in isolated, stimulated neutrophils via flow cytometry. Outcomes were assessed with mixed linear regression models accounting for repeated measures. There were no significant differences between treatments in the 4 neutrophil function outcomes. Although MFIOB varied over time, there were no interactions of treatment with time for any outcome. iCa and tCa did not differ between NEG and CON. The LSM ± SE for iCa were: −7 DIM, 1.23 ± 0.02 vs. 1.21 ± 0.02; 1 DIM, 1.07 ± 0.02 vs. 1.02 ± 0.02; 4 DIM, 1.16 ± 0.02 vs. 1.17 ± 0.02 mmol/L, respectively; and for tCa: −7 DIM 2.39 ± 0.04 vs 2.44 ± 0.05; 1 DIM, 2.01 ± 0.04 vs 1.97 ± 0.05; 4 DIM, 2.33 ± 0.04 vs 2.32 ± 0.05 mmol/L, respectively. The proportion of blood samples with tCa <2.15mmol/L at −7, 1 and 4 DIM was 5%, 76%, and 13%, respectively, with no differences between NEG and CON ($P > 0.7$). However, iCa tended to be weakly positively correlated with MFIOB at −7 DIM. There was no association between tCa and P or OB. We did not observe the hypothesized differences in aspects of innate immunity in multiparous cows fed a negative DCAD.

Key Words: hypocalcemia, immune function

M45 Factors that contribute to ketosis in early lactation Holstein dairy cattle.

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Ketosis in early lactation dairy cattle can negatively impact health events, milk yield and reproductive performance causing a loss in profit for dairy producers. The objective of this retrospective observational study was to identify factors such as management, metabolic profile and milk production that contribute to ketosis in early lactation. One hundred and thirty-two multiparous dry and lactating Holstein cows from a California commercial dairy herd were bled weekly from 3 wk before calving to the dry period via coccygeal venipuncture into evacuated sterile tubes containing sodium fluoride and potassium oxalate. Milk yield, fat and protein percentage were measured monthly by Tulare County Dairy Herd Improvement Association using a Bentley Instrument ChemSpec 150 (Chaska, MN). Blood metabolite data including nonesterified fatty acids (NEFA) using a commercial kit from Wako Chemicals Inc. (Richmond, VA), glucose and BHB using a Precision Xtra handheld meter (Abbott Diabetes Care Inc., Alameda, CA), and metabolic data using an Abaxis Vet Scan analyzer Large animal profile rotor (VetScan®, Abaxis, Inc., Union City, CA) were measured weekly. Management variables and milk production from Dairy Comp 305 (Valley Agricultural Software, Tulare, CA) were collected monthly in the previous and current lactation. Using the General Linear Model Procedure of SAS (SAS Institute v.9.4, 2018), BHB were regressed on blood metabolites, management data and milk production. Variables were eliminated from the regression if $P > 0.05$. The significant management variables that remained in the regression were total days in milk in previous lactation and days dry in previous lactation ($P < 0.05$, $R^2 = 0.70$). When metabolic variables such as aspartate amino transferase, globulin, blood urea nitrogen, NEFA, and glucose from −7 to 14 d relative to calving were added, and milk production variables such as total solids and total fat in the previous lactation were added to the regression, $P < 0.05$ and $R^2 = 0.92$. Residuals were normally distributed and unbiased. These equations indicate that cows at risk for ketosis could be identified before calving to help dairy producers manage ketosis before it negatively affects health and production performance.

Key Words: ketosis, dairy cattle

M46 Evaluation of the economic costs of ketosis and their prevention strategies in dairy cattle.

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Transition diseases result in considerable economic losses for dairy producers. The purpose of this study was to evaluate the technical consequences of subclinical ketosis (SCK), their economic impact on dairy farm, and the profitability of prevention strategies used in practice. Data from current literature was collected and summarized, and used to model the impact of different incidences (20–40–60%), and its effect on milk production (loss of 5–10% for 60 d), fertility (decrease of 25–50% for 120 d), and increased risk of displaced abomasum, clinical ketosis, metritis and the culling risk of 6, 3.6, 2 and 3.7 times, respectively. Two systematic prevention strategies applied to all cows at calving were used: propylene glycol (PG) with a total cost of €12/cow and a 47% reduction of incidence; or a slow-release monensin bolus (MON) with a total cost of €30/cow and a 60% reduction of incidence. Four simulations were carried out at 2 milk prices (€300 and €360/t): 1) a control without SCK incidence; 2) with SCK (40% incidence, a 10% loss of milk production and a 50% decrease in fertility); 3) with SCK and PG; and 4) with SCK and MON. The SCK impact was evaluated in a farm of 300 dairy cows with average 12,000 L/cow/yr and average technical performance indexes for dairy farms in Spain, using a dairy farm stochastic dynamic model (www.dairyfarm.es; Calsamiglia et al., 2018). The cost per case was €339, ranging from €277 to €373 among incidences. Economic losses due to decreased milk production (64%) were larger that losses attributed to reduced reproductive performance (36%). The PG (€63) was a more profitable strategy compared with MON (€33). Although the cost of the case was similar across incidences, when the incidence was reduced (fewer cases) or milk price decreased, the economic return of prevention treatment was lower. Subclinical ketosis has important effects on cow performance and farm profitability. The adequacy and type of prevention strategy depends on the SCK incidence, the cost of treatment and the price of milk.

Key Words: ketosis cost, propylene glycol, monensin

M47 Effect of automating health monitoring on detection of health disorders and performance of lactating dairy cows.

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Our objective was to evaluate the effect of a health monitoring program based primarily on alerts generated by automated health monitoring (AHM) systems on disease detection and performance of dairy cows. Parous and nulliparous Holstein cows at 245 ± 3 d in gestation were stratified by parity and randomly assigned to a CON (n = 622) or TRT group (n = 621). Cows from both groups were commingled. For cows in CON, clinical examination (CE) was conducted daily up to 10 DIM and in response to daily milk yield reduction (≥15% negative deviation) alerts (Afimilk) or visual observation of clinical signs of disease from 11 to 30 DIM. For cows in TRT, CE up to 30 DIM was conducted in response to one or more of the following: an alert generated with a combination of rumination time and physical activity (HID score, SCR Dairy), milk yield deviation, or visual observation of clinical signs of disease. Daily after the morning milking, cows eligible for CE were evaluated following similar procedures. Binomial and quantitative data were analyzed by logistic regression and ANOVA with repeated measures, respectively. The proportion of cows with at least one event of metritis ($P = 0.43$; CON = 12.5%; TRT = 11.1%), displaced abomasum ($P = 0.45$; CON = 1.1%; TRT = 1.6%), indigestion ($P = 0.73$; CON = 2.9%; TRT = 3.2%), pneumonia ($P = 0.3$; CON = 1.5%; TRT = 0.8%), and mastitis ($P = 0.4$; CON = 10.3%; TRT = 8.9%) did not differ. The proportion of cows with ketosis ($P = 0.09$; CON = 8.7%; TRT = 6.1%) and the total proportion of cows with at least one event of disease ($P = 0.05$; CON = 30.4%; TRT = 25.3%) tended to differ. There was no difference in the combined proportion of cows sold and dead ($P = 0.35$; CON = 15.2%; TRT = 12.3%). No difference ($P = 0.29$) was observed for average weekly milk (CON = 41.6 ± 0.3 kg; TRT = 41.2 ± 0.3 kg) for up to 35 DIM. Cows inseminated at detected estrus ($P = 0.30$; CON = 66.7%; TRT = 63.8%) and pregnancy per AI to first service did not differ ($P = 0.72$; CON = 41.7%; TRT = 40.1%). We conclude that a health-monitoring program based primarily on alerts generated by AHM systems was an effective strategy to identify cows with health disorders and did not negatively affect herd production and reproductive performance outcomes. Supported by New York Farm Viability Institute project 017-014.

Key Words: automation, health, dairy cow

M48 Characterization of peripartum circulating metabolites in cows with varying degrees of liver triglyceride accumulation.

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Identification of circulating markers that reflect liver triglyceride (lvrTG) status would provide valuable insight when liver biopsies are not available. The objective of this study was to determine if metabolites related to energy balance and hepatic health would differ between cows with varying degrees of lvTG accumulation. Liver and blood samples were collected from multiparous Holstein cows (n = 37) at −14, +1, and +14 d relative to calving (DRTC) enrolled in 2 previously reported studies with 2 treatments (TRT) each. During the dry period, cows were fed a ration that met 100% (1 TRT; n = 13) or 120% (3 TRT; n = 7, 6, 11) of energy requirements to yield a range of postpartum lvTG. Postpartum rations were similar, except for 1 ration that included ammoniated lactate (n = 6). Cows were retrospectively grouped based on maximum lvTG% (DM basis) into 3 groups (TGgrp): high (lvTG = 30.3 ± 1.1%; n = 12), med (lvTG = 15.9 ± 1.06%; n = 13), or low (lvTG = 10.4 ± 1.1%; n = 12). Samples were analyzed for serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin (alb), triglyceride (TG), β-hydroxybutyrate (BHB), and blood urea nitrogen (BUN), and plasma nonesterified fatty acids (NEFA) and glucose (glc). Data were analyzed in SAS (9.4) using Proc Mixed with fixed effects of TGgrp, DRTC, [h5] TGgrp × [SE6] DRTC, TGgrp × TRT and random effect of cow within TRT. Means were considered different at $P < 0.05$, and were separated by Tukey's adjustment. Concentrations of AST, ALT, alb, BHB, BUN, glc, NEFA, and TG all changed over time ($P < 0.01$). Blood ALT, alb, and NEFA were different ($P < 0.05$) and glc tended to differ ($P = 0.06$) between TGgrp. Notably, NEFA was greater in the high group ($P = 0.01$, 0.45 ± 0.05 mmol/L) compared with the med and low groups (0.30 and 0.30 ± 0.06 mmol/L) and ALT was greater in the low group ($P = 0.02$, 20.2 ± 0.9 U/L) than the med and high groups (16.6 and 16.3 ± 1.0 U/L). A TGgrp by time interaction ($P < 0.05$) was observed for AST and BHB. It is clear that there are differences in circulating metabolites in cows with varying postpartum lvTG%, which may be useful in characterizing hepatic health.

Key Words: transition cow, biomarker, fatty liver

M49 Prevalence of subclinical ketosis and its effects on milk production and blood chemistry in high-yielding dairy cattle.

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The objectives of this study were to determine the prevalence of subclinical ketosis (SCK) and evaluate the effects of SCK on milk production and blood chemistry of Holstein cows in Korean dairy farms. The study was carried out from 2017 to 2018 in 6 provinces of South Korea with 49 herds and 905 Holstein cows. Experimental animals were selected randomly from 5 different lactation period. Milk production, parity, and stages of lactation were recorded from each farm. Blood samples were analyzed by using Biochemical Analyzer to determine 11 biochemical indices. Statistical analysis was done using SPSS 16.0 and data were presented as mean ± SE. Animals were categorized into normal (<1.2 mmol/L), sub-clinical (1.2–2.9 mmol/L) and clinical (>2.9 mmol/L) ketosis (CK) groups based on their β-hydroxybutyric acid concentration in blood. Overall, the prevalence of SCK was 23.60%, which varied among herds. Among 49 herds, 8 herds had > 40% of SCK, and 15 herds had 20–40% SCK. The highest prevalence of SCK was observed in 1st parity with 33.33%, followed by 30.27%, 20.57%, and 5.12% in 2nd, 3rd, and 4th parities, respectively. However, the CK prevalence was highest in 3rd parity (25%). The SCK prevalence was highest (31.03%) at early lactation stages (0–49 DIM) compared with other lactation periods. The milk yield decreased significantly by 11% in SCK (normal: 38.79 ± 4.98, SCK: 34.49 ± 1.64 L/day; $P = 0.028$) animals. The animals with SCK had significantly higher aspartate aminotransferase (normal: 109.16 ± 1.53, SCK: 110.66 ± 2.29 IU/L; $P = 0.035$) and total bilirubin (normal: 0.39 ± 0.01, SCK: 0.44 ± 0.02 mg/dl; $P = 0.001$) concentrations and significantly lower blood glucose (normal: 51.08 ± 0.51, SCK: 46.15 ± 0.86 mg/dl; $P = 0.001$) concentration than normal animals. This is a recently conducted population based study that shows the nationwide prevalence of SCK in Korea. The results of this study will provide important information to farm practitioners and producers to be aware of the milk yield loss due to SCK during farm management.

Key Words: subclinical ketosis, prevalence, milk yield

M50 Haptoglobin critical thresholds for predicting health disorders during the transition period.

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The objective was to 1) establish cow-level thresholds for haptoglobin (Hp) concentrations to predict health disorders and 2) to investigate the magnitude of association between elevated Hp and health disorders within 30 DIM. Data were collected from 72 commercial dairy farms and Hp and β-hydroxybutyrate (BHB) concentrations were measured in 988 Holstein cows, 0 to 12 DIM. Associations between the health disorder of interest and Hp were assessed in SAS v. 9.4, PROC GENMOD, using a Poisson distribution, log link function, p-scale option, exchangeable correlation matrix, herd as a random effect and included biologically plausible covariates and 2-way interactions. Manual backward stepwise elimination removed covariates and interactions if $P > 0.10$. If Hp was significant, an ROC analysis with PROC LOGISTIC was used to determine the critical threshold for predicting the health disorder by identifying the point on the curve with the maximum Youden's index. Based on the threshold, Hp was categorized and the multivariable model was reassessed to determine risk ratios. Haptoglobin was not effective for predicting herd reported mastitis, DA, or ketosis ($P > 0.10$). The Hp threshold for predicting herd reported metritis (MET) was 1.52 g/L (AUC = 0.8; Se: 67%, Sp: 84%), for predicting herd removal (CULL) was 0.68 g/L (AUC = 0.7; Se: 72%, Sp: 68%), and for association with hyperketonemia (HYK; BHB ≥1.0 mmol/L) was 0.74 g/L (AUC = 0.6; Se: 45%, Sp: 72%). The Hp threshold for predicting any significant health disorder (DIS; MET, CULL, HYK) was 0.74 g/L (AUC = 0.6; Se: 45%, Sp: 74%). Risk ratios for variables remaining in the health disorder models are reported in the table. Elevated Hp was found to be associated with HYK, MET, and herd removal within 30 DIM.

Table 1 (Abstr. M50). Risk ratios (RR) for variables associated with health disorders

Disorder	Variable	Threshold	*P*-value	RR	95% CI
MET					
	Hp (g/L)	1.52	<0.01	8.6	3.0 to 24.5
HYK					
	Hp (g/L)	0.74	<0.01	1.8	1.4 to 2.3
	Parity[1]		<0.01	2.1	1.5 to 2.8
CULL					
	Hp (g/L)	0.68	<0.01	4.9	2.4 to 9.9
	Lameness[2]		<0.01	3.5	1.6 to 7.6
DIS					
	Hp (g/L)	0.74	<0.01	1.8	1.5 to 2.3
	Parity[1]		<0.01	1.6	1.2 to 2.3
	Lameness[2]		0.04	1.3	1.0 to 1.6

[1] Multiparous vs. primiparous.

[2] Lame vs. not lame.

Key Words: haptoglobin, metabolic health, transition cow

M51 Evaluation of reproduction and behavior of dairy cows treated with Pegbovigrastim.

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The aim of this study was to evaluate the effects of pegbovigrastim injection (Imrestor, Elanco Animal Health, Greenfield, IN) on behavior and time to first detected heat. The study was conducted on a commercial Mississippi dairy from August 21, 2017, to March 31, 2018. Initially, a total of 270 cows were blocked by parity group (multiparous or primiparous) and randomly assigned to control (CON, n = 144) or treatment (IMR, n = 126). Study animals were equipped with smart leg and smart neck tags (Nedap, the Netherlands). The leg tag measured lying time, walking time, and leg activity with a 3-axis accelerometer, and the neck tag measured heat activity. At 10 ± 2 d before expected calving and again at calving, treated cows received 2.7 mL of Imrestor and CON cows received 2.7 mL of 0.9% saline. Cows were body condition (BCS), hygiene (HYG), and lameness (LAM) scored weekly. The Nedap system created heat alerts based on individual behavior changes. Weekly summations of the number of steps, time spent lying down and walking (min) was then utilized to determine weekly means of these variables since calving until 60 DIM. Unfortunately, because of malfunction in the data synch between tags and the farm software, data from only 61 cows were utilized in the analysis (CON = 43 and IMR = 18). The MIXED procedure of SAS was utilized to compare the leg activity, walking time, and lying time between the 2 treatment groups. The mixed models included fixed effects of treatments and block, and random effect of cow within block. A Cox proportional hazards regression was used to determine the relative likelihood of cows displaying estrus within their first 60 DIM. Imrestor treatment did not alter the hazard ratio of estrus occurrence during the first 60 DIM (hazard ratio = 0.99, 95% CI = 0.84 to 1.7, *P* = 0.88). The median calving-to-first detected heat interval for IMR and CTR cows was 47 and 46 d, respectively (*P* = 0.45). Imrestor treatment did not affect time spent lying, walking, or amount of steps given (*P* = 0.54, 0.59, and 0.48, respectively). In conclusion, IMR treatment did not affect the behavior or time to first detected heat in study cows.

Key Words: Imrestor, precision dairy monitoring, well being

M52 Impact of a liposome-TLR agonist stimulant on macrophage bactericidal activity against *Staphylococcus aureus* and on intramammary immune responses.

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Mastitis is a major economic and welfare concern to the dairy industry and it is the most common reason for the use of antibiotics in dairy farms. The development of innovative approaches capable of enhancing udder health will contribute to the judicious use of antibiotics in dairy farms. Thus, the objective of our pilot studies was to investigate the effect of a mucosal immune stimulant (MIS; MucosImmune, Laporte Therapeutics, Fort Collins, CO) on mammary gland immune response. We hypothesized that intramammary (IMM) infusion of MIS would trigger non-specific activation of mammary gland immunity. In Experiment 1, macrophage cultures were generated from monocytes of healthy cattle (n = 3) and incubated with MIS (or PBS) for 24h, then inoculated with a clinical mastitis isolate of *S. aureus* and intracellular bactericidal activity assessed 4h later. We found that MIS activation of macrophages significantly increased intracellular killing of *S. aureus* (from 16.7% to 44.2%; *P* = 0.004), to a level comparable to that generated by IFN-g (*P* > 0.05). In Experiment 2, lactating dairy cows were randomly allocated to receive IMM administration of either MIS solution (TRT; 1mL MIS and 9mL PBS; n = 4) or 10mL of PBS (UTR; n = 3). Milk samples for TRT and UTR cows were collected at 72h after treatment. Milk samples were incubated with *S. aureus* and *E. coli* isolated from mastitis cases for 30 min to measure levels of IgA and IgG that bound to these pathogens. Milk from cows in the TRT group had higher levels of IgA and IgG antibodies against *E. coli*, but lower levels of antibodies binding to *S. aureus* (Table). Our preliminary results indicated that MIS is capable of non-specific activation of intra-mammary immunity and increases milk IgA and IgG antibodies against *E. coli,* but not against *S. aureus*.

Table 1 (Abstr. M52). Milk immunoglobulin (Ig) A and G against *S. aureus* and E. coli.

	TRT		UTR		
Item	Mean[1]	SD	Mean[1]	SD	*P*-value
Anti *S. aureus*					
IgA	55,346	13,833	100,847	30,595	<0.01
IgG	19,882	7,739	102,104	24,773	<0.005
Anti *E. coli*					
IgA	2,388	1,554	1,275	848	<0.05
IgG	2,871	1,742	1,888	1,456	<0.05

[1] Geometric mean fluorescence intensity (gMFI).

Key Words: mastitis, immune stimulant, bactericidal

M53 The probability of subclinical mastitis and isolated organisms in organic dairy herds varies between years.

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Prevention and treatment of mastitis without the use of antibiotics or synthetic products is one of the challenges organic dairies face. Greater understanding of factors affecting the probability of mastitis will aid in developing mastitis control programs. The objective was to determine if probability of subclinical mastitis and isolated organisms varied between year of 2017 and 2018 in relation to season, parity, and stage of lactation. Five organic dairies (n = 402 cows) were enrolled in the study. To determine the probability of subclinical mastitis, Dairy Herd Improvement (DHI) records for somatic cell score (SCS), days in milk and parity were assessed. Subclinical mastitis was characterized as cows having a SCS of >4. Aseptic quarter milk samples (n = 1,825) were collected from cows with subclinical mastitis and tested for microbiological identification. Organisms greater than 5% in prevelance were evaluated. Logistic regression (PROC GLIMMIX, SAS 9.4, Cary, NC) was used with fixed effects of season, parity, stage of lactation and year, and a random effect of cow(herd*yr) to test the association with mastitis and organism probabilities. Overall, the probability of subclinical mastitis was greater in 2017 than 2018 ($P = 0.007$), with cows 1.05 times more likely in the fall (OR = 1.05; 95% CI: 0.77, 1.43), 1.87 times more likely in the spring (OR = 1.87; 95% CI: 1.27, 2.76) and 2.15 times more likely in the summer (OR = 2.15; 95% CI: 1.49, 3.10) to have subclinical mastitis. 2017 also was associated with a greater probability of coagulase-negative staphylococci (CNS), *Staphylococcus hyicus* and *Corynebacterium* spp. and lower probability of *Streptococcus uberis*. Only *S. hyicus* was associated with lower probability in the spring versus the summer regardless of the year ($P = 0.03$; OR = 0.431; 95% CI: 0.19, 0.94). CNS was least common in the first parity cows ($P < 0.04$) in contrast to *Staphylococcus aureus* having greater probability in the first parity cows ($P < 0.09$) versus greater lactations. Future research targeting factors behind greater probability of *S. hyicus* in the summer and contrasting probabilities of *S. aureus* and CNS with parity will provide greater insight to developing comprehensive mastitis control programs in organic dairy systems.

Key Words: mastitis, organic

M54 Isolated microorganisms from mammary quarters milk of buffaloes in automatic milking system.

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The aim of present study was to determine the occurrence and etiology of subclinical mastitis in buffaloes submitted to an automatic milking system. A total of 146 quarter milk samples from buffaloes without clinical mastitis were analyzed. Milk collection was performed before starting the morning milking. A single milk sample was obtained from one mammary quarter of each animal. The aseptic collection of samples for microbiological analysis was performed directly from the teat, disinfected with alcohol 70% (wt/vol). The milk was stored in a 15 mL sterile polypropylene tube, frozen and sent to the laboratory to isolate and identify bacterial organisms. The samples were incubated at 37°C for 8 h for the pre-enrichment stage, and subsequently plated on sheep blood agar, brain heart infusion agar and Sabouraud agar with chloramphenicol. After incubation, morphological characteristics of the isolated colonies and individual innocuous were evaluated, then plated in selective and differential media to observe the characteristic phenotypic aspects of the genera. A total of 140 (96%) samples were culture positive, and among these, 91 (65%) mammary quarters were infected by individual pathogens. From these, 47% were *Corynebacterium* spp., 25% were *Staphylococcus* spp., 13% *Streptococcus* spp., 7% *Bacillus* spp., 2% *Klebsiella* spp., 2% *E. coli*, 2% yeast and 1% *Micrococcus* spp. *Corynebacterium bovis* was the predominant mastitogenic organism, followed by *Staphylococcus chromogenes*. Among streptococci, *Streptococcus agalactiae* was the predominant organism. The mixed infections were detected in 35% quarters in different combinations. The most common combination was *Staphylococcus* spp. and *Corynebacterium* spp., followed by *Streptococcus* spp. and *Corynebacterium* spp., while *S. aureus*, *S. equorum*, *Pseudomonas* spp., *Enterococcus* spp. and *Aerococcus viridans* were present in mixed infections with low prevalence. Buffaloes managed in intensive farms may present subclinical mastitis cases. Therefore, attention is necessary in the drying management and other mastitis prevention practices, especially when the buffaloes are submitted to an automatic milking system.

Key Words: *Bubalus bubalis*, infection, mastitis

M55 Survey of *Clostridium* populations from dairies across the United States.

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Clostridium are spore forming bacteria that are ubiquitous in fecal and environmental dairy samples. Toxin-producing *Clostridium* species such as *C. perfringens, C. septicum*, and *C. difficile* have been associated with enteric disease in ruminants. The objective of this survey was to characterize *Clostridium* populations in dairies across the United States to better understand the species richness, evenness and determine if there are regional differences in *Clostridium* populations. From November 2015 to February 2019, 14,265 fecal samples were collected from 368 farms across 26 states. Samples were transported on ice to the lab within 24 h and clostridia were enumerated on TSC agar incubated under anaerobic conditions. Counts ranged from < 1.0E1 to 1.5E8 cfu/g total clostridia with a mean of 9.2E4 cfu/g and median of 6.4E2 cfu/g. Clostridia isolates were collected from a core set of over 4,000 fecal samples to identify toxin types. Over half (52.6%) of the isolates (n = 52,322) tested from cow samples were identified as *C. perfringens*. Calf clostridia isolates (n = 6,219) had a lower percentage identified as toxin producing *C. perfringens* (38.9%) than cows. In both cows and calves *C. perfringens* isolates were mainly Type A (99.0%). Non-*C. perfringens* isolates were identified using 16S PCR. The 2 most abundant species were identified as *C. bifermentans* group (14.4%) and *C. beijerinckii* group (8.6%). These species are known for their ability to produce end products such as 1,3 propanediol, butanol and acetone. Twenty-eight species of *Clostridium* were detected including other toxin-producing species such as *C. sordellii* and *C. difficile*. *Clostridium* populations were analyzed based on region. Samples collected from California, Texas, Upper Midwest, and Wisconsin had different populations ($P < 0.05$) compared with Idaho, Great Lakes, Mid Atlantic, Florida, I-29 Corridor and Northeast; the Northeast trended toward having a different *Clostridium* population ($P = 0.09$). This data gives a better understanding of *Clostridium* species in ruminants and demonstrates regional clostridia population differences across the United States.

Key Words: *Clostridium*, microbiology

M56 Molecular characterization of *Escherichia coli* isolates obtained from bovine clinical mastitis.

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Bovine mastitis is one of the major diseases associated with the dairy industry, causing great economic losses. *Escherichia coli* is the most prevalent microorganism in clinical mastitis, causing severe symptoms and even death. In Brazil, little is known about the genetic background of *E. coli* isolates causing mastitis, and if these isolates can cause illness in the human hos, such as a zoonotic agent. In this study, we investigated the occurrence of several virulence-factor encoding genes in *E. coli* isolates from milk of cows diagnosed with clinical mastitis. This study was approved by the UNESP's Animal Use Ethics Committee (N 0136/2017). We used 10 different dairy herds, selected by convenience, and inclusion criteria were: mastitis control programs with data storage, average milk yield >20 kg, minimum of 200 lactating cows and use of machine milking. After clinical diagnosis, milk samples were collected aseptically. The isolation and identification were performed according to National Mastitis Council. The occurrence of genes frequently used for diarrheagenic *E. coli* (DEC) pathotypes identification (*escN*, *bfpA*, *stx1*, *stx2*, *elt*, *est*, *ipaH*, *aatA, aggR*, *aaiA* and *daaE*) and involved in extra-intestinal *E. coli* (ExPEC) pathogenicity (*sfaDE, fimH, iha, ecpA, papA, papC, hlyA, cnf1, sat, vat, iroN, irp2, iucD, ireA, sitA, ibe10, traT, ompT, afaBC, and cdt*) were searched by PCR in the 93 *E. coli* isolates obtained in this study. One atypical EPEC ($escN^+/bfpA^-$) and one atypical EAEC ($aaiA^+/aggR^-$) were detected. Regarding the genes associated with ExPEC pathogenicity, we observe the presence of genes encoding for: adhesins (*fimH* = 98%; *ecpA* = 48.4%), siderophores (*irp2* = 8.6%; *sitA* = 8.6%), toxins (*vat* = 3.2%; *cdt* = 2.2%; *hlyA* = 5.4%, invasin (*ibe* = 2.2%), and protactins (*ompT* = 19.4%; *traT* = 59.1%). We concluded that the majority of the isolates cannot be classified in the distinct DEC pathotypes, but possess pathogenic potential to cause extra intestinal disease.

Key Words: extra-intestinal *E. coli* (ExPEC), diarrheagenic *E. coli*, virulence

M57 Biofilm production by *Escherichia coli* isolates obtained from bovine clinical mastitis.

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Escherichia coli is an environmental pathogen causing clinical mastitis, a very important disease in dairy farms due to significant economic losses. This microorganism can use different mechanisms to colonize bovine mammary gland such as invasion cells, immune system evasion and biofilm production. The aim of this study was to investigate the biofilm formation by *E. coli*, isolated from milk of cows with clinical mastitis. This study was approved by the UNESP´s Animal Use Ethics Committee (N 0136/2017). We used 10 different dairy herds, selected by convenience, and study inclusion criteria were: mastitis control programs with data storage, average milk yield >20 kg, minimum of 200 lactating cows and use of machine milking. A total of 2,613 clinical mastitis milk samples was evaluated, during the period of September 2017 to September 2018. After clinical diagnosis, milk samples were collected aseptically. The isolation and identification were performed according to National Mastitis Council. *E. coli* was identified in 115 (4.4%) milk samples. For biofilm assay, we used 96-well polystyrene microplates and the 115 isolates were incubated in brain heart infusion (BHI) +1% glucose at 37°C for 18h without shaking. Under those conditions, 51 (44.3%) were not producers. Among the 54 remaining, 54 (47%) were classified as weak producers, 9 (7.8%) as moderate and only one (0.9%) as a strong producer. According to the results, it seems that biofilm formation is not a common characteristic among these isolates and does not play a key role in the establishment of mastitis in cows.

Key Words: mastitis, biofilm

M58 Distribution and factors associated with antimicrobial usage for cows and preweaned calves in large dairy farms.

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Use of antimicrobials in agriculture is controversial and knowledge of the factors that influence antimicrobial usage (AMU) are needed. The objectives of this study are to describe herd level AMU on dairy farms and investigate associations of AMU with selected risk factors. Eligible farms contained >250 lactating cows and met criteria for maintaining computerized animal health records. Data for one-year period was retrospectively collected on 40 farms and researchers visited all farms to validate case definitions and recording accuracy. Adult cows potential risk factors included culling rate, length of dry period, and association of early lactation AMU with total AMU. Preweaned calves risk factors included feeding method (esophageal; bottle; esophageal and bottle) and preventive usage of antimicrobials (yes; no). Herd level AMU was standardized as the number of defined daily doses (DDD) per cow and preweaned calf/yr, and AMU was also calculated as DDD/cow/yr and DDD/preweaned calf/yr. Linear regression was used to identify associations between AMU and continuous risk factors and *t*-tests were used to assess risk factors for feeding method and preventative AMU. Enrolled farms contained 52,639 cows (mean: 1,316 ± 169 SE), and 6,281 preweaned calves (mean: 180 ± 33 SE). Total herd AMU was 6.6DDD/cow and preweaned calf/yr (2.2 to 15.7). Adult cows accounted for 83% of the total herd AMU (5.5DDD). Adult cows AMU was 7.0DDD/cow/yr (2.4 to 13.2). Preweaned calves AMU was 10.4DDD/preweaned calf/yr (0.09 to 48.9). Culling rate and dry period length were not associated with AMU in adult cows ($P > 0.22$). Greater use of AMU in the first 30 DIM was associated with greater AMU in the rest of the lactation ($P < 0.001$). Dry cow therapy and AMU within the first 30 DIM represented 18% and 39% of the AMU used for adult cows respectively. Preweaned calves AMU tended to be associated with feeding method ($P = 0.08$) and were 8.6 ± 2.5DDD (esophageal feeder), 12.8 ± 5.4DDD (bottle), and 13.7 ± 3.2DDD (both methods). There was a tendency for farms that reported use of preventive antimicrobials in preweaned calves to have greater use of AMU (16.1 ± 5.3DDD vs. 9.5 ± 2.1DDD, $P = 0.10$). AMU and risk factors varied between adult cows and preweaned calves.

Key Words: antimicrobial usage, risk factors, defined daily doses

Breeding and Genetics 1

M59 Associations between serum total protein and genomic predicted transmitting abilities for production, fitness, and conformation in organic Holstein calves. I. Haagen*[1], L. Han[1], L. Hardie[1], B. Heins[2], and C. Dechow[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of Minnesota, Morris, MN.*

The objective of this study was to evaluate associations between serum total protein concentration and genomic predicted transmitting abilities (gPTA) for production, health and fertility in organic Holstein calves. Calves (n = 159) were born between January 2015 and May 2018 on 2 organic dairies. Serum total protein levels were recorded on farm and extracted from farm management software. Observations were restricted to the first 3 d following birth. Calves were genotyped (~150K markers) and received official genomic evaluations through the Council on Dairy Cattle Breeding and Holstein Association USA. Serum total protein was regressed on age (0, 1, 2, or 3 d) and the linear effect of a single gPTA; the gestation length (GL) regression also included a quadratic term. The mean serum total protein concentration for all calves was 6.54 g/dL. Production (milk, fat, and protein yields) and conformation (e.g., type, stature, strength, body depth, dairy form) gPTA were not significantly associated with serum total protein. Among fitness traits, lower gPTA for somatic cell score and higher gPTA for mastitis resistance tended ($P < 0.10$) to be associated with higher serum total protein whereas milk fever resistance was unfavorably associated ($P < 0.05$) with serum total protein. Lower daughter stillbirth (DSB) was associated ($P < 0.05$) with higher serum total protein. Linear and quadratic effects for GL were significantly ($P < 0.05$) associated with serum total protein; calves with high GL had lower serum total protein with an intermediate optimum for GL gPTA of −1. These results suggest that genetics may play a role in serum total protein concentrations in organic Holstein calves. Significant effects of traits observed near birth such as GL and DSB suggest that factors such as calf size or stress from the birthing process could influence serum total protein.

Key Words: calf health, organic, serum total protein

M60 Genetic polymorphism of κ-casein on Coalho cheese yield from Zebu cows. I. L. S. Oliveita[1], R. C. Madruga[2], R. D. S. Gomes[1], E. P. E. Silva[1], E. G. S. O. Silva[1], J. S. Bezerra[3], M. F. Bezerra[1], D. C. Sales[4], L. H. F. Borba[1], J. G. B. Galvão Jr.[5], and A. H. N. Rangel*[1], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Associação Brasileira dos Criadores de Zebu, Parnamirim, RN, Brazil,* [3]*Universidade Federal Rural de Pernambuco, Recife, PE, Brazil,* [4]*Universidade do Estado de São Paulo, Jaboticabal, SP, Brazil,* [5]*Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil.*

The objective of this study was to evaluate the influence of the genetic polymorphism of κ-casein on the yield of Coalho cheese made from Zebu cow milk. The raw milk for producing the Coalho cheese came from Guzerá (n = 3), Gir (n = 7) and Sindi (n = 10) breeds. Genomic DNA was extracted from the capillary bulb of the 20 animals and submitted to PCR (polymerase chain reaction) analysis to determine the genetic polymorphism of κ-casein. The amplified products were run in capillary electrophoresis to verify the quality and concentration of the fragments. Band reading was performed using GeneMapper Software. Finally, allele and genotype frequencies were obtained for the 3 evaluated breeds. The data were submitted to ANOVA using SAS software (version 9.0) and the differences between the means were tested by the Tukey test at 5% significance. The Sindi breed obtained higher ($P < 0.05$) performance for the Coalho cheese yield (93.68g of total solids per liter, TS/L) in relation to the Guzerá (82.25g TS/L) and Gir (83.33g TS/L) breeds. The allele frequencies found for the κ-casein genetic polymorphism were 0.75 (allele A) and 0.25 (allele B) for the Sindi breed; 1.0 (allele A) and 0.0 (allele B) for the Gir breed; and 0.83 (allele A) and 0.17 (allele B) for the Guzerá breed. The obtained genotypes for the genotype frequency were AA and AB, being AA (0.50) and AB (0.50) for the Sindi breed; AA (0.66) and AB (0.33) for Guzerá; and AA (1.0) and AB (0.0) for Gir. Thus, the Sindi breed obtained the highest frequency of the B allele (25%) when compared with the other breeds, enabling a higher percentage of total solids in the cheese, and therefore implying in a positively higher yield (g TS/L) of Coalho cheese for the breed. Therefore, the higher frequency of the κ-casein B allele has a positive influence on Coalho cheese yield.

Key Words: Zebu cattle, genotype frequencies, capillary electrophoresis.

M61 High-resolution purity analysis of sex-sorted sperm and correlation with field results. K. M. Evans, C. Heuer, C. González-Marín*, J. G. Escobar, J. F. Moreno, and R. Vishwanath, *Sexing Technologies, Navasota, TX.*

The sex of the offspring can be predetermined by flow cytometric sorting of highly purified X- or Y-chromosome-bearing sperm subpopulations. The technology has undergone several improvements in the past 10 years and it is now widely used by dairy and beef cattle, small ruminants, deer, equine and porcine industries. To ensure the quality of the sorted product, Sexing Technologies analyzes various sperm parameters at multiple checkpoints during the sex sorting process. One of these quality parameters is sperm sex purity, which is assessed using a Genesis-1 high resolution Analyzer, a modified MoFlo SX sperm sorter, developed for sperm analysis using ST proprietary technologies. Each Analyzer is calibrated using certified sperm nuclei as a standard, and has to meet predetermined specifications of number of oriented cells for the Side Fluorescence detector path and quality of resolution on DNA content on the Forward Fluorescence detector path before running any purity analysis. This confirms the instrument is well aligned and meeting the required orientation and resolution criteria to perform accurate assessment of sex sorted sperm purity. High performance sperm sorting systems known as Genesis-III have advanced digital processing, multiple heads and automation built in. These systems are verified multiple times a day to confirm that the setup is accurate for the desired sex ratio, while optimizing and maintaining productivity. Estimation of the percent of X- and Y- chromosome-bearing sperm is also performed on each batch of sex-sorted sperm produced. Typically, 3,000–5,000 cells are analyzed per sample depending on the target purity. The implementation of these technological advancements provides high confidence in the quality assessment of Sexing Technologies' sex-sorted products, which reflects in the most recent field results. Field data collected in the years of 2016–2018 confirmed that an in vitro analyzed X-chromosome-bearing sperm purity of 91.1 ± 0.18% resulted in an in vivo female calf percent of 90.3 ± 0.18%, based on 9,447 calvings.

Key Words: sex sorting, sperm quality, sex purity

M62 Impact of accounting for parent and genotyped daughters' average in the estimation of deregressed estimated breeding values used in multiple-step genomic evaluations. H. R. de Oliveira[1,2], L. F. Brito[2,3], M. Sargolzaei[2,4], F. Fonseca e Silva[1], J. Jamrozik[2,5], D. A. L. Lourenco[6], and F. S. Schenkel*[2], [1]*Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brasil,* [2]*University of Guelph, Guelph, ON, Canada,* [3]*Purdue University, West Lafayette, IN,* [4]*Select Sires Inc., Plain City, OH,* [5]*Canadian Dairy Network, Guelph, ON, Canada,* [6]*University of Georgia, Athens, GA.*

Cows have been genotyped and used to increase the size of the training population in multiple-step genomic evaluations of dairy cattle with limited number of proven bulls. However, some of the genotyped cows are daughters of progeny tested bulls, which have their estimated breeding values (EBVs) predicted based on the parent average (PA) and the phenotypic information from their daughters, which would cause double count of information. The objective of this study was to investigate the impact of accounting for PA and genotyped daughters' average (GDA; i.e., the contribution of genotyped daughters to the bull's EBV) in the estimation of deregressed EBVs (dEBVs) used as pseudo-phenotypes in genomic evaluations. In addition, an alternative deregression method was proposed (NEW). A simulated dairy cattle data set was used to compare 8 scenarios defined based on the number of bulls, genotyped bull's daughters, and genotyped cows not sired by the genotyped bulls. For all these scenarios, Genomic EBVs (GEBVs) were predicted using dEBVs estimated based on 4 methods: VR, that includes PA and GDA information in the dEBV; VRpa, that excludes PA; and JA and NEW, which exclude PA and GDA from the dEBVs using either all information available in the complete pedigree or only information from parents and genotyped daughters, respectively. The dEBVs estimated by the VR and NEW showed the lowest (0.24 to 0.36) and highest (0.33 to 0.50) validation reliabilities across scenarios, respectively. The VRpa and NEW methods produced the least biased GEBVs (inflation/deflation) and showed the most consistent bias estimates (regression coefficient) across scenarios (1.08 to 1.17). Among all methods, the JA method displayed the largest variability in bias (1.00 to 1.75) across scenarios. Therefore, it was shown that removing PA and GDA information from dEBVs can increase the reliability of genomic predictions for populations with limited number of proven bulls. In addition, the proposed NEW deregression method addresses the double counting of information and it is a feasible alternative to generate dEBVs used in multiple-step genomic evaluations.

Key Words: double-counting, genomic BLUP (GBLUP), training population

M63 Understanding the impact of technologies and novel phenotypes on breeding strategies for genetic progress in dairy cattle. C. Lynch*[1], F. Schenkel[1], K. Houlahan[1], G. de Oliveira Junior[1], L. Alcantara[1], and C. Baes[1,2], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland.*

In recent years, cutting-edge genomic technologies, methodologies and phenotype collection methods for current and novel traits have emerged. Some of these technologies are in development, some are undergoing regulatory analysis, and some have already been implemented. Each of these new technologies could potentially contribute to improving production efficiency; however, each technology has an associated cost, and potential benefits vary. The widespread adoption of these new technologies, methodologies and phenotype collection methods could potentially alter the way animals are selected within genetic and genomic evaluations. However, medium and long-term effects of incorporating these novel traits and technologies into routine breeding programs are largely unknown. Here we describe potential changes to current breeding strategies, and compare how those changes could affect the way we breed dairy cattle in the future. For example, we quantify the current use of reproductive management technologies and describe potential effects of wide-scale adoption on population structure, inbreeding, and production efficiency. A variety of comparisons are presented, and methods to compare the benefits of various schemes and tools for genetic improvement and selection are discussed. This study presents a systematic analysis of the implications of applying novel technologies and methodologies. Through analysis of current and potential future strategies, as well as outlining expected outcomes thereof, changes to dairy cattle breeding programs in short, medium and long term can be predicted, and useful insight on optimal paths forward can be gathered.

Key Words: novel traits, breeding strategies, sustainability

M64 Approximate generalized least squares method for large-scale genome-wide association study. L. Ma[1], J. Jiang[1], D. Prakapenka[2], J. Cole[3], and Y. Da*[2], [1]*University of Maryland, College Park, MD,* [2]*University of Minnesota, Saint Paul, MN,* [3]*USDA/ARS, Beltsville, MD.*

The use of genomic relationships among individuals is an effective approach for population stratification correction in the analysis of genome-wide association study (GWAS), but the matrix inversion required for the statistical testing of SNP effects limits the sample size that can be analyzed by GWAS methods using relationship matrices. We propose an approximate generalized least squares (AGLS) method for GWAS using large samples. The AGLS utilizes the mixed model result that the least squares (LS) solution to fixed SNP effects is the GLS solution or best linear unbiased estimation if the best linear unbiased prediction of polygenic effects is removed from the phenotypic observations. Since the LS method is computationally efficient, no sample size limitation for this method is expected for the foreseeable future even though dairy genomic and phenotypic data are growing at a fast pace. Combined with a previous method and computing tool for epistasis testing, the AGLS method offers capability for testing and estimating additive, dominance and epistasis effects as well as estimating allelic and genotypic effects in large-scale GWAS. AGLS was compared with BOLT-LMM that is capable of large-scale GWAS for testing additive effects. The results showed that AGLS and BOLT-LMM identified the same significant additive effects with only minor differences in a sample of 294,079 cows. For the same sample analyzed by AGLS and BOLT-LMM, the GWAS without polygenic correction lacked sensitivity, i.e., different chromosomes and different SNP within each chromosome had similar effects, except for SNP in and around the *DGAT1* gene on chromosome 14. These results showed that polygenic correction is necessary for large-scale GWAS and that AGLS is an efficient and versatile method for large-scale GWAS analysis, especially in dairy cattle where the polygenic animal effect is routinely estimated.

Key Words: genome-wide association study (GWAS), SNP, generalized least squares

M65 Computing pipeline for genomic prediction and estimation using haplotypes and SNP markers. D. Prakapenka* and Y. Da, *University of Minnesota, Saint Paul, MN.*

The haplotype analysis for genomic prediction and estimation requires considerably more data processing and has many more possible configurations of the prediction model than single-SNP analysis. To facilitate haplotype analysis for genomic prediction and estimation, we developed a computing pipeline to implement haplotype analysis. The pipeline includes 3 components, preparation of input data for haplotype analysis, genomic prediction and estimation using GVCHAP, and analysis of GVCHAP results. The input preparation starts with formatting SNP data for 2 imputing programs. A utility program with options to define haplotype blocks by a fixed number of SNPs or a fixed distance in base pairs per block then divides the haplotypes from either imputing program into haplotype blocks where each block is treated as a multi-allelic locus and is formatted as haplotype genotypes where each haplotype genotype contains 2 haplotypes. The haplotype genotypes are used as an input file for running GVCHAP. Another utility program fills in most of the parameter file required by GVCHAP as an input file. The data preparation step also contains utility programs for defining validation samples by random assignment of individuals to each validation sample or by a user provided list of individuals for assigning to validation samples. GVCHAP is the main program for genomic prediction and estimation providing GREML estimates and GBLUP for additive and dominance effects of haplotypes and single SNPs. To reduce the computing time in cross validations due to calculation of genomic relationships, GVCBLUP has a 2-step strategy to save the genomic relationship matrix during the first fold of validation and read in the genomic relationships for the remaining folds of validations. This 2-step strategy is helpful for k-fold validations and for multiple traits. The last component of the computing pipeline calculates observed prediction accuracies and produce input file for graphical analysis of haplotype and SNP heritabilities.

Key Words: genomic selection, haplotype, SNP

M66 Changes of genomic predictions with the algorithm of proven and young (APY) using different core animals in dairy cattle.

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The algorithm of proven and young (APY) can be used to predict genomic (G)EBV with a single-step genomic BLUP when the number of genotyped animals is large. Since the APY approach uses an approximation of the inverse of the genomic relationship matrix, GEBV could change when different genotyped animals are chosen as core animals. The objective of this study was to investigate what creates substantial changes in GEBV with APY when different core animals are used. Two data sets were simulated: 1) 60K genotyped animals, 70K phenotypes, 100K animals in pedigree and 2) 120K genotyped animals, 140K phenotypes, 200K animals in pedigree. In both cases, phenotypes in 12 generations and genotypes in the last 6 generations were simulated, assuming the heritability 0.5 and the additive genetic standard deviation (SD(a)) 7.1. Changes in GEBV using different core animals were compared. In addition, for udder depth (1 of 18 linear type traits), 570K genotyped animals in addition to 9M phenotypes and 10M animals in pedigree were used to investigate changes in GEBV by different core animals. Maximum Difference was selected as a potential measure of model performance among individual elite animals. Unintentionally excluding close family members from the core animals needs to be taken into consideration. In the simulation study, GEBV changed the most when the status of genotyped animals moved between core and non-core when using the smallest core size (10K in this simulation). Maximum differences in GEBV were smaller for 60K genotyped animals than those for 120K genotyped animals. Reliabilities (squared correlations between GEBV and true breeding values) were similar in any core sizes but lower than that for GEBV calculated without APY. The results from udder depth were in agreement with those from simulation, and GEBV changes decreased from 73% to 16% of SD(a) for non-core genotyped animals by increasing the core size from 10K to 60K. The extremely large change in GEBV was not found. Reliabilities were similar for any core size. For simulation and udder depth, GEBV changes asymptotically decreased by increasing the core size.

Key Words: single-step genomic BLUP, linear type traits, US Holsteins

M67 Plasma cholesterol and show-ring success of cholesterol deficiency carriers.

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Cholesterol deficiency (CD) in Holstein cattle results from an insertion in the APOB gene which traces to the bull Maughlin Storm. Calves that are homozygous for CD cannot transport cholesterol and perish before 6 mo of age. The mutation may have a partially dominant effect with lowered cholesterol in heterozygous animals. Our aims were to evaluate plasma cholesterol in heterozygous animals and to determine if CD carrier status is associated with show ring success. Blood was collected from 26 genotyped Holsteins in the Penn State dairy herd and included 12 CD carriers and 14 non-carriers. Plasma samples were analyzed for cholesterol concentration (mg/dL) using mixed models that included linear and quadratic effects of DIM. Additionally, CD carrier status of cows placing in the top 5 and top 10 in cow classes at World Dairy Expo that were sired by known CD carriers was compiled. From 2006 to 2018 there were 199 instances of 119 cows with genotypic data available and sired by a CD carrier sire placing in the top 10; of those, 114 were CD carriers and 85 were non-carriers. There were 117 instances from 75 cows for top 5 placings. Chi-squared tests for equal proportions were evaluated to determine if the following were associated with CD status: placing in the top 10 with cows allowed to contribute multiple years, placing in the top 5 with cows allowed to contribute multiple years, top 10 cows only considering individuals once, and top 5 cows only considering individuals once. There was a trend ($P = 0.09$) for non-carriers to have higher plasma cholesterol (155 mg/dL) than CD carriers (137 mg/dL). Further, there were significantly ($P < 0.05$) more CD carriers in the top 5 and 10 of classes at World Dairy Expo when cows were considered over multiple years; there was a trend ($P = 0.08$) for placement in the top 5 cows when individuals were only considered once. This data indicates that plasma cholesterol levels may be reduced in CD carriers but should be validated with a larger sample size. Moreover, reasons for an apparent advantage of CD carriers over non-carrier siblings at an elite cattle show warrants further investigation.

Key Words: cholesterol deficiency, show, cholesterol

M68 The lactation curve of the Italian river buffalo.

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The river buffalo (*Bubalus bubalis bubalis*) is currently farmed in Italy for milk production. The dairy buffalo industry is currently increasing due to the high market value of the most famous product, the mozzarella cheese. A breeding program based on AI and progeny test exist even if its impact is constrained by technical and logistic problems. Aim of this work is the study of the lactation curve of the Italian river buffalo during a 3-year period to look for possible modification and to analyze its modifications during the last 30 years. Data were test day records of 221,350 lactations of 124,015 buffalo cows, born in the period 1980–2011 recorded by the Association of Buffalo breeders (ANASB).

Lactations with at least 7 records between 6 and 300 d in milk were considered. Individual curves were fitted with 4 mathematical functions: the Wood incomplete gamma function (WD), the Wilmink function (WIL), a fourth-order Legendre orthogonal polynomials (LEG4), and a quadratic spline with one knot (QSPL). As expected, individual variability resulted in a huge variation of goodness of fit: only 25% of lactation showed an adjusted R^2 for the LEG4 larger than 0.83. According to the sign of the b parameter of WD, curves were classified as standard (88%) or atypical (12%); that is, without the lactation peak. Looking at the main parameters of the lactation curve shape, they exhibited a small variation during the considered period, even though with a quite irregular trend. The peak production showed an initial increase, from about kg 4.9 in 1980 to 5.11 in 1995, followed by a slow decrease that led to a value of 4.98 in 2011. The time at peak occurrence and the persistency of lactation did not show relevant variability in the 1980–2011 decades.

Key Words: lactation curve, river buffalo, milk yield

M69 Haplotype-based methods to select animals to sequence for later accurate imputation. A. M. Butty[1], M. Sargolzaei[1,2], F. Miglior[1], P. Stothard[3], F. S. Schenkel[1], B. Gredler-Grandl[4,5], and C. F. Baes*[1,6], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Select Sires Inc., Plain City, OH,* [3]*University of Alberta, Edmonton, AB, Canada,* [4]*Qualitas AG, Zug, Switzerland,* [5]*Wageningen University, Wageningen, the Netherlands,* [6]*University of Bern, Bern, Switzerland.*

The availability of array genotypes in dairy cattle has increased steadily in the last decade, and imputation to whole-genome sequence (WGS) has been widely studied. Although variants with minor allele frequency (MAF) below 0.05 usually represent more than half of all WGS variants identified, they are commonly excluded in most studies. Imputation of such variants is often inaccurate, impeding the use of such rare variants in further analyses. Furthermore, reference population selection also has a large impact on the accuracy of imputation. In this study, we present 2 novel methods of selection that rely on haplotype information and evaluate them in comparison with 2 previously described methods. The Genetic Diversity Index method optimizes the number of unique haplotype alleles present in the future selected group of animals, whereas the Highly Segregating Haplotype method aims to capture the most haplotype alleles possible, starting with alleles of high frequency in the population. We first simulated whole- genome sequence data of a dairy cattle population, mimicking the MAF distribution and the linkage disequilibrium pattern found in the North-American Holstein population. Reference populations of 50 to 1,200 animals were created using the 4 different selection methods. Finally, a group of target animals with simulated high-density genotypes was imputed. Accuracy of imputation was measured and compared for allelic r^2 between true and imputed genotypes for variants of different MAF. Imputation accuracy for common variants was between 0.85 and 0.99, whereas imputation accuracy of rare variants varied between 0.40 and 0.91. In general, methods based on selecting animals for their genetic diversity led to better imputation accuracy of variants with a MAF below 0.05. Methods targeting animals carrying common haplotype alleles led to higher imputation accuracies of variants with higher MAF. Therefore, the intended use of the imputed WGS must be accounted for at the time of selecting the animals comprising the future reference population.

Key Words: sequencing, imputation, haplotype

Dairy Foods: Cheese

M70 Comparison of different types of acidity values of three phases of caprine cheese milk during Cheddar cheese manufacture. R. Paswan*, A. Siddique, and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

There are several methods of measuring acidity of milk, including titratable acidity (TA), Soxhlet-Henkel (SH) value, acidity Dornic (AD) and acidity Therner (AT) values. TA is a measurement of any constituent that will react with or neutralize the 0.1 N sodium hydroxide. Fresh milk practically contains no lactic acid (LA), but still require an amount of NaOH to reach the phenolphthalein endpoint. During cheesemaking process, the cheese milk undergoes a variety of physicochemical changes. The objective of this study was to compare the differences in levels of LA, SH, AD and AT for 3 different stages of caprine cheese milk, such as fresh pasteurized milk (FPM), culture ripened milk (CRM) and whey milk (WM) during the entire procedure of Cheddar cheese manufacture. Three batches of fresh raw caprine milk were collected from the bulk tank of the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA, and pasteurized at 63°C for 30 min, cooled to 31°C, and then goat Cheddar cheeses were processed. The experimental cheese milk samples were taken at 3 different stages of cheese manufacture. The physicochemical parameters of acidity values were determined using a MilkoScan FT1, which is FTIR (Fourier Transform Infrared Spectroscopy) interferometer. The precision and stability of MilkoScan FT1 were calibrated by traditional AOAC approved fixed filter, which relies on every sine function being defined by its frequency (wavelength) and its amplitude (intensity) of IR beam. The results showed that LA, SH, AD and AT values for the FPM, CRM and WM stages were: 0.11, 0.17, 0.13; 5.34, 7.87, 5.85; 11.81, 17.83, 13.31; 12.86, 19.14, 14.71, respectively. All tested acidity values revealed that CRM samples were highest, followed by WM and FPM samples, indicating that the CRM samples cultured for one hour with Chymax starter contained significantly higher lactic acid levels generated by the starter culture bacteria. All tested acidity parameters showed the same trend of increased acidity in CRM compared with FPM and WM samples. It was concluded that the 4 tested acidity indicators of CRM had the highest values among the 3 phases of the caprine cheese milk.

Key Words: goat milk, cheese milk, acidity values

M71 Fatty acid profiles of control and iron-fortified caprine milk Cheddar cheeses stored under different time and temperature. A. Siddique* and Y. W. Park, *Fort Valley State University, Fort Valley, GA.*

Nutritional quality of a dietary fat is greatly influenced by fatty acid composition of a specific food. Caprine milk is known to have significantly high amounts of short chain and medium chain fatty acids (MCT). The objective of this study was to compare fatty acid compositions of non-fortified control (NC) with those of 2 types of iron fortified [regular ferrous sulfate (RFS) and large microencapsulated ferrous sulfate (LMFS) salts added] caprine Cheddar cheeses stored under different storage times and temperatures. Three batches of NC, RFS and LMFS cheeses were manufactured using the goat milk taken from the bulk tank of the Georgia Small Ruminant Research and Extension Center, Fort Valley State University, Fort Valley, GA. Iron was fortified for RFS and LMFS cheeses by addition of 8.23g and 9.03g Fe per 9 kg cheese, respectively at milling step, formulating 16% Fe in both forms of ferrous sulfate. Each batch of the cheeses were subdivided into 3 groups, packaged in 2”x3” plastic pouches, and stored for 0, 2 and 4 mo at 4 and −18°C. Results showed that palmitic acid (C16:0) content was the highest in all treated cheeses, followed by C18:1, C18:0, C14:0, C10:0, C8:0, C12:0 and C18:2 acids. The lauric:capric acid (C12:10) ratio was 0.40, which is unique to caprine milk and lower than that of bovine counterpart. Significant ($P < 0.05$ or $P < 0.01$) differences were found between cheese types and between storage periods in levels of all tested fatty acids except C16:1, C20:0 and C24:0 acids. Fatty acid contents of RFS and LMFS cheeses tended to be higher at longer storage time (4 mon) than those of initial NC samples. The 2-way interactions of cheese type x storage temperature, cheese type × storage period and storage period × temperature had significant ($P < 0.05$ or $P < 0.001$) effects on C10:0, C12:0; C6:0, C8:0, C18:2; and C4:0, C6:0, and C14:0 concentration, respectively. Storage temperature showed significant effect on C10, C12, C14, C14:1 and C18:1 levels. It was concluded that iron fortification and longer storage periods had significant effects on fatty acid levels than those of fresh control caprine milk Cheddar cheese samples.

Key Words: fatty acid content, iron fortification, storage

M72 Physicochemical, textural and sensory characteristics of control and rice powder-added Camembert cheeses during 4 weeks of aging. J. H. Nam[1], H. C. Bae[1], Y. W. Park*[2], and M. S. Nam[1], [1]*Chungnam National University, Daejeon, Chungnam, Korea,* [2]*Fort Valley State University, Fort Valley, GA.*

Camembert cheese (CC) is a moist, soft, creamy, surface-ripened cheese, originally made from unpasteurized cow milk in Camembert, Normandy of northern France. The surface of CC is sprayed with mold *Penicillium camemberti*, and ripened for minimum 3 weeks for its characteristic flavor. The objectives of this study were to compare physicochemical, rheological and sensory characteristics of rice powder added Camembert (RPAC) cheeses with those of non-supplemented control Camembert (NSCC) cheese during 4 weeks aging. NSCC and 3 levels (1, 3, 5%) RPAC cheeses were manufactured and evaluated for viable cell counts, and physicochemical, rheological and sensory properties of all experimental cheeses during 4 wk of ripening. Results showed that viable lactobacillus bacteria (LAB) counts after 4 wk of ripening in NSCC, 1, 3 and 5% RPAC cheeses were: $1.0 \times 10^8 \pm 0.12$, $1.3 \times 10^8 \pm 0.26$, $1.02 \times 10^8 \pm 0.15$ and $1.02 \times 10^8 \pm 0.17$ cfu/mL, respectively, indicating that 1% RPAC cheese had the highest viable LAB counts among all RPAC and NSCC cheeses. The level of water-soluble nitrogen was also the highest (115.69 μg/g) in 1% RPAC cheese among all cheese groups at 4 weeks aging. Isocitric acid, lactic acid, propionic acid, butyric acid, lactose, glucose and galactose in all cheese treatment groups were decreased as ripening period advanced. Protein degradation was most actively occurred in low molecular weight peptide at 2 weeks ripening in all RPAC and NSCC cheeses. The 1% RPAC cheese contained the lowest unpleasant flavor components, such as goaty, soapy, waxy, musty, rancid, and sour flavors. For the perspective of rheological characteristics, hardness (g) at 2–4 wk ripening was the lowest in 1% RPAC cheese compared with 2 other treated groups and NSCC. However, there were some variations in springiness, cohesiveness, gumminess and chewiness traits among different cheese groups. Sensory scores revealed that the 1% RPAC group showed the mildest taste, where its overall acceptability score was 4.10 ± 1.48. It was concluded that the 1% rice RPAC cheese displayed highest ($P < 0.05$) viable cell counts and favorable physicochemical, textural and sensory properties among all tested experimental CC cheeses.

Key Words: Camembert cheese, rice powder, physicochemical properties

M73 Effect of storage of high concentrated micellar casein on the functional properties of process cheese.

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Micellar casein is a relatively new dairy protein concentrate that can be used in process cheese products (PCP). PCP is a dairy food prepared by blending and heating various dairy and non-dairy ingredients to produce a pasteurized product with an extended shelf life. The objective of this study was to utilize highly concentrated micellar casein (HC-MC) as an ingredient in PCP and examine the effect of storage (0, 30, and 60 d at 4°C) of HC-MC on functionality of PCP. PCP formulations were prepared by mixing all ingredients (aged Cheddar cheese, HC-MC, water, unsalted butter, deproteinized whey, sodium phosphate dibasic, salt, and sodium citrate) in a kitchenaid at room temperature for 30–40 min to produce a homogeneous paste. A 25g sample of the mixture was weighed in a canister (5 replicates) and tempered at 38°C for 15–20 min and then cooked in the rapid visco analyzer (RVA) for 4 min at 90°C. The stirring speed was 1000 rpm during the first 2 min of the test and was then reduced to 160 rpm during the final 2 min. Once the PC was cooked, it was poured in molds and stored at 4°C for further analysis. This experiment was repeated 3 times using 3 different batches of HC-MC at each time point of storage (0, 30, and 60 d). The functionality of the PCP was measured by determining the cooked viscosity, texture profile analysis (TPA) hardness, and dynamic stress rheometry (DSR) melt temperature. The moisture content and pH of the PCP was not affected ($P > 0.05$) by storage of the HC-MC and ranged from 47.1 to 48.1% and 5.70–5.71 respectively. The cooked viscosity of the PCP was not affected ($P > 0.05$) by storage of the HC-MC and ranged from 755 to 769cP. However, there was a small but significant ($P < 0.05$) decrease in TPA hardness (135 to 105g) and DSR melt temperature (58.4 to 56.4°C) with HC-MC storage. This study demonstrates that HC-MC can be utilized in PCP formulations and small but significant changes in functionality were observed when the HC-MC was stored at 4°C for 60 d.

Key Words: process cheese product, micellar casein, shelf life

M74 Effect of delactose permeate fraction addition and direct acidification on low moisture part skim mozzarella composition.

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The dairy industry is interested in finding value-added uses for low value co-products of cheese manufacturing. Nanofiltered delactose permeate (DLPF) is one such product where lactose is further reduced from delactose cheese permeate, leaving higher milk mineral concentrations. The objective of this study was to evaluate the effect of this ingredient on the composition of direct-acidified low moisture part-skim mozzarella (LMPS). LMPS was produced in duplicate (~3 mo apart) by direct acidification using lactic, acetic, and citric acids at pH 5.6 and 5.4. Each batch was equally split and dry salted to achieve a salt equivalent of 1.8 (control) and 1.2% (reduced sodium). An additional split was made of the reduced sodium LMPS to which DLPF was incorporated as a hot brine during plasticization. Gross compositional analyses including: solids, fat, and ash were performed in duplicate. Multivariate Analysis of Variance using XLSTAT (Addinsoft Inc., New York, NY) was performed. Overall, DLPF treatments had lower solids and fat. Control treatments were significantly higher in ash (3.04%) than reduced sodium (2.34%) and the DLPF treatments (2.34%). With the exception of the lactic and acetic acid treatments at pH 5.6, all other DLPF treatments had significantly lower solids than non-DLPF treatments. When LMPS was manufactured using lactic or citric acid, DLPF incorporation resulted in significantly lower fat contents of the cheese. However, DLPF treatment did not affect fat contents when acetic acid was used as acidulant. Furthermore, when assessing fat on a solids basis, no differences were seen with both the acetic and lactic acid DLPF treatments and all non-DLPF treatments. Maintaining fat retention and adequate moisture is critical for profitability. The use of DLPF in acetic acid direct acidification was found to increase moisture retention while not affecting fat content. Further research should focus on effects of DLPF on LMPS sensory and functionality.

Key Words: mozzarella, cheese, permeate

M75 Effect of basil (*Ocimum basilicum* Lamiaceae) on technological properties of buffalo fresh cheeses.

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The objective of this study was evaluate the effect of basil (BA) addition, on the technological properties (texture profile and structure) of fresh cheese (FC) prepared with pasteurized buffalo milk during 21 d of storage at 4°C. Different concentrations of basil were added for FC production: 0.0% (Control without basil, CON), 0.25% (BA0.25), 0.50% (BA0.5) and 0.75% (BA0.75) of basil. These concentrations of BA were determined by previous trials. For production of control treatment (CON), the milk was heated to 35°C, calcium chloride (1g/L milk), and chymosin (0.04 g/40L) were added. Solution was rested for 45 min at 35°C to coagulation. The cheeses mass was cut, molded, and remained for 60 min at 4°C for whey expulsion before vacuum packed, the vacuum process was 15 s for not alter the cheese structure. For production with BA, it was added in the milk and homogenized (15 min) before the heating step (35°C) to coagulation. During the storage period, the FC was analyzed for hardness (g), chewiness (mJ), and cohesiveness by Brookfield Texture Analyzer CT-III with an acrylic circular probe (diameter 38.1mm and height 20mm); and the Scanning Electron Microscopy (SEM) was also realized after samples were frozen with liquid nitrogen and lyophilized. The hardness increased for FC with BA ($P < 0.05$) throughout of storage, and BA0.75 presented the higher values. The chewiness was higher ($P < 0.05$) with 15 and 21 d of storage for all treatments. The cohesiveness not present difference during the storage and between treatments. Results with scanning electron microscopy (SEM) images showed that BA altered the structure of FC. With BA addition, FC presented smaller voids spaces and the network became more compact. Thus, the compounds present in basil, such as phenolic compounds, can interact with milk proteins (phenolic-protein complex), increasing the binding force and influence in the technological properties of foods.

Key Words: dairy product, texture profile, interaction protein

M76 Effect of breed on the physicochemical and textural characteristics of South African artisanal cheese.

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Breed plays an important role on milk quality and consequently, cheese quality. Little work has been done to evaluate the quality of artisanal cheeses produced from different breeds in rural small-scale farms in South Africa. The objective of the study was to investigate the effect of 3 breeds i.e., Holstein-Friesian (HF), Jersey, and cross (cross of both), on cheese physicochemical and textural characteristics. The cheeses were manufactured in 2 different farms using raw milk under artisanal processing conditions. Aging (60 d) was conducted in 2 different chambers, that is, a control (10–12°C and humidity 80–90%) and a traditional simulation (18°C and humidity 60–70%) using a domestic air conditioner and humidifier which are affordable and accessible by rural farmers. Data were analyzed using a factorial arrangement of treatments with breed and chamber, and their interaction, as fixed factors and farm as random factor. As expected, Jerseys yielded more ($P < 0.05$) cheese (8.6 ± 0.37) compared with HF (8.1 ± 0.40) and cross (8.2 ± 0.40) per 100 L of milk. The cheeses from the 3 breeds showed differences ($P < 0.05$) on their initial (d 1) values for moisture, water activity, protein, fat, free fatty acids (FFA's), water soluble nitrogen (WSN)/total N (TN) %, salt, hardness, chewiness, yellowness, chroma and hue angle. After aging, the HF compared with Jersey and cross, had higher ($P < 0.05$) ash% (3.9 ± 0.13 vs 3.5 ± 0.10 vs 3.3 ± 0.14), fat% (36.6 ± 1.47 vs 31.5 ± 1.14 vs 32.4 ± 1.50) and salt% (2.0 ± 0.11 s 1.4 ± 0.08 vs 1.5 ± 0.13) content, whereas pH, protein, FFA's, non-protein N, WSN and WSN/TN of all cheeses were similar between breeds. For color, breed only had an effect ($P < 0.05$) on the yellowness which was higher in the Jersey (27.3 ± 0.33) compared with Holstein (23.1 ± 0.72) and cross (23.2 ± 0.51). Cheese from mixed bred had higher ($P < 0.05$) hardness and chewiness. There were interactions between the breed and aging chamber, with HF in the traditional chamber having lower ($P < 0.05$) moisture and water activity, and higher ($P < 0.05$) protein and fat content. These results are important for rural small-scale cheese producers to select ideal breeds and aging conditions for optimal production.

Key Words: breed, cheese quality, small scale farmers

M77 A survey on commercial US manufactured direct-salted block Gouda cheeses.

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Recently, cheesemakers in the United States have started making direct-salted block Gouda (DG) cheese in contrast to traditional brine-salted Gouda (TG) cheese. This approach allows cheese manufacturers to produce Gouda cheese using existing Cheddar equipment. We compared 6 DG cheeses (DG1-DG6) and 1 TG by analyzing for compositional, proteolysis, functional and sensory properties. All 7 cheeses were commercially produced from different facilities and received at 3 mo. They were ripened at 4°C and tested at 3, 6, and 9 mo. There were compositional differences between cheeses; the DG cheeses had higher moisture (39.1–44.2%), salt (1.5–1.9%), but lower fat (29.0–32.2%), and protein (21.5–24.4%) compared with TG (moisture = 34.7%, salt = 1.4%, fat = 33.8%, protein = 26.2%). TG had the highest pH values (5.44–5.56) during ripening. Cheese functionality was assessed using dynamic low-amplitude oscillatory rheology and texture profile analysis (TPA). Data were statistically evaluated using Duncan's test. There were differences ($P < 0.05$) in the crossover (melt) temperature and TPA hardness values among the DG and TG cheeses. Flavor, texture, shred properties, and pizza performance were evaluated by trained panelists using quantitative descriptive analysis. TG cheese had different sensory properties from DG cheeses; TG cheese was associated with firmness, buttery and sweet flavors. Principal component analysis (PCA) analyses on shred properties showed that TG was grouped with DG1 and DG3, and separated from others based on higher shred length and straightness values (PC1+PC2 > 95%). These cheeses also had higher TPA hardness and melt temperature. The rest of the DG cheeses were associated with more matting, surface oil and shred adhesiveness. The performance of the cheeses differed when melted on pizzas. TG had the lowest blister color value (~1.9) compared with DG (5.0–9.8) during ripening. PCA analyses showed that there were 4 groupings based on the melt properties; TG and DG3 were grouped together and associated with more skinning, higher hardness values and more free oil (PC1+PC2 > 85%). Overall, the results showed there was variation among the DG cheeses.

Key Words: commercial Gouda cheese, cheese performance

M78 Development of a rapid method using near-infrared spectroscopy to quantify starch and cellulose present in shredded Asiago, Parmesan, and Romano cheeses.

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Flow-aids consisting of starch and cellulose are added to prevent caking in grated cheeses and are used as carriers for added antimycotics. The accurate quantification of these flow-aids involves difficult wet-chemistry methods. When too little antimycotic is added, quality issues can occur. Conversely, when too much flow-aid is added to dilute the cheese for economic gain, the reputation of the dairy industry is damaged. As a way to prevent the over or underuse of these ingredients, Fourier transform near-infrared spectroscopy (NIR) could be useful. The goal of this research was to investigate whether calibrations could be made to quantify starch and cellulose in Asiago, Parmesan, and Romano cheeses, alone and separately. Samples of Asiago, Parmesan, and Romano loaves were shredded, and 0 to 5.66% of a starch/cellulose flow-aid was added. Treatments were ground, weighed, formed into a ball and pressed in the middle of the glass Petri dish with a force of 280 N for 1 min to create a homogeneous scanning surface. Samples were scanned using a Buchi NIRFlex N-500 FT-NIR spectrometer (BUCHI Labortechnik AG, CH). NIRCal 5.2 Chemometric Software (BUCHI Labortechnik) was used to analyze the spectra after first dividing the spectra of the 2,367 samples into 1,578 calibrations and 789 validation samples. The spectra were treated with standard normal variate to minimize variations and optimize the calibration. The calibration obtained has an r^2 of 0.9906 and a Sdev of 0.1504. Future research will determine if cellulose and starch can be identified and quantified separately in the same sample, and the effect of different starch and cellulose types on quantification.

Key Words: near infrared, starch, cellulose

M79 Manufacture and physicochemical characteristics of milk protein hybrid delivery systems produced by chymosin-induced gelation.

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High-temperature spray drying and extrusion method have been used for the preparation of delivery systems. However, those methods can lead to the degradation of bioactive compounds and generally allow for the production of large particle size due to high heat treatment. The objectives of this research were to manufacture of a suitable delivery system for *Lactobacillus rhamnosus* GG (LGG) by using milk protein hybrid delivery system (MPHDS) and assess the survival rate of LGG during manufacture, storage, and exposure to the simulated gastrointestinal condition. MPHDS were prepared by using chymosin at a various temperature from 25 to 40°C for 10 min and holding time from 5 to 30 min at 25°C. The initial amount of LGG added in the preparation process with approximately 9.34 log cfu/mL and enumerated by pour plate counts in MRS agar incubated at 37°C for 48 h. All measurements were performed on 3 independent samples. The microparticles obtained were rather similar in shape (globular shape) and size (around 5 to 28 μm) in confocal laser scanning microscopy images and particle size analyzer. The encapsulation efficiency of different encapsulation treatments was increased significantly ($P < 0.05$) from 66.5% to 80.3%, respectively. The viability rate during manufacture using heat temperature at 65°C for 30 min and storage at 4°C for 7 d was 71.4% to 77.6% and 76.1% to 80.3% significantly ($P < 0.05$) higher compared with free probiotics. Furthermore, the survival rate after gastrointestinal juice exposure of all prepared microcapsules was more than 70% in simulated gastrointestinal juice (pH 2.0) and 72% in simulated intestinal juice (pH 7.5) compare with free probiotics. In conclusion, encapsulation of probiotics effectively protected LGG against adverse condition such as heat treatment, storage, and gastrointestinal conditions.

Key Words: milk protein, *Lactobacillus rhamnosus* GG, microencapsulation

Dairy Foods: Chemistry

M80 Stability of algae oil against oxidation by microencapsulation with blends of caprine milk protein, β-glucan, and maltodextrin. A. Mora-Gutierrez*, R. Attaie, M. T. Nuñez de González, J. M. Kirven, and S. Woldesenbet, *Prairie View A&M University, Prairie View, TX.*

The objective of this study was to investigate the influence of microencapsulated wall material on the oxidative stability of algae oil by spray-drying. Algae oil is a rich source of docosahexaenoic acid (DHA, C22:6n-3), which is prone to oxidation. The highly hydrophobic nature of types I and II caprine caseins may improve the oxidative stability of algae oil in dairy food systems. Previous studies have reported an increase in the oxidative stability of microencapsulated borage oil, a rich source of γ-linolenic acid (GLA, 18:3n-6), by bovine casein, β-glucan and maltodextrin through spray drying. The bovine casein was useful to entrap borage oil, which is a hydrophobic bioactive compound. Blends of caprine milk protein (casein type I or casein type II), β-glucan, and maltodextrin were used as the wall material for encapsulating algae oil. The microencapsulated algae oils prepared with caprine casein type I or caprine casein type II in combination with maltodextrin and β-glucan achieved the optimal encapsulation efficiencies of 92.41% and 97.18%, respectively. The oxidative stabilities of algae oil and microencapsulated algae oils were measured at 40°C and 35% relative humidity for 28 d. The microencapsulated algae oils presented lower peroxide values than those of algae oil. The microcapsules prepared with caprine casein type II (50 g/kg), β-glucan (100 g/kg) and maltodextrin with DE = 18 (575 g/kg) improved significantly ($P < 0.05$) the oxidative stability of algae oil. The results of our studies showed that microencapsulation of algae oil with different wall materials attained high encapsulation efficiencies. The ability of caprine caseins (type I and type II) to minimize lipid oxidation of microencapsulated algae oils suggested that these caprine milk proteins exhibit effective microencapsulation properties for dairy food applications.

Key Words: algae oil, oxidation, microencapsulation

M81 An ultra-performance liquid chromatography method for furosine detection in liquid milk using microwave digestion. F. Wang[1,4], Y. Zhang[1,2], Q. Wang[1,2], G. Huang[1,2], K. Liu[1,2], J. Wang[1,2], N. Zheng[1,3], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Laboratory of Quality and Safety Risk Assessment for Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [4]*Institute of Quality Standard and Testing Technology for Agro-Products, Shandong Academy of Agricultural Sciences, Jinan, Shandong, China.*

Furosine, an indicator to assess the heat load on milk and dairy products, is closely related to the early stage of Maillard reaction. It is formed from Amadori products, with a reproducible yield after a same acid hydrolysis procedure. However, the acid hydrolysis in the standard detection method, which requires 16 to 23 h at 110°C in oven, is time-consuming. This study investigates an UPLC method using microwave digestion for furosine detection. Liquid milk was added into the high-pressure tube with hydrochloric acid and microwave digested at 160°C for 1 h, followed by filtration, dilution, UPLC separation and PDA detection at 280 nm. The results showed that the new method was time-saving, with a reproducible yield which was also investigated using the same pasteurized and UHT milk samples (n = 24). Compared with the standard method, microwave digestion showed 15–20% decrease on furosine quantitation for both pasteurized and UHT milks. Thus, a correction factor was used to normalize the results when comparing to the standard method. Moreover, method validation was taken, with the results as followed. The LOD and LOQ were 3 μg/L and 10 μg/L, respectively; the linearity range, calibrated by diluted furosine standards in matrix solvent, were 0.2–5.0 mg/L, with determination coefficient $R^2 > 0.9999$; the recoveries of various concentration of furosine in liquid milk were 84 to 106%, with RSDs ranged 0.2%~7.1%. Therefore, this UPLC method using microwave digestion is timesaving and revolutionary in the quantitation of furosine.

Key Words: furosine, microwave digestion, UPLC

M82 Evaluation of an ELISA-based visualization microarray chip technique for the detection of veterinary antibiotics in milk. B. Du[1,2], F. Wen[1], Y. Zhang[1], N. Zheng[1], S. Li[1], S. Zhao[1], H. Liu[1], L. Meng[1], F. Li[2], and J. Wang*[1], [1]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, China.*

Antibiotics such as the quinolones, tetracycline, streptomycin and lincomycin are increasingly found in dairy products. To ensure consumer safety, a rapid screening method is needed that is accurate and cost-effective. We tested a multiplexed detection technology for quantification of these 4 antibiotics simultaneously in milk. The method relies on an enzyme linked immunosorbent assay-based visualization microarray chip technique that can detect multiple target molecules simultaneously. The microarray chip was fabricated by adsorption of BSA- or OVA-conjugated small molecules as haptens on the microplates. Using competitive immunoassay, combined with silver nanoparticle (AgNP) catalyzed visualization technology to quantify multiple substances in milk at the same time. This colorimetric technology provides direct results that can be detected using common commercial microplate scanners. In this study, the LOD of the four different antibiotics were calculated by determining the mean response of 20 negative milk samples and adding 3 times the standard deviation. The LOD was defined at 3.30, 3.39, 2.42 and 4.88 μg kg^{-1} for quinolones, tetracycline, lincomycin and streptomycin, respectively, the LOQ values were 8.97, 8.43, 5.36 and 10.97 μg kg^{-1} for quinolones, tetracycline, lincomycin and streptomycin, respectively. These limits are less than the maximum residue levels established in China, the Codex Alimentarius Commission, and the European Union. The accuracy of method was also adequate for these four substances and experiments using actual samples demonstrated recovery rates ranging from 77.6% to 116.4%. The 198 liquid milk samples were analyzed using this method. This technology could achieve the simultaneous detection of analytes and will have broad applications in the food safety analysis field.

Key Words: milk, veterinary drugs, detection

M83 Detection of adulteration in milk using infrared spectroscopy and machine learning.

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Milk is one of the most important foods of humankind, and its production and commercialization in the industry require constant monitoring of its nutritional quality. Brazilian official laboratories monitor the quality of milk with analyzes that measure the composition and the microbiological quality. One of the concerns is the detection of frauds with the presence of any type of adulterants, a common illegal practice in Brazil and other countries worldwide. Among the analyses performed by the laboratory, some use Fourier transform infrared spectroscopy (FTIR) equipment. These methods generate a huge amount of data, which can be explored by computational techniques. The purpose of this work was to create a computational tool using data mining and machine learning techniques that characterize samples of infrared milk spectra to identify common adulterations in milk. More than 10 thousand samples, including 2376 were adulterated with foreign substances, which generated data from the infrared spectrum of the different milk samples. The obtained data were used to train machine learning models that could classify new and unknown milk samples as pure or adulterated. Neural network and decision tree models were trained with the spectral data from the FTIR readings and the classification task was performed considering binary and multiclass problems. In binary classification, the goal was to assert the presence or absence of an adulterant, while in the multiclass classification the specific adulterant classes were considered. The metrics obtained from each execution were classification accuracy and F1 score. The neural networks offered better results in general cases, with classification accuracies rates 84.65% to 97.16%. The carried out experiments showed the viability of the application of machine learning models for the detection of milk adulteration with the classification of samples. Training models with known sample data and their tests with different data sets provide a realistic view of the quality of the models, so one can confirm the ability of the model to classify instances correctly when submitted to unknown data sets.

Key Words: milk adulterants, Fourier transform infrared (FTIR), machine learning

M84 Oxidation kinetics of bioactive milk lipids using differential scanning calorimetry.

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The consumption of certain fatty acids naturally found in milk has been associated with several health benefits. These fatty acids are known as bioactive lipids (BML), and they provide beneficial effects beyond their basic nutrition. Examples of such fatty acids are conjugated linoleic acid (CLA, C18:2), *trans*-vaccenic acid (TVA, C18:1 t11), eicosapentaenoic acid (EPA, C20:5 ω3), and docosahexaenoic acid (DHA, C22:6 ω3). Dairy processors are actively exploring ways to incorporate BML in their formulations via fortification and enrichment protocols. Chemically, BML are unsaturated fatty acids containing at least one double bond in their structure, which makes them more prone to oxidation than the saturated fatty acids. In this work, the oxidation kinetics of DHA, EPA, CLA, and TVA was studied using differential scanning calorimetry (DSC) under different heating rates (3, 6, 9, 12, 15 and 18°C min^{-1}) in the temperature range of 50–300°C. The Kissinger-Akahira-Sunose (KAS) method was used to evaluate the kinetic triplet [activation energy, E_a; pre-exponential factor, A; and reaction model, $f(\alpha)$]. The E_a values were 59.30 ± 4.75, 47.17 ± 3.15, 55.46 ± 2.75, and 57.43 ± 3.01 kJ mol^{-1} for DHA, EPA, CLA, and TVA. The isoconversional analysis revealed that the E_a values does not vary with the degree of oxidation, indicating that the oxidation of BML can be explained by a single-step approach. Several reaction models were tested, and it was found that BML follow a first-order reaction. The calculated kinetic triplets are used to predict the oxidation, and the simulated data agreed well ($R^2 = 0.998$) with the experimental data. The use of isoconversional methods opens up opportunities in exploring the oxidation of milk fat and it offers advantages over the traditional kinetic methods.

Key Words: bioactive milk lipid, differential scanning calorimetry, oxidation

M85 Use of infrared spectroscopy to estimate the lactose content in hydrolyzed milk.

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Lactose is the main carbohydrate in milk, accounting for around 5% of its composition. It is estimated that more than half of the world population suffers from lactose maldigestion, which is the inability to digest lactose due to low production or absence of the intestinal enzyme lactase (FAO, 2013). This enzyme allows the breakdown of the lactose into glucose and galactose that will be absorbed by the intestine. Lactose accumulation in the intestinal lumen leads to fermentation by the microbiota, causing discomfort such as bloating, colic and diarrhea. Enzymatic hydrolysis, through the action of the β-galactosidase, is an efficient method to reduce the lactose content in milk and dairy products. The objective of this work was to investigate the methodology of Fourier-transform infrared spectroscopy (FTIR) to estimate residual lactose in milk. A total of 33 samples of raw milk, 20 samples of hydrolyzed milk, 52 fortified samples with 4 sugars (lactose, glucose, galactose, sucrose) were used in concentrations of 0.1; 0.5; 1.0 and 5.0%. Composition, freezing point (FP) and somatic cell count (SCC) analyses were performed. The results were provided in spreadsheets with the compositional values, FP, SCC and it were observed that lactose readings by FTIR suffer interference from the other sugars. Even hydrolyzed milk, ie lactose-free, shows a reading within the normal parameters in the lactose column. The results showed that the lactose reading by FTIR is influenced by different sugars present in the milk due to the PLS curve pre-established in the equipment, considering the same absorbance points in the infrared spectrum. The spectral data were used for the application of data mining techniques and machine learning, creating models of decision trees and artificial neural networks (ANN). The region of the spectrum that determines the sugars appears to comprise the range of 875 to 1481cm^{-1}. Preliminary data obtained by data mining of the spectra has indicated the possibility of distinguishing the different sugars present in the milk.

Key Words: residual lactose, Fourier transform infrared (FTIR), machine learning

M86 Machine learning applied to Fourier-transform infrared spectroscopy for detection of cheese whey addition to raw milk.

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Fraud in milk has a huge potential for economic damage and health risks to consumers. The addition of whey to milk is one of the major frauds, used to increase the volume provided at a reduced cost, however, its detection is labor-intensive and time-consuming. Fourier-transform infrared spectroscopy (FTIR) technique is a promising alternative for the identification of this type of fraud because a large amount of data is produced and stored using this method in an official laboratory for milk quality analysis. The artificial neural networks (ANN) is a valuable computational tool with feasible use to evaluate possible adulterations of milk. The objective of this study was to build a database of fraudulent samples of milk added with cheese whey, and to train models using machine learning algorithms applied to the results obtained from FTIR, and thereafter improve an algorithmic method to detect cheese whey illegally added to fluid milk. Addition of cheese whey to raw milk were done (0, 1, 2, 5, 10, 15, 20, 25, and 30%), and samples were kept at 7°C, 20°C and 30°C. Analyses for composition, freezing point, somatic cell count (SCC) and total bacterial count (TBC) were performed in an official laboratory (ISO/IEC 17025 accredited), after 0, 24, 48, 72 and 168 h. The machine learning process began with the preparation of the data. The records resulting from the FTIR analysis were organized to serve as input to the data mining algorithm. These records consisted of numerical data of each sample as well as the infrared spectrum obtained from each sample. The obtained data were used to generate a statistical model based on the training performed with the samples. With the results provided by the curves and the use of the ANN technique for data analysis, it was possible to observe an accuracy rate of the model with the data set described of 95.48%.

Key Words: cheese whey, Fourier transform infrared (FTIR), machine learning

M87 Quality study of UHT milk for four months of storage under laboratory conditions.

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UHT milk is the most consumed fluid milk in Brazil, due to its specific characteristics, including easy storage at room temperature for a long period of time. This prolonged storage may result in losses in quality during storage. Federal Ordinance 370 (1997) establishes the Technical Regulation on the Identity and Quality (TRIQ) of UHT milk. However, only these parameters may not be sufficient to ascertain the quality of this milk. To evaluate the alterations that occur during storage, a study was carried out to monitor changes in UHT milk throughout its shelf life (120 d). A total of 120 UHT milk samples from 2 industries in the state of Minas Gerais were stored at 2 storage temperatures (20°C/30°C) in 5 storage periods (0, 30, 60, 90 and 120 d), with 6 replicates per treatment (6 lots). When compared with the parameters required by the TRIQ of UHT milk, it was observed that the mean values of acidity, fat, and solids-not-fat (SNF) remained within the standard until the last day of storage. As for the other parameters, they are not covered by the specific legislation of UHT milk, which leads to failures during the inspection of this product. The cryoscopic index, density, somatic cell count, count of aerobic mesophilic microorganisms and SPC (standard plate count) had no changes. There was a gradual and significant increase ($P < 0.05$), over time, in the acidity, lipolysis index and CMP (caseinomacropeptide); on the other hand there was a significant ($P < 0.05$) decrease in fat, total solids (TS), SNF and total protein. Lactose remained practically constant during the storage period and had a significant decrease ($P > 0.05$) at d 120 only. The different temperatures and industries caused considerable changes in the parameters of lactose, TS, SNF, and CMP; showing that increasing the storage temperature may intensify the changes that occur in milk. The results show that there is a gradual loss of quality of UHT milk during its shelf life. In addition, UHT milk legislation needs to be complemented to establish parameters of quality control that allow its evaluation.

Key Words: UHT milk, lipolysis, proteolysis

M88 Seasonal variations of pasture fed bovine milk and their impact on heat stability of the skimmed milk.

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Seasonal variations on the milk composition have been well documented. New Zealand (NZ) is one of the key global dairy products processors; however, the detailed information about seasonal variation of NZ milk is not available to the public. As the dairy cows are pasture fed in NZ, the composition and physicochemical properties of NZ milk may be significantly altered through the weather cycle. In the current research, we demonstrated how milk composition and properties are altered through 10-mo time and if such changes have a significant impact on the heat stability of the skim milk or not. Bovine milk samples were provided by 3 dairy herds, from no less than 1000 cows, in NZ. The samples were collected 2 times a month across the 4 seasons. The principal components analysis (PCA) showed winter is a transitional period that separates the autumn milk (AM) from the milk yielded in spring and summer in terms of composition. The milk collected in autumn and early winter had significantly higher protein, fat and total solid contents. The AM associated with lower pH, a higher level of free Ca^{2+}, higher ethanol stability, and higher sedimentation rate induced by centrifugation. Regarding detailed lipid composition, AM is enriched in short-chain fatty acid, conjugated linoleic acids (CLA), and phospholipid (PL). In batch heat treatment experiment (85°C/5min), it showed that skimmed AM is more heat stable than spring and summer milk. Moreover, the skim milk heat stability is strongly correlated with ethanol stability of the raw full-fat milk (Pearson correlation, $P < 0.05$). The obtained results provided detailed insights of seasonal variations of NZ's milk. The enriched short-chain fatty acids in AM suggested that the AM derived butter or anhydrous milk fat products may have relatively softer texture. As short-chain fatty acids are relatively easier absorbed into the body and are metabolized rapidly in the liver. Also, considering the health benefits associated with the CLA and the milk PL, AM could be preferably used for manufacturing nutritional ingredients.

Key Words: milk seasonality, heat stability, milk composition

M89 Using isoconversional methods to study the effect of antioxidants on oxidation kinetics of milk fat.

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Milk fat has been used as an ingredient because of its nutritional value, functionality, and flavor. During processing and storage, milk fat may undergo oxidation which results in many undesirable changes such as unpleasant flavor and aroma, and formation of toxic compounds. A common practice to prevent the oxidation of milk fat is by the addition of antioxidants. It is worth to mention that little is known on the effect of antioxidants on the oxidation kinetics. In this work, the effect of selected antioxidants on the oxidation kinetics of anhydrous milk fat (AMF) under non-isothermal conditions was investigated. AMF with an addition of either α-Tocopherol (α-Toc), butylated hydroxytoluene (BHT), or β-carotene (β-Car) at 4 different concentrations (0.02, 0.07, 0.2, and 0.4%) were oxidized using differential scanning calorimetry (DSC) at different constant heating rates (3, 6, 9, and 12°C min^{-1}) in a temperature range of 100–400°C. DSC spectra were analyzed according to the Kissinger-Akahira-Sunose (KAS) method, from which the pre-exponential factor (A) and the apparent activation energy (E_a) were obtained. In general, the type of antioxidant, as well as its concentration, resulted in higher onset temperature of oxidation (T_{on}). For instance, the T_{on} increased from 173.8 ± 0.8, 175.5 ± 0.5, 176.4 ± 0.7, and 182.4 ± 0.4°C for AMF, BHT, β-Car, and α-Toc, respectively. The *Ea* values were 82.73 ± 5.51, 93.14 ± 6.24, 68.06 ± 3.52, and 58.51 ± 7.51 kJ mol^{-1} for AMF, BHT, β-Car, and α-Toc, respectively. Remarkably, the addition of 0.2% of α-Toc inhibited the oxidation reaction by 9-fold judging the constant rate. The obtained kinetic parameters were interpreted in term of oxidation mechanism. The implications of this study may enable off-line simulation and development of a databank.

Key Words: differential scanning calorimetry (DSC), isoconversional method, antioxidants

M90 Subcritical hydrolysis: An approach to valorize ice cream wastewater. M. Enteshari* and S. I. Martínez-Monteagudo, *South Dakota State University, Brookings, SD.*

Ice-cream manufacturers generate large volumes of wastewater (WW) through the daily cleaning programs. Identifying profitable valorization methods for WW treatments are playing a decisive role in sustainability of dairy and food industries. Treatment of ice cream WW through subcritical hydrolysis is one of the promising methods to overcome the environmental concerns deriving from dairy effluents as well as converting them with further utilization as platform chemicals. In this study, samples of ice cream WW were collected from university dairy plant during 6 weeks of production. First, physicochemical properties of WW samples were analyzed as follows: total solids (0.64 ± 0.01 to 2.05 ± 0.01 g·100 g^{-1}), total protein content (6.81 ± 0.81 and 9.67 ± 0.35 g·100 g^{-1} on dry matter basis), and pH values (from 2.99 ± 0.02 to 6.99 ± 0.03). Untreated WW samples also possess high organic load (biological and chemical oxygen demands of 38.2 ± 0.57 g·L^{-1} and 289 ± 2.83 g·L^{-1}, respectively). The hydrolysis of ice cream WW was carried out in a continuous stirred-tank at nitrogen flow of 40 bar and 230°C. After a 200 min reaction, the recovered WW hydrolysates were characterized by measuring the degree of hydrolysis (DH), antioxidant activity via measuring free radical scavenging (2,2-diphenyl-1-picrylhydrazyl or DPPH) method, as well as determining antihypertension (ACE) activity. Additionally, the peptide fractions of recovered hydrolysates were determined to attribute their proportions to relevant functional activities. The maximum value of DH (31.75 ± 0.53%), and percentage of remained DPPH (15.21 ± 0.44%) as well as ACE inhibition (96.38 ± 1.17%) showed the efficiency of subcritical hydrolysis to valorize ice-cream WW and convert it to value-added materials. The peptide identification showed the high ratio of glutamic acid and proline confirmed the relevant antiradical and antihypertensive activities of recovered hydrolysates. The outcomes present the potential of subcritical hydrolysis to convert ice-cream WW into nutraceutical compounds that can be utilized as functional food ingredients and pharmaceuticals.

Key Words: ice cream, wastewater, subcritical hydrolysis

Dairy Foods: Microbiology 1

M91 Evaluation of commercial protective cultures efficacy against yeast in cottage cheese. G. Makki* and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Early spoilage in fresh cheese contributes to food loss and negatively affects consumer experience. Traditional preservatives, like sorbates and propionates, are being removed due to consumer demand for clean labels. Protective cultures represent a potential clean label alternative for spoilage control. The study objective is to investigate bacterial cultures efficacy in bio-preserving cottage cheese against post-processing yeast contamination. Cottage curd and dressing were sourced from a manufacturer in New York. Dressing was inoculated with 3 different commercial protective cultures (of *Lactobacillus* spp. designated PC1, PC2 and PC3) following manufacturer recommended dosage. Curd was added to dressing and mixed. A positive control (PC) with no protective culture was included. Eight genera of yeast previously isolated from dairy processing environments (*Pichia fermentans, Clavispora lusitaniae, Debaryomyces hansenii, D. prosopidis, Candida zeylanoides, Rhodotorula mucilaginosa, Meyerozyma guilliermondi* and *Torulaspora delbrueckii*) were spotted on cheese surface at a rate of 100 cfu/5 g of cheese. Samples were stored at refrigeration temperature (6 ± 2°C). To enumerate yeast levels at 0, 7, 14 and 21 d post-inoculation, samples were homogenized in phosphate buffer saline, serially diluted, plated on potato dextrose agar with chloramphenicol, and incubated at 25°C for 5d. Studies were performed in duplicate with technical replicates. Average yeast counts were log-transformed, and JMP Pro was used to analyze data using least squares method and Tukey's test at significance level ($P = 0.05$). Negative control showed no growth throughout 21d. For *P. fermentans*, *C. lusitaniae*, *D. hansenii*, *D. prosodies*, *C. zeylanoides* and *R. mucilaginosa*, treatments showed no significant difference at 21d. For *M. guilliermondii,* counts were ~1 log lower at 21d in PC1 versus PC, PC2 and PC3 ($P < 0.05$). Similarly, *T. delbrueckii,* counts were ~2 log lower at 21d in PC1 compared with PC, PC2 and PC3 ($P < 0.05$). The study shows potential efficacy of PC1 against *M. guilliermondii* and *T. delbrueckii* in cottage cheese. The study demonstrates that protective cultures potential to inhibit yeast in cottage cheese is strain selective.

Key Words: cottage cheese, biopreservation, yeast

M92 A comparative study of extraction techniques for maximum recovery of lactase from *Lactobacillus delbrueckii* ssp. *bulgaricus*. R. Gyawali[1], A. Oyeniran[1], T. Zimmerman[1], A. Krastanov[2], and S. A. Ibrahim*[1], [1]*North Carolina A&T State University, Greensboro, NC,* [2]*University of Food Technologies, Plovdiv, Bulgaria.*

Lactase (β-galactosidase) is a commercially important enzyme that is used extensively in the food and pharmaceutical industries due to its capability to hydrolyze lactose. Because there is a significant market nowadays for lactose-free milk and dairy products, effective cell extraction techniques are a vital part of any industrial production line to help to meet this demand. In the present study, chemical (chloroform and toluene:acetone) and mechanical (bead-beater and sonication) techniques were used to determine the maximum recovery of lactase from 3 strains of *L. bulgaricus*. Among all extraction techniques, sonication-assisted extraction yielded the highest amount of enzyme (between 1847 and 2156 Miller Units). Interestingly, both solvent extracted lactase activities were found to be very low and not significantly different ($P > 0.05$) compared with the enzymes in the supernatant of unlysed cells (control). This result would indicate that the use of solvents is not an appropriate method for enzyme recovery. The SDS-PAGE and total protein determination also revealed that mechanical methods can completely lyse the cells, thereby releasing total protein ranging between 116 and 157 µg/mL into the supernatant. Our results thus demonstrated that the mechanical extraction technique, sonication, is the best method to recover the maximum amount of lactase from *L. bulgaricus* strains.

Key Words: *L. bulgaricus*, lactase, extraction techniques

M93 Modelling population dynamics of *Listeria monocytogenes* strain in lactic soft cheese following acid and osmotic stress exposures. T. Sibanda and E. Buys*, *University of Pretoria, Pretoria, South Africa.*

The survival responses of *Listeria monocytogenes* strains in a lactic soft cheese were studied following acid and osmotic stress exposures. Survival data of individual and mixed strains were fitted to 4 inactivation models (log-linear with tail, biphasic, Weibull and Weibull with tail). Lack of fit (LoF) analysis, showed the Weibull and log-linear with tail models provided the best fit of the data and were used to determine kinetic parameters. Analysis of inactivation kinetics showed that the susceptibility of individual strains ($P < 0.0001$) and the form of stress exposure ($P < 0.05$) have a significant effect on survival responses. Although both acid and osmotic stress exposures mainly resulted in enhanced survival of the *L. monocytogenes* strain, in one susceptible strain stress exposure caused inactivation. These findings imply that responses of *L. monocytogenes* strains in foods are dependent on the differences in the intrinsic susceptibilities of individual strains. Moreover, when introduced into the food as mixed strains, analysis of the diversity of surviving populations revealed that one strain out-lived other and remained as the only survivor after 15 d. These findings underscore the fact that predictions of pathogen responses in foods based on mixed strain cocktails as a way of accommodating strain heterogeneity must be treated with caution. Furthermore, data based on survival kinetics of stress-hardened cells could provide fail-safe models for predicting *L. monocytogenes* survival in foods.

Key Words: *Listeria monocytogenes*, modelling, survival

M94 Virulence and molecular typing of methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) *Staphylococcus aureus* isolated from bovine subclinical mastitis and Egyptian cheese samples. M. Zayda*[1,3], A. Elbagory[2], K. Honjoh[3], A. Hammad[1], Y. Masuda[3], and T. Miyamoto[3], [1]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, University of Sadat City, Sadat City, Monofiya Governorate, Egypt,* [2]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Monofiya University, Shebin Elkom, Monofiya Governorate, Egypt,* [3]*Department of Bioscience and Biotechnology, Division of Food Science and Biotechnology, Faculty of Agriculture, Kyushu University, Fukuoka city, Japan.*

In the small dairy holding settings, human, animal, environment, and awareness contribute significantly to the virulence and spreading dynamics of *S. aureus* pathogens. This study aims to trace the virulence characteristics of *S. aureus* causing bovine subclinical mastitis and the likelihood of their transmission to dairy products through apparently normal milk. One hundred and 50 California Mastitis Test positive milk samples were aseptically obtained from cows and buffalos owned by small dairy holders, as well as 75 samples of Egyptian raw milk cheese were collected from retailers at the same confined geographical district of milk sampling, in Monofiya Governorate, Egypt. Isolates were subjected to detailed biochemical characterization using SP-18 ID test followed by amplification and partial sequencing of the 16S rRNA gene to confirm isolates identities. Twenty-five *S. aureus* strains have been defined eventually. All the strains were subject to antimicrobial susceptibility testing against 18 antimicrobial compounds using the broth microdilution method. Twenty-five *S. aureus* strains showed 13 resistance patterns, involving 12 patterns with multidrug resistance. The proportion of antibiotic resistant strains was higher for ampicillin (25/25, 100%) and oxacillin (15/25, 60%). Fourteen MRSA strains were confirmed by detecting *mecA* gene and Staphylococcus Cassette Chromosome *mec* (*SCCmec)* classification. As a result, 7, 5 and 1 strains were classified into classes V, IV, and VI respectively, and one strain harbored 2 CCR genes (class 5C2&1). All isolates were examined for 11 SE genes, *pvl*, *tsst,* and 15 antimicrobial resistance genes. Moreover, the phylogenetic analysis of nucleotide sequence of Staphylococcus protein A gene (*spa* typing) revealed similarities between some MRSA and MSSA strains. This study identified a novel *spa* type "t18546" of a MSSA strain, and another MRSA strain, *SCCmec* IV/*pvl*$^+$, showing the universal characteristics of the community associated (CA)-MRSA. Transformation of human and animal-associated *S. aureus* may occur in animals udder, rendering their transmission highly incident and hazardous.

Key Words: subclinical mastitis, cheese, methicillin-resistant *Staphylococcus aureus* (MRSA)

M95 Bacteriophage identification from dairy environments.

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Bacillus spp. are within a family of bacteria known to form spores for survival in extreme environments. The spores are resistant to temperature extremes, radiation, antibiotics, and many disinfectants. When ingested by or exposed to susceptible animal hosts, the spores can germinate and create biofilms or cause infections such as endophthalmitis, bacteremia, pneumonia, or gastroenteritis. The virulence potential makes these bacteria problematic in the food industry as pathogens and spoilers. As a protective measure, dairy farmers could utilize a known bacteriophage after pasteurization to ensure dairy product quality and safety. The goal of this research is to isolate and characterize unknown bacteriophage found in dairy milk. By determining host range, DNA sequence, and morphology, phage candidates will be identified that may have promise as agents for biological control of *Bacillus* spp. Raw milk, silage, tank water, manure, and soil samples were processed for phage extraction and enrichment. Results revealed that the phage isolated in this study demonstrated a broad host range across several common *Bacillus* spp., established that levels of $CaCl_2$ were highly influential for phage uptake, indicated the presence of shared amplicons between the unknown phage and *Bacillus* phage deep blue (NC_031056) and *Bacillus* phage BCP8–2 (NC_027355), revealed that glycerol precipitation was more efficient for DNA sequencing, and unveiled that the isolated phage had an icosahedral head and presence of a sheath and tail fibers, measuring approximately 100nm together and sharing characteristics with the well-known T4 bacteriophage known to infect *Bacillus* spp. Further host range studies, genomic analyses, and PCR will reveal if this phage is novel and a possible future biological control agent, and/or whether the phage harbors antibiotic resistance genes that could have food safety and public health implications.

Key Words: bacteriophage, *Bacillus* spp., virulence

M96 Phage-based forensic tool for spatial visualization of bacterial contaminants in cheese.

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Current procedures for microbial testing typically involve a homogenizing step. These methods give valuable information on the presence/absence of a bacterial contaminant, but not where the contaminant was in the original sample. Spatial information could be useful in troubleshooting sources of bacterial contamination in a plant. For example, if the contaminant was localized on the top of a cheese, this might indicate dripping condensate along a specific processing line as its source. The objective of this proof-of-concept study was to evaluate the use of a T7 bacteriophage engineered to overexpress the luciferase NanoLuc to reveal the spatial location of *Escherichia coli* on Luria-Bertani agar (LBA) and queso fresco (QF). Four scenarios were tested to explore how phage may be applied, with a blue bioluminescent signal revealing the spatial location of contaminants: 1) Phage applied topically via molten soft agar to *E. coli*-inoculated a) LBA or b) QF and 2) Phage incorporated within a) LBA or b) QF, then inoculated with *E. coli*. Each was tested in triplicate. Cultures of BL21 *E. coli* grown for 18hr were serially diluted in phosphate-buffered saline and inoculated onto 8 ± 0.5g of LBA or QF in 6-well plates. Plates were incubated at 37C for 8hr for condition 1a and 24 h for 1b, 2a, and 2b. For 1a and 1b, stock phage was added to molten soft agar, applied topically, and incubated for 2 additional hours to allow for *E. coli* infection. After incubation, 100µL of the substrate NanoGlo was added to cover the surface of the agar or cheese, and imaged immediately in a dark box using a Canon EOS Rebel T6 camera and long exposure to capture the bioluminescent signal. Photos capture small blue spots where the incubated cfu are located. The lowest inoculum level of *E. coli* detected for each scenario was 0.64 ± 0.11, 2.73 ± 0.16, 0.67 ± 0.8, and 1.16 ± 0.54 logCFU/well, for 1a, 1b, 2a, and 2b, respectively. These data demonstrate the reporter phage proof-of-concept can be used as a forensic tool to visualize the spatial location of bacteria in a cheese matrix. Future work will translate this concept to dairy relevant phage-pathogen systems.

Key Words: bacteria, cheese, bacteriophage

M97 The effect of different ultraviolet light exposure times on microbial reduction and the components of camel milk.

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As a result of increasing interest in non-thermal technologies as possible alternative or complementary to milk pasteurization processing, this study was conducted to determine the effect of different UV-C light exposure times (5, 10, and 15 min) on the viability of bioluminescence bacteria *E. coli* O157: H7 and *Salmonella* Typhimurium in pasteurized camel milk and the chemical changes in raw camel milk components. Pasteurized camel milk samples (190 mL) were inoculated with a fixed number approximately 6×10^6 cfu/mL (6.7 logs cfu/mL) of bacterial culture and transferred into an autoclaved UV-C unit and exposed to UV-C light (39W, 115V, a germicidal lamp with a spectral peak of 254 nm), at a flow rate of 425 mL/min. The viability of bioluminescence *E. coli* O157: H7 and *Salmonella* Typhimurium evaluated first by using the in vivo imaging system (IVIS) and then by using the traditional plate count agar (PCA) method. The germicidal effect of UV-C irradiation resulted in 1.9, 3.3, 3.9-log reductions in bioluminescence *E. coli* O157: H7 and 0.9, 3, 3.9-log reductions in bioluminescence *Salmonella* Typhimurium after 5, 10, and 15 min, respectively. Compared with the untreated raw milk, the *cis*-9,*trans*-11 conjugated linoleic acid (CLA) decreased ($P < 0.01$) while the *trans*-10,*cis*-12 CLA increased in the UV-C treated milk. The TBAR values, a measurement of secondary lipid peroxidation products, showed no significant ($P > 0.05$) differences between the raw and the UV-C treated milk. Additionally, no changes ($P > 0.05$) in the protein profile (β-casein, α-casein, serum albumin, α-lactalbumin, and lactoferrin) were observed between the raw and the UV-C treated milk. In conclusion, treating camel milk with the UV-C light at wavelength 254 nm for 15 min did not meet the FDA requirements for the 5-log pathogen reduction, as well as, the UV-C light has affected the fatty acid profile of camel milk.

Key Words: camel milk, ultraviolet light, CLA

M98 The use of microbiological methods to reduce aflatoxin M_1 contamination in cheese produced in Sao Paulo State, Brazil.

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Studies have shown evidence of human exposure to aflatoxin M_1 due to the consumption of contaminated milk and dairy products (mainly cheeses). This poses a great risk to public health, since milk and milk products are frequently consumed by a portion of the population considered immunosuppressed, children and the elderly. Knowledge of the negative impacts of aflatoxins on health and economics has led to investigations of strategies to prevent their formation in food, as well as to eliminate, inactivate or reduce the bioavailability of these toxins in contaminated products This study evaluated the effect of microbiological methods using lactic acid bacteria on aflatoxin M_1 (AFM_1) reduction in Minas Frescal cheese (typical Brazilian product, being among the most consumed cheeses in Brazil) spiked with 1 µg/L AFM_1. Inactivated by heat treatment, lactic acid bacteria (0.5 vol/vol % of *L. rhamnosus* and *L. lactis*) were added during the cheese production process. Nine cheeses were produced, divided into 3 treatments: negative controls (without AFM_1 or lactic acid bacteria), positive controls (AFM_1 only), and lactic acid bacteria + AFM_1. Samples of cheese were collected on d 2, 10, 20 and 30 after the date of production and submitted to composition analyses and determination of AFM_1 by high performance liquid chromatography. The directional reductions of AFM_1 in cheese by lactic acid bacteria indicate a potential application of inactivated lactic acid bacteria in reducing the bioavailability of AFM_1 in Minas frescal cheese without physical-chemical and microbiological modifications during the 30-d experimental period.

Key Words: aflatoxin, milk, decontamination

M99 Screening, identification, and analysis of lactic acid bacterial strains grown in milk phospholipids-supplemented minimal medium and MRS medium.

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Milk phospholipids (MPLs) have gained people's attention due to their nutritional and functional properties like reducing gastrointestinal infection and supporting neurological development. They are gradually tested as ingredients for food application and human consumption. Meanwhile, lactic acid bacteria (LAB) are important components of human gut microbiome and they are commonly used in fermented dairy products. However, little is known about the effects of MPLs on the growth of LAB. The aim of this study was to select LAB strains that can grow relatively well in medium supplemented with MPLs, to sort LAB for future MPLs-LAB studies and potential applications. One hundred and 37 strains of LAB from our culture collection (OSU library) were grown in modified minimal medium (MM) and also in MRS media with 0.5% MPLs added. Strains with relatively high optical density (OD_{600nm}) and high maximum growth rates (μm_{max}) were selected and identified by 16s rRNA sequencing. Further analysis included resistance to antibiotic, pH, bile salts, hydrophobicity, auto-aggregation, and virulence factors were conducted to prove their probiotic potential. After screening and analysis, it was found out that 6 strains having relatively high OD_{600nm} and high μm_{max} in MM media supplemented with MPLs are *P. pentosaceus*, *L. pentosus*, *L. helveticus*, *L. rhamnosus*, *L. gasseri* and *L. reuteri*. Their growth kinetics changed with the presence of MPLs. Interestingly, they all had higher maximum growth rates and shorter lag phase but lower optical density growing in MPLs supplemented MRS medium than in MRS medium. However, they both had higher maximum growth rates and higher optical density in MPLs supplemented MM medium than just MM medium. This study suggests that certain LAB grew better in the presence of MPLs, and they might be used in combination with MPLs for future research and applications.

Key Words: milk phospholipids, lactic acid bacteria, kinetic growth

M100 Using nitrous acid-modified MRS medium to selectively isolate and culture lactic acid bacteria from food.

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Nitrous acid was used to modify traditional de Man, Rogosa, Sharpe medium to test our hypothesis that addition of sodium nitrite to MRS medium would improve the growth rate and density of various lactic acid bacteria while preventing the same of nontarget species. Yogurt, cheese, and sauerkraut were inoculated with individual bacterial species (*Bifidobacterium longum, Streptococcus salivarius*, and *Lactobacillus delbrueckii* ssp. *bulgaricus, Lactobacillus acidophilus, Leuconostoc mesenteroides, Enterococcus faecalis* and *Bacillus cereus*) followed by recovery and enumeration of each to compare the sensitivity between nitrous acid modified MRS (mMRS) and traditional MRS. *Lactobacillus delbrueckii* ssp. *bulgaricus* were recovered at significantly ($P < 0.05$) higher counts from cheese in nitrous acid mMRS than MRS while there was no significant difference for other species and food systems between the 2 media formulatons. To augment overall comparative recovery of species on the 2 media types, the rate of growth of each lactic acid bacterial species in MRS vs. mMRS broth was also measured. Growth curves were generated for the lactic acid bacteria as well as nonstarters in both mMRS and MRS to measure the selectivity of nitrous acid mMRS. Three of the tested bacterial species (*Bifidobacterium longum*, *Streptococcus salivarius*, and *Lactobacillus delbrueckii* ssp. *bulgaricus*) grew to significantly higher ($P < 0.05$) densities more rapidly in mMRS broth than in traditional MRS during an 18h incubation regimen. Conversely, 2 nontarget low G+C bacteria genotypically and phenotypically similar to some lactic acid bacteria, *Enterococcus faecalis* and *Bacillus cereus,* demonstrated a more prolific growth rate and significantly ($P < 0.05$) higher OD readings in traditional MRS (15–30% higher density by 18h) compared with mMRS, to validate that these nontarget species would be less competitive in the improved mMRS formulation. It was determined that nitrous acid mMRS is a viable alternative medium for culturing selected lactic acid bacteria, and offers an improved formulation of MRS for use in standard methods evaluation and optimization of lactic acid bacteria in dairy and possibly other food-grade GRAS cultures.

Key Words: MRS, lactic acid bacteria recovery, enumeration

M101 An evaluation of rep-PCR primers for the differentiation of *Lactococcus lactis* starter strains.

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Lactococcus lactis is the most commonly used cheese starter, due to its fast growth in milk, rapid production of lactic acid, and crucial role in cheese flavor and texture development. These characteristics are often strain-dependent and can result in dramatically different cheese quality, depending on which *L. lactis* strains are present during fermentation and aging. Therefore, a rapid and inexpensive tool capable of strain-level identification of *L. lactis* would be of great value to the cheese industry. One potential option, which has been successfully used to differentiate *Lactobacillus* strains, is repetitive sequence-based PCR, followed by high-resolution melt analysis (rep-PCR/HRM). Our aim was to determine if rep-PCR/HRM is suitable for strain-level differentiation of *L. lactis*. Three sets of repetitive element primers (i.e., GTG5, BOXAIR, REP1R-I/REP2-I) were investigated for their ability to distinguish 14 laboratory strains and 8 industrial strains of *Lactococcus lactis,* using hierarchal cluster analysis. The discriminatory power of each primer set was determined as the proportion of correctly paired melt curves, generated from duplicate PCR reactions. While some primer sets performed better than others, no single set was able to correctly separate all 22 *L. lactis* strains. However, the discriminatory power was improved when considering strain-clustering patterns from 2 or more primers sets. This demonstrates that a multi-assay approach to rep-PCR/HRM may be useful for confirming *L. lactis* starter strain identity.

Key Words: rep-PCR, *Lactococcus lactis*, starters

M102 Quantitative PCR coupled with sodium dodecyl sulfate and propidium monoazide for detection of viable *Escherichia coli* in milk.

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Escherichia coli has been frequently reported as a major foodborne bacteria contaminated in raw milk. Therefore, the aim of this study was to explore a quantitative real-time PCR (qPCR) technique combined with sodium dodecyl sulfate (SDS) and propidium monoaziede (PMA) to detect viable *E. coli* in milk. An internal amplification control (IAC) was also added into this reaction system as an indicator of false-negative results. The inclusivity and exclusivity of the primers were tested using DNA from 7 *E. coli* and 7 other bacterial strains. The concentrations of SDS and PMA were determined according to plate counts and Cq values of qPCR, respectively. A standard curve was established using series diluted *E. coli* DNA. The reliability and specificity of this method were further determined by the detection of *E. coli* in spiked milk. The results showed that the optimal concentrations of SDS and PMA were 100 ppm and 40 μM, respectively. A standard curve with a good linear relationship ($R^2 = 0.9925$, E = 105%) was obtained. Compared with conventional PCR and PMA–qPCR, the SDS–PMA–qPCR assay was more specific and sensitive in viable *E. coli* detection ($P < 0.001$). Therefore, we evaluated and improved the SDS–PMA–qPCR method for detecting viable *E. coli* in milk.

Key Words: propidium monoazide, sodium dodecyl sulfate, *E. coli*

M195 The protective effects of enriched citrulline fermented milk with *Lactobacillus helveticus* on the intestinal epithelium integrity against *Escherichia coli* infection.

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Citrulline is an amino acid and abundant in watermelon, which can be utilized by some strains of lactic acid bacteria (LAB). *Lactobacillus helveticus* is one of the LAB that utilizes citrulline by arginine deiminase (ADI) pathway. Fermented milk is one of the daily products used for contributing the beneficial effects of LAB. This study examined the protective effects of citrulline enriched *Lactobacillus helveticus* ASCC 511 (LH511)-fermented milk (LHFM) on intestinal epithelial barrier function and inflammatory response in IPEC-J2 cells caused by pathogenic *Escherichia coli*. The effects of citrulline enriched fermented milk with LH511 (5%LHFM_Cit-4mM) was determined by MTT assay. Adhesion assay was performed to determine the anti-adhesion effects against enterohemorrhagic (EHEC) and entero-invasive (EIEC) *Escherichia coli* in IPEC-J2 cells. The cell integrity under the challenge of lipopolysaccharide (LPS) from *E. coli* O55:B5 was examined by transepithelial electrical resistance (TEER), qPCR and Western blot assay. Five percent of 4 mM citrulline in fermented milks with LH511 (5%LHFM_Cit-4mM) significantly stimulated the population of IPEC-J2 cells by 36%. Adhesion level of LH511 was significantly increased by 9.2% when incubated with 5%LHFM_Cit-4mM and 5%LHFM_Cit-4mM reduced the adhesion of EHEC and EIEC in IPEC-J2 cells by 35.79% and 42.74%, respectively. Treatment with 5%LHFM_Cit-4mM ameliorated LPS induced activated inflammatory cytokines expression (TNF-α, IL-6 and IL-8) and concentration (IL-6 and IL-8) and early apoptosis. And it restored the TEER, and regulated the expression of tight junction (TJ) proteins (zonula occluden-1 (ZO-1), occludin and claudin-1), toll-like receptors (TLRs) (TLR2 and TLR4) and negative regulators of TLRs signaling pathway (A20 and IRAK-M). In conclusion, our findings suggested that 5%LHFM_Cit-4mM had positive effects on improving and maintaining the intestinal epithelial cell integrity and inflammatory response under both normal and pathogenic LPS-stimulated conditions.

Key Words: *Lactobacillus helveticus*, fermented milk, citrulline

Dairy Foods: Processing 1

M103 The application of supercritical CO_2 technology on modifying beta-lactoglobulin in buttermilk to reduce its antigenicity. P.-W. Yeh*, I. García-Cano, D. Rocha-Mendoza, and R. Jiménez-Flores, *The Ohio State University, Columbus, OH.*

The purpose of this study focuses on modifying β-LG (β-lactoglobulin) in buttermilk via supercritical CO_2 treatment, to reduce its antigenicity. In dairy industry, supercritical fluid extraction (SFE) is applied primarily in fat extraction. Yet it is possible to utilize SFE as a reactor in foods. Milk is an essential energy source for infants. However, formula is still needed in some instances and there are some children allergic to dairy based products. In milk proteins, β-LG is believed to be a prevalent allergen in cow's milk. Buttermilk powder was used because of its abundant milk fat globule membrane and phospholipids content. It was treated in SFE system with food grade CO_2 at 100, 150, 200, 250, 350, 400 bars, with 50 and 75°C in each condition. All analyses were completed in 10% buttermilk suspension (wt/vol). Buttermilk proteins were examined in SDS-PAGE, Western-blot, and ELISA. Orbitrap Fusion HPLC-MS/MS and periodic staining were used to check any post-translational modification. Purified β-LG was used to evaluate the cytotoxicity, viability, and inflammatory response in Caco-2 cell line by the mean of LDH, MTT, and IL-8, respectively. The SDS-PAGE quantification showed that the signal intensity of β-LG bands was reduced in a maximum of 45.8% after being processed under 250 bar/75°C, 30 min. Lighter and diffused signals were also found in Western-blot. ELISA tests proved that our treatment can significantly change the β-LG antigenicity in buttermilk ($P < 0.05$). Around 146-Da molecular weight increment was detected on treated β-LG by Orbitrap Fusion. Sugar moieties in glycoproteins were revealed by periodic staining. Treated β-LG showed 95% viability and 27% lower inflammation without any cytotoxicity in Caco-2 cells, compared with untreated β-LG. These results showed that β-LG reduction was completed by glycosylation, which has been reported as a possible pathway to reduce the allergenicity in foods. The denaturation of β-LG in supercritical fluid processing is a promising way to solve milk allergy, which is still a problem requiring more attention and further research.

Key Words: buttermilk, supercritical CO_2, β-lactoglobulin

M104 Partial demineralization and deacidification of Greek yogurt acid whey by nanofiltration for improving the drying characteristics of Greek yogurt acid whey. V. Sunkesula*, A. R. A. Hammam, and L. E. Metzger, *Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

Greek yogurt acid whey (GYW) contains high concentrations of lactic acid (LA) and minerals as compared with cheese whey. LA and the minerals, particularly the calcium (Ca) in GYW cause stickiness during spray drying thus limiting the processing and utilization of GYW. Nanofiltration (NF) has been in use to for partial removal of minerals from cheese whey and milk to produce high value added dairy ingredients. Similarly, NF can potentially be applied for partial demineralization and deacidification of GYW to improve spray drying and powder properties. The aim of this study was to evaluate the effectiveness of NF to remove minerals and LA from GYW for improving the spray drying ability of GYW and thus the drying characteristics of the powder. GYW (5.52 ± 0.2% total solids) obtained from a Greek yogurt manufacturer was pasteurized at 72°C for 15 s followed by cooling to 4°C before concentrating to 22.35 ± 0.8% using semi-industrial scale Nanofiltration (Molecular Weight Cutoff – 300 to 500Da) plant. GYW feed, NF retentate and NF permeate compositions in terms of total protein nitrogen, non protein nitrogen, total ash, minerals (sodium, potassium and calcium) and Lactic acid were analyzed. The corresponding percent reduction of each component was calculated and expressed on a dry matter basis. The lactic acid and total ash concentrations were reduced significantly ($P < 0.05$) by 34.3 ± 0.2 and 37.8 ± 0.7 respectively. The reduction of monovalent ions i.e., sodium and potassium was observed to be higher (66% and 62%) than calcium (41%). There was no significant difference ($P > 0.05$) in the total protein content and pH of GYW feed (4.13 ± 0.2, 4.44) and NF GYW retentate (3.95 ± 0.2, 4.38). The results show it is feasible to reduce the mineral content and lactic acid in GYW by approximately 38% and 34% respectively using NF. In subsequent studies, the NF retentate (treatment) and GYW (control) will be spray dried and the powder properties characterized.

Key Words: Greek yogurt acid whey, nanofiltration, demineralization

M105 Technical-scale extraction of bovine α_S-, β-, and κ-casein using decanter technology. T. Schubert*, Z. Atamer, and J. Hinrichs, *Universität Hohenheim, Stuttgart, Baden-Württemberg, Germany.*

The isolation of individual casein fractions (α_S-, β- and κ-casein) are of growing interest because of their clean-label status and their wide range of bio- and techno-functional properties. Though several isolation and purification methods have been proposed, there is still need for improvement regarding the purity and yield for the obtained casein fractions. The aim of this study was to develop a continuous process for the fractionation of caseins. The use of a decanter enables continuous separation and has the advantage of a relatively simple scale up. The fractions were obtained from micellar casein powder by means of selective precipitation and the separation process was performed using a temperature-controlled decanter centrifuge. The separation process of the fractions was optimized by changing the operational parameters of the decanter (e.g., weir bore inner diameter, rpm, differential speed, flow rate). A method for the technical-scale isolation of casein fractions from micellar casein is described in detail and the reproducibility of the method is assessed. Using micellar casein, a purified β-casein fraction can be obtained at a large scale via the proposed method outlined in this study. A purity of up to 95% for the β-casein fraction and purities of up to 61% and 54% for α_S- and κ-casein can be achieved, respectively. Further work is in progress to optimize these fractions of α_S- and κ-casein.

Key Words: casein, separation

M106 Effect of rinsing and washing time and water temperature on removal of peanut allergen from ice cream processing equipment. J. Zhang and Z. Ustunol*, *Michigan State University, East Lansing, MI.*

Ice-cream is a product which may contain all of the key food allergens, and prevention of cross contamination in a processing facility is a challenge to ice-cream manufacturers. Effective cleaning of the equipment is important to prevent food allergen cross contamination. In this study, we hypothesized that higher water temperature and longer rinsing-, washing-time will result in lower peanut allergen left on stainless steel surface. Ice cream, which contains peanut and soy, was used in this study. Thawed ice cream was filled in a stainless steel pipe for 1.5 h and then rinsed or rinsed and washed in a simulated clean-in-place system. Water pressure was 70 psi, flow rate was 1.53 L/s. Chlorinated alkaline detergent was used for washing. The effect of 3 times (10, 20 and 30 s) and 5 water temperatures (20, 30, 40, 50 and 60°C) on removal of peanut allergen from stainless steel pipe was investigated. Samples were obtained by swabbing, and tested for concentration of peanut allergen protein Ara h 1. All experiments were replicated 3 times. When equipment was only rinsed, concentrations of peanut allergen residue left on the pipe ranged from 207 ppm to 63 ppm. The overall trend suggested that higher water temperature and longer rinsing time resulted in lower peanut allergen concentration on the equipment ($P < 0.05$). When equipment was rinsed then washed, concentrations of peanut allergen residue ranged from 1.43 ppm to 0.015 ppm. The overall trend suggested that water temperature played an important role in removing peanut allergen ($P < 0.05$) while time showed a less important effect than temperature on allergen removal in this study. Only rinsing was not effective in removal of all peanut allergen. Rinsing and washing at temperatures 50°C or above, and 20 s or longer are needed to remove all peanut allergen from stainless steel equipment. Effective cleaning can reduce the chance of cross contamination as well as save time and money for the food industries. Understanding the principle of rinsing and washing is essential for effective allergen removal.

Key Words: peanut allergen, cleaning, processing equipment

M107 A conjugated whey protein hydrolysate demonstrates enhanced bioactive attributes. S. Minj*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Brookings, SD,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Whey protein hydrolysates with enhanced bioactivities may confer improved health benefits to the consumers. While some studies have shown the protein ingredients produced through conjugation with maltodextrin to have greater functionality, the effect of conjugation on the bioactivities is not clearly established. In this study, whey protein concentrate WPC80, isolate WPI90, and hydrolysates WPH10, WPH15 and WPH20 were screened for bioactivities (antimicrobial activity by agar well assay, antioxidant activity by ABTS$^+$ radical assay and antihypertensive activity by ACE inhibition assay). Hydrolysate WPH10, exhibiting the highest bioactivities was conjugated with maltodextrin to obtain a thermally stable conjugated solution. A batch of 2L conjugated solution was spray dried in a Niro drier with an inlet and outlet temperature of 200°C and 90 ± 5°C, and alternatively, freeze-dried at −80°C under 50 mTorr vacuum. The bioactivities of the conjugated samples were then assessed according to the above-mentioned assays. All experiments were conducted in triplicates and one-way ANOVA was applied to differentiate the mean values. The hydrolysates showed significantly ($P < 0.05$) higher bioactivities (10.6 ± 0.33mm, 766.72 ± 13.3 TEAC µmol/L and 67.52 ± 0.2% for antimicrobial, antioxidant and antihypertensive activity), as compared with concentrate (8.6 ± 0.33mm, 373.3 ± 21.5 TEAC µmol/L and 60.8 ± 0.1%) and isolate (9.3 ± 0.33mm, 426.9 ± 42.0 TEAC µmol/L and 62.9 ± 0.07%). Based on the highest bioactivity, hydrolysate WPH10 was selected for conjugation with maltodextrin. The conjugated WPH10 solution demonstrated higher antimicrobial (17.16 ± 0.33mm) and antioxidant activity (1044.37 ± 39.1 TEAC µmol/L) ($P < 0.05$), whereas a slight decrease in the antihypertensive activity (65.4 ± 0.2%) was observed, as compared with WPH10 alone. Subsequent spray and freeze drying of the conjugate solution exhibited even higher antimicrobial (18.5 ± 0.57mm) and antioxidant activity (1268.89 ± 41.9 TEAC µmol/L) ($P < 0.05$), while retaining the antihypertensive activity (65.6 ± 0.3%) i.e., ($P > 0.05$). Further studies are in progress to develop health formulations utilizing WPH-conjugates with enhanced bioactivity and functionality.

Key Words: bioactivities, conjugates

M108 Variation of cow milk quality traits in the dairy industry of northeast Italy in the last decades. C. L. Manuelian* and M. De Marchi, *Department of Agronomy, Food, Natural resources, Animals and Environment (DAFNAE), University of Padova, Legnaro, Italy.*

Evolution of cow bulk milk quality in northeast Italy during the last 12 years has been evaluated with in-field data from the Italian dairy industry. According to milk payment system, monthly bulk milk composition records (average of 2 samples) from 2007 to 2018 were retrieved from one of the most important dairy factories in the Veneto region (Latteria di Soligo SAC, Farra di Soligo, Italy). This dairy factory mainly transforms milk into fresh cheese (e.g., Casatella di Treviso PDO and Mozzarella cheeses). Only farms with at least 2 years of records and years with 12-mo records were retained. A total of 28,608 records from 331 farms were available for the statistical analysis. Somatic cell count (SCC) and TBC (total bacterial count) were $\log_{10}$ transformed to ensure the normality of the data. The model included year, month and their interaction as fixed effects, and multiple comparisons of the main effects were done using Bonferroni's test. All fixed effects included in the model were significant explaining the variance observed. The number of farms steadily decreased from 245 in 2007 to 135 in 2018, with an increase in the average annual milk yield/farm from 4.93×10^5 to 5.75×10^5 L during the same period. Despite the increase in production, fat (in 2007, 3.86 ± 0.004%; in 2018, 3.98 ± 0.006%; $P < 0.001$) and protein (in 2007, 3.32 ± 0.003%; in 2018, 3.36 ± 0.004%; $P < 0.001$) concentration remained quite stable across years, and SCC (−22.83%) and TBC (−7.66%) were importantly reduced between 2007 and 2018. On the other hand, month variation has a greater impact than year on fat and protein concentration, with lower values during the hot months (May–August) and an increase in TBC. The SCC were also greater from June to October respect to the other months of the year. Our results indicated that, during the last 12 years, farms have increased their farm productivity with a slight increase in fat and protein content and a significant reduction in SCC and TBC, indicating a better milk quality. The authors thank Latteria di Soligo SAC for providing the data. This project has received funding from Bando AGER 2017 – sezione Prodotti lattiero-caseari.

Key Words: dairy industry, milk quality

M109 Physical-chemical analysis of donkey milk yogurt mixed with milk added from other species. R. D. S. Gomes[1], M. F. Bezerra[1], E. G. S. O. Silva[1], I. L. S. Oliveira[1], B. K. C. Melo[1], A. F. S. Gomes[1], E. P. E. Silva[1], D. C. Sales[3], L. H. F. Borba[1], A. H. N. Rangel[1], and J. G. B. Galvão Jr.*[2], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [3]*Universidade do Estado de São Paulo, Jaboticabal, SP, Brazil.*

The objective of this study was to evaluate the physico-chemical composition of yogurts of donkey milk in a mixture with bovine, buffalo and goat milk. To do so, 3 yogurt formulations were prepared: DBV (50% donkey milk + 50% bovine milk), DBF (50% donkey milk + 50% buffalo milk) and DGO (50% donkey milk + 50% goat milk). All formulations had sugar added (8%) and were flavored with concentrated mango pulp (15%). The physico-chemical evaluation was performed on the 3first day of storage by analysis of fat, protein, casein, lactose, total solids (TS) and defatted dry extract (DDE) by DairySpec FT equipment (Bentley Instruments Inc., Chaska, MN), plus pH evaluation. The samples were diluted 1:1 with distilled water before being submitted to analysis in the equipment. The final result was obtained by multiplying the reading obtained in the equipment by 2. The data were submitted to ANOVA, complemented by Tukey's test at 5% significance using the SAS program (version 9.0). The results showed that pH (4.2 to 4.3) was similar ($P > 0.05$) for all formulations, but differences ($P < 0.05$) were observed among all formulations regarding nutritional components (fat, protein, casein, lactose, TS and DDE). Such behavior reflects the influence of the characteristics of the milks used in the composition of each yogurt. The DBF formulation had the highest protein (2.6%), lactose (13.7%), TS (19.6%) and DDE (17.9%) values and lower values for fat (1.8%), whereas DBV showed the opposite behavior (fat: 2.1%, protein: 1.9%, lactose: 12.9%, TS: 18.9%, DDE: 16.8%). The results show that the physico-chemical characteristics of donkey milk yogurts varied according to the milk of the species added in preparing the formulations. Thus, the enrichment of the donkey milk with the milk of other animal species can improve the physico-chemical characteristics of yogurts.

Key Words: physical-chemical, composition evaluation, milk derivative.

M110 Evaluating ElastoSens, a novel instrument for gelation measurements, on yogurt fermentation.

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Understanding the gelation properties of dairy products has been an important aspect in the dairy industry. Traditional methods for characterizing gelation include utilizing oscillatory rheometer, compression test, viscometer, and Formagraph. However, these methods have limitations such as the need of separate samples for each measurement, long sample preparation time, and the difficulties in short-interval measurements. This study aims to evaluate ElastoSens (Rheolution Inc., Quebec, Canada), a novel instrument that allows non-destructive, continuous measurement of elasticity (G′) and viscosity (G″) over the course of time, on yogurt fermentation that often yields a weak gel. An oscillatory rheometer was used to validate results obtained from ElastoSens for yogurt gels made with different concentrations of nonfat dry milk (0%, 2.5%, 5.0% wt/vol). The results showed no significant difference between the 2 instruments for samples below 300 Pa, the cut off G′ value for measuring soft samples using ElastoSens ($P = 0.09$). The effects of varying nonfat dry milk concentrations, types of culture, and fermentation temperatures were then investigated using ElastoSens. The results showed a positive correlation between the maximum G′ and the amount of nonfat dry milk added. Two yogurt cultures investigated yielded significant difference in their corresponding yogurt gel strength ($P < 0.001$), with results matching the description of culture properties. There was a significant difference among maximum G′ values for yogurts fermented at 41°C, 43°C and 45°C ($P < 0.05$). Six duplicate experiments were conducted for each aforementioned variable. In conclusion, our results showed that ElastoSens could be used to measure gelation properties of yogurts over time nondestructively and are comparable to traditional methods. Further validation and investigation on more dairy products are needed to explore full potentials on ElastoSens.

Key Words: ElastoSens, yogurt, gelation

M111 Effect of cavitation and nanofiltration temperature on the production of MPC80.

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It is a common practice in the dairy industry to nanofilter (NF) milk protein concentrate (MPC) to increase the solids content before drying to improve drying efficiency. Previous research has demonstrated that temperature and hydrodynamic cavitation (HC) have a significant impact on the viscosity of MPC. A reduction in viscosity could potentially increase the level of concentration that can be achieved with nanofiltration. The objective of this study was to evaluate the impact of temperature and hydrodynamic cavitation on the efficiency of nanofiltration and microbial quality of MPC. Three replicates of MPC80 (20.07, 20.61 and 20.05% total solids) were concentrated using nanofiltration membrane (Parker ATF 7938-LS02-ST, spacer 65 mil, surface area 22.5 m^2 with a 200 Da molecular weight cut off). Four different NF treatments were utilized including: NF at 22°C (NF22); NF at 50°C (NF50); HC before NF at 22°C (HCNF22); and HC before NF at 50°C (HCNF50). The HC was performed with a rotor speed of 50 Hz and flow rate 100 L/h and was applied just before NF. All treatments were nanofiltered until the permeate flux dropped to less than 0.1 LMH. The average flux of nanofiltration was 1.53, 3.20, 1.59, and 3.10 LMH and the final TS of retentate was 25.7, 29.7, 27.4, and 31.9% respectively for the NF22, NF50, HCNF22, and HCNF50 treatments. The average flux and final total solids was significantly ($P < 0.05$) higher for NF conducted at 50°C as compared with 22°C. HC did not have a significant ($P > 0.05$) impact on average flux but did significantly increase ($P < 0.05$) the final TS at 22 and 50°C. The final viscosity of the NF retentate corresponded to the final TS reached and was 67, 269, 77 and 453 cP for the NF22, NF50, HCNF22, and HCNF50 treatments, respectively. The standard plate count (SPC) of the retentate before drying was 3.9×10^4, 1.10×10^5, 7.6×10^4 and 7.3×10^5 for the NF22, NF50, HCNF22, and HCNF50 treatments, respectively. This study determined that increasing the NF temperature improves nanofiltration performance and the level of total solids achieved but also increased the SPC count of the final retentate.

Key Words: MPC80, hydrodynamic cavitation, nanofiltration

M112 Development of a benchtop method to make process cheese using a Thermomix.

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Process cheese (PC) production requires both heat and mixing to achieve an emulsion. Few benchtop methods available can provide enough sample for functional testing. Equipment that can be used include the HotMixPro, Rapid Visco Analyzer, Thermomix, Farinograph, or doughLAB. Some studies have used a Thermomix to produce PC, but specific details such as batch size, mixing speed, and heating profile were not explicitly defined. The objective of this study was to develop a benchtop production method for PC spread specifically for analysis of functional properties. Cheddar cheese of different ages (1 mo and 8 mo) was mixed with butter, instant nonfat dry milk, anhydrous disodium phosphate, cheese salt, and water and processed using varying mixing speeds (3 and 4, approximately 500 and 1100 rpm) and hold times (1 min and 3 min after reaching 80°C final temperature). Formulas (48% moisture and 750 g) were designed to allow for creation of different degrees of emulsification to test the benchtop method. PC spreads were analyzed using nuclear magnetic resonance (NMR) for volume-weighted fat droplet diameter. PC sauces (50 g cheese mixed with 10 g water) were analyzed using a micro-visco amylograph (MVAG) for viscosity (cP) at 50°C for 2 min. Two replicates of each sample were analyzed. One-way ANOVA was used to determine significance using R software. NMR analysis (Table 1) showed differences ($P < 0.05$) between cheese age and mixing speed, but not hold time. MVAG analysis (Table 1) of cheese sauces showed differences ($P < 0.05$) between age and hold time, but not mixing speed. These findings show that a benchtop Thermomix method can be used for small-batch production of PC to lead to different degrees of emulsification and viscosities.

Table 1 (Abstr. M112). Nuclear magnetic resonance and micro-visco amylograph results

Sample			Average fat droplet diameter (μm)	Average viscosity (cP)
Age (mo)	Mixing speed	Hold time (min)		
1	3	1	2.59	770.5
1	3	3	2.58	1031.1
1	4	1	2.18	670.5
1	4	3	1.73	891.5
8	3	1	3.26	378.0
8	3	3	3.16	422.3
8	4	1	2.49	345.6
8	4	3	2.36	398.0

Key Words: process cheese, Thermomix, emulsion

Dairy Foods: Products

M113 Flavor compounds and quality parameter changes during extended refrigerated storage of goat milk butter. J. H. Lee*[1], A. Discua[1,2], and B. B. Lemma[1], [1]*Fort Valley State University, Fort Valley, GA,* [2]*FreshDirect Co., New York, NY.*

Because of its nutritional and health benefits, goat milk products have been attractive to health-conscious consumers. While use of goat milk in cheese and yogurt making is well known, its use for butter has been limited. Furthermore, there is limited information available for volatile compounds in goat's milk butter. The aim of this study was to evaluate the volatile compounds and quality parameters of goat milk butter stored under refrigerated condition. Nine different batches of goat milk butter were produced by continuous churn of cream from goat milk at the university creamery. Each batch of butter was divided into 4 portions. Each portion was subdivided into 4 samples, stored in closed plastic containers at 5°C for 0, 1, 3, 6 mo. Color (CIE, L*a*b*), texture, and peroxide value (PV) were measured from each samples. Fatty acid profiles and volatile compounds of butter samples were analyzed using methylation with gas chromatography (GC) and solid phase microextraction (SPME) with GC-mass spectrometry (MS), respectively. The properties of color and texture of butter were not affected by storage time. After 6 mo, PV significantly increased ($P < 0.05$) from 1.21 to 3.03 (meq peroxide/kg fat). Among 18 isolated fatty acids, the relative weight percentages of caproic (C6:0), caprylic (C8:0), carpric (C10:0) acids significantly increased ($P < 0.05$), whereas the percentage of palmitic (C16:0) acid decreased ($P < 0.05$) after 6 mo of storage. Of 35 identified volatiles, butanoic acid, hexanoic acid, δ-octalactone, δ-decalactone, limonene, and toluene were the most intensive volatile compounds in goat milk butter, whereas these compounds did not changed during storage. Results indicated that the lipid oxidation of goat milk butter was increased over a 6-mo refrigerated storage period. However, the increment of lipid oxidation in butter was not revealed in its volatile compounds.

Key Words: goat milk butter, solid-phase microextraction (SPME), volatile compound

M114 Effects of polymerized whey protein prepared directly from cheese whey as fat replacer on physiochemical, texture, microstructure, and sensory properties of low-fat set yogurt. T. Fang[1], C. Wang*[1,3], J. Hou[2], and M. Guo[3,2], [1]*Department of Food Science, College of Food Science and Engineering, Jilin University, Changchun, Jilin Province, China,* [2]*Department of Food Science, Northeast Agriculture University, Harbin, Heilongjiang Province, China,* [3]*Department of Nutrition and Food Sciences, College of Agriculture and Life Sciences, University of Vermont, Burlington, VT.*

Polymerized whey protein has been used for yogurt making. The aim of this study was to investigate the effects of polymerized whey protein prepared directly from cheese whey on physiochemical, texture, microstructure and sensory properties of low-fat set yogurt. Cheddar cheese whey was pasteurized (60°C for 30 min) and pre-filtrated using screen mesh. The treated whey was subjected to microfiltration (MF, 0.1 µm, at 50°C for 1 h). MF whey was ultrafiltrated (UF) using a 10 kDa cut-off membrane to 25-folds. The UF treated whey was electrodialyzed (ED) to remove 90% of salt and the final protein content of the concentrated whey protein was ~10.0%. The majority (~72%) of particle size distribution of polymerized whey protein (70°C for 10 min, pH 7.0) prepared directly from whey was in the range of 1–3 µm. The PWP (1.4% protein, wt/wt) was added to skim milk as a fat replacer to make low-fat set yogurt. Samples were evaluated on sensory properties, texture, and apparent viscosity in comparison with full-fat (3.0% fat, wt/wt), low-fat (1.0% fat, wt/wt) and nonfat (0% fat, wt/wt) milk yogurt. The viscosity of low-fat yogurt incorporated with PWP (1787.98 ± 37.14 mPas) was significantly higher than low-fat yogurt (1678.76 ± 66.27 mPas), but no statistically significant difference with full fat yogurt (1826.01 ± 69.86 mPas). And the firmness of yogurts with PWP were significantly higher ($P < 0.05$) than the control yogurts without PWP separately. All yogurt samples were assessed for sensory attributes using principal component analysis. The first component explained 52.3% of total variability and was mainly related to the sensory indicators caused by cheese whey and texture attributes. And low-fat yogurt with PWP had better flavor properties among all samples. In conclusion, PWP prepared directly from whey through membrane separation technology can be used as a fat replacer to develop low-fat yogurt with desired characteristics. The polymerized whey protein (PWP) could be used as a natural and economical ingredient for formulation of low fat milk based fermented foods.

Key Words: polymerized whey protein, whey, low-fat yogurt

M115 Engineering innovative dairy emulsion droplets to mimic native milk fat globules. H. Zheng*[1], L. Ingram[2], J. A. Ortiz Salazar[3], J. Lu[4], and R. Fernando[3], [1]*Dairy Innovation Institute, Department of Animal Science, California Polytechnic State University, San Luis Obispo, CA,* [2]*Department of Biomedical Engineering, California Polytechnic State University, San Luis Obispo, CA,* [3]*Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, CA,* [4]*Dairy Processing group, Institute of Food Science and Technology, Chinese Academy of Agricultural Science, Beijing, China.*

The impact of milk fat globule membrane (MFGM)-coated emulsion droplets on lipid digestion has become an interesting research topic. Efficiently manufacturing emulsion droplets coated by MFGM materials has been a technological challenge. The MFGM materials are enriched in buttermilk, and they may be further concentrated in buttermilk concentrate (BMC) using membrane filtration technology. In the current research, the emulsification capacity of in-house manufactured BMC was investigated. Response surface methodology (RSM) was used for optimizing the volumes and ratio between BMC as the emulsifier and milk fat as the oil phase, 1–15% of BMC (variable 1) and 1–15% anhydrous milk fat (AMF, variable 2) were combined at different ratios for manufacturing emulsion systems. Emulsions were manufactured by using homogenizer at 100MPa with 5 passages. All emulsions were heat-treated at 70°C for 30min for testing the physical stability after pasteurization. Both protein and fat contents are enriched in BMC comparing with original dried buttermilk (DBM) ($P < 0.05$). The lipidomics results showed that phosphatidylcholine is about 14% enriched in BMC powder comparing with original DBM. A 6-mo shelf life study showed that BMC is an efficient emulsifier. Particle size distribution (PSD) results showed that an emulsion system made from 8% BMC + 8% AMF (namely 8/8-BMC emulsion) had D4,3 as 3.9 ± 0.14 µm. Moreover, the sample PSD profile is similar to the PSD profile of raw milk. Confocal laser scanning microscopy images confirmed that both protein and MFGM phospholipids were loaded onto to the surface of BMC stabilized emulsion droplets. Using amplitude sweep technique, the rheological characterization confirmed that the 8/8-BMC emulsion was in liquid state at both 5°C and 25°C (loss tangent >1). Stress ramp test was performed for the construction of flow behavior Ostwald/de Waele model for "8/8-BMC emulsion." All data points were covered in 95% prediction interval (95% PI) of the model. In conclusion, we developed a technology for manufacturing recombined emulsion droplets that mimic the structural assembly of native MFGs. These emulsion droplets may be applied in infant formula and in cheeses made from fully recombined milk.

Key Words: emulsion, buttermilk concentrate

M116 Effect of cavitation and nanofiltration temperature on the functionality of MPC80. A. Mishra* and L. Metzger, *South Dakota State University, Brookings, SD.*

Nanofiltration (NF) is typically utilized during MPC manufacture to concentrate ultrafiltration retentate before drying. In related research we determined that increasing the NF temperature from 22°C to 50°C and use of hydrodynamic cavitation (HC) improved nanofiltration performance and increased the level of total solids that can be achieved. In this study 3 replicates of 4 different NF retentates were spray dried and the functionality of the dried MPC was evaluated. The NF treatments utilized were: NF at 22°C (NF22); NF at 50°C (NF50); HC before NF at 22°C (HCNF22); and HC before NF at 50°C (HCNF50). All samples were spray dried using a pilot scale dryer (Niro dryer Model 1, Niro Inc., Columbia) using air atomizing-flat spray nozzle (SUE15A, external mix) with an inlet and outlet temperature 170°C and 85°C respectively. The powders produced were analyzed for physicochemical and functional quality. The moisture content of all powders was below 5% and the protein and ash content ranged from 79.81 to 81.29% and 7.23–7.35% respectively. The loose bulk density was 353, 332, 358, and 323 Kg/m^3 and the tapped bulk density was 454, 428, 456, and 421Kg/m^3 respectively for the NF22, NF50, HCNF22, and HCNF50 treatments. The loose and tapped bulk density was significantly ($P < 0.05$) higher in treatments NF at 22°C, whereas HC did not have a significant ($P > 0.05$) impact on loose or tapped bulk density. There were no significant differences in solubility at 22°C or 50°C or wetting time at 22°C; however, HC significantly ($P < 0.05$) decreased the wetting time at 50°C. There were no significant ($P > 0.05$) differences in emulsification capacity, emulsion stability, or foam stability, whereas foam capacity was significantly higher in the treatments that were nanofiltered at 50°C. The rennet coagulation time significantly ($P < 0.05$) increased and the heat coagulation time significantly ($P < 0.05$) decreased in the treatments that were nanofiltered at 50°C. This study determined that NF temperature and HC have important effects on the functionality of dried MPC80 and can be utilized to adjust the functional characteristics of MPC80.

Key Words: MPC80, hydrodynamic cavitation, nanofiltration

M117 Heat-stable whey protein isolate made without microfiltration. G. Y. Subbiah Prabhakaran*, J. A. Lucey, and M. Molitor, *University of Wisconsin, Madison, WI.*

Residual fat (RF) in products made from separated whey is detrimental for shelf life and functional applications. Objective of this study was to remove RF present in whey without utilizing microfiltration. We believe that, by demineralizing the whey via ultrafiltration, RF can be precipitated with denatured proteins and removed by centrifugation. Extensive demineralization can remove enough Ca for the resulting isolate to be heat stable. Trials were run to evaluate the effects that protein concentration (PC), pH and conductivity (CN) have on extent of RF sedimentation. Two demineralized UF retentates (UF-r) were produced from liquid sweet whey protein concentrate (WPC) 60 and 30. Respective UF-r had total solids of 8% and 5.3%; their conductivities were ~400 and ~300mS/cm. Demineralization was achieved by acidifying liquid WPCs to pH 4.5 using HCl and ultrafiltering them along with extensive diafiltration using acidified water. The UF-r were adjusted to different protein concentrations (ctrl, 5, 4, 3, 2.5, 2, 1.5%), centrifuged (for 10 min) and turbidity of the supernatant was measured (n = 2) to understand the effect of PC on RF sedimentation. The drop in turbidity (before and after centrifugation) increased with increasing PC which indicates that preconcentration of whey to about ~3% would be necessary for better sedimentation. Conductivity of UF-r was then adjusted to different levels (ctrl, 500, 750, 1000, 1250, 1500, 1750 µS/cm) and tests were repeated to understand effect of CN on RF sedimentation. Sedimentation increased with decreasing CN with maximum RF sedimentation occurring below 500 µS/cm. Similar trials were conducted for different pH levels (4.1, 4.3, 4.5, 4.7, 4.9) and max sedimentation occurred between 4.5 and 4.7. It appears that lipids in UF-r can be sedimented with conditions near the isoelectric pH of denatured proteins and the relatively low conductivity achieved by UF demineralization. Future work will determine the compositions of the sediment and supernatant to better understand the process and then scale it up to generate pilot scale WPI powder.

Key Words: WPI demineralization, isoelectric precipitation, centrifugation

M118 Sensory evaluation and intent-to-purchase of milk yogurts mixed with milk from other species. R. D. S. Gomes[1], A. F. S. Gomes[1], I. L. S. Oliveira[1], E. G. S. O. Silva[1], H. A. P. Lopes[1], I. M. Barbosa[1], D. C. Sales[3], L. H. F. Borba[1], M. F. Bezerra[1], J. G. B. Galvão Jr.[2], and A. H. N. Rangel*[1], [1]*Universidade Federal do Rio Grande do Norte, Macaíba, RN, Brazil,* [2]*Instituto Federal de Educação do Rio Grande do Norte, Ipanguaçu, RN, Brazil,* [3]*Universidade do Estado de São Paulo, Jaboticabal, SP, Brazil.*

The objective of this study was to evaluate the sensory acceptance and the intent-to-purchase of 3 yogurt formulations made with 50% donkey's milk in a mixture with bovine (DBV), buffalo (DBF) and goat's milk (DGO) supplemented with sugar (8%) and concentrated mango pulp (15%). The attributes of appearance, aroma, flavor and consistency of the yogurts were evaluated by 95 untrained tasters on the third and 3first day of shelf life by applying the 9-point hedonic scale test anchored at extremes of 1 (I highly disliked it) and 9 (I liked it very much). We also used a scale ranging from 1 (would not buy) to 5 (would buy) to evaluate intent-to-purchase. The data were submitted to variance analysis and the means were compared by Tukey test at 5% significance by the SAS program (version 9.0). The results showed that on the third day of storage, the DBV and DBF formulations were similar ($P > 0.05$) in all evaluated attributes and in the intent-to-purchase, and also presented higher mean values ($P < 0.05$) than the DGO formulation for consistency and purchase intent. On the first day of shelf life, the DBV formulation reached the highest averages ($P < 0.05$) for taste and purchase intent. Appearance and aroma were similar for all formulations during the 31 d of storage, with sensory scores between "slightly liked" (6.0) and "liked regularly" (7.0). The formulation with donkey and goat milk had lower mean values for the consistency attribute when compared with the others ($P < 0.05$). In relation to the flavor, the DGO formulation values were similar ($P > 0.05$) to the DBV on the third day of shelf life and to the DBF on the first day, in spite of presenting lower mean values. The lower means achieved by the DGO yogurt were probably influenced by the more fluid consistency of this formulation provided by the presence of goat milk. The results show that donkey milk yogurts added with bovine, buffalo and goat's milk reached good sensory acceptance up to 31 d of storage.

Key Words: sensory analysis, donkey, shelf life

M119 Influence of β-galactosidase and temperature treatment to milk on functional and reconstitution properties of low-lactose milk powders by spray drying.

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The aim of this study was to determine effects of β-galactosidase and temperature treatments to milk on functional and reconstitution properties of low-lactose milk powders (lactose <0.1%). Skim milk mixed with 5000 NTU/L of β-galactosidase were incubated at 4, 35 and 50°C, which was stirred for 1 h and then stored under refrigeration at 4°C for 20 h before spray drying at the inlet and outlet air temperatures of 170/85°C. Hydrolyzed lactose milk powder (HLMP) was darker than unhydrolyzed lactose milk powder (control) because of the greater amount of reducing sugars, which causes the Maillard reaction. HLMP had higher redness (a*) value and lower water activity compared with the control ($P < 0.05$). HLMP had no significant effect on lightness (L*) and yellowness (b*) values, moisture content and bulk density. In addition, HLMP led to a reduction in wettability and dispersibility, which means that HLMP is difficult in rehydration ($P < 0.05$). As the incubation temperature increased, the rate of glucose released also increased. Bulk density was increased and wettability and dispersibility were decreased with increase in the incubation temperature ($P < 0.05$). Incubation temperature had no significant effect on color, moisture content and water activity of HLMP. According to Carr's index and Hausner ratio values, the flowability of the control and HLMP incubated at 4°C were "poor," while HLMP incubated at 35 and 50°C were "very poor." The results revealed that the increase in the rate of lactose hydrolysis resulted in a larger agglomeration of particles. This could explain the decrease in the rehydration capacity of the powders.

Table 1 (Abstr. M119). Functional and reconstitution properties of low-lactose milk powders incubated at different temperatures (4°C, 35°C, and 50°C)

	Untreated	β-galactosidase		
	4°C	4°C	35°C	50°C
Water activity	$0.26^{a} \pm 0.02$	$0.21^{b} \pm 0.01$	$0.21^{b} \pm 0.01$	$0.23^{ab} \pm 0.02$
$L^{*\ Ns}$	91.38 ± 7.47	91.15 ± 7.45	90.66 ± 7.44	91.04 ± 7.44
a*	$-1.12^{b} \pm 0.10$	$-0.51^{a} \pm 0.05$	$-0.59^{a} \pm 0.06$	$-0.64^{a} \pm 0.07$
$b^{*\ Ns}$	7.21 ± 0.61	7.78 ± 0.65	7.60 ± 0.64	7.72 ± 0.64
Wettability (s)	$175.3^{a} \pm 11.3$	$136.0^{b} \pm 10.3$	$43.3^{c} \pm 3.7$	$44.4^{c} \pm 2.6$
Dispersibility (%)	$202.9^{a} \pm 23.5$	$135.0^{b} \pm 11.0$	$120.0^{bc} \pm 9.8$	$97.1^{c} \pm 6.4$

$^{a–c}$Means within a row with different superscripts are significantly different ($P < 0.05$).

Key Words: β-galactosidase, low-lactose milk powder, spray drying

M120 Use of polymerized whey protein as a gelation agent for symbiotic almond yogurt formulation.

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Almond milk-based products are becoming increasingly popular as milk product alternatives. In this study, a symbiotic almond yogurt containing probiotics and inulin as a prebiotic was developed using polymerized whey protein (PWP) as a gelling agent. PWP was prepared by heating 10% (wt/vol protein) of whey protein isolate solution at 85°C for 30 min at pH 7.0. The samples fortified with 0.6% PWP, 0.3% pectin and 0.07% xanthan gum have good consistency and mouth feel. The physiochemical properties of this symbiotic almond yogurt like product were analyzed for total solids 16.87 ± 0.04, protein 2.71 ± 0.17, fat 3.97 ± 0.19, carbohydrates 9.88 ± 0.08, and ash 0.26 ± 0.03 g/100g. The pH of this product was 4.47 ± 0.01 and the viscosity was 11.91 ± 2.27 mPa·s. The initial populations of the major probiotics in the samples were about 10^8 cfu/mL. The symbiotic almond set yogurt is not only a good source of protein and relatively low fat compared with a commercial sample, but has smooth texture and a refreshing taste. The results indicated that PWP may be a suitable gelation agent for formulating non-dairy fermented products. Future studies are being conducted on shelf-life tests and probiotic survivability during 10-week storage.

Key Words: whey protein, almond, yogurt

Growth and Development: Starter and Forage

M121 Chopped grass hay, cottonseed hull pellets, or oat hull pellets as roughage sources for 2- to 4-month-old calves and the effects on performance and digestion. T. S. Dennis*, F. X. Suarez-Mena, T. M. Hill, W. Hu, and J. D. Quigley, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

Fiber sources used in weaned calf diets vary widely, but effects on performance and digestibility are not fully understood. Ninety-six Holstein steers (59 d of age; initial BW = 75.9 ± 1.76 kg) were received in 2 blocks of 48 calves and started on trial between October 2017 and January 2018. Calves were housed in groups with 4 calves/pen. All diets included whole corn, protein supplement, and liquid molasses. Treatments were 25% whole oats with 5% chopped grass hay (CON), 9.5% pelleted cottonseed hulls (CSH), 9.5% pelleted oat hulls (OH), and 5% chopped grass hay (HAY). All treatments were formulated for equal CP (21% on DM basis). Starch ranged from 35 to 39% and NDF ranged from 12 to 17% on a DM basis. Calves were offered diets for ad libitum intake and orts were weighed daily. Body weight, hip width, and body condition score (BCS) were assessed initially and at 28 and 56 d. During d 19–23 (78–82 d of age), fecal samples were taken and composited by pen to estimate total-tract digestibility using acid insoluble ash. Data were analyzed as a completely randomized design with repeated measures and pen as the experimental unit. Initial measurements were similar among treatments. Calf DM intake ($P < 0.01$), ADG ($P < 0.05$), and BCS change ($P < 0.05$) were greater for calves fed CSH vs. CON and HAY, but gain:feed tended to be less for CSH vs. HAY ($P = 0.09$). Dry matter intake as a % of BW was least for calves fed CON (2.95%) and HAY (3.05%) and greatest for calves fed CSH (3.35%; $P \leq 0.05$). Hip width change was less for calves fed CON vs. other treatments ($P < 0.05$). Digestibility of NDF was greater for calves fed OH vs. CSH and HAY ($P < 0.05$), but not different from CON ($P > 0.20$). Other digestibility estimates did not differ among treatments. Calves fed CSH as a roughage source had greater ADG, but gut fill may have contributed to treatment differences as calves fed CSH consumed 10–19% more feed than other treatments resulting in reduced feed efficiency. Additionally, hay particle length in CON and HAY treatments may have limited intake compared with less physically effective fiber sources.

Key Words: calf, roughage, digestibility

M122 Effects of physical form of starter and forage provision in the diet of dairy calves during the preweaning period. A. E. Leão[1], C. F. A. Lage*[1], M. M. Campos[3], F. S. Machado[3], J. G. Laguna[4], A. L. Ferreira[3], L. G. R. Pereira[3], T. R. Tomich[3], S. F. Costa[5], M. A. Machado[3], D. R. L. Reis[3], and S. G. Coelho[1], [1]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [2]*Alta Genetics, Uberaba, MG, Brazil,* [3]*EMBRAPA, Juiz de Fora, MG, Brazil,* [4]*Michigan State University, East Lansing, MI,* [5]*Universidade Federal de Lavras, Lavras, MG, Brazil.*

The objective of this study was to evaluate the effects of physical form of starter and inclusion of hay in diet of preweaning dairy calves on the intake, performance, digestibility, ruminal development, and the mRNA expression of genes involved in the ruminal metabolism. Holstein × Gyr crossbred male calves (n = 38) were enrolled on trial between 1 and 9 wk of age, and were assigned to 3 treatments: pellet + flocculated corn starter (n = 13), ground starter (n = 12), and ground starter + 5% chopped Tifton hay (n = 13). Pellet + flocculated corn starter had 96% pellets (4 mm in diameter and 18 mm in length) and 4% steam flaked corn. The same starter of the Pellet + flocculated corn treatment was used in the treatments Ground and Ground + 5% Hay, but ground to pass a 3.2 mm sieve. Tifton hay was added at 5% as fed of the amount of starter supplied to calves of the Ground + 5% Hay treatment, chopped into 67.1% of long particles (>19 mm), 13.3% of medium particles (between 8 and 20 mm) and 19.6% of small particles (<8 mm). All calves were fed 4L/d of whole milk up to 63 d of age and were weaned at 64 d of age. Water and starter were offered ad libitum. Samples of ruminal contents were obtained from all animals at 30, 45 and 60 d of age to evaluate the pH, ammonia nitrogen and volatile fatty acids (VFA). At 55 d of age, apparent digestibility assay was performed using 18 animals (n = 6 per treatment). At 65 d of age, the 18 animals were euthanized to evaluate the development of the digestive tract. Significance was declared at $P \leq 0.05$ and tendency $P > 0.05$ and $P < 0.10$. The physical form of starter and the inclusion of hay did not influence intake, performance, feed efficiency, apparent nutrient digestibility, energy partitioning, nitrogen balance, ruminal pH, ammonia nitrogen concentration, VFA and the development of the digestive tract. The mRNA expression of monocarboxylate transporter 1 (*SLC16A1*) was higher in the dorsal sac than ventral sac. A tendency to increase the level of mRNA expression of *SLC16A3* (*MTC4*) transporter and *SLC9A2* (*NHE-2*) were observed on ground treatment. The authors thank FAPEMIG for financially supporting this research.

Key Words: digestibility, mRNA expression, ruminal metabolism

M123 Effects of fatty acid supplementation and calf starter form on intake, growth and digestion of calves from 0 to 4 months of age. L. L. Deikun*, J. D. Quigley, T. M. Hill, T. S. Dennis, and F. X. Suarez-Mena, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

Our objective was to evaluate addition of a fatty acid supplement and calf starter form on intake, growth and digestion of calves to 4 mo of age. Forty-eight male Holstein calves (2–3 d of age; initial BW = 41.9 kg ± 0.7 kg) were assigned randomly to a textured (TS) or pelleted (PS) calf starter (CS) and with (FA+) or without (FA-) fatty acid supplementation (NeoTec5g, Provimi) in milk and CS. Reconstituted whole milk powder (25% CP, 29% fat) was fed at 0.66 kg of DM/d to 39 d, then 0.33 kg of DM/d to weaning at 42 d. Calves were housed in individual pens to d 56 and in group pens (4/pen) from d 57–112. Calves were offered CS to d 56 and 95% CS + 5% chopped grass hay from d 57–112. Feed offered and refused was weighed daily. Body weight and hip width were measured at d 0, weekly to d 56, and on d 85 and 112. Fecal samples were collected during wk 4, 6, 8, 10, 13 and 16 for total-tract digestibility (TTD) measurement using acid insoluble ash as a marker. Data were analyzed as a completely randomized block design with repeated measures using calf as experimental unit from d 0–56 and pen from d 57–112. From d 0–56, CS form had no effect on intake or growth but FA+ increased average daily gain (ADG; $P = 0.02$), gain to feed ratio (G:F; $P = 0.02$) and hip width change (HWC; $P = 0.03$). Least squares means (LSM) of ADG, starter DMI (SDMI), G:F and HWC for calves fed FA+ and FA- from d 0–56 were 0.73 and 0.66 kg/d; 0.92 and 0.85 kg/d; 0.58 and 0.53; and 4.5 and 3.9 cm, respectively. Form of CS had no effect on performance from d 57–112, but FA+ increased ADG and HWC ($P < 0.01$) and tended to increase G:F ($P = 0.09$). The LSM of ADG, DMI, G:F and HWC for calves fed FA+ and FA- from d 57–112 were 1.16 and 1.05 kg/d; 3.15 and 3.30 kg/d; 0.38 and 0.33; and 5.3 and 4.6 cm, respectively. Calves fed FA+ had greater TTD of most nutrients during wk 4, 6 and 8. Starch TTD was greater in calves fed TS during wk 4, 6 and 8, but lower during wk 10, 13 and 16. These data suggest that FA+ improved animal performance at least partially by improving TTD. Feeding TS increased TTD of starch the first 8 wk, but depressed starch digestion from wk 8–16.

Key Words: calf, growth, digestion

M124 Effects of feeding calf starter mixed with hay on sorting behavior, dry matter intake, and growth performance of calves during the first 3 months of life. A. Saegusa*[1], T. Matsuba[1], K. Murayama[1], K. Inouchi[1], and M. Oba[2], [1]*Dairy Technology Research Institute, Feed-Livestock and Guidance Department, The National Federation of Dairy Co-operative Associations (ZEN-RAKU-REN), Nishi-shirakawa, Fukushima, Japan,* [2]*Department of Animal, Food and Nutritional Science, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to evaluate the effect of mixing hay in calf starter on sorting behavior, DMI, and growth performance of dairy calves during the first 3 mo of life. Forty female Holstein calves were fed milk replacer containing 28% CP and 15% fat (maximum intake: 1,105 g/d) until 56 d of age. Calves were fed a texturized calf starter (23.4% CP, 32.2% starch, 20.9% NDF on a DM basis) mixed with chopped Klein grass hay (CP 10.0%, NDF 68.6% on a DM basis) at 90:10 ratio on an as-fed basis (MIX; n = 20) or fed the same starter and hay separately (CONT; n = 20) from d 14 to 90 of age. All feeds were offered ad libitum throughout the study. Particle distribution of the MIX ration was 1.2, 14.2, 82.3, and 2.3% on the upper sieve, the second sieve, the third sieve, and the bottom pan of Penn State Particle Separator, respectively. The CONT calves consumed starter and hay at 77:23 and 96:4 ratios on an as-fed basis before and after weaning, respectively, indicating that MIX calves were offered a solid feed diet containing less hay before weaning but more hay after weaning relative to what CONT calves consumed. Treatment did not affect total DMI (sum of milk replacer, calf starter and hay), ADG, and other growth variables before weaning (d 14 to 55). However, calves on MIX treatment decreased total DMI (3,273 vs. 3,495 g/d; $P < 0.05$), ADG (1.20 vs. 1.31 kg/d; $P < 0.05$), and heart girth gain (0.39 vs. 0.44 cm/d) compared with CONT calves after weaning (d 56 to 90). Although MIX calves sorted against particles on the upper sieve of Penn State Particle Separator (Sorting index = 85.5%; $P < 0.01$) after weaning, dietary NDF content consumed by post-weaned calves (% of total DMI) was greater for MIX than CONT calves (23.6 vs. 22.8% $P < 0.05$). These results suggest that inclusion of 10% hay in a calf starter may decrease DMI and growth performance after weaning even if calves sorted against hay to some extent.

Key Words: calf starter, hay, feeding behavior

M183 Effects of fatty acid supplementation and calf starter form on starch digestion and fecal starch in calves from 0 to 4 months of age. J. D. Quigley*, T. M. Hill, T. S. Dennis, L. L. Deikun, and F. X. Suarez-Mena, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

Form of calf starter (CS) and fatty acid addition may affect total-tract starch digestion (TTSD) and concentration of fecal starch (FS), indicating differences in site and extent of TTSD. Our objective was to determine effects of form of CS and supplementation without (FA-) or with (FA+) a fatty acid blend (NeoTec5g, Provimi) on the relationship between FS and TTSD in calves to 4 mo of age. Male Holstein calves (n = 48; initial BW = 42 ± 0.7 kg, 2–3 d of age) were fed reconstituted whole milk powder (14% DM) at 0.66 kg of DM/d to 39 d, then 0.33 kg of DM/d to 42 d. Textured (TS) or pelleted (PS) CS were offered for ad libitum consumption from 0 to 56 d, then blended with 5% chopped grass hay from d 57–112. Starters contained 20% CP and 38–40% starch (DM basis). Calves were housed individually from d 0–56 and in pens (n = 4/pen) from d 57–112. Fecal starch and TTSD were measured at 4, 6, 8, 10, 13, and 16 wk using acid insoluble ash as digestion marker. Fecal starch increased with wk when calves were fed TS (2.1% to 7.2% of fecal DM at 4 and 8 wk, respectively; $P < 0.05$) but only increased from 4 to 6 wk in calves fed PS (1.2% to 4.7%). From 10 to 16 wk, FS did not change in calves fed PS, but increased ($P < 0.05$) with wk in calves fed TS. Differences in FS were independent of DM intake, which did not vary among treatments. Calves fed TS had similar TTSD at 4 and 6 wk (98.6%); however, at 8 wk, TTSD declined to 96.7% ($P < 0.05$). There was no change in TTSD in calves fed PS from wk 6–8. From wk 10–16, TTSD was greater or tended to be greater in calves fed PS (95.9 vs. 97.9%). Addition of FA blend improved TTSD ($P < 0.04$) from 4 to 8 wk, but had no effect on TTSD from 10 to 16 wk. Regression of TTSD on FS during 4–8 wk was: TTSD = 99.81 (±0.181) – 0.57 (±0.045) × FS – 0.17 (±0.261) × Form (0 = PS, 1 = TS) + 0.16 (±0.058) × (FS × Form); root mean square error (RMSE) = 0.624, $R^2 = 0.84$. During 10–16 wk the equation was: TTSD = 99.94 (±0.092) – 0.59 (±0.013) × FS; RMSE = 0.298, $R^2 = 0.98$. Form of CS influenced site and extent of starch digestion to 4 mo of age.

Key Words: calf, digestion, starter

Production, Management, and the Environment 1

M125 Daily data characteristics of automatic milking system in free-flow farms in the Upper Midwest. M. Peiter*[1], E. Irwin[2], B. Groen[3], J. A. Salfer[4], and M. I. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*Department of Animal Science, Iowa State University, Ames, IA,* [3]*Form-A-Feed, Stewart, MN,* [4]*University of Minnesota Extension, St. Cloud, MN.*

Box-style automatic milking systems (AMS) have increased in popularity over the last decade in the US. It is estimated that in the Upper Midwest (including Iowa, Minnesota, Michigan, and Wisconsin) alone there are over 400 systems in place. The objective of this study was to describe the main production parameters collected by the AMS software. We used 1 yr of retrospective daily data (summer 2017- summer 2018) from 36 free-flow AMS (Lely Astronaut, Lely, the Netherlands) farms (13,050 farm days) in Minnesota and Wisconsin for the analysis. The MEANS procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) was used to analyze the data. The average number of robots/farm was 2.8 (±1.6). The average number of cows/robot was 57.6 (±5.4). Cows were on average 47.0 (±4.9) mo old and DIM averaged 171.0 (±25.5). Daily milk production/cow was 37.3 (±4.3) kg and daily milk production/robot was 2,153.0 (±350.6) kg. A previous study conducted by our group found average daily milk production of 33.2 (±5.3) kg/cow and 1,861.1 (±380.4) kg/robot using AMS data from 2013 and 2014. It seems there has been a numeric milk production increase in AMS farms over recent years. Farms averaged 2.8 (±0.2) successful milkings/cow/d and 1.15 (±0.5) refusals/cow/d. The median number of failures per robot/d was 5.4 (±3.5). Refusals are an indication that cows are voluntarily visiting the AMS frequently. Failures should ideally be close to 0. It has been suggested by the manufacturers that there should be less than 5 failures/robot/d. Milking speed in this study was 3.07 (±0.4) L/min and milking time was 5.4 (±0.5) min. Cows with greater milking speed spend less time milking, which could allow a higher number of cows per AMS. The same parameter may also be used to decide whether cows should be culled or not. The amount of concentrate/100 kg of milk was 15.0 (±2.8) kg and concentrate offered/cow/d was on average 5.5 (±0.9) kg. Average rest feed was 0.3 (±0.14) kg. This study provides a more recent overview of AMS farm metrics in the Midwest US.

Key Words: automatic milking system, robot, milk production

M126 The association between robot time budget and milk production per robot in free-flow automatic milking system dairy farms. M. Peiter*[1], J. A. Salfer[2], and M. I. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*University of Minnesota Extension, St. Cloud, MN.*

Automatic milking systems (AMS) are becoming more common in the USA. Limited research has investigated the influence of robot time budget on milk production/robot. To investigate this relationship, we used one year of AMS software daily data (13,018 daily averages; summer 2017 to summer 2018) from 36 free-flow AMS (Lely Astronaut, Lely, the Netherlands) farms in Minnesota and Wisconsin. The MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) was used to analyze the data. Daily milk production/robot was the outcome variable and the predictors included in the initial model were failures/robot, successful milkings/robot, failure time, refusal time, milking time, treatment (prep) time, cleaning time, free time, refusals/cow, and cows/robot. Backward stepwise elimination was used to remove nonsignificant factors until all remaining factors had a $P < 0.05$ in the final model. Farm was used as random effect. Cleaning time and free time were removed from the model. Results are presented as estimates (±SE). Total failures/robot and failure time were both negatively associated with milk production/robot. For each unit increase in failures/robot, milk production/robot decreased by 2.91 (±0.85) kg/d. For each min increase in failure time, milk production/robot decreased by 0.94 (±0.17) kg/d. Refusals/cow and refusal time followed a similar pattern. For each unit increase in refusals/cow, milk production/robot decreased by 69.27 (±3.06) kg/d, and each min spent with refusals resulted in a decrease of 1.05 (±0.17) kg/d of milk production/robot. For each unit increase in milkings/robot, there was an increase in milk production/robot of 2.65 kg/d. For each extra milking min, milk production/robot increased by 0.99 (±0.02) kg/d. Treatment time (min) was negatively associated with milk production/robot (−168.84 (±5.68) kg/d). A larger number of cows/robot was associated with greater milk production/robot (15.18 (±0.36) kg/d). Robot time budget appears to influence milk production in AMS farms; therefore, robot time optimization could improve performance of AMS.

Key Words: automatic milking system, time budget, milk production

M127 The association between milking interval and milk production per visit, milk fat, and milk protein of cows housed in free-flow automated milking system farms. M. Peiter*[1], J. A. Salfer[2], and M. I. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*University of Minnesota Extension, St. Cloud, MN.*

Research has shown that milking frequency influences milk production in dairy cows. In automatic milking system (AMS) farms, not only milking frequency per cow varies, but also their milking interval. The objective of this study was to evaluate the association between milking interval and cow milk production, milk fat percent, and milk protein percent in AMS farms. We used one year of visit-level data from the AMS software (summer 2017 to summer 2018; 4,978,495 visits), from 36 free-flow AMS (Lely Astronaut, Lely, the Netherlands) farms in Minnesota and Wisconsin. The MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) was used to analyze the data. Milk production/visit, milk fat, or milk protein were the outcome variables in each of the 3 separate models. The main predictor of interest was milking interval. Days in milk (DIM), concentrate intake/visit, milking speed, and milking time were added to the model as covariates. Cow within farm was used as random effect. Milk production/visit had a median value of 12.7 (±4.51) kg. The average for milk fat was 3.78 (±0.76) %, and for milk protein 3.08 (±0.25) %. Milking interval had a median of 472.8 (±180.2) min. The median DIM was 146.0 (±106.1) d. Concentrate intake had a median of 1.69 (±0.58) kg/visit. Median for milking speed was 2.97 (±1.13) kg/min, and for milking time it was 4.32 (2.08) min. For each hour increase in milking interval, milk production increased by 0.19 kg/visit ($P < 0.0001$), fat increased by 0.02 percentage units ($P < 0.0001$), and protein increased by 0.03 percentage units ($P < 0.0001$). Milking interval appears to influence milk production/visit and milk components. However, further research investigating this association is warranted.

Key Words: automatic milking system, milking interval, milk production

M128 Updating Holstein and Jersey lactation curve parameters for the Rumination Farm System Model (RuFaS). M. Li*[1], V. E. Cabrera[1], and K. F. Reed[2], [1]*Department of Dairy Science, University*

of Wisconsin-Madison, Madison, WI, [2]Department of Animal Science, Cornell University, Ithaca, NY.

Milk productivity is believed to have risen with increased genetic progress and improved farm management last decade. However, quantification of this fact remains unclear. With the desire to better represent current animal performance in the RuFaS model, we investigated to what degree milk productivity has improved during 10 yr and how this translates into lactation curve parameters. We fitted Holstein and Jersey test-day milk production data from 2006, 2011, and 2016 to the Wood's lactation curve function ($y = at^{b}e^{-ct}$) using the least square method. As input, we used the data set provided by the Council on Dairy Cattle Breeding consisted of 12.82 million individual lactations each had at least 10 test-day records. We chose to fit separate curves for breed, state, and parity with pre-processed data. We averaged milk yield for every 10 DIM and set the cut-off point at 365 DIM to reduce variation, improve goodness of fit, and eliminate the effect of later records. For the state of Wisconsin, our results show increased 305-d milk yields and postponed, higher peaks in 2016 compared with 2011 and 2006 for each breed and parity. In general, Holstein curves had greater scale of production (*a* in the wood's model), faster rate of increase to peak (*b*), and higher rate of decline after peak (*c*) than Jersey curves. Wisconsin updated Wood's lactation curve parameters from year 2016 are reported in the table below. The RuFaS model will incorporate the parameters from this data set according to breed, parity, state, and milk component curves. These will allow possible reflection of daily production divergence caused by diet alterations, pregnancy, or health issues and will be used to predict milk yields and better inform management decisions.

Table 1 (Abstr. M128).

Parity and breed	No. of lactations	Wood's fitted parameters		
		a (kg/d)	b	c
1				
Holstein	109,534	16.13 ± 0.28	0.235 ± 0.0046	$0.0019 \pm 3.77 \times 10^{-5}$
Jersey	2,901	14.07 ± 0.39	0.186 ± 0.0076	$0.0021 \pm 6.60 \times 10^{-5}$
2				
Holstein	80,776	23.61 ± 0.54	0.227 ± 0.0064	$0.0032 \pm 5.82 \times 10^{-5}$
Jersey	2,179	19.26 ± 0.49	0.173 ± 0.0071	$0.0028 \pm 6.69 \times 10^{-5}$
>2				
Holstein	88,613	23.81 ± 0.51	0.244 ± 0.0060	$0.0036 \pm 5.54 \times 10^{-5}$
Jersey	2,826	19.21 ± 0.47	0.190 ± 0.0069	$0.0032 \pm 6.53 \times 10^{-5}$

Key Words: lactation curve, parameter, breed

M129 Modelling evaporation of water from a bedded pack using random forest regression.

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Maintaining a dry bedding is key in dairy cattle management. High bedding moisture has been related to poor cows' hygiene, increased risk of mastitis and reduced lying time. Aim of this study was to study water evaporation dynamics in a bedded pack for dairy cows. A custom method was developed to measure drying rate (DR) of different bedding materials contaminated with livestock excreta. The method involved exposing samples of bedding with defined volume (80 mL) and surface area (19.7 cm^2) to different artificially controlled conditions. Four bedding materials were included in the study (sawdust, wheat straw, wood pellets and straw pellets). For each material, bedding was collected in a real farm, dried at 65°C, and then added with a controlled amount of water to achieve 2 levels of initial moisture (40%, 70%). Experiment was carried out in a climatic chamber, which allowed recreating 2 levels of air temperature (10°C, 25°C) and 2 levels of relative humidity (40%, 80%). A custom wind tunnel was constructed to simulate 2 levels of air velocity (0.0, 1.0 m/s). Also, to reproduce the heat developed by bacterial activity in a real bedded pack, the custom wind tunnel was provided with a temperature-controlled heating plate, which allowed exposing sample to 2 levels of heating (ambient vs 35°C). A crossed treatment design with 2 repetitions was used resulting in 256 samples tested in total. Drying rate was calculated as the difference in weight before and after a 24h exposure to the test conditions. Data were analyzed using Random Forest regression. Forest Floor method was employed to visualize and interpret the model obtained. Average DR across all treatments was 0.56 kgH_2O/m^2*day, ranging from 0.08 to 1.67 kgH_2O/m^2*day. Variable importance analysis showed that initial sample moisture, air velocity and relative humidity were the most important variables affecting DR. Both air temperature and sample heating showed to produce a relatively small effect on evaporation. Also, DR resulted to be scarcely related to type of bedding material, which resulted to be the least important variable.

Key Words: bedded pack, evaporation, management

M130 Milk yield in pasture-based automatic milking systems is negatively affected by variability in concentrate fed in the robot.

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Feed is the main incentive to encourage voluntary cow traffic in automatic milking systems (AMS). Pasture-based AMS farmers utilize access to pasture and concentrate in the robot to manage cow traffic and ensure target milk yield (MY). We hypothesized that variability in concentrate intake (CI) fed in the robot affects negatively MY, which might reduce AMS profitability. A database from 17 pasture-based AMS farms from Australia, New Zealand, and Ireland was used. We analyzed 403,226 daily records over a 5-mo period (September 2018 to January 2019) including cow id, stage of lactation (SOL; early = 0 to 100, mid = 101 to 200, late = >201 d in milk), parity (primiparous vs. multiparous), MY (kg/d), milking frequency (MF, milkings/d), and concentrate intake (CI, kg/d as fed). We calculated average and coefficient of variation (CV) for each variable for a fixed 7-d period. We ran partial correlations (r; calculated using the MANOVA / PRINTE commands of PROC GLM of SAS Version 9.3, SAS Institute Inc., Cary, NC) to evaluate association between MY and CVMY and the other variables (CI, MF, CVCI, CVMF). The model included farm, month, SOL, and parity as fixed factors. As expected, MY was highly and positively correlated ($P < 0.01$) with CI (r = 0.629) and MF (r = 0.585). Milking frequency was also highly and positively correlated with CI (r = 0.529). However, CV of CI and MF affected negatively both parameters. Milk yield was highly and negatively correlated ($P < 0.01$) with CICV (r = −0.249) and MFCV (r = −0.229). Milking frequency was also highly and negatively correlated ($P < 0.01$) with CICV (r = −0.247). Our data show that milk yield in pasture-based AMS is negatively affected by variability in concentrate intake in the robot. Therefore, reducing concentrate intake variation is key to maximize milk yield output.

Key Words: automatic milking systems, milk yield, variability

M131 Interactions among pregnancy rate, turnover ratio, and herd structure. W. Li* and V. Cabrera, *University of Wisconsin-Madison, Madison, WI.*

The aim of this study was to investigate the interactions among 21-d pregnancy rate (21-d PR), herd turnover rate (TR), and herd structure demographics over time. We developed a monthly Markov chain model that mimics calf, heifer, and cow transition events according to 21-d PR and TR probabilities. The model was used to simulate a 100-adult cow herd with 16% 21-d PR and 35% TR and their corresponding calves and heifers. Monthly herd statistics were extracted and summarized by months of age for young stock, and lactation, milking, dry, pregnancy, and days in milk (DIM) for adult cows when the adult herd reached steady state after a managerial change was imposed. As expected, the overall proportion of pregnant cows increased from 48.4% to 59.5% as the 21-d PR increased to 25%, but this increase occurred only in multiparous cows (from 27.0% to 37.7%), which overcompensated a lower proportion of pregnant primiparous cows (from 22.5% to 21.8%). The percentage of dry cows increased by 1.9% points (to 11.4%). The relationship of the greater 21-d PR and lactation period was that there were 1.2% points more early lactation cows (<120 DIM), while 2.3% points less late lactation cows (>240 DIM). Increased 21-d PR decreased overall culling (0.57 heads/ mo), increased the proportion of non-reproductive culling (6.8% points to 86.9%), increased the percent of pregnant cows in DIM < 150 (14.2% points), decreased the herd average DIM (12.91 d), and increased the positive balance of replacements by 1.20 heads/mo. When decreasing TR from 35 to 25%, the non-reproductive culling proportion of primiparous cows decreased (3.9% points), but the reproductive culling proportion increased for all cows (2.5% points). With lower TR, the average DIM of the herd increased by 3.44 d together with a greater proportion of middle and late lactation cows (1.1% points). Our results indicate that the relationship of the adult herd 21-d PR versus TR to breakeven the supply and demand of replacements followed a quadratic function: $21\text{-d PR} = 0.7036(TR)^2 - 0.1198(TR) + 0.1448$ ($R^2 = 0.9998$) portraying a diminishing return of 21-d PR to compensate same change of TR at greater levels of 21-d PR.

Key Words: herd structure, 21-d pregnancy rate, turnover ratio

M132 Comparing multiple regression with two machine learning methods in a case study predicting individual survival to second lactation in Holstein cattle. E. M. M. van der Heide*[1], R. F. Veerkamp[1], M. L. Pelt[2], C. Kamphuis[1], I. Athanasiadis[3], and B. J. Ducro[1], [1]*Wageningen University and Research, Animal Breeding and Genomics, Wageningen, the Netherlands,* [2]*Cooperation CRV, Arnhem, the Netherlands,* [3]*Wageningen University, Information Technology Group, Wageningen, the Netherlands.*

In this study we compare linear multiple regression to the machine learning methods naive Bayes and random forest, to assess the added value of machine learning for the prediction of the complex trait ‘survival to second lactation’. Our dataset contained 6847 heifers born between January 2012 and June 2013, which had a known outcome for survival to second lactation and were genotyped at birth. Each heifer had 50 genomically estimated breeding values and up to 65 phenotypic records that accumulate over time. Survival to second lactation was predicted at five distinct moments in life. Methods were tested using a 20-fold validation of a randomly selected training (70%) and testing (30%) set, and then compared by various metrics, including area under the curve (AUC) value and by testing a scenario showing the realised gain in survival if the 50% highest scoring heifers were selected. At birth and 18 months, all methods had overlapping performance, with no method significantly outperforming the other. Naive Bayes has the highest average AUC at all decision moments up to 200 days past first calving. At 200 days post calving, random forest has the highest AUC. Individual heifer predictions varied between methods. Correlations of individual predictions between methods ranged from moderate to high (lowest correlation seen was r = 0.417 and highest was r = 0.700). The correlations were highest at birth and once all information was available, decreasing for prediction around first calving. In short, all three methods were able to predict survival on population level as all methods improved survival in a practical scenario. However, depending on the method used, an individual animal could be quite different between methods.

Key Words: machine learning, naive Bayes, phenotypic prediction

M133 Potential for artificial neural network application to predict the fatty acid content of feedstuffs using near-infrared spectroscopy. J. R. R. Dorea*[1], J. Goeser[3], A. L. Lock[2], and G. J. M. Rosa[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Michigan State University, East Lansing, MI,* [3]*Rock River Laboratory Inc., Watertown, WI.*

Milk fat yield and fatty acid (FA) content in lactating dairy cows can be greatly affected by dietary FA profile. The analysis of individual FA in feedstuffs has traditionally utilized gas chromatography (GC) techniques; however, this technique is costly, labor intensive, and time consuming. The objective of our study was to determine if artificial neural network (ANN) is capable of building improved near-infrared spectroscopy (NIRS) predictive models for feed FA relative to the current model approach using partial least squares (PLS). A total of 324 feed samples were scanned with an NIRS instrument and analyzed by GC (Michigan State University) for the following FA: C12:0; C14:0; C16:0; *cis*-9 C18:1; *cis*-9,*cis*-12 C18:2; *cis*-9,*cis*-12,*cis*-15 C18:3. Two predictive approaches were implemented: PLS and ANN, and all wavelengths were used as predictors in each approach. A random grid search was performed to define the best ANN architecture. Prediction quality was assessed by randomly splitting the data set into training and test sets (70% and 30% of the data set, respectively). Prediction quality of PLS and ANN were similar in terms of R^2 and Root Mean Squared Error Prediction (RMSEP), for C12:0 (PLS: $R^2 = 0.46$, RMSEP = 0.12%, ANN: $R^2 = 0.42$, RMSEP = 0.13%), C14:0 (PLS: $R^2 = 0.70$, RMSEP = 0.65%, ANN: $R^2 = 0.74$, RMSEP = 0.56%), C18:1 (PLS: $R^2 = 0.88$, RMSEP = 3.1%, ANN: $R^2 = 0.88$, RMSEP = 3.3%), C18:2 (PLS: $R^2 = 0.90$, RMSEP = 5.0%, ANN: $R^2 = 0.89$, RMSEP = 5.3%), and C18:3 (PLS: $R^2 = 0.83$, RMSEP = 6.8%, ANN: $R^2 = 0.76$, RMSEP = 8.6%). However, ANN predictions presented lower precision and accuracy for C16:0 (PLS: $R^2 = 0.80$, RMSEP = 2.93%, ANN: $R^2 = 0.65$, RMSEP = 0.74%) and C18:0 (PLS: $R^2 = 0.58$, RMSEP = 1.0%, ANN: $R^2 = 0.44$, RMSEP = 1.2%). Overall, results show that NIRS can predict feed FA with reasonable accuracy, but more accurate and precise for the major dietary FA C16:0, C18:1, C18:2, and C18:3. For this data set, the use of ANN did not improve prediction quality when compared with PLS.

Key Words: machine learning, milk fatty acids, near-infrared spectroscopy (NIRS)

M134 The use of integrated data to identify first-lactation cows at high risk of clinical mastitis. H. Delgado, L. Fadul-Pachecho, and V. E. Cabrera*, *University of Wisconsin, Madison, WI.*

The presence of clinical mastitis (CM) in first lactation cows could result in lifetime negative consequences. Therefore, constant monitoring of these cows and rapid intervention is an important management task. With the integrated information obtained from different data streams such as genomic, management and laboratory results, we used different analytical techniques to identify cows with a higher risk of contracting CM. Records from 6,218 1st lactation genomically tested cows from 2 Wisconsin herds between years 2014 and 2018 were used for the analysis. From the total, 1,179 cows presented at least one case of CM between the 1st and 10th test day (10 and 320 DIM). Different production and wellness traits were fit into a logistic regression model. Genomic Total Performance Index (GTPI) and Mastitis (Mast) traits were associated with the presence of CM. Animals with GTPI scores < 1,745 had 2.5 times higher risk of developing CM than animals with GTPI scores > 2,000 ($P < 0.0001$). Animals with Mast scores <96 had 5.3 times higher chances to develop CM than animals with Mast scores > 104 ($P < 0.0001$). We calculated SCC (cells/mL) least squares means for the previous test day to the onset of CM using mixed model analisys and compared with control. For cows with GTPI < 1,745 the result was 273,000 ± 28 cells/mL, while for cows with GTPI >2,000 it was 149,000 ± 43 cells/mL. In comparison, the SCC for animals that remained free from CM during 1st lactation the SCC was 80.38 ± 23 for GTPI < 1,745 and 38.62 ± 40 for GTPI > 2,000. Although cows under < 200,000 SCC are still under the normal threshold, our analyses indicate that farmers should be concerned with cows with SCC as low as 115,000 cells/mL if these cows have a high GTPI score. Better decisions to detect, prevent, or quickly treat CM are possible by integrating real-time data. One of such initiatives is the "Dairy Brain" project at the University of Wisconsin-Madison that allows to explore trends and associations in data that otherwise would remain unseen. Availability of real-time integrated data contributes not only to monitor CM but other health events of negative economic impact.

Key Words: data integration, Dairy Brain, dairy management

M135 Validation of on-farm milk leukocyte differential tester to identify subclinical mastitis in dairy cows.

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The objective of this study was to test the accuracy of a milk leukocyte differential tester (QScout, AAD Inc., Morrisville, NC). The study was conducted at the University of Kentucky Coldstream Dairy. Milk samples from individual quarters (n = 320) were aseptically collected into clear, 150-mL polypropylene vials. Each sample was divided into 2 replicas after mixing. One replica was analyzed for SCC immediately following milking with a SomaCount FC (Bentley Instruments, Inc., Chaska, MN) using flow cytometry. Another replica sample was evaluated with the on-farm milk leukocyte differential device directly after milking, where total leukocyte count were obtained. Somatic cell count values and leukocyte counts were used to calculate SCS (SCS = $\log_2$ (SCC/100) + 3). Subclinical mastitis thresholds of >200,000 (low) and >400,000 (high) cells/mL were set. Total cells measurements and calculated SCS from the milk leukocyte differential device were compared with SomaCount FC using correlation and regression coefficient of determination analyses. Also, sensitivity (Se), specificity (Sp), and accuracy were calculated for correct diagnosis of the 2 SCC thresholds using the SomaCount FC as reference. A correlation coefficient (r) of 0.97 ($P < 0.001$) was found for total leukocyte count, and r of 0.90 ($P < 0.001$) for SCS. In the regression the coefficient of determination was very high for SCC measures ($R^2 = 0.94$, MSE: 0.007; $P < 0.001$) and high for SCS ($R^2 = 0.80$, MSE:0.73; $P < 0.001$). The sensitivity (percentage of milk samples classified correctly) of the on-farm test was 58 [95% CI: 44 to 71]% and 72 [56 to 86]% for the low and high thresholds, respectively. The specificity (percentage of negative samples classified correctly) for the on-farm test was 100 [99 to 100]% and 100 [98 to 100]%, for the low and high thresholds, respectively. The overall accuracy (percentage of samples diagnosed correctly) was 93 [89 to 95]% and 96 [92 to 98]%, for the low and high thresholds, respectively. On-farm total leukocyte count is a promising technology that could support on farm mastitis management decisions.

Key Words: precision technology, milk quality, mastitis

M136 Effect of OmniGen-AF feeding during the dry period and early lactation on performance and health in cows.

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Recent studies with dry and lactating cows subjected to heat stress while fed OmniGen-AF (OG, Phibro Animal Health, Teaneck, NJ) were reported to have lower respiration rates and rectal temperatures than non-supplemented controls. In addition, dry matter intakes were maintained, SCC and health events were lower and milk yields were higher in the cows fed OG. In this study we evaluated the effects of feeding OmniGen-AF (OG) or placebo control (CON) during the dry period and early lactation on milk yield of dairy cows. We hypothesized that feeding OG during the dry period and through the second breeding (~150 d) would improve milk yield and fertility of cows, by promoting improvements in immune status and health. The study was conducted from July 2018 through February 2019 on a commercial dairy in Florida, with cows exposed to high THI during the dry period. To test our hypothesis, cows were fed OG (56 g/h/d; n = 140) or CON (56 g/h/d placebo; n = 132) during the dry period (~60 d) and were supplemented until pregnancy confirmation or the second breeding (~210 d total feeding length). In each treatment, cows were fed as a group and average dry matter intake (DMI) of cows fed OG and CON was recorded during the dry period and lactation. Daily milk yield was measured once each week, starting at the second week of lactation. Data were analyzed using MIXED procedure in SAS with weeks in milk (first 9 weeks) as repeated measurement and parity as covariate. Production results reveal that cows receiving OG had greater milk yield relative to CON cows (OG = 40.6 ± 0.76 kg/d, CON = 38.5 ± 0.68; $P < 0.05$). During lactation, no treatment differences were detected in DMI (OG = 26.1 ± 3.41 vs. CON = 25.6 ± 3.77 kg/d). Analysis of metritis, mastitis, retained placenta, laminitis, ketosis, pneumonia, and displaced abomasum frequencies and fertility will be performed as cows complete the trial. In conclusion, OG supplementation during the dry period and early lactation increased the milk production in dairy cows, which may indicate improved health status.

Key Words: dairy cows, dry period, early lactation

M137 Use of the Integrated Farm System Model to determine economic and environmental impacts of double cropping winter annuals with corn.

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Many dairy farms struggle to remain profitable while reducing negative environmental impacts. Double cropping may improve profitability through increased DM yields per ha and increased manure nutrient application per ha, while reducing nitrogen (N) and phosphorus (P) pollution. The objective of this study was to evaluate the impact of double cropping winter annuals and corn on nutrient cycling, total feed cost, and net return to management as feed prices and the percentage of corn land double cropped changed. Farm simulations were done using the Integrated Farm System Model (IFSM) and crop, dairy, and financial data from 3 dairy farms that practiced double cropping in Pennsylvania during 2016 and 2017. Farms ranged from 336 to 511 ha with 233 to 663 cows. Farms were simulated over a 20-year time period using weather data from DuBois, Pennsylvania. Eight scenarios measuring intensity of double cropping and change in feed price for each farm were simulated. These included: current operation, 0, 50, and 100% of corn land double cropped, 30% relative feed price increase with and without double cropping, and 30% feed price decrease with and without double cropping at current operation level. Double cropping 100% compared with 0% of corn land improved total DM yield by 19% while reducing annual N leached and P runoff losses by an average of 4.5% and 9.2%, respectively, across farms. Double cropping provided a 1.3 and 1.8% increase in net return over feed costs and net return to management, respectively, across farms. When feed prices increased 30%, use of double cropping increased net return over feed cost and net return to management by 1.6 and 2.2%, respectively, across farms. When feed prices decreased by 30%, double cropping increased both net return over feed cost and net return to management by 0.1%, averaged over all farms. Simulation with the IFSM showed that use of double cropping improved profitability on farms during times of average to high feed prices. Loss of N and P decreased with double cropping, but use of N fertilizer with winter annuals reduced this benefit.

Key Words: profitability, environment, double cropping

M138 Effect of different water source manner on performance and social behavior of dairy calves after weaning.

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The goal of the study was to find impact of water receiving method on the growth, health, and cross-sucking of calves. Sixty-three calves were kept in hutches to weaning at the age of 8 weeks. The experiment lasted from April to November. Calves drink mothers milk ad libitum from a bucket with nipple from d 2 to 4. From d 5, they received 6 kg of milk replacer per day divided into 2 portions, concentrate and alfalfa hay ad libitum. Calves were divided according to the water delivery into 3 groups: nipple sucking from bucket (A), drinking from bucket (B), and without delivery water (C). Feed and water refusals were removed and weighed. Blood samples for analysis of white and red blood pictures were taken every week. The methods of Larson (1977) for the evaluation of diarrhea and respiratory condition were used. After weaning the calves were moved to loose housing pens. Their social behavior was observed until the age of 6 mo individually. The data were analyzed using an ANOVA. We not found significant difference among groups in the average daily gains and feed intakes. Daily gains were the highest in group A (A 0.46 kg, B 0.43 kg, C 0.43 kg). A group of calves drank up more water to the weaning than B group (69.39 kg vs 50.72 kg), and group A had the highest intake of starter mixture (A 14.43 kg, B 11.30 kg, C 13.31 kg). The highest alfalfa hay consumption was found in group C (A 21.34 kg, B 22.26 kg, C 23.59 kg). Neither calf died or was culled for bad health. There were no water delivery effects for blood measurements. The faces had liquid consistency during the first weeks, rather than a firm one. Color showed a steadily trend from yellow to green and consistency changed smoothly from liquid to normal. We did not find significant differences between groups in the cross-sucking after weaning. The willingness to be sucked was the highest in calves from group A ($P < 0.001$). The results did not show a negative effect of water shortage. However, we cannot recommend the water intake just as part of the milk replacer on the basic of this experiment. This study was funded by APVV 15-0060 and QK1910438.

Key Words: calf, growth, water drinking.

M139 Association between age at first calving and productive performance in Argentinian dairy herds.

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The aim of this study was to describe the distribution of age at first calving (AFC) in dairy herds of Córdoba province and to evaluate the effect of AFC on productive indicators during the first lactation. A retrospective study was used to analyze data from 26,614 cows calved for the first time during the calendar year 2016. A total of 15,181 first-lactation cows were included in the analysis. Registers were stratified by age at first calving (AFC) into 5 groups: 1) 18–21 mo, 2) 22–25 mo, 3) 26–29 mo, 4) 30–33 mo, 5) 34–37 mo. Milk yield (MY) for first lactation cows having at least 5 milk test days was used to analyze production. The General Linear Mixed Models was applied to determine differences in start-up, peak and daily mean milk yield. The fixed effects evaluated were AFC category, calving season and herd size category, herd was fitted as a random effect. The effect of AFC on milk production during the 5 milk test days was evaluated including the random effect of cow nested within farm. The overall median and interquartile range (IQR) of AFC was 27 and 25–30 mo, respectively. Median milk yield per heifer was 27.1 kg/d (IQR = 23–31). MY, start-up and peak milk yield increased systematically from the 1st to the 5th AFC category (Table 1). Milk production adjusted by herd size and calving season for the 5 AFC categories showed lower MY for lower AFC categories at the beginning of the lactation but differences between AFC categories 3, 4

Table 1 (Abstr. M139). Estimates (mean ± SE) of productive indicators according to AFC category[1]

	1	2	3	4	5
N	202	5,569	5,579	2,389	1,442
Milk yield (MY) (kg/d)	23.0 ± 0.47[a]	24.7 ± 0.33[b]	25.3 ± 0.33[c]	25.8 ± 0.33[d]	26.5 ± 0.34[e]
Start-up MY (kg)	19.6 ± 0.56[a]	21.4 ± 0.30[b]	22.0 ± 0.29[c]	22.4 ± 0.31[d]	22.9 ± 0.33[d]
Peak MY (kg)	26.4 ± 0.51[a]	28.6 ± 0.36[b]	29.3 ± 0.36[c]	29.9 ± 0.37[d]	30.9 ± 0.38[e]

[a–e]Different letters within a row indicate significant difference at $P < 0.05$.

[1]AFC (age at first calving) categories: 1) 18-21 mo, 2) 22-25 mo, 3) 26-29 mo, 4) 30-33 mo, 5) 34-37 mo.

and 5 disappeared by the 4th test day. In conclusion, opportunities exist to reduce AFC, not much affecting MY and reducing rearing costs in dairy herds of Córdoba province.

Key Words: age at first calving, milk yield

M140 Effect of fan and showering on physiological responses and reproductive performance of Holstein Friesian bulls during subtropical summer. M. Q. Shahid*[1], M. A. Butt[1], J. A. Bhatti[2], and A. Khalique[3], [1]*Department of Livestock Production, Ravi Campus, University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*Department of Animal Sciences, College of Veterinary and Animal Sciences, Jhang, Pakistan,* [3]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Ravi Campus, Lahore, Pakistan.*

The objective of current study was to determine the effect of fan and showering on physiological responses and reproductive performance of Holstein Friesian bulls during subtropical summer in Pakistan. Thirty-six Holstein Friesian bulls balanced by age and weight were divided into 3 treatment groups. The treatments were: 1) CTL, bulls kept under shade only; 2) FN, bulls provided with fans under shade, and 3) FNS, showering along with fans under shade. The trail lasted for 6 mo from April, 2016 till September, 2016, divided into 2 seasons (dry hot and humid hot). The observations on ambient temperature, relative humidity, temperature-humidity index, DMI, water intake, rectal temperature (RT), pulse rate (PR), and respiration rate (RR) were taken daily and converted to weekly averages. Semen characteristics and selected blood metabolites were measured fortnightly. The data were subjected to ANOVA using Proc Mixed of SAS. The results indicated that DMI of bulls was similar between the treatment groups. Mean weekly water intake, RT, PR, and RR were significantly lower in FNS group compared with CTL and FN ($P < 0.001$). Semen characteristics including semen concentration, post-thaw semen motility, progressive motility, amplitude of lateral head displacement, straightness, live to dead ratio, plasma membrane integrity, normal acrosomal ridge and DNA integrity were not different between the treatment groups ($P > 0.05$). The FNS group tended to have higher semen volume compared with FN and CTL groups ($P = 0.10$). Blood glucose level was significantly lower in FNS compared with FN and CTL group ($P < 0.05$). Blood urea nitrogen and testosterone were not different among the treatment groups ($P > 0.05$). The current results indicated that showering with fans improved welfare of Holstein Friesian bulls by lowering physiological responses along with minor gain in semen volume during subtropical summer.

Key Words: heat stress, showering, bull performance

Ruminant Nutrition: Calf and Heifer Nutrition

M141 Effect of weaning program and milk feeding level on the solid feed intakes and growth of dairy calves.

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The objective of this study was to investigate how gradual weaning programs affect feed intakes and growth of dairy calves during the milk-feeding (d 1–29), weaning (d 30–45) and post-weaning phases (d 46–59) when fed different levels of milk. 84 Holstein bull calves were housed individually and randomly assigned to 1 of 4 treatments in a factorial design: high level of milk (HIGH; 35 kg of 21:19 milk replacer over 45d) or a low level of milk (LOW; 25 kg of 21:19 milk replacer over 45d) and either a 2-step weaning program (2S) or a 4-step weaning program (4S). From d 30–45, the 2S calves had 2 reductions in their milk, while 4S calves had 4 reductions in their milk. All calves received no milk starting on d 46 and were monitored until d59. All calves had ad libitum water access starting on d 1, and a starter ration from d 5. Solid feed and milk intakes were recorded daily, and BW was measured on d 2, 30, 38, 46, 52 and 59. Data were summarized by phase and analyzed using repeated measures generalized linear mixed models. There was no interaction between milk level and weaning treatments for any outcome. On average, LOW calves consumed more feed than HIGH calves during the milk-feeding (0.5 vs. 0.3 kg/d; SE = 0.04; $P < 0.01$), and weaning (1.6 vs. 1.2 kg/d; SE = 0.08; $P = 0.02$) phase. HIGH and LOW calves consumed similar amounts of feed in the post-weaning phase (HIGH = 2.2kg/d, LOW = 2.4kg/d; SE = 0.14; $P = 0.13$). Growth rates did not vary between milk treatments during the milk-feeding (HIGH = 0.6kg/d, LOW = 0.5kg/d; SE = 0.03; $P = 0.4$) and post-weaning (HIGH = 0.8kg/d, LOW = 0.8kg/d, SE = 0.10; $P = 0.6$) phases. During weaning, on average, LOW calves tended to have higher growth than HIGH calves (0.9kg/d vs 0.8kg/d; SE = 0.05; $P = 0.09$). Feed intakes were similar for weaning treatments during the weaning (2S = 1.4kg/d, 4S = 1.4kg/d; SE = 0.09; $P = 0.9$) and post-weaning (2S = 2.3kg/d, 4S = 2.3kg/d; SE = 0.14; $P = 0.92$) phases. Growth did not vary by weaning treatment for the weaning (2S = 0.8kg/d, 4S = 0.8kg/d; SE = 0.05; $P = 0.7$) or post-weaning (2S = 0.7kg/d, 4S = 0.9kg/d; SE = 0.10; $P = 0.2$) phases. Overall, the results indicate that the milk feeding level may impact solid feed consumption and growth during the weaning phase, while the weaning treatments did not affect feed consumption or growth.

Key Words: dairy calf, weaning, growth

M142 Whole corn grain or hay as fiber source on ruminal fermentation and performance of milk-fed dairy calves.

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Diets that promote the production of SCFA stimulate the ruminal development. Feeding hay may decrease energy intake of calves while whole corn grain (WCG) may be an alternative fiber source, with no TDN intake dilution, improving C4 and C3 concentration and performance. Thirty-nine Holstein calves were used in a randomized block design, considering sex, birth date and weight at 21d of age (48.7 ± 0.82 kg), when the supplementation started, to compare: 1) Control: starter concentrate (24% CP, 14% NDF, 46% NFC); 2) Hay: starter concentrate, supplemented with free choice chopped Tifton hay; and 3) Corn: starter concentrate supplemented with free choice WCG. Animals were fed 6 L/d until 21d and then 4L/d of whole milk until gradual weaning at 56d of age. The rumen fluid was collected 2 h after feeding at 6th and 8th week. Data were analyzed using the MIXED procedure of SAS and the means were compared using Tukey test ($P < 0.05$). Hay supplementation increased total DMI (Control: 365.5^{b}; Hay: 619.4^{a}; Corn: 428.3^{b} g/d), without affecting the NFC intake. There was a diet and age interaction, in which hay supplementation increased NDF intake after the 5th week. When compared with WCG, free access to hay promoted an increase in concentrate DMI (Control: 366.3^{ab}; Hay: 577.7^{a}; Corn: 320.6^{b} g/d), NDF (Control: 51.2^{ab}; Hay: 79.1^{a}; Corn: 44.3^{b} g/d) and NFC intake (Control: 170.8^{ab}; Hay: 265.4^{a}; Corn: 148.1^{b} g/d). Therefore, calves supplemented with hay presented higher ADG (Control: 483.3^{b}; Hay: 647.8^{a}; Corn: 486.2^{b} g) and final BW (Control: 61.1^{b}; Hay, 68.9^{a}; Corn: 62.9^{b} kg). Hay supplementation increased C2 (Control: 49.0^{ab}; Hay: 52.0^{a}; Corn: 46.4^{b} mM/100mM), compared with the WCG. When compared with hay, free access to whole corn grain promoted an increase in C3 (Control: 36.7^{ab}; Hay: 33.0^{b}; Corn: 37.1^{a} mM/100mM), and decreased C2:C3 ratio (Control: 1.36^{ab}; Hay: 1.60^{b}; Corn: 1.28^{a}). However, C4, total SCFA and ruminal pH were unaffected by solid diets, suggesting that WCG did not cause acidosis. In summary, the inclusion of WCG improves the energy efficiency in milk-fed calves without compromising rumen health. However, the hay inclusion can stimulate an early solid diet intake, improving performance.

Key Words: calf nutrition, performance, weight gain

M143 Behavior, ruminal and metabolic indicators of transition in calves fed different levels of NDF in the solid diet.

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The objective of this trial was to evaluate the impact of different NDF content of the solid diet on indicators of rumen development and calf behavior. Thirty-five Holstein calves were housed in suspended individual cages without bedding, blocked according to sex, birth date and weight and distributed in one of the treatments: 1) starter concentrate with 22% NDF (22NDF); 2) starter concentrate with 31% NDF, replacing part of the corn with soybean hull (31NDF); and 3) starter concentrate with 22% NDF and free access to coast-cross hay (22NDFhay). Calves received 4 L/d of milk replacer until gradual weaning at 56d. Metabolic indicators were evaluated weekly, while ruminal characteristics were evaluated at wk 4, 6, 8. Animal behavior was weekly evaluated by focal instantaneous time sampling, every 5 min, during 10 h. Data were analyzed as repeated measures over time using the MIXED procedure of SAS. Hay supplementation decreased total SCFA (22NDF = 133.3, 31NDF = 133.4 vs. 22NDFhay = 104.0 ± 10.62 mM), but molar proportion of individual SCFA were not affected ($P > 0.05$). The higher rumen N-NH3 was found in 22NDF calves (22NDF = 17.6 vs. 31NDF = 9.9, 22NDFhay = 10.7 ± 1.64 mg/dL). The 22NDFhay calves presented the longer time of solid food intake (22NDF = 45.8, 31NDF = 41.1, vs. 22NDFhay = 68.8 ± 8.25 min/10h) and rumination (22NDF = 41.8, 31NDF = 17.8, vs. 22NDFhay = 48.9 ± 8.95 min/10h). Raising the NDF content in the concentrate was not efficient in stimulating rumination. 31NDF and 22NDFhay diets may have promoted greater rumen N-NH3 utilization, resulting in decreased concentration. 22NDF diet did not cause lower ruminal pH in comparison to diets with high NDF content ($P > 0.05$). However, blood metabolites (glucose, lactate, BHB, total protein and urea) show no effect of different solid diet NDF content on rumen development ($P > 0.05$). Yet, age affected all blood parameters suggesting the metabolic transition ($P < 0.01$). The NDF increase in the concentrate can stimulate ruminal development earlier than the supply of hay. Although the supply of hay stimulates rumination.

Key Words: ruminal development, short-chain fatty acid, hay

M144 Effects of increasing dietary hay inclusion on performance and digestion of Holstein calves from 2 to 4 months of age.

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A good transition from a liquid diet to a solid diet of concentrates and forages is important, considering the small size and lack of development of the calf's rumen. However, the optimal amount of hay or other high-fiber ingredients in the diet of young calves is not well defined. Objective of this trial was to determine effects of feeding 0, 5, or 10% chopped hay (6.5% CP, 64.6% NDF) blended into a dry TMR with a textured, high starch starter (20.5% CP, 38.4% starch) on performance and digestion in Holstein steer calves from 2 to 4 mo of age. Forty-eight calves (initial BW = 90.7 ± 2.15 kg) were housed in group pens (4 per pen) and fed diets and water for ad libitum intake. Feed offered and refused was measured daily. Calf BW, hip width (HW) and body condition score (BCS; 1–5 scale) were measured initially and at 28 and 56 d. Fecal samples were collected from the pen floor with care not to sample bedding material and composited by pen during d 5–9, 26–30, and 47–51 to estimate digestibility using acid insoluble ash as digestibility marker. Data were analyzed as a completely randomized design with repeated measures and pen as the experimental unit. Initial measurements were similar, except BCS which decreased with increasing hay ($P < 0.05$). As hay increased, dry matter intake decreased linearly ($P < 0.05$). Dry matter intake as a % of BW changed quadratically with hay ($P < 0.05$), increasing from 0 to 5%, and decreasing from 5 to 10% hay. Average daily gain (1.15, 1.12, 0.95 kg/d; $P < 0.05$), feed efficiency (0.336, 0.319, 0.309 kg ADG/kg DMI; $P < 0.05$) and HW change (4.6, 4.7, 4.1; $P < 0.05$) decreased linearly with increasing hay intake. Overall estimates of DM, OM, NDF, ADF and CP digestibility changed quadratically with hay ($P < 0.05$), increasing from 0 to 5% and decreasing from 5 to 10% hay, whereas digestibility of starch, sugar, and fat decreased linearly with increasing hay ($P < 0.05$). Feeding 5% chopped hay supported optimal digestion and growth in calves 2 to 4 mo of age, which agrees with previous published research.

Key Words: calf, chopped hay, digestibility

M145 Maternal supplementation of rumen-protected lysine and methionine during the close-up period improves the nutritional status of Holstein calves.

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This study aimed to evaluate the effect of supplementation of rumen-protected lysine (RPL) and methionine (RPM) during the close-up period on nutritional status of Holstein calves. Calves used in the present study were from multiparous cows which received either a control diet (CON, n = 7, metabolizable protein (MP): 1,292 g/d, metabolizable Lysine (mLys): 87.3 g/d, metabolizable Methionine (mMet): 28.8 g/d) or a treatment diet (TRT, n = 8, MP: 1,314 g/d, mLys: 102.5 g/d, mMet: 33.8 g/d) supplemented with RPL (AjiPro-L, Ajinomoto Animal Nutrition North America, Inc.) and RPM (Met Plus, Nisso Shoji Co.,Ltd.) from 21 d before the expected calving date. After birth, all calves received the same amount of colostrum and the same diet. Blood samples were collected from the jugular vein of the calves at 2 or 3, 7, 14, 28 and 63 d of age and blood biomarkers and plasma free amino acids were measured. Data were analyzed with Welch *t*-test in each sampling points. In TRT calves, total protein at 7 and 14 d of age, albumin at 2 or 3, 7 and 14 d of age, total cholesterol at 7 and 28 d of age and glucose at 2 or 3 d of age were significantly higher ($P < 0.05$) than CON calves. β-globulin, which is synthesized mainly in the liver and indicates the status of protein nutrition, tended to be higher ($P < 0.10$) at 2 or 3, 7 d of age and significantly higher ($P < 0.05$) at 14 and 28 d of age in TRT calves than CON calves. Total amino acids at 2 or 3 d of age was significantly higher ($P < 0.05$) in TRT calves than CON calves. These results suggest that the nutritional status was improved in TRT calves. Furthermore, γ-globulin at 7 d of age and γ-glutamyltransferase (GGT) at 7 and 14 d of age, respectively, was significantly higher ($P < 0.05$) in TRT calves than CON calves. That suggests TRT calves were in a better status of passive immunity. In conclusion, maternal supplementation of RPL and RPM during the close-up period may improve the nutritional status and passive immunity in Holstein calves.

Key Words: rumen-protected lysine and methionine, close-up period, calf

M146 Fluid- and solid-associated rumen microbial ecology changes with heifer age.

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Our objectives were to determine the effects of heifer age and ruminal phase (ruminal fluid: RF; ruminal solid: RS) on rumen microbial ecology and identify when heifers become faunated with ciliate protozoa. Holstein heifers (n = 12) were enrolled into an observational study at birth, managed under commercial heifer rearing practices, and followed to 21 weeks (wk) of age. A randomized complete block design with repeated measures evaluated the effects of age, phase and age × phase. Whole rumen contents were collected via esophageal intubation at 5, 9, 13, 17, and 21 wk of age. Heifers were housed in individual hutches from 0 to 9 wk (pasteurized whole milk + texturized starter), weaned at 7 wk (starter only), moved to super hutches after sampling at 9 wk (n = 3/hutch; starter+TMR), and moved to free stalls at 13 wk of age (TMR). The pen at 21 wk included 8 additional heifers without direct contact with faunated, lactating cows. Ruminal ciliate protozoa were detected in all heifers at 21 wk, but not before. Only members of the Entodiniomorphida order were observed. Ruminal ammonia decreased ($P < 0.01$) from 5 (2.37 ± 0.28 mM) to 9 wk (1.12 mM), and did not differ at 17 (0.91 mM) and 21 wk (1.36 mM). Bacterial community structures only differed by phase at 9 wk and were dissimilar by age and age × type (ANOSIM, $P < 0.01$). The number of operational taxonomic units (OTU) and Shannon diversity (OTU evenness and abundance) differed by age ($P < 0.001$) and the interaction of age and phase ($P < 0.01$). RF Shannon diversity (2.53 *vs*. 5.22) differed at 5 and 21 wk. RF OTU increased linearly and cubically ($P < 0.01$) by age: 5 wk (218), 9 wk (191), 13 wk (582), 17 wk (1029), 21 wk (1562). Relative abundance of Fibrobacteres was greater in RF than RS ($P = 0.02$) and greatest at 17 wk (RF: 2.51%, RS:1.47% ± 0.33%). Relative abundance of Bacteroidetes linearly increased with age ($P < 0.01$), but did not differ by phase or age x phase. Relative abundance of Firmicutes (RF: 19.3 ± 3.2%, RS:40.4%, $P < 0.001$) and Proteobacteria (RF: 34.6 ± 2.8%, RS:15.8%, $P < 0.001$)

only differed by phase at 9 wk. Our findings suggest that heifers become faunated at 21 wk and rumen microbial ecology differs by heifer age and rumen phase.

Key Words: protozoa, microbiota, bacteria

M147 The effects of supplementation of sodium butyrate on growth performance of newborn calves.

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This experiment was conducted to evaluate the effects of sodium butyrate (SB) supplementation on the growth performance of newborn calves. Forty healthy Holstein calves (4-d-old; 40 ± 5 kg of body weight [BW]) were randomly allocated to 1 of 4 treatment groups (n = 10). The control group was fed 0 g/d SB (SB0), while the other groups were supplemented with 15 g/d (SB15), 30 g/d (SB30), or 45 g/d (SB45) of SB, respectively. All calves were housed in individual hutches. Before the feeding experiment, all calves were fed 4 L colostrum within 1 h after birth and were given 2 more feedings of colostrum at 6 h (2 L) and 18 h (1 L) after birth. All calves were offered milk twice daily from d 2 to d 21, and then were supplied with milk powder (it was reconstituted into milk before each feeding) from d 22 to d 60 at weaning. The intended amount of SB was mixed with a small volume of the milk and fed first at each meal. Calves accessed water and the starter freely. Bodyweight, body length, body height, chest circumference, and pH of rumen fluid of the calves were measured at d 1, 14, 28, 42, and 60, while the dietary intake and fecal scores were recorded daily. The experiment was completed at d 60. Data were analyzed using the Proc Mixed model of SAS, with linear and quadratic polynomial contrasts tested using the CONTRAST statement. The appropriate coefficients for the CONTRAST statement of this study were obtained using PROC IML's ORPOL function. DMI, chest circumference, body length, and fecal score were not affected by SB ($P > 0.05$). Average daily gain of groups SB15, SB30 and SB45 were 11.4% ($P < 0.01$), 10.1% ($P = 0.01$), and 11.4% ($P = 0.02$) higher than that of SB0, with no significant differences among the SB groups. Feed to gain ratio was significantly decreased by SB ($P < 0.01$). pH value of rumen fluid was decreased linearly ($P < 0.05$) with the increased SB supplementation at d 14. These results indicated that supplementation with sodium butyrate can improve the growth performance of newborn calves.

Key Words: sodium butyrate, dairy calf, feed-to-gain ratio

M148 Effect of supplementation of *Schizochytrium* sp. on the growth performance of dairy calves.

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Previous studies showed that *Schizochytrium* sp. (SS), a marine microalga, has positive effects on the growth and immunity of aquatic animals and poultry. This study aimed to determine the effect of SS supplementation on the growth performance and health conditions of dairy calves. Fifty newborn female Holstein calves were randomly assigned to 1 of 5 groups (n = 10). All calves were offered 4 L colostrum within 1 h after birth, and then 2 and 1 L colostrum 6 h and 18 h, respectively, after birth. The 5 groups of calves were fed milk supplemented with (per calf per d) 0 g (control, SS0), 5 g (SS5), 10 g (SS10), 20 g (SS20), or 40 g (SS40) of *Schizochytrium* sp. powder from d 4 to d 60. The supplementations ended at d 60 when the calves were weaned. The daily supplementations of SS were equally divided and fed twice at 0800 and 1500. During the experiment, DMI and fecal score were recorded daily, while bodyweight (BW), chest circumference, and body length were measured biweekly at d 1, 14, 28, 42, and 60. Data were analyzed using the Proc Mixed of SAS, with linear and quadratic polynomial contrasts tested using the CONTRAST statement. The appropriate coefficients for the CONTRAST statement of this study were obtained using PROC IML's ORPOL function. The results showed that SS had no effect on the BW, chest circumference, body length, or DMI. However, the average daily gain (ADG) increased linearly with the increased SS supplementation ($P < 0.05$), with the ADG of SS20 being 16.76% and 16.33% higher than that of SS0 and SS5, respectively ($P < 0.05$). Feed-to-gain (F:G) ratio tended to linearly decreased ($P < 0.05$) with the increased SS supplementation, and the F:G were 12.18%, 15.91% and 9.8% lower in SS10, SS20, and SS40, respectively, than in SS0 ($P < 0.05$). From d 29 to d 42, fecal scores were quadratically changed with the increased SS supplementation, with SS20 displaying a parabolic nadir. The fecal score of SS20 and SS40 was 8.2% and 13%, respectively, lower than that of SS0. These results indicate that SS, when added at an appropriate level (e.g., 20 g/d), may improve the growth performance and feed efficiency of dairy calves before weaning.

Key Words: *Schizochytrium* sp., dairy calves, feed-to-gain ratio

M149 Offering drinking water from birth increased species richness in the gut of neonate dairy heifer calves.

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The species richness is a major marker of gut health directly affecting feed efficiency (FE). We previously demonstrated that dairy calves receiving drinking water from birth (W0) had greater BW and FE than those first receiving drinking water at 17 d of age (W17). The objective of this study was to examine the impact of offering drinking water from birth on species richness and the abundance of bacterial communities in fecal microbiome of dairy calves. Thirty newborn Holstein heifer calves were randomly assigned (n = 15) to W0 and W17, fed with pasteurized milk, and weaned at 6 wk of age. Fresh feces were collected directly from the rectum at 2, 6, and 10 wk of age. The DNA was sequenced using 16S rRNA gene-amplicon sequencing on an Illumina MiSeq system. The sequences were clustered into operational taxonomic units (OTU) with a 99% similarity threshold. Treatment effects on species richness were analyzed with MIXED procedure in SAS including treatment and time (fixed effects), and calf (random effect). Treatment effects on relative abundance of the 25 most abundant OTUs were analyzed using GENMOD procedure in SAS (distribution = Poisson). At 2 wk of age, W0 had a greater number of observed species ($P = 0.033$) and species richness (the Chao1 index, $P = 0.042$) than W17. The number of species and the Chao1 index increased over time ($P < 0.01$) and became similar between W0 and W17 at 10 wk of age. At 2 wk of age, relative abundance of *Bacteroides* in W0 was 2.5-fold higher than W17, whereas *Lachnospiraceae* and *Streptococcus* in W17 were 2.0 and 8.0-fold higher than W0 ($P < 0.04$), respectively. At 6 wk of age, *Streptococcus*, *Fecalibacterium* and *Bifidobacterium* in W0 were 3.0, 7.0 and 5.0-fold greater than W17, respectively ($P < 0.04$), whereas *Bacteroides* in W17 was 2.0-fold greater than W0. At 10 wk of age,

Table 1 (Abstr. M151). Performance of calves supplemented or not with lysolecithin

	Treatment			P-value[1]		
Item	Control	Lysolecithin	SEM	T	A	T × A
Concentrate DMI, g/d	264.95	285.95	28.42	0.59	<0.01	0.32
ADG, kg	0.280	0.406	0.03	0.01	<0.01	0.58
FE	0.22	0.31	0.02	0.01	0.01	0.52
Initial BW, kg	35.95	35.67	1.48	0.68	—	—
Final BW, kg (d 56)	52.68	57.07	2.37	0.07	—	—
Heart girth, cm	80.72	81.50	0.67	0.42	<0.01	0.66
Hip width, cm	20.81	20.91	0.26	0.77	<0.01	0.29
Wither height, cm	80.71	81.38	0.54	0.39	<0.01	0.17
Fecal score	1.53	0.78	0.07	<0.01	<0.01	<0.01

[1] T = treatment effect; A = age effect; T×A = treatment vs age effect.

Succinivibrio and *Alloprevotella* in W0 were 4.5 and 5.5-fold greater than in W17, respectively ($P < 0.01$). Offering drinking water from birth was related to increased species richness in the gut of neonate calves. Higher abundance of some beneficial OTU (*Bifidobacterium, Fecalibacterium, Succinivibrio*) could partly contribute to the improved performance of W0 calves.

Key Words: fecal microbiome, gut, weaning

M150 Application of partial least squares regression to predict feed intake using feeding behavior traits in growing Holstein heifers.

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The objective of this study was to evaluate the use of partial least squares (PLS) regression models to predict feed intake of growing Holstein heifers (n = 609; Initial BW = 246 ± 43 kg), using feeding behavior traits. DMI and feeding behavior traits were measured using a GrowSafe System for 70 to 100 d (15 trials) while heifers were fed a corn-silage based ration. Nineteen feeding behavior traits were evaluated: frequency and duration of bunk visit (BV) and meal events, head-down duration (HDD), average meal length, maximum non-feeding interval, time-to bunk (TTB), corresponding day-to-day variation (SD) of these traits, and ratios of HDD per BV duration, HDD per meal duration, and BV events per meal event. Test-set validation techniques were used to calibrate and validate PLS prediction equations for DMI using performance (mid-test $BW^{0.75}$and ADG) and feeding behavior traits as independent variables. Validation groups were built by randomly selecting 3 trials to be used for validation, with results presented as the average of 5 iterations, with each trial being used for validation only once. For calibration, independent variables were excluded if variable of importance in projection (VIP) scores were less than 0.80. Overall, 12 of 19 feeding behavior traits were included (VIP >0.80) in the final model. The base model (Mid-test $BW^{0.75}$and ADG) accounted for 54% of the variation in individual-animal DMI. Inclusion of feeding behavior traits to the base model increased the R^2 of validation from 0.54 to 0.74 and reduced the model SE from 0.78 to 0.72. The R^2 and SE of model calibration and validation were similar (0.75 and 0.73 vs. 0.74 and 0.72, respectively), indicating that the PLS model was robust in predicting individual-animal DMI across trials. No differences ($P = 0.93$) were found between observed (8.59 ± 1.67 kg/d) and model predicted (8.59 ± 1.50 kg/d) DMI. Overall, performance and feeding behavior traits accounted for 74% of the variation in individual-animal DMI using PLS regression models. These results indicate that future prediction models for individual-animal DMI may benefit from the inclusion of feeding behavior traits.

Key Words: dairy cattle, partial least squares (PLS), feeding behavior

M151 Supplementation of lysolecithin in the milk replacer for dairy calves: Effects on performance.

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Lysolecithin is an emulsifier that increases the digestion and absorption of fat and may result in increased energy availability and improved animal performance. The objective of this study was to evaluate the effects of the inclusion of lysolecithin in the milk replacer (24% CP, 19% Fat) and its influence on calves performance. Thirty-two newborn and colostrum-fed calves were blocked according to sex, date and weight of birth and distributed in 2 treatments: 1) Control: milk replacer; 2) Lysolecithin: milk replacer supplemented with lysolecithin (Lysoforte, 4 g/d). All calves were bucket fed 6 L/d of milk replacer diluted to 14% solids and had free access to water and concentrated. Body weight was measured every week, growth parameters were measured biweekly and feed intake and fecal score were registered daily. Data were analyazed as repeated measures using PROC Mixed of SAS. Even though the inclusion of lysolecithin had no effect on concentrate intake, supplemented calves presented higher ADG and feed efficiency (FE), resulting in a tendency for higher final body weight. However, body measurements were not affected by lysolecithin supplementation. Lysolecithin supplementation in the milk replacer decreased average fecal score, suggesting lower incidence of diarrhea. All parameters were affected by age, with increasing values for concentrate DMI, ADG and FE and fecal scores. Fecal scores were affected by the interaction of supplementation and age, with increasing differences between groups as calves aged. These results indicate that the emulsifier increases weight gain and efficiency of nutrients utilization without affecting feed intake.

Key Words: average daily gain, liquid diet, emulsifier

M152 Effects of dietary 25-hydroxyvitamin D_3 on vitamin D status and growth of dairy heifer calves.

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Vitamin D plays a role in maintaining calcium and phosphorous homeostasis and is required for normal growth and development of calves. We hypothesized that supplementing dairy calves with 25-hydroxyvitamin D_3 [25(OH)D] would improve development and immunity of dairy calves. The objective of this experiment was to test the effects of dietary 25-hydroxyvitamin D_3 on growth and immune status of dairy heifer calves. One-hundred 43 Holstein heifer calves were assigned to one of 2 treatments, control (CON) and 25-hydroxyvitamin D_3-supplemented (25D), starting at approximately 14 d of age. The control diet was manufactured to provide vitamin D_3 at a rate of 1.5 µg vitamin D_3/kg BW and 25D heifers were supplemented with an additional 1.7 µg 25D/kg BW. The 25D was administered orally. Calves were kept in group pens and fed ad libitum milk replacer and starter grain with an automated feeding system. Treatments were applied daily to individual calves. Bodyweight, whither height, and blood samples were collected weekly from start of treatment until weaning, then every 2 weeks until 16 weeks of age. Data were analyzed with mixed models that included fixed effects of treatment and time and random effects of calf nested within treatment and pen. Serum 25(OH)D concentrations of 25D heifers were greater compared with CON (123 vs. 44 ± 6 ng/mL, $P < 0.001$). The 25D heifers grew faster compared with CON (ADG = 0.82 vs. 0.78 ± 0.13 kg/d, $P = 0.03$) such that 25D heifers were 4 kg heavier ($P = 0.01$) and tended ($P = 0.07$) to be 1 cm taller at 16 weeks. Milk intake of the 25D heifers was greater than the CON heifers (9 L/d vs 8 L/d, $P = 0.03$). Intake of starter grain did not differ between treatments. Concentrations of leukocytes in blood, neutrophil phagocytosis and oxidative burst capacity, and concentrations of IgG1 and IgG2 in serum did not differ between treatments. Feeding dairy heifer calves 25-hydroxyvitamin D_3 at 1.7 µg/kg BW plus 1.5 µg vitamin D_3/kg BW increased weight gain compared with just feeding 1.5 µg vitamin D_3/kg BW.

Key Words: vitamin D, calf, growth

M153 Effect of different times of super-conditioning in pelleted starter on nutrient digestibility and performance of pre-weaned calves.

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The objective of this study was to investigate the effects of conditioning time during pelleting process on performance, apparent digestibility, rumen fermentation, and blood metabolites of pre-weaned calves fed with pelleted starter diet. Thirty-six 3-d-old Holstein dairy calves (41 ± 0.5 kg of BW) were randomly assigned to one of 3 experimental diets: T1) starter without super- conditioning (Control); T2) starter with super-conditioning at 85°C for 2 min; and T3) starter with super-conditioning at 85°C for 4 min. All calves were weaned on age 56 d and remained in the study until d 70 of experiment. Data were analyzed by GLM procedure of SAS ($P < 0.05$) using a completely randomized design with 12 replications for every treatment. Starter intake, feed efficiency and fecal score were not affected ($P > 0.05$) by super-conditioning time during the pre- or post-weaning periods, but average daily gain (ADG) significantly increased ($P < 0.05$) in calves fed with T3 during the post-weaning period (0.92, 0.98 and 1.16 kg/d in T1, T2 and T3, respectively). The pH, ammonia nitrogen, total VFA and the molar proportions of acetate, propionate and butyrate were not different ($P > 0.05$) among treatments, but ammonia nitrogen (6.76, 5.81 and 5.44 mg/dL in T1, T2 and T3, respectively) and molar proportions of propionate (33.81, 36.12 and 39.72 mol/100 mol total VFA in T1, T2 and T3, respectively) were shown high tendency ($P = 0.085$) in calves fed with T3 during the post-weaning period. Body measurements were not affected ($P > 0.05$) by the treatments. The concentration of blood glucose and insulin was higher ($P < 0.05$) in calves fed with T3. Apparent digestibility of dry matter, starch, crude protein, NDF and ADF were not different ($P > 0.05$) among treatments during the pre-weaning period, but starch and dry matter digestibility during the post-weaning period were significantly increased ($P < 0.05$) in starter T3 (76.44, 78.83 and 79.59% for dry mater and 94.71, 96.63 and 97.4% for starch digestibility in T1, T2 and T3, respectively). Results of the present study indicated that enhancing of super-conditioning time from 2 to 4 min during pelleting process improved ADG and apparent nutrient digestibility in post-weaning period of dairy calves.

Key Words: performance of calf, nutrient digestibility, super-conditioning time

M154 Effects of feeding 25-hydroxyvitamin D_3 versus vitamin D_3 to dairy calves: Effects on growth and serum concentrations of vitamin D metabolites and minerals.

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The objectives of this study were to test the effects of dietary vitamin D_3 compared with 25-hydroxyvitamin D_3 on growth and serum concentrations of vitamin D metabolites, minerals, and energy metabolites of dairy calves. Forty-five Holstein bull calves (<7 d of age) were blocked into weekly cohorts and randomly assigned to one of 5 daily supplements; control (CON, 0.25 µg of vitamin D_3/kg BW), and 2 levels (1.5 or 3 µg/kg BW) of vitamin D_3 (VitD), or 25-hydroxyvitamin D_3 (25D) added to the CON treatment resulting in the following treatment groups: CON, VitD1.5, VitD3.0, 25D1.5, 25D3.0. Calves were fed milk replacer until weaning at 56 d of age and had ad libitum access to water and starter grain throughout the experiment. Treatments were added to the milk replacer until weaning and then to starter grain thereafter. Bodyweight and blood samples were collected weekly. Data were analyzed with mixed models that included fixed effects treatment and time and random effects of calf nested within block and treatment. The CON and 25D-treated calves gained more ($P < 0.05$) weight from birth to weaning compared with VitD-treated calves (CON = 0.57, 25D = 0.50 VitD = 0.41 ± 0.04 kg/d), but height and feed intake did not differ between treatments. Supplementing VitD and 25D increased ($P < 0.001$) concentrations of 25(OH)D in serum compared with CON but the increment was greater ($P < 0.01$) for 25D compared with VitD (CON = 13.3, VitD1.5 = 22.9, VitD3.0 = 30.2, 25D1.5 = 40.8, 25D3.0 = 74.2 ± 4.0 ng/mL). Concentrations of Ca, P, Mg, NEFA, glucose, IgG1 and IgG2 did not differ between treatments, however, BHBA was greater ($P < 0.01$) in VitD and 25D supplemented calves compared with CON (CON = 0.19 VitD = 0.23 and 25D = 0.25 ± 0.02 mM). Although serum P did not differ among treatments, it tended ($P = 0.08$) to increase with serum 25(OH)D of calves. Feeding 25D to dairy calves was more effective at increasing serum 25(OH)D concentrations and BW gain compared with feeding VitD.

Key Words: 25-hydroxyvitamin D_3, calf

M155 Evaluation of milk replacer feeding rates on Holstein calves via a meta-analytical approach: 1. Effect on digestion and growth from 0 to 2 months of age. W. Hu*, T. M. Hill, T. S. Dennis, F. X. Suarez-Mena, and J. D. Quigley, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

The objective of this study was to evaluate the effects of the milk replacer at 2 feeding rates on growth performance of dairy calves via a meta-analytic approach. A database was developed from 10 studies conducted at the Nurture Research Center, Provimi (Brookville, OH) from 2014 to 2016. Male Holstein calves (n = 491; 42.8 ± 4.9 kg BW initially; < 1 wk of age) were randomly assigned to 1 of 2 treatments: total milk replacer intakes of 29.7 ± 0.6 kg DM (MOD) and 48.8 ± 4.5 kg DM (HIGH). Meals were divided into 2 equal a.m. and p.m. feedings; during weaning half the allotment per d was offered for 3–7 d in the a.m. feeding only. Calves were weaned at 6–7 wk. Milk replacer ranged from 24.8 to 28.6% CP and 17.6–20.2% fat; starter ranged from 17.3 to 22.2% CP and 3.0–4.3% fat on a DM basis. Statistical analysis was conducted using mixed-effects models. From 0 to 35 d, calves fed HIGH had greater ADG (0.642 vs. 0.447 kg/d; $P < 0.001$) and gain/DMI (0.537 vs. 0.490 kg/kg; $P < 0.001$) but consumed less starter (0.104 vs. 0.208 kg/d; $P < 0.001$) compared with calves fed MOD. From 0 to 56 d, feeding HIGH resulted in greater ADG (0.634 vs. 0.545 kg/d; $P < 0.001$), gain/DMI (0.491 vs. 0.469 kg/kg; $P = 0.001$), and hip width change (0.069 vs. 0.064 cm/d; $P = 0.001$), but lower starter intake (0.467 vs. 0.690 kg/d; $P < 0.001$) compared with calves fed MOD. Apparent digestibility of DM (73.3 vs. 78.4%; $P < 0.001$), OM (74.1 vs. 79.0%; $P < 0.001$), CP (74.8 vs. 78.9%; $P = 0.001$), and NDF (40.8 vs. 53.9%; $P < 0.001$) were lower for calves fed HIGH vs. MOD when estimated during a 5-d period in wk 8. Feeding calves more than 0.681 kg/d (as-fed basis) of milk replacer improved BW gain and structural growth in the first 56 d of life; however, digestibility of DM, OM, CP, and NDF was depressed after weaning. Growth advantage from feeding more milk replacer was partially lost during weaning transition, which could be due to the reductions in nutrient digestibility.

Key Words: calf, performance, digestibility

M156 Body weight and skeletal growth in pre-weaned dairy calves fed organic-certified milk replacer. C. A. LeCuyer[1], O. M. Gorman[1], M. Ghelichkhan*[1], R. C. R. Tinini[2], J. G. Dessbesell[2], M. A. Zambom[2], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Universidade Estadual do Oeste do Paraná, Marechal Cándido Rondon, PR, Brazil.*

Calf management is a critical aspect of dairy farming and proper nutrition in the preweaning phase is essential to produce healthy and productive cows. In the first few weeks of life calves depend entirely on milk or milk replacer (MR) to meet their nutritional requirements. Milk replacer provides several benefits to dairy producers including improved biosecurity, calf performance, and profitability. However, organic producers typically feed whole milk (WM) to their calves partially because no organic-certified MR was available in the market until recently. Our goal was to compare WM vs. MR (Organi-Calf; MilkSpecialties Global, MN) on BW and skeletal growth of pre-weaned dairy calves. Thirty organic-certified Jersey calves (18 female, 12 male) were assigned to 1 or 2 treatments: 4 L of WM or MR from birth until weaning (8 wk of age) in a randomized complete block design. Water and starter grain were offered ad libitum throughout the study. Body weight and skeletal measurements were taken weekly. Data were analyzed using the MIXED procedure of SAS with repeated measures over time; birth BW or individual body measurements were used as covariate. The starter grain averaged 24.9% CP and 5% crude fat, while mean true protein and fat concentrations of WM were 3.7 and 4.64%, respectively; MR had 21.3% CP and 22.1% crude fat. Body weight of calves fed WM (mean = 40.5 kg) was 3.6 kg greater ($P < 0.001$) than that of calves fed MR (mean = 36.9 kg; SEM = 0.70 kg). Significant effects were also observed for wither height (73.9 vs. 72.2 cm; SEM = 0.35 cm), hip height (76.6 vs. 75.2 cm; SEM = 0.30 cm), heart girth (82.8 vs. 80.8 cm; SEM = 0.44), and body length (75.6 vs. 72.9 cm; SEM = 0.69 cm) with feeding WM vs. MR, respectively. In contrast, hip width (mean = 18.4 cm; SEM = 0.49 cm) did not differ in calves fed WM or MR. It can be concluded that MR was not comparable to WM regarding BW and skeletal growth over time. Profitability analyses are needed to determine the feasibility of adopting MR in organic

Key Words: calf, organic dairy, whole milk

M157 Evaluation of milk replacer feeding rates on Holstein calves via a meta-analytical approach: 2. Carry-over effect on digestion and growth from 2 to 4 months of age. W. Hu*, T. M. Hill, T. S. Dennis, F. X. Suarez-Mena, and J. D. Quigley, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

Objective of this study was to evaluate carry-over effects of feeding milk replacer at 2 rates on growth performance of dairy calves from 2 to 4 mo of age via a meta-analytical approach. A database was developed from 10 studies conducted at the Nurture Research Center, Provimi (Brookville, OH) from 2014 to 2016. Male Holstein calves (n = 485; 76.1 ± 7.0 kg BW at 2 mo of age) were previously assigned to 1 of 2 treatments: total milk replacer intakes of 29.7 ± 0.6 kg DM (MOD) and 48.8 ± 4.5 kg DM (HIGH). Milk replacer contained 24.8–28.6% CP and 17.6–20.2% fat on a DM basis. Calves were weaned at 6–7 wk, moved into pens by treatment on d 56, and then fed calf starter (19.4–22.3% CP and 3.4–4.6% fat, DM basis) blended with 5% hay until d 112. Statistical analysis was conducted using mixed-effects models. From 56 to 84 d, ADG (0.864 vs. 0.885 kg/d; $P = 0.480$) or DM intake (2.361 vs. 2.431 kg/d; $P = 0.204$) did not differ, but apparent digestibility of DM (78.6 vs. 75.9%; $P < 0.001$), OM (79.6 vs. 76.8%; $P < 0.001$), CP (80.9 vs. 78.5%; $P < 0.001$), and NDF (55.2 vs. 46.6%; $P < 0.001$) was greater for calves fed MOD vs. HIGH when estimated at a 5-d period between wk 11–13. From 56 to 112 d, ADG (1.015 vs. 0.976 kg/d; $P < 0.001$), gain/DMI (0.375 vs. 0.349 kg/kg; $P < 0.001$), and hip width change (0.089 vs. 0.081 cm/d; $P < 0.001$) was greater, but dry feed intake was less (2.797 vs. 2.923 kg/d; $P = 0.008$) for calves previously fed MOD vs. HIGH. Over the entire period of calf early life (d 0–112), ADG was 0.784 vs. 0805 kg/d ($P = 0.004$) and hip width change was 0.077 vs. 0.075 cm/d ($P = 0.081$) for calves previously fed MOD vs. HIGH. Feeding calves more than 0.681 kg/d (as-fed basis) of milk replacer in the nursery decreased BW gain and structural growth in the grower period (d 56–112). Reduced digestibility of DM, OM, CP, and NDF as result of more milk replacer in the nursery could cause calves to grow slower from 2 to 4 mo of age.

Key Words: calf, performance, digestibility

M158 Effects of dietary vitamin D$_3$ or 25-hydroxyvitamin D$_3$ on mineral metabolism in growing calves. R. Zimpel*[1], M. B. Poindexter[1], A. Vieira-Neto[1], A. Husnain[1], S. Buoniconti[1], P. Celi[2], C. Cortinhas[2], C. D. Nelson[2], and J. E. P. Santos[1], [1]*University of Florida, Gainesville, FL,* [2]*DSM Nutritional Products, Columbia, MD.*

Objectives were to determine the effects of dose and source of vitamin D (vitamin D$_3$ or 25-hydroxyvitamin D$_3$ [25OHD$_3$]) on mineral metabolism and bone mineral density (BMD) in calves. The hypothesis was that dietary 25OHD$_3$ would enhance Ca accretion and BMD. Forty-five Holstein male calves were enrolled within the first week of age in a randomized complete block design. Calves were assigned to 1 of 5 treatments: T1 (0.25 mg of vitamin D$_3$/kg BW), T2 (0.25 mg of vitamin D$_3$ + 1.5 mg of 25OHD$_3$/kg BW), T3 (0.25 mg of vitamin D$_3$ + 3.0 mg of 25OHD$_3$/kg BW), T4 (1.75 mg of vitamin D$_3$/kg BW) and T5 (3.25 mg of vitamin D$_3$/kg BW). Treatment were supplied from birth to 131 ± 9 d of age when calves were euthanized and tissues collected. Calves were fed milk replacer until 49 d of age and had ad libitum access to starter grain and water. Blood was sampled on d −15, −14, −13, −12, −11, −9, −7, −5, −3 and −1 relative to slaughter. Intake of DM was evaluated in the last 15 d before slaughter. Total fecal collection and spot urine was sampled on d −4 to −2 relative to slaughter. The BMD was determined in the right metacarpus. Data were analyzed by ANOVA with mixed models using the MIXED procedure of SAS. Contrasts evaluated included the effects of supplementing vitamin D (Sup: T1 vs. T2+T3+T4+T5), source of vitamin D (Source: T2+T3 vs. T4+T5), dose of vitamin D (Dose: T2+T4 vs. T3+T5), or the interaction between Source and Dose (Int: T2 + T5 vs. T3 + T4) (Table 1). Intake of DM or Ca did not differ among treatments. Supplementing vitamin D reduced blood Ca; however, supplemental 25OHD$_3$ increased blood Ca compared with vitamin D$_3$. Treatment did not affect digestibility or retention of Ca, but calves fed 25OHD$_3$ tended to have increased BMD.

Table 1 (Abstr. M158).

Item	T1	T2	T3	T4	T5	SE
Intake						
DM, kg/d	4.3	4.0	4.0	4.0	4.3	0.25
Ca, g/d	61.9	55.1	58.6	56.7	62.2	4.0
BW gain, kg/d	1.32	1.20	1.19	1.25	1.34	0.12
Blood						
tCa, m*M* *†	2.868	2.858	2.840	2.788	2.808	0.024
iCa, m*M* *	1.473	1.454	1.437	1.446	1.429	0.014
Calcitriol, pg/mL	51.4	53.8	55.2	53.4	57.0	3.2
BMD, g/cm^2 †‡	0.986	1.033	1.054	0.967	1.038	0.024
Ca digestibility, %	50.5	53.2	51.6	52.1	52.9	2.5
Ca retention, g/d	31.1	29.0	30.3	29.4	33.1	2.9

*Sup ($P < 0.09$); †Source ($P < 0.10$); ‡Dose ($P < 0.07$).

Key Words: bone density, calcium, vitamin D

M159 The effects of supplementation of *Yucca* on growth performance and health condition of newborn calves. W. H. Liu[1], L. Ma[1], Y. Ma[1], Z. T. Yu[2], and D. P. Bu*[1,3], [1]*Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [3]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China.*

Previous studies showed that *Yucca* supplementation could improve growth performance and immunological functions in poultry. This study aimed to evaluate the effects of supplementation of *Yucca* on the growth performance of newborn calves. A total of 40 healthy Holstein calves (4-d old; 40 ± 5 kg of body weight) were randomly allocated to 1 of 4 treatment groups (n = 10). The control group was fed 0 g/d *Yucca* (Y0), while the other groups were supplemented with 3 g/d (Y3), 6 g/d (Y6), or 9 g/d (Y9) of *Yucca*. All calves were housed in individual hunches. Before the feeding experiment, all calves had been each fed 4 L colostrum within 1 h after birth and given 2 more feedings of colostrum at 6 h (2 L) and 18 h (1 L) after birth. All calves were offered milk twice daily from d 2 to d 21, and then a milk powder (it was reconstituted into milk before each feeding) from d 22 to d 60 at weaning. The intended amount of *Yucca* was mixed into a small volume of the milk that was fed first at each meal. Calves had free access to water and a starter. Bodyweight (BW), body length, body height, chest circumference of the calves were measured fortnightly, while the dietary intake and fecal scores were recorded daily. The experiment ended at d 60. Data were analyzed using the mixed model procedure of SAS, with linear and quadratic polynomial contrasts tested using the CONTRAST statement of SAS with coefficients estimated. The *Yucca* did not affect DMI, chest circumference, body length, or average daily gain ($P > 0.05$). The feed-to-gain ratio of groups Y3, Y6 and Y9 was 15.2%, 18.4%, and 13.3% significantly lower ($P < 0.01$) than that of Y0, with no significant differences among the Y3, Y6, and Y9. The fecal score decreased linearly ($P < 0.05$) with the increased *Yucca* supplementation, and the fecal score of group Y9 was 7.1% ($P < 0.05$) lower than that of Y0. These results indicate that supplementation with *Yucca* can improve the growth performance, and the health status of newborn calves.

Key Words: *Yucca*, dairy calf, fecal score

M160 Effects of feeding a flaxseed supplement in the transition period on milk production, fatty acid concentration in milk and plasma, incidence of disease postpartum and reproductive function in dairy cows and heifers. F. A. Gambonini*[1], D. M. Cunningham[1], R. C. Fry[2], K. J. Harvatine[1], J. L. Pate[1], J. Moats[3], and T. L. Ott[1], [1]*Pennsylvania State University, State College, PA,* [2]*Atlantic Dairy Consulting, Kennedyville, MD,* [3]*O&T Farms Inc., Regina, SK, Canada.*

During the transition period, cows exhibit reduced immune function that can lead to postpartum disease and reduced fertility. Dietary fatty acids (FA) modulate immune function. This study determined the effects of feeding a supplement high in n-3 (omega-3) FA on milk production, FA concentration in milk and plasma, postpartum disease and fertility. Six sequential, 60-d periods (n = 3 control (C), n = 3 treated (FL)) were conducted over 12 mo on a commercial dairy. A subset of cows (2+ lactations) and heifers (1st lactation) receiving the C (n = 292) or FL (n = 271) diets for the entire transition period were used. Diets were fed starting ~3 weeks pre-calving and for ~3 weeks after calving. The C diet was isonitrogenous and isocaloric to the FL diet, which contained a commercial flaxseed supplement (LinPROR) formulated to 3% of dry matter intake. Individual cow production, health and fertility data were collected. Blood was taken from a subset of cows and heifers (n = 12/period) at entrance into the close-up pen, the week of calving and for 2 following weeks. Plasma and milk were analyzed for FA content. Data were analyzed using the MIXED procedure of SAS with repeated measures or proc GLIMMIX, with fixed effects of treatment and lactation and random effects of cow within treatment. Seasonal effects were accounted for by blocking. Both milk and plasma total n-3 concentration was greater ($P < 0.01$) in the FL group, while the n-6:n-3 ratio was lower ($P < 0.01$). Milk yield tended to be greater in the FL group at 5 ($P = 0.06$) and 10 weeks ($P = 0.1$) postpartum. Milk fat and protein percent were greater ($P < 0.01$) in the FL group during the first month of lactation. First service conception rates in cows (C 54% vs. FL 55%) and heifers (57% vs. 67%) did not differ nor did pregnancy loss. Incidence of postpartum disease did not differ between groups. In summary, the FL diet increased n-3 FA in milk and plasma and tended to increase milk yield while having no detrimental effect on conception rates, embryo loss or disease postpartum.

Key Words: n-3, flaxseed, fertility

M161 Development of a model to predict nutrient requirements in pre-ruminant dairy calves up 105 kg of BW.

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Data on growth and composition of dairy calves are available and could be used to better describe nutrient requirements. The objective of this work was to estimate maintenance energy requirements and develop equations to predict energy and protein requirements for growth. Data from 5 comparative harvest studies, 4 with Holstein and one with Jersey calves, representing 206 individuals and 31 different treatments, were compiled and split in 2 sets, for equation development (85%) and evaluation (15%). Average final full body weight (FBW) ranged from 31 to 105 kg. Treatments represented diets with ranges in nutrient supply in protein (14.3–31.2%), fat (14.8–33.4%) and feeding rates (148–353 kcal of metabolizable energy (ME)/d/ $kg^{0.75}$ FBW). Data included nutrient intake, weight gain and chemical composition of gain in an empty body weight (EBW) basis. Maintenance energy coefficients were estimated regressing ME intake and the logarithm of the estimated heat production (HP, kcal/d/$kg^{0.75}$ EBW). The antilog of the intercept at zero intake was considered the fasting HP, and the value at which the function and the unity line were equal constituted the ME requirement for maintenance (ME_m). Maintenance requirements for metabolizable protein (MP) were calculated using published data. Maintenance requirements were subtracted from nutrient intake to estimate energy (ME_g, Mcal/d/$kg^{0.75}$) and protein (MP_g, kg/d/$kg^{0.75}$) available for growth. Parameters for predicting retained fat (EBFG, kg/d) and protein (EBPG, kg/d) were estimated using multiple linear regression. Fasting HP was 76 and 85 whereas ME_m was 105 and 129 kcal/$kg^{0.75}$ EBW for Holsteins and Jerseys, respectively. The equations to predict EBPG (0.183 × EBW gain (kg/d) + 4.52 x MP_g − 0.344 × ME_g) and EBFG (0.099 × EBW gain (kg/d) + 0.228 × fat intake (kg/d) + 0.152 x ME_g – 8.59 × MP_g + 27.22 × ME_g × MP_g) accounted for 89 and 88% of the variation. Predicted values for EBFP and EBPG were multiplied by their energy constants (9,367 and 5,686 kcal/kg, respectively) and added together to estimate retained energy, which captured 88% of the variation in the evaluated data set.

Key Words: calf, nutrient, requirement

M162 Performance and health of dairy calves fed milk replacers at a conventional or accelerated feeding rate supplemented with different fat sources.

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One-hundred (2 to 5 d old) individually fed Holstein heifer calves (39.5 ± 0.63 kg) were randomly assigned to 1 of 4 milk replacers (MR), 24% crude protein (CP):24% fat as-fed, formulated with different fat sources to evaluate calf performance and health through 56 d. The study was conducted between December 2017 and April 2018. All calves were fed ad libitum water and a texturized calf starter, 18% CP as-fed, from d 1 to 56. Calf MR treatments were as follows: 1) MR formulated with 100% animal fat (AF) fed at a low feeding rate (FR) of 0.28 kg MR powder in 2 L of water 2× daily from d 1 to 35, and 1x daily from d 36 to weaning at d 42, (LFNC); 2) MR formulated with 85% AF and 15% coconut oil (CO) fed as for Trt 1, (LFWC); 3) MR formulated with 100% AF fed at a high FR of 0.43 kg MR powder in 3 L water 2× daily from d 1 to 42, and 1× daily from d 43 to weaning at d 49, (HFNC); and 4) formulated with 85% AF and 15% CO MR fed as for Trt 3 (HFWC). Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate.There were no interactions between fat source and FR for any parameters. Feeding rate ($P < 0.01$) and fat source ($P < 0.04$) improved feed:gain (d 1 to 42) for LFWC and HFWC (0.60 and 0.62, respectively) when compared with LFNC and HFNC (0.57 and 0.61, respectively). Feeding rate ($P < 0.01$) and fat source ($P < 0.07$) increased d 56 hip height (HH) with average gains of 10.6, 12.1, 12.7, and 13.1 cm for LFNC, LFWC, HFNC, and HFWC, respectively. Fat source did not affect d 1 to 56 ADG ($P > 0.1$). Total (d 1 to 56) starter intake was significantly lower ($P < 0.01$) for HF calves, averaging 44.0 kg compared with 59.9 kg for LF calves. There were no differences ($P > 0.15$) in overall DM intake from d 1 to 56. Scouring days (days >3, where 1 = normal and 4 = watery) were affected by FS (1.55 vs 0.83 d, $P < 0.01$) and d 1 to 42 treatment costs ($0.47 vs 0.14, $P < 0.001$) for LFNC+HFNC vs LFWC+HFWC. Under the conditions of this study, fat source did not affect 56 d ADG, although calves fed a MR with a blend of AF and CO had improved 56 d HH gain, 42 d feed:gain and better health status compared with calves fed MR formulated with AF only.

Key Words: calf performance, milk replacer, fat source

M194 Unprotected choline chloride in a dual-flow continuous culture system improves propionate concentration from low NDF diets.

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Choline may be degraded to trimethylamine and methane by ruminal microbes; however, its effects on ruminal fermentation are still uncertain. Due to its final degradation to methane, we hypothesized that unprotected choline chloride effects on ruminal fermentation would depend on dietary NDF concentration. We used 8 fermenters of a dual-flow continuous culture system in a 4x4 duplicated Latin-square with a 2x2 factorial arrangement; factors being: choline chloride supplementation (0 and 2.5 g/kg DM) and dietary NDF% (30 and 40). Resulting treatments were: 1) 30% NDF + choline; 2) 30% NDF, no choline; 3) 40% NDF + choline, 4) 40% NDF, no choline. Basal diets (30 and 40% NDF) were fed at 0800 and 1800, and choline chloride was supplemented 4 times/d. Each experimental period lasted for 10 d (7 d adaptation, 3 d sample collection). At 0, 1, 2, 4, 6, and 8 h post morning feeding, pH was measured and samples collected for diurnal kinetics analysis of pH, VFA, and NH_3-N. Also, one sample of pooled digesta effluents was collected before morning feeding for pooled daily VFA and NH_3-N concentrations. Main effects of choline supplementation (Cho), dietary NDF% (NDF), and their interaction (Cho*NDF) were tested for all response variables. Effect of time (h post morning feeding) was considered for diurnal kinetics and data analyzed as repeated measures. For molar proportions of VFA daily pool, a 1.6% reduction in acetate ($P = 0.03$) resulted from choline supplementation regardless of dietary NDF%. However; only when 30% NDF diet was fed, a 8.0% increase in propionate ($P = 0.006$), and decreases of 8.2% in isobutyrate ($P = 0.02$) and 0.22 units in acetate:propionate ($P = 0.04$) were observed as a result of choline supplementation. For molar proportions of VFA kinetics, addition of choline tended to decrease acetate:propionate in 0.12 units ($P = 0.1$); but propionate tended to increase 7.8% only when choline was added to 30% NDF diet ($P = 0.09$). Our results suggest that feeding unprotected choline chloride at 2.5 g/kg DM may enhance efficiency of VFA synthesis, particularly when supplemented to lower NDF diets.

Key Words: in vitro, ruminal fermentation, fiber

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M163 Pre- and post-weaning performance and health of dairy calves fed milk replacers formulated with alternative blended protein and fat sources at different levels of protein. D. Ziegler*[1], H. Chester-Jones[1], C. Soderholm[2], and B. Hansen[2], [1]*University of Minnesota, Waseca, MN,* [2]*Milk Specialities Global, Eden Prairie, MN.*

One-hundred 3 (2 to 5 d old) individually fed Holstein heifer calves (39.1 ± 0.63 kg) from 2 commercial dairies were randomly assigned to 1 of 4 milk replacer (MR) treatments formulated with blended protein and fat sources to evaluate pre- (d 1 to 49) and post-weaning (d 50 to 56) calf performance and health. The study was conducted between May and August 2018. All calves were fed a medicated (Decoquinate at 45.4 g/ton) texturized calf starter (CS; 18% crude protein CP as fed). Water and CS were offered free choice from (d 1 to 56). All MR were fed at 0.34 kg in 2 L of water 2× daily from d 1 to d 42 and 1× daily from d 43 to weaning at d 49; supplemented daily with neomycin sulfate and oxytetracycline at 22 mg/kg BW/day (d 1 to 14). Milk Replacer treatments were as follows: 1) 20% CP:20% fat (all milk protein, MP, all animal fat, AF; CONAM20); 2) 25% CP:20% fat MP AF, (CONAM25); 3) 25% CP:20% fat AF formulated with blend of bovine plasma, soy isolate and hydrolyzed wheat gluten proteins (BP) replacing 35.6% of MP, (BP25); and 4) 25% CP:25% fat MR formulated with BP as in TRT 3 and a blend of AF and coconut oil (BF) replacing 15% of total fat, (BPBF25). There was an increase in ADG ($P = 0.10$) trend for calves fed CONAM25 and BPBP25 vs. those fed CONAM20 and BP25 (d 1 to 56) averaging 0.68, 0.65, 0.61 and 0.60 kg/d respectively. Hip height gain was similar across treatment groups averaging 10.95 cm gain (d 1 to 56). There were no differences in CS or dry matter intake (d 1 to 56) averaging 30.7 and 60.5 kg total intake respectively. No differences in gain:feed ratio (d 1 to 56) were observed. There were no differences in health costs or daily fecal scores across treatments. Scouring days (days ≥3, 1 = normal to 4 = watery) tended to be higher ($P = 0.07$) for calves fed BP25 vs. other treatment groups. Under conditions of this study there were no benefits to feeding a 25:20 MR BP25 compared with calves fed a 20:20 a.m. MR CONAM20. The substitution of coconut oil for animal fat did not benefit overall performance (d 1 to 56).

Key Words: calf performance, milk replacer protein sources, fat source

M164 Gene expression analysis of milk proteins and fatty acid synthesis genes in goat milk fat fed with linseed and chia oils. R. K. Choudhary[1], J. S. Hundal[2], M. Wadhwa[2], S. Choudhary*[1], and Neetika[2], [1]*School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India,* [2]*Department of Animal Nutrition, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.*

Plant oils supplementation in animal diet has been used as a reliable feeding strategy to enhance omega-3 fatty acids content in milk toward increasing milk nutraceutical value. To investigate diet induced differences gene expression of milk protein *LALBA* and *CSN2* and milk fat synthesis (*CD36, XHD, ACACA, SCD* and *FABP3*), linseed (*Linum usitatissimum*) and chia (*Salvia hispanica*) oils have been fed as additive at 1% of diet for 60 d to lactating goats (n = 12). The milk from Beetal goats (*Capra aegagrus hircus*) at d 0 (before treatment) and after treatment during 2 stages of lactation (d 15 and d 34) was collected. The milk fat, after centrifugation, was processed for total RNA isolation and subjected to gene expression analysis. The RNA quality (OD ratios) and quantity (concentration), as measured by NanoDrop spectrophotometer, did not differ ($P > 0.05$; 2-way ANOVA) either by treatments or by days in lactation. The mRNA expression of *ACACA*, *FABP3* and *CD36* at d 15 and *SCD*, *XDH*, *LALBA* and *CSN2* at d 34 was upregulated at d 34, in comparison to d 0, as the main effects of stage of lactation. Supplementation of feed with both the oils increased ($P < 0.05$) expression of *CD36* at d 34, with more pronounced expression by linseed. In relation to control, abundance of *XDH* was increased on d 34 (18-fold; $P < 0.01$) in linseed oil fed milk. However, abundance of milk protein genes was not affected by the treatment ($P > 0.05$), indicating that milk production is unlikely affected by oil supplementation. There were statistically significant interactions observed among all the genes of our study with the lactation stage. Data suggest that linseed supplementation is more effective in increasing gene expression of milk fat synthesis genes than chia oil. Since the percent of omega-3 fatty acids in milk of linseed oil fed animals is more (1.26) than the chia oil (0.95), indicating that linseed oil could be a choice for fatty acid augmentation in milk.

Key Words: goat, plant oil, mammary gene expression

M165 Interactions between iodine and protein sources: Effects on milk yield, milk components, and urinary N excretion in Jersey cows. M. Ghelichkhan*, D. Williams, L. H. P. Silva, and A. F. Brito, *University of New Hampshire, Durham, NH.*

Kelp meal (KM) is a supplement made from the brown seaweed *Ascophyllum nodosum* known to bioaccumulate iodine (I). *A. nodosum* is also the richest of phlorotannins and contains other bioactive compounds that may boost animal production. However, we are not aware of any study that evaluated the effects of KM compared with another I source in cows fed soybean meal (SBM) or canola meal (CM). Our goal was to assess the interactions between 2 sources of I and RDP on production and urinary excretion of nitrogenous metabolites. Sixteen multiparous Jersey cows (93 ± 58 DIM) were used in a factorial replicated 4 × 4 Latin square design (21-d periods). Diets were formulated to yield similar concentrations of CP and I and consisted (DM basis) of 55% baleage and 45% concentrate. Dietary CP and NDF concentrations averaged 18.5 and 35.6%, respectively. Experimental treatments were: 10% SBM + 110 mg/d of ethylenediamine dihydroiodide (EDDI) or 114 g/d of KM and 12.5% CM + 110 mg/d of EDDI or 114 g/d of KM. Kelp contained 775 mg/kg of I. Data analysis included the main effects of RDP and I source and interaction. Compared with CM, SBM significantly decreased DMI (20.7 vs. 21.7 ± 0.82 kg/d) and MUN (10.4 vs. 12.9 ± 0.4 mg/dL) and increased milk yield (22.7 vs. 21.5 ± 0.81 kg/d), thus improving feed efficiency (1.19 vs. 0.99 ± 0.03 kg/kg). Milk N efficiency was also greater ($P < 0.01$) in SBM than CM cows. Reduced milk yield and MUN was likely caused by atypical concentrations of NDICP (23.6%) and ADICP (15.5%) in the CM used. Concentrations of milk fat (5.44 vs. 5.08 ± 0.14%) and protein (3.64 vs. 3.52 ± 0.05%) were significantly greater in cows fed CM vs. SBM. Feeding KM significantly increased milk yield compared with EDDI (22.4 vs. 21.8 ± 0.81 kg/d), while DMI tended ($P = 0.09$) to improve in KM cows. Neither RDP nor I source affected the urinary excretion of total purine derivatives. In contrast, urinary excretion of N (% of N intake) decreased (26.2 vs. 31.2 ± 1.92%; $P < 0.05$) with feeding CM rather than SBM diets. Overall, SBM and KM increased DMI and milk yield, and feeding SBM further improved feed and milk N efficiency.

Key Words: iodine, protein, seaweed

Table 1 (Abstr. M166). Iodine metabolism in Jersey cows

	CM		SBM			*P*-value		
Item	EDDI	KM	EDDI	KM	SEM	RDP	I	RDP × I
I intake mg/d	96.3	93.3	95.7	92.8	0.30	<0.001	<0.001	0.83
Milk I, ng/ml	786	471	799	652	56.53	0.009	<0.001	0.02
Milk I, mg/d	16.7	10.2	17.8	15.1	0.58	<0.001	<0.001	<0.001
Plasma TSH, µIU/mL	0.393	0.388	0.396	0.396	0.003	0.01	0.25	0.19
Plasma total T_3, ng/mL	1.07	0.98	1.18	1.13	0.09	0.05	0.30	0.81
Plasma total T_4, ng/mL	40.5	37.9	46.0	42.7	3.10	0.05	0.26	0.88

M166 Interactions between iodine and protein sources: Effects on milk iodine and thyroid hormones in Jersey cows.

M. Ghelichkhan*, D. Williams, L. H. P. Silva, and A. F. Brito, *University of New Hampshire, Durham, NH.*

Kelp meal (KM) is a supplement made from the brown seaweed *Ascophyllum nodosum* known to bioaccumulate iodine (I). However, feeding KM may result in milk I above the recommended concentration of 500 µg/L. Canola meal (CM) contain goitrogenic glucosinolates (GLS) that affect I distribution in tissues through competitive inhibition of iodide transportation by the Na-iodide symporter. Our goal was to assess the interactions between 2 sources of I and RDP on I metabolism in dairy cows. Sixteen multiparous Jersey cows (93 ± 58 DIM) were used in a factorial replicated 4 × 4 Latin square design (21-d periods). Diets were formulated to yield similar concentrations of CP and I and consisted (DM basis) of 55% baleage and 45% concentrate. Dietary CP and NDF concentrations averaged 18.5 and 35.6%, respectively. Experimental treatments were: 10% soybean meal (SBM) + 110 mg/d of ethylenediamine dihydroiodide (EDDI) or 114 g/d of KM and 12.5% CM + 110 mg/d of EDDI or 114 g/d of KM. Kelp contained 775 mg/kg of I. Data analysis included the main effects of RDP and I source and interaction. Although I intake was greatest in SBM and EDDI diets, the actual difference between treatments was small. The drop in the concentration and yield of milk I was more pronounced in cows fed CM plus KM than CM plus EDDI (RDP x I interaction). Cows fed CM had greater concentrations of plasma thyroid stimulating hormone (TSH), triiodothyronine (T_3), and thyroxine (T_4) than those fed SBM possibly in response to GLS found in CM. Milk I decreased below 500 µg/L only in CM plus KM diet, which was also linked to CM-GLS. The fact that milk I did not drop below 500 500 µg/L with feeding CM plus EDDI was unexpected and requires further investigations.

Key Words: iodine, protein, seaweed

M167 Effect of cobalt source on nutrient digestibility, microbial protein production and efficiency and B_{12} synthesis in dual-flow continuous culture fermentors—Summary of 4 studies.

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Four dual-flow continuous culture fermenter studies were summarized to determine effects of Co source, (1 ppm Co) on nutrient digestibility, microbial protein production and efficiency and vitamin B_{12} synthesis. Three Co sources, $CoSO_4$, cobalt glucoheptonate (COPRO, Zinpro Corp., Eden Prairie, MN) and Co pectin (CoPectin, Zinpro Corp.), were evaluated in 3 to 4 studies. Rumen inoculum was obtained and pooled from 2 ruminally cannulated Holstein cows consuming a lactation diet absent of Co. In each completely randomized study, treatments were run in triplicate over a 17 d fermentation period, sampled d 15–17. A standard lactation diet (100 g DM/d) was fed in 4 meals/d. Artificial saliva was continuously infused into the fermenters (1162 mL volume), for a 13%/h liquid dilution rate and 22 h solid retention time. Select data were analyzed using linear mixed effect regression model of R. Random effects were included in the model to account for different repeated measures intercepts. The 3 Co sources had similar ($P \geq 0.15$) total VFA production (376 mmol/d), and DM, OM, ADF, NDF, and NSC digestibilities (65, 57, 42, 41 and 81% respectively). Vitamin B_{12} production was similar ($P \geq 0.15$) among the Co sources but numerically greatest for CoPectin (10870, 10557, 10281 pmol/d; CoPectin, COPRO and $CoSO_4$ respectively). CoPectin tended to have greater CP digestibility and microbial N production than $CoSO_4$, with COPRO intermediate ($P \leq 0.15$; 78.6, 72.4 and 76.5% and 2.11, 1.91 and 2.04 g/d, respectively). Greater ($P \leq 0.05$) amounts of feed N were converted to microbial N with CoPectin than $CoSO_4$, with COPRO intermediate (93.8, 92.4 and 93.5 respectively). CoPectin and COPRO produced greater amounts of microbial N/kg OM digested than $CoSO_4$ ($P \leq 0.05$; 38.9, 37.8 and 35.1 respectively). Replacing 1 ppm Co from $CoSO_4$ with 1 ppm Co from CoPectin or COPRO increased microbial protein production and efficiency and numerically increased vitamin B_{12} production in continuous culture. CoPectin improved microbial protein production and efficiency over COPRO.

Key Words: cobalt, B_{12}, microbial efficiency

M168 Partial replacement of starch and protein sources in dairy cows rations with sustainable feeds: Effects on milk production, rumen health, and digestibility of fiber.

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To increase sustainability of cow nutrition and reduce food wastage, we partially replaced protein and energy sources of dairy cows rations with 2 sustainable feeds coming from grain industry. Four isoproteic diets were formulated including condensed wheat distillers soluble (D), former foodstuff (F) or both (DF), as protein (D) or starch source (F). These diets were compared with a standard dairy cows ration (CTR). The effect of these diets on milk yield, rumen health and degradability of fiber was evaluated in a replicated 4 × 4 Latin square trial with 8 mid-lactating Holstein cows. Cows were blocked by parity and average milk production, and received dietary treatments for 4 21-d periods, composed of 14 d of adaptation and 7 of data recording. Individual TMR delivered and residual was analyzed 3 times/period, while individual feed and water intake, milk yield, rumination time and rumen pH were recorded daily. In each period, milk was sampled 4 times for composition, SCC and urea content. Fecal composition was analyzed 4 times/period to evaluate Total-tract Fiber Degradability (TTDpdNDF) of diets. Data were analyzed with a mixed model with diet and period as fixed effects and cow within block as random effect. Data recorded daily were analyzed with repeated measures including in the model the time within cow effect. The treatments did not influence DMI (23 kg/d on average) or TTDpdNDF of the diets. The F diet tended to increase milk yield compared with the CTR and the D, while the DF significantly increased production of 1 kg/d ($P < 0.05$) without affecting milk quality. Rumination time was not influenced by the treatments, as well as average daily rumen pH and time with pH < 5.5. Conversely, the DF diet significantly increased the time with rumen pH < 5.8 (min. 172 vs 56, 105 and 109, $P < 0.05$ for DV, CTR, D, and V diet, respectively). The inclusion of former foodstuff in the diets can increase sustainability of cow nutrition without impairing cow performance and health nor DMI or digestibility of the diets. However, further studies are needed to explain possible interactions of these feeds on rumen fermentations.

M169 Feeding rumen-protected choline to prepartum Holstein cows in negative energy balance increases circulating lipoprotein phosphatidylcholine and triglyceride levels while preventing hepatic triglyceride accrual. W. A. Myers*[1], M. G. Zenobi[2], B. A. Barton[3], C. R. Staples[2], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Florida, Gainesville, FL,* [3]*Balchem Corp., New Hampton, NY.*

Rumen-protected choline (RPC) supplementation may increase hepatic phosphatidylcholine (PC) synthesis to promote triglyceride (TG) secretion within very-low density proteins. To assess whether RPC enhances lipoprotein PC and TG levels in dairy cows, 41 pregnant, nonlactating, multiparous Holstein cows were fed a RPC (ReaShure, Balchem Corp., New Hampton, NY) that provided 0 (control), 6.5, 12.9, 19.4, and 25.8 g/d of choline ions, respectively. Diets were fed to exceed nutrient requirements for 5 d, then cows were restricted to consume ~31% of their net energy requirements for 9 d. Preprandial plasma and liver were collected on d 9 of feed restriction. Plasma TG-rich and low-density lipoprotein (LDL) fractions were isolated using liquid chromatography. Lipoprotein fraction total TG, cholesterol and phospholipids were quantified. Lipoprotein fractions and liver were processed for lipidomics. Statistical analyses were done using the Mixed procedure of SAS. Birth weight of the calves and number of days prepartum at enrollment were covariates. Normalized omic data were natural log-transformed. A significant linear increase ($P < 0.01$) in TG-rich lipoprotein total TG levels was observed with choline ion supplementation. Likewise, RPC linearly increased TG levels within LDL fractions ($P = 0.02$). Total LDL fraction phospholipids tended to be modified by treatment (quadratic, $P = 0.09$). The majority of PC within the TG-rich lipoprotein fraction increased linearly with increasing RPC (40 PC out of 45 PC detected; e.g., PC 38:5; $P < 0.01$). A similar linear outcome was observed for select TG-rich lipoprotein TG (168 TG out of 317 TG detected; $P < 0.05$). In LDL, RPC increased majority of PC detected (control vs. RPC [all levels], $P < 0.05$); however, TG was not overtly modified. In liver, RPC increased a limited number of PC (<15% detected; control vs. RPC, $P < 0.05$). Hepatic total TG was lowered by RPC (17.5 vs. 13.6% of tissue DM; control vs. RPC, $P < 0.05$). We conclude that RPC increased lipoprotein PC and TG concentrations, and reduced hepatic TG deposition in dairy cows.

Key Words: choline, liver, phosphatidylcholine

M170 Characterization of the very low density lipoprotein lipidome exported from primary bovine hepatocytes supplemented with choline and methionine. T. L. Chandler*[1], S. J. Erb[2], W. A. Myers[1], B. A. Barton[3], J. W. McFadden[1], and H. M. White[2], [1]*Cornell University, Ithaca, NY,* [2]*University of Wisconsin-Madison, Madison, WI,* [3]*Balchem Corp., New Hampton, NY.*

Supplementing choline in dairy cattle decreases liver triglyceride (TG), potentially through increased very low density lipoprotein (VLDL) export. Our objectives were to determine the effect of choline chloride (CC) and D,L-methionine (DLM) on VLDL TG export from primary bovine hepatocytes and characterize the lipidome of exported VLDL. Hepatocyte monolayers (n = 3 preps) were treated (24 h) with CC (0, 0.01, 0.1, or 1.0 m*M*) and DLM (0, 0.1, or 0.3 m*M*; 4 × 3 factorial) in the presence of 1.0 m*M* fatty acids. Hepatocytes were then harvested to quantify cellular TG and VLDL isolated from media by size exclusion chromatography. Total VLDL TG was measured by colorimetric assay and untargeted lipidomics using mass spectrometry. Data were normalized to DNA, expressed relative to the 0 m*M* CC and 0 m*M* DLM treatment within each prep, and analyzed by PROC MIXED with fixed effect of CC, DLM, the interaction, and random effect of prep. Pearson correlations between cell TG, lipoprotein TG, and lipid species were calculated. Contrasts evaluated for CC were 0 m*M* vs. (0.01, 0.1, 1.0 m*M*), linear, and quadratic; and for DLM were 0 m*M* vs. (0.1, 0.3 m*M*) and 0.1 vs. 0.3 m*M*. Cell TG tended to be decreased ($P = 0.06$) in the presence of CC. Increasing CC quadratically affected (0.96, 1.07, 0.88, 0.98 ± 0.05 arbitrary units (AU); $P < 0.01$), but DLM did not alter (1.0, 0.98, 0.93 ± 0.05 AU; $P = 0.31$), VLDL TG. Lipidomics revealed 168 TG, 28 diacylglycerol, 32 phosphatidylcholine (PC), 19 phosphatidylethanolamine, 18 phosphatidylserine, and 10 sphingomyelin species but total of each species was not altered ($P > 0.10$) by CC or DLM, nor were cell TG correlated ($P > 0.10$) to these lipids. Although total PC was unaltered, increasing CC linearly decreased ($P = 0.05$) lyso-PC (LPC; e.g., 18:0), a lipid involved in inflammation. Cell TG were correlated (r = 0.54; $P < 0.01$) to LPC. Overall, supplying CC and DLM minimally altered the VLDL lipidome. Decreased cell TG and LPC with increased CC should be further examined, especially in the context of liver fat accumulation and inflammation in vivo.

Key Words: liver triglyceride, lipid export, methyl donor

M171 Fast determination of intestinal protein digestibility with vibrational molecular spectroscopic techniques for dairy cows. H. Shi[1,2] and P. Yu*[1], [1]*Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada* [2]*College of Life Science and Engineering, Foshan University, Foshan, Guangdong, China.*

Information on applying different IR techniques to the prediction of nutrients digestibility in feed is limited and no literature is available on the comparison of NIR and MIR spectroscopy for the prediction of protein digestibility in feed or food samples. The objective of this study was to evaluate the potential of using non-invasive vibrational molecular spectroscopy (near IR vs. ATR-FT/MIR) to rapidly determinate intestinal crude protein (CP) digestibility (IPD). The model feed used in this study was wheat. The CP and IPD reference values along with NIR and MIR spectra data were exported to the Unscrambler X v10.4 (CAMO). The raw spectra were preprocessed by 10 different preprocessing algorithms, including baseline offset correction, multiplicative scattering correction (MSC), detrending, first derivative (FD), second derivative (SD), standard normal variate (SNV), first derivative + SNV (FD-SNV), SNV + first derivative (SNV-SD), second derivative + SNV (SD-SNV) and SNV + detrending (SNV-detrending). For CP, the best near-IR model showed an excellent prediction performance (R2 = 0.98); the best ATR-FT/Mid-IR model also gave an excellent prediction performance (R2 = 0.96). Regarding to IPD, the best model obtained by Near-IR technique showed approximate quantitative predictive ability (R2 = 0.68), and the best model generated by ATR-FT/Mid-IR technique obtained similar prediction performance (R2 = 0.67). ATR-FT/Mid-IR models generally showed better predictive abilities than near-IR models, which may be due to the ATR-FT/Mid-IR spectra record fundamental molecular vibrations and can be more easily affected by multiple interferences. The amide I and II bands played important roles in the development of PLS models for CP and IPD. Results from this study demonstrated the potential of using IR spectroscopy for the prediction of nutrient digestibility while more efforts are required to improve the performance of near-IR and ATR-FT/Mid-IR spectroscopy in predicting the IPD.

Key Words: protein digestibility, dairy cow, vibrational molecular spectroscopy

M172 Pre- and post-weaning performance and health of dairy calves fed texturized calf starters formulated with a combination of sunflower meal, linseed meal, or soybean meal compared with a starter with multiple protein sources. D. Ziegler*[1], H. Chester-Jones[1], B. Ziegler[2], and A. Manthey[2], [1]*University of Minnesota, Waseca, MN,* [2]*Hubbard Feeds Inc., Mankato, MN.*

One-hundred eight (2 to 5 d old) individually fed Holstein heifer calves (39.6 ± 0.65 kg) from 3 commercial dairies were randomly assigned to 1 of 4 texturized starters (TS) 18% crude protein (CP) as fed, formulated with different protein sources to evaluate pre- (d 1 to 42) and post-weaning (d 43 to 56) calf performance and health. The study was conducted between October 2017 and January 2018. All calves were fed a non-medicated 20% CP:20% fat milk replacer (all milk protein) fed at 0.28 kg in 2 L of water 2× daily from (d 1 to 35) and 1× daily from d 36 to weaning at d 42; supplemented daily with neomycin sulfate and oxytetracycline at 22 mg/kg BW/day (d 1 to 14). Calf starter treatments were as follows: 1) TS formulated with multiple protein sources, (CON); 2) TS formulated with 25% sunflower meal, 75% soybean meal, (TSSF); 3) TS formulated with 25% linseed meal, 75% soybean meal, (TSLN); and 4) TS formulated with 100% soybean meal, (TSSBM). Water and TS were offered free choice from (d 1 to 56). Data were analyzed using the PROC mixed procedure of SAS and repeated measures analyses applied where appropriate. There were no differences in pre-weaning (d 1 to 42), post-weaning (d 43 to 56) or total gains (d 1 to 56) averaging 0.65, 1.15 and 0.77 kg/d respectively ($P > 0.05$). Hip height gain was similar across treatments averaging 11.7 cm. Post-weaning TS intake tended to be greater for TSLN 37.3 kg vs. CON 34.4 kg and TSSF 34.6 kg with TSSBM being similar at 35.2 kg ($P = 0.08$). Total TS intake (d 1–56) was similar across treatments averaging 56.9 kg ($P > 0.05$). There were no differences in gain:feed ratio, pre- or post-weaning or overall (d 1 to 56) across treatments averaging 0.62, 0.46 and 0.57 respectively ($P > 0.05$). There were no differences in scouring days, or health costs across treatments. Under conditions of this study calves fed a TS with a combination of sunflower meal, linseed meal, soybean meal performed as well as a TS formulated with multiple protein sources.

Key Words: calf performance, calf starter, protein sources.

M173 Effects of the interaction between cow phenotypic milk urea nitrogen and dietary crude protein on milk production responses and efficiency. P. Letelier*[1], F. Contreras-Góvea[1], B. Albarrán-Portillo[2], F. Gomes[1], and M. Wattiaux[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Universidad Autónoma del Estado de Méjico, Temascaltepec, Mexico.*

Milk urea nitrogen (MUN) is a monitoring tool to evaluate dietary crude protein (CP) and predict urinary urea-N (UUN). However, MUN is also heritable ($h^2 = 0.21–0.41$) suggesting possible genetic effects. In this study we evaluated the interaction between dietary CP (14.9 vs. 16.5%, dry matter basis) and cow phenotypic MUN group on performance, milk protein yield, and feed conversion efficiency using 18 cows grouped in 9 pairs consisting of 1 high MUN cow (HMUNC; 15.5 ± 1.5 mg/dL) and 1 low MUN cow (LMUNC; 12.2 ± 1.3 mg/dL) of same parity, similar DIM (89 ± 18 DIM), and identical milk protein yield (1.39 ± 0.22 and 1.38 ± 0.21 kg/d for HMUNC and LMUNC, respectively). The experiment was designed as a 2-period crossover (26 d with last 3 d for sampling) with varying levels of soyhulls and soy Plus in the diets. Dry matter intake (DMI) was recorded using an "e-gate" system. Dry matter intake (27.7 ± 3.7 kg/d), fat-protein-corrected milk (FPCM; 41.6 ± 7.6 kg/d) and yield of milk protein (1.36 ± 0.26 kg/d), fat (1.5 ± 0.4 kg/d) and lactose (2.4 ± 0.5 kg/d) were not influenced by dietary CP and cow phenotypic MUN group. Reducing dietary CP reduced MUN (10.7 vs. 14.1 mg/dL; $P < 0.01$) and MUN yield (5.0 vs. 6.9 g/d; $P < 0.01$), and it tended to reduce feed conversion efficiency (kg FPCM/kg DMI; 1.14 vs. 1.18; $P = 0.09$). The LMUNC had lower MUN (11.4 vs. 13.4 mg/dL) and MUN yield (5.51 vs. 6.35 g/d) than HMUNC. No interactions were found between cow phenotypic MUN group and dietary CP for MUN ($P = 0.88$), MUN yield ($P = 0.85$), FPCM ($P = 0.78$), milk protein yield ($P = 0.60$) and feed conversion efficiency ($P = 0.49$). Although more research is needed, the lack of interactions suggested that current recommended MUN target values should apply to cows (or a herd) regardless of their potential for milk protein production. Results suggested also that genetic selection could be applied to reduce MUN (and presumably UUN excretion) and MUN yield without penalizing DMI, milk production, and yields of milk protein, fat and lactose.

Key Words: milk urea N, dietary crude protein, milk protein production

M174 Impact of replacing canola meal with solvent-extracted distillers grain with solubles as a protein source on milk production. R. Edwards*[1], D. Ledgerwood[2], D. Waldner[3], and H. Rossow[1], [1]*University of California, Davis, Tulare, CA,* [2]*Novita, Brookings, SD,* [3]*Valley Nutrition, Visalia, CA.*

Because of the high unsaturated fatty acid content of dried distillers grains with solubles (DDGS), usage of DDGS as a protein supplement for dairy cows is limited. However, new extraction processes can remove corn oil from distillers grains and produce solvent extracted distillers grain with solubles (NM; NovaMeal, Brookings, SD). The objective of this study was to evaluate the impact of replacing canola meal with NM and urea as protein sources on milk yield and components in a commercial dairy herd. A total of 2,776 Holstein cows averaging 63 DIM were enrolled to one of 4 dietary treatment pens: 1) primiparous control (C) group with 3.1 kg CM, 0.058 kg urea; 2) primiparous treatment (NT) group replaced CM with 2.1 kg NM, 0.11 kg urea; 3) multiparous C group 3.5 kg CM, 0.057 kg urea; and 4) multiparous NT group replaced CM with 2.4 kg NM, 0.11 kg urea. The TMR were formulated to be isonitrogenous and isoenergetic with similar fatty acid profiles and averaged 17% CP, 4.5% fat and contained 45% forage and 55% concentrate. Pens were fed for 6 wk, then TMR for C and NT pens were switched within parity groups for a total of 2 pens per treatment-parity with 694 cows per treatment-parity group. Then, following a 2 wk adjustment period, data collection resumed. Data were analyzed using the Mixed procedure of SAS (v.9.4) with random effects DIM and days on treatment. Multiparous cows had greater milk yield, ECM, milk fat yield, milk protein yield, and DMI but, lower milk fat %, milk protein % and MUN than primiparous cows ($P < 0.05$) due to their higher milk yield. There were no differences between treatment least squares means

(LSM) for milk yield, ECM, milk protein %, DMI and MUN but, milk fat yield and % were greater with treatment NT (2.05 kg vs. 2.01 kg and 5.29% vs. 5.07% LSM for NT and C treatments, respectively). Milk protein yield was lower in treatment NT (1.25 kg vs. 1.28 kg LSM for NT and C treatments, respectively). These results show that NM with supplemented urea can be used to replace CM as a protein supplement in a lactating dairy cattle TMR.

Key Words: solvent-extracted distillers grain with solubles, canola meal, bypass protein

M175 Effects of a yeast-derived microbial protein supplement on transition dairy cow performance.

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The objective of this study was to evaluate the effects of partial substitution of soybean meal with yeast-derived microbial protein (YMP) on transition dairy cow performance. The study was conducted at the UK Coldstream Dairy from August 2017 to June 2018. Seventy-one Holstein dairy cows were enrolled in the study from 28 ± 3 d before expected calving until 21 d post-calving. At enrollment, cows were randomly assigned to either a treatment (YMP) or a control (CON) diet. Isonitrogenous and isoenergetic diets were formulated with corn silage, alfalfa silage, grass hay, cottonseed, and a grain mix with or without YMP. Treatment diets were formulated to supply animals with 100 and 300 g of YMP/d (DEMP; Alltech Inc., Nicholasville, KY) during the dry and fresh periods, respectively. Dry and fresh cows were housed in a compost bedded pack barn equipped with automatic feed bins that recorded individual feed intake of which DMI was calculated. Milk yield and composition were recorded daily using an automatic meter until 100 DIM; 4% fat corrected milk (FCM) and energy corrected milk (ECM) were also calculated. A mixed linear model was used to determine the effects of YMP on milk yield, 4% FCM, ECM, and DMI. The model included the fixed effects of treatment, parity, body weight, milk yield, and disease occurrence, with lactation wk as a repeated measure. There were no significant differences in milk yield between YMP and CON cows during the fresh period (0 to 21 DIM) (YMP = 30.91 ± 1.34, CON = 28.87 ± 1.32 kg/d; $P = 0.14$) and for ECM (YMP = 35.36 ± 1.43, CON = 32.98 ± 1.40 kg/d; $P = 0.11$). However, cows fed YMP tended to produce more 4% FCM (YMP = 33.15 ± 1.32, CON = 30.81 ± 1.30 kg/d; $P = 0.09$). During the first 3 wk of lactation, YMP cows had lower DMI compared with CON cows (YMP = 17.04 ± 0.63, CON = 20.91 ± 0.61 kg DM; $P < 0.01$). Total milk produced until 100 DIM was not different between treatments (YMP = 4,020 ± 170.83, CON = 4,122.15 ± 165.49 kg; $P = 0.56$). Overall, feeding YMP during the transition period did not affect daily milk yield but cows fed YMP had decreased DMI when at a similar production level as CON cows.

Key Words: protein supplement, bypass protein, microbial CP

M176 Differential gene expression analysis of the effects of a negative energy and protein balance induced by feed restriction in mid-lactation dairy cows.

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The aim of the study was to assess the effects of a short-term period of negative energy balance (NEB) induced by feed restriction on expression of genes in liver and muscle. Seven multiparous Holstein cows (93 ± 15 DIM) were randomly assigned to 7 treatments in a 7 × 4 incomplete Latin square design with 5-d periods. In 6 treatments, including a restricted control (RC), daily DMI was restricted to provide 60% of calculated net energy requirements in the 7th treatment cows were fed for ad libitum (AL) DMI. Liver and muscle tissue was biopsied on the last day of the period. Total RNA was sequenced on an Ilumina HiSeq2500. Transcripts were assembled from the reads aligned to NCBI Bos taurus UMD_3.1.1. Statistical analysis was done separately for each tissue using the "voom" method from the limma package in R. Data of differentially expressed genes (DEG) between RC and AL with their associated P values were imported into DAVID and DIA for enrichment (↑ up- and ↓ downregulated) and functional analysis. A total of 284 genes in liver (FDR $P < 0.25$) and 261 in muscle (uncorrected $P < 0.01$) were detected. The top most significant categories among the 3 gene ontology (GO), KEGG and Reactome pathways were related to regulation of metabolism and signaling [insulin, AMPK and FoxO signaling, cell death and apoptosis, PPAR (↑) and glucagon signaling, synaptic plasticity (↓)], lipid metabolism [cell lipid transporters, peroxisome, cholesterol transport, fat digestion and absorption (↑)], AA metabolism [biosynthesis of AA (Lys ↑), urea cycle (↑)], vitamin metabolism [vitamin absorption and digestion, folate biosynthesis (↓)], and carbohydrate metabolism (Liver: ↑; muscle ↓) [pentose, ascorbate and aldarate], immune system (↓), calcium homeostasis (↑), and endocrine metabolism (estrogen, thyroid and oxytocin signaling). The genes with the greatest log2 fold change emphasized the vital roles of lipid metabolism [*APOA1*, *HMGCS2*, *BREH1*, and *FABP7* (↑)], immune system [*SAA1* (↓)], protein and bone mobilization [*SERPINA3* (↓), *PI16* (↑), *SPP2* (↑)], and vitamin B metabolism [*TCN1* (↓)].

Key Words: feed restriction, gene expression

M177 Effects of abomasal infusions of amino acids or glucose on expression of genes during an induced negative energy and protein balance.

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The aim of the study was to assess the effects of 5 supplements during a short-term period of negative energy and protein balance induced by feed restriction on expression of genes in liver and muscle. Seven multiparous Holstein cows (93 ± 15 DIM) were randomly assigned to 7 treatments in a 7 × 4 incomplete Latin square design with 5-d periods. Daily DMI was restricted to provide 60% of net energy requirements. Treatments were 4-h abomasal infusions of amino acid (AA) or glucose (0.4 mol/kg BW) initiated at feeding time (0900 h): monosodium glutamate (MSG), lysine (LYS), glutamine (GLN), valine (VAL), and water (CON) as control. Liver and muscle tissue was biopsied on the last day of the period. Total RNA was sequenced on an Ilumina HiSeq2500. Transcripts were assembled from the reads aligned to NCBI Bos taurus UMD_3.1.1. Statistical analysis was done using the "voom" method from the limma package in R. Data of differentially expressed genes between each treatment and CON with their associated P values were imported into DAVID and DIA for enrichment (↑ up- and ↓ downregulated) and functional analysis. Infusion of GLN overexpressed pathways for metabolism of AA (↑), gluconeogenesis (↑) [*SDS*], synthesis of pantothenate and CoA (↑), circadian rhythm regulation (↑) [*PER1, PER2*], immune system (↑) [*S100A9, CXCR2*]. Synthesis of serotonin and melatonin (↓) [*TPH1*], Tyr metabolism (↑), cholesterol transport (↑) [*LDLR*] and glucose metabolism (↑) [*HK2*, *GFOD1*] were the most significant pathways during MSG. Infusion of LYS affected TOR signaling (↑) [*PRKG1*, *GPAT3*], cholesterol synthesis (↓) [*DHCR24*], FA transport (↓) [*FABP7*], and immune system (↓) [*GPR39*, *PADI2*, *C3*]. Treatment of VAL upregulated mineral tissue turnover [*ALPL*, *MN1*] and possibly reduced muscle tissue mobilization [*SESN2* (↑), *CHSY3* (↓), *ADAMTS5* (↓), *MEOX2* (↓), *LAMA2* (↓), *CCNG1* (↑), *DPP4* (↓), *GTSE1* (↓)]. No relevant functional pathway was found during GLC. Our results identify some of the possible mechanisms of action that supplementation of these AA provided to counteract a period of negative energy and protein balance.

Key Words: negative energy and protein balance, amino acid, gene expression

M178 Milk production of organic dairy cattle is influenced by altering supplemental feed protein content.

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As demand for organic dairy products grows, the industry must focus on feeding strategies that optimize milk production. Dietary crude protein (CP) is variable across farms, and affects animal health and production during the grazing season. As a result of diet CP variability, milk urea nitrogen (MUN) profiles of grazing herds are often outside of the optimal range (9–12 mg/dL). This study evaluated the impact of altering the CP content of dietary supplements on milk production and the MUN profile of grazing cattle. Six Vermont organic dairy farms participated in a 6-week trial during the 2018 summer grazing season. Farms were paired by their 2017 summer MUN profile, and farms within each pair were assigned to either 1) continuation of their regular supplements (n = 3, CON), or 2) a 16% CP (% of DM) supplement formulated using an organic barley and roasted soybean mix (n = 3, TRT). All farms were maintained on their regular supplements during the first 2 weeks of the trial (baseline), and then continued on either the CON or TRT supplements for the last 4 weeks of the trial (experimental period). Each week, milk samples were collected at 2 consecutive milkings. Individual milk yields were recorded at each sampling, and samples were collected and commercially analyzed for protein, fat, and MUN content. All parameters were analyzed using PROC MIXED of SAS, and effects of treatment, week, and treatment x week were determined. Milk weight, fat percent, protein percent, and MUN content were all affected by treatment x week ($P < 0.0001$). Across the experimental period, milk yield was higher in the TRT group versus the CON group (11.89 kg vs 9.92 kg, respectively). Fat percent was higher in the CON group versus TRT group (4.20% vs 3.71%), as was protein percent (3.15% vs 3.07%). The MUN was higher in the TRT versus the CON group (13.24 mg/dL vs 11.62 mg/dL). Modifying the supplement CP effectively increased milk yield, indicating that protein intake was limiting milk production. However, further formulation must be done to ensure that MUN remains within the optimal range and that milk fat and protein are not negatively affected as observed in the TRT group in this study.

M179 Meta-analysis of the effects of prepartum dietary metabolizable protein (MP) on performance of dairy cows.

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Objectives were to use meta-analytic methods to determine the effects of dietary supply of MP prepartum on performance of dairy cows. The literature was systematically reviewed and 27 randomized experiments that manipulated prepartum dietary MP supply, including 125 treatment means and 1,801 cows, were included in the meta-analysis. Eight experiments with 27 treatment means reported responses for 510 nulliparous cows. Data collected included the ingredient composition and chemical analyses of prepartum diets, parity group, days on prepartum treatment, and means and respective SEM for DMI and productive responses. Diets were entered into NRC (2001) software to predict supplies metabolizable protein (MP) and metabolizable amino acids prepartum. Mixed effects meta-analysis was conducted and statistical models investigated the effects of dietary prepartum MP supply (g/d) on performance. Models included the random effect of experiment and were weighted by the inverse of SEM squared to account for the precision of each experiment. The estimated supply of MP resulted in distinct ranges in nulliparous (517 to 1,094 g/d) and parous cows (463 to 1,733 g/d); therefore, data were analyzed separately for each parity group. In nulliparous cows, increasing the prepartum MP supply linearly ($P < 0.05$) increased postpartum DMI ($\beta = 0.0089 \pm 0.0006$; increasing MP supply from 800 to 1,000 g/d increased DMI by 1.7 kg/d), milk yield ($\beta = 0.0058 \pm 0.0023$; increment = 1.1 kg/d), FCM ($\beta = 0.0072 \pm 0.0022$; increment = 1.4 kg/d) fat yield ($\beta = 0.0002 \pm 0.0001$; increment = 0.05 kg/d) and protein yield ($\beta = 0.00005 \pm 0.00002$; increment = 0.03 kg/d). However, in parous cows, increasing prepartum MP supply had no effects on DMI, yields of milk, FCM, and milk fat, but linearly increased ($P < 0.05$) protein yield only in cows producing more than 36 kg/d of milk ($\beta = 0.00006 \pm 0.00002$; increment by increasing MP supply from 900 to 1,200 g/d = 0.02 kg/d). Prepartum diets for nulliparous cows should supply 1,100 g of MP that was upper limit in the database, whereas minor benefits were observed of feeding parous cows more than 800 g/d MP.

Key Words: metabolizable protein, prepartum, transition cow

M180 In vitro ruminal dry matter degradability and volatile fatty acid and gas production of carinata meal compared with other protein sources.

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Our objective was to evaluate in vitro rumen fermentation of carinata meal from different processing methods (cold pressed or solvent extracted) and compare it to other common protein sources. Treatments were: 1) solvent extracted carinata meal (CAR-SE) 2) cold pressed carinata meal (CAR-CP) 3) solvent extracted linseed meal (LIN-SE), 4) cold pressed linseed meal (LIN-CP), 5) solvent extracted soybean meal (SOY-SE) and 6) cold pressed soybean meal (SOY-CP). The 24 h in vitro trial used the Ankom RF gas production system, with rumen fluid from 3 dry dairy cows. Duplicate samples were weighed in F57 fiber bags and placed in triplicate bottles. Samples for VFA analysis were collected at 24 h. Data were analyzed with MIXED procedures in SAS 9.4. Orthogonal contrasts were used to evaluate processing. The DM degradation and gas production were greatest for SOY meals (Table 1). Total VFA and isovalerate were greatest for SOY-CP. Solvent extracted meals had greater acetate. Cold pressed meals had greater propionate. Greater butyrate was found for SOY-CP and CAR-CP. Solvent extracted meals had greater acetate to propionate ratio. Differences in rumen utilization and fermentation of the different plant sources were found, with varied impacts within meal source related to oil extraction methods.

Key Words: carinata meal, rumen fermentation, oilseed meals

M181 Effects of a high-protein corn product on production responses in mid-lactation dairy cows.

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An experiment was conducted to assess the effects of a high-protein corn product (56% crude protein; CP) relative to other sources of protein on the lactation performance of dairy cows. Twenty-four Holstein cows (620 ± 47.7 kg of body weight, 98 ± 34 d in milk, 2.28 ± 0.46 lactations; mean ± SD) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows were individually fed one of 4 diets with a different protein concentrate

Table 1 (Abstr. M180).

Item	Feedstuff							*P*-value	
	CAR-SE	CAR-CP	LIN-SE	LIN-CP	SOY-SE	SOY-CP	SEM	Feed	SE vs. CP[1]
DM deg, %	46.3^{c}	49.3^{b}	30.8^{d}	50.4^{b}	58.9^{a}	61.4^{a}	0.74	<0.01	<0.01
24-h gas production, mL/100 mg of DM	41.8^{b}	38.8^{bc}	27.0^{c}	45.5^{b}	72.4^{a}	77.3^{a}	4.50^{6}	<0.01	0.28
Total VFA, m*M*/g of DM	16.8^{c}	14.2^{c}	15.9^{c}	19.6^{ab}	18.8^{b}	26.1^{a}	1.32	<0.01	0.05
Acetate, % of total VFA	63.2^{ab}	57.3^{d}	64.8^{a}	58.5^{b}	62.6^{a}	58.1^{bc}	0.90	<0.01	<0.01
Propionate, % of total VFA	25.2^{b}	29.5^{a}	25.4^{b}	30.6^{a}	26.3^{b}	28.9^{a}	0.64	<0.01	<0.01
Butyrate, % of total VFA	8.4^{b}	9.4^{a}	8.0^{b}	8.4^{b}	8.2^{b}	9.3^{a}	0.18	<0.01	<0.01
Isovalerate, % of total VFA	2.55^{bc}	2.37^{d}	1.80^{e}	2.40^{dc}	2.76^{b}	3.56^{a}	0.097	<0.01	<0.01
Valerate, % of total VFA	0.68	0.18	0.08	0.13	0.14	0.18	0.210	0.36	0.44
Acetate:propionate	2.77^{a}	1.95^{b}	2.84^{a}	2.09^{b}	2.75^{a}	2.01^{b}	0.134	<0.01	<0.01

[a–d]Values with unlike superscripts differ by $P < 0.05$ using Tukey's test.

[1]Contrast *P*-value for solvent-extracted versus cold-pressed processing.

source during each 28-d period, including: soybean meal (SBM), high-protein corn product (HPCP), soybean meal with rumen-bypass protein (SBMBP), and canola meal with rumen-bypass protein (CANBP). Diets were formulated for equal concentrations of CP (17%) and balanced to meet lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. Data were analyzed with mixed models accounting for diet, period, and cow (random), and differences between treatment means were determined using Tukey's HSD. The CANBP diet increased dry matter intake ($P < 0.01$) compared with SBM and HPCP (28.9 vs. 27.6 and 27.3 ± 0.46 kg/d). Treatment affected milk yield ($P < 0.001$), as SBMBP and CANBP increased yield compared with SBM (42.0 and 42.4 vs. 39.9 ± 1.79 kg/d), but HPCP decreased milk yield compared with other treatments (37.4 ± 1.79 kg/d). The SBM diet increased milk protein concentration relative to HPCP (3.08 vs. 3.00 ± 0.04%; $P < 0.01$). Both SBM and SBMBP increased milk urea nitrogen (MUN) compared with CANBP (11.7 and 11.6 vs. 10.8 ± 0.34 mg/dL, $P < 0.01$), but HPCP decreased MUN compared with other treatments (10.0 ± 0.34 mg/dL). The HPCP diet reduced protein, lactose, and solids nonfat yields compared with all other treatments ($P < 0.001$). There was no evidence of treatment effects on body weight ($P = 0.89$) or body condition score ($P = 0.19$) change. In conclusion, the HPCP diet reduced milk and components yields and MUN, possibly due to lower amino acid content as a percentage of crude protein, lower protein digestibility, or both.

Key Words: milk yield, dietary protein, formulation

M182 Assessment of the capacity of certain mycotoxin binders to adsorb amino acids. A. Kihal, M. Rodriguez-Prado, C. Godoy, C. Cristofol, and S. Calsamiglia*, *Universitat Autonoma de Barcelona, Bellaterra, Spain.*

The objective of the study was to evaluate the capacity of 6 mycotoxin binders to adsorb 4 different essential amino acids. The experiment was conducted in in vitro conditions to simulate a post ruminal digestion model in 2 phases: first phase to simulate the gastric digestion with pepsin, malic acid, citric acid, acetic acid and lactic acid at pH 3; and a second phase to simulate intestinal digestion with bile salts and pancreatin extract at pH 6.5. The experimental design was a factorial 6 × 4 with main factors being mycotoxin binders (bentonite, clinoptiolite, sepiolite, montmorillonite, active carbon and yeast cell walls) and amino acids (lysine, methionine, threonine and tryptophan). Amino acids were incubated separately (Study 1) and together (Study 2) with each mycotoxin binder. The average adsorption in Study 1 was high (49%), with the highest adsorption for clinoptiolite (56%) and the lowest for sepiolite (44%). For the adsorption of amino acids, tryptophan (63%) was adsorbed the highest and lysine (36%) the lowest. Adsorption of threonine was different among mycotoxin binders being lowest for active carbon (20%), and for tryptophan being lowest in montmorillonite (40%), and highest in clinoptiolite (78%) and active carbon (77%). In Study 2, the average adsorption was lower than in Study 1 (19%), suggesting that there was competition for the adsorption surface. Adsorption was highest for the yeast cell wall (22%) and lowest for clinoptiolite (4%). For the adsorption of amino acids, threonine (22%) was the highest and methionine (16%) the lowest. Adsorption of tryptophan was different among mycotoxin binders being lowest for clinoptiolite (0%), bentonite (2%) and sepiolite (10%), and highest for yeast cell wall (49%). For methionine, adsorption was lowest for clinoptiolite (5%) and highest for montmorillonite (25%). Mycotoxin binders have a high degree of adsorption of amino acids, which may limit their bioavailability. Results also suggest that the adsorption is competitive among amino acids.

Key Words: mycotoxin binders, amino acid

M184 The effect of supplemental amino acids and fatty acids on the yield of milk and milk components of mid- to late-lactation dairy cows. A. N. Negreiro*[1], M. M. Western[1], J. de Souza[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined the relationship between amino acid (Met and Lys) and fat supplementation on the yield of milk and milk components of mid- to late-lactation dairy cows. Thirty-six Holstein cows (154 ± 52 DIM) were used in a truncated Latin square with a 2x2 factorial arrangement of treatments with 2 consecutive 35 d periods, with the final 5 d used for sample and data collection. Treatment diets were: 1) Control diet containing no supplemental fat or amino acids (diet contained 86 and 89% of predicted Met and Lys requirements, respectively; CON); 2) Diet containing supplemental fat (1.5% diet DM; 83% C16:0-enriched supplement) and no amino acid supplementation (FA); 3) Diet containing amino acid supplementation (105 and 98% of predicted Met and Lys requirements, respectively) and no supplemental fat (AA); 4) Diet containing supplemental fat and supplemental amino acids (FA+AA). The statistical model included the random effect of cow and fixed effects of supplemental fat, supplemental amino acids, period, and their interactions. There was no effect of treatment on body weight, body condition score change, protein yield, or energy-corrected milk. No interactions between amino acid and fat supplementation were observed for DMI and yields of milk and milk components. Overall, compared with CON, amino acid supplemented diets decreased milk yield (43.6 vs 45.1 kg/d, $P < 0.01$) and increased DMI (31.9 vs 31.3 kg/d, $P < 0.01$), milk fat content (3.68% vs 3.52%, $P < 0.01$), and milk protein content (3.25% vs 3.17%, $P < 0.01$). Compared with CON, fatty acid supplemented diets increased milk fat content (3.66% vs 3.52%, $P < 0.01$) and milk fat yield (1.60 vs 1.56 kg/d, $P = 0.03$). Compared with CON, fatty acid supplemented diets tended to increase 3.5% fat-corrected milk (45.2 vs 44.8, $P = 0.09$). Both amino acid and fatty acid supplemented diets tended to increase body condition score compared with CON (3.42 vs 3.38, $P = 0.09$; and 3.42 vs 3.38, $P = 0.06$, respectively). In conclusion, amino acid supplementation to mid- to late-lactation cows increased milk fat and protein content but did not increase yields. Fatty acid supplementation increased milk fat content and yield and tended to increase 3.5% fat-corrected milk yield.

Key Words: amino acid, dairy cow, fatty acid

M185 Effects of precision essential amino acid formulation on a metabolizable energy basis for lactating dairy cows.

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To optimize N utilization in dairy cattle, AA balancing is an approach to improve productive efficiency. Relationships between absorbed AA supply (AAS) and metabolizable energy (ME) intake have been described and can be further derived to create an essential AA (EAA) requirement relative to ME. Variation exists around the EAA to ME values, and questions involve both methodological and biological variance. The objective of the study was to evaluate differences in animal performance when grams of AAS to ME are varied around the optimum value for each EAA. Lactating Holstein cattle (n = 144; 2.9 ± 1.4 lactations, 92 ± 24 DIM) were blocked in 16 cow pens (n = 9) by DIM and parity and enrolled in a 114-d longitudinal study. Pens were fed a common diet during a 14-d covariate period and randomly assigned to 1 of 3 treatment diets (Negative [NEG], Neutral [NEU], and Positive [POS] treatments) over the 100 d treatment period. Diets were formulated to be isocaloric, varying only in the level of EAA fed and rumen N balance was positive for all diets using CNCPS v7. Cattle on NEU (14.5% crude protein [CP]) were fed according to previously calculated optimal grams of AAS to ME, whereas NEG (14% CP) and POS (16% CP) were formulated for ± 1 standard deviation relative to NEU, respectively. Results were analyzed using an ANOVA mixed model in SAS (v.9.4). Cattle fed POS made more energy corrected milk (ECM, 45.2 ± 0.5 kg) than NEG cattle (41.1 ± 0.5 kg, $P < 0.01$) and tended to make more than NEU cattle (43.8 ± 0.5 kg, $P = 0.10$). Milk true protein yield was greater in both NEU (1.26 ± 0.01 kg) and POS (1.29 ± 0.01 kg) relative to NEG (1.16 ± 0.01 kg, $P < 0.01$), but similar for NEU and POS ($P = 0.21$). Yield of milk fat was greater in POS (1.65 ± 0.03 kg) compared with NEG (1.54 ± 0.03 kg, $P < 0.01$) but not NEU (1.60 ± 0.03 kg, *$P = 0.24$).* Dry matter intake for POS (28.5 ± 0.2 kg) tended to be higher than NEG (27.4 ± 0.2 kg, $P = 0.10$), and similar to NEU (28.2 ± 0.2 kg, $P = 0.68$). Results indicate an increase in ECM and protein yield when cattle were fed NEU compared with NEG, and a marginal increase when fed POS compared with NEU. Results suggest a greater efficiency of nitrogen at NEU level over NEG treatment.

Key Words: amino acid, energy, nutritional model

M186 Rumen-protected methionine supplementation on productive performance and plasma amino acids concentration on early lactation of high-producing dairy cows.

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Methionine (MET) is the first limiting amino acid (AA) in corn silage-based diets when metabolizable protein is supplied with soybean meal. The aim of this study was to determine the effect of rumen-protected MET (RPM) supplementation on productive performance and plasma AA concentration of lactating cows. Seventy-six multiparous Holstein lactating cows, housed in a freestall barn and milked 3× daily were divided in 2 groups and stratified by milk yield (MY) and DIM (39.1 ± 6.8 kg/d of milk and 65 ± 28 DIM). Cows were assigned to 1 of 2 treatments in a randomized complete block design with a 2-wk covariate period followed by a 10-wk treatment. Treatment diets were CON (n = 38) basal diet (3.77LYS:1MET); and MET balanced with an RPMet source, RPM (n = 38; basal diet + 23 g of Smartamine M, Adisseo SA; 2.97LYS:1MET). MY was recorded daily and individual milk samples were taken every 2 weeks throughout the experiment to determine milk composition and milk components yield. A subset of 24 cows had blood samples collected on +30 d to measure plasma AA concentration. Body weight (BW) and body condition score (BCS) were measured at the beginning and the end of the experiment. Data were analyzed using the MIXED procedure of SAS with repeated measures. MY (41.7 vs. 40.1 kg/d; $P = 0.026$), ECM (41.0 vs. 38 kg/d; $P \leq 0.01$), milk protein yield (1.30 vs. 1.18 kg/d; $P \leq 0.01$) and milk fat yield (1.42 vs. 1.29 kg/d;$P = 0.02$) were greater for RPM compared with CON group. Milk percentage of protein (3.14 vs. 2.97%; $P \leq 0.01$) and casein (2.39 vs. 2.28%; $P \leq 0.01$) were also greater for RPM with tendency ($P = 0.06$) for higher milk fat percentage compared with control (3.41 vs. 3.21%). Supplementation with RPM did not affect MUN and SCC. Both treatments had similar BW, but the CON tended ($P = 0.08$) to have higher BCS. Plasma Met concentration was greater for RPM compared with CON (29.6 vs. 18.4 µ*M*; $P < 0.01$), with no differences observed in Lys, and His concentration. Supplementation with RPM improved productive performance by increasing milk yield and components, and this data suggests that dietary AA utilization with adequate level of LYS is improved by adjusting the MET level.

Key Words: amino acid, milk protein, milk yield

M187 Effect of biochanin A on urea and amino acid hydrolysis and bacteria community in rumen.

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Plant bioactive compounds have been chosen as alternatives to antibiotics to promote animal productivity. Biochanin A is a type naturally occurring bioactive compound. It is O-methylated isoflavone and found in red clover, alfalfa sprouts, and other legumes. The aim of this study was to determine the effect of biochanin A on rumen microbial fermentation and composition. The experimental design consisted of a control (no additive) and biochanin A (0.03 mg/mL) and each treatment was triplicated. Anaerobic medium was prepared with sterile rumen fluid and McDougall buffer in tubes. The tubes containing anaerobic medium were inoculated with rumen mixed microbes, and cultured at 39°C for 24 h as the first generation. Then, the culture from the first generation was transferred by inoculation to new anaerobic tubes with the medium incubated as the second generation. The transferring was continued until the fourth generation. The fermentation fluid was sampled for the detection of VFA, ammonia and amino acids at 0, 12, 16, 24 h during culturing. The gas production in the tube was detected by pressure sensor. Different doses of biochanin A were mixed with urea to evaluate its inhibition of urease activity and calculate half-maximal inhibitory concentration (IC50). The bacterial composition was carried out by 16S rRNA gene sequencing. The significant differences between 2 treatments were analyzed by ANOVA in SAS. Results showed that biochanin A increase microbial gas production by 2.5% ($P < 0.05$), but no effect on VFA production. Microbial urease activity was inhibited ($P < 0.05$) by biochanin A with the IC50 of 319.8 μmol/L. Biochanin A also inhibited ($P < 0.05$) the degradation rate of Val, Lys, Met, Leu and total amino acids by 55.84, 37.03, 12.63, 11.74, and 18.00%, respectively. Inhibition of urease activity and amino acid decomposition by biochanin A resulted in a reduction ($P < 0.05$) of ammonia by 28%. The 16S rRNA gene sequencing showed that biochanin A reduced ($P < 0.05$) the abundance of proteolytic bacteria *Prevotella* and *Streptococcus*. Therefore, biochanin A reduced the production of ammonia by inhibiting proteolytic bacteria and its decomposition of urea and amino acids activity.

Key Words: biochanin A, rumen, amino acid

M188 Effects of rumen-protected amino acids supplementation during prepartum and postpartum periods on performance of transition dairy cows.

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We examined effects of prepartum (3 wk) and postpartum (3 wk) supplementation of rumen-protected lysine (RPLys; USA Lysine; Kemin Industries Inc.; 42% Lys bioavailability) and methionine (RPMet; MetiPEARL; Kemin Industries Inc.; 29% Met bioavailability) on performance of transition cows. Holstein prepartum cows (88) were blocked by parity and expected calving and assigned to 1 of 4 treatments arranged factorially. Treatments were a prepartum diet with (Pre+) or without (Pre−) RPLys (10 g digestible Lys) and RPMet (4 g digestible Met) followed by postpartum diet with (Post+) or without (Post−) RPLys (26 g digestible Lys) and RPMet (11 g digestible). Prepartum, only 2 treatments were applied (Pre+ and Pre−; n = 44/treatment), but postpartum cows received treatments of Pre−Post−, Pre−Post+, Pre+Post−, or Pre+Post+ (n = 22/treatment). Data were analyzed using the MIXED procedure of SAS where block within parity was random effect and treatment, parity, day, and their interactions were fixed effects. During the prepartum period, treatment did not affect DMI and BW. During the postpartum period, milk protein content was greater (3.23 vs. 3.11%; $P = 0.01$) for Post+ compared with Post- independent of prepartum treatment. However, DMI, BW, milk yield, and yields of milk components were not affected by Post+ vs. Post−. No effects of prepartum treatment on postpartum performance were observed. However, Pre+ decreased logSCC (4.60 vs. 4.83/mL; $P = 0.01$) compared with Pre− and this effect continued ($P = 0.04$) for 10 wk after supplementation of RPLys and RPMet ceased. Prepartum cows had or tended to have greater ($P < 0.10$) plasma concentrations of Lys, Met, and branched-chain amino acids for Pre+ vs. Pre−. Cows on Post+ tended to have greater ($P = 0.09$) plasma Lys but have similar Met compared with Post−. In conclusion, effects of prepartum and postpartum supplementation of RPLys and RPMet on performance were minimal except milk protein content. However, prepartum supply of RPLys and RPMet may have potential to lower SCC postpartum.

Key Words: rumen-protected lysine, rumen-protected methionine, transition cow

M189 Further evaluating the plasma free amino acid dose-response technique as a method to assess the metabolizable methionine contributions of analogues of 2-hydroxy-4-(methylthio) butanoic acid.

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A dose-response trial involving abomasal infusion of 2-hydroxy-4-(methylthio)butanoic acid (HMTBa) demonstrated linear responses for plasma methionine (Met), total sulfur amino acids (TSAA) and HMTBa. This trial was to further evaluate and identify remaining weakness in the approach by comparing abomasal infusion of HMTBa (Rhodimet AT88) vs feeding DL-2-hydroxy-4-(methylthio)butanoic acid isopropyl (HMBi) (MetaSmart), a known source of metabolizable Met. Five lactating Holstein cows were used in a 5 × 5 Latin square with 7-d periods. Treatments were negative control, 12 and 24 g/d of infused Met equivalents from HMTBa, and 24 and 48 g/d of fed Met equivalents from HMBi. Infusions were continuous except during milking. Cows were fed at 8-h intervals; HMBi treatments were divided into 3 equal portions, mixed with 1.5 kg of the basal TMR and eaten within 20 min. Blood samples were collected the last 3 d of each period at 2, 4, 6 and 8 h after the morning feeding, cooled, and centrifuged. Plasma was used for HMTBa analysis and deproteinized plasma for AA analysis. Data were analyzed using the PROC MIXED and PROC REG procedures of SAS with period and treatment by level as main effects. Increasing amounts of infused HMTBa resulted in linear increases in plasma Met, TSAA, and HMTBa, while increasing amounts of fed HMBi resulted in only a trend for linear increases in these metabolites (Table 1). Previous research indicated undetectable concentrations of HMTBa in plasma of control cows when infused with increasing amounts of HMTBa. Others have observed rapid increases in plasma HMTBa after bolus meals of HMTBa, with concentrations not always returning to baseline values by

Table 1 (Abstr. M189). Effect of infused HMTBa and fed HMBi treatments on plasma sulfur metabolites (μ*M*)

	HMTBa			HMBi			Linear contrast *P* =	
Item	0	12	24	24	48	SEM	HMTBa	HMBi
Met	23.5	25.4	27.8	24.2	25.6	1.16	0.02	0.07
HMTBa	0.266	2.086	4.968	1.396	2.308	0.936	0.003	0.13
TSAA	99.9	105.2	108.5	103.9	107.6	2.95	0.02	0.06

8 h after the meal. We suggest a need for longer experimental periods and for mixing dietary treatments with the entire diet.

Key Words: dose-response, 2-hydroxy-4-(methylthio)butanoic acid (HMTBa), DL-2-hydroxy-4-(methylthio)butanoic acid isopropyl (HMBi)

M191 Effects of absorbed amino acids on the milk fat yield: A meta-analytic approach.

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Absorbed AA can be used for maintenance, growth, pregnancy, and milk yield. We hypothesized that certain AA may also affect milk fat yield in dairy cows. The objective was to evaluate the effects of individual absorbed amino acids (AA) on milk fat yield of lactating dairy cows. A database of 175 studies (724 treatment means) conducted with dairy cows (BW = 604 ± 46 kg, days in milk = 135 ± 53.6) fed diets containing 50.9 ± 10.2% forage (starch = 27.7 ± 6.96, neutral detergent fiber = 32.1 ± 5.50, crude protein = 16.5 ± 2.502, rumen degradable protein = 11.39 ± 1.725, % DM) was used. Dietary composition of each AA was calculated using the Cornell Net Carbohydrate and Protein System feed library. The absorbed amount of each AA (g/d) was calculated using equations for estimating 1. AA supplied by the microbial protein using rumen degradable protein, 2. AA supplied by rumen undegradable protein, and 3. the intestinal digestibility of each AA (Myers et al., 2019). Statistical analysis was conducted using R (v. 3.5.1, 2019). A linear mixed model was fitted (lme4) using a random effect of study and weighted using the square root of the number of animals represented in each treatment. Milk fat was positively related to absorbed Met and Ile yielding more than 2 g/d of milk fat per g of absorbed AA, while absorbed Lys was associated with a milk fat response of 0.9 g/d of milk fat per g of Lys ($P < 0.05$). No other essential AA evaluated (Arg, His, Leu, Phe, Thr, Trp, Val) significantly affected milk fat yield (Table 1). Our results showed that absorbed Met, Lys, and Ile should be considered as potential drivers of milk fat yield in dairy cows.

Table 1 (Abstr. M191).

Predictor (g/d)	Estimate	SE	95% CI	*P*-value
Intercept	426.251	43.348	341.29–511.21	<0.001
Absorbed Arg	−0.482	0.884	−2.22–1.24	0.580
Absorbed His	−2.127	1.571	−5.21–0.95	0.176
Absorbed Ile	2.185	0.962	0.30–4.07	0.024
Absorbed Leu	−0.538	0.361	−1.25–0.17	0.137
Absorbed Lys	0.927	0.469	0.01–1.85	0.049
Absorbed Met	2.066	0.628	0.84–3.30	0.001
Absorbed Phe	−0.388	0.709	−1.78–1.00	0.585
Absorbed Thr	2.003	1.360	−0.66–4.67	0.141
Absorbed Trp	6.034	4.223	−2.24–14.31	0.154
Absorbed Val	0.695	1.348	−1.95–3.34	0.606

Key Words: absorbed amino acid, methionine, lysine

M192 Comparison of two sources and levels of rumen-protected methionine on plasma concentration and mammary clearance of methionine and milk production in dairy cows.

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The objective of this study was to compare the effect of 2 levels of rumen-protected Met (RPM) from a prototype (72% D,L Met; Balchem Corp.) with a commercial product on plasma concentration and mammary clearance of Met and milk production in dairy cows. Twenty multiparous, lactating cows were used in a 5 × 20 Latin rectangle (period as rows and cow as columns) with 14 d periods. Cows were randomly assigned to the following treatments: 1) control (CTL; 0 g of RPM), 2) 6.0 g of MP-Met from Smartamine M (SM6), 3) 12.0 g of MP-Met from Smartamine M (SM12), 4) 6.0 g of MP-Met from RPM prototype (PT6), and 5) 12.0 g of MP-Met from RPM prototype (PT12). The basal diet was formulated for −9.5 g/d in MP-Met balance (2.02% of MP-Met supply), 16.0% CP, and 52% forage (corn and ryegrass silages) to 48% concentrate (corn grain and soybean meal). Treatments were top-dressed twice daily. Milk and blood samples were collected and analyzed. The RPM treatments increased ($P = 0.01$) milk protein percent compared with the CTL treatment. Relative to SM12, the SM6, PT6, and PT12 treatments did not differ in milk protein percent (3.19, 3.17, and 3.17 vs. 3.27%). Relative to CTL, the SM6, PT6, and PT12 treatments numerically improved milk protein percent. The SM6, SM12, and PT6 treatments showed no differences in milk fat percent. Relative to PT12, the SM12 treatment increased ($P = 0.03$) milk fat percent (4.70 vs. 4.42 ± 0.10%). Relative to SM12, the PT12 treatment tended to increase ($P = 0.08$) milk lactose yield (1.83 vs. 1.78 ± 0.03 kg/d). Relative to CTL, the RPM treatments increased ($P = 0.01$) Met concentration in coccygeal vessels and the abdominal mammary vein. Relative to RPM treatments, the CTL treatment increased ($P < 0.01$) mammary clearance rate of Met. In summary, the RPM treatments improved milk protein percent. The prototype at both levels sustained milk protein percent at the same level as SM6 and SM12. The RPM treatments increased plasma concentration and mammary capture of Met, which may have contributed to greater milk protein percent.

Key Words: dairy cow, milk protein, rumen-protected methionine

Teaching/Undergraduate and Graduate Education

M193 Overcoming language barriers in animal and veterinary science education: Practical and innovative solutions. E. Vargas-Bello-Pérez*[1] and L. E. Hernández-Castellano[2], [1]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark,* [2]*Department of Animal Science, AU Foulum, Aarhus University, Tjele, Denmark.*

In recent years academic mobility and cultural diversity have constantly increased in higher education institutions. Consequently, language barrier has become an important issue in the learning process in the cases of English-speaking regions such as the United States and Europe, where there are 24 official languages. In animal and veterinary sciences, the complexity of the vocabulary used during the learning process exacerbates this problem. For instance, species names (e.g., *Bos taurus*, *Staphylococcus aureus*, *Fasciola hepatica*), muscle names (e.g., longissimus dorsi, latissimus dorsi, biceps brachii), organ parts (e.g., corpus callosum, hippocampus) or terms such as ad libitum, in vivo, in vitro, in situ, postpartum, which are Latin. In addition, the use of either technical words (e.g., methylenedioxymethamphetamine, depolarizability, gluconeogenesis), words uncommonly used in English (e.g., aliquot, supernatant, centrifuge, rumination) or technical concepts (e.g., fresh cow, dry cow, white veal) can also challenge the transfer of knowledge from lecturers to students. To overcome this problem, lecturers must create an interactive and engaging classroom atmosphere, provide a reduced and concise lecture and promote the interaction among students through the promotion of discussion. In our experience, the use of e-tools, electronic polling, and quiz tools (such as Poll Everywhere, Glogster, Kahoot and Spreaker) also contributes to student's interaction. We have observed that when teaching complex biological processes, hands-on laboratory exercises (HLE) and case-based activities (CBA) also contribute to overcome language barriers between students and lecturers when teaching complex biological process. For example, in farm animal reproduction, students may study semen evaluation by HLE, whereas in CBA students can learn specific parameters of a real case to understand estrus cycle, manipulation, and synchronization. In conclusion, lecturers need to create an interactive classroom by the use of direct and concise language and the use of new technologies to promote learning in an international classroom.

Key Words: education, e-tools, language

CSAS Symposium: From Data to Decisions—The Next Step for Technology in Dairy Production

16 Precision feeding in automated milking systems: Current gaps and future needs. G. B. Penner*, *University of Saskatchewan, Saskatoon, SK, Canada.*

Adoption rates of automated milking systems (AMS) continue to rapidly increase in North America. There is no doubt that feeding management for cows housed in facilities with AMS is different than for herds using parlor-based milking systems, yet there are few studies that have critically evaluated feeding management practices for AMS systems. The central dogma for feeding management for cows milked with AMS is that a pelleted concentrate must to entice cows to enter the AMS with greater quantities having a stimulatory effect. Moreover, feeding a basal PMR that has a low nutrient density not only stimulates cows to seek the pellet in the AMS, but also allows for precision feeding approaches by allocating the type or quantity of pellet provided at a cow-level in the AMS. While these concepts are appealing, the ability to stimulate voluntary visits to the AMS and increase milk yield or milk component yield with greater concentrate allocation in the AMS has been variable. As a result, studies evaluating precision feeding management have not consistently reported positive responses. The lack of a positive response for voluntary visits and increased milk yield may be explained as follows: (1) there is a difference between the quantity of pellet programmed for delivery and that actually delivered in the AMS; (2) that not all concentrate delivered is consumed; and (3) that dairy cattle reduce intake of the partial mixed ration with increasing consumption of the concentrate delivered in the AMS. Further development of AMS to capture information related to the amount of AMS concentrate delivered, refused, and partial mixed ration eating behavior and or consumption is necessary to move forward toward precision feeding approaches.

17 The use of precision technologies to monitor, selectively treat, and manage nutrition of dairy calves. J. H. Costa*[1], H. W. Neave[2], M. M. Woodrum[1], and M. C. Cantor[1], [1]*Dairy Science Program, Animal and Food Sciences, University of Kentucky, Lexington, KY,* [2]*Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada.*

Monitoring individual calves continuously using precision dairy technologies is rapidly becoming an important management tool on-farm for dairy calves; automated monitoring of feed intake and feeding behavior is particularly common. We present some of the technologies available, and 2 key uses of technology that show promise to refine dairy calf management through individual monitoring and decision-making that we argue will improve calf health, performance and welfare on farms. First, bovine respiratory disease complex (BRD) and diarrhea are prevalent illnesses in dairy calves that are critically important to detect early to avoid significant mortality risk. Studies in dairy cattle have shown that feeding behavior and intake change in the days before clinical signs of illness are visually detected (such as metritis, ketosis, and mastitis). Currently, treatment of sick dairy calves relies on caretaker experience to correctly visually identify signs of disease that are already present. We discuss how changes in feed intake and behavior using data from automated feeders can identify pre-clinical signs of illness in dairy calves, and how this data may also reveal the extent of treatment or recovery success. Second, data from technologies can be used to support nutritional management decisions and selection of calves from an early age. Automated feeders can be used to wean calves automatically based on their intakes of grain, rather than weaning calves at a set age. We describe the large variability in grain intakes and weaning ages when weaned using this method, and how individual characteristics may be used to identify those calves that will successfully wean early. This individualized feeding and weaning method offers the best chance of success for all calves, by allocating milk away from calves ready to wean and toward calves requiring more time on milk before weaning. Overall, the potential uses of precision technology tools are likely to become more common on farms, and consequently improve calf management strategies that affect the success of future dairy replacement animals.

Key Words: precision nutrition, weaning, personality traits

18 The integration of sensor technologies to optimize reproductive management on dairy farms. R. L. A. Cerri*[1], T. A. Burnett[1], A. M. L. Madureira[1], J. Bauer[1], B. F. Silper[1], L. B. Polsky[1], M. Kaur[1], P. P. Souto[1], W. A. Gomes[1], K. Pohler[2], R. F. Cooke[2], S. J. LeBlanc[4], and J. L. M. Vasconcelos[3], [1]*Applied Animal Biology, University of British Columbia, Vancouver, BC, Canada,* [2]*Department of Animal Sciences, Texas A&M University, College Station, TX,* [3]*Department of Animal Production, São Paulo State University, Botucatu, Brazil,* [4]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

Studies from the past 6 years have demonstrated how estrus events and intensity are associated with ovulation, ovarian and uterine function, and fertility. Data from recent studies involving spontaneous and induced estrus have shown that greater relative increase and longer duration of estrus, captured by different activity monitors, significantly improve pregnancy/AI (around 10 to 15% units across different studies) and decrease pregnancy losses. Intensity and duration of estrus were surprisingly only weakly associated with follicle diameter and concentration of estradiol at estrus. The likelihood of ovulation was greater for estrus episodes with high vs. low relative increase in physical activity. Data from 2 studies showed that estrus behavior near AI also modified the expression of genes related to the immune system, adhesion molecules and prostaglandin synthesis in the endometrium and those related to apoptosis, progesterone synthesis and prostaglandin receptor in the corpus luteum. Most recently, studies from the United States and Canada demonstrated that reproductive programs using activity monitors and emphasizing detection of estrus can be successful and comparable to intensive timed AI protocol-based programs. One last study from our laboratory also concluded that the inclusion of GnRH at AI greatly improved P/AI from cows bred upon estrus events, but only those of low intensity, which showed the potential to use activity monitors as a tool in targeted protocols. Quantitative information from estrus events could be used to improve estrous detection quality and develop decision-making strategies at the farm level. Further studies in this field should aim to (1) better understand ovarian, embryo and endometrium mechanisms associated with either the expression or intensity of estrus and, 2) refine the collection of phenotypes related to estrus (i.e., relative increase, absolute increase, baseline levels, duration, and repeatability within cow) to improve estrous detection and possibly genetic selection.

Key Words: activity monitors, dairy cow, estrous expression

19 Integration of big data from multiple sources to improve dairy herd performance and sustainability.

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The amount of data collected on dairy farms will expand exponentially with computerized milking systems and sensors, including cameras and scene recognition algorithms to capture animal behavior. In addition, the volume of data generated by various organizations such as milk recording (DHI) and genetic evaluation organisations, veterinary clinics and feed suppliers will keep increasing. For example, mid-infrared analysis of milk samples produces data points for more than 1000 wavelengths, allowing for new information such as fatty acid profiles. Building data pipelines between these silos and connecting them to on-farm sensing devices data has a potential to improve herd performance and sustainability. Although redundant to a certain extent, all sources can contribute on their own. For example, spectral signatures of bulk tank milk samples provide precise herd information every other day, while milk recording leads to detailed data on each cow about 10 times per year. On-farm devices produce data in a less controlled way but at a much higher frequency, increasing considerably the volume of phenotypic information. Once integrated, all this data has the potential to improve relevant traits (e.g., feed efficiency, milk quality, and animal health and welfare) while improving labor efficiency, decreasing environmental footprint, and increasing ROI on digital investments. Data integration can also lead to new benchmarks, research, and innovation, large-scale monitoring such as health issues, and transparency for consumers. However, with so much data, producers will need assistance and guidance toward the most critical aspects of their business to focus on. This can be achieved through advanced analytics. First, farm specific, highly dimensional predictive algorithms based on machine learning will detect and diagnose anomalies, weaknesses, or opportunities for process improvement. The models and their predictions will need to be explainable. Second, and even more challenging, prescriptive algorithms using artificial intelligence techniques will recommend ways to remediate problems or improve upon a situation. Additional challenges for the dairy industry will include the adoption of advanced computational platform to process massive data sets, and protocols for cybersecurity and ethical data handling.

20 Monitoring dairy cow feeding behavior to optimize nutritional management.

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The dairy industry has an increasing availability of equipment that is readily available for the automation of management tasks, including milking and feeding, as well as the monitoring of dairy cow behavior. Such automation not only has the ability to improve production and time efficiency on farm, but also increases our ability to monitor individual cows. Much research has been focused on the use of individual behavioral monitoring to detect health disorders, both in occurrence and in advance of clinical symptoms. This, in turn, has the potential to allow producers to identify and implement prevention and treatment protocols at earlier time points. There is, however, also an opportunity to use behavioral monitoring to inform management decisions on farm. Given the inherent relationship between feeding behavior, feed intake, dietary composition, and nutritional management, there is opportunity to use information of feeding behavior to optimize nutritional management. This presentation will, thus, specifically focus on how when, and what cows eat and ruminate their feed and the relationship of that behavior with nutrient intake, health, and production. Specific examples will then be provided to demonstrate the utility of monitoring changes in said behavior, with the goal of informing decisions related to nutritional management. Limitations and challenges associated with such monitoring will also be discussed. Long-term, it is anticipated that through precision monitoring of feeding behavior, particularly with the aid of automation, dairy producers will be able to make more timely decisions for altering and adjusting nutritional programs, both at a cow and herd level, to optimize cow health, welfare, and production.

Key Words: automation, feeding behavior, nutrition

ADSA Graduate Student Dairy Foods Oral Competition

21 Hydrodynamic cavitation: A clean label approach for ice-cream formulation. J. Sim*, H. Bonnemann, L. Metzger, and S. I. Martinez-Monteagudo, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Trends in consumer's health- and wellness-lifestyle have perceived stabilizers as undesirable ingredients. Stabilizer plays an essential role in the manufacturing of ice cream because it reduces the growth of ice crystals and prevents migration of free water. Some stabilizer has been chemically derivatized to improve its solubility in water. This derivatization dose not meet consumer's demands for the clean and natural ingredients. Our research aims was to utilize hydrodynamic cavitation (HC) to reduce the concentration of stabilizers in ice-cream mix. The central hypothesis of this research is that by appropriate choice of temperature, flow rate, and rotor speed, it is possible to regulate fat destabilization (partial coalescence and agglomeration of fat globules), and thus create an emulsion, utilizing protein rather than relying on stabilizers. An ice-cream formulation of industrial interest was used for our experiments that contained different the concentrations of the stabilizer mix (0, 0.07, 0.14, 0.21, 0.28%). The stabilizer mix consisted of guar gum, mono- and diglycerides, locust bean gum, carrageenan, and polysorbate 80. The manufactured formulations were analyzed in terms of particle size distribution, microscopy and dynamic mechanical spectra. A control formula was manufactured with conventional homogenization and heat treatment and was compared with the treatments. The obtained strain sweep showed a viscoelastic region within the same range of strain (0.05–28%). No difference was observed in the viscoelastic region for samples with 0.28% of the stabilizer mix and treated with HC (100 L/h and 3600 rpm) when compared with the control formulations. In contrast, an increase of 6-fold in the linear section of the viscoelastic region was observed in formulations with 0.14% of the stabilizer mix and treated with HC. Interestingly, the viscosity did not significantly change different within formulation varied from 180 to 200 cP at a shear rate of 10 s^{-1}. Dynamic rheological measurements as strain sweeps exhibited increased product stability with the application of HC. The information generated in this study may help to develop strategies for the formulation and manufacture of ice cream with reduced concentration of stabilizers

Key Words: clean label, hydrodynamic cavitation, ice-cream formulation

22 Improving the functionality of lower protein dairy streams for enhanced emulsion stability. C. Hinnenkamp*[1], S. Drusch[2], G. Reineccius[1], and B. P. Ismail[1], [1]*University of Minnesota, Saint Paul, MN,* [2]*Technical University of Berlin, Berlin, Germany.*

The industry solution to the increased consumer appetite for protein enriched products has been to utilize whey protein isolate and high whey protein concentrates (WPC) in formulations. However, the production of these high whey protein ingredients generates lower protein streams that are underutilized due to lower functionality. A potential solution is the utilization of targeted modification of WPC in combination with whey protein phospholipid concentrate (WPLC), a lower protein, coproduct, to stabilize emulsions. The use of strategically hydrolyzed whey protein along with WPLC, will enhance antioxidant activity and improve emulsion properties resulting in a low cost, clean label protein solution for high value applications, such as encapsulation of bioactives. Our objective was to enhance the emulsification properties and antioxidant activity of WPC through limited hydrolysis and combine with WPLC to improve the oxidative and physical stability of fish oil emulsions. WPC was hydrolyzed (HWPC) by two enzymes, and the protein/peptide profiles were characterized by gel electrophoresis and size exclusion HPLC. Changes in structural properties, namely surface hydrophobicity and zeta potential, were monitored. Antioxidant activity and emulsification properties were assessed. Fish oil was then emulsified with combinations of WPC, HWPC, and WPLC and stored for one week at elevated temperatures to test physical characteristics and hydroperoxide formation. Limited hydrolysis of WPC (7–12% degree of hydrolysis) improved both antioxidant activity and emulsification properties. Protease M hydrolysates had enhanced emulsification properties due to targeted hydrolysis of α-lactalbumin. Whereas, trypsin targeted β-lactoglobulin reducing the surface hydrophobicity, which maintained the emulsification capacity and improved emulsion stability. Finally, antioxidant activity was improved notably for Protease M hydrolysates. Targeted hydrolysis of WPC combined with WPLC can improve the oxidative and physical stability of fish oil emulsions, thus improving the value of these underutilized dairy products. Future work will focus on expanding utilization of these whey protein systems to microencapsulating fish oil.

Key Words: whey protein phospholipid concentrate, whey protein concentrate (WPC), emulsion stability

23 Manufacturing of process cheese without emulsifying salt using acid curd. A. R. A. Hammam* and L. E. Metzger, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Process cheese products (PCP) are a dairy food prepared by blending dairy ingredients (such as natural cheese, protein concentrates, butter, NFDM, whey powder, and permeate) with nondairy ingredients (such as sodium chloride, water, emulsifying salts, color, and flavors) and then heating the mixture to get a product with an extended shelf-life. Emulsifying salts (e.g., sodium citrate, disodium phosphate) are critical for the functional characteristics of PCP because they improve the emulsification characteristic of casein by displacing the calcium phosphate complexes that are present in the insoluble calcium-paracaseinate-phosphate network in natural cheese. The objective of this study was to manufacture PCP using a combination of acid curd cheese and micellar casein that would provide the required emulsion capacity without the use of emulsifying salts. The acid curd utilized was produced from micellar casein concentrate that was standardized to 3, 6, and 9% protein. In the formulation, the acid curd was blended with micellar casein so that the formula contained a 2:1 ratio of protein from acid curd relative to micellar casein. Additional dairy and non-dairy ingredients (Cheddar cheese, butter, and salt) were also used in the formulation. The PCP was prepared by mixing all ingredients in a kitchen aid to produce a homogeneous paste. Approximately 25 g of the mixture was cooked in a RVA for 3 min at 95°Cwith a 1000 rpm stirring speed during the first 2 min and 160 rpm for the final min. The cooked PCP was then transferred into molds and refrigerated until further analysis. This trial was repeated 3 times using acid curd (produced from 3, 6, and 9% protein MCC). No significant differences ($P > 0.05$) were detected in the cooked viscosity (402–483 cP), hardness (354–384 g), melting area (30.0–31.4 mm) and melting temperature (48–51°C) between PCP made from different acid curd and was similar to typical process cheese produced with conventional ingredients and emulsifying salt. We conclude that PCP can be made with no emulsifying salts when the formulation utilizes a 2:1 ratio of protein from acid curd relative to micellar casein.

Key Words: process cheese, micellar casein, acid curd

24 Clean label: The naturalness perception and acceptance of dairy ingredients. S. Maruyama*, N. Streletskaya, L. Goddik, and J. Lim, *Oregon State University, Corvallis, OR.*

Consumer demand for natural foods continues to grow and dairy processors have answered the calling offering many clean label products. However, regulatory agencies have not clearly defined “natural” labeling, and accordingly the industry and consumers may have different interpretations of the term. The current study aims to measure consumers’ naturalness perception of dairy ingredients and to investigate factors affecting their responses. An online-administered survey asked over 500 frequent yogurt consumers to evaluate naturalness, acceptability, and familiarity of common yogurt ingredients including various sugars, stabilizers, preservatives, and coloring agents. Respondents also rated the acceptability of the ingredients when their functions and sources were given. Importantly, socio-demographic (e.g., gender, income, education, household size) and personal preference (e.g., purchasing habits, ingredient knowledge) information was collected at the end of the survey. In terms of perceived naturalness, we found that coloring agents and preservatives varied substantially within each category, with some ingredients perceived as natural and others as unnatural. In contrast, sugars were perceived as generally natural, whereas all stabilizers were perceived as less natural, with rankings statistically not different within the ingredient category. Further examination of the interquartile ranges for each ingredient revealed that consumers strongly agreed on the degree of naturalness for some ingredients (e.g., vegetable juice, red 40), but not others (e.g., pectin, fructose). Considering acceptability of each ingredient, providing ingredient function had little impact on the ratings. However, providing ingredient source significantly improved the degree of ingredient acceptability. We will discuss these findings along with the impact of heterogeneity in socio-demographic and personal preference on the perceived naturalness and acceptability of common yogurt ingredients.

Key Words: clean label, dairy ingredients, consumer behavior

25 Cloudy vs. foggy: Mystery behind high pressure-induced aggregates of milk proteins for the valorization of cheese whey. A. Marciniak*[2,1], S. Suwal[4,1], G. Brisson[1], M. Britten[3,1], Y. Pouliot[1], and A. Doyen[1], [1]*Department of Food Sciences, Université Laval, Institute of Nutrition and Functional Foods (INAF)/Dairy Science and Technology Research Centre (STELA), Quebec, QC, Canada,* [2]*Department of Food Science and Technologies, Ohio State University, Columbus, OH,* [3]*Saint-Hyacinthe Research and Development Centre, St Hyacinthe, QC, Canada,* [4]*Department of Food Science, Faculty of Science, University of Copenhagen, Copenhagen, Denmark.*

Protein aggregation can be used to improve functionality in certain food systems, especially in gelled systems. However, in beverages application, this phenomenon is generally undesirable because it is usually related to protein insolubility and turbidity. Nonetheless, some research has demonstrated a molecular chaperone-like property of certain milk proteins that helps avoid protein aggregation. Here, we investigated the effect of β-casein (β-CN) on pressure-induced aggregation of whey proteins: β-lactoglobulin (β-LG) and α-lactalbumin (α-LA) using various qualitative and quantitative analyses (turbidity, SDS-PAGE, HPSEC and TEM). Protein model solutions containing different ratios of α-LA, β-LG and β-CN were pressurized by high hydrostatic pressure (HHP). Pressure treatment of β-LG alone generated a highly turbid solution (foggy) containing large aggregates while the addition of both proteins (α-LA and β-CN) at different ratios led to a drastic decrease in turbidity, despite the presence of larger aggregates (cloudy). In fact, TEM analysis showed larger and amorphous aggregates for β-LG with α-LA and β-CN, and globular, denser aggregates for β-LG alone. Further analysis of these aggregates by fractionation (HPSEC) followed by SDS-PAGE showed no β-CN directly involved in β-LG aggregation, suggesting a chaperone-like effect of β-CN under HHP. Our experiments, performed on model dairy solutions, demonstrated that α-LA and β-CN inhibits the formation of insoluble aggregates (decreases turbidity) under HHP treatment of β-LG that could be relevant in milk protein fortified beverages.

Key Words: chaperone protein, dairy protein, high hydrostatic pressure

26 Developing a dairy-based health formulation by combining the bioactive properties of whey protein hydrolysates and probiotic organisms. S. Minj*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Brookings, SD,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

With the increased consumer demand for nutritional foods, it is important to develop novel products with enhanced health benefits and functionality. We developed a spray dried health formulation by encapsulating probiotics in a conjugated whey protein matrix. Samples of whey protein concentrate, isolate and hydrolysates were screened for bioactivities (antimicrobial, antioxidant and antihypertensive activity) and based on highest bioactivities hydrolysate WPH10, was conjugated with maltodextrin to obtain a thermally stable conjugated solution. Probiotic cultures *Bifidobacterium animalis* ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356 were added in the conjugated solution at ratio of 1:1, with spiking level of $\log_{10}$ cfu/mL. The mixture was spray dried in 2-L batch using a Niro drier with an inlet and outlet temperature of 200°C and 90 ± 5°C. To conduct the storage stability, the dried formulation was stored in airtight bottles for 10 weeks at 4, 25, and −18°C, to monitor cell viability, moisture and functionality. The experiments were done in triplicates and one-way ANOVA was applied to differentiate the mean values. In comparison to WPH10 alone, the conjugated WPH10 solution demonstrated higher antimicrobial and antioxidant activities. Following spray drying a higher antimicrobial (18.5 ± 0.57 mm) and antioxidant activity (1268.89 ± 41.9 TEAC μmol/L) was observed with retention in the antihypertensive activity (65.6 ± 0.3%ACE inhibition). The mean probiotic counts in conjugated WPH10 matrix before and after spray drying were 10.37 log cfu/mL and 8.50 log cfu/g, respectively. These counts were maintained for at least 4 weeks under all 3 storage conditions. Beyond that, the viability gradually decreased and at 10 weeks of storage the counts were 3.79 and 4.03 cfu/g at 4 and −18°C, respectively. At this point, the moisture content was 0.94 ± 0.14%, with a slight increase in the wetting time (from 47 ± 2min to 61 ± 3min) and decrease in the solubility (from 90.7 ± 0.13% to 82.2 ± 0.4%). Such formulation can be applied in functional foods to harness their enhanced health benefits and functionality.

Key Words: conjugation, functional

27 Study of the interaction between whey proteins and anthocyanins using fluorescence spectroscopy. G. Miyagusuku-Cruzado*, R. Jimenez-Flores, and M. M. Giusti, *The Ohio State University, Columbus, OH.*

The food industry is moving away from synthetic colorants in favor of natural alternatives. Anthocyanins (ACNs) are plant pigments with vivid colors, but their application is restricted by their limited stability, particularly at pH close to neutral. Previous research showed that addition of whey protein isolate (WPI) to model juices colored with ACNs improved their color characteristics and increased their thermal resistance when compared with model juices without whey proteins (WP). It was hypothesized that WP may be interacting with ACNs, leading to enhancements in tinctorial strength and thermal stability. In this study, we performed fluorescence quenching (FQ) experiments to assess the level of interaction between anthocyanins and whey proteins. Eleven different anthocyanins were isolated from *Berberis boliviana*, purple corn and black carrot using semi-preparative HPLC. Identification and purity analyses were performed using uHPLC-MS. Model solutions were prepared by diluting WPI until a concentration of 1 mg/mL (0.1%, 54 μM) was reached using pH 3 citric acid-Na_2HPO_4 buffer, followed by the addition of purified ACNs (>90% purity) at different concentrations (1–100 μM of Cy-3-glu eq.). FQ by ACN addition was measured using a microplate reader with an excitation wavelength of 280nm. Results were adjusted to the Stern-Volmer (SV) equation for analysis. Furthermore, to stablish whether the FQ phenomena was a result of collisional quenching or binding-related quenching, measurements were conducted at different holding temperatures (30, 35 and 40°C). Results showed that addition of anthocyanins at concentrations as small as 1 μM resulted in significant FQ of WP ($P < 0.05$); and that Pt-3-glu, Mv-3-glu and Cy-3-glu were the most efficient quenchers. Measurements at different holding temperatures showed good linearity ($r^2 > 0.9$) and no significant changes in the SV constant with increasing temperature, suggesting that the type of FQ phenomena was a binding-related one. Therefore, WP can interact with anthocyanins in a binding-related manner, improving the color characteristics and thermal resistance of the pigment.

Key Words: natural colorant, whey protein isolate (WPI), fluorescence quenching

28 Impact of stretching temperature in a waterless cooker on the functionality of Mozzarella cheese. E. K. Aversa*[1], S. Govindasamy-Lucey[2], M. E. Johnson[2], J. J. Jaeggi[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

Use of high stretch temperatures during manufacture of low moisture part-skim (LMPS) Mozzarella cheese decreases residual rennet activity (RRA) and microbial counts. High stretch temperatures could help extend the shelf-life of LMPS destined for export. The aim of this study was to determine the impact of stretching temperatures (60, 74, and 88°C) in a waterless cooker on the composition, texture, flavor, and functional properties of LMPS Mozzarella. Four LMPS Mozzarella cheeses (n = 4) were manufactured using camel chymosin and an Airdus waterless cooker. Cheeses were stored at 4°C and analyzed at 2 wk, 3 and 6 mo. Cheeses were analyzed for RRA and primary proteolysis. Changes in flavor, shred properties, and pizza performance were evaluated using quantitative descriptive analysis (QDA). Cheeses stretched at ≤74°C had similar and typical composition. Cheese stretched at 88°C had reduced fat and moisture contents due to losses during the heating process. Cheese stretched at ≥74°C had higher pH values ($P < 0.05$) because of low acid development due to reduced microbial starter counts. Cheese stretched at ≥ 74°C had decreased RRA compared with cheese stretched at 60°C, resulting in lower proteolysis during storage. Stretching temperature affected the shred and pizza characteristics of the cheeses. Cheese stretched at ≥74°C had lower shred adhesiveness during storage compared with cheese stretched at 60°C, which had high adhesiveness ($P < 0.05$). Cheeses stretched at ≥74°C were characterized by higher blister quantity, hardness values, and more skinning when baked. During storage, the blister quantity increased for cheeses stretched at 60°C but not for cheeses stretched ≥74°C. Proteolysis in the cheeses stretched at 60°C caused a decrease in strand length and thickness. Cheese stretched at ≥74°C retained firmness and chewiness. This was consistent with TPA data where hardness values were maintained in cheese stretched at 88°C due to decreased proteolysis and higher protein content. Use of high stretch temperatures could be a useful method for helping to extend the performance shelf life of LMPS Mozzarella.

Key Words: low moisture part-skim (LMPS) Mozzarella, performance shelf life, high stretching temperature

29 Relationship between probiotics, their lipolytic activity on milk phospholipid in buttermilk fermentation and potential health contribution. K. Wang*, I. García-Cano, D. Rocha-Mendoza, J. Ortega-Anaya, and R. Jiménez-Flores, *Department of Food Science and Technology, The Ohio State University, Columbus, OH.*

The objective of this project is to demonstrate the lipolytic activity of probiotics in buttermilk fermentation with added milk phospholipids (MP), yielding products that can enhance MP absorption, providing additional health benefits through higher digestibility in intestine and possible modification of gut bacteria. Probiotics are beneficial for health, improving gut function, regulating immune response and the metabolites: lipases and proteases produced by them can hydrolysate lipids and proteins, which can lead to higher absorption of dairy nutrients. However, MP are known for its low absorption in intestine. Studies showed that metabolites produced by lactic acid bacteria (LAB) are associated with increased absorption of sphingomyelin and potentially all MP. Our hypothesis is that selected LAB would work better in helping digest MP. Bacteria isolation was performed from the LAB collection from OSU-Dr. Jimenez's laboratory and further identified by 16S rDNA sequencing. Quantitative lipolysis activity was tested using α-naphthyl acetate. Eight promising strains were tested for probiotic potential by screening the ability to resist low pH and bile salts, high values in auto-aggregation and hydrophobicity, also must have antimicrobial activity, be susceptible to antibiotics and, screened their virulence factors by PCR. Three selected strains were grown in skim milk (as control) and compared with those grown in buttermilk with or without MP added. Lipolysis products were analyzed by TLC, HPLC, SDS-PAGE, zymograms and fatty acid sequencing after fermentation. The results showed that 7 of the promising strains with high lipolytic activity were probiotics. Three selected strains (*Lactobacillus casei*, *Lactobacillus helveticus*, and *Lactobacillus acidophilus*) showed to grow better in buttermilk medium with added MP than other medium. They also have the enzymes to break down MP and produce lipolysis products. The results of this work can be used to increase bioavailability of MP and its application in fortified dairy products and pharmaceuticals.

Key Words: lactic acid bacteria, milk phospholipid, buttermilk

30 A cross-cultural study of the sensory perception of skim milk powder between Ireland, America, and China. C. Zeng*[1], K. Kilcawley[1], M. O'Sullivan[2], M. Drake[3], and S. Miao[1,4], [1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*The Sensory Group, School of Food and Nutritional Sciences, University College Cork, University College Cork, Cork, Ireland,* [3]*Department of Bioprocessing and Nutrition Science, Southeast Dairy Foods Research Centre, Raleigh, NC,* [4]*College of Food Science, Fujian Agriculture* *and Forestry University, Fujian Agriculture and Forestry University, Fuzhou, Fujian, China.*

Understanding potential cross-cultural sensory differences in the perception of Irish dairy products is important to gain market share, especially in key target areas such as the United States and China. The objectives of this study were to investigate if consumers and sensory panellists in Ireland, United States and China could discern differences in skim milk powder (SMP) produced from different bovine diets (ryegrass [GRS], ryegrass/white clover [CLV], and trial mixed rations [TMR]). The volatile profile of each SMP sample was analyzed by SPME-GCMS. SMP samples were evaluated by Irish (n = 78), American (n = 100) and Chinese (n = 106) consumers using a hedonic sensory acceptance test. Ranking Descriptive analysis was performed using untrained panellists (n = 20) familiar with dairy products in Ireland and China and Spectrum Descriptive analysis was undertaken by a trained American panel (n = 8). Twenty 6 volatile compounds were identified in the SMP samples, with 10 statistically different based on diet. The SMP samples could be discriminated based on their volatile profile with acetoin (3-hydroxy butanone) having the greatest impact. It was most abundant in the SMP produced from TMR and is derived from carbohydrate metabolism. US consumers preferred SMP derived from TMR, and Irish consumers preferred SMP from either GRS or CLV. Chinese consumers perceived SMP samples differently to USA and Irish consumers, but preference was not influenced by diet (GRS, CLV or TMR). Both Irish and Chinese untrained assessors found it difficult to discern differences between SMP produced from either GRS or CLV, but could differentiate SMP produced from TMR. Trained US panellists found significant differences in barny aftertaste, grassy/hay, salty taste, cardboard and sweet aromatic attributes between the samples. This study has shown that bovine diet affects the sensory perception of SMP and that cultural differences in perception exist. These sensory differences result from mainly indirect (rumen metabolism and lipid oxidation) and direct (absorption and inhalation) transfer from the diet into milk and subsequently into the SMP.

Key Words: cross-cultural study, skim milk powder, bovine diets

ADSA Graduate Student (MS) Production Oral Competition

31 Effects of a methionine analog and branch chain volatile fatty acids on rumen fermentation and biohydrogenation of linoleic acid in vitro. J. E. Copelin*[1], P. A. Dieter[1], J. L. Firkins[2], M. T. Socha[3], and C. Lee[1], [1]*Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [3]*Zinpro Corporation, Eden Prairie, MN.*

The objectives of this experiment were to examine rumen fermentation and biohydrogenation altered by a methionine analog, branched-chain volatile fatty acids, or their combination in a condition of feeding high polyunsaturated fatty acids. An in vitro batch culture was conducted to determine the biohydrogenation of linoleic acid at 2, 4, 8, and 24 h and pH and VFA at 24 h. Dietary treatments included a typical diet (50:50 of forage to concentrate on a DM basis; CON), CON with 3.0% linoleic acid (DM basis; LA), LA with 0.1% of a methionine analog (HMTBa; Rhodiment, Adisseo Inc.), LA with isobutyrate, iso-valerate, and 2-methyl-butyrate (1 mmol/L of each; BCVFA), and a combination of HMTBa and BCVFA (COMBO). Data were analyzed using MIXED procedures of SAS with incubation as a random effect and treatment, time, and their interaction were fixed effects. At 24 h incubation, LA did not affect pH compared with CON. Compared with LA, BCVFA, and COMBO decreased ($P < 0.01$) pH. Total VFA tended to be lower for LA vs. CON but greater ($P < 0.10$) for HMTBa, BCVFA, and COMBO compared with LA. Linoleic acid supplementation (i.e., LA, HMTBa, BCVFA, and COMBO) decreased acetate and increased ($P < 0.01$) propionate concentration compared with CON. Compared with LA, there were minimal changes in VFA for HMTBa and BCVFA. Dry matter and NDF digestibility was lower ($P < 0.01$) for LA vs. CON without a difference among LA, HMTBa, BCVFA, and COMBO. Compared with LA, BCVFA had minimal effects on profile of long chain fatty acids. However, HMTBa and COMBO increased ($P < 0.01$) 18:1 t11 and 18:2 c9t11 at 4 h of incubation and tended to increase ($P < 0.10$) these intermediates at 8, 12, and 24 h compared with LA. However, 18:1 t10 and 18:2 t10c12 were not affected by HMTBa vs. LA. In conclusion, linoleic acid at 3% of DM depressed feed fermentation, which was alleviated by supplementation of BCVFA according to decreased pH and increased total VFA. Supplementation of a methionine analog altered microbial biohydrogenation pathways of linoleic acid.

Key Words: methionine analog, branched-chain VFA, biohydrogenation

32 Palmitic, stearic, and oleic acids differently alter NDF digestibility in a continuous culture system. A. Sears*[1], J. de Souza[2], B. Wenner[3], and F. Batistel[1], [1]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT,* [2]*Perdue Agribusiness, Salisbury, MD,* [3]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

The objective of this study was to evaluate the effect of palmitic, stearic and oleic acid on NDF digestibility and rumen fermentation. Continuous culture fermenters (n = 8) were used in a replicated 4 × 4 Latin square design with 7 d of adaptation and 4 d of sampling. Treatments were: 1) control diet without fatty acids; 2) control diet plus 1.5% of palmitic acid (99% C16:0); 3) control diet plus 1.5% of stearic acid (99% C16:0); and 4) control diet plus 1.5% oleic acid (99% *cis*-9 C18:1). The control diet (60 g DM/day) was a 50:50 orchardgrass hay:concentrate mixture that provided 8.7 g CP, 21 g NDF, 11 g starch, and 1.5 g fatty acids fed twice daily. The fatty acid treatments maintained the same nutrient input into the fermenters as the control except for fatty acids. Daily fermenter effluent was collected over 24 h post-feeding and a 30% subsample was pooled by fermenter within period. Buffer solution was delivered continuously at rate of 10%/hour. Data were analyzed using a mixed model including the fixed effect of treatment, and the random effects of period and fermenter. Data are reported as least squares means with differences declared at $P \leq 0.05$ and tendencies at $0.05 < P \leq 0.10$. Compared with control, oleic acid tended to decrease NDF digestibility (36.6% vs. 43%, $P = 0.09$), whereas no effect was observed for palmitic (46.6% vs. 43%, $P = 0.33$) and stearic acid (44.5% vs. 43%, $P = 0.68$). Palmitic ($P = 0.01$) and stearic ($P = 0.04$) increased NDF digestibility compared with oleic acid; whereas no difference ($P = 0.57$) was observed between palmitic and stearic acid on NDF digestibility. Compared with control, total production of VFA was not affected ($P > 0.10$) by the fatty acid treatments (161.4, 168.9, 151.0, and 146.7 mmol for control, palmitic, stearic, and oleic, respectively); however, palmitic acid increased VFA production ($P = 0.03$) compared with oleic and tended to increase ($P = 0.08$) compared with stearic acid. Ammonia concentration was not affected by fatty acids ($P > 0.10$), nor was molar proportion of VFA ($P \geq 0.40$). In conclusion, palmitic and stearic did not affect NDF digestibility nor VFA production compared with control diet, while oleic acid impaired both variables.

Key Words: fermenter, fiber digestibility, rumen fermentation

33 Effects of orally dosed lipopolysaccharide and sodium butyrate, in combination and alone, on rumen development in dairy calves. C. A. Ceh*, C. M. Parsons, T. T. Yohe, M. L. McGilliard, M. D. Ellett, H. H. Hanling, S. E. Baynard, and K. M. Daniels, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

The objective was to investigate the effects of orally dosed lipopolysaccharide (LPS) and Na-butyrate on rumen cell proliferation in Holstein dairy calves. The hypothesis was that LPS and butyrate synergize to promote rumen development. Twenty-two bull calves arrived in one of 2 groups, spaced 2 wk apart. Within each group, calves were assigned to one of 4 treatments: control (CON; n = 5), butyrate (BUTY; n = 5), LPS (n = 6), or LPS plus butyrate (LPSB; n = 6). All treatments were administered orally twice daily and consisted of either: 0.9% saline (CON); 11mM Na-butyrate (BUTY); LPS ranging from 2.5 to 40 µg/kg body weight $(BW)^{0.75}$ (LPS), or both butyrate and LPS (LPSB). LPS dosage volume increased across weeks, ranging from 10 to 40 mL per dose. Calves were fed milk replacer (22%CP, 20% fat) and starter (20% CP, 3% fat) twice daily based on metabolic BW. Feed intake, fecal and respiratory scores, and rectal temperature were recorded daily. Calf BW, hip height, blood samples, and rumen content samples were collected weekly. Calves were weaned at 6 wk of age and euthanized at 8 wk of age, whereupon ruminal weights and ruminal samples for papillae area and epithelial thickness were collected. Blood and rumen samples were analyzed for blood metabolites (BHBA and glucose) and VFA concentrations, respectively. Feed intake, health measures, and blood metabolites did not differ by treatment. Calf BW increased by week ($P < 0.0001$). Irrespective of week, LPS weighed more and had higher ADG than BUTY ($P = 0.020$). Irrespective of week, withers height was higher in LPS compared with CON ($P = 0.006$). Rumen pH and rumen VFA concentrations did not differ by treatment but did decrease and increase, respectively, with week in conjunction with increased starter intake. Total empty stomach ($P = 0.014$) and reticulorumen weights

($P = 0.012$) were higher in LPSB compared with BUTY. Here, LPS and BUTY appeared to have synergistically affected some, but not all rumen measurements without affecting calf growth, intake, or health. This suggests that frequent oral exposure to LPS may have a direct effect on calf rumen growth.

Key Words: calf, rumen development, lipopolysaccharide

34 Colostrum supplementation with omega-3 fatty acids and α-tocopherol decreases indicators of oxidative stress and alters plasma fatty acid profile in newborn calves during the first week of life.

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Oxidative stress (OS) occurs when antioxidants fail to neutralize an overabundant concentration of reactive oxygen species, resulting in damage to cellular components. This phenomenon is prevalent in neonatal calves, potentially causing disease vulnerability and immune dysfunction. Past studies have shown the benefits of fish and flax oil on calf health and growth due to their omega-3 fatty acids (n-3 FA); these metabolites may mediate inflammation and OS through anti-inflammatory and antioxidant properties. We hypothesized a 60 mL fish and flax oil colostrum supplement would improve indicators of calf health and plasma concentrations of n-3 FA during the first week of life. Sixteen Holstein calves were blocked by sex and birth date and randomly assigned to control (no supplement; Con), or fish and flax oil (FFtrt) supplemented in first colostrum (3 L within 6 h, > 22% on Brix). FFtrt was a 60 mL 1:1 blend of oils with 200 mg α-tocopherol. Blood was sampled on d 1, 2, 4, 7, 14, and 21 after birth for assessment of passive transfer, oxidant status, and FA profile. Health was scored daily. Hip height and body weight were recorded weekly. Data were analyzed with a mixed procedure in SAS 9.4 including treatment, sex, and day as fixed effects and calf and block as random effects. FFTrt did not alter concentration of total protein in blood serum, prevalence of diarrhea, or rate of growth ($P > 0.10$), but tended to improve nasal scores ($P = 0.07$). FFtrt increased plasma concentrations of n-3 FA as much as 90% by 1 d of age ($P < 0.01$). FFtrt decreased oxidant status index (OSi) by 55% by 2 d of age (Con: 73, FFtrt: 32 OSi; $P < 0.01$) and remained decreased overall in the first week of life (Con: 74, FFtrt: 50 OSi; $P < 0.01$). OSi and FA concentrations returned to control values by d 14. In conclusion, a colostrum supplement of n-3 FA and α-tocopherol decreased oxidant status and increased plasma n-3 FA concentrations in the first week of life and has the potential to improve health of neonatal calves.

Key Words: omega-3, oxidative stress

35 Metabolic profile of dairy calves supplemented with flax oil or soy oil.

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Flax meal has been commercially used in the livestock industry as an alternative feeding ingredient; however, the oil extruded during milling has limited research in livestock feeding. Our objective was to investigate the effects of supplementing flax oil or soy oil in calves on metabolic profile. Thirty-six Holstein heifer calves in individual hutches were used in a 12-wk randomized complete block design study. Treatments were: 1) control (CON) with no oil, 2) 80 g/d of flax oil (FLAX), and 3) and 80 g/d of soy oil (SOY). Pre-weaning oils were fed with the milk and post-weaning they were top-dressed on starter pellets. Calves were fed 2.83 L of pasteurized milk 2 × /d during wk 1 to 5 and 1 × /d during wk 6. All calves were weaned at d 42. Pellets and water were fed ad libitum. Once weekly at 3 to 4 h post-feeding blood was collected from the jugular vein for metabolite analyses. Results were analyzed using the MIXED procedure of SAS 9.4 with repeated measures. Significant differences were declared at $P < 0.05$. Dry matter intakes (1792, 1487 and 1650 g/d; SEM = 79.5 for CON, FLAX, and SOY, respectively) were greater ($P < 0.01$) in CON than FLAX with SOY similar to both. There was a treatment × wk interaction ($P < 0.01$) with calves on FLAX eating less in the last 2 weeks of the study. Beta hydroxyl butyrate (BHB; 69.2, 69.5, and 67.4 mg/dL; SEM = 9.14) and plasma urea nitrogen (PUN; 11.4, 10.5, and 10.7 mg/dL; SEM 0.36) were not different among treatments. Cholesterol (36.9, 52.3, and 56.0 mg/dL; SEM = 2.29) was greater ($P < 0.01$) in the oil treatments compared with CON. Triglycerides (26.3, 27.0, and 32.1 mg/dL; SEM = 1.25) were greater ($P < 0.01$) in SOY. A tendency ($P = 0.05$) was observed for glucose (106.9, 105.6, and 99.9 mg/dL; SEM = 2.15) to be less in SOY. Supplementing flax oil and soy oil maintained BHB and PUN concentrations, increased cholesterol concentrations, but caused different responses compared with CON for Triglycerides and Glucose. This study demonstrates that the supplementation of plant-based oils to young calves affects their metabolic profile when compared with a diet with no oil supplementation.

Key Words: flax oil, Holstein dairy calf, metabolic profile

36 Factors affecting dairy cattle protective grouping behavior, also known as bunching, against *Stomoxys calcitrans* (L.) on California dairies.

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Bunching is the protective aggregating behavior of cattle against *Stomoxys calcitrans* (stable fly), where cattle bunch in a group with their heads to the center. Stable flies have a painful bite leading to stress which impacts productivity and welfare. Our objectives were to estimate stable fly intensity on dairies, threshold required to induce bunching, and the association of bunching with management and environmental factors. Between April and July 2017 we enrolled a convenience sample of 20 California dairies (herd size 2466 ± 1050), 13 Holstein, 4 Jersey and 3 were mixed. Data about feeding, manure management and cow cooling were collected using an in-person survey. Stable fly activity was recorded weekly using Alsynite traps and counts on cows. Bunching behavior was recorded weekly while recording fly activity. Data was analyzed using linear mixed models. At the dairy level bunching was associated with mean stable fly counts ≥150 flies/trap/week, months May and June (versus July), feeding wet distiller grains, and presence of wheat/corn or alfalfa crops on >2 sides of the dairy. Higher weekly mean ambient temperatures and cleaning the fence line manure were protective against bunching. At the pen level bunching was associated with a stable fly count >1 fly/cow leg, >50 flies/trap-week on traps closest to the pen, ambient temperature ≤30°C, freestall pens (versus open lot pens), dry and lactating cows (versus close-up cows), pens fed a ration containing molasses (versus not) all had higher odds of bunching; however relative humidity >50% was protective. Findings from our study show that bunching on the study dairies varied by stable fly activity, environment, facility design, dairy surroundings and management factors, including feeding and manure management.

Key Words: bunching, dairy cattle, stable fly

37 Effect of molasses-based liquid feed supplementation through robotic milking systems on fresh cow behavior, health, and production.

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The objective of this study was to determine the effect of molasses-based liquid feed (LF) supplementation on the behavior, health, and production of early lactation cows in robotic herds. In 6 commercial robotic dairy herds, 400 dairy cows were randomly assigned, within farm, balanced by parity, to receive at calving 1 of 2 treatments: 1) control group (C) receiving standardized robotic pellet (mean = 4.3 kg/d, n = 200), or 2) standardized robotic pellet feeding (mean = 4.0 kg/d, n = 200) plus 1 kg DM/d of LF for MP cows (1.6 kg/d as fed) and 0.88 kg DM/d for PP cows (1.4 kg/d as fed). Across farms, cows were fed a partial mixed ration that were similar in ingredient and nutrient composition. Cows on LF received supplementation for the first 60 d post-calving. Production, rumination time, and health status were monitored for 100 d post-calving. Blood samples were taken 2×/wk for the first 4 wk post-calving to assess energy balance (BHB). Samples with BHB ≥ 1.2 mmol/L were classified as a case of subclinical ketosis (SCK). Using mixed-effect linear regression models, we analyzed the effect of treatment, parity, and their interaction (only if significant $P < 0.05$) on linear outcomes. Chi-squared tests were used to establish associations between treatment and health status. Milk yield (LF = 36.8 kg/d, C = 36.6 kg/d; SE = 1.2; $P = 0.88$) and milking frequency (LF = 3.2×/d, C = 3.2×/d; SE = 0.07; $P = 0.48$) did not differ by treatment. Control cows had more daily robot visits overall compared with LF cows (LF = 5.1×/d, C = 5.8×/d; SE = 0.36; $P = 0.02$). Treatment affected the number of times cows tested positive for SCK ($P = 0.05$); cows on LF had fewer repeated occurrences of SCK, such that 15% of cows on LF had ≥3 cases of SCK out of 5 tests, compared with 27% of control cows. Overall, the results of this study suggest that supplementing molasses-based LF to robot-milked cows may help support the energy demands of milk production in early lactation and, thus, reduce the incidence of repeat SCK cases during that period.

Key Words: molasses, robotic milking, subclinical ketosis

38 Impact of commercial direct-fed microbial on cow performance during the calving transition.

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The objective of this study was to investigate the impact of feeding a commercial direct-fed microbial on primiparous and multiparous cows in the calving transition period. Primiparous (n = 22) and multiparous (n = 19) cows were fed a close-up TMR before calving and a lactation TMR after calving. Three weeks before expected calving, all animals were blocked to balance parity and body weight, then assigned to control group (CTRL; n = 21) or a direct fed microbial (DFM; n = 20). The DFM animals received a top-dressed DFM fed daily at 12.5 g per head. Weekly, DMI and milk production were measured. Data were analyzed using treatment as a fixed effect, block and parity as random effects, and week as a repeated measure. Pre-planned contrasts compared treatments weeks −3, 1, 5, and 9. Between primiparous and multiparous cows, there was no difference in DMI (29.2 ± 2.15 vs. 33.9 ± 2.32 kg/d, respectively, $P = 0.13$), but milk production was higher in multiparous cows (30.4 ± 1.18 vs. 41.2 ± 1.31 kg/d, respectively, $P < 0.01$). There was no difference in DMI between CTRL and DFM in week −3 (19.9 ± 2.01 vs. 18.7 ± 1.89 kg/d, $P = 0.64$), wk 1 (29.9 ± 1.71 vs. 30.8 ± 1.56 kg/d, $P = 0.64$), wk 5 (42.8 ± 1.72 vs. 43.7 ± 1.58 kg/d, $P = 0.65$), or wk 9 (44.0 ± 2.30 vs. 40.5 ± 2.33 kg/d, $P = 0.23$). There was no difference in milk production between CTRL and DFM in wk 1 (30.7 ± 1.43 vs. 31.5 ± 1.47 kg/d, $P = 0.68$), wk 5 (37.3 ± 1.40 vs. 39.4 ± 1.41 kg/d, $P = 0.27$), and wk 9 (39.2 ± 1.78 vs. 39.1 ± 1.87 kg/d, $P = 0.97$). Milk efficiency between CTRL and DFM tended to be higher for DFM in wk 1 (1.07 ± 0.09 vs. 1.20 ± 0.09 kg/kg, $P = 0.09$), but not wk 5 (0.93 ± 0.09 vs. 0.97 ± 0.09 kg/kg, $P = 0.60$), and wk 9 (1.01 ± 0.12 vs. 1.01 ± 0.11 kg/kg, $P = 0.99$). Supplementation of close-up TMR and early lactation rations did not affect DMI or milk production but tends to improve milk efficiency in the first week of lactation.

Key Words: direct-fed microbial (DFM), calving transition

ADSA-SAD Undergraduate Dairy Production Oral Competition

39 Immune response to *Escherichia coli*-induced bovine mastitis in lactating dairy cows.

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An active compound of citrus oils (CO), citral, contributes to the bactericidal activity of CO and serves as a non-antibiotic alternative candidate for the treatment of bovine *Escherichia coli* mastitis. During times of inflammation, monitoring the immune response can generate economic and welfare benefits due to the high incidence of mastitis across the dairy industry. The objective of this study was to evaluate the immune response after being challenged with mastitis pathogen *E. coli* strain P4. Eighteen healthy, multiparous Holstein cows in mid-lactation (>100 d in milk) were challenged with 800 cfu of *E. coli* strain P4 into one rear mammary quarter. One of 3 intramammary treatments were administered into the infected quarter ~24 h post-infection including: 1) CON: sterile phosphate buffer solution twice daily, 2) CO: 1.0% (vol/vol) citral twice daily or 3) AB: ceftiofur hydrochloride once daily (based on label instructions). Coccygeal blood was collected daily through d 7 post-challenge where serum was harvested. Serum haptoglobin was analyzed using a commercially available kit (Tridelta Development Limited, Dublin, Ireland). Data were analyzed using ANOVA of the PROC MIXED procedure of SAS v.9.4. The results indicated that serum haptoglobin was not different by treatment ($P = 0.55$) or for the interaction of treatment by day of challenge ($P = 0.97$). In conclusion, CO does not alter serum haptoglobin response even when compared with the control during *E. coli* bovine mastitis and CO may not serve as an alternative treatment for bovine mastitis infection with a gram-negative organism.

Key Words: mastitis, citrus oil, alternative treatment

40 Twinning in dairy cattle.

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The dairy industry continues to push for greater milk production through greater reproductive performance in our animals. A negative effect of this is increased twinning rates. Factors that contribute to twinning in dairy cattle include double ovulation, genetics, season, and management. Cows that experience an extended period in a negative energy balance can have atypical ovarian activities, such as double ovulation and anovulatory repeated follicular waves that can lead to the occurrence of twins (Lopes et al., 2003). High producing dairy cattle are in a negative energy balance for a longer period compared with their lower producing counterparts (Fricke 2001). Twin pregnancies have a negative economic impact. Depending on the type of twin pregnancy, parity, and days in milk when the twin pregnancy occurs, the cost of the vet services per pregnancy increased from $97 to $225 per cow when compared with singletons. The overall negative economic impact of twinning on dairy farm profitability in the United States was estimated to be $96 million per year (Novales et al., 2018). Twinning poses a variety of pre- and postpartum health risks, including a decrease in the number of days on a transition diet, increased dystocia occurrences, decreased milk yield, and increased days to conception. In one study, monozygotic (same sex) twins made up 5.5% of all twin births in the population. Dizygotic twins (a male and female) have a 90% chance of the heifer calf being a freemartin, due to the exchange of hormones across the blood barrier from fetus to fetus (López-Gatius et al., 2017). Twins struggle to thrive in the first few days after birth, and lag in growth when compared with singleton calves. The dairy industry must be prepared for the challenges that come along with twins, whether it be early termination of the pregnancy or better management of the cow during the pregnancy. Dairy producers can help their cows throughout the pregnancy by giving moving cows to the transition group 3 weeks earlier and monitoring and maintaining the body condition score of the cow during the dry period (Frickle, 2001). Producers can achieve this by monitoring the ration of the group of cows and feeding them the appropriate ration for their stage in lactation.

Key Words: twinnings, high production, twin

41 Evaluating the potential impact of a slick gene on reducing heat stress in dairy cattle.

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Heat stress is a major challenge to many dairy farmers, especially those in the southeastern United States where months of high temperature in conjunction with high humidity wreak havoc on productivity. The detrimental impact is seen most commonly in youngstock performance, reproductive efficiency, and output in the milking parlor. Estimates for yearly economic loss due to heat stress on dairies range widely, with the low end estimating a total loss of 900 million to the high end putting that estimate at just over 5 billion. Producers have long strategized on facility modifications to maximize heat abatement. Others have found advantages in simple alteration in herd makeup through crossbreeding. However, a new and finally tangible approach may be to further change the animal's genetic makeup through specific gene selection. Cows with the "slick" gene, first identified in Senepol cattle, display a very short, slick coat. This slick coat is controlled by a single dominant gene located on bovine chromosome 20, which contains the prolactin receptor. Cows displaying the slick phenotype have a truncated prolactin receptor, caused by a single base deletion and an accompanying frame shift that results in an earlier stop codon on the prolactin receptor. Slick coated cattle are shown to have lower vaginal temperatures, lower respiration rates, and higher rates of sweat production. Most importantly to a producer's bottom line, slick coated cattle experience a less dramatic drop in production during the warmer months. Two heterozygous registered Holstein bulls are currently being marketed to producers to introduce the phenotype into US herds. There is no evidence to suggest that the gene exists naturally in the base female Holstein population, so calves produced from the heterozygous bulls have a 50% chance of displaying the slick phenotype. As the breeding program continues, it is anticipated that homozygous bulls and females will quickly develop allowing producers to have a cow that, along with facilities, can maximize heat abatement and hopefully the producer's financial profit potential.

Key Words: slick gene, heat tolerance, productivity

42 Balancing production and rumen health: Implementing precision technologies to manage subacute rumen acidosis.

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A wide variety of metabolic diseases cause economic loss in the dairy industry. Even well-managed, high-yielding herds are subject to metabolic diseases because of high dry matter intake and high grain content in lactation diets. Though concentrate feeding is known to increase milk production, high grain intake levels may cause a decrease in rumen pH, an imbalance of rumination, and a decrease in milk fat (Dohme, 2008;

Beauchemin, 2018; Harvatine, 2016). Abundantly feeding concentrates can result in rapid increases of volatile fatty acid (VFA) production. This in turn can lead to subacute rumen acidosis (SARA). SARA is characterized by bouts when ruminal pH drops between 5.2 and 5.6 for at least 3 h, over a 24 h period (Gozho, 2005). To prevent the onset of SARA and other associated conditions, efforts have been made to improve rumen environment using precision feeding technologies. The term "precision feeding" is used to describe efforts in ration formulation to manage nutritional requirements of dairy cows in specific life stages. Many researchers are developing nutritional management strategies that can be used to decrease the incidence of SARA. For example, the inclusion of physically effective fiber has been shown to maintain rumen pH by limiting the fermentation rates of non-fiber carbohydrates (Allen, 1997). Likewise, adding rumen modifiers and buffers in lactating diets can help maintain rumen pH, increase feed efficiency, and limit SARA (Krause, 2008). A recent advancement proven to be effective in limiting metabolic disorders such as SARA is the dynamic concentrate parlor feeder (DCPF). This conglomerate feeding technology is capable of calculating the nutrient needs of individual cows and supplementing ingredients with a focus on enrichment of TMR diets (Bach, 2014). Further development of precision feeding technologies can allow for reduced occurrences of metabolic disease, increased feed efficiency, and increased milk production.

Key Words: precision dairy technology, rumen health, subacute rumen acidosis

43 Probiotic supplements as a low-cost solution to bolster calf performance.

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Dairy calves are born with sterile gastrointestinal tracts (Uyeno et al., 2015). Microflora ingested during the birth process are the first colonizers of the calf gastrointestinal tract (GIT). Thereafter, bacteria from the calf's environment are ingested. Ingested bacteria can be one of 2 types: pathogenic or commensal. In dairy calves, pathogenic bacteria cause illness and diminish efficiency of digestion and overall growth. Commensal bacteria, on the other hand, pose no such problem and often have favorable outcomes on calf growth. Probiotics are living organisms that, when consumed, maintain or restore beneficial bacteria to the digestive tract. Strains from the *Lactobacillus*, *Enterococcus*, and *Bifidobacterium* genera have been used efficaciously as probiotics in dairy calves (Uyeno et al., 2015). Times where probiotics may be especially beneficial to dairy calves include after a course of antibiotics during heat stress (Ruppert et al., 1998) and during the weeks surrounding weaning. Calves that consume probiotics tend to consume higher amounts of milk, grain, and forage earlier than non-treated calves, resulting in higher average daily gains (Quigley et al., 2014). Probiotics can be purchased over the counter at feed stores and anywhere that sells veterinary medicine. They are available in many forms to make administration easier. When used as a preventative, probiotics are an affordable first line of defense for dairy calves.

Key Words: calf, probiotics, immunocompetency

44 *Salmonella* Heidelberg in dairy calves.

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The most sensitive period in raising dairy calves is from birth to 3 mo of age. The most prevalent causes of morbidity and mortality from birth to weaning are scours and pneumonia, with 75% of calf deaths occurring due to these illnesses. Scours, or diarrhea, is a common illness in young dairy calves, accounting for approximately 50 to 75% of calf death during the first 3 weeks of life. The most common causes of calf scours are bacteria, viruses, and protozoa. Treatment of scours requires identification of the causative organism so that the proper intervention plan can be selected. It is important to note that many organisms that cause scours are zoonotic, so individuals caring for these animals must be extremely conscientious. Antibiotics are most useful when bacteria are the causative agent. *Salmonella* Heidelberg is a bacterium that causes severe illness in calves and humans. Calves infected with *Salmonella* Heidelberg may develop diarrhea or die abruptly without any clinical signs. People can also become infected with *Salmonella* Heidelberg with symptoms including diarrhea, fever, and abdominal cramps, which may last 4 to 7 d. From January 2015 through November 2017, a multi-drug-resistant *Salmonella* Heidelberg was linked to contact with dairy calves. A total of 56 people in 15 states were infected with this organism. Surveillance in veterinary diagnostic laboratories showed that calves in several states were infected with the outbreak strains of multidrug-resistant *Salmonella* Heidelberg, and a CDC investigation showed that the clinical isolates from affected individuals were resistant to multiple antibiotics. Currently, no approved antimicrobial drugs are effective against the *Salmonella* Heidelberg strains isolated from calves. Since periodic occurrence of *Salmonella* Heidelberg continues to be of concern, proper sanitation and hygiene are of utmost importance in preventing scours in calves and protecting human health.

Key Words: dairy calf, *Salmonella*, human health

ADSA-SAD Undergraduate Original Research Oral Competition

45 Impact of maternal direct-fed microbial on immunoglobulin concentration in dairy calves. M. N. Degenshein*[1], R. L. Hiltz[1], M. R. Steelreath[1], H. Nielsen[2], A. Aguilar[2], and A. H. Laarman[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Lallemand Animal Nutrition, Milwaukee, WI.*

This study examined the impact of maternal direct-fed microbial supplementation on transfer of passive immunity and development of active immunity in dairy calves from birth to weaning. Primiparous (n = 10) and multiparous (n = 11) cows were blocked by parity and body weight and fed a close-up TMR before calving. The dams' TMR was either not supplemented (CTRL; n = 11) or supplemented with a commercial direct-fed microbial (DFM; n = 10). Within 4 h of birth, bull calves (n = 12) and heifer calves (n = 10) were fed 4L of maternal colostrum. Blood serum was collected from Holstein bull calves (n = 12) and heifer calves (n = 10) at 0, 24, and 72 h and then weekly until weaning. Also, calves were weighed at birth and weekly until weaning. Data were analyzed in SAS with fixed effect of treatment and, where appropriate, repeated effect of week. Average daily gain (ADG) did not differ between CTRL and DFM (1.34 ± 0.06 vs. 1.44 ± 0.07 kg/d, respectively; $P = 0.29$) or between bulls and heifers (1.33 ± 0.06 vs. 1.45 ± 0.07 kg/d, respectively; $P = 0.20$). Serum IgG concentrations at birth were not different between CTRL and DFM (1753 ± 290 vs. 2325 ± 372 mg/dL, respectively; $P = 0.95$). At 24 h, IgG peaked for CTRL and DFM (1562 ± 666 vs. 2176 ± 744 mg/dL, respectively; $P > 0.74$) and then subsided, at 72 h, for both CTRL and DFM (514 ± 666 vs. 855 ± 744, respectively; $P = 0.43$). Serum IgG concentrations increased for both CTRL and DFM between wk 4 (1681 ± 666 vs. 2813 ± 744 mg/dL, respectively; $P = 0.43$) and wk 7 (2636 ± 666 vs. 2653 ± 744 mg/dL, respectively; $P = 0.43$). These data suggest feeding a direct-fed microbial to dams may not impact passive transfer of immunity or weight gains in young calves. Also, calf endogenous IgG production appears to exceed maternal IgG denaturation between 4 and 7 weeks of age.

Key Words: calf, immunoglobulin, direct-fed microbial

46 Semen quality in lost Holstein Y-chromosome lineages. S. E. Jewell*[1], J. M. DeJarnette[2], H. Blackburn[3], W. S. Liu[1], C. G. Sattler[2], and C. D. Dechow[1], [1]*Pennsylvania State University, University Park, University Park, PA,* [2]*Select Sires Inc., Plain City, OH,* [3]*National Animal Germplasm Program, Fort Collins, CO.*

Since the introduction of artificial insemination, Holstein bulls have been reduced to 2 Y-chromosome lineages. One possible reason is that modern Y-lineages (MY) had better semen quality than lost Y-chromosome lineages (LY). The objective of this study is to compare semen quality of young bulls from LY to semen quality in MY. Two LY were resurrected through in vitro embryo production using semen from ZIMMERMAN ALSTAR PILOT (born in 1954) and U-OF-MINN W CALIBN CUTHBERT, who was a ROSAFE CALIBAN (born in 1953) son born during a long-term selection experiment; semen from both bulls was procured from the National Animal Germplasm Program repository. Two sons of CUTHBERT born in March 2017 and 3 sons of PILOT born in November 2017 were transferred to Select Sires Inc. Semen was collected in the summer of 2018 and in January 2019, respectively. Two ejaculates were taken each date of collection and measured for semen volume, sperm concentration, total number of sperm, and visually scored motility. The 5 LY bulls were compared with 58 age-matched MY bulls collected at the same time. Analyses were conducted to compare fixed effects of LY with MY, or with each Y-chromosome lineage treated as a separate effect (n = 4) in a mixed model. The explanatory variables considered were fixed effects of week of year, daily ejaculate number (1 or 2), and interval between collection days; bull identity was included as a random effect. Interval between collections was not significant for any trait and ejaculate number was not significant for motility. Significant ($P < 0.05$) differences between LY and MY were evident for total number of sperm (4.6 billion versus 3.4 billion, respectively) and sperm concentration (1.3 billion/ml versus 1.0 billion/ml, respectively). For motility, Y-lineage treated as a separate effect tended toward significance ($P < 0.10$) with CUTHBERT sons (74.8%) having lower motility than sons of the MY bull ELEVATION (77.3%) and PILOT (77.6%). There were no significant differences among lineages for semen volume. This research suggests that initial semen quality may not be the primary reason Y-chromosome lineages were lost from the Holstein breed.

Key Words: Y chromosome, semen, genetic diversity

47 Are implantable microchips a reliable way to continuously measure body temperature in dairy calves? M. Woodrum*, M. Cantor, and J. H. Costa, *Dairy Science Program, Animal and Food Sciences, University of Kentucky, Lexington, KY.*

Four consecutive studies aimed to test the validity of an implantable passive radio frequency identification (RFID) microchip (Bio-thermo, Allflex USA) in various implant sites in a calf as an alternative to rectal temperature (RT) measurements. Preimplantation, microchips were validated in a water bath against a rectal thermometer, and we found a very high correlation to the rectal thermometer (r > 0.95) with no measurement bias found in Bland-Altman plots. Three microchips were implanted per calf: subcutaneously behind the ear (EAR), subcutaneously by the upper scapula (SCAP), and intramuscularly in the neck (NECK). Microchips were tested for repeatability of measurements when implanted; each calf (n = 11) had an average of 24 observations per site all taken within 2 min. The average coefficient of variance for microchips were low (0.12 ± 0.03% CV) and the difference between measurements were negligible (0.1 ± 0.04°C, range 0–0.4°C). Following, calves (n = 12) were enrolled in a 24-h study, where each calf had their RT, microchips, and tympanic temperature measured hourly. Correlations were high between SCAP vs NECK (individual animal correlation; median [Q1, Q3] r = 0.75 [0.60, 0.84]; $P \leq 0.02$) and EAR vs NECK (r = 0.78 [0.73, 0.84]; $P \leq 0.01$). From the 24 h RT had negligible correlations with the 3 microchips sites and tympanic temperature, RT's highest correlation was with tympanic (r = 0.19 [0.07, 0.22]; $P \leq 0.81$). Finally, calves (n = 10) were enrolled in a long-term study where daily for 30d RT and the microchips were measured. Rectal temperature had negligible correlations with EAR and NECK, and RT had a low correlation with SCAP (r = 0.33; $P < 0.001$). Also, correlations were high for EAR vs NECK (r = 0.79 [0.73, 0.89]; $P \leq 0.02$); the weakest correlation was between EAR vs RT (median r = 0.06 [0.03, 0.14]; $P \leq 0.99$). Our results suggest that microchip temperature readings are consistent among microchips, but readings vary by implant site and has a weak linear relationship with RT. Microchips are promising technologies, but further testing is necessary to support the use to determine body temperature and its deviations in dairy calves.

Key Words: calf, temperature, precision technology

48 Impact of concentrate location on the behavior and performance of dairy cows milked in a free-traffic robotic system.

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The objective of this study was to determine if the location of concentrate allowance in a free-traffic automated milking system (AMS) affects intake, sorting behavior, milking activity, and production of lactating dairy cows. Fifteen primiparous Holstein cows (47.1 ± 15.0 DIM) were exposed to each of 2 treatments in a crossover design with 28-d periods: 1) a higher concentrate partial mixed ration (PMR) with an AMS concentrate allowance of 3.0 kg/d (H-PMR), and 2) a lower concentrate PMR with an AMS concentrate allowance of 6.0 kg/d (L-PMR). To assess sorting, offered and refused feed samples were separated into: long (>19mm), medium (8–19mm), short (4–8mm), and fine (<4mm) particles. Feed sorting (%) was calculated as: actual DMI of each particle fraction/predicted DMI. PMR DMI, milk yield, milking activity, and lying behavior were recorded electronically. Data were summarized by cow and day and analyzed in mixed-effect linear regression models. Cows on the L-PMR consumed more AMS concentrate (6.3 kg/d vs. 3.1 kg/d; SE = 0.04; $P < 0.01$). PMR intake was reduced when on the L-PMR, (17.1 vs. 19.1 kg/d; SE = 0.4; $P < 0.01$), while total DMI was greater (23.6 vs. 22.3 kg/d; SE = 0.4; $P = 0.03$). Selection for medium and against fine particles increased when cows were on the H-PMR ($P < 0.01$), while selection against long ($P = 0.03$) and for short particles ($P = 0.04$) tended to increase. Time spent eating PMR (199 min/d; SE = 8.8; $P = 0.81$) was similar between treatments, however meal size tended to be larger when cows were on the H-PMR (2.2 vs 2.1 kg/meal; SE = 0.6; $P = 0.06$). Cows tended to spend 30.8 min/d more time lying down when on the L-PMR ($P = 0.08$). On the L-PMR, cows tended to have more voluntary AMS visits (5.9 vs. 4.6 visits/d; SE = 0.5; $P = 0.08$), were fetched less (0.1 vs. 0.5 x/d; SE = 0.09; $P < 0.01$) and had a greater milking frequency (3.5 vs. 3.0 x/d; SE = 0.1; $P < 0.01$). On the L-PMR, cows produced 40.6 kg/d, while producing 39.1 kg/d on the H-PMR; a statistical difference between these was not detected (SE = 0.7; $P = 0.13$). Overall, allocating a greater proportion of total dietary concentrate to a free-traffic AMS may improve milking activity and decrease the need for fetching, while reducing sorting of the PMR.

Key Words: automatic milking system, concentrate, feeding management

49 Effects of feeding alfalfa or grass hay and corn or wheat grain on production performance and income over feed cost of high-producing Holstein cows.

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The objective of this study was to evaluate the effects of feeding diets containing alfalfa ($380/ton) or grass ($140/ton) hays and corn or wheat grains on production performance and income over feed cost (IOFC) of dairy cows. Eight primiparous (587 ± 49 kg BW; 66 ± 16 DIM) and 16 multiparous (683 ± 56 kg BW; 66 ± 19 DIM) Holstein cows were assigned to 1 of 4 diets in a replicated 4 × 4 Latin square design with 21-d periods. Treatments included: 1) diet containing corn grain and alfalfa hay (CA), 2) diet containing corn grain and grass hay (CG), 3) diet containing wheat grain and alfalfa hay (WA), and 4) diet containing wheat grain and grass hay (WG). All diets were formulated using least-cost formulation while meeting energy and protein requirements for a 620-kg lactating cow producing 40 kg/d of milk (3.85% fat; 3.05% protein) and providing, ≥50% forage, ≥28% NDF, and ≤30% starch. Theoretical dietary costs (per kg DM) were $0.285, $0.283, $0.252, and $0.260 for CA, CG, WA, and WG, respectively. The statistical model included the effects of square (fixed), treatment (fixed), square by treatment interaction (fixed), period (random), and cow within square (random), and the random residual error. Orthogonal contrasts were used to test the main effects hay and grain, and their interaction. Dry matter intake (27.1 vs 24.4 kg/d; $P < 0.01$) and milk yield (47.5 vs 44.7 kg/d; $P < 0.01$) were greater for alfalfa- than for grass-based diets. While milk fat concentration was greater for grass- than for alfalfa-based diets (4.23 vs 3.89%; $P < 0.01$), milk fat yield was not affected by hay type (1.85 kg/d; $P > 0.65$). Milk yield tended to be greater for wheat- than for corn-based diets (46.9 vs. 45.3 kg/d; $P < 0.06$) but corn-based diets increased milk fat concentration relative to wheat-based diets (4.36 vs. 3.76%; $P < 0.01$). The resulting daily IOFC (per cow) were $14.27, $13.48, $13.14, and $12.53 for GC, GW, AC, and AW, respectively. In conclusion, feeding grass-based diets resulted in cheaper diets, a greater milk fat concentration, a greater milk price, and a greater IOFC than feeding alfalfa-based diets.

Key Words: alfalfa hay, grass hay, income over feed costs

Animal Behavior and Well-Being: Focus on Behavior

50 Measurements of behavior are essential components in the assessment of animal welfare. J. Rushen*, *University of British Columbia, Vancouver, BC, Canada.*

In this talk, I argue that the measurement of cattle behavior is a necessary aspect in the assessment of their welfare. Issues of behavioral deprivation have been central to the concern about animal welfare since the beginning and surveys have shown repeatedly that the public / consumers are particularly concerned about the inability of farm animals to perform natural behavior in intensive housing systems. Furthermore, early research showed that even farm animals subject to generations of artificial selection still show much of their natural behavior when given the opportunity to do so. Some of the most contentious issues in dairy cattle welfare involve primarily behavioral issues. Measures of animal behavior also add precision in animal welfare assessment. For example, common behavioral responses to illness provide one means for us to assess the relative impact on dairy cow welfare of various diseases. Behavioral based measures provide the best means we have at present of judging the acute emotional response of dairy cattle to short-term husbandry procedures and the longer term emotional state of the animals. However, to best use behavioral measures in animal welfare assessment, we need to better understand the causal and motivational bases of the behaviors. Finally, exploiting dairy cattle's behavior provides a novel means of solving some husbandry problems, while giving animals a sense of control over their environment, potentially improving their welfare. Because of these matters, assessments of animal welfare that do not include behavioral measures do not provide a complete picture of the welfare of the animals.

51 Making stall beds more comfortable: The effect of longitudinal space on lying behavior and leg injuries on dairy cows housed in deep-bedded tie-stalls. S. McPherson* and E. Vasseur, *McGill University, Sainte-Anne-de-Bellevue, QC, Canada.*

Poor stall comfort impairs lying behavior and leads to injuries. Stall bed comfort is affected by the amount of longitudinal space, defined by the front restriction of the stall (manger wall) and the stall length, and bedding depth. This project aimed to maximize cow comfort by investigating the combined effect of 3 aspects of the stall bed: stall length, manger wall height, and bedding depth. Two rows of 12 tie-stalls were modified. Each row was a different length: short (178 cm; length commonly found in Quebec) or long (188 cm). Two manger wall treatments were applied randomly to the stalls in each row: high (20 cm, upper limit recommended) or low (5 cm). A 7.6 cm deep straw bedding layer was maintained by a bedding guard. Cows were randomly divided into 4 groups ($n = 6$/group), blocked by parity (2.7 ± 0.32) and DIM (115 ± 13.2 d). Two groups were assigned to each row and subjected to both manger wall treatments in a crossover design (1 wk habituation, 6 wk data collection/treatment). Lying behaviors were recorded continuously via leg-mounted accelerometers. Hock injury was scored 1x/wk and analyzed as a difference from baseline for each period. Data were analyzed using a mixed model with length, sequence, block, treatment, and period as fixed effects, week as a repeated measure, and cow as a random effect. Cows in long stalls were found to spend more time lying (848.5 vs. 797.9 min/d; $P < 0.05$) and had longer lying bouts than cows in short stalls (74.1 vs. 52.9 min/bout; $P < 0.05$). Improvement in hock injury was observed from wk 1 to 6 for all treatments ($P \leq 0.001$, lateral tarsal; $P \leq 0.01$, lateral calcanei). Manger wall height did not affect injury or lying time. Higher lying times in our study were comparable to those reported in deep-bedded loose-pens, indicating that cows with more bedding, especially those in long stalls, were more comfortable. Deep-bedded straw stalls with bedding guards are applicable on tie-stall farms and result in cows that are more comfortable, spend more time lying, and have fewer leg injuries.

Key Words: stall length, manger wall height, deep bedding

52 Effect of pre-milking waiting time on the activity behavior of dairy cows. D. Manriquez*[1], S. Zuniga[1], G. Solano[1], S. Paudyal[1,2], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Texas A&M University, College Station, TX.*

The objective was to assess the effect of pre-milking waiting time in the holding area on the subsequent lying behavior and number of steps of dairy cows. Holstein cows (parity 1, $n = 17$; parity ≥ 2, $n = 111$) from a dairy in northern Colorado were affixed with a pedometer (IceQube, IceRobotics, Edinburgh, UK) in one rear leg providing number of steps (STP), lying time (LY; min), and standing time (STD; min). Cows were milked 3× in a 60-stalls rotary milking parlor (DeLaval). Time of entrance to the milking stall for each individual cow was automatically recorded from January to June 2018 (average DIM at enrollment = 15.3 ± 7.1). Starting time for each milking pen was also available. Individual waiting time was calculated for each milking as the difference between the pen starting time and the time of cow entrance to the milking stall. Waiting times were categorized (WaitCat) in low (≤ 30 min) and high (>30 min). STP, STD, and LY were added until the beginning of the subsequent milking and standardized in minutes/hour. General linear models were created to test the associations between waiting time categories and activity parameters. Parity (1; ≥ 2), shift (morning, afternoon, night), and their interactions with WaitCat were also included. Table 1 presents activity values by waiting category, as well as P-values for variables included in the models. Our preliminary analyses determined a tendency toward significance for the effect of WaitCat and parity on STP. A significant interaction between shift and Waitcat was observed for all the outcomes, suggesting that daily activities may modify the effect of waiting time on the behavior of dairy cows. Further analyses considering other potential confounding factors are required to clarify the significance of pre-milking waiting time on cow behavior.

Table 1 (Abstr. 52). Summary of activity parameters (mean ± SE) by pre-milking waiting category

	Steps (no./h)	Standing time (min/h)	Lying time (min/h)
WaitCat (min)			
$\leq$30	107.5 ± 4.24	34.8 ± 0.54	25.2 ± 0.54
>30	112.0 ± 4.25	34.7 ± 0.54	25.3 ± 0.54
P-value			
WaitCat	0.10	0.70	0.70
Parity	0.002	0.80	0.80
WaitCat × Parity	0.01	0.60	0.60
Shift	<0.001	<0.001	<0.001
Shift × WaitCat	0.60	0.03	0.03

Key Words: lying, milking, behavior

53 Rumination time and metritis in grazing dairy cows. R. Held*[1] and P. Sepúlveda-Varas[2], [1]*Escuela de Graduados, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile* [2]*Instituto de Ciencias Clínicas Veterinarias, Universidad Austral de Chile, Valdivia, Chile.*

Changes in rumination time (RT) can be used as an indicator of illness, yet no work to date has evaluated this relationship in dairy cows on pasture. The objectives of this study were to describe the RT of grazing dairy cows during the transition period and to determine the relationship between metritis and RT. Our sample included 53 multiparous and 17 primiparous Holstein cows that calved during the spring season at the Experimental Dairy Farm of the Universidad Austral de Chile (Valdivia, Chile). Cows were assessed every 2 or 3 d between 3 and 21 d in milk, and metritis was diagnosed by inspection of the vaginal discharge. Any presence of other clinical disease was recorded, and cows were subsequently categorized into 2 health categories: (1) healthy, no metritis and had no other signs of clinical (retained placenta, milk fever, metritis, mastitis) postpartum diseases; and (2) metritis, diagnosed as having metritis with no other signs of clinical postpartum disease with the exception of retained placenta. RT was recorded daily using an automated monitoring system from 3 wk before calving to 3 wk after calving. Data were analyzed using mixed linear models (SAS v9.4). The RT differed between healthy primiparous and multiparous cows; primiparous cows had lower RT during the precalving (d −21 to d −2; 504 ± 41 min/d vs. 559 ± 19 min/d; $P < 0.001$) and postcalving period (d 2 to d 21; 423 ± 9 min/d vs. 483 ± 5 min/d; $P < 0.001$) compared with multiparous cows, but RT did not differ between these parity classes at calving (d −1 to d 1; 412 ± 33 min/d vs. 426 ± 18 min/d; $P = 0.60$). The average number of days from calving to the first signs of metritis was 7.5 ± 0.7 d for primiparous cows and 6.7 ± 0.6 d for multiparous cows. A health status × day interaction was seen for the RT in primiparous cows ($P = 0.02$), but not for multiparous cows ($P = 0.95$) during d 3 to d 7 after calving. Primiparous cows that developed metritis spent less time ruminating ($P < 0.05$) during d 6 and d 7 compared with healthy cows. Our results suggest that changes in RT is not a good early indicator of metritis but is affected when the disease is already present in grazing dairy cows. Project funded by FONDECYT #11170820.

Key Words: transition, pasture, illness

54 Effect of hot-iron disbudding on rest and rumination in dairy calves. S. Adcock, B. Downey, C. Owens, and C. Tucker*, *Center for Animal Welfare, Department of Animal Science, University of California Davis, Davis, CA.*

Hot-iron disbudding, a husbandry procedure that prevents horn bud growth through tissue cauterization, is painful for calves. The resulting burns remain sensitive to mechanical stimulation for weeks, but the procedure's influence on behaviors that involve movement of the head, such as resting with the head down and ruminating, is largely unknown. We assigned female Holstein calves to 1 of 2 treatments: disbudded with a heated iron at 4 to 10 d of age (n = 11) or not disbudded (n = 11). Disbudded calves received a lidocaine cornual nerve block and oral meloxicam at the time of the procedure. All calves were provided starter from 5 d of age and half of the animals had access to hay, balanced across disbudding treatments. We recorded resting and ruminating behavior using 5-s scans taken every 5 min for 24 h once a week capturing the window from 3 to 21 d after the procedure. In addition to scan sampling, we used ear tag accelerometers (eSense by Allflex) to monitor behavior in 1-min intervals throughout the experiment. Mixed β regressions were used to test the effect of treatment and its interaction with age on the daily proportions of time the calf spent lying and ruminating based on the live scan observations thus far. Calf was fitted as a random effect in the models. Compared with controls, disbudded calves ruminated less in the first 2 wks after disbudding (mean ± SE: 10 ± 1% vs 18 ± 2% of total time; $P = 0.003$) and were more likely to lie with their head down and still across all weeks (31 ± 1% vs 26 ± 1% of total lying time; $P = 0.012$). A decrease in ruminating and increase in lying with the head down and still may reflect an avoidance of moving the head in ways that could aggravate disbudding wounds. We conclude that disbudding, in addition to resulting in prolonged sensitivity of the wounds, is severe enough to alter daily behavior patterns for at least 3 wks, raising additional welfare concerns about the procedure.

Key Words: cautery disbudding, pain, animal welfare

55 Age affects Holstein cow behavioral responses to hoof disorders. C. Toet[1] and A. Adams-Progar*[2], [1]*Wageningen University and Research Centre, Wageningen, the Netherlands,* [2]*Washington State University, Pullman, WA.*

Early detection and treatment of hoof disorders in dairy cattle is imperative for animal well-being and production. Changes in cow behavioral patterns may assist with the early detection of hoof disorders. The objective of this study was to evaluate how hoof disorders affect behavioral patterns in Holstein cows. Hoof trimming records collected over 2 yr at the Washington State University Knott Dairy Center (Pullman, WA) were used to identify cows diagnosed with hoof disorders (H; n = 69) and sound cows (S; n = 69), matched by age and hoof trimming date. Hoof disorder diagnoses included digital dermatitis, white line disease, and sole ulcers. The CowManager system was used to collect cow ear temperature, activity, rumination, and eating behaviors for 7 d before hoof trimming, day of hoof trimming, and 7 d after hoof trimming. Data were analyzed to compare differences between cows using PROC MIXED in SAS. A 3-way interaction among cow age, hoof health status, and day of study was detected for ear temperature ($P < 0.0001$), active ($P < 0.0001$), non-active ($P = 0.0002$), rumination, ($P = 0.008$), and eating ($P = 0.0003$) behaviors. All cow groups had the lowest ear temperatures before hoof trimming and highest ear temperatures after hoof trimming, with the greatest difference occurring in the 5-yr-old cows ($P = 0.03$). The oldest cows (7 yr of age and older) with hoof disorders showed more variation in all behaviors over the 15-d period than sound counterparts. The youngest cows (3 yr of age) with hoof disorders spent less time being active (H: 2.14 ± 0.01 h vs. S: 2.61 ± 0.01 h) or eating (H: 2.12 ± 0.02 h vs. S: 2.70 ± 0.02 h) than sound counterparts. A significant difference ($P = 0.01$) in non-active behavior occurred between 4-yr-old cows with hoof disorders (6.85 ± 0.03 h) and sound counterparts (8.30 ± 0.04 h). The greatest difference in rumination between cows with hoof disorders and sound cows occurred in the oldest cows (H: 7.91 ± 0.05 h vs. S: 9.97 ± 0.04 h). The use of activity monitoring technologies to identify changes in behavior could aid in the early detection of hoof disorders in dairy cows; however, the age of the animals being monitored should be considered, as demonstrated in this study.

Key Words: activity monitor, cow behavior, hoof disorder

56 The effect of access to an outdoor pack on lameness and estrus expression. A. Smid*, M. von Keyserlingk, and D. Weary, *University of British Columbia, Vancouver, BC, Canada.*

Pasture access can help prevent lameness and facilitate the expression of estrus but providing access to quality pasture is not always feasible. An alternative form of outdoor access is a deep-bedded pack, but it is unknown if access to an outdoor open pack is beneficial in these ways.

The aims of this study were to investigate if access to an outdoor open pack can: 1) prevent the development of lameness, and 2) facilitate the expression of estrus behavior. A total of 36 cows were enrolled directly after calving and randomly assigned to either the OUTDOOR or INDOOR treatment and followed for a total of 12 weeks. All cows were housed in the same pen, but only OUTDOOR cows had access to an outdoor open pack. Each week, cows were gait scored from video observations. Cow behavior in the pen and on the outdoor pack was continuously followed by video recordings. Following an alert from the activity monitoring system, cows were checked for the presence of a preovulatory follicle and the absence of a mature corpus luteum (CL) by rectal ultrasonography following milking as well as 48 h and 7 d thereafter to confirm ovulation. Both suspected estrus and false alerts were scanned 7 d post-alert to confirm estrus classification (i.e., if the alert was correctly classified as a true or false estrus). For each true estrus event, the number of visits to the outdoor pack by OUTDOOR cows was investigated. The effect of treatment on gait score was assessed using a mixed model with treatment specified as fixed effect and cow as repeated effect. The effect of estrus status on visits to the outdoor pack was assessed using a mixed model with estrus status (i.e., in estrus or not) as fixed effect. No effect of treatment was found on mean gait score: INDOOR: 2.46 ± 0.15; OUTDOOR: 2.61 ± 0.15, but OUTDOOR cows visited the outdoor pack more often on days when they were in estrus (1.78 vs 6.07 visits/d). These results indicate that access to an outdoor pack has a positive effect on the expression of estrus behavior for freestall-housed dairy cows.

Key Words: outdoor area, bedded pack, exercise

57 Development of an automated computer vision system to monitor behavior of dairy calves.

J. R. R. Dorea*, S. Cheong, D. K. Combs, and G. J. M. Rosa, *University of Wisconsin-Madison, Madison, WI.*

Calfhood diseases during early stages of growth can detrimentally affect productive performance in dairy cattle, resulting in considerable economic losses to the dairy industry. Health issues can alter calf behavior, so that change on behavior pattern could be used as an earlier indicator to prevent diseases. However, in large dairy operations, the daily monitoring of calf behavior is laborious, and the large number of animals becomes a limiting factor for such evaluation. Thus, the objective of this study was to develop an automated computer vision system to individually monitor behavior of dairy calves housed in groups. The experiment was conducted at the Dairy Research Cattle Center of the University of Wisconsin-Madison. Five calves were housed in a group from the 4th to 8th weeks of age. All calves had ad libitum access to water and calf starter from the first week of life until the end of the trial. A Wi-Fi camera with night vision capability (Amcrest Outdoor Wi-Fi Camera, model: IP3M-956EW) was installed 4 m above the pen. Images were acquired every 5 s and sent to a data storage in the cloud platform. For each image, calves were spatially located and then labeled for their respective identification (ID, from Calf 1 to Calf 5) and classified for animal behavior (lying, drinking, eating, and standing). A total of 650 images were used for training purpose and 100 images were used for validation. A deep neural network approach called Mask RCNN was implemented to generate the predictions. The algorithm was trained by adopting the strategy of transfer learning, for which the weights from COCO data set were used. All analyzes were implemented in Python using the open source frameworks TensorFlow and Keras. The accuracy to recognize a specific calf were: 77% (Calf 1), 70% (Calf 2), 80% (Calf 3), 92% (Calf 4), 80% (Calf 5). The accuracy to predict the behavior activity of lying, drinking, eating, and standing were: 91, 86, 85, and 100%, respectively. These results indicate that a computer vision system can be a powerful tool to monitor behavior of dairy calves housed in groups.

Key Words: calf, computer vision, deep learning

58 Deriving intake from multiple 3-dimensional accelerations in peripartal Holstein dairy cows.

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Dry matter intake is commonly observed to decreased around calving in dairy cows and this can have severe health consequence depending how acute and prolonged this effect occurs. The objective was to evaluate the use of 3-dimensional accelerometer sensors to estimate DMI in peripartal dairy cows. Twelve Holstein dairy cows housed in bedded pack pens during close-up were fitted with 3 sensors that record acceleration in the 3-axis (i.e., x, y, and z), one sensor on the lateral side of the left hind leg and 2 attached to a halter directly superpose over the jaw and nose. After calving, cows were moved to a free-stall barn bedded with straw. Cows were assigned 2 groups, a data collection group (A; n = 6) and a validation group (B; n = 6). Accelerations and individual intakes were collected from −7 to 7 d relative to parturition. Cows were trained to use Calan gates at least 7 d before data collection. Sensors were set to record the accelerations at 1 min intervals. Acceleration characteristics highly associated with DMI determine in a previous study were used to cross-reference accelerometer data and DMI in group A. Six new variables were derived based on lag-times in jaw and nose accelerations. The REG procedure of SAS was used in group B derive DMI from acceleration combinations (DMIA) and compared this against the actual DMI using the CORR and MIXED procedures of SAS. Previously, 921 acceleration combinations were deemed relevant for DMI estimation, and these were tested in the current study. LegX+NoseX model had the strongest positive correlation ($r = 0.54$), and its DMIA was similar ($P = 0.26$) than the actual DMI (14.5 vs 15.9 kg/d), but it did not follow the rapid decreased observed in actual DMI around calving ($P = 0.07$). In contrast, LegZ+JawX+LagNoseZ+LagNoseX model had the closest actual DMI estimation ($P = 0.99$), but a not significant correlation ($P = 0.46$). The intermediate model LegZ+JawZ+JawY+LagNoseY+LagJawY had a weak correlation ($r = 0.30$) but described a similar decreased ($P = 0.53$) between DMIA and actual DMI around calving. Accelerometer sensors have a great potential to estimate peripartal DMI, could be a future approach utilize by commercial dairy farms to flag cows at risk to develop a postpartal disease.

Key Words: accelerometer, intake, sensor technology

Animal Health: ADSA-NMC Platform Session: Milk Quality and Mastitis Control in a Changing Dairy Industry

59 Rethinking milking efficiency in a dynamic dairy industry. R. Erskine*, *Michigan State University, East Lansing, MI.*

The dairy industry is consolidating, but also becoming more diverse in employment organization. Many dairy managers have limited human resource knowledge and experience; this often leads to frustration with protocol drift and a sense that employees are not engaged in the success of the farm beyond prescribed instructions. Additionally, the role of immigrant labor can complicate communication barriers and management–employee relationships. Education, training and translation tools have been developed by land grant universities, consultants and agricultural agencies. However, these programs were developed from a management-directed perspective with minimal input from employees. Furthermore, the effectiveness of employee training, or education programs, relative to farm protocols and productivity, has not been evaluated for short or long-term success. Dairy herds that ensure strict compliance of milking protocols have lower bulk tank somatic cell counts than herds that have difficulties with protocol compliance. Thus, further advances in milk quality must address the gap between human resource needs and the capacity of producers and managers to address them. Superimposed on these labor issues, many dairy operations increase the pressure on employee performance by striving to maintain a high level of milking parlor throughput. Thus, milking efficiency is often defined in terms of cows milked per hour, or milk produced per hour. While this definition of milking efficiency is pervasive, recent research by our Quality Milk Alliance (http://qualitymilkalliance.com/) project team suggests that we may need to reconsider the definition of milking efficiency to include milk quality, harvest, and employee performance.

Key Words: human resources, milking protocol, milking efficiency

60 Automated mastitis detection for robotic milking systems using deep learning and recurrent neural networks. M. T. M. King*[1], S. A. Naqvi[2], M. Champigny[3], R. Deardon[2], H. W. Barkema[2], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Production Animal Health, University of Calgary, Calgary, AB, Canada,* [3]*PhenoLogic Co., Toronto, ON, Canada.*

With the abundance of data collected by automated milking systems (AMS) comes the need for reliable, validated algorithms for disease detection. The objectives of this study were to (1) integrate AMS data to develop accurate mastitis detection models using recurrent neural networks; (2) determine the relative importance of variables and their effect on model performance; and (3) assess the accuracy of our models. Milking data (including milk yield, visit frequency, duration, temperature, conductivity), cow behavior (rumination time, activity), cow data (DIM, parity), and mastitis records were collected from 13 commercial AMS dairy herds in Ontario, Canada for the first 30 d of lactation for 822 cows. Clinical mastitis was diagnosed when a cow had poor quality or quantity of milk production, as measured by the AMS, and abnormal milk or udder upon visual examination, and the cow was treated using an antimicrobial. Deep learning models were used to predict the daily probability of a cow being diagnosed with mastitis, based on 54 possible input variables (i.e., milking, behavior, and cow data, for each milking and variance for each day). Initial models were run only using healthy cows (no recorded health disorder) and cows having only mastitis. Recurrent neural networks, with varying numbers of long short-term memory cells, were trained using different lengths of time windows when cows were classified as sick for 3, 5, 7, and 15 d centered around diagnoses. Farms were divided into 3 groups: 9 farms for model training and development (n = 240 cows, 20 mastitis cases), 2 farms for model testing (n = 81 cows, 6 mastitis cases), and 2 farms for hold-out validation. Using a combination of milk and behavior data and prediction windows of 3, 5, 7, and 15 d centered around the day of diagnosis, models achieved 82, 85, 79, and 93% accuracy during testing, respectively. Excluding behavior data reduced prediction accuracy by 5% units. Excluding daily variances reduced prediction accuracy by 7% units. Overall, these methods and resulting algorithms have great potential to improve the reliability and timeliness of automated mastitis detection for dairy producers using AMS.

Key Words: robotic milking, mastitis detection, machine learning

61 Detection of bovine mastitis biomarkers in milk by porous silicon optical biosensors. N. Pinker*[1] and G. Shtenberg[2], [1]*the Hebrew University, Rehovot, Israel,* [2]*The Volcani Center, Bet Dagan, Israel.*

The proposed research specifically addresses the most prevalent health problem in dairy cows, the bovine mastitis (BM), by developing a generic biosensing platform for on-site monitoring applications. Traditional diagnostic methods check the quality of milk through the detection of mammary gland inflammation, infection diagnosis and its causative pathogens. However, all those techniques lack the ability to detect analyses in real-time or outside the laboratory boundaries. Therefore, there is an urgent need for a rapid, accurate, cost effective, simple and portable method to evaluate in real-time new cases of BM, followed by corrective and preventive actions, leading to a positive effect on animal health and overall economy of the dairy farms. Herein, we have designed and fabricated an optical biosensing platform, based on porous silicon nanostructures, Fabry-Pérot film, for the detection of BM-predicting biomarkers, haptoglobin (Hp) and *N*-acetyl-β-D-glucosaminidase, which are monitored in real-time by reflective interferometric Fourier transform spectroscopy. Indeed, increased Hp levels cause a profound increase in the relative optical signal as specific hemoglobin-Hp complexes are formed. Thus, for clinical and subclinical BM values a red shift of 0.36 ± 0.04% and 0.24 ± 0.006% in the effective optical thickness are obtained. These results correlate to Hp concentrations obtained by ELISA test in which values of 9.6 ± 0.3 and 2.3 ± 0.1μg/mL are received for clinical and sub-clinical milk samples (both positive to *S. dysgalactiae* pathogen), respectively. Control experiment of healthy milk (SCC < 100,000, with negative microbial contamination) presented a minor increase of 0.06% in optical output as insignificant values of Hp are obtained (1.1 ± 0.1 μg/mL). Similar differentiation is shown for the latter biomarker. Our studies reveal that the optical nanostructure capable of multitasking: immobilization, size-exclusion, rapid and sensitive detection of specific biological targets in real milk samples, offering simultaneous real-time detection based on physical characteristics. Thus, the main advantage of the presented biosensing concept is the ability to detect BM predicting biomarkers in milk, using a simple and portable experimental setup.

Key Words: bovine mastitis, optical biosensor, porous Si

62 Insights into the relationships among dairy farmers, milk quality, and farm closure in the southeastern United States.

S. M. Schexnayder*, K. L. DeLong, J. Ellis, P. Krawczel, G. M. Pighetti, and S. P. Oliver, *University of Tennessee, Knoxville, TN.*

The decline in dairy farms in the Southeast United States has outpaced the decline in the United States overall. Although the inventory of dairy cows has remained roughly unchanged in the last decade, the number of US dairy herds has declined by 58% while the southeast lost 71% of its herds. Challenges of consistently producing high quality milk have likely contributed to this decline. The focus of this study was to identify farm/farmer characteristics and farmers' attitudes and perceptions about mastitis and mastitis management related to SCC (as a marker of milk quality) and to dairy farm closure. A mail survey of dairy farms in operation, or which had ceased milk production over the previous 3 years, in 7 Southeast states captured information about BTSCC levels, farm characteristics, operator characteristics, farm management practices, information sources accessed by the operators, and operators' perceptions of and attitudes about mastitis and mastitis management. A probit regression model was used to determine factors were associated with the dairy's operational status (closed or operational). Closed dairies had lower average milk production per cow, smaller herd size and greater age of the farm's primary operator. Operators of closed dairies reported that the lowest level of bulk tank SCC (BTSCC) that caused them concern was on average 418,000 cells/mL, while operators of dairies still in business reported concern at 338,000 cells/mL. Several variables reveal a relationship between milk quality and dairies' operational status. The positive influence of the presences of both incentives for high quality milk and penalties for milk of quality below a specific threshold demonstrates that milk payments supplemented by incentives and/or penalties are contributing to dairies remaining operational. Some management practices also contribute to dairies' operational status. Having the primary dairy operator in the parlor routinely for milking is associated with dairy closure and may reflect either small-sized dairies or understaffed dairies. Routine bacterial culturing, participation in DHIA testing, and using antibiotic therapy to treat clinical mastitis each have negative marginal effects and are associated with dairy closure. While each of these dairy practices has a role in good herd and dairy management, this finding may suggest that poor performing dairies are turning to these practices because of consistently high BTSCCs and/or high rates of clinical mastitis, both of which could be associated with poor awareness of or poor execution of other mastitis prevention and management practices.

63 Comparative efficacy of teat sealant protocols: A systematic review and network meta-analysis.

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Treatment and prevention of intramammary infections (IMI) represent a large portion of antibiotic use in the dairy industry. Dairy producers have multiple intervention choices to prevent IMI in the dry period, including use of internal teat sealants with or without antibiotics. Generally, producers and advisors are interested in relative efficacy, as opposed to pair-wise comparisons to non-treated controls or a single comparator. Network meta-analysis assesses relative efficacy of multiple interventions through direct and indirect evidence. The objective of this review was to assess the efficacy of teat sealants to prevent new IMI and clinical mastitis in the subsequent lactation. Five databases, relevant conference proceedings, and FDA NADA summaries were searched. Eligible studies assessed teat sealants with or without antibiotics, compared with no treatment or other treatment (e.g., antibiotics alone) in pre-partum heifers or dairy cows at dry-off, and assessed one or more of the following critical outcomes: incidence of IMI at calving, incidence of IMI in the first 30 DIM, or incidence of clinical mastitis in the first 30 DIM. Study screening and data extraction were done independently in duplicate. From 2280 initially identified records, 199 were assessed at full text, with 75 studies containing 82 trials passing this stage. 40 trials had full data extracted, with network meta-analysis performed using 23 trials reporting incidence of IMI at calving. Use of an internal teat sealant significantly reduced the risk of IMI at calving compared with non-treated controls (RR = 0.36, 95% CI = 0.25, 0.72), but the use of any category of antibiotic in addition to teat sealants did not reduce the risk beyond that of teat sealant alone. Exploration of sources of heterogeneity is ongoing. This work revealed important challenges with comparable outcomes, replication and connection of interventions, and quality of reporting of study conduct. Consideration of use of reporting guidelines, standardized outcomes, and inclusion of at least one intervention arm used in other research would increase the value of primary research in this area.

Key Words: mastitis, udder health, mixed treatment comparison

64 Antimicrobial resistance of *Klebsiella* species from milk submitted to Wisconsin Veterinary Diagnostic Laboratory for mastitis testing, 2008–2018.

E. Furmaga*, N. Aulik, and M. F. Valenzuela, *Wisconsin Veterinary Diagnostic Laboratory, Madison, WI.*

The purpose of this retrospective study was to evaluate trends in antimicrobial resistance (AMR) in *Klebsiella* from milk samples submitted to the Wisconsin Veterinary Diagnostic Laboratory (WVDL) for bovine mastitis testing. At WVDL, the CMV1AMAF panel by the Thermo Scientific Sensititer system is used to run minimum inhibitory concentration (MIC) panels. The Sensititer database was reviewed for milk submitted for mastitis testing where *Klebsiella* species was determined to be the primary pathogen. The mastitis cases were finalized from January 1, 2008 through December 31, 2017. Clinical Laboratory Standards Institute (CLSI) guidelines were used to determine MIC breakpoints. Due to the lack of *Klebsiella*-specific breakpoints in CLSI, MIC cut-offs for antibiotics on the mastitis panel were used from *E. coli*; both bacteria are members of the Enterobacteriaceae family. The percent of samples resistant to the antibiotics was calculated; *Klebsiella* was found to be completely resistant to most antibiotics on the panel except for ceftiofur and tetracycline. For ceftiofur, resistance ranged from 0% (2010) to 22.7% (2017), and for tetracycline, from 13.8% (2011) to 40.9% (2013) (Table 1). Overall, there was no significant change in antimicrobial resistance of *Klebsiella* over the 10-year period of data.

Table 1 (Abstr. 64). Percent of *Klebsiella* samples resistant to ceftiofur and tetracycline, 2008–2017

Antibiotic[1]	2008 (n = 71)	2009 (n = 59)	2010 (n = 57)	2011 (n = 29)	2012 (n = 39)	2013 (n = 44)	2014 (n = 43)	2015 (n = 40)	2016 (n = 30)	2017 (n = 75)
Ceftiofur[2]	1.4	6.8	0	0	7.7	11.4	7.0	7.5	3.3	22.7
Tetracycline[2]	21.1	23.7	26.3	13.8	41.0	40.9	25.6	30.0	26.7	25.3

[1] CLSI breakpoint concentrations (µg/mL) used to classify samples as antibiotic resistant (R).

[2] Bacteria species/sample source for antibiotic: for ceftiofur, *E. coli*/bovine mastitis (R >4), and for tetracycline, *Enterobacteriaceae* family/humans (R >8).

Breeding and Genetics Symposium: Joint ADSA/Interbull Session: Ten Years of Genomic Selection

65 From sequence of Dominette to 10K and 50K SNP chips. D. Bickhart*, *USDA Agricultural Research Service Dairy Forage Research Center, Madison, WI.*

Genetic markers based on interrogated nucleotide variant sites have been used in cattle genetics since the late 1990s. Owing partly to the excessive cost and labor-intensive means of assessing marker sites, such as microsatellite repeats, their commercial use was relatively limited. Additionally, genotyping accuracy was error-prone and did not cover the cattle genome evenly. In this presentation, we highlight 2 specific improvements that started the modern genomics era. The development of high-resolution DNA sequence maps of the cattle genome and the adaptation of microarrays for high throughput genotyping served as the catalysts for data collection for modern cattle genomic selection. Simultaneously, the development of computational methods for associating animal SNP genotypes with productive trait phenotypes improved the accuracy of animal selective breeding values by providing higher resolution of allelic transmission to offspring. The efficacy of these tandem improvements has generated incredible value for the modern cattle breeder; however, many challenges and questions persist. Linkage between genetic markers and their associated causal genetic mutations can be further improved by genotyping the actual mutation itself. There is additional novel sequence within the cattle pan-genome that is currently not represented in the cattle reference genome assembly, which may not be tracked by current genetic markers. Finally, methods need to be developed that efficiently incorporate whole genome DNA sequence data into genomic selection. Future techniques and technologies that may address these challenges will be discussed.

Key Words: SNP chip, cattle reference, genomics

66 How to implement genomic selection. P. M. VanRaden*, *USDA Animal Genomics and Improvement Laboratory, Beltsville, MD.*

Key features of genomic selection remain essential, and new features continue to be added 10 years after first implementation. Breeding values with higher reliability earlier in life are estimated by combining DNA genotypes for many thousands of loci using existing identification, pedigree, and phenotype databases for millions of animals. Quality control for both new and previous data greatly improves by comparing genomic and pedigree relationships to fix parent-progeny conflicts and discover many additional ancestors. Many quantitative trait loci and gene tests have been added to previous assays that used only evenly spaced, highly polymorphic markers. Imputation now combines genotypes from many assays of differing marker density. Prediction models have gradually advanced from normal or Bayesian distributions within breed and trait to single-step, multitrait, multibreed, or other more complex models. Genomic selection was initially applied to males to predict progeny performance but now is widely applied to females or even embryos to predict their own later performance. The initial focus on additive merit has expanded to include mating programs, genomic inbreeding, and recessive alleles. Many producers now use DNA testing to decide which heifers should be inseminated with elite dairy, beef, or sex-sorted semen; be embryo donors or recipients; or be sold or kept for breeding. Because some of these decisions are expensive to delay, predictions are now provided weekly instead of every few months. International genomic databases and predictions are often more accurate than within-country genetic evaluations previously designed for progeny testing. Obtaining predictions from an international database is usually more effective than computing predictions from only local data unless local breeds, conditions, or traits differ greatly from the large database. Selection indexes include many new traits, often with lower heritability or requiring large initial investments to obtain phenotypes, which provide further incentive to cooperate internationally. The genomic prediction methods developed for dairy cattle are now applied widely to many other animal, human, and plant populations.

Key Words: genomic prediction, DNA testing, dairy cattle

67 The role of Interbull in the successful implementation of genomic selection in breeds other than Holstein. E. C. Santus*, *ANARB, Bussolengo, Italy.*

The use of DNA information in dairy cattle is not a complete novelty as specific markers for interesting genes have been used in selection schemes for specific needs since the late 1980s. As we all know, the breakthrough occurred at the turn of the millennium. First with the envision and later with the practical application of the massive analysis of thousands of markers on cattle genome. Since then, the approach has changed completely passing from the Mendelian identification of desirable alleles to the studies on the associations between markers and phenotypes. At that time, numbers started to matter and the risk of facing the "new opportunities, old inequalities" paradox became a real scenario as the new mechanisms of exploitation of the new technology called for a minimum size population and set a limit for entry into this new market. The Brown Swiss breed is a small breed compared with the Holstein breed, but all breeds are small if you use the Holstein breed as the term of reference. Thus, it is of some relevance to use the Brown Swiss example as a possible model. The greatest advantage of this breed in Europe was the existence of a very active international community. Another advantage was the fact that no single country had alone the size of the population needed for a reliable implementation of genomic selection. Political aspects were not, as always, of secondary importance when the discussion about sharing valuable information started. Since the very beginning, Interbull—recognized by all as an independent, reliable and competent third party—was identified as a natural platform that could solve most of the political issues. The development of an agreement among all parties, called "Intergenomics," was the first necessary step, which was followed by specific agreements between each party and Interbull. Thanks to that approach, genomic selection has become a reality for the Brown Swiss breed and has revolutionized the way the genetic management of the population has been carried out since then. After only a few years, in 2014, Intergenomics evolved into Intergenomics 2.0 and now it has become a routine service provided by Interbull to the member countries that joined the agreement.

Key Words: Interbull, genomics, Brown Swiss

68 Exploiting homozygosity in the era of genomics—Runs of homozygosity, inbreeding, and genomic mating programs. C. Maltecca*[1], C. Baes[2], and F. Tiezza[1], [1]*North Carolina State University, Raleigh, NC,* [2]*University of Guelph, Guelph, ON, Canada.*

Theory and early simulation results suggested that the implementation of genomic selection should result in a lower rate of inbreeding per generation. However, current experience has shown that sires selected on GEBV have a higher inbreeding coefficient than those selected using conventional approaches. With a large proportion of dairy populations genotyped genomic information offers a unique opportunity to understand and manage genomic diversity. With this work, we will show different approaches aimed at constraining inbreeding accumulation while accounting for the heterogeneity of inbreeding depression across the genome. In most cases characterizing and managing diversity and inbreeding using autozygous segments, either homozygous by descent (HBD) or approximated by runs of homozygosity (ROH) is advantageous compared with using average genomic inbreeding metrics. In simulated data constraining the accumulation of long HBD (ROH) allow more efficient recruitment of new potential beneficial haplotypes as well as limiting the accumulation of deleterious variants in the population more efficiently. Identification of specific variants related to recessive load remains difficult. Using a heuristic approach, an average of 244 deleterious haplotypes are flagged as potentially partially detrimental per each combination of chromosome-trait of interest. Many of these haplotypes are shared across traits and have a small overall effect, in accordance with the partial recessive theory of inbreeding depression.

Key Words: homozygosity, genomic selection, genomic diversity

69 **Single-step genomic evaluations.** E. A. Mäntysaari*, M. Koivula, and I. Strandén, *Natural Resources Institute Finland (Luke), Jokioinen, Finland.*

During the last decade genomic selection has revolutionized the dairy cattle breeding programs. For example, the Nordic dairy cows (Denmark, Sweden, Finland) born in 2018 were >90% sired by young genomic tested bulls. The average age of sires for Red Dairy Cattle (RDC) cows born 2018 was 3.1. While before the key driver of genetic progress was the selection of progeny tested sires, it is now the young sire preselection. This leads into difficulties in estimation of genetic progress by the traditional genetic evaluations. The only long-term solution is to include the genomic information into national animal model evaluations. Although means for this; that is, single-step evaluation models, have been available since 2010, they have not been yet implemented in large-scale national dairy cattle evaluations. At the first, single-step evaluations were hindered by computational cost. This has been largely solved by sparse presentations of $\mathbf{G}^{-1}$ (genomic relationship) and $\mathbf{A}_{22}{}^{-1}$ in single-step GTBLUP, or in APY approach, or using single-step marker models. In our test runs with 10.3 M beef cows and 1.5 M genotypes, each ssGTBLUP iteration of 6-trait calving difficulty model took roughly 4 times longer than pedigree animal model iteration. Concurrently with algorithm development, the computing resources have evolved both in availability of RAM and CPUs. The problems actively studied now are the same for the both single-step approaches (GBLUP and marker models). In both the convergence in iterative solving seems to get worse with increasing number of genotypes. Problems are clearer with low heritability traits, and in multi-trait models with high genetic correlations among traits. Additionally, it seems that they interact with unbalancedness of pedigrees and diverse genetic groups. Standard iteration approach is preconditioned conjugate gradient, in which the convergence has been improved with better preconditioning matrices. Another difficulty to be considered is the inflation in genomic predictions for selection candidates. The genomic models seem to overvalue the genomic relationship (or SNP) information. The problem is usually smaller in single-step evaluations than in multi-step evaluations but is more difficult to cover by "tune-up" parameters.

Key Words: genomic evaluation, single-step, cross-validation

70 **What's next for dairy cattle breeding?** G. Gorjanc*, *The Roslin Institute, University of Edinburgh, Edinburgh, UK.*

This contribution builds on current trends in dairy cattle breeding and discusses continuing and new avenues in genomics, phenomics, breeding and molecular genetics. Last decade has seen an immense growth in genome-wide genotyping with SNP arrays, which has enabled breeders and producers to quickly genetically improve their herds. The number of genotyped animals is now in the millions and it is possible that in the near future all breeding, if not all production, dairy animals will be genotyped. At the same time genome re-sequencing is becoming cheaper. Appropriate sequencing strategies and imputation will give us whole-genome sequence for the millions of genotyped animals. Investment in sequencing is not there yet, but some breeding programs are already sequencing all AI bulls. Although SNP array information is sufficient for genomic selection within breeds, sequence information will facilitate studies and breeding across breeds through increased mapping resolution. Sequence data will provide information on short insertions and deletions and large-scale structural variants. New data formats will be required, such as genome graphs. Modeling the vast amounts of data will pose a significant challenge. Recent studies on population and evolutionary genetics in human proposed a data structure (based on evolutionary trees with recombination) that enables almost perfect data compression. I will show how to use this data structure for quantitative genetic modeling of haplotype effects, either with SNP array or sequence data. When applied to a genome region the model has a sparse structure similar to the pedigree model. Such a model could facilitate within- and across-breed data analysis. The volume of phenotype data will also increase through the use of existing and new recording technologies, for example, activity sensors, automatic milking systems, infrared spectroscopy, gene-expression, etc. These high-throughput phenotypes are often high-dimensional and can be used to infer various properties of an animal with deep learning methods. For example, routine infrared spectroscopy data from milk samples is used in the UK to predict cow pregnancy status, which is in turn used as phenotype data for genetic evaluation of fertility. The sheer volume of these data will pose a question of how different breeding entities and activities contribute to genetic gain. Blockchain-like technologies could be used to track the flow of information in genetic evaluations and flow of genome segments in a breeding program to create a transparent tracking system. Such a system is required as current public-private breeding landscape drives rapid changes in genetic diversity. Introduction of genomic selection increased the loss in genetic diversity despite the potential to enlarge the breeding pool. I will show with simulation of a phenotypic and genomic dairy breeding program that optimal contribution selection should be used to increase the efficiency of converting genetic diversity into genetic gain. Finally, molecular genetics will drive new developments via genome editing, gene drives and potentially also cell-line breeding.

Dairy Foods Symposium: Advances in Spore Control Throughout the US Dairy Value Chain

71 How the US dairy industry increased production of low-spore dairy products and ingredients. A. Bienvenue*[1] and R. Kapoor*[2], [1]*US Dairy Export Council, Arlington, VA,* [2]*National Dairy Council, Rosemont, IL.*

The demand for milk and dairy ingredients is growing globally. To capitalize on this opportunity, US dairy exporters must become a supplier of choice to end-users in international markets. Spore-levels are top of mind for end-users that will manufacture ultra-high temperature (UHT) and shelf stable beverages, foods and nutritional formulas, because the presence of spores limits end-users' ability to use ingredients in those applications. The ability to control spore levels is crucial for ingredient suppliers targeting high-value applications. The US Dairy Export Council (USDEC) and National Dairy Council (NDC) teamed up to lead an effort to enhance the U.S. dairy industry's capabilities to consistently produce milk powders that meet international customer specifications. Our initiative has advanced knowledge of best practices and delivered solutions for spore-control on the farm, in the plant, and during cleaning. In addition, we have gained a better understanding of the impact of spore-testing methodologies. Our research efforts have been amplified by the support from other farmer-funded state and regional organizations and industry partners. The transfer of technical knowledge catalyzed by this initiative led to the adoption of new practices, upgrades to current facilities, and major capital investments to build new milk powder plants. All these efforts have increased the US dairy industry capacity to supply greater volumes of low-spore milk powder to the global market. This talk will focus on why low-spore dairy ingredients are important for export markets and the specific objectives related to the USDEC-NDC milk powder quality enhancement program. This talk will also summarize some of the key findings from various projects that were supported through this initiative.

72 Spores in milk powders—Practical solutions for improved detection and important insights for predictability of spoilage of reconstituted products. R. T. Eijlander[1], R. van Hekezen[1], A. Bienvenue[2], V. Girard[3], E. Hoornstra[4], N. Johnson[5], R. Meyer[5], A. Wagendorp[1], D. C. Walker[6], and M. H. J. Wells-Bennik*[1], [1]*NIZO, Ede, the Netherlands,* [2]*US Dairy Export Council, Arlington, VA,* [3]*BioMérieux, R&D Microbiology, La Balme-les-Grottes, France,* [4]*FrieslandCampina, Laboratory & Quality Services, Leeuwarden, the Netherlands,* [5]*Nestec Ltd., Nestlé Research, Konolfingen, Switzerland,* [6]*Abbott Laboratories, Columbus, OH,*

Various bacteria produce spores as a survival mechanism under adverse conditions. Bacterial spores that are present in foods and ingredients may survive heat processing conditions that are applied for preservation of the finished product. When spores are exposed to conditions that allow for spore germination and outgrowth, spoilage of such products can result in costly product losses, or, in the case of outgrowth of pathogens, consumption of such products may result in food borne illness. Food or food ingredient companies rely on practical and fast microbiological methods to determine the amount of spores that are present in their products for routine risk assessments. The current ISO method aimed at enumeration of especially heat resistant spore in dried milk (ISO/TS27265; 2009) is not routinely applied, as it requires heating at an impractical temperature of 106°C. The efficacy and reliability of alternative methods rely on the actual heat load applied, the ability of the cultivation medium to support growth of all surviving spore species, and on the interpretation of the analytical results. In a collaborative effort of NIZO, Nestlé, BioMérieux, FrieslandCampina, Abbott and USDEC, the options for a practical and reliable spore enumeration method were investigated. In addition, the outcomes of the analyses of spore concentrations in powders were evaluated in relation to spoilage of reconstituted UHT treated milk by highly heat resistant bacterial spores that survived the heat treatment. This applied method involves a heat treatment at 100°C and plating on TSA, which was found to best support the recovery of commonly encountered spore forming species in dairy products. The study provides tools to standardize practical spore tests and enables improved interpretation of spore count test results in relation to spoilage risks of UHT-treated products made from milk powder.

73 Interventions for reduction of spore-forming bacteria at the farm level. A. Bianchini*, *University of Nebraska-Lincoln, Lincoln, NE.*

Bacillus and Paenibacillus spp. are spore-forming bacteria with the ability to survive the pasteurization process due to their spore structure. These bacteria can produce different enzymes that negatively affect the quality of dairy products, reducing the shelf life of fluid milk and limiting the market for powdered milk. Therefore, the control of sporeformers is crucial to improve the quality of fluid products and to reach specific international powder markets thus benefiting the US dairy industry. In this presentation, I will present an overview of sporeformers associated with the supply chain (fluid, condensed milk, and dry powder) along with potential interventions that may be applied at the farm level to control these microorganisms. It has been previously reported that effective cleaning of teats, changes in bedding material, and CIP procedures could be potential interventions to decrease sporeformers in milk. Additionally, the type of sanitizers (i.e., iodine or chlorine based) used in the parlor could have an effect in this microbial population. These potential interventions have been the subject of research at the University of Nebraska–Lincoln (UNL), with some of the interventions showing to be successful in improving the quality of raw milk. This presentation will provide a brief overview of spore research done at UNL in the last 5 years, along with results obtained so far from interventions at farm level to reduce spore-forming bacteria in raw fluid milk.

74 Strategies for minimizing sporeformers and spores during milk powder processing. S. Anand*, *Midwest Dairy Foods Research Center, Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

High counts of thermoduric sporeformers and their endospores in milk powders offer a major challenge in their marketability and utilization in further processing and product development. These organisms reduce the shelf life of products and cause many spoilages. This presentation includes strategies that we researched in our lab to minimize sporeformers in skim milk powders. In a typical dairy processing plant, the first step starts at the raw milk reception and storage stage. Based on the spore former population dynamics, regression models and contour plots were developed, which helped us choose specific temperature and storage duration combinations that would keep the population more toward vegetative cells. Our other research indicated that having a shift toward vegetative cells would result in lower biofilms on plate heat exchangers (PHE) during pasteurization. Such biofilms are a source of contamination of milk being pasteurized. Certain stainless steel modifications were also tested for reduced biofilm formation on PHEs, which demonstrated reduced biofilm formation even during extended pasteurization runs up to 17h. By combining the above 2 approaches, it was possible to keep the sporeformers and spores counts low in milk and eventually in powders. Another approach that was found to be effective was to apply cavitation, combined with pasteurization, as an alternative processing step during the manufacturing of skim milk powder. Hydrodynamic cavitation was more effective, compared with ultrasonication, in reducing the counts of thermoduric sporeformers and their endospores. Pilot-scale trials successfully demonstrated that a 2 stage cavitation, when combined with pasteurization and followed through evaporation and spray drying, resulted in producing skim milk powder with reduced counts of sporeformers and spores. Further, combining optimized raw milk holding conditions based on regression models with that of hydrodynamic cavitation, as in line process step before pasteurization, was most effective in producing much lower spore count skim milk powder.

Key Words: sporeformers, spore, skim milk powder

75 Recurrence frequency and required intervention cleaning in place (CIP) of persistent populations of thermophilic sporeformers in milk powder production.

T. Erickson*, *Ecolab, Eagan, MN.*

There is strong evidence that in many dairy processing systems a persistent population of spoilage organisms, often thermophilic sporeformers, survives cleaning in place (CIP) and can lead to high spore counts during subsequent runs. Deep-cleaning intervention CIP programs have proven to provide a tool for controlling these populations. However, added cost and potential impact on gasket lifetime point toward a need to limit the frequency of Intervention CIP. The optimal frequency varies by application, product, and plant design but can be characterized through evaluation of quality, production and sanitation records. Once established and confirmed, these periodic intervention CIP cycles help to optimize overall quality and drive consistency in production. Intervention frequency vary from days to weeks in plants of varying age and design, including purpose-built, low-spore plants.

Extension Education 1

76 Consumer perception and willingness to pay for milk in Tennessee. E. A. Eckelkamp*, E. A. Paskewitz, H. S. Wallace, K. B. Johnson, and S. Upendram, *University of Tennessee, Knoxville, TN.*

The dairy industry in Tennessee faces challenges with fluid milk oversupply, lower prices, and dairy farms struggling to survive. While many challenges revolve around supply, an overall decline in consumption of fluid milk is also occurring. Many states, including Tennessee, have pushed for a marketing shift toward local milk and dairy products in an effort to increase product demand and farm revenue. This project was focused on understanding the underlying reasons for these trends. The primary study objective was to understand consumer preferences and willingness to pay for local, organic, generic brand, and fluid milk in general. A secondary study objective was to understand consumer perceptions on milk production, safety, health, and retail markets. This study occurred from January 29 to February 2, 2019, in the form of a survey to elicit information on consumer preferences, milk preferences, local choices for dairy, health, and purchaser demographics. A 42-question survey was disseminated through Qualtrics, returning 440 completed surveys. The FREQ procedure of SAS 9.4 was used to assess consumer responses. All respondents resided in Tennessee. Mean age was 42 ± 15 yr with 81% being female. Price (35%), taste (31%), and expiration date (26%) were considered the first, second, and third most important considerations when purchasing milk. This was further reflected in the price consumers were willing to pay for generic ($3.20 ± 1.00), local ($4.20 ± 1.60), or organic ($4.20 ± 3.60) branded milk. Respondents considered milk “local” if it was produced within the surrounding counties (32%) or within the state (25%). For the secondary objective, 77% of respondents indicated they knew nothing to some about cow’s milk. The majority of respondents used personal physicians or pediatricians (23%) and online articles (21%) to learn about milk. Milk safety was not a concern. Although a small price discrepancy existed between generic and specialty branded fluid milk, more research is needed on the economic consequences to dairy producers. Educating physicians and pediatricians could better reach consumer audiences and potentially increase milk purchases.

Key Words: dairy production, local, consumer preference

77 A survey of US dairy nutritionist perceptions and methods of balancing lower crude protein rations for lactating cows. J. Prestegaard*[1], V. Daley[1,2], and M. Hanigan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*National Animal Nutrition Program, University of Kentucky, Lexington, KY.*

To optimize dietary protein efficiency, nutritionists should balance rations to meet dairy cow amino acid (AA) requirements. The objective of this study was to gather information on ration balancing in regards to dietary protein in lactating dairy cow diets. In 2019, a 33-question electronic survey was sent to dairy nutritionists through the American Registry of Professional Animal Scientists (ARPAS). The survey featured questions related to balancing diets for AAs, levels of dietary crude protein (CP) content recommended over the last 5 years, and opinion on future environmental regulations related to N excretion by cows. Incomplete answers or missing responses to individual questions were removed from the data set. Frequency tables were generated using the FREQ procedure of SAS. A total of 61 dairy nutritionists completed the survey. Most dairy nutritionists considered AA balancing important (89%); of these, 7% considered methionine only, 68% considered methionine and lysine, and 24% considered other AAs. Only 11% of nutritionists did not consider AA requirements at all. The majority of nutritionists decreased their recommendations for dietary CP content over the last 5 years (79%). Nutritionists were split on whether they believed N waste on dairy farms would become more tightly regulated within the next 5 years; 48% believed it would, 33% believed it would not and 19% were unsure. Overall, dairy nutritionists are increasingly conscious about AA requirements of dairy cows. Therefore, an increase in N efficiency may be expected, but this trend is financially, rather than environmentally, motivated. While the formulation of rations for metabolizable methionine and lysine is a step in the right direction, future considerations should include balancing of rations based on all essential AAs. Survey results are being used to develop educational strategies (workshops, lectures, and other materials) to disseminate updated information on balancing rations for AAs and the benefits of low CP rations for dairy cows.

Key Words: survey, amino acid, ration formulation

78 Microlearning courses are effective at increasing the feeling of confidence and the feeling of accuracy in the work of dairy personnel. W. Heuwieser*[1], P. Ospina[2], M. Wieland[2], F. Leal Yepes[3], B. Nguyen[3], and A. Hesse[1], [1]*Clinic for Animal Reproduction, Faculty of Veterinary Medicine, Freie Universität Berlin, Berlin, Germany,* [2]*Quality Milk Production Services, Cornell University, College of Veterinary Medicine, Ithaca, NY,* [3]*Department of Population Medicine and Diagnostic Sciences, Cornell University, College of Veterinary Medicine, Ithaca, NY.*

The objective of the study was to determine if microlearning courses were effective at building the feeling of confidence and accuracy in the work of dairy personnel. Three courses on colostrum management were developed with a cloud-based software. The courses had 4 topics (objectives, standard operating procedure, background information, quiz), a contact time of 10 min, and were accessible via PC or smartphone. With a 12-item survey, we collected relevant data on course perception and feedback. Learner’s experiences such as access, page visits, time spent, and completion were observed with a learning management system. From February to May 2018 interested farms (convenience sample) were contacted by email or Whatsapp and invited to participate free of charge. The email contained an introduction, the URL, and instructions on how to launch the course. On the first 3 screens an informed consent of the participants was obtained, a disclaimer shown, and the navigation explained. The menu page provided buttons linked to the 4 topics. A total of 80 people (employees, trainees, herdsmen) were invited of which the majority (50 to 62%) participated. Overall, the course quality was rated high (1.4 on a 5-point scale; 1 being the best). The overall response rate was 40% and almost all participants accessed the SOP within the course (i.e., 92, 90, and 96% in the first, second, and third course, respectively). Most employees reported that after the training they felt more confident to conduct the task described (77%) and were convinced to work more accurately (89%). Actual contact time was 5 to 7 min longer than the perceived contact time indicating a high level of engagement. Furthermore, most participants confirmed that repeating relevant steps even of a simple and well-known task was also worthwhile. Two thirds of the participants took the courses in their leisure time and one third accessed the courses more than once. Almost all participants (89%) accessed the background information (“why should I do it”). The majority (55% to 92%) of the participants provided feedback in open text fields. User generated data can be used for future course developments and to improve employee engagement.

Key Words: training, e-learning, SOP

79 The impact of corn silage harvesting and feeding decisions on milk margins.

T. J. Beck[2], R. C. Goodling*[1], M. A. Haan[3], V. A. Ishler[1], and A. M. Rosales[4], [1]*the Pennsylvania State University, University Park, PA,* [2]*Penn State Extension, Carlisle, PA,* [3]*Penn State Extension, Leesport, PA,* [4]*Penn State Extension, Lancaster, PA.*

Feeding and cropping management practices are critical to the profitability of a dairy business. Twenty-five Pennsylvania dairies were sampled 4 times for corn silage and total mixed ration (TMR) for the harvest years 2016 and 2017. These farms monitored monthly milk margin and income over feed cost and completed an annual financial analysis. The objective was to demonstrate how cropping and feeding management influence financial performance annually as well as monthly. Project farms were categorized by their net return over labor and management (profit) and then compared for differences in milk margin (Table 1). High net return groups averaged greater milk production, average components, and average gross milk price when compared with medium and low groups. The high group had lower feed costs regardless if based on home raised costs or standardized market costs. Medium net return farms had the lowest average milk production by group, but also had the highest milk components, which improved their overall milk price. No direct relationship between corn silage harvest performance and feeding management with annual financials was detected. Producers can implement good management practices related to forage quality and feeding management but if hired labor or loan payments are excessive, profitability will be challenged.

Table 1 (Abstr. 79). Monthly milk price and feed cost data combined for years 2016 and 2017 grouped by return over labor and management

Value	1-Low	2-Med	3-High	Average
Milk/milk cow per day, lbs.	73.7	72.3	79.9	75.9
ECM/milk cow per day, lbs.	74.7	75.6	81.3	77.7
Milk fat, %	3.7	3.9	3.7	3.8
Milk protein, %	3.0	3.1	3.0	3.1
Cost of production w/ labor and mgmt., $/cwt	22.22	20.25	17.39	19.76
Gross milk price, $/cwt	18.11	18.63	18.24	18.31
Milk cow feed cost, $/cwt				
Using home raised costs	6.65	7.24	6.59	6.80
Using standard monthly market costs	7.98	7.93	7.68	7.84
Milk margin (gross milk price – feed cost), $/cwt	11.46	11.39	11.65	11.51

Key Words: feed cost, milk price, profitability

80 Research and educational priorities of California's North Bay dairies.

R. Black*, *University of California Agriculture and Natural Resources, Santa Rosa, CA.*

California's North Bay region contains predominately organic, pasture-based dairies, likely requiring different educational resources and research compared with other California dairy regions. The objective of this research was to determine the educational and research needs of California's North Bay dairies. In September 2018, surveys were mailed to 124 dairy producers in Sonoma, Marin, and Mendocino Counties. Surveys asked producers to rank educational and research priorities in the areas of animal health, organic certification, nutrition, business, and general management from 1 to 5 (1 = not a priority, 5 = high priority) and information sources and methods for distribution of future educational information from 1 to 5 (1 = no value, 5 = a lot of value). Forty-one dairy producers responded (33.1%), with 34 managing dairy cattle and 7 managing small ruminants. Of the respondents, 27 were certified organic (68.9%). Dairy producers' highest ranked priorities for research and education included (1) pasture management, (2) disease prevention, (3) state and federal funding opportunities, (4) labor management, and (5) water quality. Organic producers ranked (mean ± SD) aspects of organic certification to be medium-high priority, including the certifier's dairy knowledge/expertise (4.3 ± 0.8), compliance with pasture access regulations (4.1 ± 1.1), and their ability to report dry matter intake for pastured animals (3.9 ± 1.2). Dairy producers found greatest value in information from veterinarians (4.1 ± 1.1), University of California Cooperative Extension (3.9 ± 1.0), and other dairy producers (3.6 ± 1.2), and less value in information from webinars (2.1 ± 1.2), podcasts (2.1 ± 1.2), and social media (2.7 ± 1.3). The preferred methods of education delivery for future extension materials included on-farm demonstrations (3.7 ± 1.2), educational meetings (3.7 ± 1.2), and individual farm visits (3.5 ± 1.3), with the majority of producers preferring quarterly (39.0%) or twice per year (31.7%) meetings. Survey results inform extension and allied industry professionals in ways to best assist North Bay dairy producers through future programing and research.

Key Words: needs assessment, cooperative extension, dairy

Forages and Pastures 1

81 Influence of cutting height on nutrient composition and yield of whole-plant corn silage through a meta-analysis. E. M. Paula*[1], B. A. Saylor[1], J. Goeser[2,3], and L. F. Ferraretto[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Dairy Science, University of Wisconsin, Madison, WI,* [3]*Rock River Laboratory Inc., Watertown, WI.*

Harvesting practices such as cutting height are known to alter whole-plant corn silage nutritive value and biomass yield. However, there is no consensus in the literature about the optimum cutting height threshold to balance nutritive value and biomass yield. Therefore, the objective of this study was to evaluate the influence of cutting height on nutrient composition and yield of whole-plant corn silage through a meta-analytic approach using a data set comprised of 10 published studies and 64 treatments means. Cutting height was the sole independent variable used in the present study. Dependent variables were DM, starch, and NDF, concentrations, as well as ruminal in vitro or in situ NDF digestibility (NDFD), DM yield, and milk production per area or per Mg of fresh silage (estimated using the Milk2006 predictive equations). Data were analyzed using PROC MIXED in SAS; the model included the fixed effect of treatment and the random effect of trial. Means and standard deviations for dependent and independent variables were as follows: cutting height (36 ± 21, cm), DM (36.4 ± 4.3, % of as fed), NDF (40.2 ± 4.7, % of DM), starch (25.3 ± 9.3, % of DM), NDFD (58.9 ± 12.8, % of DM), DM yield (14.9 ± 2.5 t/ha), milk production/Mg (1682, ± 289, kg/Mg), and milk production per area (19317 ± 3522, Mg/ha). Yield of DM was reduced ($P = 0.001$) by 0.05 Mg/ha for each cm of increased cutting height. However, for each cm increase in cutting height there was an increase ($P < 0.05$) of 0.09, 0.08, and 0.08%-units in DM, starch, and NDFD, respectively. Conversely, there was a negative linear effect ($P < 0.05$) for NDF, with a 0.10%-unit decrease per cm increase in cutting height. There was a decrease ($P < 0.05$) in milk production per area of 37.8 kg/ha for each cm increase in cutting height, whereas there was a tendency ($P = 0.09$) to increase milk production by 1.868 kg/Mg of fresh silage for each cm increase in cutting height. These equations indicate that increasing cutting height improves forage quality, at the expense of DM biomass yield. Therefore, caution is needed when planting area is limited.

Key Words: biomass DM yield, cutting height, NDF digestibility

82 The effects of a silage inoculant on aerobic stability and quality of grass/legume mixture fermented for 8 days and the impact on total mixed ration stability. G. Copani*[1], K. Witt[1], I. Eisner[1], V. Vrotniakiene[2], and J. Jatkauskas[2], [1]*Chr. Hansen Animal Health & Nutrition, Hørsholm, Denmark,* [2]*Institute of Animal Science of Lithuanian University of Health Sciences, Baisogala, Lithuania.*

Sometimes, due to feed shortage, farmers need to open the silage bunker early. Silages are mixed with other feeds and formulated into a total mixed ration (TMR) satisfying the animal's requirement. The aerobic stability (AS) of the silages will impact the quality of the TMR ration and subsequently the animal performance. Silage inoculants can be used for enhancing hygiene and AS of the silages. The objective of this study was to evaluate the effects of a silage inoculant on hygiene, AS and subsequent impact on TMR stability after 8 d of silage fermentation. Wilted grass/legume mixture was ensiled into cylindrical bales (total n = 20, 500–600 kg/bale) and wrapped in 6 layers of plastic film. The Control bales (C; n = 10), with no inoculant, and the experimental bales, inoculated with SiloSolve FC (150,000 total cfu/g of forage) (FC; n = 10) containing 50:50 of *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037), were stored outside for 8 d. Bales (n = 5/treatment (trt)) were tested for fermentation parameters, hygiene and AS. The AS, tracked for 8 d, was performed on unwrapped bales by monitoring the temperature increase inside the mass (until they reached +3°C above T ambient). The remaining bales (n = 5/trt) were used for TMR preparation. Three different TMRs were prepared after 1, 3, and 5 d of aerobic exposure of the bales, respectively. TMR temperature was monitored for 24 h. Data were analyzed in a randomized block design using the GLM procedure (SAS, 9.4), fixed effects of the model were trt, aerobic exposure duration and their first order interaction. Compared with C, FC treated bales reported higher acetate level ($P < 0.05$), lower yeast and molds ($P < 0.01$) and improved AS of the TMR after 3 and 5 d of aerobic exposure of the bales ($P < 0.05$) (no effect after 1 d), while no differences were observed on temperature of the silages before TMR preparation. These results demonstrate that use of SiloSolve FC improves silage hygiene and TMR stability after a short period of fermentation.

Key Words: aerobic stability, TMR stability, silage inoculant.

83 Effect of irrigation on fiber concentration and digestibility of corn plant tissues. L. Martin*[1], G. Ferreira[2], C. L. Teets[2], S. Hines[3], G. Shewmaker[4], M. de Haro Marti[5], and M. Chahine[1], [1]*Department of Animal and Veterinary Science, University of Idaho-Twin Falls, Twin Falls, ID,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [3]*University of Idaho-Jerome, Jerome, ID,* [4]*Kimberly R & E Center, University of Idaho, Kimberly, ID,* [5]*Universtiy of Idaho-Gooding, Gooding, ID.*

The objective of this study was to determine the effect of irrigation on neutral detergent fiber (NDF) and lignin (LIG) concentrations and on in vitro apparent dry matter digestibility (IVDMD), and in vitro neutral detergent fiber digestibility (IVNDFD) of stems, leaf-sheaths, and leaf-blades of corn. Five commercial corn hybrids for silage (one of them showing the brown midrib phenotype) were planted in a split-plot setting within a randomized complete block design (4 replicates). Treatments consisted of a control treatment with furrow irrigation at planting and 3 more times during crop growth (WATERED) and a non-irrigated treatment with furrow irrigation only at planting (NON-WATERED). When the corn was between 1/4 and 3/4 milk-line stage of maturity, 5 plants from each plot were cut by hand, and stems, leaf-sheaths, and leaf-blades from the second phytomer below (LOWER) and the second phytomer above (UPPER) the ear insertion were dissected and frozen for analysis. Tissues were analyzed for NDF concentration, IVDMD, and IVNDFD. Data were analyzed using Proc Mixed of SAS, and the model included the effects of block (random, df = 3), treatment (fixed, df = 1), whole-plot error (random, df = 3), hybrid (fixed, df = 4), treatment by hybrid interaction (fixed, df = 4), and the residual or split-plot error (random, df = 25). WATERED contained lower NDF concentrations (64.6 vs. 67.6% NDF; $P < 0.01$) and greater IVDMD than NON-WATERED plots (56.7 vs. 54.8% IVDMD; $P < 0.05$). IVNDFD tended to be greater for WATERED than for the NON-WATERED plots (51.7 vs. 50.1% IVNDFD; $P < 0.10$). Irrigation did not affect LIG concentration in the cell wall ($P < 0.11$), which averaged 19.9%. UPPER phytomers had a lower NDF concentration (64.4 vs. 67.7%; $P < 0.01$) and a greater IVNDFD than LOWER phytomers (52.8 vs. 49.0%; $P < 0.01$). In conclusion, under the conditions of this study, limited water supply does not affect lignin concentration in the cell wall and does not increase the in vitro digestibility of fiber in corn for silage. The latter observation is contrary to the general industry belief that water-stress increases fiber digestibility in forages.

Key Words: drought, fiber digestibility, environment

84 Milk yield and CH_4 emission in Jersey cows grazing forage canola.

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Forage canola is an annual crop that can be used to extend the fall grazing season. Along with high mass yield, canola also has high DM digestibility and recent in vitro research showed decreased in CH_4 production. We aimed to evaluate the effect of canola as a grazing resource on milk yield and enteric CH_4 emissions in dairy cows. Twelve multiparous and 6 primiparous mid-lactating organic-certified Jersey cows were blocked by parity and, within block, assigned to 1 of 2 treatments (control = CTRL or canola = CAN) in a randomized complete block design. Cows in the CTRL group were fed indoors and had no access to pasture, while CAN cows stayed in the barn during the day and grazed at night (from 1800 to 0500 h). The experiment last 6 wk with sample collection done during wk 3 and 5 and wk 0 used as covariate. Diets were formulated to yield a 60:40 forage-to-concentrate ratio with half of the baleage replaced by canola in cows assigned to CAN. Data were analyzed using SAS with repeated measures over time. Daily herbage allowance was set at 7.5 kg of DM/cow. Canola averaged 4,000 kg of DM/ha, 24.8% CP, and 15.5% NDF. Cows in the CTRL group consumed 21 kg/d of DMI. Estimated herbage DMI (pre- minus post-grazing mass) averaged 3.2 kg/d and mean baleage plus concentrate DMI was 15.5 kg/d in CAN cows. Milk yield was lower in CAN than CTRL diet (20.8 vs 22.3 kg/d; $P < 0.01$) due to decreased DMI and increased energy expenditure for grazing. However, yields of FCM and ECM and concentration and yield of milk fat did not differ between treatments. While milk protein concentration increased in CAN cows ($P < 0.001$), milk protein yield did not change. Cows grazing canola had elevated MUN compared with those in the CTRL diet (13.5 vs. 11.1 mg/dL; $P < 0.001$) possibly because of greater concentration of soluble protein in canola (mean = 55%) vs. baleage (mean = 38.3%). Enteric CH_4 production (415 vs. 469 g/d; $P < 0.01$) and intensity (15.0 vs 16.4 g/kg of ECM) were lower in cows fed CAN vs. the CTRL diet, which may be associated with decreased DMI and presence of glucosinolates in canola. Overall, forage canola has potential to be used for grazing during the fall season.

Key Words: CH_4 emission, brassica, grazing

85 Effect of microbial inoculation and particle size on fermentation profile, aerobic stability, and ruminal in situ starch disappearance of high-moisture corn.

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The objective of this study was to assess the effects of microbial inoculation and particle size on fermentation profile, aerobic stability, and ruminal in situ starch disappearance of high-moisture corn. High-moisture corn (HMC) was harvested, either coarsely or finely ground (3797.8 vs. 984.1 ± 42.4 μm, on average, respectively), and inoculated with distilled water (CON), *Lactobacillus plantarum* CH6072 at 1×10^5 cfu and *Enterococcus faecium* CH212 at 5×10^4 cfu/g of fresh forage (LPEF), or *L. buchneri* LB1819 and *Lactococcus lactis* O224 at 1.5×10^5 cfu/g (LBLL). Silos were ensiled in quadruplicate vacuum pouches for 0, 14, or 28 d. Data were analyzed as a completely randomized design with a $2 \times 3 \times 3$ factorial arrangement of treatments using PROC GLIMMIX. The model included particle size, microbial inoculation, storage length, and their interactions as fixed effects. An interaction between microbial inoculation and storage length was observed for lactic acid ($P = 0.001$). Concentrations of lactic acid were similar among all inoculant treatments at 0 d, greatest in LPEF and lowest in LBLL at 14 d, and lower in LBLL compared with CON and LPEF (0.93 vs. 1.73 ± 0.04% of DM) at 28 d. A 3-way interaction between particle size, microbial inoculation, and storage length was observed ($P < 0.05$) for acetic acid. At 14 and 28 d, acetic acid concentrations were greatest in Fine LBLL, followed by Coarse LBLL, Fine CON, and Fine LPEF, and lowest for Coarse CON and Coarse LPEF. Ruminal in situ starch disappearance was greater ($P < 0.05$; 57.5 vs. 51.5% of starch, on average, respectively) with LBLL compared with CON and LPEF. Likewise, greater aerobic stability was observed ($P < 0.05$; 224.8 vs. 44.5 h, on average, respectively) with LBLL compared with CON and LPEF. These results suggest that certain strains of *L. buchneri* produce acetic acid, improve aerobic stability, and increase starch disappearance of HMC within 28 d of fermentation.

Key Words: microbial inoculation, high-moisture corn, aerobic stability

86 Effect of a dual-purpose bacterial inoculant and recombinant bacterial expansin-like protein on fermentation profile and digestibility of whole-plant corn silage.

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This study aimed to examine the effects of a dual-purpose bacterial inoculant (DPI) and a recombinant bacterial expansin-like protein (BsEXLX1) on whole-plant corn silage fermentation and digestibility. The dose of BsEXLX1 was estimated based on endoglucanase (2410 μmol/min/ml) and xylanase (16887 μmol/min/ml) activities in the inoculant. The hydrolytic capacity of DPI and freshly purified BsEXLX1 was examined using filter paper (FP) and oat-spell xylan using 4 treatments: 1) Control (Distilled water), 2) BsEXLX1 (37 mg/g), 3) DPI (120,000 cfu/g of lactic acid bacteria and *Propionibacterium freudenreichii* + fibrolytic enzymes) and 4) DPI + BsEXLX1. In addition, whole-plant corn was harvested at 34% DM, ensiled in quadruplicate for 0, 3 and 60 d in 0.7 kg mini-silos after it was sprayed with or without the treatments. Samples were analyzed for fermentation profile and incubated in quadruplicate to determine 24 h gas production (GP) and in vitro true DM and NDF digestibility. Hydrolytic capacity data were analyzed with a model that included effects of DPI, BsEXLX1 and the interaction, whereas for corn silage samples, the model included effects of DPI, BsEXLX1, day and interactions. Although, DPI and BsEXLX1 synergistically increased ($P < 0.01$) hydrolysis of FP (7.1 vs 5.5 mg/g), BsEXLX1 decreased hydrolysis of xylan compared with DPI alone (10.3 vs 12.2 mg/g). After 3 d of ensiling, no treatment effects were observed on silage DM, DM recovery, CP concentration or yeast and mold counts ($P > 0.10$) but BsEXLX1 decreased pH (3.94 vs 3.88, $P < 0.01$), compared with the Control. After 60 d, lactate concentrations were increased (6.3 vs 8.3% DM, $P < 0.05$) by DPI, whereas BsEXLX1 decreased NDF concentration (47.1 vs 45.1%, $P = 0.03$). Consequently, compared with DPI alone, BsEXLX1 and DPI increased starch concentration (20.4 vs 24.9% DM) and tended to increase ($P < 0.07$) GP (66.2 vs 70.2 mL/g OM), and DMD (48.2 vs 50.2%) but not NDFD (29.6 vs 31.9%) at 60 d.

These results suggest that BsEXLX1 + DPI could increase fermentation and digestibility of whole-plant corn silage.

Key Words: corn silage, expansin, inoculant

87 The impact of zero-grazing versus conventional grazing in early lactation autumn-calving dairy cows in Ireland.

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Zero-grazing, also referred to as 'cut and carry' or 'green-chop', is a feeding system where fresh grass is cut daily and fed to housed cows. Interest in zero-grazing has increased in recent years in temperate seasonal grass-based systems of milk production, particularly in spring and autumn. Optimising the proportion of grass utilized can increase profitability due to its lower cost compared with alternative feeds. Zero-grazing can potentially help achieve this, particularly where climatic and soil conditions limit grazing opportunities. However, there is a paucity of information on the impact of zero-grazing on cow performance. This study aimed to determine the effect of zero-grazing versus conventional grazing in early lactation on the performance of autumn-calved dairy cows. Twenty-four Holstein-Friesian cows were blocked on days in milk (28DIM ± 13), parity, predicted 305-d yield, and body condition score (BCS), and assigned to one of 2 treatments in a randomized complete block design (n = 12). The 2 treatments were; zero-grazing (ZG) and grazing (G). The diets were fed for a 14d acclimatization period and then for a further 35d. The ZG group were housed full-time and fed zero-grazed grass, while the G group grazed outdoors at pasture. Both treatment groups were offered ad libitum quantities of perennial ryegrass (*Lolium perenne* L.) from within the same paddock. Average pre-grazing/pre-cutting yield was 2024 kg of DM/ha (±557) (>4cm). Both treatments received a daily buffer feed of 2.5 kg DM grass silage and 1.8 kg DM maize meal before milking and 7.2 kg DM concentrates in-parlor. Average milk yield in the ZG treatment (31.67 kg) did not significantly differ from the G treatment (30.54 kg) ($P > 0.05$). Likewise, method of offering pasture did not have an effect on milk composition or somatic cell count ($P > 0.05$). Furthermore, BCS, body weight, locomotion score, and rumen pH were not affected by treatment ($P > 0.05$). Results suggest that zero-grazing did not negatively impact cow performance and could be used as a means of utilizing grass in the diet where conventional grazing is not possible.

Key Words: zero-grazing, early lactation, cow performance

88 Diagnostics tool for corn silage: Development, validation, and characterization index using principal component analysis from Québec, Canada.

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The development of a multivariate diagnostic tool relating corn silage (CS) quality traits and herd productivity or profitability could be helpful for producers. Despite the fact that CS analyses are commonly available, the capacity of the producers to relate the information to herd performance is complicated due to the high number of chemical, biological and fermentative parameters (>20 variables). These parameters could also be affected by harvested years (HY), fermentation length (FL) and farms, making interpretation and decision process complicated for the producers. Thus, our aim was to formulate comprehensive quality parameters for CS that will help producers understand the quality assessment formulated by the analytic report and validated interpretation consistency over different HY, FL and growing regions (GR). A principal component (PC) analysis was performed using SAS 9.4 with 2,124 CS samples harvested from 2014 to 2018 HY in Québec, which were analyzed by near-infrared spectroscopy for nutritional and fermentation characteristics. Six PC were retained and were interpreted based on loading vectors of each variables on specific extracted PC, where loading are the correlation between latent component and original variables. PC corresponds to: PC1) carbohydrates and maturity of CS; PC2) homolactic fermentation, protein solubilization and starch digestibility (DEG); PC3) FL, heterolactic fermentation, or other secondary fermentations; PC4) NDF DEG; PC5) protein degradation and heating; and PC6) metabolites associated with L. buchneri group. The interpretation consistency validation of the PC was performed from a subset (n = 2061) containing GR (n = 7), HY (n = 4) and FL. The statistical model included: farm origin (random effect), HY, GR, FL and the following interactions: HY × GR, HY × FL. The PC were respectively affected ($P < 0.05$) by: HY, FL (PC2); HY, GR, FL (PC1, PC3, PC4, PC5, and PC6); HY × GR (PC1, PC2, PC4, and PC5); HY × FL (PC3, PC4, PC5, and PC6). The relationship between the PC and HY, GR or FL allows to validate PC interpretation and moving forward to connecting the PC to dairy herd productivity and profitability using farm historical database.

Key Words: corn silage, near infrared (NIR), fermentation profile

89 Effect of hybrid on performance, digestion, and nitrogen balance of lactating cows fed whole-plant corn silage.

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Content and digestibility of fiber and starch affect the nutritive value of corn silage. The objective was to evaluate lactation performance, digestion, and N balance of cows fed silage of hybrids (Limagrain, Brazil) selected for grain (3040) or silage (6030, 3055). Hybrids were cultivated in duplicate (0.8 ha/plot) and harvested 133 d after seedling at the 3/4 milk line stage of maturity. Productivity (ton of DM/ha) and kernel vitreousness (% of endosperm) were: 22 and 59.6 for 3040; 22 and 49.5 for 6030; 18.5 and 62.3 for 3055. Composition of silages 3040, 6030, and 3055 were 42.6, 41.2, 46.9% DM; 44.4, 41.4, 42% NDF; 41.7, 44.5, 43.1% starch; 27.3, 29.7, 22.2% of starch <4.75 mm; 26.9, 29.2, 26% particles <8 mm. After 143 d of storage, silages were individually fed to 18 Holstein cows (135 ± 50 DIM) in 3 × 3 Latin squares (28-d periods). Diets contained (% of DM): 49% silage, 20.8% NDF from silage, 32.9% NDF, 21% starch from silage, 30.1% starch. Hybrid did not affect DMI (24.6 kg/d. $P = 0.54$). Milk yield (kg/d) was lower ($P < 0.05$) for 3055 (35.9) than 3040 (37.2) and 6030 (37.1). Protein and casein secretions were higher for 6030 than 3055 ($P < 0.05$). The proportion of consumed N going into milk (29.1%), urine (39.1%), feces (28%), or retention (3.8%) did not differ ($P \geq 0.16$). Urea-N in blood and milk were reduced by 6030 ($P < 0.05$). Total-tract starch digestibility (% of intake) was lower ($P < 0.05$) for 3055 (87.2) than 3040 (91.8) and 6030 (93.3). Total-tract NDF digestibility (43.5% of NDF intake) did not differ ($P = 0.73$), nor did ruminal fermentation profile ($P \geq 0.22$). Rumination and ingestion behaviors did not differ ($P \geq 0.25$). The proportion of daily intake in the morning was increased and the proportion in the afternoon was reduced by 3055 ($P < 0.05$). Hybrid

3055 induced the highest preferential intake of particles <8 mm (128% of predicted intake. $P < 0.05$) and more refusal of particles >19 mm than 6030 (64 vs 85% of predicted intake. $P < 0.05$). Lactation performance was reduced by hybrid 3055, the one with highest vitreousness of the endosperm and lowest kernel processing score and starch digestibility. The digestibility of NDF did not explain differences among hybrids.

Key Words: corn silage, starch digestibility, fiber digestibility

90 A meta-analysis examining the effects of herb species inclusion in grazing swards for dairy cows.

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A meta-analysis was conducted which compared grazing swards containing a grass species and at least one herb species to grazing swards without herb species. Effects on milk production and urinary nitrogen excretion were determined. A literature search was completed on the online databases CAB Direct, Web of Science and Google Scholar using the search strategy 'dairy cow', 'herb OR forb OR phorb' and 'grazing'. Studies were screened by title and abstract. They were included for further screening if they were reported in English, the population in the study was dairy cows and the treatment included at least one herb species in a grazing sward with a grass. The comparator was taken as a sward containing grass only or grass and clover. Milk yield, variance, and sample size were required for eligibility. In all, 116 studies were identified. A total of 41 duplicates were removed. After eligibility screening, 11 papers from 6 publications, published between 2006 and 2018, remained. Studies were from New Zealand (n = 7), Australia (n = 3) and the US (n = 1). The population were either HF or HFxJe with a range in mean daily milk yield from 12.05 to 34.7 kg (mean = 18.6kg). A total of 324 and 284 cows were included in treatment and control groups respectively for milk production analysis. Data analysis was conducted in R using a random effects model. Heterogeneity was reported using the I^2 statistic. Analysis of milk yield resulted in a point estimate = +1.04 kg/cow/day (CI = 0.64, 1.45; $I^2 = 17\%$). Milk solids (fat + protein) analysis generated a point estimate of +0.03 kg/cow/day (CI = −0.01, 0.08) with significantly higher heterogeneity ($I^2 = 68\%$). Urinary nitrogen was estimated from MUN when reported (n = 6). A point estimate of −34.87 mg/dL (CI = −67.15, −2.6) was generated although heterogeneity was high among studies ($I^2 = 59\%$). This meta-analysis shows the potential benefits of multispecies swards. Despite heterogeneity, the negative confidence interval indicates a reduction in urinary nitrogen excretion. In addition, a potential increase in milk yield was shown.

Key Words: dairy cow, mixed swards, milk production

91 Establishment of perennial forages with annual sudangrass or sorghum-sudangrass hybrids improved forage yields and in vitro total-tract NDF digestibility.

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The objective of this study was to evaluate the effects of underseeding a mixture of perennial forages (alfalfa, clover and tall fescue; control) with different annual companion forages on forage yields, chemical composition and total-tract NDF digestibility (TTNDFD) using Daisy^{II} incubator. Treatments included the control seeded alone or with a companion forage [Sudangrass (SG), Sudangrass brown midrib (BMR) gene 12 (BSG), Sorghum-Sudangrass BMR gene 6 (BSSG) or oat]. Experimental plots (7 replicates / treatment) were harvested at d 60 (1st cut) and d 90 (2nd cut) at the bud stage of alfalfa. Forage indigestible NDF (iNDF) was calculated by in vitro incubation at 240 h whereas potentially degradable NDF (pdNDF) was calculated by subtracting iNDF from total NDF. Digestion rate (kd) of pdNDF was estimated by in vitro incubation at 24, 30 and 48 h. Data were analyzed as repeated measures using the MIXED procedure of SAS with fixed effects of treatment, cut and treatment x cut interaction. Total forage yields (cuts 1 and 2; DM basis) were higher ($P < 0.001$) with SG (6.56 T/ha), BSG (5.50 T/ha) and BSSG (5.37 T/ha) compared with control (2.82 T/ha). Oat produced higher forage yield in the first cut only. In presence of companion crops, yields of individual perennial forages and weeds were reduced ($P < 0.001$). Companion crop yield was lowest ($P < 0.001$) with oat (3.49 T/ha), intermediate with BSG (4.72 T/ha) and highest with SG (5.86 T/ha) or BSSG (5.89 T/ha). Companion forages reduced ($P < 0.001$) CP and ADL but increased ($P < 0.001$) NDF and ADF concentrations of harvested forages. In vitro TTNDFD followed the order ($P < 0.001$): BSG and BSSG (average 59.2%) > SG (55.7%) > control (49.9%) > oat (46.0%). Evidently, iNDF values were lower ($P < 0.001$) with SG, BSG and BSSG (average 21.0%) than control (35.6%) and oat (34.0%). First cuts had lower ($P < 0.001$) TTNDFD but higher ($P < 0.001$) kd than second cuts. In conclusion, seeding perennial forages with annual companion forages (SG, BSG, or BSSG) may improve forage yields and fiber digestibility in dairy cows.

Key Words: dairy cow, forage, fiber digestibility

92 Effect of using fresh ryegrass-berseem clover as forage basis for TMR during the winter period on dairy cow performance and nitrogen utilization.

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Alfalfa hay-corn silage mixture are the common forages used for winter TMR in Mediterranean dairy systems. Fresh forages can improve milk quality and systems sustainability. Berseem clover is a productive winter forage commonly sown with annual ryegrass. The objective of the study was to evaluate the effect of using fresh ryegrass-berseem clover (MIX) and alfalfa hay-corn silage (CON) as forage basis for TMR during the winter period on dairy cows' performance and metabolic N. Lactating Holstein Friesian cows (n = 24) were blocked according to productive traits and allocated to each TMR according to a complete block design. Cows were individually stalled for 10 weeks and received the TMR ad libitum. TMRs were formulated as a 50:50 forage-to-concentrate ratio. TMR samples (2/wk) were analyzed for dry mater (DM), crude protein (CP) and neutral detergent fiber (NDF) contents and in vitro DM digestibility (DMD). Feed intake and milk yield were measured daily. Individual milk composition was measured once every other week. Cow blood serum, urine and fecal samples were collected on wk 10 and were analyzed for N contents. Data were analyzed using repeated measures over time with treatment, week, and treatment by week interaction included in the model. The time effect was excluded from the model for N utilization variables. Offered TMRs compositions were: DM, CON:

518, MIX: 408; SD 57.4 g/kg; CP, CON 170, MIX 148; SD 12.7 g/kg DM; CP; NDF CON: 325, MIX: 330; SD 16.1 g/kg DM and DMD, CON: 79, MIX: 82; SD 1.9. Cows had similar feed intake 24.1 ± 1.45 kg DM/d, but CON cows had greater milk yield (24.9 vs 23.3; SEM 1.45 kg/d; $P < 0.05$), milk solids yield (3.19 vs 2.64; SEM 0.089 kg/d; $P < 0.001$), milk urea nitrogen (17 vs 14; SEM 1.2mg/dL; $P < 0.05$), serum urea N (12.7 vs 6.9; SEM 0.49 mg/dL; $P < 0.001$), urine urea N (202 vs 76; SEM 15.4 g/d; $P < 0.001$) and fecal N (240 vs 180; SEM 8.9 g/d; $P < 0.001$) than MIX cows. Cows' had similar milk composition (3.45 ± 0.207% fat, 3.45 ± 0.082% protein and 5.28 ± 0.039% lactose). The use of fresh forage in dairy cows' TMR decreased milk production, did not affect milk composition but decreased N excretion. The study was supported by FONDECYT 11160697

Key Words: fresh forage, milk, berseem clover

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93 Early lifetime information enhances calf selection by improving accuracy of predictions with machine learning algorithms and regression.

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Calf information collected early in life (genetics, growth, health) can inform dairy farmers about raising the best replacement heifers and culling surplus heifers if it improves the accuracy to predict future performance. Raising the best replacement heifers increases the expected milk sales if better selection decisions are made, but this increased revenue is offset by the cost to gather the information. Therefore, our objective was to find the break-even cost of information generated through the first lactation from culling surplus heifers based on predictions from early lifetime information with methods of gradient boosting, random forest, and regression. Survival to first calving and up to 305-d first-lactation milk production conditional upon first calving were predicted for 4,850 calves at 120 d of age for calves born between April 2012 and November 2015 on a single farm. The survival and conditional milk predictions were multiplied to obtain the prediction of expected milk sales used for heifer selection. The value of marginal milk was set at $0.29/kg. Predictions were generated through 10-fold cross-validation with 5 new random split replicates. The break-even cost of the information was the cost that could be spent per born calf. If 30% of heifers were culled, the genomic genetic estimates, growth and health data combined together predicted by gradient boosting, random forest and regression resulted in a break-even information cost of $116, $109 and $105, respectively. When parent average genetic estimates, growth and health were used together, gradient boosting generated the highest value of information ($98), followed by random forest ($80) and regression ($70). We conclude that gradient boosting, followed by random forest and regression predictions based on early lifetime information increases prediction accuracy. Genetic information was more valuable than growth and health data for prediction. This methodology could be extended to predict lifetime cow revenue for a more complete picture of break-even information costs to guide heifer selection decisions.

Key Words: machine learning, prediction, information value

94 Machine learning algorithms for early prediction of clinical mastitis.

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Somatic cell count is the most used metric to detect CM. However, SCC data are only available once a month. Integrated data on a continuous basis for every milking are available through the "Dairy Brain" project at the University of Wisconsin-Madison. These data could be used to more accurately predict the onset of CM on a permanent basis. We analyzed records from 2 different data streams in a Wisconsin farm (2016–2018): 1) milking system (milk production [kg] and milk conductivity [mS/cm], and 2) management system (CM, metritis, and retained placenta cases, lactation, and DIM). Days in milk were limited to 1 to 150 and lactations were grouped between 1, 2 and 3+ (n = 681,759 records from n = 3,319 cows). One of the limitations of the data was the low number of CM cases (981), which accounted for only 1% of the records. Therefore, balancing the data was necessary. The SMOTE technique gave the best results for balancing the data. With the resulting data set, various classification machine learning algorithms were tested using 75% of the data as training data. Included variables were the difference of milk production and milk conductivity between milkings, lactation groups, DIM, and previous cases of CM, retained placenta, and metritis. Significant variables were the difference of milk production and milk conductivity between milkings, lactation groups, and DIM. Two algorithms, random forest and gradient boosting, showed the best performance. Also, best results were achieved using data from the 5 previous milkings before the reported case of CM. For random forest the specificity was 0.74 and the sensitivity was 0.70, whereas for the gradient boosting results were the opposite, a lower specificity, but a higher sensitivity (0.58 and 0.82, respectively). Cows with CM compared with healthy cows had higher absolute mean difference for milk conductivity (0.92 vs. 0.86 mS/cm) and milk production (2.22 vs. 1.95 kg), respectively. Results show that the algorithms can predict well cases of CM; however, additional validation is required. Likewise, the integration of other data streams such as genetics, sensors and diet changes, could help improve the prediction accuracy.

Key Words: data integration, clinical mastitis, machine learning

95 Development of a prediction equation for body weight change in early-lactating dairy cows by Fourier-transform infrared predicted fatty acid profiles in milk.

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Most cows undergo a state of negative energy balance in early lactation. Body reserves then play a major role in milk fat production, whereas less fatty acids (FA) are synthesized de novo in the mammary gland. This is reflected in the milk FA composition. The objective of this study was to validate if the milk FA profile can be used to predict body weight change (BWC) in early lactating cows in commercial dairy farms. Data originated from 17,067 Danish Holstein cows of 7–35 d in milk across 166 herds in Denmark between March 2015 and March 2017. Body weight was recorded by floor scales in Lely automatic milking systems at each milking. Dairy Herd Improvement milk samples were analyzed by Fourier-transform infrared spectroscopy. Data for BWC prediction included stage of lactation, parity, and test day data for milk production and components (fat, protein, somatic cell counts, and FA concentrations). Daily median BWC was −0.43 (−8.80 to 5.53; 1st to 99th percentile) g/kg of body weight across lactations. Predictions of daily BWC were based on a random forest algorithm with 10-fold repeated cross-validation for which 20% of the herds were randomly withhold for cross-validation. The overall root mean square error of prediction was 1.66 g/kg of body weight at a coefficient of determination after cross-validation of 0.896. The 5 most important variables considered by the model were the short-chain FA group (C4:0–C10:0), C18:1, the medium-chain FA group (C12:0–C16:1), the saturated FA group, and C16:0. While short-chain and some medium-chain FA are synthesized in the mammary gland, C18:1 originate from body reserves (e.g., during energy deficits), and C16:0 and C16:1 derive from either the mammary gland or body reserves. These results suggest that the milk FA profile may be used as an early indicator of BWC in early lactation. Further work is needed to validate the model with North American data, and to investigate possible associations with production, fertility, and health traits.

Key Words: body weight loss, Dairy Herd Improvement (DHI), Fourier-transform infrared (FT-IR)

96 A stochastic animal life-cycle simulation model and its herd structure. M. Li*[1], V. E. Cabrera[1], and K. F. Reed[2], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Animal Science, Cornell University, Ithaca, NY.*

We are building a stochastic Monte Carlo model to represent real farm scenarios by simulating individual animal life events. This animal life cycle model simulates events according to reproduction protocols, genetic merit, production curves, and culling decisions for individual cow on a daily basis. Events follow herd-level stochastic distributions with animal-specific modifiers. There are 6 groups of animals in the model: Calves, Heifers I, Heifers II, Heifers III, Cows, and Culled animals. Calves are from birth to weaning with particular facility and nutrition care, and decisions of whether to keep calves on the farm are made during this stage. The Heifers I group are from weaning to breeding with stochastic growth rates. Heifers II group are from breeding initiation to close to calving, where estrus cycles, user-defined breeding programs, and continued growth are simulated. The Heifers III group are less than one month before parturition. In this period, they either enter the herd or are sold; replacements may also be purchased. Production events in the Cows group are determined by breed, parity, production-level lactation curves, stage of pregnancy, and health status. Also, events initiated by breeding protocols like insemination and drying-off. Culling may occur in any of the 5 stages and is determined by stochastic culling rates for a variety of reasons. The Culled group have maintenance and slaughter value calculated. The stages and events were programmed using well-established distributions from literature and expert opinion. Last day of a 3000-d simulation of a 1000-cow herd possibly resulted in a herd composed of 138 Calves; 808, 1203, 54 in Heifers I, II, and III, respectively, 1000 Cows and 3 Culled animals. The high number of young stocks illustrates the combined effect of using sexed semen and keeping all female calves. This animal life cycle model is a sub-model of the whole farm Ruminant Farm Systems (RuFaS) model, which will be able to simulate the effects of individual animal management on whole farm efficiency and productivity.

Key Words: simulation, stochastic model, herd structure

97 Environmental benefits of alternatives in dairy farm management in the changing climate of the northeastern United States. K. Veltman[1], A. Rotz*[2], L. Chase[3], J. Copper[4], C. Forest[5], P. Ingraham[6], C. Izaurralde[7], C. Jones[7], R. Nicholas[5], M. Ruark[8], W. Salas[6], G. Thoma[9], and O. Jolliet[1], [1]*University of Michigan, Ann Arbor, MI,* [2]*USDA-ARS, University Park, PA,* [3]*Cornell University, Ithaca, NY,* [4]*University of Washington, Seattle, WA,* [5]*Penn State University, University Park, PA,* [6]*Applied Geosolutions, Durham, NH,* [7]*University of Maryland, College, Park, MD,* [8]*University of Wisconsin, Madison, WI,* [9]*University of Arkansas, Fayetteville, AR.*

To meet the nutritional needs of a growing population, dairy producers must increase milk production while minimizing farm-gate environmental impacts. As we look to the future, management practices must also be adapted to projected climate change. A comprehensive assessment was made of the effects of climate change on both the productivity and environmental performance of farms as influenced by strategies to adapt to the changing climate. Production systems were evaluated using 3 representative northern US dairy farms: a 1500-cow farm in New York, a 150-cow farm in Wisconsin, and a 50-cow farm in southern Pennsylvania. The farms were simulated using farm-scale process-based modeling and climate projections for high and low greenhouse gas emission scenarios. Environmental impacts of the farms, which included farm-gate reactive nitrogen and carbon footprints and phosphorous runoff, generally increased in the near future (2050) if no mitigation measures were taken. Overall, feed production was maintained as decreases in corn grain yields were compensated by increases in forage yields. Adaptation of the cropping systems through changes in planting and harvest dates and crop varieties reduced the reduction in corn grain yields, but the detrimental effects of climate change were not fully negated. Adoption of farm-specific beneficial management practices, including changes in diet, manure management and cropping practices, substantially reduced the greenhouse gas emissions and nutrient losses of dairy farms in current climate conditions and stabilized the environmental impact in future climate conditions, while maintaining feed and milk production. This modeling exercise illustrates that more sustainable dairy production systems can be maintained in the future climate of the Northeast with appropriate management changes.

Key Words: Integrated Farm System Model, climate change, farm management

98 Executing a better nutritional grouping strategy in commercial dairy farms. J. A. Barrientos Blanco*, V. E. Cabrera, and R. D. Shaver, *University of Wisconsin-Madison, Madison, WI.*

The objective of this study was to evaluate the economic benefits of a nutritional grouping strategy (NGS) in a lactating herd from a commercial dairy farm. Seventy-eight days (June–August 2017) of data from lactating cows from a commercial Wisconsin dairy farm were used for the study. Lactating cows (n = 1898 ± 115) are weekly regrouped in 14 pens according to lactation, days in milk (DIM), or a combination of both for which 8 diets are provided. Diets are seldom reformulated and nutritional requirements are not factored to allocate cows to pens. The same 14 pens were used to simulate the implementation of NGS following closely current farm criteria, but also including nutritional requirements (net energy (NE), metabolizable protein (MP)), and milk yield in an attempt to generate more homogeneous groups for improved diet accuracy. The goal was to implement a continuous weekly system of cows' pen allocation and diet reformulation. The NE and MP requirements from the NGS where used to formulate the diets with the NDS software using the same feed ingredients and prices as the current farm diets. Diet MP and NE density were adjusted to the nutritional group requirements. Milk yield prediction, adjusted DMI, diet costs, and income over feed cost (IOFC) were used to compare actual farm feeding strategy vs. NGS. Results indicate that NGS improves nutritional accuracy of the diets related to better diet nutrients allocation among groups. Therefore, the diet cost of NGS decreased for low nutritional requirements and increased for high nutritional requirements groups compared with current farm groups. The total cost of 78 d of feeding lactating cows for current farm groups was $485,638, whereas for NGS was $478,252, which resulted in $7,386 diet cost savings. Predicted total milk production increased 21,081 kg for the 78-d period. Adjustments in nutrient density of diets for NGS increased the percentage of crude protein which resulted in higher prediction of milk yield. Diet cost savings plus higher income from milk in NGS results in higher IOFC. Current total IOFC of farm groups was $769,314, while for NGS was $783,810, which resulted in an increase of $14,496.

Key Words: feeding costs, nutritional accuracy, income over feed cost

99 Keeping the most profitable cow and not the most yielding one: Lifetime cost-benefit assessment as a decision-making support tool in dairy management. D. Warner*[1,2], R. Lacroix[1], R. I. Cue[2], and E. Vasseur[2], [1]*Valacta, Dairy Production Centre of Expertise Quebec-Atlantic, Sainte-Anne-de-Bellevue, QC, Canada,* [2]*Department of Animal Science, McGill University, Sainte-Anne-de-Bellevue, QC, Canada.*

Farm profitability and efficiency are essential factors for sustainable dairy production. Previous studies indicate that dairy producers often underestimate the cumulative costs and preferentially consider current events for making decisions especially in keeping cows in the herd. The objective of this study was to investigate the main criteria used by producers for a cow to stay in the herd for an additional lactation. Data originated from production and health records from Valacta DHI and herd health files for cows up to the fourth lactation and born between February 1997 and March 2012 across 114 herds in Quebec, Canada. The data set included production costs (feed costs, costs of health events) and revenues (milk value, margin over all costs), accumulated on lactation (LAC) and productive lifetime (PLT) basis. A cohort of cows in a herd that stayed in production for an additional lactation (ST) was compared with a cohort that left by the end of lactation (LF) through paired *t*-test analyses. LAC costs of health events were higher ($P < 0.01$) for the LF than ST cohort in each lactation, in particular as of lactation 3. LAC feed costs were higher ($P < 0.05$) for the LF cohort only in lactation 1–2. LAC revenues were considerably lower for the LF cohort, in particular for margin over all costs ($P < 0.01$). These results suggest that revenues generated at the current lactation largely influenced the stayability in the herd. Profitable cows appeared to be more feed-efficient until lactation 2, and less likely to encounter diseases at later lactations. PLT costs progressively increased at a larger rate ($P < 0.01$) for the LF cohort, in particular for feed costs. Yet, PLT milk value was higher ($P < 0.05$) for the LF cohort, whereas PLT margin over all costs did not differ ($P > 0.1$) between cohorts. These results suggest that cows that are more profitable could be retained in the herd if cumulative costs over a lifetime are considered. Lifetime cost-benefit assessment has the potential to inform producers on keeping the most profitable instead of the most yielding cow in making a decision to retain an animal in the herd. This is a powerful tool to improve herd profitability and longevity.

Key Words: profitability, longevity, culling

100 Development of a decision support tool for optimal allocation of nutritional resources in a dairy herd. A. Bellingeri*[1,2], A. Gallo[1], D. Liang[2], F. Masoero[1], and V. Cabrera[2], [1]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*University of Wisconsin-Madison, Madison, WI.*

The objective of the present study was to examine the effect of a Linear Programming (LP) model developed to concomitantly optimize cropping plans and animal diets to minimize whole dairy farm feed costs. The optimizations were carried out on data from 15 Italian dairy farms. All herds were high yielding Holstein-Friesian cows (37.5 ± 2.3 kg of ECM), housed in freestall barns, fed TMRs, and without access to pasture. Nutritional requirements were kept equal to the actual farm practice. These included DMI, RDP, RUP, NEL, NDF, ADF, f-NDF, which were group calculated according to NRC (2001) equations. Production levels were considered to remain constant as the nutritional requirement remained unchanged. Other farm characteristics such as herd structure, nutritional grouping strategies, feed consumption, cropping plan, intrinsic farm limitations (e.g., silage and hay storage availability, water for irrigation, manure storage) and on-farm-produced forage costs of production were collected from each farm for year 2017. The optimized scenario resulted in different diets and cropping plans with different feed allocation. The total feed cost (€/100 kg of milk) was greater in the actual (€20.4 ± 2.3) than the optimized scenario (€19.0 ± 1.9), resulting in a 6.7% feed cost reduction with a range between 0.49% and 21.6%. This means €109 ± 96.9 greater net return per cow per year. The implementation of our proposed LP decision support tool for the optimal allocation of the nutritional resource and crops in a dairy herd has the potential to reduce feed cost of diets and improve the farm feed self-sufficiency.

Table 1 (Abstr. 100). Differences in feed costs, energy, and protein feed self-sufficiency among farms between optimized scenario minus actual situation, expressed as % of herd requirement (mean ± SD)

Variable	Difference (%)	*P*-value
Feed costs (€/lactating cow per day)	−6.65 ± 4.32	0.057
Feed self-sufficiency (energy)	18.25 ± 20.59	<0.05
Feed self-sufficiency (protein)	10.34 ± 9.29	<0.05

Key Words: feeding cost, cropping plan, optimization

101 Economics of embryo transfer use to improve reproductive performance during heat stress. F. Ferreira*[1,2], M. Marcondes[1,3], and A. De Vries[1], [1]*University of Florida, Gainesville, FL,* [2]*University of California, Davis, CA,* [3]*Universidade Federal de Viçosa, Viçosa, MG, Brazil.*

Heat stress (HT) impairs reproductive performance. The use of in vitro produced embryos (IVP-ET) can be an aid to improve conception rates (CR) during HT, but economic studies on this practice that consider the seasonality of production are scarce. Our objective was to estimate how an IVP-ET program during HT affects annual herd profitability under a parlor capacity (PCAP) and a maximum number of calving per month (MCALV) constraints. We developed a Markov chain model with 4 HT months, which impaired milk production, mortality, and CR. Heifers were bred with conventional semen only, and cows either conventional semen or IVP-ET. We used a nonlinear model to maximize the annual herd profit by changing the number of heifers calving each mo, subject to the constraints. IVP-ET prices were $100 or $200, the duration of the IVP-ET during HT was 2 or 4 mo, and the breeding in which the IVP-ET started was first or third. No genetic gain was considered. Mean CR for conventional semen during cool and HT mo was 0.57 and 0.52 (heifers) and 0.37 and 0.31 (cows), and mean CR for IVP-ET was 0.33 and 0.30 (cool and HT mo). Seasonality in milk production was measured by low-to-peak ratios (LP, lowest mo value divided by the greatest). When PCAP was the only constraint, the profitability decreased from $970,319 when no IVP-ET was used (999 average milking cows, LP 0.93) to $824,970 (4 mo of $200 IVP-ET, starting on the first breeding, 911 average milking cows, LP 0.56). Less milking cows explain the reduction in profitability, as the model avoided expensive IVP-ET breeding and became more seasonal. When both PCAP and MCALV were constraints, profitability decreased from $962,099 (no IVP-ET, average 991 milking cows, LP 0.92) to $772,718 (4 mo of $200 IVP-ET, starting on the first breeding, 982 average milking cows, LP 0.91) due mainly to the use of expensive IVP-ET breedings during HT. An average of $20 per IVP-ET would reach the same profitability as scenarios without IVP-ET, regardless of the constraints. In conclusion, the use of IVP-ET during HT under the current market prices of IVP-ET was not feasible. Using other herd constraints or considering additional negative effects of HT might increase the value of IVP-ET during HT.

Key Words: economics, embryo transfer, heat stress

102 Economics of using beef semen. W. Li* and V. Cabrera, *University of Wisconsin-Madison, Madison, WI.*

The objective of this study was to comprehensively analyze the economics of using beef semen under different reproductive performance, management, and market conditions. We used an improved version of the Premium Beef on Dairy tool from the University of Wisconsin-Madison (available at http://DairyMGT.info). A brand-new module for simulating calves and heifers was developed and connected with the existing model. Then, we simulated farms with 35% turnover ratio under 3 reproductive levels (15%, 20%, 30% 21-d pregnancy rate) to generate eligible animals for breeding at different services. Five incremental sexed semen strategies were studied including no sexed semen use to 2 services on adult cows and some combinations with top genetic animals. Beef semen utilization was restricted to the remaining adult cows and used at 5 levels (0%, 25%, 50%, 75%, and 100%). Scenarios of distinct market conditions were determined by changing calf prices and semen costs to analyze income from calves over semen costs (ICOSC). As expected, results showed that the higher beef crossbred calf price or lower the cost beef semen, the more beef semen use and higher ICOSC, but more replacement needed to maintain the herd size. Thus, the optimal semen combinations with positive replacement balance usually coupled with increased sexed semen utilization. Reproduction was still a limiting factor of farm profitability considering higher reproductive farms had higher ICOSC and more positive replacement balance, which enabled them to continue using sexed semen even if expensive. Under current Wisconsin market conditions, a medium reproductive farm could benefit from using a combination of sexed semen for first- and second-service heifers, first-service primiparous, first-service secondiparous and 20% genetically superior adult cows; conventional semen for all other heifers and 25% of other adults; and beef semen for the rest of eligible cows. This will procure enough replacements and $2,418/mo ICOSC in a 1,000-cow farm. The updated model and the decision support tool provide a comprehensive decision-making opportunity for farms in terms of optimal ICOSC under projected replacement balance.

Key Words: income from calves over semen costs, herd simulation, semen strategy

103 Impact of health status at arrival to a milk-fed veal facility on breakeven purchase price of calves. D. L. Renaud*[1], K. C. Dhuyvetter[2], S. J. LeBlanc[1], D. F. Kelton[1], T. F. Duffield[1], and M. W. Overton[2], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Elanco Animal Health, Greenfield, IN.*

Male calves are purchased from the dairy industry in North America to produce red meat. There is wide variation in the price paid for male calves, and it is unclear which variables influence the price that could be paid for each calf. The objective of this study was to assess how the health traits of calves at the time of arrival and demographic variables affect the breakeven purchase price of a male calf entering the veal industry. A deterministic model was constructed using the prevalence of health abnormalities, weight at arrival, source of the calf, number of days in the barn, base carcass price, days to mortality, feed costs, season at arrival, interest rate, housing location, carcass dressing percentage, and costs associated with housing, labor, utilities, trucking, and health to calculate the breakeven purchase price and an estimate of profit. Sensitivity analysis was conducted using health variables measured at arrival and demographic variables including season at arrival and housing location to identify the factors with the largest impact on the predicted average daily gain, early and late mortality risk, breakeven purchase price, and profit. At the baseline inputs, the average predicted profit was −$5.36 per calf and it was most sensitive to the location of housing where the calves were fed and the body weight of the arriving calf. The mortality risk in the first 21 d after arrival was predicted to be 2.2%, whereas the risk of mortality after 21 d was 3.7%. The risks of early and late mortality were most sensitive to the level of dehydration measured at arrival and the season of arrival for the purchased calves, respectively. The predicted average daily gain was 1.12 kg/d and it was most sensitive to the housing location. The breakeven purchase price was estimated to be $242.49 per calf, which was most sensitive to the housing location where the calves were fed. The results of this analysis demonstrate that veal producers need to consider many variables before the purchase of calves. In addition to overall market conditions, veal producers should factor health characteristics and the expected performance of the calves they purchase into what they are willing to pay for them.

Key Words: male dairy calf, economics, breakeven purchase price

Reproduction 1

104 Using automated data collection systems to identify factors that affect the magnitude and duration of increased activity at estrus in Holstein cows. M. C. Lucy*[1], B. Liao[2], J. M. Christensen[2], and F. G. Kumro[1], [1]*University of Missouri, Columbia, MO,* [2]*DeLaval International AB, Tumba, Sweden.*

Automated data collection systems were used to identify periods of increased activity at estrus and to assess factors affecting the magnitude and duration of estrous activity. In total, 4,172 estrous periods in 1,454 cows across 5 herds in Europe and Canada were studied. Each herd used automated management systems manufactured by DeLaval (Tumba, Sweden) including a milk progesterone (MP4) measurement and analysis system (Herd Navigator), a body condition scoring system (BCS Camera) and an activity system (DelPro). A "heat alarm" (HA) was defined as 2 consecutive MP4 samples below 5 ng/mL following a luteal phase. Estrus was defined as an increase in activity within 7 d after HA. Peaks in activity (estrus) were detected with an algorithm and the magnitude (fold change above baseline), duration, and area were determined. Cows were classified according to parity, month, BCS (≤2.75, 2.75–3.0, 3.0–3.25, >3.25), days in milk (DIM; ≤56, 57–84, 85–140, and >140), and daily milk production (<40, 40–50, and >50 kg/d) at the time of HA. Data were analyzed using a mixed model (PROC MIXED; SAS) with animal nested within herd defined as random. There was an effect of herd (range: 65.7 to 78.7%; $P < 0.001$), month (range: 69.0% for May–Jun to 78.7% for Nov-Dec; $P < 0.002$) and BCS (range: 70.0% for ≤2.75 to 77.1% for >3.25; $P < 0.004$) on the percentage of cows with an activity peak after HA. Neither DIM, parity, nor milk affected peak detection. For cows with an activity peak, the peak area was affected by herd ($P < 0.001$), DIM ($P < 0.001$), month ($P < 0.001$) and BCS ($P < 0.001$). Differences in peak area were primarily associated with greater peak duration and not peak magnitude. Peak duration (h) differed for herds (range: 16.9 ± 0.3 to 19.2 ± 0.2; $P < 0.001$), DIM (range: 17.4 ± 0.2 for ≤56 to 19.3 ± 0.3 for >140; $P < 0.001$), month (range: 17.3 ± 0.3 for May–June to 19.1 ± 0.2 for Nov-Dec) and BCS (range: 17.6 ± 0.3 for ≤2.75 to 18.9 ± 0.2 for >3.25; $P < 0.001$). Neither parity nor milk affected peak area or duration. Conclusions were that estrous activity is affected by herd, season (lowest in summer), BCS (lowest in low BCS cows) and DIM (lowest in early lactation cows).

Key Words: estrus, activity, BCS

105 Activity before artificial insemination and conception in dairy cows in grazing and nongrazing conditions. S. Paudyal*[1,2] and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Texas A&M University, College Station, TX.*

The objective was to characterize activity before artificial insemination (AI) in cows that did or did not conceive in grazing and non-grazing conditions. Holstein cows (n = 310) from a herd in Northern Colorado (2,800 milking cows) were affixed with a pedometer (IceQube IceRobotics, Edinburgh, UK) on one leg at 12 ± 8 DIM to monitor steps (ST; n/d), lying time (LT; min/d), and lying bouts (LB; n/d) for 7 mo. Milk yield (MY; kg/d) and reproductive data were obtained from on-farm software. Reproduction was managed through heat detection by use of tail head painting and AI technicians were unaware of activity data. Cows were maintained in free stall barns and had access to pasture during the final 58 d of the study. Activity parameters were analyzed as continuous and categorical variables (high vs. low relative to the median). Generalized linear models and logistic regression analysis were used to evaluate activity on the day before AI (d-1) and subsequent pregnancy. Models included DIM, parity, THI, technician, and sire as covariables. Overall, 67% of the cows were pregnant at the end of the study. There were 551 and 221 AI during the non-grazing and the grazing period (27.6% vs. 30.8% conception). A significant effect on ST was determined for the interaction between grazing and AI outcome ($P = 0.02$), as conception was associated with greater or lower ST, depending on access to grazing. Subsequent analyses by grazing category indicated no differences in ST for cows that conceived or remained open. LT and LB were not affected by access to grazing. LT at d-1 was greater in cows that conceived (537 ± 16 vs. 498 ± 9; $P < 0.05$). Similarly, LB at d-1 was greater in cows that conceived (9.26 ± 0.33 vs. 8.5 ± 0.21; $P = 0.05$). Daily MY was not associated with AI outcome. The logistic regression analyses considering activity variables divided in categories indicated that the only parameter partially associated with conception was ST: During the non-grazing season, the odds (95% CI) of pregnancy in cows in the high ST category were 2 (1.2–3.33) times the odds of cows in the low ST category. The level of variation on activity behavior before AI was partially different for cows that conceived or failed to conceive in grazing and non-grazing conditions.

Key Words: activity, reproduction

106 Characterization of behavior biometrics using 3D-kinematic analysis as ovulation approaches in dairy cows in tiestall housing. H. J. Perez Marquez*, M. J. Guesgen, and C. J. Bench, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

The objective of this study was to investigate 3D-kinematics as a method to determine if primiparous dairy cows display differences in movement biomechanics during the estimated estrus period as ovulation approaches. Fourteen primiparous naturally cycling dairy cows (n = 14) were used in a split-plot overtime design. 3D-Kinematic assessments were undertaken and milk samples were collected from d 14 post-ovulation until 2 d after each cow's second ovulation. Frequency of event (Events/5min) data were collected for Pelvic tilt (Pt) and shift angles (Ps), Feet strikes (Fs), and Micro (Mic; 85–95°), Middle (Mid; 95–100°) and Macro (Mac; >100°) tail angle movements (T). Statistical analysis (Proc Glimmix in SAS) compared behavioral data at baseline (luteal phase), estimated estrus period (−24 h) and ovulation day within EU and between EUs (Sample time and Cow effect). The largest follicular diameter and estradiol concentration occurred at −24 h (Table 1). The frequency of almost all behavioral parameters measured significantly decreased during the estimated estrus time (−24 h) compared with baseline and ovulation with the exception of Pt and Ps (Table 1). The accuracy of behavioral biometrics as estrus indicators were analyzed using sensitivity (Se), specificity (Sp) and Youden index (J index) levels (ROC curves). Feet strikes had the highest J index and Se-Sp level followed by Pt, Ps, TMid, and TMicro movements as estrus indicators. Our data indicates that primiparous tie-stall housed dairy cows exhibit movement-biomechanic changes as spontaneous ovulation approaches.

Key Words: estrus, movement biomechanics, pipeline housing

107 Investigating reproductive traits in cows with high and low genetic merit for fertility in a seasonal, pasture-based system. S. Meier*[1], B. Kuhn-Sherlock[1], P. R. Amer[2], J. Bryant[1,3], J. R. Roche[1,4], and C. R. Burke[1], [1]*DairyNZ Limited, Hamilton, New Zealand,*

Table 1 (Abstr. 106). Comparison between physiological and behavioral parameters (LSM ± SEM), significance level, and receiver operating characteristic curve results

Parameter	Baseline	−24 H	Ovulation	P-value	Se[1]	Sp[2]	J index[3]
Follicle diameter (mm)	13.9 ± 0.5	17 ± 0.5	—	0.01	1.0	0.6	0.6
E2 (pg/mL)	15.7 ± 1.6	17.4 ± 1.7	16.0 ± 1.6	0.99	0.9	0.6	0.4
Pt	13.1 ± 8.3	17.4 ± 11.0	11.7 ± 7.4	0.01	0.7	0.5	0.4
Ps	3.5 ± 2.5	10.6 ± 7.1	3.4 ± 2.3	0.01	0.7	0.6	0.3
Fs	14.4 ± 2.7	9.9 ± 1.9	12.4 ± 2.4	0.07	0.9	0.6	0.5
Tmac	1.8 ± 1.1	1.7 ± 1.0	1.9 ± 1.1	0.41	1.0	0.3	0.3
Tmid	6.1 ± 3.2	1.8 ± 1.0	3.8 ± 2.0	0.01	1.0	0.3	0.3
Tmic	14.6 ± 7.2	7.3 ± 3.6	15.3 ± 7.5	0.01	0.9	0.4	0.3

[1] Sensitivity.

[2] Specificity.

[3] Youden index.

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We investigated novel, early reproductive phenotypes to accelerate the rate of genetic gain for fertility by examining reproductive phenotypes in dairy cows with divergent genetic merit for fertility in a seasonal, pasture-based dairy system. Two groups of heifers (n = 550) with high (+5.0%) or low (−5.1%) fertility breeding values (FertBV) via custom mating. The FertBV were based on parent averages for submission rates in lactations 1 to 3, calving rate in lactations 2 to 4, with body condition and milk production in lactation 1 as correlated traits. The high and low FertBV groups were managed together from 9 d old and were balanced for live weight and milk production trait breeding values, and % North American ancestry. We report heifer reproductive traits to seasonal breeding, and submission and pregnancy rates during lactations 1 and 2. Heifers with high FertBV attained puberty at an earlier age (358 d vs 379 d old, SED 6.0 d; $P < 0.01$) and a lighter live weight (271 kg vs. 296 kg, SED 4.3 kg; $P < 0.01$), and had a 9% greater 6-wk pregnancy rate, compared with low FertBV heifers (90% vs. 81%, SED 3.1%, $P < 0.02$). Lactation 1 had 257 high and 224 low FertBV cows and lactation 2 had 203 high and 121 low FertBV cows. During lactation 1, we mated more high FertBV cows during the first 3-wk of breeding compared with low FertBV cows (87% vs 48%; SEM 3.7%; $P < 0.01$). The 6-wk pregnancy rate was 67% for the high and 33% for the low FertBV cows (SEM 3.3%; $P < 0.01$), respectively. In lactation 2, the average calving date for the low FertBV cows was10 d later than the high FertBV cows, reflecting their poorer reproductive performance in lactation 1. The 3-wk submission (88% vs 63%; SEM 5.4%; $P < 0.01$) and 6-wk pregnancy rates (74% vs 44%; SEM 5.1%; $P = 0.13$) were greater in the high compared with low FertBV cows, respectively. In the absence of reproductive interventions, the differencesin reproductive performance between groups in their first and second lactation were greater than predicted from their difference in Fert BV. The difference in reproductive performance between the FertBV groups validates the relevance of the FertBV and indicates puberty attainment is a candidate trait for genetic selection.

Key Words: puberty, reproduction, genetic fertility

108 Early genomic prediction of daughter pregnancy rate is associated with improved fertility outcomes in Holstein dairy cows. F. S. Lima*[1], F. T. Silvestre[2], F. Penagaricano[3], and W. W. Thatcher[3], [1]*Department of Veterinary Medicine, University of Illinois, Urbana, IL,* [2]*Zoetis Inc., Kalamazoo, MI,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The use of genomic testing for selecting replacement heifers in commercial farms has recently attracted much attention. Genomic prediction of daughter pregnancy rate (GDPR) is one of the low heritability traits that have the potential to benefit the most from the genomic selection. Our objectives were to assess the relationships between GDPR and pregnancy for the first service (P1), pregnancy at the end of lactation (PEND), number of services for conception (NS), days from calving to first service (TP1), and days open (TPEND). Data for GDPR, reproductive parameters, and milk production (MP) from 1401 primiparous and 3044 multiparous Holstein cows, located in 4 farms with the same reproductive management, were used in the analyses. Data for GDPR and MP (first 2 mo for first service or 305 milk-equivalent for pregnancy at the end of lactation) were categorized in quartiles. Data were analyzed separately for primiparous and multiparous cows. Generalized linear models for P1, PEND, NS, TP1 and TPEND included the effects of GDPR, farm, MP, and the interaction between GDPR and MP. There were positive effects of GDPR ($P < 0.01$) in primiparous and multiparous cows for P1, PEND, NS, TP1, and TPEND. An effect of farm ($P < 0.01$) was detected for primiparous and multiparous for PEND, NS, TP1, and TPEND, and P1 in multiparous. An effect of MP was found ($P < 0.01$) for PEND in primiparous and multiparous, and NS for primiparous. Positive GDPR effects include a 22.5% higher P1 (55.5% vs. 33.0%), 15.3% higher PEND (80.9% vs. 65.6%), and 48.7 d shorter TPEND (129.3 vs. 178.0 d) for the highest GDPR quartile compared with the lowest GDPR quartile in multiparous cows. An interaction between GDPR and MP was detected for NS in primiparous cows only, where high-producing cows had fewer NS as GDPR increased, whereas no relationship between GDPR and NS was observed in low-producing cows. In summary, GDPR was a strong predictor for all reproductive parameters measured in both primiparous and multiparous cows, and interactions with milk production were restricted to NS in primiparous cows.

Key Words: genomic, daughter pregnancy rate, fertility

109 Genetic merit for fertility and type of reproductive management strategy affected the reproductive performance of primiparous lactating Holstein cows. E. M. Sitko*[1], M. M. Pérez[1], G. E. Granados[1], M. Masello[1], F. Dicroce[2], A. McNeel[2], D. Weigel[2], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Zoetis, Kalamazoo, MI.*

Our objective was to evaluate first service reproductive performance of primiparous lactating dairy cows of different genetic merit for fertility submitted to AI through a timed AI (TAI) protocol or a combination of AI at detected estrus and TAI (AIE+TAI). Cows from 6 commercial farms were stratified into High (H), Medium (M), and Low (L) fertility groups based on a Reproductive Index (RI) value calculated from multiple genomic predictions generated by a commercially available genomic test (CLARIFIDE, Zoetis). Within each fertility group cows were randomly assigned to a TAI (n = 1,155) or AIE+TAI (n = 1,245) treatment group. Cows in TAI received the Double-Ovsynch protocol and TAI at 84 ± 3 DIM. Cows in AIE+TAI were eligible for AIE after a PGF2α treatment at 53 ± 3 DIM. Cows not AIE within 21 d of the PGF treatment received TAI after the Ovsynch protocol with progesterone supplementation. Binary and quantitative outcomes were analyzed using logistic regression and ANOVA, respectively. Farm was included as random effect in all models. Differences in LSM were analyzed by the LSD method. Mean RI was lowest ($P < 0.001$) for HF (93.4 ± 0.8), intermediate for MF (98.6 ± 0.8), and highest for LF (105.6 ± 0.8). Conversely, RI did not differ for TAI (99.3 ± 0.8) and AIE+TAI (99.2 ± 0.8) for all cows or within fertility group ($P > 0.10$). For AIE+TAI, the percentage of cows AIE was similar ($P = 0.45$) for HF (75.8%), MF (73.5%) and LF (72.0%). Overall pregnancy per AI (P/AI) irrespective of reproductive program was greater ($P < 0.01$) for HF (59.7%; n = 784) than MF (52.4%; n = 812) and LF (49.5%; n = 804) but MF and LF did not differ. Overall P/AI was greater for TAI than EDAI ($P < 0.0001$; TAI = 58.4% vs. AIE+TAI = 48.9%). For AIE+TAI, P/AI for cows AIE was greater for HF (53.9%; n = 307) than LF (40.0%; n = 303) and MF (47.8%; n = 307) but MF was similar than HF and LF. Overall P/AI was greater ($P < 0.05$) for TAI services within the HF (54.4 vs 65.4% for AIE and TAI) and LF (43.4 vs 56.1% for AIE and TAI) groups but was not different for the MF group. In conclusion, genetic merit for fertility and the type of reproductive management strategy for first service affected the reproductive performance of lactating primiparous cows.

Key Words: reproductive performance, genomics, dairy cow

110 Plasma progesterone is associated with individual genetic traits and likelihood of conception in seasonal-calving pasture-based dairy cows. E. Rojas Canadas[1,2], M. M. Herlihy[1], J. Kenneally[1], F. Kearney[3], P. Lonergan[2], and S. T. Butler*[1], [1]*1Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland,,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [3]*Irish Cattle Breeding Association, Bandon, Co. Cork, Ireland.*

The objectives were to (1) evaluate the associations between plasma progesterone (P4) concentration after first service and reproductive outcomes and (2) examine the associations between genetic traits and plasma P4 concentration. First and second lactation spring calving dairy cows (n = 915) from 35 dairy farms located in Ireland were enrolled in the study. Plasma P4 (ng/mL) was determined from a single blood sample collected at either 7 (n = 475) or 13 (n = 440) d after first insemination at detected estrus (FAI). The fertility sub-index of the Economic Breeding Index and the predicted transmitting ability (PTA) values for the individual traits calving interval (CIV), survival, milk kg and percentage protein in milk were obtained from Irish Breeding Cattle Federation, and cows were stratified into quartiles. For continuous data, the normality of both the raw data and residuals was tested, and where transformation was necessary the most appropriate Box-Cox transformation was implemented. GLIMMIX procedure was used to determine the associations between plasma P4 concentration, reproductive outcomes and individual genetic traits including herd, year and parity as fixed effects. Plasma P4 at 7 and 13 d after FAI was positively associated with likelihood of pregnancy after FAI ($P = 0.05$ and $P = 0.001$, respectively). The PTAs for milk kg and survival were positively associated with plasma P4 at d 7 ($P = 0.02$ and $P = 0.01$, respectively) and d 13 after FAI ($P = 0.05$ and $P = 0.004$, respectively), and cows in the quartile with greatest PTAs for milk kg and survival had greater likelihood of pregnancy after FAI compared with the other quartiles. Cows in the quartile with greatest fertility sub-index and smallest CIV tended (both $P = 0.10$) to have greater plasma P4 at d 7 and 13 after FAI compared with the other 3 quartiles. In conclusion, plasma P4 at 7 and 13 d after FAI were associated with individual genetic traits and reproductive outcomes. Supported by Irish Department of Agriculture, Food and the Marine (Grant 13S528).

Key Words: reproductive phenotype, genetic merit for fertility traits, serum progesterone

111 Fertility of fresh and frozen sex-sorted semen in dairy cows and heifers in seasonal-calving pasture-based herds. C. Maicas*[1,2], I. Hutchinson[1], J. Kenneally[1], J. Grant[3], A. Cromie[4], J. Moreno[5], R. Vishwanath[5], E. Maia[5], P. Lonergan[2], and S. Butler[1], [1]*Animal and Grassland Research and Innovation Centre, Teagasc, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Dublin, Ireland,* [3]*Research Operations Group, Teagasc, Ashtown, Dublin, Ireland,* [4]*Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland,* [5]*Sexing Technologies, Navasota, TX.*

The objective was to evaluate the reproductive performance of dairy heifers and cows inseminated with fresh and frozen sex-sorted semen (SS) in seasonal-calving pasture-based dairy herds. Ejaculates from 10 Holstein-Friesian bulls were split and processed to provide 1 of 4 semen treatments: (1) fresh conventional semen at 3×10^6 sperm per straw (CONV); (2) fresh SS at 1×10^6 sperm per straw (SS-1M); (3) fresh SS semen at 2×10^6 sperm per straw (SS-2M); and (4) frozen SS at 2×10^6 sperm per straw (SS-FROZEN). Semen from all treatments was used to inseminate cows and heifers following detection of spontaneous estrus during the 2 d following processing. Pregnancy per artificial insemination (P/AI) was determined using subsequent calving records supplemented with pregnancy diagnosis data when available. Separate generalized linear mixed models for heifers (n = 3,214) and cows (n = 5,493) were used to examine effects on pregnancy per artificial insemination (P/AI), with semen treatment (n = 4), bull (n = 10) and treatment by bull interaction as fixed effects, and herd as a random effect. Additional relevant variables for cows and heifers were also tested (e.g., DIM, parity, genetic traits), and retained where significant. CONV semen had greater P/AI than SS-FROZEN in heifers and greater P/AI than any SS treatment in cows. None of the SS treatments differed from each other (Table 1). P/AI increased with greater DIM, Economic Breeding Index and Body condition score, and decreased as parity number increased, but none of these variables interacted with semen treatment. The relative P/AI for frozen SS compared with conventional semen was greater (>84%) than previously reported in lactating cows, but fresh SS semen did not have greater P/AI compared with frozen SS.

Table 1 (Abstr. 111). Pregnancy/AI (P/AI, %) and performance of sex-sorted semen relative to conventional semen (SS/CONV, %) in dairy heifers and cows

	Heifers		Cows	
Treatment	P/AI	SS/CONV	P/AI	SS/CONV
CONV	60.9^{a}	—	48.0^{a}	—
SS-1M	54.2^{ab}	88.9	37.6^{b}	78.4
SS-2M	53.5^{ab}	87.8	38.9^{b}	81.0
SS-FROZEN	52.8^{b}	86.7	40.6^{b}	84.7

^{a,b}LSM within a column with different superscripts differ ($P < 0.05$).

Key Words: sex-sorted semen, pasture-based system

112 Fertility of frozen sex-sorted semen at 4 x 10^6 sperm per dose in lactating dairy cows in seasonal-calving pasture-based herds.

C. Maicas*[1,2], S. Holden[1], E. Drake[1], A. Cromie[3], P. Lonergan[2], and S. Butler[1], [1]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland,* [3]*Irish Cattle Breeding Federation, Bandon, Co. Cork, Ireland.*

The objective was to evaluate the reproductive performance of frozen sex-sorted semen (SexedUltra 4M) relative to conventional frozen semen in seasonal-calving pasture-based dairy cows. Semen from Holstein-Friesian (n = 8) and Jersey (n = 2) bulls was used. Four of the Holstein bulls were resident at or near a sex-sorting laboratory (Cogent, UK or ST Benelux, Netherlands). Ejaculates from the remaining bulls were collected in Ireland, diluted with transport medium and couriered to Cogent in temperature-controlled parcel shippers. Transit time from ejaculation to arrival at the sorting lab was 5 to 6 h. Ejaculates were split and processed to provide frozen conventional semen at 15×10^6 sperm per straw (CONV) and frozen sex-sorted semen at 4×10^6 sperm per straw (SS), and used for inseminating lactating dairy cows after spontaneous estrus. Pregnancy diagnosis was performed by ultrasound scanning (n = 7246 records available for analysis). Generalized linear mixed models were used to examine effects on pregnancy per artificial insemination (P/AI), with semen treatment (CONV vs. SS), bull (n = 10) and treatment by bull interaction as the fixed effects, and herd (n = 142) as a random effect. Overall, P/AI was greater ($P < 0.001$) for CONV than for SS (59.9% vs. 45.5%; 76.0% relative to CONV). This study was not designed to compare resident bulls vs. shipped ejaculates, but the magnitude of the difference between CONV and SS was apparently less for resident bulls (60.3% vs. 50.2%, $P < 0.001$; 83.3% relative to CONV) than for shipped ejaculates (58.6% vs. 40.7%, $P < 0.001$; 69.5% relative to CONV). The treatment x bull interaction effect was significant for shipped ejaculates ($P = 0.006$; range 45 to 86% P/AI relative to CONV) but not for the resident bulls ($P = 0.70$; range 81 to 87% P/AI relative to CONV). In conclusion, frozen SS at 4×10^6 sperm per dose had lesser P/AI compared with CONV at spontaneous estrus in pasture-based dairy cows. Strategies to improve the P/AI with SS (e.g., timing of AI) require further research. Assistance from Sexing Technologies is gratefully acknowledged.

Key Words: frozen sex-sorted semen, pasture-based system

113 The genetic components of sexed semen production.

C. Heuer*, T. Gilligan, J. Moreno, and R. Vishwanath, *STgenetics, Navasota, TX.*

Sexed semen has gained importance in the dairy cattle breeding industry over the last decade which brings about the possibility of a set of complex new traits on bull selection tailored toward the production of sexed semen. The main trait of interest is sort-rate, which defines the number of semen cells that are being sorted per second. The sort-rate is the result of parameter adjustments in the sorting process while maintaining a workable split of the semen populations to achieve a certain purity in the process. The objective of this study was to estimate (co)variance components of sort-rate and semen quality traits of sexed sorted Holstein sperm. A data set comprising 311,337 sorting periods for 2,058 Holstein bulls was used in a pedigree based animal model that included pen-day and age-group as fixed effects as well as a permanent environmental and additive genetic effect of the bull. The repeatability and heritability of sort-rate had estimates of 0.26 and 0.07 with standard errors of 0.01 and 0.02, respectively. Breeding values estimated from the model had a range of 1,800 cells per second, which illustrates tremendous potential for selection and optimization of machine workload. Besides the core phenotype of sort-rate, semen quality is of paramount importance in the production of sexed sorted semen. A data set on 97,895 individual semen freeze codes was used to estimate heritabilities and genetic correlations of post thaw motility of sex sorted semen at zero and 3 h after incubation at 37°C, acrosomes, ejaculate volume and initial motility. Heritabilities (standard errors) ranged from 0.027 (0.008) for initial motility to 0.146 (0.026) for ejaculate volume. Zero and 3 h motility had estimated heritabilities of 0.1 (0.022) and 0.117 (0.024), respectively. The highest genetic correlations were found between 3 h motility and acrosomes at 0.906 (0.035) and zero and 3 h motility at 0.88 (0.037). Ejaculate volume was negatively genetically correlated with all other traits with a maximum at −0.47 (0.176) with initial motility. The results align with findings from conventional semen. Sort-rate was found to have a significant genetic component that can be utilized to select for more efficient bulls with regard to semen sorting.

Key Words: sexed semen, semen quality, reproduction

Ruminant Nutrition 1: Protein and Amino Acid 1

114 Assessing essential amino acid availability in lactating dairy cows infused with methionine, lysine, and histidine or leucine and isoleucine. X. Huang*[1], P. Yoder[1,2], and M. Hanigan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*Perdue Agri-Busines LLC, Salisbury, MD.*

The objective of this study was to evaluate the effects of jugular infusion of 2 groups of AA on essential AA availability and utilization by the mammary glands. Four cows (78 ± 10 DIM) were assigned to 4 jugular infusion treatments: saline (CON), methionine plus lysine plus histidine (MKH), isoleucine plus leucine (IL), or MKH plus IL (MKH-IL) in a 4 × 4 Latin square design. Each period was 16 d in length with 8 d of adaption followed by 8 d of jugular AA infusion. Infusion rates were 10 g of methionine, 38 g of lysine, 20 g of histidine, 50 g of leucine and 22 g of isoleucine per day. On last day of each period, a ^{13}C labeled AA mix was infused into the jugular vein over a 6 h period, and blood samples were collected hourly and assessed for AA enrichment. Cows were fed a basal diet consisting of 15.2% crude protein with adequate rumen degradable protein but 15% deficient in metabolizable protein. Milk production increased significantly with infused IL ($P = 0.04$), but did not change in response to MKH ($P = 0.44$). Milk protein concentration and yield increased for the MKH infusion ($P < 0.01$) whereas milk protein yield tended to increase for IL ($P = 0.08$). Total plasma AA entry rates were estimated for each EAA by fitting a 6-pool, dynamic model to observed plasma, ^{13}C AA enrichment. The blood Met entry rates for CON, MKH, IL and MKH-IL were 57, 82, 62, 90 g/d respectively after subtracting infused Met. MKH infusion significantly increased Met entry ($P < 0.01$) and IL infusion tended to increase Met entry ($P = 0.06$), which was related to greater Met intake ($P = 0.09$). The increase in Met entry with MKH infusion reflected increased Met availability from the diet or increased recycling of Met in the body. Essential AA transport and metabolism by the mammary glands will be reported.

Key Words: dairy cow, essential AA, whole-body entry rates

115 Effects of jugular infused methionine, lysine, and histidine or leucine and isoleucine on protein expression and post-translational modification. P. S. Yoder*[1,2], W. K. Ray[3], R. F. Helm[3], X. Huang[1], C. Schumacher[1], E. Huang[1], I. A. M. A. Teixeira[4], and M. D. Hanigan[1], [1]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Perdue AgriBusiness LLC, Salisbury, MD,* [3]*Department of Biochemistry, Virginia Tech, Blacksburg, VA,* [4]*UNESP-Universidade Estadual Paulista, Jaboticabal, SP, Brazil.*

The objective was to evaluate protein expression and post-translation modifications in mammary and muscle tissues of cows supplemented with 2 AA groups. Four lactating cows were assigned to treatments of saline (CON), methionine plus lysine plus histidine (MKH), isoleucine plus leucine (IL), or MKH plus IL in a 4 × 4 Latin square. Milk protein yield increased with MKH and IL independently with the MKH$^+$IL treatment yielding a 9.6% increase versus CON. Mammary and muscle proteomes were assessed for total and phosphorylated abundance and the respective ratio using proteomics. Total and site-specific phosphorylated abundances of mammalian target of rapamycin (mTOR), ribosomal protein S6 kinase (S6K1), ribosomal protein S6 (rpS6), and eukaryotic initiation factor 2a (eIF2a) were assessed by western immunoblotting. The treatments both independently affected the phosphorylation ratio of mTOR pathway proteins in the udder as indicated by multivariate analyses. For the udder proteome, there was a tendency for a treatment interaction on total and phosphorylated abundance as well as the phosphorylation ratio. Univariate proteomic analysis revealed an enhanced phosphorylation ratio of mitogen-activated protein kinase 1 (MAP2K1) by MKH and IL and insulin receptor substrate 1 (IRS1) by MKH treatment only which may indicate negative mTOR feedback. Western immunoblotting indicated decreased total and phosphorylated abundance of S6K1 as well as phosphorylation ratio for the MKH infusion. There was a positive treatment interaction for total and phosphorylated abundance of rpS6, S6K1, and eIF2a as well as the phosphorylation ratio of rpS6. No significant changes were observed in muscle. Results indicate that supplementing MKH or IL affects protein expression and phosphorylation ratio of many udder proteins. However, the changes in mTOR signaling proteins were generally opposite of expectations. The latter appears to be the result of negative feedback arising from infusion of MKH and IL alone and when infused in combination, less negative feedback and positive treatment interaction on mTORC1 targets.

Key Words: milk protein, mammalian target of rapamycin (mTOR)

116 Effect of grouping by genetic merit on the response to dietary protein in first-lactation cows. G. I. Zanton* and D. M. Bickhart, *USDA-Agricultural Research Service, Madison, WI.*

The objective of this study was to characterize the effects of genomic predicted transmitting ability values for protein yield (gPTA) and concentration of metabolizable protein (MP) on milk production in first lactation cows. It was hypothesized that cows of different gPTA would respond differently to diets differing in MP concentration. To test this hypothesis, 56 genomically tested, first lactation Holstein cows were blocked into quartiles by gPTA and randomly assigned to a diet containing an adequate (ADMP; 16.5%CP, 30.3%NDF) or low (LOMP; 14.5%CP, 32.8%NDF) concentration of MP. Diets were formulated to contain identical base ingredients of corn silage (40%), alfalfa silage (20%), ground high moisture shell corn (15.5%), canola meal (8%), roasted soybeans (4%), dried distillers grains with solubles (2%), soybean hulls (1.5%), and a vitamin-mineral mix (2.5%). To these base ingredients, ADMP had an additional 6.5% canola meal and LOMP had an additional 6.5% soyhulls added. Cows were fed individually once daily and milked thrice daily, with intake and milk yield measured daily. Milk was sampled at 6 consecutive milkings weekly and body weight (BW) was measured twice weekly for 2 wk before and for 12 wk while consuming treatment diets. Pretreatment measurements were used for covariate adjustment in the statistical model, contrasts for diet and diet by gPTA block interactions were calculated, and $P \leq 0.05$ was considered significant with $0.05 < P \leq 0.10$ trending toward significance. Cows consuming ADMP had higher DMI, BW, and production of milk and milk components than LOMP. Milk and milk protein yield increased linearly with gPTA block for cows fed ADMP, but there were very small differences in these measurements when fed LOMP (linear gPTA block × diet interaction $P < 0.08$). Yield of fat and energy increased and BW decreased linearly with gPTA block irrespective of diet. Feed and nitrogen efficiencies were higher with ADMP and with increasing gPTA, but these factors did not interact with each other. In conclusion, the expression of genetic differences in milk and protein yield was blunted by feeding LOMP, but not ADMP, in first-lactation Holstein cows.

Key Words: milk, protein, genetics

117 Effect of rumen-protected lysine supplementation to total mixed rations differing in crude protein concentration in lactating cows. S. W. Gee*[1], N. E. Lobos[2], and A. J. Carpenter[1], [1]*University of Guelph, Ridgetown, ON, Canada,* [2]*Kemin Industries, Inc., Des Moines, IA.*

A feeding trial was performed to determine the effects of rumen-protected lysine supplementation in lactating cattle diets differing in CP content. Twelve multiparous mid-lactation Holstein cows were blocked by DIM into a triplicate 4 × 4 Latin Square. Diets were arranged in 2 × 2 factorial design with 2 levels of CP (LO = 15%, HI = 17%) with or without lysine supplementation (+L, -L). Periods consisted of 14 d of adaptation followed by a sampling period of 3 d. During sampling periods, daily milk yield and DMI were recorded, and total collections of urine and feces were taken. Milk, urine, fecal, and plasma samples were collected for additional analysis. No significant interactions were found between CP content and Lys supplementation ($P \geq 0.14$). DMI was affected by dietary CP ($P \leq 0.01$) but not Lys supplementation ($P = 0.21$; LO-L = 25.3 kg/d; LO+L = 26.0; Hi-Ly = 27.5; Hi+L = 28.2; SEM = 1.42). Milk yield increased with CP content ($P = 0.03$) and Lys supplementation ($P \leq 0.01$; LO-L = 34.6 kg/d; LO+L = 36.6; Hi-L = 36.1; Hi+L = 38.0; SEM = 1.87). Energy-corrected milk yields increased with dietary CP ($P = 0.02$) and supplemental Lys ($P \leq 0.01$; LO-L = 34.98; LO+L = 37.06; Hi-Lys = 36.71; Hi+L = 39.02; SEM = 1.79). Milk fat (LO-L = 1.44 kg/d; LO+L = 1.52; Hi-Lys = 1.49; Hi+L = 1.60; SEM = 0.08) and milk protein (LO-L = 1.09 kg/d; LO+L = 1.18; Hi-Lys = 1.19; Hi+L = 1.25; SEM = 0.056) yields were raised by dietary CP ($P = 0.05$ and $P \leq 0.01$ for fat and protein, respectively) and supplemental Lys ($P = 0.01$ for both fat and protein). Increased CP content increased milk protein %, milk urea nitrogen, and fecal N output ($P \leq 0.01$), but there was no effect of Lys ($P \geq 0.56$). No changes were seen in plasma glucose, NEFA, or BHBA as a result of either dietary CP or Lys supplementation ($P > 0.10$). The analysis shows that supplementing Lys in low CP diets can result in similar milk production to high CP diets without Lys supplementation.

Key Words: lysine, low CP diet, N output

118 Amino acid utilization by mammary glands in response to dietary protein and insulin. L. M. Campos*[1], A. G. Rius[1], J. A. D. R. N. Appuhamy[1], D. Kirovski[2], T. F. V. Bompadre[1,3], and M. D. Hanigan[1], [1]*Virginia Polytechnic Institute and State University, Blacksburg, VA,* [2]*University of Belgrade, Beograd, Serbia,* [3]*University of Sao Paulo, Piracicaba, Sao Paulo, Brazil.*

Amino acid supply is an important determinant of milk production. In addition, insulin is a hormone that indirectly regulates protein synthesis. It has been previously observed that hyperinsulinemia stimulates amino acid utilization. The aim of this study was to investigate mammary gland efficiency (MGE) of use of essential amino acids (EAA) in response to dietary protein and hyperinsulinemia. Six lactating Holstein (590 kg BW; 33.0 kg milk/day) cows were randomly assigned in a 2 × 2 factorial arrangement crossover design. The diet was formulated based on NRC requirements for milking cows to be protein sufficient (17.5%; SCP) or deficient (14.0%; DCP). The diets were composed of corn silage, mixed grass and legume silage, and a concentrate mix. Milk samples were collected daily. Arterial and venous blood were sampled via catheters once an hour for 8 h without infused insulin (NI), and again after 4 d of an intravenous infusion of 1 µg of insulin/kg of BW per h plus varying glucose to achieve hyperinsulinemic-euglycemic status (HI). Plasma was prepared by centrifugation, and the supernatant analyzed for EAA concentrations by isotope dilution. MGE was calculated as: $\text{Milk\_EAA}_{(g/d)}/(\text{AV\_EAA}_{(g/L)} \times \text{Plasma Flow}_{(L/d)})$. Statistical analyses were performed using R Studio. Milk yield output was not affected by dietary protein and insulin. There was an interaction between dietary CP and insulin for MGE of Leu, Lys, and Phe. Hyperinsulinemia decreased the efficiency of use of Thr, but did not affect others. MGE for Ile and Val tended to increase and for Met and Thr tended to decrease with decreasing dietary CP.

Key Words: efficiency, essential, hormone

Table 1 (Abstr. 118). Amino acid net uptake in the mammary gland

	NI		HI			*P*-value		
	SCP	DCP	SCP	DCP	SE	CP	Insulin	CP × I
Milk yield, kg/8 h	11.4	11.7	12.7	10.9	1.11	0.22	0.66	0.06
Essential AA, g/g								
Arg	0.20	0.20	0.19	0.27	0.04	0.44	0.60	0.41
Ile	0.34	0.36	0.32	0.33	0.03	0.12	0.59	0.27
Leu	0.41^{a}	0.40^{a}	0.22^{b}	0.49^{a}	0.06	<0.01	0.22	<0.01
Lys	0.65^{b}	0.51^{bc}	0.41^{c}	0.89^{a}	0.11	0.06	0.37	<0.01
Met	1.02	0.95	1.14	1.02	0.08	0.17	0.17	0.66
Phe	0.56^{a}	0.50^{ab}	0.39^{b}	0.58^{a}	0.06	0.26	0.42	0.03
Thr	0.63^{a}	0.44^{a}	0.32^{b}	0.18^{b}	0.07	0.09	<0.01	0.78
Val	0.27	0.32	0.25	0.40	0.08	0.12	0.62	0.48

119 Evaluation of rumen-protected lysine prototypes on plasma amino acid concentrations of lactating Holstein cows. M. I. Rivelli*[1], M. J. Cecava[2], P. H. Doane[2], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*ADM Research Division, Decatur, IL.*

The objective of this study was to determine the short-term effects of targeted rumen-protected and post-ruminal Lys supplementation to dairy cows on protein and AA in blood; and production of milk and milk components. A total of 18 multiparous Holstein cows [BW = 687 ± 68 kg; DIM = 151 ± 57 (mean ± SD)] were assigned to 1 of 3 treatments in a replicated 3 × 3 Latin Square design balanced to measure carryover effects. Total length of the experiment was 73 d including a 10 d diet adaptation period before the start of the trial. Experimental periods were

21 d in length with each divided by adaptation phase (d 1 to 14) and sample phase for statistical inferences (d 15 to 20). Treatments were as follows: cows fed a basal TMR + 150g of dried ground molasses (CON); basal TMR+ 150g of dried ground molasses + a commercially available rumen-protected lysine source (AjiPro-L, Ajinomoto Heartland Inc., Tokyo, Japan) (AJP, positive control); and basal TMR + 150g of dried ground molasses + a rumen-protected lysine prototype source (NPL, prototype B, NutraPass 50, ADM Animal Nutrition, Quincy, IL). Protected lysine sources were each included at a rate of 0.51% [w:w] of the DM. Data were analyzed using the MIXED procedure of SAS using 2 treatment orthogonal contrasts. Contrast 1 (CONT1): CON compared with NPL and contrast 2 (CONT2): AJP compared with NPL. Blood was sampled from the coccygeal vein or artery 4 and 8 h after feeding on 3 consecutive days before the first period and at the end of each period from each cow. There were no differences among treatments for either contrasts for total AA concentrations ($P > 0.10$). Plasma Lys concentration as a percentage of total AA (4.02 vs 4.30 ± 0.09) and plasma Lys concentration as a percentage indispensable AA (8.67 vs 9.30 ± 0.14) was greater for cows fed NPL than cows fed CON ($P < 0.01$, CONT1). Plasma Lys concentration as a percentage of total AA tended to be greater for cows fed AJP than cows fed NPL (4.43 vs 4.30 ± 0.014;$P = 0.08$,CONT2). Plasma 3-methyl-histidine concentration tended to be greater for cows fed CON than cows fed NPL (3.362 vs 3.39 ± 0.14 µM/L*;* $P = 0.08$, CONT1). In conclusion, Lys lipid protection seems to be an effective method of protection.

Key Words: rumen-protected lysine, MUN, milk protein

120 Succinate dehydrogenase activity and protein levels in liver of dairy cows fed TMR or in a pasture-based system. M. García-Roche*[1,2], M. Carriquiry[1], D. A. Mattiauda[1], M. Ceriani[1], A. Jasinsky[1], A. Cassina[2], and C. Quijano[2], [1]*Departamento de Producción Animal y Pasturas, Facultad de Agronomía, Universidad de la República, Montevideo, Montevideo, Uruguay,* [2]*Centro de Investigaciones Biomédicas, Departamento de Bioquímica, Facultad de Medicina, Universidad de la República, Montevideo, Montevideo, Uruguay.*

Succinate dehydrogenase (SDH) is a mitochondrial enzyme involved in the respiratory chain, Krebs cycle and gluconeogenesis. Hence, a drop in its activity may lead to energy deficit or decreased gluconeogenesis, which could in turn affect milk yield and metabolic status. Our aim was to study the effect of 2 feeding strategies on the activity and protein expression of SDH in liver biopsies during early (35 DPP) and late lactation (180 DPP). Multiparous Holstein cows (n = 24, 664 ± 65 kgBW, 3.0 ± 0.4 BCS, spring calving) were assigned in a randomized block design to (G0) a total mixed ration (TMR) fed ad libitum (70% forage: 30% concentrate) or (G1) grazing plus supplementation from 0 to 180 d postpartum (DPP). The G1 cows grazed *Festuca arundinacea* or *Medicago sativa* in 2 (18 h) or 1 (6 h) session depending on heat stress (30 or 20 kg DM/cow/day) and were supplemented with 5.4 kg DM of a commercial concentrate or offered TMR (50% of G0 offer). From 180 to 250 DPP, all cows grazed *Festuca arundinacea* (10h; 30 kg DM/cow/day) and were offered TMR (50% of G0 offer). Data were analyzed with a mixed model using repeated measures. Milk yield was greater at 35 DPP (36 vs. 20 ± 0.8 kg/d, $P < 0.001$) and BCS was greater at 250 DPP (2.45 v*s*. 2.70 ± 0.05, $P < 0.001$). SDH subunit A (SDHA) protein levels and specific activity were studied in liver biopsies during early (35 DPP) and late lactation (250 DPP). Protein levels of SDHA tended to be higher for cows in the pasture-based strategy (1.10 vs. 0.98 ± 0.05 relative intensity, $P = 0.096$; for G1 vs. G0). However, no differences in activity were observed between treatments. Nonetheless, SDH specific activity tended ($P = 0.07$) to be greater during early lactation (159 vs. 129 ± 17 mU/mg, 35 vs. 250 DPP, respectively) and it correlated positively (r = 0.3, $P < 0.05$) with milk yield, suggesting a link between this enzyme and increased milk production in early lactation.

Key Words: dairy cattle, respiratory chain, gluconeogenesis

121 Essential amino acids influence milk fat synthesis in mammary epithelial cells. I. A. M. A. Teixeira*[1,2], P. S. Yoder[2,3], E. Huang[2], X. Huang[2], and M. D. Hanigan[2], [1]*Departament of Animal Science, Unesp, Jaboticabal campus, Jaboticabal, SP, Brazil,* [2]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [3]*Perdue AgriBusiness LLC, Salisbury, MD.*

Increased milk fat yield has been observed when dairy cows are supplemented with amino acids (AA). The mechanism of this phenomenon is still unclear. Previously, amino acids have been reported to regulate mechanistic target of rapamycin (mTOR). This regulation may extend to de novo fat synthesis as mTOR is linked to activation of transcription factor sterol-regulatory-element-binding protein 1 (SREBP1). Activation of the latter will increase de novo fat synthesis. The objective of this study was to evaluate the effects of individual essential amino acids (EAA) on milk fat synthesis and regulation of the related transcription factors. The research was performed in 2 studies. In the first study, we measured de novo fatty acid synthesis in primary bovine mammary epithelial cells using isotopically labeled acetate as a tracer. The cells were subjected to 13 treatments varying in AA profile. Omission of L-leucine (Leu), L-methionine (Met), L-phenylalanine (Phe), all of the EAA, or all of the AA reduced ($P < 0.05$) the isotopic enrichment of C14:0, C16:0, and C18:0. Removal of these AA were associated with reductions in de novo synthesis of C14:0. Synthesis of C16:0 appeared to be more responsive with removal of any of the EAA causing a significant reduction in the isotope ratio. In the second study, we evaluated the effects of individual EAA on cellular signaling involved in milk fat synthesis using primary bovine mammary epithelial cells subjected to similar treatments. Omission of L-arginine, Leu, Met, or all of the EAA reduced ($P < 0.05$) the phosphorylated-to-total signaling ratio of mTOR (Ser2448) and ribosomal protein S6 (rpS6; Ser235/236) in primary bovine mammary epithelial cells. Omission of Leu, Met, and Phe influenced fat synthesis in the primary mammary epithelial cells. Understanding the link between AA and fat synthesis in the mammary gland has practical application in formulating diets to enhance milk fat production.

Key Words: amino acid, fatty acid, milk fat

122 Modeling milk protein yield responses to amino acid supply of dairy cows fed silage-based diets. A. Vanhatalo[1], T. Kokkonen[1], and P. Huhtanen*[2], [1]*University of Helsinki, Helsinki, Finland,* [2]*Swedish University of Agricultural Sciences, Umeå, Sweden.*

Production responses to changes in nutrient supply in dairy cows can be predicted with a reasonably accuracy from a large data set of milk production studies using a mixed model regression analysis. In this study, we extended this approach to a level of using individual AA supply for predicting milk protein yield (MPY). Our hypothesis was that the predictions of MPY could be improved by including the supply of individual AA supply compared with the basal model based on ME and Feed-MP supply. Treatment mean data were collected from feeding trials with cows fed silage-based diets supplemented with concentrates. The total data set included 1102 diets in 246 studies. The ME and MP supplies were calculated according to the Finnish system. For calculating the individual AA supply from RUP, the AA profiles were adopted from tabulated values. Microbial AA profile was derived from studies in dairy cows fed silage-based diets. PROC MIXED of SAS was used to develop models predicting MPY. Linear and quadratic effects of the supply of individual AA (g/kg MP or g/kg EAA) were included in the basal model with ME intake and supply of Feed-MP as independent variables. Variation in estimated supply of AA was small (mean CV 2.3% for EAA/MP) .The basal model predicted MPY well with adjusted root mean squared error 16.8 g/d. Individual AA (g/kg MP) only marginally improved predictions. The effect was significant for Arg ($P < 0.01$), His ($P = 0.04$) and Leu ($P < 0.001$), but quantitatively the effects were small. Quadratic effects were significant for Leu and Met with maximum MPY at 79 and 25 g/kg MP, respectively. The effects of Ile, Thr and Val were negative ($P < 0.01$) when included as a second AA with Leu. When AA were expressed as g/kg EAA Arg ($P < 0.01$) and Leu had positive ($P < 0.001$) effects on MPY, whereas the effects of Ile, Thr and Val were negative ($P < 0.001$). Quadratic effects were significant for Leu and Met with maximum MPY at 174 and 53 g/kg EAA, respectively. The models imply that potential for increasing MPY of cows fed silage-based diets is small reflecting balanced AA profile from microbial protein and RUP.

Key Words: milk protein yield, amino acid supply, modeling

123 Effects of oscillating dietary crude protein concentration on production in lactating cows.

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Oscillating the dietary crude protein (CP) from adequate to deficient levels compared with continuously feeding adequate CP has increased N use efficiency in sheep and beef cattle. We hypothesized oscillating dietary CP to dairy cows could maintain milk protein production while feeding a lower average dietary CP content. Our objective was to determine whether oscillating CP content of a diet marginally deficient in metabolizable protein compared with feeding a constant CP concentration alters milk production and N utilization. Thirty Holstein cows (119 ± 21 DIM) were used in a randomized block experiment with a 10-d covariate period and a 50-d treatment period. Diets were adequate CP fed continuously (CP+; 16.2% CP of DM); marginally deficient CP fed continuously (CP−; 14.1% CP); or 24-h oscillations from adequate (16.2% CP) to deficient CP (11.9% CP) to be on average equal to CP− (OSC). Data were analyzed with a mixed model with fixed effects of diet, week, the interaction and random effects of block and block by diet. Compared with CP−, dry matter intake tended to be lower for OSC (23.2 vs. 22.2 kg/d; $P < 0.11$) but similar to CP+ (22.9 kg/d; $P < 0.59$). Milk yield was greater for CP+ compared with CP− (36.6 vs 35.1 kg/d; $P < 0.02$) and similar between CP− and OSC (35.3 kg/d; $P < 0.78$). Milk protein yield was similar among treatments (avg. 1.07 kg/d; $P \geq 0.14$). Milk urea-N (MUN) was higher for CP+ (12.8 mg/dL; $P < 0.01$) and tended to be higher for OSC (10.9 mg/dL; $P < 0.10$) compared with CP− (10.2 mg/dL). Higher average MUN for OSC could be from enhanced urea recycling. Cyclical patterns in milk and protein yields and MUN occurred with OSC. On days cows received adequate CP, milk and milk protein yields and MUN were lower ($P \leq 0.04$) compared with days cows received deficient CP (milk: 34.6 vs. 36.1 kg/d; protein: 1.00 vs. 1.05 kg/d; MUN: 9.3 vs 12.5 mg/dL), which indicates production lagged 1 d in response to changes in dietary CP. Overall, the results suggest oscillating CP may improve N utilization through enhanced urea recycling, but additional nutrients may be needed to compensate for reduced intake and to enhance production.

Key Words: oscillating protein, nitrogen utilization

124 Histidine dose-response effects on lactational performance and plasma amino acid concentrations in lactating dairy cows.

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The objective of this experiment was to determine the effect of increasing histidine (His) doses on milk production, milk composition and plasma amino acid (AA) concentrations in lactating dairy cows fed a diet that meets or exceeds their energy and metabolizable protein (MP) requirements. Sixteen Holstein cows [72 ± 15 d in milk, 46.7 ± 7.8 kg milk yield (MY)] were used in a replicated 4 × 4 Latin square design experiment with 4, 28-d periods. The targeted treatments were: (1) total mixed ration (TMR) with 1.8% digestible (d)His of MP, (2) TMR with 2.2% dHis (His2.2), (3) His2.2 supplemented with rumen-protected (RP)His to supply 2.6% dHis, and (4) His2.2 supplemented with RPHis to supply 3.0% dHis of MP. The actual dHis intakes were 1.83, 2.27, 2.95, and 3.2% dHis of MP, respectively. The diets supplied similar MP (2,695 ± 422 g/cow/d) and other nutrients, except dHis. Dry matter intake (DMI) and MY were recorded daily, and milk and blood sampling was performed on the last wk of each period. All data were analyzed using PROC MIXED of SAS with treatment, period and period × treatment in the model. Square and cow within square were random effects and DMI and MY were analyzed as repeated measures with ar(1) covariance structure. Linear and quadratic effects of His inclusion rate were tested. DMI was not affected by His dose ($P = 0.52$; averaging 23.4 kg/d; SEM = 1.42). There was a quadratic tendency for increased MY ($P = 0.08$; 41.5, 42.7, 43.3, and 42.3 kg/d, respectively). Lactose concentration ($P < 0.01$; quadratic effect) and yield ($P < 0.001$; linear effect) were increased by His. Milk true protein concentration was lower ($P = 0.02$) for His2.2 compared with all other treatments. True protein yield and milk fat concentration and yield were not affected ($P \geq 0.10$) by treatment. Plasma concentration of His increased quadratically ($P < 0.001$) by His supplementation, as did Lys ($P < 0.01$) and the branched-chain AA ($P < 0.02$). In the conditions of this experiment, lactational performance of dairy cows fed a MP-adequate diet was optimized at dHis supply of 80 g/d, or 2.95% of MP.

Key Words: histidine, milk production, plasma amino acid

125 Effects of soybean meals versus canola meal on the lactational performance, enteric gas emission, and plasma amino acid profile in dairy cows.

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This study investigated the effects of feeding solvent-extracted soybean meal (SSBM), canola meal (CM), or extruded soybean meal (ESBM) on an equivalent crude protein (CP) basis on lactational performance, enteric gas emission and plasma AA profile in dairy cows. Fifteen Holstein cows [95 (SD = 20) days in milk] were used in a replicated 3 × 3 Latin square design experiment with 3, 28-d periods. The diets contained (DM basis): 13.6% SSBM (52% CP, 67.8% ruminal degradability, RDP), 14.2% ESBM (49% CP, 43.7% RDP), or 17.1% CM (40.7% CP, 41.5% RDP). The diets met or exceeded the nutrient requirements of the cows for net energy of lactation and metabolizable protein. Veg-

etable oil (canola oil for CM or soybean oil for SSBM) was added to equalize the ether extract of the diets. Rumen-protected Met was used to ensure a digestible Met supply of 2.2% of MP in all diets. Data were analyzed using the MIXED procedure of SAS as repeated measures with the ar(1) covariance structure and square and cow within square as random effects. CM increased ($P < 0.001$) dry matter intake (DMI; 26.9 kg/d; SEM = 0.82) compared with ESBM and SSBM (25.3 and 24.5 kg/d, respectively). CM and ESBM increased ($P = 0.002$) milk yield (43.8 and 42.6 kg/d; SEM = 1.82) compared with SSBM (41.1 kg/d). Treatment did not affect milk composition or feed efficiency ($P > 0.05$). Milk urea N concentration was lowest ($P < 0.001$) in CM, followed by SSBM and was highest for ESBM. Compared with CM and SSBM, ESBM increased ($P \leq 0.003$) plasma concentrations of Ile, Leu, Val, and the sum of EAA and decreased ($P < 0.001$) Met and Cys. Animals fed CM produced less ($P = 0.008$) enteric methane per kg of DMI (15.0 g/kg) than both ESBM (16.9 g/kg) and SSBM (17.0 g/kg), but methane emission intensity was similar ($P = 0.14$) among treatments (average 9.9 g/kg energy-corrected milk; SEM = 0.60). In summary, data suggest that substitution of soybean meals with CM, on an equal CP basis, may enhance DMI. The substitution of SSBM with ESBM or CM may enhance lactational performance in dairy cows.

Key Words: soybean meal, canola meal, dairy cattle

Ruminant Nutrition Symposium: Mycotoxins—Recognizing Their Presence and Dealing with Them in Ruminant Nutrition

126 Ruminants—Are they as resilient to mycotoxicosis as we think? D. Diaz*, *University of Arizona, Tucson, AZ.*

Mycotoxins are mold produced feed contaminants that although undesirable are mostly unavoidable consequences of feed production. The level of mycotoxins in foods and feed can fluctuate widely and vary significantly from year to year. Although several hundred mycotoxins have been described in the scientific literature, less than 10 have been extensively studied since the discovery of aflatoxin in the early 1960s. Ruminant diets include both forages and concentrate grains, which may increase the risk of mycotoxins in comparison with grain-based monogastric diets. The multiplicity of ingredients in complex diets may increase the probability of multiple mycotoxin contamination but decrease the risk of high mycotoxin concentrations. It has been widely accepted that ruminants, due to microbial degradation, are less susceptible to mycotoxin poisoning than non-ruminants. However, rumen metabolites of the parent mycotoxin may be equally or more toxic. Because of this, differences between parent mycotoxins and their metabolites are much greater in ruminants than non-ruminants. Mycotoxins can increase disease incidence and reduce production efficiency in ruminants. They can cause dermal toxicity, reproductive effects, carcinogenicity, neurotoxicity, teratogenicity, nephrotoxicity, and hepatoxicity. Additionally, mycotoxins may affect immune function and cause lipid peroxidation. In spite of current research advances, applied aspects of mycotoxicology are either limiting or difficult to extrapolate into the real world. This review will attempt to discuss some of the most common problems related to presence of mycotoxin in ruminant diets.

Key Words: mycotoxin, ruminant, toxicology

127 Use of technology to better understand multi-mycotoxin and emerging mycotoxin challenges. A. Weaver*, *Alltech Inc., Nicholasville, KY.*

Mycotoxins can increase disease susceptibility as well as reduce performance, and therefore are a challenge faced by agricultural industries worldwide. Additionally, many scientific works have demonstrated that mycotoxin co-contamination can further increase risk. Despite the important role mycotoxins play in dairy cow productivity, mycotoxin occurrence and concentration are difficult to track without the support of monitoring programs. Thankfully, technology in the agricultural sector is evolving and on the rise. Various methods such as weather and soil mapping have been developed for early prediction of mold presence and may be used to assess the link between environmental conditions, soil quality, crop growth and health, mold spore transfer and mold colonization. Advances in mycotoxin analysis have also occurred. Through the advent of rapid, precise and accurate detection methods the characterization and identification of multiple mycotoxins, their metabolites, conjugated forms and emerging mycotoxins is now possible. Quantification using LC-MS/MS may provide the most accurate picture of mycotoxin risk as it allows for structural diversity, varying extraction efficiencies, interferences with complex feed matrices and potential for multi-mycotoxins. These challenges had been successfully met by developing 37+ analysis, a research backed multi-mycotoxin method applied to animal feed matrices that has been extended, optimized, and validated for 50 mycotoxins in a cost-effective manner. Over the past 6 years, the evaluation of more than 25,000 samples from around the world have demonstrated that more than 96% of samples contain mycotoxins with 89% of samples containing 2 or more mycotoxins (average 5 mycotoxins per sample). These results clearly demonstrate the need for management programs that account for multi-mycotoxin challenges. Overall, mycotoxins can be one challenge holding the dairy cow back from reaching her genetic potential. However, techniques that assess, monitor and minimize mycotoxin risk from the field to the cow are available that can manage mycotoxins and reduce effects on cow performance and profitability.

Key Words: mold, mycotoxin, technology

128 Applying fungicide on corn plants to improve the composition of corn silage for dairy cows diets. F. Cardoso*, *University of Illinois, Urbana, IL.*

An increasing global population, decreasing amount of arable land available for crop production in the United States, and an increased global demand for protein in the human diet encourage crop and livestock producers to seek solutions to improve the efficiency of producing large crop yields. Corn silage is one of the most commonly used forages included in dairy diets in the United States. For producers, feed costs are often the most expensive part of the operating budget. Corn yield losses may increase the cost of feed and limit herd size. The complex interaction of fungi and corn plants in the field threaten yields, decreasing the efficiency of food production and, also, the nutritive quality of feedstuffs for ruminants. By metabolizing sugar compounds within the plant cell, fungal infections on corn plants reduce the nutritional contents available for ruminant diets. Applications of fungicides can aid in protecting corn plants from fungal infection, therefore, limiting yield losses and increasing the nutritive quality of the plant material. The field of knowledge of feeding ruminants corn silage from corn treated with foliar fungicide is still narrow, but findings from previous research highlight the negatives of making and feeding silage from diseased corn plants. This presentation will summarize the knowledge available on fungi and plant relationship, limiting plant infection by applying fungicide, and how corn silage from corn with fungicide application affects dairy cow performance. It is concluded fungicide application on corn used to make corn silage for dairy cows may improve the nutritional composition of the feedstuff, as defined by increases in milk components and feed conversion, reductions in fiber concentrations, and improvements in ruminal digestibility.

Key Words: fungicide, corn silage, mycotoxin

129 *Fusarium* mycotoxins deoxynivalenol and fumonisins affect milk production and liver health in dairy cows. A. Gallo[1], A. Minuti[1], F. Piccioli Cappelli[1], B. Doupovec[2], J. Faas*[2], D. Schatzmayr[2], and E. Trevisi[1], [1]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*BIOMIN Research Center, Tulln, Austria.*

The *Fusarium* mycotoxins deoxynivalenol (DON) and fumonisins (FUM) are common contaminants of dairy cow rations. Despite the rumen's ability to degrade these mycotoxins to some extent, especially high producing cows are sensitive to these mycotoxins. The main site of DON and FUM detoxification besides the rumen is the liver. Therefore, the aim of this study was to investigate the effect of a contamination level of DON and FUM below US and European Union guidance levels on the performance and liver enzyme activities of dairy cows. In a randomized block design, 12 Holstein cows in mid-lactation (114 ± 16 d in milk) were fed either a negative control TMR (CTR) or a TMR with a low contamination level of *Fusarium* mycotoxins (0.4 mg/kg DON, 1.1 mg/kg FUM) (MTX). Each of the 3 experimental periods consisted of a 3-week challenge period followed by a 2-week clearance period. Milk production was recorded daily and blood samples were collected from the jugular vein at the beginning (d 0), on d 14 and d 21 of each experimental period. Data were analyzed as repeated measurements in a completely randomized design using the MIXED procedure of SAS (SAS 9.4 TS, 2018). The mycotoxin treatment had a significant effect ($P < 0.05$) on the milk production. CTR animals produced on average 37.94 kg/d whereas MTX animals produced on average 36.37 kg/d (−1.57 kg/d). Liver enzyme activities in blood increased in MTX-treated cows after 3 weeks. Aspartate amino transferase (AST) activity was significantly higher ($P < 0.05$) in MTX treated animals (117.1 U/L) compared with CTR (106.6 U/L). While bilirubin was significantly lower in the MTX-group (1.39 µmol/L compared with 1.61 µmol/L in CTR), both alkaline phosphatase (ALP) and albumin were not affected by the MTX treatment. γ-Glutamyl transferase (GGT) activity tended to be higher ($P = 0.059$) in MTX-treated animals (30.7 U/L) compared with CTR animals (29.1 U/L). Considering that AST and GGT are commonly used as an indicator for hepatic lesions and function it can be concluded that dietary DON and FUM levels that comply with legal limits showed a negative effect on the milk yield and liver enzyme activities in blood of dairy cows.

Key Words: mycotoxin, liver enzyme, milk yield

130 Silicoglycidol clay for the reduction of aflatoxin M_1 in urine and its effects on inflammatory biomarkers in dairy cows.

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Aflatoxins produced by *Aspergillus flavus* and *A. parasiticus* can be biotransformed into AFM_1, a carcinogenic metabolite excreted via urine and milk. Thus, study objectives were to determine the effects of dietary adsorbent, silicoglycidol clay (ATX), on urine AFM_1 concentration, total AF fluid excretion (urine + milk), and inflammatory biomarkers in dairy cows. Twelve primiparous lactating Holstein cows (279 ± 88 DIM and 675 ± 19 kg BW) were used in a replicated 3 × 3 Latin square design with 21-d periods: (1) Control (CON) consisting of basal TMR; (2) aflatoxin diet (AF) consisting of 100 µg of AFB_1/kg DM intake with basal TMR; and (3) an AF challenge similar to AF but supplemented with 0.10% clay in the diet (AF+ATX), top dressed on the basal TMR. For each period, urine and blood samples were collected on d 21. Statistical analysis was performed using the MIXED procedures of SAS (SAS Institute Inc., Cary, NC) with square, period within square, and treatment as fixed effects and cow within square as a random effect. Immunoglobulin G (158.0 ± 7.1 mg/L; $P = 0.75$) and haptoglobin (1.1 ± 0.4 µg/mL; $P = 0.57$) were similar across treatments. Concentration of AFM_1 in urine was 9.9 ± 1.2 µg/L for the AF diet whereas the inclusion of ATX resulted in 41% less AFM_1 in urine ($P \leq 0.04$). Urine output was similar across treatments but, due to differences in effective AFM_1 exposure, urinary excretion was 2.33 fold greater in the AF challenged cows compared with those consuming ATX. Comparing AF to AF+ATX, excretion of AFM_1 via milk and urine was 28% and 57% lower, respectively. Total fluid transfer of AFM_1 was decreased from 26 ± 5.6% in the AF group to 11.7 ± 6.0% for the AF+ATX group. Cows that consumed AF had numerically 25.2% lower concentration of circulating haptoglobin compared with the AF+ATX diet, possibly indicating immunosuppression whereas the clay supplement seemed to have a restorative effect. These results suggest that AF may be immunosuppressive in the liver and it is primarily excreted via urine. Silicoglycidol clay effectively reduced AF transfer and excretion via urine and milk.

Key Words: adsorbent, animal health, hepatotoxic

131 Reduction of aflatoxin M_1 in milk of Holstein cows administered an aluminosilicate clay.

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Thirty-five Holstein cows were utilized in a randomized complete block design to evaluate the efficacy of an aluminosilicate clay (adsorbent, FloMatrix, PMI Nutritional Additives, Arden Hills, MN) at reducing aflatoxin M_1 transfer into the milk. Cows were stratified by parity, stage of lactation, and previous milk production. Cows were randomly assigned to 1 of 5 dietary treatments, and treatment was administered for 13 d (n = 7): (1) control (CON), basal TMR with no AF or clay; (2) clay control (4C), basal TMR plus 4 oz clay; (3) AF control (AFC), basal TMR plus 113 ppb AF; (4) AF diet with smaller clay dose (4C+AF), basal TMR plus 4 oz clay and 113 ppb AF; (5) AF diet with greater clay dose (8C+AF) basal TMR plus 8 oz clay and 113 ppb AF. Data were analyzed using the GLM procedure of SAS. Treatment and day were considered independent variables, and milk yield, DM and nutrient intakes, aflatoxin M_1 variables, milk composition, body weight and condition, locomotion, and respiratory rate were dependent variables. A covariate adjustment was used to analyze milk yield and composition from previous milk yield records and DHIA records taken 3 d before the start of the treatment period. Means were separated using Fisher's Least Significant Difference, and significance was declared when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$. Data were presented as mean ± the largest standard error. Milk yield averaged 29.63, 31.85, 29.95, 29.93, and 31.30 ± 1.762 kg/d for CON, 4C, AFC, 4C+AF, and 8C+AF diets, respectively, and was greatest in cows consuming 4C and 8C+AF diets ($P = 0.013$). Respiratory rate and BCS were similar across treatments, and 8C+AF cows tended to have a greater locomotion score compared with other treatments (1.71 v. 1.23, $P = 0.057$). Aflatoxin M_1 concentration, secretion, and transfer were reduced by the addition of clay ($P < 0.001$), and a dose response was observed. Aflatoxin M_1 concentration averaged < 0.01, 0.00, 1.64, 1.26, and 0.90 ± 0.383 ppb for CON, 4C, AFC, 4C+AF, and 8C+AF cows, respectively. A reduction of transfer of 21.88 and 40.63% was observed for 4C+AF and 8C+AF diets, respectively.

Key Words: clay adsorbent, aflatoxin, milk production

Small Ruminant Platform Session: Omics Application in Small Ruminants—Current Situation, Limitations, and Opportunities for the Future

132 The use of different omic tools: Applications and benefits to production and health. J. S. Osorio*, *South Dakota State University, Brookings, SD.*

The exponential advances in cutting-edge technologies and informatics tools utilized in biomedical science to generate and process high-throughput biological data sets (omics data) have permeated into the realm of animal science, and more specifically dairy cattle nutrition and physiology. Omic tools including genomics, epigenomics, transcriptomics, proteomics, and metabolomics produce large amounts of data that need appropriate bioinformatic analyses to derive meaningful conclusions. Omic technologies will help in the discovery of biological markers due to various physiological, management, and environmental factors such as lactation, nutrition, and heat stress. The merging of omics and bioinformatics represent the foundation of systems biology, a field of study broadly use in modeling organisms to enhance the understanding of the complex biological interactions occurring within cells and tissues at the gene, protein, and metabolite level. These complex interactions can be orchestrated by major factors such as nutrition and physiology during critical life stages including late pregnancy, in utero development, or postnatal growth. Therefore, this review will give examples of the applications and benefits of the use of different omic tools in aspects related to the health and performance of dairy animals. For instance, transcriptomics in small ruminants revealed the negative impact of undernutrition on genes regulating milk synthesis and mammary cell proliferation while activating apoptosis and involution. Changes in the bovine mammary gland transcriptome during the transition from late pregnancy to lactation has revealed the contrasting upregulation of genes associated with milk synthesis while downregulating those related to cell proliferation. Studies on miRNAs from goat and sheep mammary gland have shed light on the potential regulatory mechanisms these non-coding RNAs have during the onset of lactation. Omics research is limited in ruminants, especially in small ruminants. However, the increasing adoption of these technologies will provide new avenues for integrative research across and within tissues with the goal to develop new nutritional management approaches to achieve better animal health and production.

Key Words: omics, bioinformatics, ruminant

133 Maternal nicotinamide supplementation during the perinatal period affects milk metabolomics and modifies the development and transcriptomics of small intestine of their kids. X. S. Wei*, Q. Y. Yin, J. J. He, H. H. Zhao, and J. H. Yao, *Northwest Agriculture and Forest University, Yangling, Shaanxi, China.*

Nicotinamide (NAM) was evidenced to improve energy metabolism and attenuate oxidative stress during perinatal period. Maternal nutrition programs both short- and long-term development of offspring. This study was aimed to investigate the effect of maternal NAM on milk metabolomics and small intestinal development of kids. Fifteen multiparous does were paired and allocated into 3 treatments: control (C), postpartum (P, supplemented post-kidding), and entire-perinatal (EP, supplemented from d −21 to 28 around kidding). The NAM (5 g/d) was drenched to does at 0700 h. After kidding, the kids were named L_C, L_P, and L_{EP}, respectively, and fed milk from their mothers until d 28 of life. Individual milk was sampled at 2 consecutive milkings once a week, and blood of kids was sampled on d 14 and 28. At d 28, does and kids were slaughtered to collect tissue samples. The data were analyzed using PROC MIXED of SAS. For kids, the villus height in the duodenum was 24.6% higher in L_P than in L_C, and that in the ileum was 39.7% higher in L_{EP} than in L_C ($P < 0.05$). The ratio of villus height to crypt depth of intestine was all increased in L_{EP} ($P < 0.05$). The serum IgG tended to increase by maternal NAM ($P = 0.078$), and the total antioxidant capacity was 56.5% and 62.8% decreased in both L_P and L_{EP} ($P = 0.002$). Transcriptome profile of jejunal mucosa showed that glucose and amino acid transporters were improved. The does supplemented with NAM had an improved antioxidative status ($P < 0.001$), and lower level of milk IL-1β was found in EP ($P = 0.033$). The MDA of mammary tissue was decreased by NAM ($P < 0.05$). Moreover, the milk metabolomics profile showed that glycerol was decreased while citramalic acid and sucrose were elevated with NAM supplementation. Metabolism of glycerolipid, galactose, and starch and sucrose were enriched. In conclusion, maternal NAM improved the small intestinal development of kids partially by affecting the milk antioxidative status and metabolites, further suggesting the importance of nutritional strategy from prepartum period and milk feeding in early life for kids.

Key Words: maternal nicotinamide, intestinal development, mother-kid relationship

134 Rumen microbiome and fermentation interaction in dairy ewes under different supplementation using 16S rRNA Illumina amplicon sequencing. F. E. Miccoli*[1,2], D. Colombatto[2,3], and R. A. Palladino[1,3], [1]*School of Agriculture Science, National University of Lomas de Zamora, Buenos Aires, Argentina,* [2]*Department of Animal Production, University of Buenos Aires, Buenos Aires, Argentina,* [3]*Consejo Nacional de Investigaciones Cientificas, (CONICET), Buenos Aires, Argentina.*

Phylogenetic analysis of the 16S rRNA gene by next generation sequencing is widely used to study the microbiome, and the role of nutrition on rumen fermentation and microbiota communities. This study aimed to analyze the interaction between rumen microbiome and fermentation of dairy ewes supplemented with corn grain (CG) or soybean hulls (SH). Eighteen samples of rumen liquor (solid and liquid) were taken from 6 fistulated dairy ewes (30 DIM; 78.2 ± 13.53 kg) in a crossover design, fed either CG or SH, in a 50:50 forage to concentrate ratio (fresh annual ryegrass, *L. multiflorum*). Genomic DNA was extracted and quantified on the Nanodrop 1000. DNA quality was assessed by electrophoresis on 2% agarose gel. The V4 region of the 16S rRNA gene in bacteria and archaea from each sample (5 ng/ µL) was PCR amplified with Illumina adapters to generate 18 amplicon libraries. Illumina Nextera XT indexes were added by a second PCR. The gel-purified library pool was measured on the Nanodrop 1000, and then quantified by qPCR (KAPA SYBR FAST Universal qPCR kit with Illumina Primer Premix). Six pM of pooled library was combined with 12.5 pM denetaured PhiX V3 control library (6:4 vol/vol) and run on a MiSeq 500 cycle version 2 reagent cartridge on an Illumina MiSeq for 500 sequencing cycles. OTU identification was assessed by QIIME and multivariate statistical analysis by Infostat (2009). There was a negative relation between rumen pH and 3 of the main genus increased in CG ($P < 0.05$), *Butyrivibrio* ($P < 0.10$; $R^2 = -0.18$), *Ruminococcus* ($P < 0.05$; $R^2 = -0.32$) and *Shuttleworthia* ($P < 0.05$; $R^2 = -0.33$). Additionally, acetate/propionate ratio was also negative related to these genus (*Butyrivibrio*, $R^2 = -0.44$; *Ruminococcus*, $R^2 = -0.21$; *Shuttleworthia*, $R^2 = -0.45$). *Firmicutes*:*Bacteroidetes* ratio (4.8CG: 1.67SH; $P < 0.05$) was negative associated with rumen pH ($P < 0.01$, $R^2 = -0.43$) and milk fat (5.98% CG vs. 7.78% SH; $P < 0.05$, $R^2 = -0.39$). Investigating microbiome-fermentation interactions may provide new insights to understand host efficiency.

Key Words: rumen microbiome, fermentation, dairy ewe

135 Identification of mastitis pathogens using MALDI-ToF and its association with somatic cell count in dairy goats.

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Mastitis is defined as an inflammation of the mammary gland, which widely affects ruminants, such as cows, sheep and goats. Worldly recognized as an indicator of milk quality and udder health, assessment of somatic cell count (SCC) still the gold standard for recognition of subclinical mastitis cases in dairy cows farms. The objective of this work was to describe the relationship between pathogens identified from goat milk by matrix-assisted laser desorption/ ionization-time of flight (MALDI-ToF) and SCC values. Total of 98 aseptic milk samples were collected (15 mL) during morning milking from 47 Toggenburg and 4 Alpine goats at a commercial dairy goat farm in South Dakota. Goats were between 2 and 8 years-old, at the end of lactation stage. To perform the microbiological test, 10 µL of milk were spread onto 5% sheep blood agar and incubated for 24/48 h. The isolates were submitted to MALDI-TOF for species identification and SCC was determined through a service provider (Eurofins DQCI, Mounds View – MN). A total of 74% (n = 73; SCC ranging from 42 to $13{,}186 \times 10^3$ cells/mL) of all milk samples were bacteriologically negative. Among the positive milk samples, the distribution of bacteria identified and range of SCC were as follows: 42% *Staphylococcus simulans* (n = 11; 958 to $8{,}164 \times 10^3$ cells/mL), 15% *Staphylococcus aureus* (n = 4; 1,828 to $25{,}392 \times 10^3$ cells/mL), 11.5% *Staphylococcus chromogenes* (n = 3; 507 to $1{,}825 \times 10^3$ cells/mL), 11.5% *Bacillus* spp. (n = 3; 501 to $1{,}196 \times 10^3$ cells/mL), 8% *Staphylococcus epidermidis* (n = 2; 1,552 to $3{,}163 \times 10^3$ cells/mL), 8% *Staphylococcus caprae* (n = 1; $1{,}828 \times 10^3$ cells/mL) and 4% *Staphylococcus warneri* (n = 1; 375×10^3 cells/mL). Chi-squared test was done and a significant difference was observed when SCC values from positive and negative samples were compared ($P < 0.001$). However, values of SCC show high variability within and between pathogens. Since non-infectious factors also impact SCC in dairy goats the interpretation of this test requires attention, thus caution should be used when using solely SCC as a diagnostic tool.

Key Words: goat mastitis pathogens, MALDI-ToF, somatic cell count

136 ^{1}H-NMR based-metabolomics of milk produced from heat-stressed goats with induced mammary inflammation.

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The aim of the present study was to evaluate the effect of both heat stress and simulated intramammary infection on milk metabolomics in dairy goats. Eight multiparous Murciano-Granadina dairy goats (2.2 ± 0.1 L/d; 100 ± 5 DIM, 42 ± 2 kg BW) were maintained under 2 environmental conditions varying in temperature, relative humidity (RH) and temperature-humidity index (THI): 1) 4 goats under thermoneutral (TN; 15 to 20°C, RH = 50 ± 5%, THI = 59 to 65), and 2) 4 goats under heat stress conditions (HS; 35°C from 0900 to 2100 and 28°C from 2100 to 0900, RH = 45 ± 5%, THI = 75 to 83). Adaptation of 11 d to the experimental treatments was allowed. On d 12, each animal had one udder half infused with 10 mg *E. Coli* lipopolysaccharide (LPS) and the other udder half as the control with 0.9% saline (CON). This resulted in 4 treatment combinations: TN-CON, TN-LPS, HS-CON, and HS-LPS. Milk samples (0, 4, 6, 12, and 24 h) were collected and analyzed by ^{1}H NMR spectroscopy operating at 600 MHz. Data were processed by the R program (pls package), in which the principal component analysis and partial least square–discriminant analysis were used to identify possible metabolite markers in milk. Citrate increased ($\log_2$ fold change = 1.79; $P = 0.010$) in milk of HS goats. The increment in milk citrate might explain the known deteriorated coagulation properties during the cheese-making from the milk of heat-stressed animals. On the other hand, the LPS challenge resulted in an increment in milk acetone, β-hydroxybutyrate, alanine, choline, lactate, and isobutyrate. In conclusion, high ambient temperatures and udder inflammation dramatically affected the metabolomic profile of milk. Milk citrate and ketone bodies were detected as potential markers for heat stress and udder inflammation, respectively. Project AGL-2013-44061-R (Plan Nacional, MINECO, Spain).

Key Words: metabolomics, heat stress, intramammary infection

137 Current situation and new insights of omics applied to small ruminants.

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Omics technologies (mainly, proteomics, transcriptomics and metabolomics) have been highly improved over the last years. The development of these tools has allowed important advances in the field of livestock science. In small ruminants (goats and sheep), omics approaches are a unique tool to increase the understanding of the different physiological and biological processes. Advanced and more sensitive proteomics techniques have provided crucial information about proteins present in colostrum from goats and sheep, especially those in low abundance. Differences in the colostrum proteome of both species suggest that the use of colostrum replacer or colostrum from other species may not ensure a correct passive immune transfer to the offspring. Furthermore,

proteomics revealed that not only immunoglobulins are increased in blood of newborn lambs after colostrum feeding, but also several other immune proteins. Omics studies on small ruminants' mammary gland are still scarce and mainly based on transcriptomic approaches. Mastitis is one of the most important diseases in dairy animals, and in this field, proteomics has been used to describe several inflammatory proteins in both mammary gland and milk. The adaptation of different breeds to harsh conditions (e.g., feed restriction) has been also studied using proteomics, although similar metabolomics-based studies did not show these differences between breeds. Additionally, transcriptomics and nuclear magnetic resonance-based metabolomics of blood, milk and urine in goats increased our understanding of how goats respond to heat and cold stress. Therefore, it seems that omics are powerful techniques to assess the effect of stressor factors. The general insight suggests that omics approaches are a very useful tool that contributes to explore different processes. The constant development of these techniques and the integration of several omics are recommended to clarify and accelerate the acquisition of knowledge. Finally, the versatility of omics approaches would contribute in the near future in the discovery of new biomarkers for fundamental processes, such as, welfare, health, metabolism, among others, in small ruminants.

Key Words: omics, goat, sheep

ADSA-SAD Undergraduate Dairy Foods Oral Competition

138 A2 Milk: Where are we now? E. Brown* and C. Williams, *Louisiana State University, Baton Rouge, LA.*

Milk, often considered nature's most nearly perfect food, is an excellent source of many nutrients. It is an excellent source of protein, containing 8 g per serving. The 2 major proteins in milk are casein and whey, with casein accounting for 80% of milk protein. β-Casein comprises about 30% of protein in cow's milk, with the 2 variants being A1 and A2. Commercially available milk contains mostly A1 proteins. In 2000, a New Zealand scientist discovered that cows produce both A1 and A2 milk proteins, and then he developed the a2 Milk Company. By identifying A2 protein producing cows with DNA tests and selling their milk, the a2 Milk Company built a successful business that expanded to the United States in 2015. Research has shown that a2 Milk is easier to digest, resulting in less stomach discomfort for consumers. While a2 Milk is not a new development, the product continues to gain in popularity as consumers desire a dairy based milk that they can easily digest rather than a plant-based beverage alternative such as soy or almond milk. As consumer interest has grown, a2 Milk has become more available throughout the United States and is available in over 6,000 locations. In fall 2018 the a2 Milk Company began distributing their products to Costco stores across the southeast United States. As the demand for this product continues to grow, the company continues to build retailer partnerships as it promotes brand awareness and national distribution to provide consumers the opportunity to enjoy the nutritional benefits of real milk.

Key Words: a2 milk, milk protein, consumer demand

139 Don't ditch the dairy: Lactose-free milk. H. Torrealba* and J. Laporta, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

After infancy, it is estimated that 65% of the population experiences a reduced ability to digest lactose, one of the sugars found in milk. Some symptoms of lactose intolerance include bloat, abdominal pain, gas, and diarrhea. Due to the uncomfortable nature of these symptoms when consuming milk and milk derived products, consumers have turned to other non-dairy options primarily plant-based alternatives such as almond or soy beverages referred to as "milk" to help relieve these symptoms. Lactose-free milk is a hidden gem in the dairy industry that has not received as much attention or promotion. The science behind it is simple, the lactase enzyme is added to the milk cooling tank to break down the disaccharide lactose into glucose and galactose, 2 sugar molecules. The milk is then tested to ensure it is free of lactose before going through the re-pasteurizing and packing process. Like regular milk, lactose-free milk comes in whole, reduced, low-fat, skim, and even chocolate flavors, allowing consumers to choose from a variety of options as with standard milk. The price of lactose-free milk is within the same range of almond and soy "milk" and mostly varies depending on brand (e.g., Lactaid vs Silk vs generic brands). Each serving of the original version of "Almondmilk" from Silk contains only 1 g of protein, 7 g of sugar, and 2.5 g of total fat and it has 10 added ingredients, including vitamin and mineral blends. In comparison, lactose-free milk contains similar nutritional value from regular conventional milk, with 8 g of protein, 13 g of sugar, and 0 to 8 g of total fat. In addition, lactose-free milk only adds 3 ingredients: vitamin A and D, and the lactase enzyme. Lactose intolerance affects people worldwide. When lactose-intolerant consumers look for the most nutritionally sound product with minimal additives, lactose-free milk is an option that should be highly considered.

Key Words: lactase, nutrition, milk alternatives

140 Cheese processing: Opportunities and considerations for dairy farmers. B. Jones*, A. Hawkins, and J. Costa, *University of Kentucky, Lexington, KY.*

The current fluid milk market creates a difficult financial environment for many dairy producers, driving them to investigate alternatives for their milk with the objective of increasing profit margins. In the United States, cheese consumption per capita has steadily increased, while fluid milk consumption has decreased from 1995. This change in consumer preference provides an opportunity for dairy farmers to invest in milk processing, as cheese manufacturing. In fact, an online survey of farmers that process milk on-farm, found that 64% of respondents have started in the past 10 years, with cheese being the most popular commodity produced (69% of respondents; Smith et al., 2013). The advantages of starting a cheese operation in comparison to other dairy products include creating a less perishable product, maintaining control of the quality, and by-product whey can be used in animal feed or sold. Unlike other on-farm processing options, cheese requires patience and skill. This usually results in developing your own ability through continued education or hiring skilled labor. Cheese is not exempt from considering the cow in the development of a product. The quality and components of milk influence cheese quality. Raw milk with a somatic cell >100,000 has been found to reduce cheese yields and those >400,000 can have an effect on texture and flavor of the cheese (Murphy et al., 2016). The percent of the total herd milk that would be utilized for cheese making can also be a limiting factor. Establishing a mission and goals through a business plan and funding are critical in creating a successful business. Startup costs for a facility can be substantial and strict government regulations on inspections must be followed. A set plan for marketing must also be developed before the cheese operation begins. Direct sale through farmers markets, on farm retail locations or distribution through local restaurants, educational institutions, or agritourism stops may be used. Starting an on-farm processing facility and business can be challenging but also presents a unique opportunity for the producer to have control of their market and product.

Key Words: milk price, production alternatives, family farm

141 Benefits of enzyme-modified cheese production. K. M. MacRae* and D. R. Olver, *Pennsylvania State University, University Park, PA.*

In recent years consumers have shown increased interest in low-fat cheeses and have fueled demand for a wider variety of cheese-flavored products. According to the US Dairy Export Council (USDEC), the best method for producing economical and consistent cheese flavors is through enzyme-modified cheese production. Through this technology, enzymes turn milk into curds and whey to create different textures and tastes of cheeses. Common cheeses modified with enzymes include Cheddar, Mozzarella, Romano, Parmesan, Bleu, Gouda, and others. Lipases are used to break down milk fats and give characteristic flavors to cheeses, while proteases are used to produce hydrolyzed whey protein. Together the use of lipolytic and proteolytic enzymes allow for the formation of a strong flavored cheese product in 1–2 d compared with traditional aging methods that often take years. For example, Wilkinson and Kilcawley (1998) stated that the flavor intensity of enzyme-modified cheese was 5–30 times that of natural cheese. Using young enzyme-modified cheese is less expensive than using mature cheese and provides the same aged cheese flavor. Reduced-fat or low-fat products can also be made with this technology without reducing flavor. Additionally, enzyme-modified cheese products can be dried to extend shelf life and allow for easier transport. According to USDEC, the low moisture of these dried products such as dehydrated cheese and cheese powders allows for easy application to snacks and cereal-based products. Overall, the use of enzyme-modified cheeses allows for a cheaper and faster alternative to traditional methods while providing additional cheese varieties through taste and texture differences.

Key Words: enzyme-modified cheese

142 A2 milk and the potential impact on consumer markets.

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In the last decade, consumer markets have been moving further and further away from traditional dairy milk as the number of available milk substitutes have increased drastically. What began as a niche market for lactose intolerant consumers as well as vegan consumers has snowballed into a market in close competition with typical dairy products. However, in recent years, researchers, geneticists, and farmers have started taking steps toward a niche product of their own to bring consumers back to the dairy market. Traditional dairy is known for providing a significant amount of biologically relevant nutrients including fats, carbohydrates, and proteins. One protein found in milk known as casein can occur in several genetically determined forms. The most common forms of this protein are known as A1 and A2 β-casein with most dairy cows producing a combination of both. The difference between the 2 is relatively small but can cause a dramatic difference in the way the protein is digested. Digestion of A1 β-casein protein yields an amino-acid peptide called β-casomorphin-7 (BCM-7), which has been linked to health issues including but not limited to lactose intolerance-like symptoms, heart disease, and type 1 diabetes. Unlike the former, digestion of A2 β-casein produces much less BCM-7 and thus less associated adverse health effects. The segment of the population that this product targets is those with perceived lactose intolerance, which is actually a negative reaction to the A1 protein. Though it does not answer all questions with regards to declines in fluid milk consumption, the discovery that simple genetic selection for cows homozygous A2A2 could yield a more digestible dairy product may provide a resurgence in dairy product consumption. Overall, an increase in the prevalence of A2 milk has the potential to combat newly found competition for dairy products while potentially also increasing overall consumer preference for traditional dairy.

Key Words: A2 β-casein, consumer market, perceived lactose intolerance

ADSA Graduate Student (PhD) Production Oral Competition

143 Effect of supplementing rumen-protected methionine or methionine analogs in calf starter on growth and efficiency of Holstein calves from 14 to 91 days of age. R. A. Molano*[1], A. Saito[2], N. D. Luchini[3], and M. E. Van Amburgh[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Zen-Raku-Ren, Tokyo, Japan,* [3]*Adisseo North and Central America, Alpharetta, GA.*

During weaning, methionine (Met) supply decreases as liquid feed is reduced and rumen function is developing. During this transition, calf starter should both promote rumen development and provide adequate nutrients postruminally. Rumen-protected forms of Met (RPM), and Met analogs, such as HMTBa or HMBi are used to increase Met supply, stimulate ruminal fermentation, or both. Our hypothesis was Met is a limiting AA and Met analogs might facilitate rumen development during this period. To test this hypothesis, 74 14 d old Holstein calves were individually housed from birth to 91 d of age, in 2 enrollment periods. Milk replacer (28% CP, 15% fat) was offered up to 1.6 kg DM/d. Calves were balanced for sex and randomly assigned to receive a control starter (CTRL, n = 20) or one supplemented with RPM (n = 16), HMTBa (n = 19), or HMBi (n = 19). Starters (23% CP, 2.5 Mcal ME/kg) were offered ad libitum and supplement inclusion was set to provide an additional 0.16% DM of Met equivalents. Weaning occurred from d 49 to 63. Body weight (BW) was measured weekly. Data were analyzed as a randomized block design with repeated measures over time, using enrollment period as block. Pre-planned contrasts were conducted against the CTRL. RPM and HMBi inclusion enhanced starter intake preweaning and HMBi tended to increase intake postweaning (Table 1). However, there were no differences from CTRL in postweaning BW or BW gain. Post-hoc contrasts among Met supplements indicated HMBi improved feed intake and BW gain postweaning ($P < 0.05$) with a tendency for greater final BW ($P = 0.06$).

Table 1 (Abstr. 143). Effects of different forms of methionine or Met analogs[1] in Holstein calves

Item	Control	RPM	HMTBa	HMBi	SEM
Preweaning					
Milk replacer intake, kg/d	1.37	1.35	1.38	1.37	0.02
Starter intake, kg/d	0.09	0.13*	0.09	0.12*	0.03
BW gain, kg/d	0.99	1.00	0.98	1.02	0.05
BW at d 49, kg	84.7	84.2	85.3	86.0	1.56
Postweaning					
Starter intake, kg/d	3.27	3.20	3.05	3.40†	0.02
BW gain, kg/d	1.33	1.26	1.18*	1.42	0.07
Final BW, kg	133.2	130.2	129.5†	136.9	2.63

[1]RPM = rumen-protected methionine; HMTBa = 2-hydroxy-4-(methylthio)-butanoic acid; HMBi = HMBTa isopropyl ester.

Comparisons against control: *$P < 0.05$, †$P < 0.1$.

Key Words: calf, weaning, methionine

144 Effect of pre- and postnatal heat stress on dairy calf thermoregulation and productivity. B. Dado-Senn*[1], L. Vega Acosta[2], M. Torres Rivera[2], S. L. Field[1], M. G. Marrero[1], S. Tao[3], T. F. Fabris[1], G. Ortiz-Colon[2], G. E. Dahl[1], and J. Laporta[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Puerto Rico, Mayagüez,* [3]*Department of Animal and Dairy Science, University of Georgia, Tifton, GA.*

Dairy calves exposed to prenatal heat stress experience compromised growth and immunity compared with those provided with prenatal heat abatement. We hypothesize that heat abatement postnatally will improve calf thermoregulation and growth, particularly if dairy calves were also exposed to heat abatement prenatally. Holstein calves born to heat-stressed (prenatal HT, shade) or cooled (prenatal CL, shade, fans and soakers) dams during late gestation (~46 d, temperature-humidity index; THI ≥ 68) were postnatally exposed to heat stress (postnatal HT, shade) or cooling (postnatal CL, shade and fans at ~4 m/s) for 56 d (THI ≥ 68; HTHT, HTCL, CLHT, and CLCL; n = 12/group). Skin temperature (ST, shaved rump), rectal temperature (RT), and respiration rate (RR) were recorded daily (0700, AM; 1300, AF; and 1900 h, PM) and BW weekly. Feed intake (milk replacer [MR] and grain) was averaged weekly. Calves were allotted up to 10 L of MR and up to 3 g grain via automatic feeder group pens with MR weaning beginning at 42 d. Data were analyzed as a 2 × 2 factorial arrangement by PROC MIXED in SAS. There was an interaction for ST in the AM and PM; CLCL calves had the lowest ST but CLHT calves had the highest (AM: 29.94 *vs* 32.66 ± 0.12°C; PM: 33.16 *vs* 35.26 ± 0.14°C, $P < 0.01$). Rectal temperature was lowered by both pre- and postnatal cooling AM and AF (AM: 38.67 *vs* 38.84 ± 0.02°C; AF: 38.97 *vs* 39.12 ± 0.02°C, for CLCL *vs* HTHT respectively, $P < 0.01$), whereas only postnatal cooling reduced RT PM (39.18 *vs* 39.29 ± 0.02°C, $P < 0.01$). At all times of day, HTCL calves had the lowest RR but HTHT calves exhibited the highest RR (42.64 *vs* 52.51 ± 0.92 bpm, $P < 0.01$). Prenatal CL calves tended to weigh more, particularly at birth (42.38 *vs* 40.51 ± 0.76 kg, $P = 0.09$), and weaning (80.51 *vs* 75.84 ± 1.77 kg, $P = 0.07$) compared with prenatal HT. Milk intake tended to be higher for postnatal CL calves pre-weaning (7.15 *vs* 6.69 ± 0.18 L/d, $P = 0.07$), and grain intake increased in postnatal CL calves during weaning (1.39 *vs* 1.11 ± 0.09 g/d, $P = 0.03$). This study indicates that a combination of pre- and postnatal heat abatement altered calf thermoregulation accompanied by slight improvements in productivity.

Key Words: heat abatement, calf, growth

145 Early-life fecal microbiota transplantation affects systemic and polymorphonuclear leukocyte mRNA biomarkers of inflammation and liver function in neonatal dairy calves. F. Rosa*[1], E. Trevisi[2], and J. S. Osorio[1], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Department of Animal Science, Food and Nutrition, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

The objective of this study was to evaluate the effects of early life fecal microbiota transplantation (FMT) on health and performance of neonatal dairy calves. The adult donor was selected based on health and production records as well as fecal samples testing negative for infectious pathogens. Sixteen healthy newborn Holstein calves (n = 8/trt) were used in a completely randomized design and housed in individual hutches from birth to 7 wk of age. Calves were fed 2.8 L/d of antibiotic-free milk replacer 2 × /d during wk 1 to 5, 1 × /d on wk 6, and weaned at wk 7. Antibiotic-free starter and water were fed ad libitum. Calves were assigned to either a baseline nutritional program (CON) or 1 × /d inoculations with 25 g of fecal donor material (FMT) mixed in the milk replacer from 8 to 12 d of age. Blood samples were collected weekly for immunometabolic profiling and polymorphonuclear leukocytes (PMNL) isolation for gene expression analysis. Data were analyzed using the MIXED procedure of SAS, where treatment, wk, and their interaction were the fixed effects in the model, and calf the random effect. Orthogonal contrasts were used to determine linear and quadratic effects over time. There was a trend ($P = 0.09$) for greater BW (50.8 vs 52.7 ± 0.7 kg) in FMT calves. A TRT × Wk ($P = 0.02$) was observed in haptoglobin, that was reflected in a positive quadratic effect ($P = 0.04$) in FMT calves but not in CON. Similarly, a TRT × Wk ($P = 0.07$) in IL1B resulted in a positive quadratic effect ($P = 0.07$) over time in FMT, and a linear increase ($P = 0.07$) in CON. A trend for a TRT × Wk ($P = 0.06$) was observed in the liver function biomarker paraoxonase, which resulted in greater ($P < 0.01$) paraoxonase in FMT calves than CON at 3 wk of age. The TRT × Wk ($P = 0.09$) in *NFKB1* mRNA expression in PMNL was associated with a greater ($P = 0.03$) expression in FMT calves at wk 1 (or 7 d) before the FMT was performed, but *NFKB1* expression was similar between FMT and CON at wk 2 and 3. These results suggest that early life FMT in neonatal calves have positive effects in their neonatal stage not only on growth performance but also in mediating the inflammatory response and liver function.

Key Words: calf, immunity, microbiota

146 Effects of a high-protein corn product on production responses in mid-lactation dairy cows. W. E. Brown* and B. J. Bradford, *Kansas State University, Manhattan, KS.*

An experiment was conducted to assess the effects of a high-protein corn product (56% crude protein; CP) relative to other sources of protein on the lactation performance of dairy cows. Twenty-four Holstein cows (620 ± 47.7 kg of body weight, 98 ± 34 d in milk, 2.28 ± 0.46 lactations; mean ± SD) were randomly assigned to treatment sequence in a replicated 4 × 4 Latin square design balanced for carryover effects. Cows were individually fed one of 4 diets with a different protein concentrate source during each 28-d period, including: soybean meal (SBM), high-protein corn product (HPCP), soybean meal with rumen-bypass protein (SBMBP), and canola meal with rumen-bypass protein (CANBP). Diets were formulated for equal concentrations of CP (17%) and balanced to meet lysine and methionine requirements. The SBM diet was formulated to provide 5.7% rumen-undegradable protein (RUP), while SBMBP and CANBP diets were formulated for 6.8% RUP to match HPCP. Data were analyzed with mixed models accounting for diet, period, and cow (random), and differences between treatment means were determined using Tukey's HSD. The CANBP diet increased dry matter intake ($P < 0.01$) compared with SBM and HPCP (28.9 vs. 27.6 and 27.3 ± 0.46 kg/d). Treatment affected milk yield ($P < 0.001$), as SBMBP and CANBP increased yield compared with SBM (42.0 and 42.4 vs. 39.9 ± 1.79 kg/d), but HPCP decreased milk yield compared with other treatments (37.4 ± 1.79 kg/d). The SBM diet increased milk protein concentration relative to HPCP (3.08 vs. 3.00 ± 0.04%; $P < 0.01$). Both SBM and SBMBP increased milk urea nitrogen (MUN) compared with CANBP (11.7 and 11.6 vs. 10.8 ± 0.34 mg/dL, $P < 0.01$), but HPCP decreased MUN compared with other treatments (10.0 ± 0.34 mg/dL). The HPCP diet reduced protein, lactose, and solids nonfat yields compared with all other treatments ($P < 0.001$). There was no evidence of treatment effects on body weight ($P = 0.89$) or body condition score ($P = 0.19$) change. In conclusion, the HPCP diet reduced milk and components yields and MUN, possibly due to lower amino acid content as a percentage of crude protein, lower protein digestibility, or both.

Key Words: milk yield, dietary protein, formulation

147 Association of mid-infrared predicted milk and blood constituents with early lactation negative outcomes in Holstein cows. K. Bach*[1], D. Barbano[2], and J. McArt[1], [1]*Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY,* [2]*Department of Food Science, College of Agriculture and Life Sciences, Cornell University, Ithaca, NY.*

Partial least square regression estimates of milk and blood constituents using Fourier transform mid-infrared (FTIR) analysis have shown promise as a tool for monitoring early lactation excessive energy deficit in dairy herds. Our objective was to analyze milk via mid-FTIR to determine the association of early lactation predicted milk β-hydroxybutyrate (BHB) concentrations, predicted blood nonesterified fatty acid (NEFA) concentrations, and predicted milk de novo fatty acid (FA) percentages relative to total FA concentrations with the risk of negative outcome diagnosis (hyperketonemia, displaced abomasum, metritis, culling, and/or death). We enrolled 517 multiparous Holstein cows from 2 dairy farms in New York State. Proportional, composite milk samples were collected twice weekly from 3 to 18 DIM for a total of 4 time points (T1: 3 to 7 DIM; T2: 6 to 11 DIM; T3: 10 to 14 DIM; T4: 13 to 18 DIM) and analyzed using mid-FTIR spectrometry for milk BHB and FA composition and predicted blood NEFA. Blood samples were collected for hyperketonemia determination (blood BHB ≥ 1.2 mmol/L) using a hand-held meter within 1h of milk sample collection, and farm-diagnosed occurrence of negative outcomes during the first 30 DIM was collected from herd management software. The incidence of negative outcomes between 3 and 18 DIM was 20.2%. Explanatory models were developed for each predicted milk constituent of interest at each time point using fixed effect multivariable Poisson regression for negative outcome prediction. For all time points, an increased risk of negative outcome diagnosis was associated with higher predicted milk BHB (Relative Risk $(\mathrm{RR})_{\mathrm{T1}} = 2.0$; $\mathrm{RR}_{\mathrm{T2}} = 3.4$; $\mathrm{RR}_{\mathrm{T3}} = 5.2$; $\mathrm{RR}_{\mathrm{T4}} = 9.1$; all $P \leq 0.002$), higher predicted blood NEFA ($\mathrm{RR}_{\mathrm{T1}} = 2.7$; $\mathrm{RR}_{\mathrm{T2}} = 2.5$; $\mathrm{RR}_{\mathrm{T3}} = 3.8$; $\mathrm{RR}_{\mathrm{T4}} = 10.0$; all $P \leq 0.001$), and lower predicted milk de novo FA relative percentages ($\mathrm{RR}_{\mathrm{T1}} = 2.9$; $\mathrm{RR}_{\mathrm{T2}} = 3.3$; $\mathrm{RR}_{\mathrm{T3}} = 5.8$; $\mathrm{RR}_{\mathrm{T4}} = 7.2$; all $P \leq 0.001$). Our results suggest that mid-FTIR predicted milk BHB, blood NEFA, and milk de novo FA relative percentages are promising indicators of subsequent negative outcome diagnosis in early lactation.

Key Words: milk, β-hydroxybutyrate, Fourier transform mid-infrared (mid-FTIR)

148 Relationship between serum lipid-soluble vitamins during the periparturient period and health measures in the Pacific Northwest dairy herds. C. Y. Tsai*[1], H. H. Hung[1], W. J. Price[2], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Statistical Programs, College of Agricultural and Life Sciences, Moscow, ID.*

During the periparturient period, dairy cows mobilize stored nutrients to support fetal development and milk production. An objective of the present study was to determine the relationship between newborn calves' serum lipid-soluble vitamins and the dams' health status during the periparturient period in a commercial dairy farm in the western United States. Blood sample from jugular vein was obtained from a total of 459 calves within the first 4 d of life. Serum were collected and stored at −80 Celsius for analysis of lipid-soluble vitamins (α-tocopherol, β-carotene, and retinol) via HPLC, running 6 min by 78% acetonitrile, 13% dichloromethane, 8.9% methanol, and 0.1% n-butanol mobile phase. Health data were collected for each animal on sampling day. Dam's health status was recorded to determine how metabolic disorders and diseases might be affecting calves' lipid soluble vitamins. The data were analyzed using the generalized linear mixed model with diseases as fixed effect and calves as random effect; significance was declared at $P = 0.05$. Retinol and β-carotene were normally distributed while α-tocopherol data were log-transformed for statistical analysis. Results showed that calves from dams with pneumonia (1893 vs. 2464 ± 199 ng/mL) and milk fever (1440 vs. 2447 ± 44 ng/mL) during the periparturient period had significantly lower serum retinol compared with that for calves from healthy dams. The calves from mastetic dams however tended to have greater serum retinol concentration that that for calves from healthy dams (2766 vs. 2419 ± 191 ng/mL, $P = 0.08$). Similar result was observed regarding metritis; calves from dams with metritis had greater a-tocopherol than that for calves from healthy dams (3345 vs. 2940 ± 1.1 ng/mL). In summary, the dams' metabolic disorders may affect the calves' lipid soluble vitamins status and may be associated with calves' health issues in the future. Further investigation should focus on dams' health and metabolic indices to determine relationship between their health status during the periparturient period and calves' metabolic state.

Key Words: epidemiology, vitamin, dairy cow

149 Dietary restriction improved feed efficiency of inefficient lactating cows. Y. A. Ben Meir*[1,2], I. Halachmi[3], S. J. Mabjeesh[2], and J. Miron[1], [1]*Hebrew University of Jerusalem, Jerusalem, Israel,* [2]*Agriculture Research Organization, Reashon Lezion, Israel,* [3]*Institute of Agricultural Engineering, Reashon Lezion, Israel.*

The aim of this study was to reduce voluntary dry matter intake (DMI) to increase feeding efficiency of pre-classified low efficient (LE) dairy cows through restricted feeding. We studied the effects of feed restriction on eating behavior, milk and energy corrected milk (ECM) production, in vivo digestibility, energy balance, and measures of feed efficiency [residual feed intake (RFI) and ECM/DMI]. Previously to the present study, the individual efficiency of 220 cows in ARO herd was estimated. Average RFI (actual DMI - predicted DMI according to NRC 2001 equation) of ARO lactating cows was 1.5, LE cows were characterized by RFI > 2.7 and efficient cows by RFI < 0.5 (20% least and most efficient). Based on this characterization, 24 LE and 12 E cows selected for this study. Follow 2 wk adaptation, the 24 LE cows were paired and divided into 2 groups with similar BW, DIM, ECM yield, and RFI (average ± SE, 710 ± 12, 213.1 ± 11.3 d, 38.4 ± 0.90 kg/d, and 3.92 ± 0.27, respectively). In parallel, the 12 E cows were also paired (668 ± 16.5, 129.3 ± 8.83 d, 49.5 ± 1.08 kg/d, and 0.21 ± 0.23). The 2 dietary treatments consisted of ad libitum feeding vs. restricted feeding (13% of ad libitum) of the same 36.5% roughage diet, for 5 wk. Comparisons between restricted and ad libitum LE cows and between restricted and ad libitum E cows analyzed by using Mixed Model with treatment, date and date × treatment as fixed effects and animal as random effect. Restricted LE cows had shorter eating time, lower meal and visit frequency, but similar rate of eating; meal size and meal duration compared with LE cows fed ad libitum. Their DM and neutral detergent fiber digestibility were similar, and their ECM yield decreased by 5.3%, compared with the LE cows fed ad libitum. Feed efficiency (RFI, ECM/DMI and net energy retained / digestible energy intake (NE_T/DEI)) was improved in the LE restricted cows compared with the ad libitum LE cows. Similar increase in feed efficiency (RFI and ECM/DMI) found also in restricted E cows vs. E cows fed ad libitum. Our results show the potential of improving feed efficiency of LE and E cows by moderate restriction. However, TMR restriction reduced ECM yield of E cows by 9.3% while their energy efficiency (NE_T/DEI) remained similar to that of E cows fed ad libitum.

Key Words: eating behavior, energy balance, residual feed intake

150 Development of a robust, net energy-based measure of feed efficiency in dairy cattle. D. J. Seymour*[1,2], A. Cánovas[2], T. C. S. Chud[2], J. P. Cant[1], V. R. Osborne[1], F. S. Schenkel[2], and F. Miglior[2], [1]*Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Residual feed intake (RFI), along with the related residual net energy intake (REI), are some of the most widely used measures of feed efficiency in dairy cattle. As these methods are regression-based, efficiency is expressed relative to the animals used to estimate the regression coefficients. Furthermore, measures of feed efficiency are highly influenced by the mobilization of body reserves to support lactation, leading to the inflation of efficiency estimates particularly in the early stages of lactation when animals are in negative energy balance. The objective of this study was to develop a measure of feed efficiency based on the NRC net energy model that can be estimated irrespective of contemporary group and account for endogenous energy. Daily records of feed intake, milk production, body weight and body condition score were collected on first-lactation Holstein heifers (n = 40) over the first 150 DIM. Smoothing splines were used to generate smooth curves for each input and interpolate any missing records. Based on the smoothed records, daily measures of gross feed efficiency (GFE), return over feed costs (ROFC), RFI, and REI were estimated. All net energy modeling was based on the NRC net energy model. The amount of net energy for lactation (NEL) released or captured from body reserves was calculated using the daily change in bodyweight and body condition score. The available NEL per day was estimated as the amount consumed in the diet plus or minus the amount released or sequestered by body reserves, respectively. Lactation efficiency was then estimated as the proportion of available NEL captured in milk. Lactation efficiency was well correlated ($r > 0.65$; $P < 0.001$) with GFE, ROFC, RFI and REI and did not exhibit inflation in early lactation as seen with other measures. With the increased adoption of precision management technologies, this measure of feed efficiency represents a promising method of determining an individual animal's feed efficiency at any stage of lactation and independent of other animals.

Key Words: dairy cattle, feed efficiency, net energy

Animal Behavior and Well-Being: Focus on Physiological Response

151 Utilizing a multidisciplinary approach to assess livestock welfare. J. Johnson*, *USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.*

Animal agriculture sustainability is being challenged by a rapidly increasing global human population, environmental pressures such as climate change, and a push toward reducing or eliminating antibiotic use. As a result, livestock producers are tasked with producing more animal products with less inputs and technologies while ensuring that appropriate animal welfare standards are maintained. The belief that distress should be limited for livestock to improve welfare is widely accepted by producers, scientists, and the public, and as such, farm animal welfare is an important societal issue. However, the correct way to assess the welfare state of livestock is often debated and using sound science to define what constitutes “good” animal welfare is a key component of developing animal husbandry practices and mitigation strategies to improve animal welfare while maintaining economic sustainability. The study of animal welfare is unique in that it often incorporates multiple scientific disciplines (i.e., stress physiology, behavior, immunology, nutrition) to evaluate the impact of various stressors on animal welfare and understand the interactions between differing biological systems under stressful conditions. Therefore, a multidisciplinary assessment of livestock welfare allows scientists to determine the relationships between animal behavior and physiology that can affect welfare states and ultimately influence animal performance and production efficiency.

Key Words: animal welfare, livestock, multidisciplinary

152 Using drool as an indicator of heat load in dairy cattle. A. M. Drwencke*[1], G. Tresoldi[2], and C. B. Tucker[1], [1]*Center for Animal Welfare, Department of Animal Science, University of California, Davis, Davis, CA,* [2]*College of Agriculture, California State University, Chico, Chico, CA.*

Heat stress is a prominent issue in the dairy industry that results in approximately $800 million in production losses each year. Signs of heat stress include higher respiration rates and body temperature as well as panting, a combination of drooling and breathing through an open mouth. Identification of early signs of heat stress is important for effective abatement, but the thresholds of when onset occurs are not well defined. Drooling could be a way to identify early signs of increasing heat load. Our objective was to identify the respiration rate and body temperature of cattle at 3 time points: 1) when cows initially began drooling in a day (ID), 2) during other drooling events following the first occurrence in a day (OD) and 3) when they were not drooling (ND). Twenty-four Holstein cows averaging (±SD) 37.5 ± 4.5 kg/d of milk were observed over the course of a summer (12 d/cow; 6 cows at a time; total of 48 d of observation). Respiration rate and signs of drooling, breathing with their mouth open and tongue extended past the teeth were taken every 30 min from 1000 to 1900 h. Body temperature was recorded every 3 min 24 h/d using vaginal loggers. To evaluate body temperature alongside drooling, all readings of vaginal temperature within 10 min of a respiration rate and panting data collection event were averaged and assigned to that time point. During the time period of observation, air temperature averaged 33.3 ± 4.0°C or THI 79 ± 3.2. Least squares means within a mixed model were used to obtain average values. Respiration rates in breaths/min were: ID = 73 ± 2; OD = 76 ± 2; ND = 60 ± 2 ($P < 0.001$). Body temperature averaged: ID = 38.8 ± 0.05; OD = 39.0 ± 0.05; ND = 38.7 ± 0.05°C ($P < 0.001$). Taken together, these results indicate that the first occurrence of drool in a day is an early indicator of increased heat load. Drooling occurs at higher respiration rates and body temperature than when no drool is present. Finally, within this data set, any occurrence of drooling could have been utilized by producers as an indicator of increased heat load in cattle.

Key Words: heat stress, drooling, panting

153 Efficacy of pain control for caustic paste disbudding in very young calves. C. N. Reedman*[1], T. F. Duffield[1], T. J. DeVries[2], K. D. Lissemore[1], N. Karrow[2], Z. Li[2], and C. B. Winder[1], [1]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Dairy producers disbudding calves with caustic paste are less likely to provide pain control than those using cautery. Little research has been conducted on pain control for this method and no studies have specifically examined calves under a week of age although producers will commonly apply this product at this time. The objective of this study was to evaluate the efficacy of local anesthesia and nonsteroidal anti-inflammatory drug (NSAID) analgesia in very young dairy calves. 140 heifer calves aged 1–9 d were enrolled into 28 blocks and randomly allocated to 1 of 5 interventions: sham control; positive control (no pain control); lidocaine cornual block; meloxicam; and lidocaine cornual block and meloxicam. Data were analyzed using mixed models with a fixed effect for baseline values and a random effect for trial block. Compared with no local anesthetic, lidocaine reduced serum cortisol at 15, 30, 45, and 60 min post-disbudding (60 min; −138 pg/mL, 95% CI: −200 to −76 pg/mL). Cortisol values were not different between lidocaine treated calves and sham controls at these time points. At 60, 90, 120, and 180 min post-disbudding, calves treated with lidocaine and meloxicam had reduced cortisol compared with lidocaine alone (180 min post disbudding, −61 pg/mL, 95% CI −112 to −10 pg/mL), and values did not differ between lidocaine/meloxicam treated calves and sham controls at these time points. At 3–4 d post-disbudding, treatment with lidocaine and meloxicam tended to reduce haptoglobin (+0.16 mg/mL, 95% CI 0.00 to 0.32), but no differences were found between groups at 3 h and 6–7 d post-disbudding. At 60, 90, and 120 min post-disbudding, lidocaine treated calves had decreased pressure sensitivity (90 min, −2.26 kgf, 95% CI −3.15 to −1.37). No differences were seen in pressure sensitivity between groups at 180 min, 3–4- or 6–7-d post-disbudding. These findings suggest that the combination of local anesthesia with NSAID analgesia are beneficial at reducing pain indicators and inflammation in very young calves disbudded with caustic paste.

Key Words: welfare, anesthesia, analgesia

154 Effects of environmental enrichment on behaviors, growth, and stress in limit fed Holstein heifers. K. M. Kelly*[1], S. H. Ward[1], J. H. C. Costa[2], G. W. Smith[1], and A. J. Geiger[3], [1]*North Carolina State University, Raleigh, NC,* [2]*University of Kentucky, Lexington, KY,* [3]*Zinpro Corporation, Eden Prairie, MN.*

The objective of this study is to determine if the addition of environmental enrichment will prevent the negative behaviors exhibited by limit-fed heifers. Twenty-four Holstein heifers (161.9 ± 33d of age) were randomly assigned to a split-plot design and housed in 2 sets of 3 pens containing a brush (BR), ball (BA) or no enrichment (NO).

Within each pen, 4 Calan gates were used to individually feed heifers with control (CON, n = 2) or limit-fed (LIM, n = 2) diet. LIM heifers were fed 85% of the NRC (2001) DM % recommendation and the CON was fed 100% of NRC (2001) DM% recommendations. Both diets were balanced to provide the same nutritional content. Weekly rumen samples, blood samples, and body measures were taken. Rumination events were recorded every 5 min over 6 h after feeding. Individual heifer behaviors such as feed aggression, feeding, use of enrichment, and social play behavior were recorded for 24 h each week. Lying time was recorded every hour for 6 weeks using an accelerometer. A linear regression in MIXED procedure of SAS was used to analyze the effects of treatment, date, and all 2-way interactions for all models on social play, object interactions, rumination events, and lying time. Pen was included in the random statement. Enrichment or diet did not affect the average daily gain. LIM heifers consumed less (0.68 kg/d DM, $P = 0.003$). CON heifers with a BR or NO had 4 and 2.8 more rumination events than the LIM in those groups (respectively, $P < 0.001$). Providing a BA increased social play by 8 min per day ($P = 0.01$). Heifers, regardless of diet, spent 30 min more using the BR then heifers using the BA ($P = 0.03$). Treatment did not affect feed aggression or visits to the feed bunk. When the pen was enriched with a BA, LIM heifers laid 72 more mins/day compared with CON ($P = 0.01$). BR did not affect lying time. Enrichment use in limit fed heifers shows some improvement in lying times and social play.

Key Words: limit feeding, environmental enrichment

155 Integration of productive, reproductive, and health variables with activity behavior data as welfare indicators in dairy cows.

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This study aimed to investigate the associations between activity behavior data and 2 proposed welfare categories (WC) based on productive performance, cyclicity, and absence of clinical disease. A single cohort of 202 Holstein dairy cows (Parity 1; n = 55; Parity ≥ 2; n = 147) was followed from 10 d pre-calving to 150 DIM. All cows were ear-tagged with a device measuring activity (AT), rumination (RT), and eating (ET) time (min/h). Daily milk yield and health data were retrieved from on-farm software. Ovarian cyclicity was evaluated by transrectal ultrasonography at 35 DIM. Upon completion of the follow up period, study cows were classified into one of 2 WC: (1) WC1 = milk yield ≥ study group mean, absence of clinical disease, and cyclic at d35; and (2) WC2 = cows that failed in at least one of the WC1 conditions. Differences in behavior data between WC were determined by repeated measures analysis, including, parity, DIM and the interaction term WC × DIM in the models. Subsequently, odds ratios were calculated using 5 min increments in behavioral variables to evaluate their ability on predicting WC categorization. Overall, 59 cows were classified in WC1 and 141 in WC2. A significant effect on AT was found for WC × DIM ($P < 0.0001$), but no differences were found in AT between WC. A significant effect on RT was determined for WC × DIM ($P < 0.0001$) and the effect of WC showed a tendency for significance ($P = 0.08$), where RT was greater in WC1 (20.0 ± 0.4 vs. 19.2 ± 0.23 min/h). Overall, ET did not differ between WC (WC1: 14.8 ± 0.5 vs. WC2: 13.7 ± 0.3 min/h; $P = 0.25$). As interaction terms were significant, multiple comparison analysis resulted in significant differences within the first 21 DIM, where WC1 had greater RT and ET. RT and ET were adequate predictors for WC; increases by 5 min in RT and ET increased by 2.2 (1.3 – 4.2; $P < 0.001$) and 1.6 (1.1 – 2.5; $P = 0.02$) times the odds of being classified as WC1. These associations could be used to implement welfare evaluations based on this behavioral parameters.

Key Words: activity, rumination, health

156 The impact of heat stress on individual cows in a Pennsylvania dairy herd.

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The objective of this study was to estimate the impact of heat stress on milk and component yields at the Penn State University research farm. Data included 703,106 daily records from 1156 Holstein cows that calved from 2004 to 2016. Temperature-humidity index (THI) was calculated with daily average temperature (°C) and average relative humidity (%) retrieved from the nearest weather station. Milk, fat, protein, and energy corrected milk (ECM) yields were evaluated with a model that included the fixed effects of lactation, year-month of calving, biweekly days in milk (DIM), age at calving; random effects included cow, date, and random regression on THI within cow. This model assumes that, in the same herd, cows respond differently to the same severity of heat stress, which was termed the heat-resistance coefficient (HRC). For every THI unit above 72, there was a significant ($P < 0.05$) reduction of 0.282 kg, 0.011 kg, 0.009 kg, and 0.301 kg for milk, fat, protein, and ECM yields, respectfully. Production for the least heat resistant quartile of cows and the most heat resistant quartile of cows based on HRC for ECM was compared. The heat tolerant group did not decline in production as THI rose, whereas it declined steadily for the least heat tolerant group. Production in the most extreme THI groups (not heat stressed = THI <72; highly heat stressed = THI >78) are shown in Table 1. Production for intermediate THI levels were in-between the extremes presented in the table. Cow HRC for milk yield was positively correlated with HRC for ECM (0.95), fat (0.15), and protein (0.59) yields. The HRC of fat yield was positively correlated with ECM (0.38) and protein (0.36) yields. In conclusion, there is significant variation among cows within the same herd for reaction to heat stress.

Table 1 (Abstr. 156). Production for the top and bottom quartiles of heat-resistant cows for ECM

	Least heat resistant group (n=132)		Most heat resistant group (n=132)	
	Not heat stressed	Heat-stressed (THI >78)	Not heat stressed	Heat-stressed (THI >78)
Milk, kg	37.08^{a}	31.86^{b}	35.60^{c}	36.52^{a}
Fat, kg	1.40^{ac}	1.34^{bd}	1.42^{a}	1.36^{cd}
Protein, kg	1.15^{a}	1.07^{b}	1.13^{a}	1.10^{b}
ECM, kg	39.17^{ac}	36.02^{b}	38.81^{a}	38.15^{c}

$^{a–d}$Different letters within the same row indicate a significant difference ($P < 0.05$).

Key Words: heat stress, temperature-humidity index, milk yield

157 Hot weather increases competition between dairy cows at the drinker.

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Heat stressed dairy cows on pasture will compete for resources that aid cooling, but it is not known how heat stress affects the competition for water by indoor housed cows. The objective of this study was to evaluate how heat stress affects the behavior of indoor, loose-housed dairy cows at the drinker. For 3 wk after calving, cows were housed in a dynamic group (n = 20) in a pen equipped with 12 electronic feed bins, 2 electronic water bins, and 24 freestalls. A total of 68 lactating, Holstein dairy cows were enrolled over a 59-d period. The electronic water bins recorded time spent at the drinker, frequency of visits, water intake, and competitive events for 24 h/d. Competitive events were quantified using the number of replacements (when one cow displaced another cow from the drinker and took her place). Heat stress was defined as a temperature humidity index (THI) ≥ 72. THI parameters examined were daily mean, minimum, maximum, and number of h ≥68 or 72. To determine if there was a lag effect of heat stress on drinking behavior, we examined the effects of this on the day of behavioral recordings and on the previous 1 to 3 d. For the analysis of time spent at the drinker, frequency of visits, and water intake, the measures from all cows in the pen (n = 20) were averaged to create one observation per day. For the analysis of competitive events, the number of replacements at the drinker was summed from all cows in the pen (n = 20) to create one observation per day. A linear regression analysis was performed to determine the relationship between heat stress and behavior at the drinker. We found that, with increasing numbers of hours in heat stress conditions (THI ≥72) over a 3-d period, cows drank more water, spent more time at the drinker, made more visits to the drinker, and engaged in more competition (P <0.0001 for all). These results indicate that behavior at the drinker may provide a biomarker of heat stress. This measure may be of practical value, especially for farms where attendance at the drinker can be monitored electronically.

Key Words: heat stress, aggressive behavior, water intake

158 Provision of shelter during the prepartum period: Effects on behavior and non-esterified fatty acid concentrations of dairy cows in a pasture-based system. D. Cartes*[1], A. Strappini[2], R. Held[1], and P. Sepúlveda-Varas[3], [1]*Escuela de Graduados, Facultad de Ciencias Veterinarias, Universidad Austral de Chile, Valdivia, Chile,* [2]*Instituto de Ciencia Animal, Universidad Austral de Chile, Valdivia, Chile,* [3]*Instituto de Ciencias Clínicas Veterinarias, Universidad Austral de Chile, Valdivia, Chile.*

In pasture-based systems where seasonal calving predominates, many farmers use open dirt corrals to keep cows before calving. Our objective was to examine whether shelter provision under winter conditions influences behavior and body fat mobilization in outdoor-housed prepartum dairy cows. Two cohorts of 12 clinically healthy multiparous Holstein prepartum cows were housed in open dirt corrals at the Experimental Dairy Farm of the Universidad Austral de Chile (Valdivia, Chile) during the winter months (July and August). Twenty-one days before the expecting calving date, the cows in each cohort were paired. Each pair was randomly assigned to either a corral without shelter, or to a corral with access to an artificial shelter until calving. The use of shelter was measured by scan sampling every 10 min from video data (2 cameras/corral) during the 3 weeks study period. The lying time were measured with data loggers and rumination time was recorded using an automated monitoring system over the study period. A blood sample was taken from the coccygeal vein of each cow for measurement of nonesterified fatty acids (NEFA) the same day once a week. Daily lying and rumination time were summarized by week and comparisons were made between groups with and without access to shelter. Data were analyzed using mixed linear models (SAS v9.4). Cows spent 62% of their daily time in the shelters and 75% of this time they were lying down. Furthermore, the cows that had access to shelter during the prepartum spent more time lying down during the wk 3(706 min/d vs. 559 min/d; $P < 0.001$) and wk 2 (742 min/d vs. 566 min/d; $P < 0.001$) before calving compared with cows without shelter access. They also had lower NEFA concentrations on wk 2 (272 μmol/L vs.554 μmol/L; $P < 0.001$) and wk 1 (460 μmol/L vs. 646 μmol/L; $P = 0.05$) before calving. The daily rumination time was not different between groups. These results demonstrate the importance of granting a protected area for the welfare of prepartum dairy cows exposed to winter climate conditions. Project funded by FONDECYT #11170820.

Key Words: shelter, winter, dairy cow

159 Validation of a multiple accelerometer sensor system to estimate dry matter intake in lactating dairy cows. N. Carpinelli*, F. Rosa, R. C. B. Grazziotin, and J. Osorio, *South Dakota State University, Brookings, SD.*

Dry matter intake is a valuable parameter that can be used to evaluate health and milk efficiency in dairy cows. The objective was to evaluate the use of 3-dimensional accelerometer sensors to estimate DMI in lactating dairy cows. Twenty-four late-lactation Holstein dairy cows housed in a free-stall barn were fitted with 3 sensors that record acceleration in the 3-axis (i.e., x, y, and z), one sensor on the lateral side of the left hind leg and 2 attached to a halter directly superpose over the jaw and nose. Cows were assigned 2 groups, a data collection group (A; n = 12) and a validation group (B; n = 12). Cows were trained to use Calan gates during a 7-d period followed by 10 d of data collection of acceleration and individual intakes for both groups. Four cameras were used to continuously video record cows, then eating times for each cow were generated. Sensors were set to record the accelerations at 10-s intervals. Eating times and accelerometer data from group A was cross-reference based on date and time. Six new variables were derived from jaw and nose accelerations by measuring the change in acceleration between 2 consecutive time points (lag-time). The REG procedure of SAS was used to regress each acceleration against the DMI during 24h period in group A to select the highest R^2 data. In group B, DMI derived from acceleration combinations (DMIA) was compared against the actual DMI using the CORR and MIXED procedures of SAS. All 32,767 acceleration combinations were tested, and only 921 were deemed relevant (>80% data coverage). LagNoseZ+LagNoseX model had the strongest positive correlation ($r = 0.42$), but its DMIA was greater ($P < 0.01$) than the actual DMI (36.2 vs 21.4 kg/d). In contrast, LegY+JawZ+LagNoseY model had similar DMIA and actual DMI ($P = 0.99$) but a weaker correlation ($r = -0.11$). The LegX+JawX+LagNoseZ model had a weak correlation ($r = 0.28$), but an accurate ($P = 0.77$) estimation of actual DMI (21.8 vs 21.4 kg/d). Accelerometer sensors have a great potential to estimate actual DMI in dairy cows, but a superior correlation will be required to improved robustness and reliability in a commercial farm.

Key Words: accelerometer, intake, sensor technology

160 Nutrient intake, feeding patterns of growing bulls fed different concentrate levels and a single fiber source. A. u. R. Muhammad*[1,2], C. Q. Xia[1], B. Cao[1], and H. Su[1], [1]*State Key Laboratory of Animal Nutrition, Beijing, China,* [2]*Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad, Faisalabad, Pakistan.*

The objective of the study was to determine the effect of concentrate levels with corn stover silage (CSS) on the nutrient intake and feeding behaviors of growing Chinese Holstein bulls. Twenty-eight bulls (weighing 227 ± 6.8 kg) were selected and fed randomly 4 concentrate levels (1–1.5 kg (LC), 1.5–2 kg (MC), 2–2.5 kg (HC), and 2.5–3 kg (H^+C)) and CSS ad libitum. The concentrate and CSS consumption were recorded daily. Video recordings were performed with an infrared anti-nozzle camera to record the feeding behavior. Three minutes scan

sampling method was used to measure eating time for concentrate, CSS and chewing time over 24-h time period. Alterations in DMI and nutrient intake between the concentrate levels for 3 mo were analyzed using the MIXED process in SAS, with the month as a repeated measure. For feeding behavior, data were averaged over 5 d (focal animal sampling; 4 calves/treatment). Differences among the concentrate levels according to intake time of concentrate, silage and chewing time for 24 h were analyzed using the MIXED process in SAS, while hour was considered as a repeated measure. After 3 mo, increases in the concentrate level resulted in increased ADF in LC (2.26 kg/d, SE = 0.07, $P < 0.001$) and MC (2.81 kg/d, SE = 0.07, $P < 0.001$) treatments. Similarly, NDF intake were also increased in LC (3.97 kg/d, SE = 0.09, $P < 0.001$) and MC (4.43 kg/d, $P < 0.001$) treatments. Whereas the ADF intake was decreased in HC (2.61 kg/d, $P < 0.001$) and H^+C (2.41 kg/d, $P < 0.001$) treatment. Similar with ADF, NDF intake was decreased in HC (4.35 kg/d, $P < 0.001$) and H^+C (4.04 kg/d, $P < 0.001$) treatment. The longest time required to consume the concentrate was observed in the H^+C (29 min, $P < 0.001$) treatment compare with LC treatment (23 min, $P < 0.001$). The highest silage consumption was observed for the LC treatment at 16:00 (25 min, $P < 0.001$). Chewing was highest at 04:00 (33 min, $P = 0.03$) and 18:00 (36 min, $P = 0.03$) for the H^+C treatment. Overall, increasing the concentrate in the diet altered specific behaviors, which could compromise the calves' welfare.

Key Words: corn stover, bull, behavior

Animal Health 1: Metabolic Health and Disease

161 The dynamics of BCS during the far-off and close-up period impacts postpartum diseases in Holstein cows. P. Melendez*[1], F. Bargo[2], G. Tuñon[3], and J. Grigera[4], [1]*College of Veterinary Medicine, University of Georgia, Tifton, GA,* [2]*University of Buenos Aires, Buenos Aires, Argentina,* [3]*INIA, Uruguay, Montevideo, Uruguay,* [4]*Bovine Practitioner, Buenos Aires, Argentina.*

Body condition score (BCS) is a quick, cheap and reliable tool that helps determine fat reserves. BCS at calving and changes in BCS after calving have been consistently associated with milk yield, diseases, and fertility in dairy cows, yet, the dynamics of BCS during the dry period has been less studied. The objective was to assess the change in BCS within the far-off (dryoff to −21 d prepartum) and close-up (−21 d prepartum to calving) and its association with postpartum diseases. The study analyzed 22,000 lactations from 28 dairies from Argentina. BCS was assessed with a scale of a 1/4 unit from 1 (emaciated) to 5 (obese). BCS was carried out by 2 of the authors creating 4 groups. G1: cows that maintained or gained BCS during far-off and close-up; G2: cows that maintained or gained BCS during far-off and lost BCS during close-up; G3: cows that lost BCS during far-off and maintained or gained BCS during close-up; G4: cows that lost BCS during far-off and close-up. Diseases were compared among groups by conducting logistic regression. Dependent variable was the incidence of the disease (yes, no). Three models were run, comparing G1 vs G2, G1 vs G3, and G1 vs G4, correcting for year, BCS at calving, lactation and farm. Group was not associated with the incidence of milk fever and lameness; however, cows gaining or maintaining BCS across the entire dry period (G1) were less likely to experience RFM, metritis, subclinical ketosis (BHB ≥1.2 mmol/L), clinical mastitis, and culling than cows losing BCS during the close-up period (G1 vs. G2; and G1 vs. G4; Table 1). The impact of losses of BCS during the far-off period was less dramatic (G1 vs. G3) than during the close-up period.

Key Words: BCS, postpartum disease, dry period

162 Incidence of subclinical and clinical ketosis in the California Central Valley: Similarities among commercial herds. M. Wukadinovich* and H. Rossow, *University of California, Davis, Davis, CA.*

Ketosis is a common metabolic disease in postpartum dairy cows due to negative energy balance at the onset of lactation resulting from low dry matter intake and disorders of energy metabolism. Ketosis results in hyperketonemia, hypoglycemia, decreased milk yield, and is often associated with other primary health disorders. To estimate the incidence of subclinical (SCK) and clinical ketosis (CK) in the California Central Valley, blood samples and milk production data were collected from 10 commercial dairy herds in Tulare, Kings, and Kern counties. In February 2018, an average of 17 multiparous Holstein cows from each herd were bled during wk 1 and wk 2 postpartum and whole blood was analyzed for glucose and acetoacetate (AcAc) levels using NovaMax meters (Nova Diabetes Care, Inc., Billerica, MA). Ketosis was defined as SCK at 1.0–1.4 mmol/L and CK > 1.4 mmol/L AcAc in whole blood. Previous lactation milk fat yield and 305-d equivalent milk yield were collected from DairyComp305 (Valley Ag Software, Tulare, CA). All data were analyzed using the Mixed Procedure of SAS with repeated measures by cow or a Generalized Linear Model Procedure (v. 9.4, SAS Institute 2015). Across all herds, average SCK was 12% (repeat cases 5%) and CK was 11% (repeat 61%). All repeat CK cases progressed from SCK in wk 1. Across herds, the lowest incidence of hyperketonemia (>1.0 mmol/L AcAc) was 0% and the highest incidence was 44%. Glucose and AcAc were inversely related ($P < 0.001$; $R^2 = 0.12$). Hyperketonemic cows produced 393 kg more milk ($P = 0.1$) and 44 kg more milk fat ($P = 0.02$) in their previous lactation compared with non-ketotic cows. Five dairies housed fresh cows in free stall corrals and 5 dairies housed fresh cows in free stall corrals with an attached exercise corral or in an open lot style corral. Cows with access to an exercise corral or housed in an open lot had lower blood AcAc levels compared with cows housed in free stalls (0.55 vs. 0.64 mmol/L; $P = 0.02$, $R^2 = 0.17$) and had a decreased recurrence of a ketotic event in the same lactation (5% vs. 26%). Therefore, factors affecting blood AcAc levels on these commercial herds were glucose, previous lactation milk and fat yield, and access to an exercise corral.

163 β-Hydroxybutyrate measurements in bovine milk compared between known standards, a clinical pathology analyzer, and Fossomatic high-throughput testing. D. Wilson*[1] and G. Goodell[2], [1]*Utah State University, Logan, UT,* [2]*The Dairy Authority, Greeley, CO.*

β-Hydroxybutyrate (BHB) is measured in dairy cow milk to detect ketosis, usually associated with negative energy balance. The objective was blinded comparison of spiked milk samples resulting in known concentrations of BHB to BHB measurements using a clinical pathology analyzer and Fossomatic Fourier transform infrared (FTIR) high throughput testing. Milk meter collected samples from Holstein cows with low (<0.02 m*M*) BHB were spiked with DL-BHB reagent to concentrations of 100 μ*M* (0.1 mmol)/L, 200, 300, 400, 500, 600, 800, 1000, 1200, 1500, and 2000 μ*M*/L. Two aliquots of 17 milks at each concentration were tested, blinded to operator and randomized in concentration

Table 1 (Abstr. 161).

Model	Milk fever	RFM	Metritis	Ketosis	Mastitis	Lame	Culling	Disease
G1 vs. G2	0.89	0.76	0.68	0.75	0.78	1.02	0.86	0.82
	0.72–1.12	0.63–0.92	0.54–0.86	0.54–1.06	0.68–0.90	0.83–1.27	0.74–1.00	0.74–0.92
G1 vs. G3	1.22	0.85	1.20	0.85	0.92	0.95	0.84	0.97
	0.93–1.60	0.68–1.06	0.68–1.84	0.60–1.20	0.79–1.09	0.74–1.22	0.70–1.00	0.86–1.11
G1 vs. G4	1.02	0.74	0.91	1.02	0.80	0.89	0.66	0.72
	0.65–1.60	0.53–1.04	0.59–1.41	0.54–1.92	0.62–1.03	0.60–1.30	0.50–0.86	0.58–0.89

order. Calibration was per manufacturer's standards, as was that milk was centrifuged to milk sera for testing with the ChemWell-T 460 analyzer, and whole milk was tested using the Fossomatic. Thresholds of 200 and 300 μM/L for classifying cows as ketotic (K) vs. non-ketotic (N) were tested for test agreement and accuracy. Results were 374 runs on ChemWell and 352 runs on Fossomatic (one outlier run excluded). ChemWell results: mean correlation with standards 0.991 (range 0.970 to 0.999). 200 μM/L threshold: 97.6% agreement (K vs. N, Kappa 0.848 very good) with standards, if spiked milks considered "gold standard," sensitivity (sens) 99.1%, specificity (spec) 82.4%. 300 μM/L threshold: 98.7% agreement (K vs. N, Kappa 0.956, very good), sens 98.4%, spec 100%. Fossomatic results: mean correlation with standards 0.920 (range 0.784 to 0.984). 200 μM/L threshold: 85.8% agreement (K vs. N, Kappa 0.355 fair) with standards, if spiked milks considered "gold standard," sens 94.6%, spec 35.8%. 300 μ*M*/L threshold: 91.5% agreement (K vs. N, Kappa 0.656, good), sens 96.0%, spec 66.7%. The FTIR Fossomatic BHB testing requires recalibration multiple times per day and results are not as accurate as those with the clinical pathology analyzer, but are more accurate than many other milk high throughput diagnostic tests currently used in the dairy industry. Continued improvement in BHB milk calibration standards for the Fossomatic is needed.

Key Words: β-hydroxybutyrate (BHB), milk, laboratory

164 Association between hyperketonemia during the first 10 days postpartum and productive parameters throughout lactation in dairy cows.

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Hyperketonemia (HYK) is frequently observed in high-producing dairy cattle and has been associated with suboptimal health and performance. Traditionally, HYK has been defined by blood β-hydroxybutyrate (BHB) of >1.2 mmol/L, although cows vary in their clinical response to HYK. Thus, our objective was to evaluate the association between HYK occurring in early lactation with milk yield and reproductive performance in Holstein dairy cows throughout an entire lactation. We hypothesized that elevated BHB in the first 10 d postpartum is not necessarily detrimental to all cows. Blood BHB was measured in 1,108 cows from 6 herds in Minnesota between 3 and 10DIM. Cows were followed for their entire lactation, until next calving or culling. Health and performance data were collected from herd management software. In addition to classification based on blood BHB (HYK+ and HYK−), cows were further classified into remaining (REM, cows that reach subsequent calving) or culled (CUL, cows that were sold or died during lactation). Cox proportional hazard was performed to analyze time to pregnancy, adjusting for herd and parity effects. Milk yield was analyzed by ANOVA accounting for repeated measures and adjusting for herd effect; separate models were created for primiparous and multiparous cows. Disease events and DIM at culling were applied to all models and removed using backward stepwise elimination. Overall prevalence of HYK was 11.2% (range 3.2% to 20.3%). Although the hazard ratio (HR) for pregnancy by 300 DIM for HYK+ (REM and CUL combined) cows was 0.64 (95% CI: 0.47–0.86) when compared with HYK− (REM and CUL combined) cows, the REM HYK+ HR was 5.65 (95% CI: 4.65 - 6.87) times higher than CUL HYK− cows. Similarly, no difference was observed when comparing milk production for combined HYK+ and HYK− multiparous cows. However, REM HYK+ cows produced, on average, 5.1kg/day (95% CI: 3.47 - 6.11) more milk than the CUL HYK+ cows. These findings suggest that HYK does not affect all cows in a similar fashion.

Key Words: ketosis, reproductive performance, milk yield

165 High concentrations of fatty acids induce hepatic lipid accumulation by activating endoplasmic reticulum stress in dairy cows with severe fatty liver.

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Disruption of endoplasmic reticulum (ER) homeostasis is intrinsically linked with lipid metabolism disorder in humans and mice. Whether ER homeostasis is affected in cows with fatty liver is unknown. The aim of this study was to investigate ER status and the potential role of ER stress in the progression of fatty liver in dairy cows. Liver and blood samples were collected from cows diagnosed as healthy (n = 15) or with severe fatty liver (n = 15). Hepatocytes were isolated from calves and treated with various concentrations of fatty acids and/or tauroursodeoxycholic acid (TUDCA), a canonical inhibitor of ER stress. Milk production, dry matter intake, and glucose concentration were lower, and concentrations of fatty acids and β-hydroxybutyrate were greater in dairy cows with severe fatty liver. The phosphorylation level of protein kinase RNA-like ER kinase (PERK) and inositol requiring protein-1a (IRE1a), the cleavage of activating transcription factor-6 (ATF6) protein, and the abundance of several unfolded protein response (UPR) genes (78 kDa glucose-regulated protein, AMP-dependent transcription factor 4, and spliced X-box binding protein 1) were greater in liver of cows with severe fatty liver. Alterations in ER homeostasis with severe fatty liver were associated with greater abundance of sterol regulatory element-binding protein 1c (SREBP-1c) and its target genes acetyl-CoA carboxylase-α (*ACACA*), fatty acid synthase (*FASN*), and diacylglycerol acyltransferase 1 (*DGAT1*). The increase in phosphorylation level of PERK and IRE1a proteins, and the cleavage of ATF6 protein in response to increasing doses of fatty acids (which were reversed by TUDCA treatment) in hepatocytes underscored a mechanistic link between fatty acids and ER stress. Importantly, fatty acids treatment upregulated the abundance of *SREBP-1c*, *ACACA*, *FASN*, and *DGAT1* and lipid accumulation in calf hepatocytes, while inhibition of ER stress by TUDCA significantly weakened these effects. Overall, results indicate that an activated ER stress and the ensuing UPR in response to increased influx of fatty acids into hepatocytes are causative of severe fatty liver in dairy cows.

Key Words: fatty liver, endoplasmic reticulum stress, unfolded protein response

166 Herbal formula CHF03 attenuates high-fat diet-induced nonalcoholic fatty liver disease by regulating nuclear factor-κB in mice.

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Nonalcoholic fatty liver disease (NAFLD) is a hepatic ailment with a rapidly increasing incidence due to dietary hyper nutrition and subsequent obesity. Discovering effective natural materials and herbs can provide alternative and complementary medical treatments to current chemical pharmaceuticals. To develop an effective natural agent for NAFLD, we formulated a combination of 10 herb mixtures and observed lipid-lowering efficacy and to investigate the preventive effects of a compound of Chinese Herbal Formula (CHF03) on a high-fat diet (HFD) induced model of NAFLD in mice in vitro and in vivo. The CHF03 groups were fed with HFD and orally administered 10 g/kg CHF03 once a day orally. HE staining was performed to analyze pathologic changes of the liver; a Transmission Electron Microscopy assay was performed to measure the ultrastructural alterations of the mitochondria, and Western blotting was performed to detect the expression of gene proteins related to lipid metabolism and inflammation. To further examine the safety of CHF03, the composition of CHF03 was analyzed by liquid chromatography-mass spectrometry (LC/MS) and acute toxicity and maximum tolerable dose was performed in mice. The results of histomorphological and ultra-structural changes showed that CHF03 could effectively inhibit the occurrence of non-alcoholic fatty liver induced by high fat. CHF03 attenuated high-fat diet-induced oxidative stress, which was confirmed by measuring the level of oxidative stress markers (GSH, GSH-Px, MDA, SOD and CAT). The preventive effects of CHF03 against lipogenesis and inflammatory were mediated by the inhibition of protein acetyl-CoA carboxylase (ACC1), fatty acid synthase (FAS) and nuclear factor κB (NFκB). Moreover, CHF03 dose-dependently inhibited lipid accumulation and gene expressions involved in lipogenesis and related regulators (SREBP-1c, CPT1, FAS, ACC1, ApoA and NFκB) and prevented the PA-induced lipid accumulation in hepatocytes. Additionally, CHF03 inhibited the PA-induced increase in the expression, nuclear localization, and transcriptional activities of NFκB. These results suggest that CHF03 to suppress HFD-induced NAFLD in part through the activation of NF-κB.

Key Words: nonalcoholic fatty liver disease, herbal formula, hepatocytes

167 Effect of hypocalcemia prevention using an anion-supplemented diet on rumination behavior in periparturient dairy cows. J. Goff*, A. Hohman, and L. Timms, *Iowa State University, Ames, IA.*

Accelerometers can be used to gauge rumination activity (RumAct). The purpose of this study was to determine if hypocalcemia affected RumAct in older periparturient cows fed a high or low DCAD diet to induce or prevent periparturient hypocalcemia. Twenty-six Holstein cows entering their 3rd or greater lactation were assigned to an Anion Supplemented (AS) or No Anion precalving diet and fed behind Calan gates. After calving, all cows were fed the same lactation diet. DMI were determined for each cow from 14 d before calving until d 5 of lactation. Blood was sampled daily the wk before calving, at calving, and at 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4 and 10 d after calving. Urine sample pH determined before calving was significantly decreased in cows fed the AS diet (6.99 + 0.16 vs. 8.26 + 0.06). The effects of diet, d around calving and their interaction on plasma Ca, DMI, and RumAct were assessed by the Mixed Procedure of SAS, with d around calving as the repeated measure. Cows fed AS diet had significantly higher blood Ca concentrations from 24 h before calving thru the first 36 h of lactation ($P < 0.05$). Four No Anion cows developed milk fever necessitating IV Ca treatment. On the 1st and 2nd d of lactation, cows fed AS diet consumed significantly more DM (3.85 kg on d 1, $P < 0.01$; 3.39 kg on d 2, $P < 0.025$) than cows fed the No Anion diet. RumAct decreased in all cows at parturition. Diet had no effect on 24 h RumAct until the day before calving. During that 24-h period, cows fed AS spent 457 m ruminating while No Anion cows spent only 356 m ruminating; a 22% decrease ($P = 0.005$). RumAct was greater in cows fed anions during the 1st and 2nd 24 h periods of lactation with anion fed cows ruminating 127 and 115 more min/d during the 1st and 2nd 24 h periods after calving respectively ($P < 0.002$). RumAct data were also compiled into min of RumAct per 2 h period. Cows fed AS had significantly greater RumAct ($P < 0.05$) than No Anion cows during 13 of the 24 2-h periods between 12 h before calving thru the first 36 h after calving. Milk fever cows had extended periods where the rumination rate / 2 h was undetectable, even after treatment restored blood Ca concentrations to normal levels.

Key Words: rumination, anions, hypocalcemia

168 Effects of carprofen in a pain management protocol in dairy cows with abomasal displacement undergoing left flank omentopexy. H. Meyer[1], S. Kaestner[2], and J. Rehage*[1], [1]*Clinic for Cattle, University of Veterinary Medicine Hannover, Hannover, Germany,* [2]*Clinic for Small Animals, University of Veterinary Medicine Hannover, Hannover, Germany.*

Aim of the study was to investigate the efficacy of the nonsteroidal anti-inflammatory drug carprofen for pain management during and after surgical correction of left sided abomasal displacement (LDA) and possible adverse effects on integrity of abomasal mucosa. The blinded study included 24 lactating Holstein cows with LDA corrected by left flank omentopexy. Cows were randomly assigned to one of 2 treatments, carprofen (C; n = 12; 1.4 mg/kg of Rimadyl, Zoetis, IV) or placebo (P; saline; n = 12) one h before and 72 h after surgery. Local paralumbar nerve blocks and infiltration of the incision line (160 mL Procaine 2%) induced anesthesia. Cows were investigated from the day before until 4 d after surgery. Daily feed intake and milk yield were assessed, and behavior monitored by video recordings. Heart and respiratory rate, body temperature, mean arterial blood pressure as well as blood concentrations of glucose, lactate, β-hydroxybutyrate, nonesterified fatty acids and cortisol were determined in regular pre-set intervals. Macroscopic fecal examination, hemoFec testing for occult fecal blood, analysis of pepsinogen in serum and hematological parameters were carried out to determine adverse effects on abomasal mucosa. Results were evaluated in a mixed model with repeated statement (SAS statistical package; fixed effects: group, time, random effect: cow). A significantly decreased mean cortisol response was detected in cows of group C during the entire period from 20 min before the start of the surgical procedure until 10 h after the operation was completed (time × group $P < 0.05$). According to results of video recordings after surgery in average cows of C spent significantly ($P < 0.05$) more time ruminating and presented less pain associated behavior (determined by visual analog scale and multiple pain discomfort scale) than cows of P. Based on macroscopic fecal examination, blood count, the hemoFec-Test and serum pepsinogen concentrations, there was no indication for adverse effects on integrity of the abomasal mucosa. The study demonstrated that the use of carprofen in pre-emptive multimodal pain management increased the wellbeing of the patients without evoking adverse effects.

Key Words: carprofen, analgesia, cows

169 Impaired hepatic autophagic activity in dairy cows with severe fatty liver. X. Du, G. Liu, and X. Li*, *Jilin University, Changchun, Jilin, China.*

The ability of liver to respond to changes in nutrient availability is essential for the maintenance of metabolic homeostasis. Autophagy is a conserved catabolic process that mobilizes intracellular nutrients to meet energy requirements in the event of nutrient deficiency. Dairy cows with severe fatty liver generally have a severe negative energy balance (NEB). Therefore, the aim of this study was to investigate the hepatic autophagy status in dairy cows with severe fatty liver. Liver and blood samples were collected from healthy (n = 15) and severe fatty liver (n = 15) cows. Liver tissue was biopsied and serum samples were collected on 3 consecutive days. Dairy cows with severe fatty liver displayed significant hepatic lipid accumulation. Activities of liver injury indicators (aspartate aminotransferase, alanine aminotransferase, glutamate dehydrogenase, and gamma-glutamyl transferase) were all greater in cows with severe fatty liver. The blood concentrations of haptoglobin and serum amyloid A were also markedly higher in dairy cows with severe fatty liver. The mRNA expression of autophagosome formation related gene *ULK1* was lower in the liver of dairy cows with severe fatty liver than in healthy cows. However, the expressions of *Becn1*, vacuolar protein sorting 34 (*Vps34*), autophagy-related gene (*ATG*) *3*, *ATG5*, *ATG12*, were comparable between these 2 groups. More importantly, the ubiquitinated proteins, protein expressions of sequestosome-1 (SQSTM1, also called p62) and microtubule-associated protein 1 light chan 3 (MAP1LC3, also called LC3)-II were significantly higher in the liver of dairy cows with severe fatty liver than in healthy cows. Moreover, transmission electron microscopy observation showed increased number of autophagosomes in the liver of dairy cows with severe fatty liver. Taken together, these results indicate that dairy cows with severe fatty liver display liver damage, systemic inflammation and impaired hepatic autophagic flux. Furthermore, impaired autophagic flux may result in liver damage and inflammation and further promote the occurrence and development of fatty liver. This study also demonstrates that the hepatic adaptive capacity is impaired in dairy cows with severe fatty liver.

Key Words: fatty liver, autophagic activity, dairy cow

170 A fluorescence resonance energy transfer approach to determine intracellular zinc bioavailability in bovine mammary epithelial cells. R. Mohan*, F. Rosa, and J. S. Osorio, *Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Zinc is a key micronutrient involved in many cellular processes and biological pathways including oxidative stress and inflammation. Therefore, we evaluated the effect of the intracellular bioavailability of Zn in bovine mammary epithelial alveolar cells (MacT) incubated at 0, 10, and 50 µ*M* concentrations of Zn. Before transfection, MacT cells were cultivated in high glucose Dulbecco modified Eagle's medium (DMEM) with sodium pyruvate and supplemented with 10% fetal bovine serum (FBS), penicillin/streptomycin and Fungizone Antimycotic. The plasmid used in this study was the peZinCh-NB (Addgene) designed to detect intracellular Zn through a fluorescence resonance energy transfer (FRET) technology. Cells were seeded 24 h before transfection at 30,000 cells/well in a 96-well plate. Additional cells were seeded at 300,000 cells/well in a 6-well plate for gene expression analysis. Cells were transfected with the transfection reagent Lipofectamine 3000 at 0.3 µL/well and at 150 ng/well of plasmid in a reduced serum medium (OptiMEM) deprived of FBS. Transfected cells were treated for 24h in triplicates with 0, 10, and 50 µ*M* Zn. An inverted fluorescent microscope for live imagining (EVOS FL Auto) equipped with a motorized scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4× magnification at 0, 12, and 24 h post-treatment. Quantification of Zn and cell viability were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS. Overall intracellular availability of Zn increased ($P < 0.01$) in cells incubated with both 10 µ*M* and 50 µ*M* of Zn as early as 12 h post-treatment. At 12 and 24 h post-treatment, the greatest ($P < 0.01$) Zn intracellular bioavailability was observed with 50 µ*M* of Zn, when compared with 10 µ*M* of Zn and control. The cell viability at 24 h was similar ($P \geq 0.26$) across treatments with 81.59, 80.92, and 75.06% for control, 10 µ*M* and 50 µ*M* of Zn, respectively. These preliminary data indicate that intracellular Zn can be detected via a fluorescent protein system in real-time in bovine cells. To expand on these effects, gene expression analysis will be performed.

Key Words: zinc, bovine cells, fluorescent protein

Breeding and Genetics Symposium: Joint ADSA/Interbull Session: Data Pipelines for Implementation of Genomic Evaluation of Novel Traits

171 International collaborations for breeding for novel traits. Y. de Haas*, *Animal Breeding and Genomics of Wageningen University and Research, Wageningen, the Netherlands.*

With the successful incorporation of genomic information into breeding schemes the reliance on very large populations of phenotyped animals is relaxed. However, a reference population of several thousand animals is still required to estimate the contribution of each genomic region to expression of the phenotype under investigation. For novel traits, like feed efficiency and enteric methane emissions of dairy cattle, a reference population of this size cannot be easily established within a country, but requires international collaboration. METHAGENE and the Global Dry Matter Initiative (gDMI) are 2 examples of such intensive and successful collaborations. METHAGENE showed that even when enteric methane emissions of individual animals was recorded with different sensors and with different protocols, there are ways to combine the data for joint analyses, so that partners can take advantage of each other. METHAGENE not only looked at direct records of enteric methane, but also at proxies for methane related to (1) ingestion, (2) rumen, (3) milk composition, (4) hindgut, and (5) the animal itself. No single proxy was found to accurately predict enteric methane emissions, while combinations of 2 or more proxies are likely to be a better solution. Combining proxies can increase the accuracy of predictions, mainly because different proxies describe independent sources of variation in enteric methane emissions and one proxy can correct for shortcomings in the other(s). In gDMI, we demonstrated that using dairy cattle dry matter intake (DMI) phenotypes and genotypes from multiple populations increase the accuracy of genomic breeding values (gEBVs) for this important trait, provided a multi-trait approach is used. Data from research herds in Europe, North America, and Australasia were combined to estimate the accuracy of genomic prediction for DMI using multi-trait models. The mean accuracy of prediction was 0.44, ranging from 0.37 (Denmark) to 0.54 (the Netherlands). Overall, for novel traits, there is benefit of collaboration, as phenotypic information from other countries can be used to augment the accuracy of genomic evaluations of individual countries.

Key Words: breeding, novel trait, dairy cattle

172 Genetic relationships between different measures of feed efficiency and the implications for dairy cattle selection indexes. R. J. Tempelman*[1] and Y. Lu[2], [1]*Michigan State University, East Lansing, MI,* [2]*Axio Research, Seattle, WA.*

Dairy profit selection indexes have increasingly incorporated one of the various measures of feed efficiency (FE) as a key component trait. Definitions of FE traits range from DMI to residual feed intake (RFI), noting that RFI is effectively DMI adjusted for various energy sink traits such as body weight (BW) and milk energy (MILKE). Other definitions of FE fall between these 2 extremes such as feed saved (FS), which combines RFI and the portion of DMI required to maintain BW. The use of different FE traits can create confusion as to how to meaningfully compare their heritabilities or estimated breeding values (EBV) and their corresponding accuracies, or even how to differentially incorporate these EBV into selection indexes. If RFI and FS are merely linear functions of DMI, BW, and MilkE with known genetic variances and covariances between the 3 traits, there is no need to directly compute RFI or FS phenotypes to determine their heritabilities, genetic correlations, EBV and their respective accuracies. We demonstrate how the estimated aggregate genetic merits and corresponding accuracies are invariant to the specification of a FE trait within a selection index. That is, economic weights for a selection index involving one particular measure of FE readily convert into the economic weights for a selection index involving a different measure of FE. We use these different specifications of FE to provide insight as to the impact of the degree of missingness (i.e., paucity of DMI or BW relative to milk yield records) on the EBV accuracies of the various derivative FE traits. We particularly highlight that the generally observed higher EBV accuracies for DMI, then for FS, and lastly for RFI are partly driven by the typically greater genetic relative to residual correlations between DMI with RFI and FS and by the higher genetic correlations of DMI with BW and MILKE. Finally, we advocate a genetic regression approach to deriving FS and RFI recognizing that genetic versus residual relationships between FE component traits may differ substantially from each other.

Key Words: feed efficiency, multiple trait analysis, selection index

173 Multiple-trait single-step genomic evaluation for hoof health. F. Malchiodi*[1,2], J. Jamrozik[2,3], A.-M. Christen[4], A. Fleming[3], G. J. Kistemaker[3], C. Richardson[2,5], V. Daniel[6], D. F. Kelton[7], F. S. Schenkel[2], and F. Miglior[2], [1]*Semex Alliance, Guelph, ON, Canada,* [2]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Canadian Dairy Network, Guelph, ON, Canada,* [4]*Valacta, Sainte-Anne-De-Bellevue, QC, Canada,* [5]*School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia,* [6]*Vic's Custom Clips, Arva, ON, Canada,* [7]*Department of Population Medicine, OVC, University of Guelph, Guelph, ON, Canada.*

Hoof lesions represent an important issue in modern dairy herds, with prevalence reported in different countries ranging from 25 to 80%. This high prevalence of hoof lesions has both economic and social consequences, resulting in increased labor expenses while decreasing animal production, longevity, reproduction, health, and welfare. Therefore, a key goal of dairy herds is to reduce the incidence of hoof lesions, which can be achieved both by improving management practices and through genetic selection. In Canada, a hoof health sub index has recently been released based on a national genetic evaluation program for hoof health, which was achieved by the creation of a centralized data collection system that routinely transfers data recorded by hoof trimmers into a coherent and sustainable national database. The 8 most prevalent lesions in Holsteins (digital dermatitis, interdigital dermatitis, interdigital hyperplasia, heel horn erosion, sole hemorrhage, sole ulcer, toe ulcer, and white line lesion) are analyzed with a multiple-trait model using single-step GBLUP method. Estimated genomic breeding values for each lesion are then combined into a sub-index (Hoof Health) according to their economic value and prevalence. In addition, data recorded within this system are used to create an interactive management report for dairy producers by Canadian DHI, including the prevalence of lesions on farm, their trends over time, and benchmarks with provincial and national averages.

Key Words: hoof lesions, genomic evaluation, dairy

174 Pooling data for international evaluations for feed intake and efficiency. J. Lassen*, *Viking Genetics, Randers, Denmark.*

Genetic evaluation of feed intake and efficiency is a hot topic worldwide. Lack of data to make genetic evaluation for feed intake and efficiency is an equally hot topic worldwide. Therefore, many initiatives have been made to exchange data. As an example the Efficient Dairy Genome Project led by University of Guelph and University of Alberta institutions from several countries have put up data on feed intake, methane and related traits to exploit the opportunities to make national genetic evaluations. One of the main ideas behind the data sharing in this project is that institutions that contribute data have full access to all the data that is uploaded. For a country to be able to publish breeding values, having access to the data that is the foundation of the estimation is essential. If an institution does not upload data on a specific trait, the institution does not have access to the data from the other institutions on this trait. Each country has their own protocol to make phenotypic and genomic registrations of data. This can be a challenge for setting up an appropriate genomic evaluation. Phenotypes are not measured in the same way between countries and genotypes are from very different panels. In addition, the genetic background of the populations in the participating countries can be very different. For a trait like feed intake where relatively huge genotype by environment interactions are expected, this is a big challenge when number of animals as well as number of records are limited. An alternative would be to avoid having genomic evaluations in such a case; however, this is not an option. New methods to measure feed intake in commercial farms are needed for several reasons: 1) to be able to use individual feed intake as an on-farm management tool, 2) to get more data from cows that are not in experiments and genetically close to the current reference group, 3) to be able to actually demonstrate that selection has an effect. Therefore, more effort should be put into developing tools and technologies that are profitable to farmers.

Key Words: data pooling, feed intake, feed efficiency

175 Development, implementation, and future perspectives of health evaluations in the United States. K. L. Parker Gaddis*[1], P. M. VanRaden[2], J. B. Cole[2], E. Nicolazzi[1], and J. W. Dürr[1], [1]*Council on Dairy Cattle Breeding, Bowie, MD,* [2]*Animal Genomics and Improvement Laboratory, Agricultural Research Service, USDA, Beltsville, MD.*

The rate at which new traits are being developed is increasing, leading to an expanding number of evaluations provided to producers, especially for functional traits. The objectives of this presentation include discussion of development and implementation of health evaluations in the US, as well as potential future work. Beginning in April 2018, routine official genomic evaluations for 6 direct health traits were made available to US producers from the Council on Dairy Cattle Breeding (Bowie, MD). Traits include resistance to milk fever, displaced abomasum, ketosis, mastitis, metritis, and retained placenta. These health traits were incorporated into net merit indices beginning in August 2018 with a total weight of approximately 2%. Previously, improvement of cow health was primarily made through changes to management practices or selection on indicator traits, such as somatic cell score (SCS) and productive life. Widespread genomic testing now allows for improvement of traits with low heritabilities such as health; however, phenotypes remain essential to the success of genomic evaluations. Establishment and maintenance of data pipelines is a critical component of health trait evaluations, as well as appropriate data quality control standards. Data standardization is a necessary process when multiple sources are involved. Model refinement continues, including implementation of variance adjustments beginning with the April 2019 evaluation. Mastitis evaluations were submitted to Interbull along with SCS for international evaluation of udder health. Possible future developments include multiple-trait models, evaluation of other breeds, and evaluations for additional functional traits such as calf health, feed efficiency, locomotion, or lameness. Future developments will require new and continued cooperation among numerous industry stakeholders. Producers and the dairy industry as a whole must decide how to handle similar evaluations from multiple sources, including proprietary traits from private companies. There is more information available than ever before with which to make better selection decisions; however, this also makes it increasingly important to discern accurate and unbiased information.

Key Words: functional trait, genetic evaluation, health trait

176 Implementation of genomic selection for heat tolerance. T. T. T. Nguyen[1], P. J. Bowman[1,2], M. Haile-Mariam[1], B. J. Hayes[3], and J. E. Pryce*[1,2], [1]*Agriculture Victoria, Bundoora, VIC, Australia,* [2]*La Trobe University, Bundoora, VIC, Australia,* [3]*University of Queensland, Brisbane, QLD, Australia.*

Heat stress is an issue of growing concern for many livestock production systems worldwide affecting not only animal welfare, but also farm profitability. In December 2017, genomic estimated breeding values for heat tolerance in dairy cattle were released for the first time in Australia. The data set was constructed by merging herd-test production records with weather station data. Heat tolerance phenotypes were defined as the rates of decline in milk, fat and protein yield after a heat stress event (i.e., temperature-humidity index exceeds 60), and were estimated using a reaction norm model. The genomic prediction equation was developed from a reference population of 2,236 sires (with heat tolerance phenotypes on daughters) + 11,853 cows for Holsteins and 506 sires + 4,268 cows for Jerseys. These sires and cows were genotyped with 46,276 SNP. Each component of heat tolerance (genomically predicted decline in fat, protein, and milk) is weighted by its economic value, which is assumed to be the same as their weights in the Australian selection indices. The genomic breeding values are then standardised within breed to have a mean of 100 and standard deviation of 5. Although the reliability of this new trait is moderate (on average around 38%), it is expected that this will improve as the reference populations are increased. The genetic trend for heat tolerance has worsened, which is consistent with the correlation with the Australian national selection index (Balanced Performance Index; BPI) which is −0.20. Heat tolerance is currently not part of the BPI, however, its inclusion will be considered as part of the next review of the index.

Key Words: heat tolerance, genomic selection

Dairy Foods: Cheese, Yogurt, and Ice Cream

177 Effect of different aging conditions on the physicochemical and textural characteristics of an artisanal cheese produced in South African rural communities. F. Nyamakwere[1], M. Busti[2], E. Raffrenato*[1], P. A. Gouws[3], K. Dzama[1], and G. Esposito[1], [1]*Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,* [2]*Caseificio della Famiglia Busti, Fauglia, Italy,* [3]*Department of Food Science, Stellenbosch South Africa.*

Small-scale dairy farmers in African rural communities might fail to invest in cheese making process due to limited resources to acquire temperature and humidity controlled aging chambers. The study evaluated the effect of different aging conditions on physicochemical and textural characteristics of an artisanal hard-pressed cheese during ripening. The cheeses were manufactured in summer in 2 different farms using raw milk. Aging was conducted for 60 d using either a temperature and humidity controlled chamber (control: 10–12°C, humidity 80–90%) or a traditional simulation using portable air conditioner and humidifier. Data were analyzed using a factorial arrangement of treatments with age and chamber, and their interaction, as fixed factors and farm as a random factor. The portable systems created a chamber at 18°C and 60–70% humidity. Cheese moisture, fat, pH, water activity, and non-protein nitrogen (N) were reduced during ripening ($P < 0.05$). The ripening index [soluble N/Total N%] was higher in the control (6.4 ± 0.38 vs 5.6 ± 0.38; $P < 0.05$) compared with the traditional chamber (TC). The control yielded cheeses with higher ($P < 0.05$) moisture% (34.05 ± 0.65) and water activity (0.96 ± 0.002). Compared with the TC, the control had lower ($P < 0.05$) values for total solids (65.9 ± 0.63 vs 69.9 ± 0.63), protein% (25.6 ± 0.15 vs 27.6 ± 0.15) and ash% (3.44 ± 0.11 vs 3.70 ± 0.11). Neither chamber showed differences in cheeses textural properties (chewiness, springiness, cohesiveness, and hardness) and color. There were interactions between the aging chamber and ripening age; ash% (4.00 ± 0.12), protein% (27.7 ± 0.21), fat% (34.1 ± 1.35), hardness (149.67 ± 30.72), and chewiness (463.67 ± 15.15) were higher ($P < 0.05$) at d 60 in the traditional chamber. The results show that the traditional chamber could be ideal for on-farm cheese ripening, yielding good quality cheese for resource limited cheese producers. This study forms a baseline for the development of cheese production at an artisanal level for small-scale farmers in South Africa based on their environmental conditions and resources.

Key Words: ripening, temperature, humidity

178 Nanofiltration as sustainable approach to controlling cheese acidity by adjusting lactose to casein content of milk. J. A. Stankey, Y. Lu*, S. Govindasamy-Lucey, M. Molitor, J. J. Jaeggi, M. E. Johnson, and J. A. Lucey, *Center for Dairy Research, University of Wisconsin-Madison, Madison, WI.*

Previously we developed a technique to standardize milk lactose content while maintaining a constant casein (CN) level through the addition of reverse osmosis (RO) water to ultrafiltered (UF) milk. This process dilutes the water-soluble minerals, particularly shifting the Ca equilibrium, which can impact the clotting process unless cheesemakers add $CaCl_2$ to milk to aid gelation. In regions with water scarcity a more sustainable approach could be to use water that was generated through processing of UF permeate (nanofiltered permeate; NF). Although NF permeate contains some monovalent ions, $CaCl_2$ could be added to assist renneting. Milks were standardized with either RO or NF permeate to achieve a high (HL; 1.7% lactose) or low (LL; 0.9% lactose) lactose-to-CN ratios: LLRO, HLRO, LLNF, HLNF; additionally both NF treatments were also fortified with 0.04% $CaCl_2$: LLNFC and HLNFC. Total CN (2.5%) and fat (2.5%) contents were similar in all 6 milks. All 6 milk treatments were used to make low-moisture part-skim (LMPS) Mozzarella cheeses (n = 4), were aged at 4°C for 84 d, and evaluated for composition, texture, functional, and sensory properties during ripening. Cheeses had similar moisture contents (47.9% ± 0.5). Throughout storage cheeses made with RO water were harder (texture profile analysis), firmer (sensory), and less meltable (rheology) than cheeses made with NF permeate; addition of $CaCl_2$ to NF also decreased firmness and meltability ($P < 0.05$). At 28 d, HL cheeses baked on pizzas had darker blister color, more cohesive, higher strand length, and more acid and lower pH ($P < 0.05$) than LL cheeses. Pizzas made with LL cheeses at 28 d had lighter blister color and were less acidic ($P < 0.05$) than HL cheeses. Pizzas with LLRO cheese were chewy, had more blisters, higher strand thickness than pizzas with LLNF or LLNFC cheeses. Adjusting the lactose content of the milk by standardizing with NF permeate was a useful, sustainable alternative to use of RO permeate for controlling cheese pH which also positively impacted texture, functionality, and sensory properties of LMPS Mozzarella.

Key Words: lactose, casein, nanofiltration

179 Minimizing moisture migration in large 291-kg blocks of Cheddar cheese. C. E. Collins*[1], M. E. Johnson[2], S. Govindasamy-Lucey[2], J. J. Jaeggi[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

In the industry, 291-kg blocks (known as 640-blocks) of Cheddar cheese are manufactured for ease of handling and reducing trim loss during conversion. Significant moisture (MC) migration occurs within blocks due to temperature gradients during cooling. Some manufacturers also concentrate cheese milk, resulting in higher amounts of casein and thus higher amounts of colloidal calcium phosphate (CCP). To reduce MC variation within 640-blocks, we explored the impact of pre-acidifying milk to solubilize some CCP and varying cooling rates after pressing to reduce temperature gradients. Four replicate 640-blocks of stirred curd Cheddar cheese were manufactured at a commercial facility. Milk was ultrafiltered to 14.8 ± 0.6% solids and 3.2 ± 0.1% casein. Vats were pre-acidified to pH levels (no pre-acidification, pH 6.35, 6.20) using lactic acid. Blocks were stored in different coolers for 15 d (4 or 21°C for 2 d followed by 4°C). All blocks were transferred to a 1°C warehouse at 15 d. After 30 d, 640-blocks were cut into sixteen 40-lb portions. Inner core and outer samples were cut from four 40-lb blocks. Inside of 640-blocks was lower in MC than outside, as expected. Treatments reduced MC variability between inside and outside less than expected, likely due to low pH (<5.0) observed in pre-acidified samples. Slow cooling resulted in lowered pH. Functionality was monitored by texture profile analysis and small amplitude oscillatory rheology. Cheese hardness was higher in inside of the 640-blocks compared with outside, but slightly less variation was observed with pre-acidification and slow cooling. Higher temperatures for maximum loss tangent (an index of meltability) were observed in inside samples compared with its outside. Less variation between inside and outside samples was observed in melting point (LT = 1) for pre-acidified and slow cooled cheeses. A split-split plot design was used for statistical analysis, and a comparison of means was carried out ($P < 0.05$). Pre-acidifying cheese milk and slowing the cooling rate appeared to reduce some functionality variation in 640-blocks of

Cheddar cheese, but more control is needed over final cheese pH to reduce MC migration.

Key Words: pre-acidification, cooling, moisture

180 From'Innov: A new concept to improve efficiency and sustainability of the cheesemaking process. J. Chamberland*[1], S. Benoit[2], M. Harel-Oger[1], Y. Pouliot[2], R. Jeantet[3], and G. Garric[1], [1]*INRA, UMR 1253 Science and Technology of Milk and Eggs, Rennes, France,* [2]*2STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Quebec City, QC, Canada,* [3]*Agrocampus Ouest, UMR 1253 Science and Technology of Milk and Eggs, Rennes, France.*

The From'Innov process was recently proposed to increase cheesemaking control and flexibility while reducing its environmental impact. The process consists in developing cheese aroma and texture apart, thus replacing the traditional ripening process by supplementing a liquid pre-cheese with aromatic matrices fermented by traditional ripening microorganisms. Conceptually, it was hypothesized that it could improve both economic and environmental performances of cheesemaking plants. However, economic balance and electrical energy, natural gas and fresh water consumptions of industrial plants needed to be known to assess the real benefit of this process. Consequently, a process simulation was performed in 3 virtual plants processing 100,000 kg of milk per day. It enabled to compare the From'Innov process to the traditional and the MMV processes (another liquid pre-cheese-based process) for the production of industrial bloomy soft cheeses (Camembert-type), from the milk reception to the end of ripening. Globally, the virtual plants using liquid pre-cheese technologies required more energy and fresh water. However, their specific energy consumption were lower because of their higher cheese yield (20.7% and 18.3% in the From'Innov and the MMV plants, respectively) compared with the traditional process (12.1%). The From'Innov and the MMV plants needed 3.1 ± 0.1 and 3.7 ± 0.1 MJ per kg of cheese, respectively, while 5.2 ± 0.2 MJ per kg of cheese was required in the traditional plant. A similar pattern was observed for the fresh water consumption, where 6.6 ± 0.1, 8.0 ± 0.6 and 10.9 ± 0.8 L were required to produce one kg of cheese in the From'Innov, the MMV and the traditional plants, respectively. Economic balances including costs associated to building construction, industrial equipment or human resources allowed to calculate the margin of the From'Innov process, which was 2.4% and 39.0% higher than that of the MMV and the traditional processes, respectively. Being more profitable and less energy demanding, this From'Innov process should enable to produce a wide variety of cheese on demand, thus contributing to improve the ecoefficiency of the industry.

Key Words: cheese, liquid pre-cheese, efficiency

181 Emotions evoked by cheese odors. C. Licon*[2,1], C. Manesse[1], A. Fournel[1], and M. Bensafi[1], [1]*Lyon Neuroscience Research Center, Lyon, France.,* [2]*Fresno State University, Fresno, CA.*

Cheese quality is defined in part by its aroma; however, the consumer's perception of these attributes is not often recognized as a critical component. From a fundamental perspective, consumer's hedonic ratings are linked to emotions and memories, which are often characterized by changes in bodily responses like skin conductance and heart rate. Nowadays, methodology for measuring emotions and their relation to cheese perception use arbitrary scaling methods, which is a drawback because each person typically uses scales differently and replication and precision are hardly achieved, mainly when dealing with cheese evaluation. In this sense, applying emerging tools to measure psychophysiological responses represent an exciting method to obtain more accurate and complementary representations of how cheese aromas are perceived by consumers. The aim of this work was to better understand the sensory perception of cheese aroma in terms of emotional responses evoked by behavioral and psychophysiological responses. Data from 105 odors were collected in a previous study (Licon et al., 2018) from which 50 cheese aromas were analyzed here. Two sessions were necessary, the first consisted of recording physiological responses to odors (skin conductance, skin temperature, respiration, and heart rate) together with psychophysical ratings (pleasantness, intensity). The second consisted of psychophysical tests only (pleasure, happiness, sadness, fear, anger, disgust, surprise and neutral; scale 0 to 1). We found that skin conductance (latency and rise time) was the physiological response better correlated with emotions: especially with pleasantness ($r = -0.442$, $P = 0.001$; $r = -0.297$, $P = 0.036$) and disgust ($r = 0.476$, $P < 0.001$; $r = 0.478$, $P < 0.001$). Some of the emotions evoked by cheese aromas were disgust by butanoic acid and propyl butyrate, pleasure, and happiness by 2,3-butanedione, 2-methyl-propanal, ethyl octanoate, and 1-octen-3-ol and surprise by dimetylsulphide and propyl acetate. In conclusion, the present findings sustain the physiology of aroma perception, showing that perceptual dimensions such as pleasantness and disgust enable consumers to decide whether a cheese aroma is attractive or repulsive.

Key Words: cheese aroma, emotions, physiology

182 Application of partial calcium-depleted milk protein concentrate in protein-fortified stirred yogurt for improving the gel water-holding capacity and product processability. J. A. Ortiz Salazar*[1,2], R. H. Fernando[2], and H. Zheng[1], [1]*Dairy Innovation Institute, Department of Animal Science, California Polytechnic State University, San Luis Obispo, CA,* [2]*Department of Chemistry and Biochemistry, California Polytechnic State University, San Luis Obispo, CA.*

Higher protein fortification may result in a stiffer yogurt gel. Such effect limits the protein fortification level, because a gel with high stiffness may cause poor textural sensory attributes and poor product processability. In the current research, a partial calcium depleted milk protein concentrate 80 (CD-MPC) was manufactured. It may be used as protein fortifier in high protein yogurt without increasing gel firmness as much as a standard MPC80 (Std-MPC). Textural, rheological and stability properties of the fermented yogurt gels were characterized for presenting the functionalities of CD-MPC. The calcium content of CD-MPC is lower than a Std-MPC ($P < 0.05$). CD-MPC and Std-MPC were applied into 4 different fat-free model yogurt gels respectively; the 4 different models are discriminated by 4 protein contents: 4%, 6%, 8%, 10% (wt/wt). In each model, nonfat dry milk powder was used as the base ingredient for contributing 3.5% protein content and either CD-MPC or std-MPC was used for compensating the rest protein content for each model. Textural analysis results showed that using CD-MPC instead of using Std-MPC may result in relatively lower firmness, consistency, cohesiveness, and viscosity ($P < 0.05$) in the models containing no less than 6% protein. Steady-state shear viscosity and dynamic oscillatory strain sweep measurements were used for characterizing rheological properties of yogurt gels. For the higher protein models, the flow behavior curves were well separated between the test and control samples, the test samples were relatively less viscous. A similar trend was observed in the strain sweep tests, CD-MPC containing gels showed lower storage modulus and loss modulus. Both textual and rheological results confirmed that CD-MPC may result in a weaker gel when compared with Std-MPC. In the stability study, the results showed that CD-MPC may result in improved water holding capacity (WHC) compared with std-MPC. Overall, the current results suggest that the CD-MPC is a promising protein fortifier which may be used for improving gel stability without highly increasing gel firmness of protein fortified yogurts.

Key Words: milk protein concentrate, yogurt

183 Texture defects of fermented milk products caused by vibrations—Impact of amplitude and different process parameters. A. Körzendörfer*[1], P. Temme[2], E. Schlücker[2], and J. Hinrichs[1], [1]*University of Hohenheim, Stuttgart, BW, Germany,* [2]*University of Erlangen–Nuremberg, Erlangen, BY, Germany.*

Machinery like pumps used for the commercial production of yogurt and related fermented milks generate vibrations that can spread to the fermentation tanks. Such vibrations can disturb the gelation by causing texture defects including lumpiness and syneresis. The aim of this study was to research the effect of vibrations on the aggregating milk proteins and evaluate their relevance with regards to the manufacture of fermented milk products. To generate defined vibrational states, an experimental setup was developed consisting of a jacketed stainless steel tank (cylindric, $d = 100$ mm, $l = 600$ mm). Vibrations are directly introduced by a piston at the bottom, which is oscillated by an electrodynamic vibration exciter. Pressure sensors are used to describe the propagation of specific vibrations within the liquid. At a frequency of 30 Hz, a linear decrease of the pressure was observed. In contrast, a standing wave was formed at 1000 Hz. Vibrations ($f = 30$ Hz) were then applied during yogurt fermentation at 43°C by varying the amplitude from 0 – 10 m/s^2. After acidification, set milk gels were processed and analyzed (rheology, water-holding capacity). A method based on image analysis was applied to quantify visible particles and evaluate textures. The number of independent repetitions of the experiments was $i \geq 3$. Vibrations of lower amplitudes ($a = 2.5$ m/s^2) increased the number of visible particles by a factor of 2 ($P < 0.05$). Higher amplitudes ($a \geq 5$ m/s^2) resulted in considerable syneresis of the set gel and a less homogeneous appearance of the final product. The particle number was increased by a factor of 10 to 20 ($P < 0.001$), however, deeper analysis revealed that particle formation was more pronounced in the lower and upper area within the fermenter. A high particle number was also linked to a reduced product viscosity and increased whey separation. In conclusion, the magnitude of a specific vibration depends on both its characteristics and other process parameters. Manufacturers of fermented milk products should consider vibrations as a further cause for quality defects.

Key Words: graininess, fermented milk product, yogurt structure

184 Dynamic structural breakdown behavior of a model Maasdam-style cheese under tensile deformation as studied using confocal scanning laser microscopy. P. Lamichhane[1,2], M. A. E. Auty[3], A. L. Kelly[2], and J. J. Sheehan*[1], [1]*Teagasc Food Research Centre Moorepark, Fermoy, Cork, Ireland,* [2]*University College Cork, Cork, Ireland,* [3]*Mondelez International Ltd., Reading, UK.*

Knowledge of the breakdown behavior of cheese is important for gaining insights into texture perception, flavor and nutrient release, as well as the origin of undesirable texture defects within the cheese matrix such as slits and cracks. In this novel study, changes in the microstructure of model Maasdam-style cheeses were observed in situ during tensile deformation by placing a microtensile stage directly under a confocal scanning laser microscope (CSLM), and recording force/displacement data simultaneously. A small indentation (called a notch) was made at a center point on the test specimens, and growth of the notch was observed under tensile deformation using CSLM. Widening of the notch, stretching of the protein network near the leading point of the notch, detachment of fat globules, and their subsequent release from the cheese matrix, as well as fracturing of the cheeses, partly along curd granule junctions, were all observed during tensile deformation. Moreover, an inherent micro-defect was observed at a curd granule junction within the cheese matrix and this micro-defect fractured along the curd granule junction under tensile deformation, suggesting that the micro-defects present within the cheese matrix could be a key underlying factor in the formation of undesirable slits or cracks. Further work showed that the fracture behavior of cheese was altered by changing ripening temperature, using different coagulant types, or by inhibition of residual chymosin. Such approaches could be applied to designing cheeses with specific texture profiles or for designing optimal cheese textures to withstand increased gas pressure during ripening in eye-type cheeses, which may help to prevent the formation of undesirable splits and cracks. Overall, this study demonstrated the potential of in situ imaging of cheese microstructure for developing a greater understanding of the breakdown behavior of cheese matrices.

Key Words: cheese, fracture behavior, in situ imaging

185 Differentiating between the effects of chymosin-mediated proteolysis, coagulant type, ripening temperature and calcium solubilization on fracture behavior of Maasdam-style cheese. P. Lamichhane[1,2], P. Sharma[1], D. Kennedy[1], A. L. Kelly[2], and J. J. Sheehan*[1], [1]*Teagasc Food Research Centre Moorepark, Fermoy, Cork, Ireland,* [2]*University College Cork, Cork, Ireland.*

Ripening-related changes within the cheese matrix influence cheese properties, such as texture perception and development of undesirable slits and cracks. However, the individual contribution of various factors on cheese properties remains unclear; therefore, the aim of this study was to decouple and explore the individual roles of chymosin-mediated proteolysis, coagulant-type, ripening temperature and calcium solubilisation on the fracture behavior of Maasdam-style cheese. Addition of a chymosin inhibitor, i.e., pepstatin A, to the curd/whey mixture during cheese manufacture completely inhibited the breakdown of α_{S1}-casein over 90 d of ripening, while substitution of fermentation-produced bovine chymosin with fermentation-produced camel chymosin decreased breakdown of α_{S1}-casein by ~40%. However, neither treatment influenced the hydrolysis of β-casein or the solubilisation of colloidal calcium. Ripening of cheese at a consistent low temperature (8°C) decreased the rate of breakdown of α_{S1}-casein and β-casein and solubilisation of colloidal calcium as compared with cheeses ripened for a period at higher temperature (warm-room stage at 23°C between 20 and 48 d ripening). A significant positive correlation was found between intact α_{S1}-casein content and fracture stress, suggesting that the hydrolysis of α_{S1}-casein has an important role in the softening of cheese texture. However, no significant association between levels of intact α_{S1}-casein and strain at fracture was observed, suggesting that breakdown of α_{S1}-casein had no pronounced influence on the brittleness of Maasdam-style cheese. In contrast, the strain at fracture was significantly positively correlated with the level of intact β-casein and insoluble calcium content. Results from this study provide a new perspective on potential opportunities for cheese-makers to reduce the incidence of slits and cracks in cheese.

Key Words: proteolysis, insoluble calcium, fracture properties

186 Prototype protein characterization unit (PCU) to study the dissociation of casein micelles by sodium and potassium citrates

and tartrates. E. Mahdinia*, C. Hettiarachchi, and F. Harte, *Pennsylvania State University, University Park, PA.*

There is a growing demand for drinks and shakes containing high dairy protein contents. The effect of ionic environments in complex protein quaternary structures (e.g., casein micelles), can only be transformed into technological applications using an empirical approach where a broad set of environmental conditions are tested (e.g., emulsifying salt, salt concentration, casein concentration, temperature, and pH). For this purpose, a prototype protein characterization unit (PCU) was designed and assembled to autonomously look thorough a wide range of sample conditions rapidly to generate a clear picture of how the parameters effect salt-protein interactions. The PCU was tested for environmental conditions including pH (6.0, 6.5 and 7.0), temperature (5, 15, 25, 35, 45 and 55°C), salt concentration (0–200 m*M*), exposure time (0–240 s), and measuring absorbance (400 nm), fluorescence (excitation: 380 nm; emission: 470 nm) and viscosity (gauge pressure) of the liquid systems containing milk proteins and salts. The absorbance values were then interpreted as micelle dissociation through the critical salt concentration (C*) using an exponential decay model [absorbance = absorbance(c = 0)×Exp(−C/C*); where C is the salt concentration]. Sodium and potassium citrates were effective in dissociating casein micelles with critical concentrations leading to micellar dissociation ~2 to 4 m*M*. An increase in temperature shortened the required exposure time for dissociation and decreased system viscosity by nearly 50%. Increase in pH however, increased the critical concentration values from nearly 5 m*M* at pH = 6.0 to 7 m*M* at pH = 7.0. Furthermore, tartrates were also able to deliver the similar effects but at higher concentrations (~15–50 m*M*). Effects of anions were significantly more profound compared with cations where the average initial critical concentration for citrates was ~3 to 4 m*M* and for tartrates was ~20 m*M*. In summary, the mapping of protein-salt interactions will allow for better understanding of protein functionality toward liquid system containing high protein contents.

Key Words: casein, ionic environment, emulsifying salt

187 Ice cream from milk from cows supplemented with unsaturated fatty acid sources: Physicochemical and sensory characteristics. E. Vargas-Bello-Pérez*[1,2], N. Cancino-Padilla[1], C. Geldsetzer-Mendoza[1], M. S. Morales[3], J. Romero[5], P. C. Garnsworthy[5], and R. A. Ibáñez[1], [1]*Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg C, Denmark,* [3]*Departamento de Fomento de la Producción Animal, Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile.,* [4]*Laboratorio de Biotecnología, Instituto de Nutrición y Tecnología de los Alimentos, Universidad de Chile, Santiago, Chile,* [5]*Sutton Bonington Campus, The University of Nottingham, Loughborough, UK.*

The objective of this study was to evaluate effects of supplementation of dairy cows with different fatty acid sources [soybean oil (SO) and fish oil (FO)] on the physicochemical and sensory characteristics of ice cream. Fifteen Holstein cows averaging 198 ± 35 d in milk were assigned to 3 groups: control diet with no added lipid (n = 5 cows); and supplemented diets with SO (n = 5 cows; unrefined SO; 30 g/kg DM) or FO (n = 5 cows; FO from unrefined salmon oil; 30 g/kg DM). Diets were based on corn silage and alfalfa silage. Cows received treatments during 63 d, milk was registered daily, and individual milk samples were taken on d 21, 42 and 63 for milk composition. At the sampling days, milk collected from individual cows from the same treatment was pooled and made into ice cream. Sensory evaluation of ice creams was carried out in relation to 7 attributes: appearance, texture, melting resistance, taste, aroma, milkfat and generally acceptability. All parameters were analyzed using the PROC MIXED of SAS. Milk production (42.9 ± 1.5 kg/d), milk fat (1.5 ± 0.10 kg/d) and milk protein (1.5 ± 0.06 kg/d) were not affected by treatments. Fat (16.1 ± 0.001 g/100g), lactose (3.99 ± 0.001 g/100g), and sucrose (17.1 ± 0.01 g/100g) in ice creams were not affected by treatments ($P > 0.05$). Protein contents in ice creams were higher ($P < 0.05$) with SO. Milk fat (g/100g total FAME) saturated fatty acids were decrease ($P < 0.05$) with SO and FO compared with control. C18:2n6t, C18:2n6c and C18:2c9,t11 were increased ($P < 0.05$) with SO whereas C20:3n3, C20:3n6, C20:5n3 and C22:6n3 were higher ($P < 0.05$) with FO. Draw temperature and firmness were higher ($P < 0.05$) in SO compared with control and FO ice creams. Overrun and melting rate were higher ($P < 0.05$) in SO and FO ice creams. Melting resistance was higher ($P < 0.05$) in FO compared with control and SO ice creams. Overall, supplementation of dairy cow diets with SO and FO did not have detrimental effects on milk production, ice cream physicochemical and sensory characteristics. From a human standpoint, SO and FO improved the FA profile of milk. This study was sponsored by a research grant from FONDECYT 1170400, Chile.

Key Words: ice cream, fatty acids, oil supplements

Dairy Foods: Products

188 Catalytic synthesis of lactose derivatives from whey permeate. M. Enteshari* and S. I. Martínez-Monteagudo, *South Dakota State University, Brookings, SD.*

The current surplus of lactose has promoted global research efforts seeking for alternate uses. Lactose presents technologically challenges such as low solubility and sweetness, as well as malabsorption by a certain population. There is a considerable industrial interest to further utilize lactose (LA) as a feedstock for the production of lactose-based ingredients. In this work, the catalytic conversion of LA to produce lactobionic acid (LBA) and lactulose (LAU) was studied. The reaction parameters of LA conversion were optimized in a continuous stirred tank reactor using a LA monohydrate solution over commercial ruthenium supported on activated carbon (5%-Ru/C). Then, the feasibility of the conversion of LA directly from sweet and acid whey permeate (SWP and AWP) was investigated under optimized catalytic reaction (stirring rate of 600 rpm, 60 bar oxygen pressure, and 70°C). The SWP was collected from a local cheese plant, while AWP was obtained from a Greek yogurt plant. Overall, the conversion of LA increased gradually within the reaction time. After 210 min, the values of LA conversion reached to 83.3, 36.2 and 33.4% for the LA solution, SWP, and AWP, respectively. Regardless of reaction time, the SWP showed the highest production yield of LA derivatives due to its high initial LA content (87.44 ± 4.93 g 100 g^{-1} on dry matter) and higher pH level (6.23 ± 0.01). Furthermore, after 210 min catalytic reaction, LBA yield reached to its maximum values (4.99, 2.06, and 2.45% for SWP, AWP, and LA solution, respectively). During 30 min, the LAU yield reached to highest value (22.99, 15.28, and 17.91%, for SWP, AWP, and LA solution, respectively). Prolonged reaction time may induce degradation of produced derivate. Moreover, the acidic nature of LA solution and AWP did not favor formation of LAU. These results revealed that chemical conversion of LA can be a promising approach for the simultaneously production of LBA and LAU directly from the whey stream.

Key Words: lactose, catalytic reaction, whey permeate

189 A two-step process for the production of sweetening syrup from lactose. S. Cheng, L. Metzger, and S. Martinez-Monteagduo*, *South Dakota State University, Brooking, SD.*

Lactose is the most underutilized dairy ingredient. Current applications of lactose are insufficient to use the recovered lactose from the manufacture of dairy products (cheese, Greek yogurt, and protein concentrate). As an ingredient, lactose offers technological challenges (poor solubility and low sweetness strength) and health concerns (malabsorption and digestive problems). It is critical to develop technological approaches that can help to expand lactose utilization. The objective of this work is to develop process for producing a mixture of natural sweeteners derived from lactose using a 2-step process. Aqueous lactose was converted into a sweetening syrup via enzymatic hydrolysis followed by catalytic isomerization over MgO/SiO_2. First, the enzymatic hydrolysis using β-galactosidase was performed at room temperature, and it converted 95.77 ± 0.67% of lactose into glucose and galactose. Second, the hydrolysed lactose solution was catalytically isomerized at 100°C for 2 h in the presence of MgO/SiO_2 containing different MgO loading ratios (10, 20, 30, and 40 wt.%). The prepared MgO/SiO_2 catalysts were characterized by BET, XRD, FTIR, CO2-TPD, and TEM. The highest isomerization yield of glucose and galactose to produce fructose and D-tagatose (26.8 ± 0.5 and 17.5 ± 0.5%, respectively) was obtained with 20% of MgO/SiO_2. The overall process (enzymatic hydrolysis followed by isomerization over MgO/SiO_2) converted 99.3 ± 0.2% of lactose into a sweetening syrup made of glucose (~30.48%), galactose (~33.51%), fructose (~16.92%), D-tagatose (~10.54%), lactulose (~3.62%), and unidentified byproducts (<0.69%). A reaction mechanism for the formation of a sweetening syrup from lactose via 2-step process was proposed. The outcomes of this research present an opportunity for expanding the utilization of lactose.

Key Words: sweetening syrup, D-tagatose, catalytic isomerization

190 Valorization of sweet and acid whey: Enzymatic generation of prebiotic lactulose for the scale up towards robust processing. C. Schmidt*, A.-K. Nedele, F. Balinger, and J. Hinrichs, *Universität Hohenheim, Stuttgart, Germany.*

Utilization of sweet and acid whey is a frequently researched and discussed topic. Especially, the non-protein fraction of acid whey is seldom utilized in food applications. Hence, the aim of this study was to valorize the non-protein fraction of sweet and acid whey by transgalactosylation of lactose to prebiotic lactulose. Therefore, 7 commercial β-galactosidases were tested regarding their ability to produce lactulose in sweet and acid whey UF-permeates (pH 4.5 to 6.5). Additional performance parameters characterizing the transgalactosylation reaction were defined and evaluated to select enzyme preparations for robust processing. Three promising enzyme preparations were identified, which provide a high lactulose yield ($Y \geq 0.2$) after 48 h at 6°C. Solely Enzeco is usable for sweet and acid whey, while Biolactase and Lactozym are only usable with sweet whey. The degree of hydrolysis exceeded 0.8 only with the bacterial enzyme preparations, which showed no affinity toward the transgalactosylation reaction. Yeast β-galactosidases provided overall higher isomeric ratios of 1-lactulose, compared with fungal β-galactosidases. Subsequently, the feed composition was optimized for the enzyme preparations using a central composite design. Concentration of lactose and fructose as well as enzymatic activity were varied on 5 levels. Using a response surface analysis the optimal feed composition for sweet (13% wt/wt fructose and 6.5% wt/wt lactose) and acid (23% wt/wt fructose and 6.0% wt/wt lactose) whey could be determined.

191 Separation of phospholipids from whey protein phospholipid concentrate. A. V. Swaminathan*[1], D. Otter[2], M. Molitor[2], K. J. Burrington[2], and J. A. Lucey[1,2], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Wisconsin Center for Dairy Research, Madison, WI.*

Phospholipids are emerging as a food ingredient due to their functional and nutritional properties. Our goal was to develop a new (non-solvent) separation process to produce an enriched dairy phospholipid (lecithin) fraction from whey protein phospholipid concentrate (WPPC). Two chemical treatments: (1) calcium-induced protein aggregation (pH 4.8) and (2) thermocalcic aggregation (pH 7.3) at 2 temperatures (50 and 80°C) were applied to rehydrated WPPC solutions. These experiments were done on a bench top scale to aid in precipitation of proteins and phospholipids. Chemically treated solutions were centrifuged and fractionated into 3 layers: (1) top fat layer (2) supernatant in middle zone and (3) sediment at bottom of centrifuge tubes. Compositional analysis was done on each layer to understand how the components (protein, phospholipid) fractionated. Treatment 1 did not aid in precipitation of proteins and about 70% of the proteins fractionated into the supernatant. Treatment 2 precipitated about 90% of the phospholipids at 80°C but the proteins precipitated along with the phospholipids. With both the treatments, proteins, fat and phospholipids always fractionated together in similar ratios. The proteins in each layer were characterized using SDS-PAGE and a range of proteins including whey proteins, caseins and milk fat globule membrane proteins were identified. The microstructure of protein and fat in WPPC was investigated using confocal laser scanning microscopy (CLSM). Dual staining of rehydrated WPPC solution with Fast Green FCF (proteins) and Nile Red (lipids) showed the presence of very large protein aggregates that varied in size from 20 to 150 µm with most fat trapped or associated with these aggregates, which explained why fat and protein co-separated with our chemical treatments. We are exploring other treatments to separate the components in these aggregates to identify a suitable separation process.

Key Words: whey protein phospholipid concentrate (WPPC), phospholipids, separations

192 Extraction of dairy phospholipids using switchable solvents: A feasibility study. K. Rathnakumar*, S. Cheng, and S. I. M. Monteagudo, *South Dakota State University, Brookings, SD.*

Phospholipids (PLs) are found as lipid bilayers in all plant and animals cells membranes. Dairy PLs are located in the milk fat globule membrane, and they account for about 1–4% of the total milk fat depending on the season and lactation stage. The consumption of PLs has been associated with numerous health benefits including reducing heart disease, cholesterol adsorption, and improved immunological functions. Dairy foods and their byproducts represent a natural source of PLs with great potential for isolation and further commercialization. The current extraction of dairy PLs involves various steps (concentration, solvent separation, lipid recovery and fractionation) within the entire process, which results in low overall efficiency and economically unviable. This study aimed at evaluating the feasibility of a primary amine (N,N-dimethylcyclohexylamine) as a switchable hydrophilicity solvent (SHS) for extracting dairy PLs. For comparison, PLs were also extracted using the Folch method (FM) consisted of chloroform and methanol mixture, and Mojonnier method (MM). PLs were extracted from 4 different matrix (raw cream, buttermilk, b-serum and buttermilk concentrate), and they were qualitatively characterized by thin layer chromatography. The extraction was performed at room temperature using different SHS ratios (3/1, 6/1, and 12/1 wt/wt). After extraction, the solvent was removed from the extract by bubbling CO_2 at atmospheric pressure. The lipid yield obtained from SHS method for raw cream was found to be higher (28.9 ± 1.3%) than FM and MM, 27.4 ± 1.6% and 29.2 ± 1.3%, respectively. Contrary, higher values of lipid yield were obtained for buttermilk, concentrated buttermilk, and B-serum using MM. Interestingly, the recovered fraction of PLs using the SHS extraction at a ratio of 12/1 was substantially higher than FM and MM. The recovered fraction of PLs for raw cream, b-serum, and buttermilk concentrated were 0.33, 7.5, 77.2, and 99.9%, respectively. These results demonstrated the feasibility of using SHS such as *N,N*-dimethylcyclohexylamine for the extraction of dairy PLs.

Key Words: phospholipid, switchable solvent, byproduct

193 Characterization of high-pressure-jet-dried skim milk powders. C. A. Hettiarachchi, G. L. Voronin*, and F. Harte, *Pennsylvania State University, University Park, PA.*

Milk powders are utilized in a variety of industrial applications as concentrated protein sources. Due to their lengthened shelf life and ease of storage and transportation, they are often preferred over liquid ingredients. Recently, we have shown that application of high-pressure-jet (HPJ) technology leads to enhance interfacial properties of fluid milk at pressures over 300 MPa. Capturing these novel properties in a dried powder form was the objective of present work, with a view to broaden the applications of milk powders. For this purpose, we combined a HPJ system with a spray dryer modified to accommodate the spray cone produced by the HPJ nozzle. Using this setup, condensed skim milk (CSM, 25% TS) was HPJ-dried into powders (termed ‘HPJD’) at 100 (control), 300, 400, and 500 MPa and they were evaluated for physiochemical properties. The powders obtained from CSM processed using HPJ at 500 MPa exhibited significantly ($P < 0.05$) increased particle density (1.482 g/cm^3) compared with the 100 MPa HPJD powders (1.328 g/cm^3), but particle size analysis reflected no significant differences between the powers obtained from CSM processed at 100 MPa (9.31 µm diam) vs 500 MPa (9.60 µm diam). The viscosity of the reconstituted HPJD powders (9% wt/wt, dry basis) increased from 2.31 mPa.s (100 MPa) to 6.03 mPa.s (500 MPa). Foam stability was determined using a foam volume stability index (FVSI), which compares the initial foam expansion to the foam remaining after a specified time. The foam stability of the 500 MPa reconstituted sample was exceptional with a foam head that remained stationary for 8 h (FVSI of 100%), while the foam in the 100 MPa sample diminished almost entirely in 2 h (FVSI of 21.3%). Emulsification ability (1:9 oil in 2% wt/wt diluted reconstitute) was determined using an automated backscattering device that describes stability as the combined difference in backscattering intensity at all heights divided by the total sample height or Turbiscan Stability Index (TSI). Plotting time vs. TSI, the reconstituted 500 MPa HPJ sample had a lower average destabilization rate (6.4 TSI/hr) than the 100 MPa sample (10.2 TSI/hr). HPJD powders have physiochemical properties that can be utilized in a variety of applications such as ice cream or specialty beverages.

Key Words: high pressure, powder, foam

194 The effect of milk predicted transmitting ability and concentrate supplementation level on selected milk quality and processability parameters in late-lactation spring-calving grazing dairy cows. M. J. Doran*[1], F. J. Mulligan[2], M. B. Lynch[1], A. G. Fahey[1], E. L. Brady[2], C. Grace[1], M. O’Sullivan[3], M. O’Rourke[3], G. Rajauria[1], and K. M. Pierce[1], [1]*School of Agriculture and Food Science, University College Dublin Lyons Farm, Naas, Co. Kildare, Ireland,* [2]*School of Veterinary Medicine, University College Dublin, Veterinary Science Centre, Belfield, Dublin, Ireland,* [3]*Institute of Food and Health, School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

Irish dairy production is based on a spring calving grazing system with the use of CS when there is a shortage of grass. Milk quality and processability deteriorates in late lactation and the issue is more pronounced in seasonal production systems. Genetics and supplementation strategies may affect MQ and MP. Therefore, the objective of this study was to investigate the effect of milk PTA, CS level and their interaction on late lactation MQ (composition) and selected MP (rennet coagulation time (RCT), ethanol stability (ES) and milk pH) parameters. A factorial experiment, with 2 milk PTA groups [high (+191 kg) versus low (−53kg)] and 2 CS levels (0 (Grass-only; GO) versus 2.7 kg DM CS) was carried out over a 51-d period. Forty-eight Holstein Friesian (24 low milk (LM) and 24 high milk (HM) PTA) dairy cows were blocked on parity and balanced on DIM, BCS and Economic Breeding Index. Cows within PTA groups were randomly assigned to one of 2 CS levels, resulting in 4 treatment groups (n = 12): 1) LM PTA offered GO (LG); 2) LM PTA plus 2.7 kg DM CS (LC); 3) HM PTA offered GO (HG) and 4)

HM PTA plus 2.7 kg DM CS (HC). All cows were grazing full time and were offered 17 kg DM grass/cow per d. There was no interaction for any parameter measured. Milk pH, ES and RCT did not differ between treatment groups. However, cows offered CS had higher ES (+2.07%, $P < 0.05$) than cows offered GO. There were no milk compositional differences between LG and LC. Casein % was higher in HG (+0.15%, $P < 0.05$) compared with HC and was also higher in LC (+0.15%, $P < 0.05$) compared with HC. Fat % tended to be higher in LG (+0.21 kg, $P = 0.06$) compared with HG. Cows in the HM PTA group had decreased fat % (−0.22%, $P < 0.01$), protein % (−0.12%, $P < 0.05$) and casein % (−0.11%, $P < 0.01$), while CS also reduced fat % (−0.17%, $P < 0.05$), protein % (−0.14%, $P < 0.05$) and casein % (−0.11%, $P < 0.01$). In conclusion, CS and HM PTA negatively affected milk composition. Offering CS increased ES suggesting CS may increase colloidal stability of milk.

Key Words: dairy cow, late lactation, milk processability

195 High-pressure-jet alterations in ice cream mix physiochemical properties and its potential to replace non-dairy emulsifiers and stabilizers. G. L. Voronin*, R. Roberts, and F. Harte, *Pennsylvania State University, University Park, PA.*

The current consumer base is driving a movement for ‘clean label’ foods, i.e., foods with few and familiar ingredients. High pressure jet technology has recently shown potential in the development of clean functional ingredients by increasing foaming, viscosity, and emulsifying properties in skim and whole milk. The objective of this study was to utilize high pressure jet-induced functional properties in a low fat (6% fat) ice cream mix to create properties similar to those seen with the addition of emulsifiers and hydrocolloids. Ice cream mix or serum (nonfat portion of the ice cream mix) were subjected to 0.1 MPa (control), 200 MPa and 400 MPa high pressure jet processing. A similar formulation but containing polysorbate 80 (0.075% wt/wt) was also used as a control. The treated samples were evaluated for particle size, density, emulsifying and foaming properties, flow properties, crystallization kinetics, and fat-protein interactions. Foaming properties were evaluated by a foam expansion index (FEI), which compares the foam expansion to the initial liquid volume. The ice cream mix made with serum processed at 400 MPa exhibited significantly ($P < 0.05$) enhanced foam potential, foam stability, and mix stability, with FEI at 0 h and 8 h of 90% (~18% above control) and 78% (~78% above control), respectively. The ice cream mix processed at 400MPa showed increased viscosity (170 mPa.s vs. 3 mPa.s in control), increased foam stability (FEI = 46% at 8 h), increased particle size (D90 = 30 um vs 0.9 um in controls). These differences were attributed to an increase in casein-fat interactions and casein-casein interactions caused by the 400 MPa high pressure jet treatment. An increase in protein concentration in the sediment after ultracentrifugation (100 × kG for 30 min) supported this conclusion. Interestingly, the density of the ice cream mix processed at 400 MPa was significantly ($P < 0.05$) lower (0.69 g/mL) than the control (1.04 g/mL) as micro-bubbles were trapped in the mix matrix. These findings support the potential to use high pressure jet technology to reduce the need for non-dairy emulsifiers and hydrocolloids in low fat ice cream formulations.

Key Words: high pressure, ice cream, casein

196 Coating spent coffee grounds with whey protein: A valorization study. J. Osorio-Arias*[1], O. Vega-Castro[2,1], and S. Martínez-Monteagudo[3], [1]*Universidad de Antioquia, Medellín, Antioquia, Colombia,* [2]*Corporación Universitaria Americana, Medellín, Antioquia, Colombia,* [3]*South Dakota State University, Brookings, SD.*

Food and dairy industry are now considering waste streams as important actors in the transition to sustainable manufacture. It is crucial to develop cost-effective processing alternatives for the utilization of such streams that not only creates value from waste materials but also reduces the environmental impact. In this work, we studied the valorization of spent coffee ground (SCG), a rich source of fatty acids, amino acids, polyphenols, minerals, and polysaccharides. Our objective is to evaluate the structural properties of a powder obtained by spray drying containing different percentages of whey protein concentrated. Different percentages of SCG (4–16%) were mixed (2500 rpm for 5min) with concentrated whey (total solids 39%). Afterward, the mixture was spray dried using an inlet temperature of 170°C. The moisture content and water activity of obtained powder were 3.25 ± 0.18 and 0.252 ± 0.01, while the bulk density was 0.495 ± 0.10 g/cm^3, showing a significant correlation ($P < 0.05$) with the particle size (43.87 µm). This observation is due to the fact that small particles can occupy spaces between large particles, increasing the apparent density of the system. Such an effect was confirmed by Scanning electron microscope. Moisture-sorption isotherms were determined (17–45°C) and the moisture content at the monolayer was between 0.043 and 0.086 kg of water/g dry basis, and the isosteric heat was 27.65 kJ/mol, which decreased as a function of the increase in moisture content. On the other hand, some amino acids were determinate in the powder samples phenylalanine (135.97), lysine (68.91), leucine (406.12), isoleucine (613.07) and proline (192.79) all in mg/100g. In general, the addition of whey concentrated as a coating medium for SCG particles shows great potential for the fortification of dairy products.

Key Words: whey protein concentrate, spent coffee grounds, valorization of by-products

197 Comparison of micellar casein isolate and nonfat dry milk for the production of acid milk drinks. D. Wilbanks*[1], M. Lee[2], J. Lucey[1], and Y. Rahimi[3], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Daegu University, Gyeongsan, North Gyeongsan, South Korea,* [3]*Arla Foods, Viby J, Denmark.*

Acid milk drinks (AMD), such as drinking yogurt and kefir, are popular throughout the world. Over time, instability of these drinks can occur by aggregation of protein particles and whey separation. The objectives of this research were to investigate physio-chemical properties of high protein AMDs and their stability over time. Micellar casein isolate (MCI) is a dairy powder that contains a high ratio of casein to whey proteins and is obtained by microfiltration of milk. The first phase of this research was to compare 2 types of milk powders: MCI and nonfat dry milk (NFDM) – to observe how the use of different dairy proteins impact gel strength and stability of the fermented products. Milk bases were prepared (n = 2) at 8% protein, heated to 85°C for 30 min, cooled, and inoculated with starter culture and incubated at 40°C to pH < 4.3. Acidification rates were measured during fermentation along with small amplitude oscillatory rheology (SAOR) measurements using a rheometer. After incubation for 14 h gels were sheared at high and low speeds for 60 s in a blender and rheology, particle size (Malvern Mastersizer 2000), and pH were measured immediately post-shear and at 4 weeks of ambient storage. Acidification was faster for MCI due to less buffering compared with NFDM. SAOR performed during fermentation revealed that gels produced from MCI had lower storage and loss moduli compared to samples made with NFDM. The time-dependent recovery of gel structure after shear was observed in both AMDs and neither products remained fluid throughout storage, although MCI had lower gel stiffness immediately after shearing and after 4 weeks of storage. The yield stress of sheared products was higher for samples made from NFDM compared with MCI. Protein aggregation as measured by particle size was greater in NFDM, likely due to the higher concentration of denatured whey proteins. In conclusion, the weaker gel produced by MCI makes it an interesting choice for high protein AMDs, though a hydrocolloid like pectin may still be needed for long-term stabilization of the beverage at ambient temperature.

Key Words: micellar casein, stability, high protein

Forages and Pastures Symposium: Silage Conservation Practices and Management—Effects on Forage Quality, Farm Profitability, and Feed Efficiency

198 Silage additives: Effects on silage fermentation and animal production. L. Kung Jr.*, *University of Delaware, Newark, DE.*

Silage fermentation is an uncontrolled process affected by a multitude of forage factors and management practices. In general, undesirable fermentations result in excessive losses of DM and energy, and can result in an accumulation of unwanted end products that can negatively affect animal performance. Various types of additives including enzymes, organic acids, and microbial inoculants have been used to maintain and improve forage quality and improve aerobic stability during ensiling. For example, plant cell wall degrading enzymes have been added to silage to improve digestibility. However, true pre-digestion of nutrients in the silo is a questionable goal. Instead, the goal should be to improve the digestibility of silage when it reaches the rumen and or intestine of the cow. Short chain organic acids such as propionic acid, potassium sorbate, and sodium benzoate have good antifungal properties that can suppress yeasts that are responsible for initiating aerobic spoilage. Various microbial organisms have also been added to silages to improve fermentation. Originally classified as homolactic acid bacteria, such organisms were used to hasten silage fermentation and result in fermentations that recovered more DM and energy. Ironically, extremely efficient fermentations can lead to poor aerobic stability of silages. Over a decade ago, the introduction of L. buchneri, a heterolactic acid bacteria, resulted in a paradigm shift because it was able to anaerobically convert moderate amounts of lactic acid to acetic acid, which is highly antifungal in nature and thus improved aerobic stability. Changes in silage fermentation and aerobic stability from the use of additives can have direct and indirect effects on animal performance. This review will give a history of various silage additives, their modes of action and effects on animal production.

Key Words: silage, additive, inoculant

199 Quantification of the emission reduction benefits of mitigation strategies for dairy silage. F. M. Mitloehner*, *Department of Animal Science, University of California, Davis, Davis, CA.*

Dairy silages can be a major air emission source of volatile organic compounds (VOCs). In general, emission of VOCs from silage can be mitigated by either 1) reducing VOC production in the liquid/solid phase of the silage pile, or 2) reducing relative emission from the face of the silage pile or the feedlane. The focus of the present research was on monitoring and modeling of VOC production, as well as emissions mitigation via various silage storage methods, de-facing practices, and feed management approaches. For the field monitoring of emissions from different silage storage and defacing methods, we used flux chambers and wind tunnels that were attached vertically on the silage face, immediately after de-facing. Different storage methods (i.e., conventional standard pile vs silage bag), and defacing methods (e.g., perpendicular, lateral, and rake extraction) were compared aiming at reducing emissions. The monitoring data was used to inform and validate a new VOC process-based model that was developed to predict VOC emissions from silage sources on farms using theoretical relationships of mass transfer and parameters determined through our earlier laboratory experiments and numerical modeling. The results for silage storage indicated that silage bags vs. conventional silage piles emit considerably fewer VOC emissions. Furthermore, lateral defacing versus perpendicular- and rake defacing reduced emissions of most VOCs. Simulations of all relevant silage mitigation options that were studied on the commercial dairies were conducted using the VOC modeling tool. These simulations clearly showed that most of the reactive VOC emissions on a California dairy occur from feed lying in feed lanes during feeding as opposed to the silage storage pile or bag. However, one shall not view those monitoring results in isolation, because only the integration of other parts of the feed's life cycle, using whole farm modeling, explains not just the relative but also the absolute effectiveness of mitigation techniques in reducing VOCs on the entire dairy. The whole farm modeling clearly showed that mitigation efforts should be applied to reducing VOC emissions from feeding rather than focusing solely on those from the exposed face of silage piles.

200 Utilization of silages in the diets of high-producing dairy cows: Effects on milk production and feed efficiency. K. F. Kalscheur*, *USDA-ARS, US Dairy Forage Research Center, Madison, WI.*

Silage is an integral ingredient in the diets of high-producing dairy cows throughout the world. In North America, silages formulated into the diets of high-producing dairy cows are predominantly corn silage and alfalfa silage along with smaller usage of other forages such as small grain silage and grass silage. Silages can be one of the least expensive feedstuffs to include in dairy cow diets, therefore it can be advantageous to include as much forage into the lactating dairy cow diet if it does not limit intake and does not result in decreased milk production. Typically, diets with increasing forage NDF concentration decrease DMI attributed to greater forage NDF concentration and bulk density of the diet. While increased forage NDF concentration may decrease milk production, with proper diet formulation and the inclusion of high quality silages, milk production may be maintained and feed efficiency may be improved. Implementation of harvesting techniques such as increased cutting length can improve nutrient digestibility of the resulting silage. In addition, selection of forage varieties containing the brown midrib (BMR) gene results in silage with lower lignin, higher fiber digestibility, greater intake, and potentially higher milk production. Lower lignin alfalfa varieties are now available that may result in greater fiber digestibility and potentially improved production. Utilizing both BMR silages and lower-lignin alfalfa varieties into one diet may help push the upper limit of how much forage can be included while maintaining high milk production and improving feed efficiency.

Key Words: dairy cow, silage, feed efficiency

201 Baled silage management: Effects on silage fermentation, forage quality, and animal production. W. Coblentz*, *US Dairy Forage Research Center, Marshfield, WI.*

The production of baled silages is increasingly popular, particularly with small and mid-sized dairy and beef producers. There are several reasons this silage preservation technique is attractive to producers, but the primary advantage is a reduced risk of weather damage to valuable forage crops compared with preservation as dry hay. Most core principles for making high-quality baled silages are similar to those for precision-chopped silages; among these, maintaining anaerobiosis is a priority. However, there are some notable differences between silage types. Principal among these is the restriction of rate and extent of fermentation within baled silages, which often results in less production of desirable fermentation acids and a greater (less-acidic) final pH. For baled silages, fermentation restrictions occur in part because recommended moisture concentrations (45 to 55%) are drier, and particle-length is much longer. Preservation of baled silages is optimized by applying polyethylene (PE) film wraps promptly, using an appropriate number of film layers (6 to 8), selecting an appropriate storage site, and by close monitoring for evidence of puncture, particularly by birds or vermin. The heterogeneous nature of baled silages, coupled with a restricted rate and extent of fermentation, may increase clostridial activity relative to precision-chopped forages ensiled at comparable moisture concentrations. To date, research evaluating inoculants or other additives designed to improve the fermentation has been limited in scope. Embedding PE films with an oxygen-limiting barrier has been beneficial in some trials, but most differences between these novel plastic formulations and commercial PE films have been limited to decreased yeast and mold counts at the surface layer; whole-bale assessments of fermentation or nutritive value have been less conclusive. Recent evaluations of bale-cutting mechanisms have demonstrated modest improvements in fermentation, but bale-cutting is difficult to justify solely on that basis. Baled silages can be produced successfully by adhering to straightforward management principles, and is likely to remain popular in the well into the future.

Key Words: baled silage, fermentation, pH

Lactation Biology 1

202 Attenuation of melatonin and serotonin circadian rhythms during the dry period is related to increased milk yield. A. Suarez-Trujillo*[1], H. Sun[2], J. Franco[3], K. Huff[1], T. Steckler[1], J. Boerman[1], M. Grott[4], J. Townsend[5], J. Jhonson[6], K. Plaut[1], and T. Casey[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Department of Statistics, Purdue University, West Lafayette, IN,* [3]*Metabolite Profiling Facility, Bindley Bioscience Center, Purdue University, West Lafayette, IN,* [4]*Animal Sciences Research and Education Center, Purdue University, West Lafayette, IN,* [5]*Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN,* [6]*USDA-ARS Livestock Behavior Research Unit, West Lafayette, IN.*

The circadian timing system (CTS) synchronizes internal physiology with regularly occurring cues such as light-dark cycle. To understand the role of circadian rhythms in regulation of lactogenesis we exposed cows to chronic phase shifts in light-dark cycle (PS) to disrupt the CTS during the dry period. Milk production efficiency increased in PS exposed cattle with no detrimental effects on blood NEFA or BHB levels. Our objective was to measure effect of PS exposure on melatonin (MEL) and serotonin (5HT) levels and circadian rhythms in cows to determine if alterations were associated with changes in production efficiency. Briefly, 5 wks before expected calving (BEC) multiparous cows were moved to tie-stalls and divided into 2 treatments: control (C, n = 16; 16 h light: 8 h dark) or phase-shifted (PS, n = 16; exposed 6 h light-dark phase shifts every 3 d). All cows were exposed to control lighting after calving. Blood samples were taken at 0600 on d 35 BEC, 21 BEC, 2 before calving (BC), and d 0, 2, 9, 15 and 22 postpartum (PP). A subset of cows (n = 6/group) were sampled every 4 h over 48 h at 3 time points: d 23 BEC, 9 BEC, and 5 PP. LC-MS/MS was used to measure MEL and 5HT. Body temperature was recorded every 30 min vaginally using calibrated iButton data loggers at d 23 BEC and d 9 BEC. PS ($P < 0.05$) shifted phase and attenuated circadian rhythms of core body temperature. The overall effect of PS was to increase MEL and decrease 5HT ($P < 0.05$) at 0600 sampling. MEL was > 1.5-fold higher on d 15 PP and 22 PP in PS than C; whereas 5HT was 1.3-fold lower ($P < 0.05$). Cosine fit analysis found MEL circadian rhythms were significantly affected by treatment with C cows exhibiting strong rhythms across 3 time points ($r^2 \geq 0.72$; $P < 0.01$) and PS cows only fitting 24 h rhythm at d 23 BEC ($r^2 \geq 0.72$; $P < 0.01$). Circadian rhythms of blood 5HT was only evident at d 9 BEC in C cows ($r^2 = 0.68$; $P < 0.01$), area under the curve of 5HT (170 ± 29 and 235 ± 29 mg/ml; $P = 0.13$) and MEL (1.62 ± 0.15 and 1.94 ± 0.14 ng/mL; $P = 0.14$) was increased from d 9 BEC to 5 PP, respectively across the treatments. These data led to hypothesis that the attenuation of circadian rhythms facilitates lactogenesis to increase milk production efficiency.

Key Words: circadian rhythms, melatonin, serotonin

203 Circadian clock gene *BMAL1* regulates mammary epithelial cell growth and milk protein expression. K. Huff*[1], S. Cummings[2], M. Brown[2], A. Suarez-Trujillo[2], K. Plaut[2], and T. Casey[2], [1]*Department of Biochemistry, Purdue University, West Lafayette, IN,* [2]*Department of Animal Sciences, Purdue University, West Lafayette, IN.*

There is growing evidence that the mammary circadian clock regulates milk production, but the mechanism of regulation is poorly understood. *BMAL1* is a core clock gene that acts as a transcription factor when it dimerizes with CLOCK to regulate the molecular circadian rhythm. We hypothesized that *BMAL1* regulates mammary cell proliferation and metabolic activity. To test this hypothesis, *BMAL1* was knocked out (BMAL1-KO) of the mouse mammary epithelial cell line HC11 using CRISPR-Cas9. A monoclonal colony was selected for study based on PCR and Western blot analysis indicative of homozygous knockout of the *BMAL1* gene. Growth and differentiation of BMAL1-KO was compared with the wild-type (WT) line. Two-way ANOVA found line and day had an effect on 8-d growth curve (n = 5 experiments). Log phase doubling time was not different ($P > 0.05$) between WT and BMAL1-KO. However, by d 6 and 8, the BMAL1-KO cells reached stationary phase at a lower cell density ($P < 0.05$) than WT HC11. BMAL1-KO lines exhibited lower ($P < 0.05$) MTT absorbance on d 2 and 4. Images of the MTT-stained cells showed denser staining within the cytoplasm of HC11 compared with BMAL1 KO. Previous studies found BMAL1 KO mice exhibited accelerated aging attributed to reactive oxygen species (ROS). Initial study with commercial assay suggests that BMAL1-KO cells have higher ROS levels, and thus build up may be causing cell death. Q-PCR analysis found BMAL1-KO cells expressed significantly lower ($P < 0.05$) β-casein mRNA, which was confirmed with Western blot analysis. These data support that mammary clock plays a central role in regulating metabolic activity and cellular detoxification.

204 Effect of a single high dose of dexamethasone on milk production, composition, and mammary gland gene expression. A. Sadovnikova[1], A. Mathews[1], S. Garcia[2], J. Trott[1], and R. Hovey*[1], [1]*University of California, Davis, Davis, CA,* [2]*University of Sydney, Sydney, NSW, Australia.*

While chronic stress can decrease milk production, an acute, high-dose of a synthetic glucocorticoid such as dexamethasone (DEX) can abruptly and transiently suppress milk yield. The mechanism underlying this response, and whether it is realized systemically or locally within the mammary gland, has not been established. We sought to define the effect of acute DEX on the yield and composition of milk produced by dairy cows and the accompanying changes in mammary gland gene expression. A single, high dose of DEX (40 mg, IM) was administered to 4 multiparous lactating Holstein cows (55.7 ± 5.3 DIM, 746 kg average body weight). Mammary gland biopsies were performed on alternating quarters at 0, 12, 24, and 72 h post-DEX. To define changes in milk volume and composition, cows were quarter-milked every 12 h for 7 d before through 5 d after DEX. Oxytocin (IV, 5 IU) was administered after each milking to ensure complete milkout, and milk samples were collected both before and after oxytocin. Milk was analyzed for lactose, protein, fat, casein, and solids. Total RNA was isolated from biopsied mammary tissue and subjected to 3′-TAG RNA sequencing for differential gene expression analysis. Data were analyzed with a mixed effects repeated measures ANOVA. Within 12 h of DEX, average 12-hourly milk yield decreased from 29.1 to 22.5 kg ($P < 0.05$) and returned to baseline by 72 h. Lactose concentration decreased from 4.96% at 0 h to 4.84% at 72 h ($P < 0.05$). There were no differences in the concentration of casein, fat, total protein, or solids in milk. Based on 3′-TAG RNA sequencing data, α-lactalbumin *(LALBA)* gene expression was suppressed within 12 h of DEX ($P < 0.05$). Complete differential gene expression and KEGG pathway analyses will define which genes and pathways are stimulated or suppressed in the lactating mammary gland in response to DEX. These data show that DEX induced a transient suppression of milk production, *LALBA* expression, and lactose production, without affecting fat, total protein, or casein content.

Key Words: mammary gland, lactation, gene expression

205 The mechanistic target of rapamycin complex 1 plays a central role in dietary amino acid regulation of murine lactation.

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Dietary AA, the building blocks of proteins, drive synthesis of milk protein in the mammary gland. The mechanistic target of rapamycin complex 1 (mTORC1) is a ubiquitously expressed, highly conserved kinase complex that integrates nutrient and endocrine signals to regulate metabolic functions, including synthesis of milk components. We hypothesized that mTORC1 indeed plays a crucial role in the regulation of murine lactation by dietary AAs.To test this hypothesis, C57BL/6J dams were randomly assigned to 1 of 3 treatment groups (n = 7 dams, litters standardized to 5 pups/dam) on lactation day (LD) 1: adequate protein (HP, 18% CP), protein restricted (LP, 9% CP), or HP plus every other day IP injection of 4 mg/kg rapamycin (HPR). On LD13, mice were killed and tissues harvested for analysis. Live animal data, including litter and dam weights, milk production, and dam daily food consumption were analyzed by 2-way ANOVA with repeated measures in RStudio. Tissue Western blot data were analyzed with one-way ANOVA with Dunnett post hoc multiple comparisons against HP treatment. By LD13, milk production was 66% less for LP and 48% less for HPR than HP ($P < 0.05$). Similarly, litter weight was 31% less for LP and 28% less for HPR than HP ($P < 0.05$). Dam weight remained unchanged despite differences in lactation performance. Phosphorylation of mTORC1 substrates S6K1(T389), S6(S240/244), and 4E-BP1(S65) in the mammary glands decreased, respectively, 30, 37, and 66% for LP, and 61, 85, and 33% for HPR ($P < 0.1$), compared with HP. Overall, pharmacological inhibition of mTORC1 closely mimicks the effect of severe dietary AA restriction on lactation performance, demonstrating a central causal role of mammary mTORC1 in the regulation of milk and milk protein production by dietary AAs. Although, systemic effects of rapamycin may have contributed to the lactation phenotype of HPR mice.

Key Words: amino acid, mammary mechanistic target of rapamycin complex 1 (mTORC1), milk protein

206 Heat stress reduces total mammary blood flow and trans-mammary disappearance of metabolites in lactating dairy cows.

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We profiled total mammary blood flow and trans-mammary disappearance of metabolites in heat-stressed lactating dairy cows. Eighteen mid-lactation Holstein cows were housed in tie-stalls in environmental chambers and subjected to one of the 3 treatments: (1) hyperthermia and ad libitum feed intake (HS), (2) normothermia and pair-feeding relative to HS (PF), or (3) normothermia and ad libitum feed intake (CT). All cows were housed under CT for 5 d before onset of treatments. Hyperthermia was modulated over 14 d of constant heat stress by increasing THI to ~80, thereby elevating rectal temperatures (RT) to ~40C, then maintaining RT at that level. Cows on normothermia were exposed to constant thermoneutrality (THI~66). After hyperthermia, HS cows were returned to CT conditions for 8 d to evaluate recovery. Total mammary blood flow was measured throughout the trial by transrectal scanning of both right and left external pudic arteries using color Doppler ultrasound equipped with a convex probe. Mammary arteriovenous blood samples were collected, analyzed for concentration of metabolites, and trans-mammary metabolite disappearance was calculated. During hyperthermia, mean RT was higher ($P < 0.001$) in HS than PF and CT (40.1, 38.4 and 38.5 ± 0.1°C, respectively), DMI was reduced similarly ($P < 0.001$) in HS and PF, relative to CT (11.0, 11.2 and 19.8 ± 0.8 kg/d, respectively), and milk yield was reduced more ($P < 0.10$) in HS than in PF, relative to CT (18.2, 21.1 and 27.8 ± 1.4 kg/d, respectively). Similar to DMI, total mammary blood flow decreased ($P < 0.002$) in HS and PF compared with CT (405, 363 and 557 ± 36 L/h, respectively). Relative trans-mammary disappearance of glucose was similar between PF and CT but higher ($P < 0.01$) than HS (23.0, 22.4, 16.5 ± 1.8%, respectively). Relative trans-mammary disappearance of triglycerides did not differ between treatments, averaging 46.5 ± 2.6%. For all variables measured, HS and PF cows returned to CT levels within 8 d of recovery. Heat stress reduces total mammary blood flow and trans-mammary disappearance of metabolites in lactating dairy cows.

Key Words: mammary uptake, nutritive blood flow, non-nutritive blood flow

207 The mechanistic target of rapamycin complex 1 controls lipid and lactose synthesis in bovine mammary epithelial cells.

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The mechanistic target of rapamycin complex 1 (mTORC1) is a nutrient-sensing pathway that plays a central role in the regulation of growth and metabolism. Previous work has implicated mTORC1 in the control of protein synthesis in bovine mammary epithelial cells (BMEC), but whether it is involved in regulation of other milk components is not known. The objective of this study was to assess the role of mTORC1 on lipid synthesis and lactose synthesis in BMEC. BMEC were isolated by enzymatic digestion of lactating mammary tissue and induced to differentiate by incubation with lactogenic hormones (5 µg/mL insulin, 5 µg/mL prolactin and 5 µg/mL hydrocortisone). Protein abundance and site-specific phosphorylation was measured by immunoblotting. Relative mRNA transcript levels were measured by real-time quantitative PCR. Lipid and lactose synthesis were measured by the incorporation of radiolabeled precursors. Data were analyzed by ANOVA using a randomized complete block design with PROC MIXED in SAS and differences were consider significant at $P < 0.05$. To establish the role of mTORC1 on milk synthesis, we first established that our cell model was capable of lactogenic differentiation. *CSN1S1* ($P = 0.02$) and *LALBA* ($P = 0.03$), 2 important genes encoding for milk protein were significantly increased by our lactogenic treatment. To confirm the role of mTORC1 in milk component synthesis we used CRISPR/Cas9-mediated knockout (KO) of tuberous sclerosis complex 2 (TSC2), an upstream negative regulator of mTORC1, as gain-of-function genetic model in primary BMEC. TSC2 KO cells had higher phosphorylation ($P < 0.05$) of both 4EBP1 (Thr70) and rpS6 (ser240/244) compared with cells transduced with a non-targeting (CTR) guide RNA, confirming mTORC1 hyper activation. TSC2 KO cells had higher gene expression on both *SREBF1* ($P = 0.01$) and *FABP3* ($P = 0.001$) compared with CTR cells. Infected BMEC also showed an increase gene expression of 35% ($P = 0.03$) for *PGM1* and an increase of 44% ($P = 0.006$) for *B4GALT1*. In conclusion, these results demonstrate a critical role for mTORC1 in the regulation of both lipid and lactose synthesis in BMEC. M.-A. G. was funded by Op$^+$LAIT. This work was supported by FRQNT and NSERC.

Key Words: mechanistic target of rapamycin complex 1 (mTORC1), bovine mammary epithelial cells (BMEC), CRISPR

208 Quantitative histological changes in lactating bovine mammary gland after endotoxin challenge.

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Infection of mammary glands by pathogenic bacteria causes inflammation and elicits structural and functional regression of mammary alveoli. The aim of the study was to evaluate quantitative changes in mammary alveolar structure, leukocyte infiltration into alveoli, and mammary apoptosis in response to infusion of endotoxin (lipopolysaccharide; LPS) in lactating cows. Ten multiparous cows, blocked by days in milk, parity and milk yield, were divided into treatment and control groups. In treatment (T) cows, both the front and rear quarters on the right or left side of the udder were assigned randomly to receive either LPS (50 µg in 10 mL, TL) or saline (10 mL, TS); the contralateral quarters received the other treatment. Udder-halves of control (C) cows were similarly assigned to receive either saline (10 mL, CS) or no infusion (untreated; CU). Mammary biopsies were obtained at 0 h (0600), 3 h (0900) and 12 h (1800) from each hind quarter of treatment and control cows. Endotoxin treatment induced 5.2- and 7.2- fold increases in number of neutrophils in alveolar lumina at 3 h and 12 h, respectively ($P < 0.01$), whereas neutrophils were rarely observed in the saline-infused control glands (TS and CS). Although the alveolar size was increased at 3 h and 12 h in comparison to 0 h ($P < 0.05$), it was not affected by LPS challenge. At 12 h, there was a decrease in the mean area stained positively for E-cadherin, an epithelial cell marker, in mammary glands treated with endotoxin (6.89 + 0.28% vs. 8.75 + 0.30%, or 8.1 + 0.29%, $P < 0.01$) for TL vs TS or CS, respectively. In addition, the percentage of cells stained with cleaved caspase-3, a marker of apoptosis, was increased by 3 h post-LPS infusion and showed significant time by treatment effects (15.9 + 0.92% vs. 8.1 + 0.92%; $P < 0.01$) for TL vs. TS. The majority of cleaved caspase-3-positive cells were stromal cells. These results demonstrate that LPS induces neutrophil infiltration into the alveolar lumen within 3 h, decreases E-cadherin expression, and promotes apoptosis in the mammary gland of lactating cows.

Key Words: apoptosis, E-cadherin, lipopolysaccharide

209 Omega-3 and omega-6 fatty acids are more abundant in colostrum than in transition and whole milk.

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Consistent increases in colostrum and transition milk fatty acids (FA) will indicate a biological necessity for the neonatal calf. The objective of this study was to infer neonatal requirements from the persistent elevation of specific FA in primiparous (PP) and multiparous (MP) colostrum. Colostrum was milked (M1, 5.3 ± 0.7 h post-calving) from 10 PP and 10 MP (parity = 3.1 ± 0.4; Breevliet Farms Ltd., Wetaskiwin, AB, Canada) Holsteins that consumed the same pre- and postpartum rations. Milkings 2 to 5 (M2–5; inter-milking interval = 12.0 ± 0.3 h) and 12 (M12) were respectively termed transition and whole milk. Fatty acids were determined by gas chromatography (AAFC, Lacombe, AB, Canada). Data were analyzed by parity, milking (repeated), the parity-milking interaction (P × M), and cow (random). Differences were understood as $P < 0.05$ and trends were noted when $0.05 \leq P < 0.10$. Overall, PP C18:2n-6 was 0.39%-units greater ($P < 0.05$) and C18:3n-6 tended to be 0.008%-units greater ($P = 0.06$) than MP, while C20:4n-6, C20:5n-3, and C22n-3 increased ($P < 0.01$) by 0.08-, 0.02-, and 0.008%-units for MP compared with PP cows. The n-3 and n-6 FA (% total) were uniformly elevated ($P < 0.01$) at M1 compared with all other milkings. Their proportions declined ($P < 0.01$) abruptly by 21.5 (C18:2n-6), 30.4 (C18:3n-6), 22.4 (C20:4n-6), 36.3 (C20:5n-3), and 35.7% (C22:6n-3) at M2 and they were respectively 76.8, 53.6, 28.4, 27.4, and 35.7% of their M1 abundance at M12. C22:6n-3 was proportionally greatest at M1 due to MP colostrum having 1.6, 2.3, 2.7, and 4.0 × more (P × M, $P < 0.01$) C22:6n-3 at M1 than M3, M4, M5, and M12, whereas PP C22:6n-3 was 378.6, 458.9, and 296.8% less at M1, M2, and M3 than MP and did not differ between milkings. Otherwise, increased colostral n-FA was consistent between parities regardless of P × M interactions (P × M, $P < 0.01$: C18:2n-6, C20:4n-6, and C20:5n-3; P × M, $P = 0.08$: C18:3n-6). C20:4n-6, C20:5n-3, and C22:6n-3 yields were 1.46 ± 0.17, 0.32 ± 0.03, and 0.041 ± 0.007 g less ($P \leq 0.01$) during M12 than M1 but stable from M1 to M5. Greater proportions and yields of n-3 and n-6 FA in colostrum indicate a neonatal requirement for n-FA.

Key Words: colostrum, neonate, omega fatty acids

210 Milk fat-to-protein ratio as a proxy for energy balance in early lactation dairy cows.

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This study used a meta-analysis approach to evaluate relationships between milk fat-to-protein ratio (FPR) and calculated daily energy balance (EB) in early lactation dairy cows. The data set was collected from 20 experiments (67 treatments, 736 cows – mostly Holstein Friesian). Perennial ryegrass silage was the predominant forage source in all experiments (mean forage-to-concentrate ratio on a DM basis was 47:53). Daily EB (MJ of metabolizable energy; ME/d) was calculated for each cow using equations in 'Feed into Milk' (UK dairy cow rationing system), daily intakes and milk yields, weekly live-weights and either weekly or fortnightly milk composition data. Concentrate ME contents were determined from 'book values' (FeedByte - SAC Consulting) for individual ingredients, while the ME content of forages offered were mostly determined via NIRS predictions. A mean EB for each week (MJ of ME/d) was subsequently calculated. Data were split into 3 periods (wk 1–4, 5–8 and 9–12 post calving) and analyzed separately for parities 1, 2 and 3. Relationships between FPR and EB were examined on the same occasions (n = 894) that blood samples were collected for each individual cow as described in a companion abstract. The data were analyzed using a Linear Mixed Model (REML estimation method). Study and cow within study were fitted as random effects in the model. The mean FPR (±SD) for parity 1, 2, and 3 were 1.29 ± 0.205, 1.26 ± 0.209, and 1.28 ± 0.227 respectively. The FPR was negatively related to EB and this trend was consistent across all parities and periods. All regression slopes of the linear equations were statistically different ($P \leq 0.01$). Daily EB responses for wk 1–4 were −5.71, −8.15, and −6.68 MJ of ME/d per 0.1 units of FPR, whereas values for wk 5–8 were −4.73, −8.25, and −6.14 MJ of ME/d per 0.1 units of FPR (for parity 1, 2, and 3 respectively). At 9–12 weeks post calving, EB responses to FPR became more negative as parity increased: −4.36, −6.85, and −8.77 MJ of ME/d per 0.1 units of FPR (for parity 1, 2, and 3, respectively). This meta-analysis confirms that FPR is a good proxy for EB in dairy cows.

Key Words: dairy cow, energy balance, fat-to-protein ratio

211 Milking interval did not affect the increased milk yield carry-over effect of increased milking frequency in early lactation.

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Increasing milking frequency (MF) increases milk yield (MY) and farm profit. Early lactation increased milking frequency (IMF) involves milking cows 4 times daily (4×) for the first 21 d post-calving followed by twice-daily milking (2×) for the remainder of lactation. Increasing MF in early lactation increases production during and after the IMF period. Milking intervals (MI) describes the timing of daily milkings. Uneven 4× MI in early lactation may be easier to apply because employees can milk 4× cows at the beginning and end of 2× milking sessions. The objective of this experiment was to compare the MY effect of even and uneven 4× MI in early lactation IMF. Fourteen multiparous and 6 primiparous cows were milked using unilateral frequent milking with right udder halves milked 4× and left udder halves milked 2× for 20 d in early lactation. Ten (7 multiparous and 3 primiparous) cows were allocated evenly based on parity and assigned to either the even or uneven MI groups. The uneven and even MI groups were assigned to 9:3:9:3 h or 6:6:6:6 h interval respectively. Milk from each udder half was weighed and sampled for components on the final day of treatment and at 60, 120, 180, 240, and 300 DIM. On d 20, 4× udder halves produced 4.78 ± 1.02 kg and 7.31 ± 0.99 kg more milk than the 2× halves for the even and uneven MI groups. There was no significant difference between MI groups for udder half difference on d 20 ($P = 0.07$) or throughout lactation. The increased MY carry-over effect in 4× udder halves averaged 1.07 ± 0.55 kg/d for the even MI group and 0.63 ± 0.28 kg/d for the uneven group through 300 DIM. There was no significant MY difference between udder halves after cows were returned to 2× milking. IMF increases labor costs and uneven MI may optimize net income from increased MY minus elevated labor costs. This project was supported by Agriculture and Food Research Initiative Competitive Grant 2017-67015-26538 from the USDA National Institute of Food and Agriculture.

Key Words: increased milking frequency, milking interval, lactation

212 Fitting extended lactation curves of Holsteins.

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There is potential to optimize lactation lengths on an individual cow basis to allow for extended lactations in cows that exhibit high production and/or persistency at 305 DIM. This requires producers to make delayed breeding decisions within the voluntary waiting period. To identify which cows might be suitable for extended lactation, models can be used to forecast milk yields (MY). In this study, 3 models (Wood, Wilmink and Dijkstra) were fitted to a data set of daily MY (n = 651) from extended lactations >305 DIM. Lactations were grouped by parity (first and greater) and lactation length (305–404 DIM, 405–505 DIM and 505+ DIM). Within each group, the models were fitted to individual curves. Each model had a high goodness of fit for each group, with a mean root mean square prediction error (RMSPE) of 9.3% of the milk yield average. There were no significant difference of goodness of fit between models ($P = 0.05$) in the parity and lactation length groups, although in each group the Dijkstra model yielded numerically higher fit statistics. Due to this advantage and the mechanistic nature of its parameters, the Dijkstra model was used to predict 305-d MY after fitting to data from only the first i DIM, where i was incremented from 30 to 300 DIM in weekly intervals. Residuals from each 305-d MY prediction were used to calculate RMSPE. In the 30 DIM group, RMSPE was 61136 kg but it declined rapidly to 11.9 kg at 111 DIM, after which additional data only marginally improved the RMSPE to 9.7 kg at 300 DIM. The probability that 305-d MY for an individual cow would be above a given target for inclusion in an extended lactation program was estimable from best-fit parameters and MSPE. In conclusion, the Dijkstra model fitted extended lactations of a variety of lengths in both primiparous and multiparous cows. Furthermore, this model can be fitted to MY data from the first 111 DIM to accurately forecast 305-d MY. These forecasts can be used to identify which individual cows in a herd may be suitable to undergo extended lactation.

Key Words: extended lactation, modelling

Physiology and Endocrinology 1

213 Choline supply during negative nutrient balance alters molecular components and intermediate metabolites in the hepatic methionine cycle and transsulfuration pathway. D. N. Coleman*[1], A. Alharthi[1], V. Lopreiato[2], E. Trevisi[3], M. Miura[4], Y. X. Pan[1], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Magna Græcia University, Catanzaro, Italy,* [3]*Università Cattolica del Sacro Cuore, Piacenza, Italy,* [4]*Ajinomoto Co. Inc., Tokyo, Japan.*

Enhanced post-ruminal supply of choline (CHL) may increase flux through the methionine (Met) cycle to improve immunometabolic status during a negative nutrient balance (NNB). The objective was to investigate effects of post-ruminal CHL supply during a feed restriction-induced negative NNB on: 1) hepatic activity of cystathionine β-synthase (CBS) and methionine synthase (MTR) and expression of enzymes and metabolite concentrations in the transsulfuration pathway and Met cycle and 2) plasma biomarkers of liver function, inflammation and oxidative stress. Ten primiparous rumen-cannulated Holstein cows (158 ± 24 DIM) were used in a replicated 5 × 5 Latin square design with 4-d treatment periods and 10 d of recovery (14 d/period). Treatments were unrestricted intake with abomasal infusion of water (A0), restricted intake (R; 60% of NE_L requirements) with abomasal infusion of water (R0) or R plus abomasal infusion of 6.25, 12.5, or 25g/d CHL ion. Liver tissue was collected on d 5 when infusions ended for metabolomics and radioactive enzyme assays, and blood on d 1, 3 and 5 for measurement of plasma biomarkers via commercial kits. Statistical contrasts were A0 vs. R0, R vs. the average of CHL doses and tests of linear and quadratic effects. While R decreased MTR activity ($P = 0.02$), it tended to increase linearly with CHL ($P = 0.07$). Activity of CBS tended to be lower with R ($P = 0.07$) and decreased linearly with CHL ($P = 0.03$). Hepatic glutathione content was not different with R or CHL ($P > 0.10$) but taurine tended to be greater with CHL ($P = 0.09$). Betaine and carnitine were greater with R ($P < 0.05$) and further increased with CHL ($P < 0.05$). Plasma aspartate aminotransferase and bilirubin increased with R ($P < 0.05$) but decreased with CHL ($P < 0.05$). Data suggest that enhanced supply of CHL during NNB decreases entry of homocysteine to the transsulfuration pathway, potentially favoring remethylation to Met by acquiring a methyl group from betaine. As such, Met may provide methyl groups for synthesis of carnitine. Changes in blood biomarkers suggest a beneficial effect of CHL on liver function during NNB.

Key Words: lactation, methyl donor

214 Hepatic 1-carbon metabolism, methionine cycle, and transsulfuration pathway intermediates in neonatal Holstein calves are altered by maternal postruminal supply of methionine during late pregnancy. A. S. Alharthi*[1], M. Abdelmegeid[2], R. Yambo[1], A. Elolimy[1], Y.-X. Pan[3], C. Parys[4], and J. J. Loor[1], [1]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL,* [2]*Department of Animal Medicine, Faculty of Veterinary Medicine, Kafrelsheikh University, Kafr El-Shaikh, Egypt,* [3]*Food Science and Human Nutrition, Division of Nutritional Sciences, University of Illinois, Urbana, IL,* [4]*Evonik Nutrition & Care GmbH, Wolfgang, Hanau, Germany.*

Pregnancy and early life are critical periods of plasticity during which the fetus and neonate may be influenced by environmental factors such as nutrition. The aim of this study was to investigate if increasing Met supply during late-pregnancy affects profiles of 1-carbon metabolism, methionine cycle, and transsulfuration pathway intermediates as well as enzyme activities in neonatal calf liver. Twenty-eight calves born to cows fed a basal control [CON, n = 14; 1.47 Mcal/kg dry matter and 15.3% crude protein] or CON plus rumen-protected methionine (MET, n = 14; Mepron at 0.09% of diet DM; Evonik Nutrition & Care GmbH, Germany) were used. Liver biopsies were harvested at 4, 14, 28, and 50 d of age and used for metabolomics via GS-MS. Daily starter intake and weekly body weight were recorded. Statistical analysis was performed using PROC MIXED of SAS (SAS Institute Inc., Cary, NC). Maternal supplementation with Met led to greater ($P \leq 0.05$) BW (59.8 vs. 55.6 ± 1.4 kg). However, daily starter intake, average daily gain, and feed efficiency were not affected by maternal treatment. There was greater ($P \leq 0.05$) overall activity of cystathionine-β-synthase (CBS) in MET calves, while betaine-homocysteine S-methyltransferase (BHMT) and cystathionine-β-synthase (CBS) increased (interaction $P \leq 0.05$) in MET calves between d 4 and 14 with a peak at 28 d. Despite a linear increase from d 4 to 28, activity of methionine synthase (MTR) in MET calves was lower on d 4 and 50 d. Greater ($P \leq 0.05$) hepatic concentrations of the 1-carbon metabolism and methionine cycle intermediates adenosine, betaine, choline, and N,N-dimethylglycine were detected in MET calves. Among transsulfuration pathway metabolites, concentrations of cystathionine, cysteinesulfinic acid, hypotaurine, serine, and taurine were greater ($P \leq 0.05$) in MET calves. Overall, data suggest that increasing the maternal supply of Met during late-pregnancy benefits calf growth in part through alterations in hepatic metabolism.

Key Words: methyl donor, epigenetics, nutritional programming

215 Influence of prepartum dietary cation-anion difference and the decline of calcium at the onset of lactation. M. K. Connelly*[1], R. M. R. Harris[5], F. S. Andrade[6], J. P. Nascimento Andrade[6], J. Kuehnl[1], A. Beard[1], E. Block[4], I. J. Lean[2,3], and L. L. Hernandez[1], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Scibus, Camden, NSW, Australia,* [3]*School of Life and Environmental Sciences, Faculty of Veterinary Science, University of Sydney, Camden, NSW, Australia,* [4]*Arm & Hammer Animal Nutrition, Princeton, NJ,* [5]*National Centre for Epidemiology and Population Health, Research School of Population Health, Australian National University, Canberra, Australia,* [6]*Federal Rural University of Rio de Janeiro, Seropedica, RJ, Brazil.*

The onset of lactation results in a large and sudden increase in calcium (Ca) demand by the mammary gland to synthesize milk. The ensuing drop in circulating Ca plays a factor in the cow's metabolic adaptation to lactation. When Ca maintenance fails, cow health and productivity is affected. Negative dietary cation-anion difference (DCAD) diets have been utilized to create metabolic conditions favorable to maintaining blood Ca concentrations. The objective of this study was to determine how the decline in Ca at the onset of lactation, in combination with positive or negative DCAD diets, alters Ca metabolism postpartum. Thirty-two multiparous Holstein cows were blocked by parity and 305d production in a randomized complete block design. Cows were fed a negative (−120 mEq/kg; −DCAD) or positive (+120 mEq/kg; +DCAD) DCAD diet from 251 d of gestation until parturition (n = 16/diet). Immediately after calving cows were continuously infused for 24 h with (1) an intravenous solution of 10% dextrose or (2) Ca gluconate (CaGlc) to maintain blood ionized Ca (iCa) concentration at 1.2 m*M* (normal calcemic) forming 4 treatment groups (n = 8/treatment). From 3 d pre- to 3 d postpartum, blood was collected every 6 h to monitor iCa concentrations. Groups were compared using a mixed model ANOVA with time included as a repeated measure. Cows fed −DCAD had increased iCa concentrations compared with +DCAD cows during the 72 h prepartum ($P = 0.01$) and immediately before start of infusion ($P < 0.05$). Twelve hours after termination of infusion (36 h post parturition) cows infused with CaGlc had decreased iCa concentrations compared with cows infused with dextrose ($P = 0.0003$). Cows in +DCAD/CaGlc group had decreased blood iCa compared with all other treatments 36 h postpartum (0.88 ± 0.03 m*M*; $P < 0.05$). Treatment with CaGlc during the 24 h after parturition negatively affected the immediate postpartum Ca homeostasis, regardless of prepartum dietary treatment. Additionally, feeding -DCAD better equipped cows to respond to Ca requirements at the start of lactation.

Key Words: calcium, dietary cation-anion difference (DCAD)

216 Maternal body condition score during late-pregnancy is associated with in utero development and neonatal growth of Holstein calves.

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We investigates the association between cow body condition score (BCS) during late-pregnancy on developmental parameters and blood biomarkers of neonatal calves. Forty-nine multiparous Holstein cows were retrospectively divided by prepartal BCS into BCS ≤3.25 (n = 30; LoBCS) and BCS ≥3.75 (n = 19; HiBCS) groups. Plasma samples were collected from cows at −10 d relative to parturition. Body weight, hip and wither height, hip width and body length were measured at birth and weekly through weaning (42 d of age) and until 9 weeks of age. Calf blood samples were collected from the jugular vein at birth (before receiving colostrum, 0 d), 24 h after first colostrum and at 7, 21, 42 and 50 d of age. The data were subjected to ANOVA using the mixed procedure of SAS. The statistical model included Day, BCS, and their interactions. Dry matter intake during the last 4 wk of pregnancy was lower overall ($P = 0.06$) for HiBCS cows (12.0 vs. 12.9 ± 0.3 kg/d). Concentrations of fatty acids ($P = 0.01$; 0.30 vs. 0.19 ± 0.03 mmol/L), ceruloplasmin ($P = 0.05$; 2.54 vs. 2.30 ± 0.09 μmol/L), and nitric oxide ($P = 0.05$; 31.53 vs. 29.95 ± 0.64 μmol/L) were greater overall at −10 d in HiBCS cows. A negative correlation was observed between calf birth weight and the concentrations at −10 d in HiBCS cows of haptoglobin (r = −0.76; $P < 0.01$), ceruloplasmin (r = −0. 57; $P = 0.05$), and reactive oxygen metabolites (r = −0.79; $P < 0.01$). Birth body weight was lower ($P = 0.03$; 42.5 vs. 44.7 ± 0.72 kg) in calves born to dams with HiBCS. Although calves born to cows with LoBCS maintained greater postnatal body weight ($P = 0.04$; 63.3 vs. 59.7 ± 1.33 kg), hip and wither height, hip width, body length, daily starter intake and average daily gain did not differ due to maternal BCS. Overall, results highlight an association between BCS during late-gestation on calves development and postnatal growth.

Key Words: BCS, offspring, dairy cow

217 Association of prepartum urine pH and postpartum disorders in Holstein cows fed anionic diets.

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Anionic salts impose a metabolic acidosis which improve calcium homeostasis. Monitoring urine pH (UpH) is key to establishing effective use of anionic diets in prepartum cows. UpH between 6.0 and 7.0 can reduce hypocalcemia, however, it has often been suggested lowering the pH <6.0 for even better control of hypocalcemia. The pH is a log scale of the [H$^+$]. Reducing the UpH from 8.5 to ~5.5 indicates increasing proton excretion by the kidney of about 1000 fold. The objective was to establish the association between UpH of prepartum cows fed anionic diets and the incidence of postpartum disorders. The study was conducted between Jul 2018 and Jan 2019 in a herd from Argentina. Prepartum diet had a potential DCAD of – 90 mEq/kg DM. UpH of 200 prepartum cows were evaluated. UpH was categorized as <6.0 (n = 22); between 6.0 and 6.9 (n = 46); ≥ 7.0 (n = 135). The frequency in each UpH category for stillborn, dystocia, RFM, metritis, ketosis, LDA and mastitis was calculated. Logistic regression models were carried out for each disorder, considering as the main effect the UpH category, adjusting for days from pH evaluation to calving, parity, sex of the calf, type of parturition, season of urine pH evaluation, and BCS at calving. Models for all diseases, except stillborn, showed that the UpH was not associated with the incidence of the disorder. Cows with UpH <6.0 were 2.39 times more likely to have a stillborn than cows with UpH ≥6.0. These findings reinforce the importance of not lowering the urine pH to <6 in prepartum cows fed anionic salts. The marginal gain in the reduction

Table 1 (Abstr. 217).

Urine pH	Stillbirth	Milk fever	Dystocia	RFM	Metritis	Ketosis	LDA	Mastitis
<6.0 (n=22)	13.6^a (3)	0.0 (0)	18.2 (4)	4.6 (1)	13.6 (3)	27.3 (6)	0.0 (0)	22.7 (5)
6.0-6.9 (n=46)	8.7^b (4)	4.2 (2)	10.9 (5)	2.2 (1)	13.0 (6)	26.1 (12)	4.3 (2)	23.9 (11)
≥7.0 (n=135)	4.4^b (6)	2.3 (3)	20.7 (28)	8.1 (11)	14.0 (19)	23.0 (31)	3.0 (4)	14.8 (20)
Comparison urine pH								
<6.0 vs. ≥6.0	2.39	0.68	0.89	0.65	1.05	1.16	1.55	1.09
AOR (95% CI)	1.06-5.40	0.15-3.17	0.49-1.63	0.34-1.27	0.58-1.92	0.71-1.89	0.68-3.52	0.67-1.74
P-value	0.035	0.62	0.72	0.21	0.86	0.53	0.29	0.73

of milk fever by lowering the pH to <6.0 is minimal, compared with an increase in the incidence of stillborn.

Key Words: urine pH, anionic diet, stillbirth

218 SexedULTRA sperm sorting: A method to improve the quality of ejaculates with below standard characteristics.

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SexedULTRA is a method of sperm sorting and processing that provides a benign environment to improve the quality of ejaculates and retain sperm cell integrity. The aim of this study was to evaluate if the in vitro characteristics of below standard quality ejaculates can be improved by SexedULTRA processing. Two low quality ejaculates from 6 bulls were split, processed, and cryopreserved as non-sorted (NS), SexedULTRA bulk-sorted (SBS) and sex-sorted (SSS). Percent head and tail morphologies were evaluated visually after thawing (0 h). Sperm motility was estimated visually (MOT) and classified into total (TOT) and progressive (PROG) using a computer assisted sperm analyzer after thawing and post-incubation (3 h at 36°C). Percent viability (VIA) and acrosome integrity (PIA) were assessed using flow cytometry after thawing and post-incubation. Sperm DNA fragmentation index (DFI) was estimated using the Halomax Kit immediately after thawing and after 6, 24, 48 and 72 h of incubation at 36°C. In vitro fertilization (IVF) was performed as a measure of sperm competence using NS and SSS straws from 4 of the ejaculates. Two hundred oocytes per treatment group (ejaculate × treatment) were included in the comparison for embryo production. Data were analyzed by a mixed model in conjunction with a Tukey contrast with the fixed effect of treatment and time, and random effect of ejaculate. SSS and SBS sperm head and tail abnormal morphologies were significantly lower ($P < 0.05$) when compared with NS sperm. SexedULTRA had lower ($P < 0.05$) DFI than NS sperm at every time of incubation. SSS and SBS sperm also presented numerically higher MOT, TOT, PROG, VIA and PIA than NS sperm, but differences were not significant. During incubation of NS sperm, a strong time by treatment effect ($P < 0.05$) was seen for MOT, TOT and PROG. This effect was not present for SSS or SBS sperm. The percent of embryos produced was significantly higher ($P < 0.05$) for SexedULTRA sperm. The results show that SexedULTRA processing can remove morphological issues, improve sperm quality and increase embryo production of low-quality ejaculates, allowing for samples that would otherwise be discarded, to be processed as a bulk- or sex-sorted product.

Key Words: SexedULTRA, sperm sorting, sperm quality

Production, Management, and the Environment 2

219 Validation of the RumiWatchSystem to monitor feeding and locomotive behaviors in a grazing dairy herd. G. M. Pereira*, B. J. Heins, and K. T. Sharpe, *University of Minnesota, West Central Research and Outreach Center, Morris, MN.*

The objective of the study was to validate a halter and pedometer for monitoring feeding and locomotive behaviors. The study was conducted at the University of Minnesota grazing dairy in Morris, Minnesota, from May to June 2018. Lactating crossbred dairy cows (n = 12) were offered pasture for 22 h per day during the study. The RumiWatchSystem (Rumiwatch, Itin and Hoch GmbH, Liestal, Switzerland) classified data with a halter as ruminating, eating, drinking and other, and classified data with a pedometer as standing, lying and walking behaviors. In addition, the halter classified jaw movements as grazing bites or rumination chews. Observational data were recorded on Samsung tablets using the Pocket observer app (The Observer XT, Version 14.0, Noldus Information Technology, Leesburg, VA). Agreement was determined between visual observation and the halter and pedometer for 144 h of feeding and locomotive behaviors. Additionally, grazing bites and rumination chews during 1,205 min were evaluated between direct visual observation and the halter. Pearson correlations and concordance correlation coefficient (PROC CORR of SAS), bias correction factors (Cb), location shift (V) and scale shift (μ) (epiR package of R software) evaluated associations between direct visual observations and the halter and pedometer. Correlations between visual observations and the halter ($P < 0.01$) were 0.84 for ruminating, 0.76 for eating, 0.39 for drinking, and 0.57 for other behaviors. Correlations between visual observations and the pedometer ($P < 0.01$) were 0.83 for standing, 0.91 for lying, and 0.38 for walking behaviors. The correlations for grazing bites and rumination chews were 0.46 ($P < 0.01$) and −0.04 ($P = 0.79$), respectively. For grazing bites and rumination chews analyzed together, the correlation was 0.68 ($P < 0.01$). The results suggest that the RumiWatchSystem may accurately monitor rumination and eating, as well as standing and lying behaviors in a grazing system. Behaviors such as drinking and walking were seldom observed and may be difficult to accurately monitor in grazing dairy cattle.

Key Words: precision technology, grazing, rumination

220 Validation of an ear tag for grazing behavior in Minnesota and Ireland. G. M. Pereira[1], B. J. Heins*[1], B. O'Brien[2], A. McDonagh[2], L. Lidauer[3], and F. Kickinger[3], [1]*University of Minnesota, West Central Research and Outreach Center, Morris, MN,* [2]*Teagasc, Animal & Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland,* [3]*Smartbow GmbH, Weibern, Austria.*

The objective of the study was to validate the ear tag (Smartbow GmbH, Weibern, Austria) for grazing behavior. The Smartbow ear tag includes an acceleration sensor, a radio chip, and temperature sensor for calibration. Smartbow can monitor estrus detection and rumination by acceleration data from ear and head movements. To validate Smartbow, a halter system (Rumiwatch, Itin and Hoch GmbH, Liestal, Switzerland) was used. The halter is comprised of a 3-axis accelerometer that records acceleration patterns by a noseband pressure sensor and detects jaw movements according to chewing activities. The study was conducted at the University of Minnesota grazing dairy in Morris, Minnesota, and the Teagasc Animal and Grassland Research Centre in Ireland. During May and June of 2017, Smartbow ear tags were attached to grazing cows and 3 observers visually recorded behaviors for a total of 90 h in Minnesota. Observational data from Minnesota and additional data from Ireland were used to develop a grazing algorithm. During September of 2018, data were collected by Smartbow and Rumiwatch with 12 crossbred cows in Minnesota (n = 248 h) and 10 Holstein Friesian cows in Ireland (n = 248 h). A 2-sided paired *t*-test compared the percentage of time recorded for grazing behaviors and Pearson correlations (PROC CORR of SAS) evaluated associations between Smartbow and Rumiwatch. For total recorded grazing time in Minnesota, the percentage of time recorded by Smartbow was 37.0% (CI 32.1 to 42.0) and by Rumiwatch was 40.5% (CI 35.5 to 45.6). For total recorded grazing time in Ireland, the percentage of time recorded by Smartbow was 35.4% (CI 30.6 to 40.2) and by Rumiwatch was 36.9% (CI 32.1 to 41.8). Smartbow and Rumiwatch agreed strongly for monitoring grazing in Minnesota (r = 0.96; CI 0.94–0.97; $P < 0.01$) and in Ireland (r = 0.92; CI 0.90–0.94; $P < 0.01$). The results suggest that there is great potential for Smartbow to be utilized in pasture-based dairy production systems to support farm management decision making.

Key Words: ear tag, validation, grazing

221 Association of management practices, housing, milking speed and robot visits with milk production per cow on free-flow automatic milking system farms. M. Peiter*[1], E. Irwin[2], B. Groen[3], J. A. Salfer[4], and M. I. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*Department of Animal Science, Iowa State University, Ames, IA,* [3]*Form-A-Feed, Stewart, MN,* [4]*University of Minnesota Extension, St. Cloud, MN.*

Automatic milking systems (AMS) are common in Europe and have grown in popularity recently in the USA. The objective of this study was to investigate the association between management practices, housing, milking speed and robot visits with milk production/cow on free-flow cow traffic farms. We visited 36 AMS (Lely Astronaut, Lely, the Netherlands) farms in Minnesota and Wisconsin over the summer of 2018. Producers answered a survey about general farm management practices and barn characteristics. In addition, we collected retrospective daily data from the AMS software. We used data for the 30 d (1,080 daily averages) before the farm visit to evaluate the association of management and housing factors with milk production/cow (kg/d). The MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC) was used to analyze the data. Backward stepwise elimination was used to remove nonsignificant factors until all remaining factors had a $P < 0.05$ in the final model. Farm was used as random effect. Average milk production/cow was 37.6 (±4.4) kg/d. Results from the multivariable analysis are presented as least squares means (±SE). Amount of daily concentrate offered in the robot was associated with increased milk production/cow. For every additional kg of concentrate offered, cows increased milk production by 1.3 (±0.2) kg/d. Successful milkings and refusals were also associated with daily milk production/cow. For each 1-unit increase in milkings/d, cows produced 8.9 (±0.3) kg more milk/d. Conversely, refusals had a negative association with milk production. For each unit increase in refusals, there was a decrease in milk production of 0.4 (±0.1) kg. As expected, higher milking speed resulted in higher milk production. For each additional kg of milk milked per minute, milk production per day increased by 4.2 (±0.2) kg. Milking time (sec) was also associated with higher milk production (0.06 (±0.003) kg/d), potentially a result of proportion of high producing cows in the herd. Results indicate that feeding practices and cow visit behavior can influence cow productivity in AMS.

Key Words: automatic milking system, management, housing

222 Utilizing data collected via automated sensors as proxies for feed intake in dairy cattle and the impact of health status. C. Siberski*[1], M. S. Mayes[1], P. Gorden[2], A. Copeland[2], B. M. Goetz[1], L. H. Baumgard[1], and J. E. Koltes[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Vet Diagnostic & Production Animal Medicine, Iowa State University, Ames, IA.*

Genetic selection for feed efficiency (FE) could decrease feed costs on dairy farms. Currently, measuring individual feed intake on commercial dairies is impractical due to high equipment and labor costs. Development of portable, affordable technologies as indicator traits would help make selection for FE feasible. The objective of this study was to determine if traits collected by automated sensor technologies were associated with feed intake. Furthermore, the impact of an animal's health status on proxies was examined. Data were collected on 108 lactating Holstein cows (parity 1–4, DIM 50–215) in 2 seasonal groups; summer (n = 48) and fall (n = 60). All animals were fit with eartags tracking activity (ETACT) and inner ear temperature (ETTEMP). Fall cows also received an additional eartag measuring rumination and rumen boluses recording rumen pH (RBpH), temperature (RBTEMP) and cow activity (RBACT). Individual feed intake, milk weights, components, body weight (BW), BCS, and health events were recorded. Data were analyzed with PROC GLIMMIX in SAS. Models included dry matter intake (DMI) as the response variable and fixed effects of DIM, milk weight, fat, protein, and lactose, metabolic BW, sensor measure, and health event (HE). Random effects included parity, pen and seasonal group. When animals were classified by their HE(s) for the entirety of the trial, RBpH and RBTEMP were associated with DMI ($P < 0.01$), while RBACT tended toward significance ($P < 0.1$). Each interaction between HE and RBpH, RBTEMP and RBACT were also significant ($P < 0.0001$). When mastitis was included at the time of illness, ETTEMP, RBpH and RBACT were associated with DMI ($P < 0.05$). When lameness was examined at the time of the event, ETTEMP and RBACT were significant ($P < 0.05$), while RBpH tended toward significance ($P < 0.1$) Results indicate automated sensor traits act as indicators of feed intake. Health events appear to have long lasting influence on sensor trait and feed intake phenotypes. Future work will focus on development of models to predict feed intake from these data.

Key Words: precision livestock farming, indicator trait, feed intake and efficiency

223 Association of management practices, housing, milking speed, and robot visits with milk production per robot on free-flow automatic milking farms. M. Peiter*[1], E. Irwin[2], B. Groen[3], J. A. Salfer[4], and M. I. Endres[1], [1]*Department of Animal Science, University of Minnesota, St. Paul, MN,* [2]*Department of Animal Science, Iowa State University, Ames, IA,* [3]*Form-A-Feed, Stewart, MN,* [4]*University of Minnesota Extension, St. Cloud, MN.*

According to automatic milking system (AMS) manufacturers, a goal for milk production is 2,300 kg/robot/d or greater. The objective of this study was to investigate the association of management and housing factors with milk production/robot on free-flow cow traffic farms. We visited 36 AMS (Lely Astronaut, Lely, the Netherlands) farms in MN and WI over the summer of 2018. Producers answered a survey about general farm management practices and barn design. In addition, we collected retrospective daily data from the AMS software. We used data for the 30 d (1,080 daily averages) before the farm visit for the analysis with the MIXED procedure of SAS 9.4 (SAS Institute, Inc., Cary, NC). Backward stepwise elimination was used to remove nonsignificant factors until all remaining factors had a $P < 0.05$ in the final model. Farm was used as random effect. Milk production/robot was 2224.2 (±340.1) kg/d. Results are presented as least squares means (±SE). Multiple robots/pen tended to be associated with lower milk production/robot (−58.2 (±34.3) kg/d, $P = 0.09$). A higher number of cows/robot resulted in greater milk production/robot (35.6 (±1.25) kg/d). Using an automatic alley scraper resulted in an increase of 145.6 (±62.7) kg of milk/robot compared with manual scraping. To our knowledge, this is the first study to look at number of feeds offered in the robot in the US. There was a tendency for a positive association ($P = 0.09$) between number of feeds/robot and milk production/robot (69.9 (±40.8) kg/d). For each additional kg of concentrate offered in the robot, there was an increase of 66.2 (±10.5) kg of milk/robot. Successful milkings/cow/d was positively associated with milk/robot (503.1 (±15.0) kg/d). Refusals were negatively associated with milk production/robot (−24.5 (±5.1) kg/d). A higher milking speed resulted in more milk/robot (245.3 (±14.3) kg/d). Similar pattern was observed for milking time (sec) (3.3 (±0.2) kg/d). Results indicate that certain feeding practices and housing factors can influence robot performance.

Key Words: automatic milking system, management, housing

224 Growth, health, and economics of dairy calves fed organic milk replacer versus whole milk in an automated feeding system. K. Sharpe* and B. Heins, *University of Minnesota, West Central Research and Outreach Center, Morris, MN.*

The objective of this study was to determine growth, health, and profitability of organic dairy calves fed an organic milk replacer (MR) versus pasteurized whole milk (WM) in an automated group feeding system. The study was conducted at the University of Minnesota West Central Research and Outreach Center's, Morris, MN, organic dairy. Eighty-one Holstein and crossbred calves were assigned to feeding groups by birth order during 2 calving seasons from March to July 2018 and from September to December 2018. Calves were introduced to the Holm & Laue HL100 Programmable Calf Feeder (Holm & Laue GmbH & Co KG, Westerronfeld, Germany) at 5 d and were allowed to drink up to 8 L/d at the maximum allowance. Calves were weaned from the automated feeder at 56 d. Data were analyzed using PROC MIXED of SAS. Independent variables for analyses were the fixed effects of breed group, season of birth, treatment group, the interaction of season and treatment group, along with pen as a random effect. No differences ($P < 0.05$) were found between MR or WM groups for average daily gain, weaning weight, hip height, and heart girth. The WM calves had shorter ($P < 0.05$) feeding station visit durations (2.44 min) than MR calves (3.01 min), slower ($P < 0.05$) consumption rates (1.85 L/min) than MR calves (2.48 L/min), and higher ($P < 0.05$) consumption amounts (1.52 L/visit) than MR calves (1.32 L/visit). On a daily basis, WM calves had more unrewarded visits to the feeding station (16.07) than MR calves (12.07), fewer unfulfilled visits (3.02 visits) than MR calves (10.34), and fewer fulfilled visits (3.73) than MR calves (5.05). Drinking speeds of WM calves were higher ($P < 0.05$) (1,301.4 mL/min) than the MR calves (581.0 mL/min). The MR calves had higher ($P < 0.05$) fecal scores than WM calves. The cost per kg of gain for MR ($8.82/kg) calves was ($P < 0.05$) higher compared with the WM ($6.35/kg) calves. The results from this study indicate that there may be both health and economic advantages to feeding organic dairy calves whole milk during the pre-weaning period.

Key Words: automated feeder, calf, organic

225 Milk and reproductive performance in Holstein cows experiencing hyperketonemia in early lactation. Y. Schuermann[1,2], D. Warner[1,2], R. Cue[2], L. Fadul-Pacheco[1], R. Lacroix[1], R. Moore[1], V.

Ouellet[3], E. Charbonneau[3], F. Miglior[4], D. Lefebvre[1], and D. Santschi*[1], [1]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [2]*McGill University, , Ste-Anne De Bellevue, QC, Canada,* [3]*Université Laval, Québec, QC, Canada,* [4]*University of Guelph, Guelph, ON, Canada.*

Hyperketonemia has a relatively high prevalence of 22.6% on Quebec Holstein farms. The objectives of this study were to identify the consequences of elevated milk β-hydroxybutyrate (BHB) on whole-lactation milk performance and on reproductive success. Records from 505,412 Holstein cow observations (159,525 first parity, 132,694 s parity and 213,193 third and later parity) from 3,551 herds were included in the analysis. Data were retrieved from Valacta's database from cows that calved between 2011 and 2017. Cows were grouped based on parity and according to the milk BHB concentration on first test-day between 5 and 35 d in milk (DIM) into negative (NEG; <0.15m*M*), suspect (SUS; 0.15 to 0.19 m*M*), positive (POS; ≥20 m*M*). Mixed model analyses with PROC MIXED of SAS including herd as a random effect revealed that POS cows produced greater milk fat (376 vs 361 kg SEM 6.4; $P < 0.01$), as well as more energy-corrected milk (9,419 vs 9,153 kg SEM 146; $P < 0.01$) over a 305-d lactation compared with NEG cows. However, analysis of reproductive performance revealed that POS cows experienced more days open (149 vs 126 d SEM 3.6; $P < 0.01$), had a greater interval from first service to conception (41 vs 31 d SEM 1.7; $P < 0.05$) and required more services per conception (2.1 vs 1.8 SEM 0.1; $P < 0.01$) than NEG cows. These changes in reproductive parameters were observed across parities. Additionally, a survival analysis with PROC LIFETEST and PROC PHREG of SAS revealed reduced pregnancy rates for POS cows, especially for multiparous cows. Lastly, a frequency analysis with PROC GENMOD of SAS was used to assess culling rates, where by 60 DIM, the culling rates were higher for POS cows (3.4 vs 2.6% ± 0.3; $P < 0.01$) compared with NEG cows. Overall, high milk performance was associated with high milk BHB levels. Elevated BHB levels significantly hindered the reproductive performance of these cows and increased their chances of being culled from the herd.

Key Words: hyperketonemia, first service to conception, culling rate

226 Combining milk and body weight perturbations as proxies for robustness in primiparous dairy cows.

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Livestock husbandry aims at managing the environment in which animals are reared to enable them to express their production potential. However, animals are often confronted with perturbations that affect their performance. Evaluating effects of these perturbations on animal performance could provide metrics to quantify and understand how animals cope with their environment, and therefore better manage them. Automation of body weight (BW) and milk yield (MY) measurements in dairy herds is increasingly widespread in commercial farms. BW and MY dynamics over reproductive cycles can together be informative about animal health status and effects on potential performance. The goal of this study was to use a differential smoothing approach on both MY and BW time series of dairy cattle to extract quantitative features of the profiles of perturbations. Daily MY and BW records from 491 primiparous Holstein cows from 33 commercial French herds were used. These cows were not involved in experiments and did not experience any particular controlled challenge. Perturbations were solely expected to occur at a standard level in commercial farms, e.g., mastitis decreasing milk production, diarrhea decreasing digestive tract contents, nutritional disorder enhancing body reserve mobilization. After the fitting procedure, 2711 deviations were detected in MY time series and 1539 were detected in BW time series for all cows. Fifty-seven percent of these deviations were detected in the same period (±10 d) in both MY and BW time series. Clustering methods were applied on all individual deviations and identified contrasted types of cows in terms of responses to perturbations. These responses involved either only MY, or only BW or both. Results suggest that combing various individual dynamic measures can be of great interest to obtain reliable estimates of robustness components in large populations

Key Words: robustness, perturbation, dairy cow

227 The association between MUN and protein efficiency across protein content and lactation stage.

E. Liu* and M. J. VandeHaar, *Michigan State University, East Lansing, MI.*

Most of the data used to study the association between MUN and protein efficiency was from cows fed excessive protein. Few studies examined how the relationship between MUN and protein efficiency was affected by dietary protein content and lactation stage. In this study, 69 Holstein cows (in 8 experiments) were fed diets containing sufficient and deficient protein in both mid and late lactation. In each lactation stage, cows were included in the crossover experiment with two 28-d treatment periods. The diets contained 18% or 14% CP in mid lactation, and 16% or 12% CP in late lactation. All 4 diets contained sufficient RDP for rumen function with expeller soybean meal added to achieve the high protein diets. Cows were milked 2× daily; intake and MY were recorded daily. Milk composition was measured during 4 consecutive milkings weekly and BW was measured 3× weekly. Protein efficiency was calculated as dietary protein captured in milk protein (milk protein efficiency, MPE), and dietary protein captured in milk protein and body tissues (gross protein efficiency, GPE), respectively. MUN, MPE and GPE were calculated for each cow in mid lactation averaged across both diets to compare against values in late lactation averaged across both diets and for the high-protein diets compared with the low protein diets averaged across both stages of lactation. Pearson correlation coefficients among MUN, MPE and GPE across lactation stage and dietary protein content were calculated after accounting for effects of parity, cohort and experiment. MUN of individual cows was repeatable across dietary protein content ($r = 0.8$, $P < 0.05$) and lactation stage ($r = 0.5$, $P < 0.05$). MPE was repeatable across dietary protein content ($r = 0.8$, $P < 0.05$) but not lactation stage ($r = 0.2$, $P = 0.2$). GPE was repeatable across dietary protein content ($r = 0.7$, $P < 0.05$) but not lactation stage ($r = 0.2$, $P = 0.2$). On average, protein efficiency was greatest for low-protein diets that produced lowest MUN regardless of lactation stage; however, protein efficiency of individual cows was not correlated with MUN within diets. In conclusion, some cows consistently have lower MUN regardless of diets or stage of lactation; however, they are not necessarily using protein more efficiently.

Key Words: protein efficiency, MUN

Reproduction Symposium: The Etiology of Pregnancy Failure in Cattle—The When and Why

228 Pivotal periods of pregnancy loss in cattle. M. C. Wiltbank*[1,2], M. A. Mezera[1,2], A. Garcia-Guerra[3], G. M. Baez[4], J. N. Drum[1,5], and R. Sartori[1,5], [1]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI,* [2]*Endocrinology & Reproductive Physiology Program, University of Wisconsin-Madison, Madison, WI,* [3]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [4]*Department of Agricultural and Animal Sciences, Universidad Francisco de Paula Santander, Cucuta, Colombia,* [5]*Department of Animal Science, University of Sao Paulo, ESALQ, Piracicaba, SP, Brazil.*

Pregnancy failure occurs at different stages for a variety of causes with major impacts on reproductive and economic performance of cattle operations. Four pivotal periods during the first trimester of pregnancy are discussed. The first period occurs in the first week of pregnancy with lack of fertilization and degeneration of the early embryo producing major losses, particularly under specific environmental and hormonal conditions. In general, 20–50% of high-producing lactating dairy cows have already experienced pregnancy loss during the first week though there are some methods available to decrease pregnancy loss during this period. These involve increasing oocyte quality by methods such as decreasing heat stress, increasing progesterone concentrations during preovulatory follicle development, and reducing loss of body condition during the early postpartum period. Additionally, the effect of the oviductal environment on early embryonic development is an area of active investigation. The second pivotal period (d 8–27) encompasses embryo elongation and maintenance of the corpus luteum (CL) by maternal signals. There is surprising variation in reported losses during this period with insufficient or inappropriate timing of embryonic interferon-tau or inadequate uterine histotroph as underlying causes of ~30% losses. The third pivotal period is during the second month of pregnancy (d 28 to 60) with losses averaging 11.7% in US dairy cows with ~40% greater losses in multiparous (13.4%) than primiparous (9.5%) cows. Recipients of in vitro produced embryos undergo greater losses (15.6%) in second month. Finally, a fourth pivotal period during the third month of pregnancy has lower pregnancy losses (~2%), compared with the first 3 periods. Twins in the same uterine horn can be an important cause of pregnancy loss in second and third months. Nevertheless, conclusive determination of underlying causes of most pregnancy losses remains elusive. Each pivotal period corresponds to physiological changes in the embryo, uterine environment, and ovary that may allow development of strategies that rationally target the fundamental reason for pregnancy loss in each pivotal period

Key Words: corpus luteum, pregnancy

229 Progesterone effects on early embryo development. P. Lonergan*, *University College Dublin, Dublin, Ireland.*

A significant proportion of pregnancy loss in dairy cows occurs during the first 3 weeks after conception, particularly during the period of conceptus elongation that occurs before implantation. Progesterone (P4) from the corpus luteum plays a major role in regulating endometrial function including conceptus–maternal interaction, pregnancy recognition, and uterine receptivity to implantation. Numerous studies have demonstrated an association between systemic P4 concentrations in the very early stages of pregnancy and likelihood of pregnancy establishment. Low P4 concentrations have been implicated as a causative factor in low pregnancy rates observed in high-producing dairy cows. Elevated concentrations of P4 in the immediate post-conception period have been associated with an advancement of conceptus elongation, an increase in conceptus-derived interferon-tau production and, in some cases, higher pregnancy rates. Elevated P4 advances the transcriptomic changes in the endometrium which normally occur during early pregnancy, resulting in enhanced conceptus elongation; interestingly, the embryo does not have to be present in the uterus during the period of elevated P4 to benefit from it, supporting the concept that the positive effect on conceptus growth is mediated via P4-induced changes in the endometrial transcriptome. Recent studies have demonstrated that the bovine endometrium responds differently to age-matched conceptuses of varying size in both an IFNT-dependent and -independent manner, which may be reflective of the likelihood of successful pregnancy establishment. Multiple strategies have been devised to augment P4 including treatments which provide exogenous P4 (e.g., intravaginal P4 pessaries, P4 injections) or those which enhance the ability of the CL to produce P4 (e.g., administration of human chorionic gonadotrophin). However, despite such effects on conceptus elongation, data on the benefits of post-insemination supplementation with P4 on pregnancy rates are conflicting and, at best, relatively modest. Increased knowledge of the regulation of conceptus-endometrial interactions is necessary to understand and elucidate the causes of pregnancy loss and provide a basis for new strategies to improve pregnancy outcomes and reproductive efficiency.

Key Words: bovine, conceptus, endometrium

230 Maternal control of embryo competence for pregnancy success—The role of embryokines. P. Hansen*, *University of Florida, Gainesville, FL.*

The mammalian embryo possesses a great deal of autonomy in early development: embryos can develop to the blastocyst stage when cultured in a solution of salts, amino acids, energy substrates, and a surfactant. Nonetheless, the maternal environment modulates developmental events to improve blastocyst competence for maintenance of pregnancy and to alter characteristics of the resultant offspring. Regulation of preimplantation embryonic development is mediated in part by cell-signaling molecules produced by the endometrium. These molecules, termed embryokines, include insulin-like growth factor 1 (IGF1), colony stimulating factor 2 (CSF2), dickkopf WNT signaling pathway inhibitor 1 (DKK1), and activin A. Some of the variation in fertility between females is likely caused by differences in secretion of specific embryokines by the maternal endometrium. The best example is for DKK1. Gene expression for *DKK1* in the endometrium was reduced in lactating cows as compared with nonlactating cows and in heifers that were inherently infertile as compared with heifers that were inherently fertile. Actions of embryokines on the preimplantation embryo have the potential to program long-term changes in fetal development and postnatal phenotype. In the cow, this has been observed for DKK1 (reduced birth weight) and CSF2 (increased postnatal growth). Developmental programming of postnatal phenotype often is characterized by sexual dimorphism. At least in part, sex-dependent effects of maternal environment during the preimplantation period could be due to sex-specific actions of specific embryokines. Thus, for example, actions of CSF2 on the morula- and blastocyst-stage embryo affect trophoblast elongation at d 15 of gestation differently for female embryos than male embryos. Further elucidation of the function of embryokines may lead to novel methods for improving fertility and altering postnatal function.

Key Words: embryokine, embryo, blastocyst

231 Uterine influences on pregnancy success. T. E. Spencer*[1], J. G. N. Moraes[1], S. Ortega[1], T. W. Geary[2], H. L. Neibergs[3], and P. J. Hansen[4], [1]*Division of Animal Sciences, University of Missouri, Columbia, MO,* [2]*Fort Keogh Livestock and Range Research Laboratory, USDA Agricultural Research Service, Miles City, MT,* [3]*Department of Animal Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA,* [4]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

In cattle, establishment of pregnancy in cattle begins at the conceptus stage (embryo/fetus and associated extraembryonic membranes) and includes pregnancy recognition signaling, implantation, and the onset of placentation. Survival and growth of the preimplantation blastocyst and elongating conceptus requires embryotrophic factors provided by endometrium of the uterus. However, the critical physiological pathways and genes that govern pregnancy establishment and success are not well elucidated. Serial embryo transfer was previously used to classify heifers as high-fertile (HF), subfertile (SF), or infertile (IF). Pregnancy rate was substantially higher in HF and SF than IF heifers on d 17, and elongating conceptuses were about 2-fold longer in HF than SF heifers. Transcriptional profiling detected relatively few differences in the endometrium of nonpregnant HF, SF, and IF heifers. In contrast, there was a substantial difference in the transcriptome response of the endometrium to pregnancy between HF and SF heifers. Considerable deficiencies in pregnancy-dependent biological pathways associated with extracellular matrix structure and organization as well as cell adhesion were found in the endometrium of SF animals. Distinct gene expression differences were also observed in conceptuses from HF and SF animals, with many of the genes decreased in SF conceptuses known to be embryonic lethal in mice due to defects in embryo and/or placental development. Analyses of biological pathways, key players, and ligand-receptor interactions based on transcriptome data divulged substantial evidence for dysregulation of conceptus-endometrial interactions in SF animals. These results support the ideas that the uterus impacts conceptus survival and programs conceptus development, and ripple effects of dysregulated conceptus-endometrial interactions elicit loss of the post-elongation conceptus in SF cattle during the implantation period of pregnancy. These studies provide an important foundation to understand implantation and early placentation-phase pregnancy loss and develop genetic and physiological approaches to improve the outcome of natural and assisted pregnancies.

Key Words: uterine influences, pregnancy success

232 Early diagnosis of pregnancy loss. A. Ealy*, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Pregnancy losses are substantial in lactating dairy cattle. The majority of pregnancy losses occur during embryonic development (before d 45 post-insemination), before rectal palpation can be utilized effectively for pregnancy diagnosis. Earlier determination of pregnancy status can benefit producers by allowing for earlier re-insemination and can benefit researchers seeking to understand the physiological causes of pregnancy losses. This presentation will explore current methods used to predict pregnancy status and likelihood of pregnancy loss before d 45 of gestation. Transrectal ultrasonography is the 'gold standard' for detecting embryos, amnionic sacs and embryonic heart beats by d 24–28 post-insemination. However, this method requires technical skill and up-front equipment costs. An alternative to ultrasonography is to examine circulating concentrations of pregnancy-associated glycoproteins (PAGs). These placenta-produced proteins can be detected in the maternal circulation from d 24–28 onward. Cow-side tests are not currently available for PAG testing, but local and regional labs permit rapid turnaround times for pregnancy determination (usually 1–2 d). Quantifying circulating PAG concentrations is also being used in research settings to predict impending pregnancy losses. Earlier prediction of pregnancy status is possible. Plasma and milk progesterone concentrations at d 17–24 post-insemination are used to identify open cows. Commercially available qualitative progesterone kits are available for on-farm testing. A more recent development is the use of peripheral blood leukocyte gene expression to predict pregnancy status. Conceptus production of interferon-tau in early pregnancy alters interferon-stimulated gene (ISG) expression within maternal cells, including circulating immune cells. These pregnancy-dependent ISG responses can be detected at d 17–22 post-insemination in cattle. However, these same gene responses are also observed in disease states, thus ISG-based tests predict open cows with greater accuracy than pregnant cows. Collectively, these technologies benefit dairy herd fertility by increasing the number of breeding opportunities during early and mid-lactation, thus reducing days open.

Key Words: pregnancy, embryo, placenta

Ruminant Nutrition 2: Protein and Amino Acid 2

233 Effect of dietary forage source and rumen undegradable protein on growth and nutrient use in dairy heifers. E. E. Corea-Guillén*[1], M. V. Mendoza[1], F. López[1], A. Martinez[1], M. Alvarado[1], C. Moreno[1], G. A. Broderick[2], J. Castro-Montoya[3], and U. Dickhöfer[3], [1]*Facultad de Ciencias Agronómicas, Universidad de El Salvador, San Salvador, El Salvador,* [2]*Broderick Nutrition and Research LLC, Madison, WI,* [3]*Institute of Agricultural Sciences in the Tropics, University of Hohenheim, Stuttgart, Germany.*

Our objective was to access effects of dietary forage and rumen undegradable protein (RUP) on intake and performance of growing heifers. Holstein heifers with mean 6.5 mo of age and 166 kg of body weight were randomly assigned to 4 groups of 8 and fed diets with 2 forages and 2 RUP levels in a 2 × 2 factorial design. Forage sources were cowpea (Vigna sinensis) or pangola grass (*Digitaria decumbens*) hay added at 250 g/kg dietary dry matter (DM). Diets contained 260 (RUP260) or 360 (RUP360) g RUP/kg crude protein (CP). Heifers were housed in individual metabolism cages. The trial lasted 10 wk: 2 wk for adaptation and 8 wk for data collection. Diets were formulated for 175-kg heifers at 0.8 kg/d of average daily gain (ADG). In wk 10, microbial protein synthesis (MPS) was estimated from urinary purine derivative excretion; apparent digestibility was estimated by total fecal collection. Fish meal or urea were fed to equalize dietary N and to produce contrasting RUP concentrations. Data were analyzed as a 2x2 factorial using the mixed methods of SAS; results are in Table 1. Replacing dietary pangola hay with cowpea hay increased ($P < 0.05$) intake of DM and CP, apparent digestibility of OM and NDF, and ADG. There were trends ($P = 0.06$) for increased NDF digestibility, ADG and feed conversion efficiency (FCE; g ADG/kg DMI) with increased dietary RUP; however, increasing RUP reduced MPS. No forage by RUP interactions were detected. Replacing dietary pangola hay with cowpea hay increased intake and digestibility and improved performance of growing Holstein heifers.

Key Words: cowpea hay, RUP, Holstein heifer

234 Quantifying the relation between diet branched-chain AA and production responses: A meta-analysis. Y. Roman-Garcia*[1], L. E. Moraes[1], M. Socha[2], and J. L. Firkins[1], [1]*Department of Animal Science, The Ohio State University, Columbus, OH,* [2]*Zinpro Corporation, Eden Prairie, MN.*

We studied the relationship between the intake of branched chain AA and the production of lactating dairy cows. A meta-analysis of 274 studies and 1036 treatment means from published dairy cow experiments was conducted using a Bayesian hierarchical modeling approach. Response variables were: milk yield (MY; kg/d), energy-corrected milk (ECM; kg/d), milk protein yield (kg/d) and milk fat yield (kg/d). Three levels of models were developed. Level 1 included the diet variables dry matter intake (DMI; kg/d), rumen degradable protein (RDP; % DM), rumen undegradable protein (RUP; % DM), starch (% DM), neutral detergent fiber (NDF; % DM), fatty acids (FA; % DM), and ratio of acid detergent fiber and NDF (ADF/NDF). Level 2 variables included variables in level 1 + Leu (% DM), Ile (%DM), and Val (% DM). Level 3 included level 1 and 2 variables + rumen NH3-N (mg/dl), rumen pH (pH), rumen isovalerate (Ival, % of VFA), and rumen isobutyrate (Ibut, % of VFA). Initially, we used an all possible models selection approach using linear mixed effects models with the random effect of study. The model with the lowest Akaike Information Criterion was then fitted as a Bayesian hierarchical model with minimally informative priors. Models were evaluated using 5-fold cross validation and variance inflation factors. In general, level 2 models always improved compared with level 1 models. For instance, the MY model (CCC = 0.61), predicts $\text{MY} = 1.78 + 0.891(\text{DMI}) + 0.857(\text{RUP}) - 0.0415(\text{RUP}^2) - 0.0744(\text{NDF}) + 0.449(\text{FA}) + 16.4(\text{Ile}) - 12.3(\text{Ile}^2) + 2.73(\text{Val})$. MY had a positive response to Ival and Ibut in the level 3. Likewise, the model for ECM (CCC = 0.68), predicted responses as $\text{ECM} = -4.26 + 1.02(\text{DMI}) + 1.16(\text{RUP}) - 0.0606(\text{RUP}^2) + 4.67(\text{ADF/NDF}) + 15.4(\text{Ile}) - 8.51(\text{Ile}^2)$. Due to high correlation between RDP and RUP they were interchangeable in model selection without much change the response of BCAA. For MY and ECM RUP yielded a better model. Overall, utilizing the diet branched chain AA as predictors improved models over other diet variables. Ile was present in all the models with a positive coefficient at a decreasing rate, suggesting an important role of Ile for dairy production.

Key Words: branched-chain VFA, Bayesian

235 Effects of feeding floury and brown midrib whole-plant corn silage varieties from corn treated with foliar fungicide to lactating Holstein cows on nitrogen utilization. L. J. Wente*[1], M. S. Akins[2], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Marshfield, WI.*

Table 1 (Abstr. 233). Effect of dietary forage and RUP on intake and performance of dairy heifers

	Pangola hay		Cowpea hay			*P*-value		
Item	RUP260	RUP360	RUP260	RUP360	SEM	Forage	RUP	Forage × RUP
DMI, kg/d	6.88^{b}	7.00^{b}	7.39^{a}	7.36^{a}	0.073	<0.01	0.54	0.33
CP intake, kg/d	0.89^{b}	0.90^{b}	0.95^{a}	0.95^{a}	0.007	<0.01	0.20	0.71
OM digestibility, %	65.5^{c}	67.6^{bc}	70.6^{a}	69.7^{ab}	0.97	<0.01	0.56	0.13
NDF digestibility, %	54.2^{b}	57.6^{ab}	58.7^{a}	60.8^{a}	1.37	0.01	0.06	0.64
MPS, g/d	568^{ab}	476^{bc}	615^{a}	445^{c}	41.3	0.84	<0.01	0.36
ADG, g/d	785^{b}	831^{b}	845^{ab}	956^{a}	39.2	0.03	0.06	0.41
FCE, g/kg DM	114^{b}	118^{ab}	115^{b}	130^{a}	5.0	0.26	0.06	0.29

^{abc}Means with different superscripts differ ($P < 0.05$).

Diets fed to lactating dairy cows can contain 50% or more of whole-plant corn silage (WPCS). Fungicide can be applied to help minimize disease incidence. With decreasing agronomic prices, producers may try to reduce cost by not applying fungicide. The purpose of this experiment was to determine the effects of foliar fungicide application on floury and brown midrib (BMR) varieties of corn for WPCS on nitrogen utilization of lactating dairy cows. A 4 × 4 Latin square design balanced to measure carryover effects was used with 32 lactating, Holstein cows [BW = 649 ± 74 kg; DIM = 189 ± 49 d]. Treatments were floury WPCS with fungicide application (FF), floury WPCS with no fungicide application (FC), BMR WPCS with fungicide application (BF), and BMR WPCS with no fungicide application (BC). Fungicide (pyraclostrobin, $C_{19}H_{18}ClN_3O_4$ + metconazole, $C_{17}H_{22}ClN_3O$; Headline AMP, BASF, Florham Park, NJ) was applied at vegetative tassel (VT). WPCS was inoculated (Silo-King; Agri-King, Fulton, IL) and ensiled for 295 d before feeding. Statistical analysis was performed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Nitrogen intake was greater for cows in non-fungicide treatments (635.76 ± 15.3 g/d) than for cows in fungicide treatments (591.86 ± 15.3 g/d; $P < 0.01$). Milk protein nitrogen was greater for cows in non-fungicide treatments (159.83 ± 3.62 g/d) than for cows in fungicide treatments (143.98 ± 3.62 g/d; $P < 0.01$). Urinary urea nitrogen was greater for cows in non-fungicide treatments (236.33 ± 5.46 g/d) than for cows in fungicide treatments (209.68 ± 5.46 g/d; $P < 0.01$). Cows in FF excreted less nitrogen in manure (3.68 ± 0.15 g/d) than cows in FC (4.10 ± 0.15 g/d; $P = 0.01$). In conclusion, cows in fungicide treatments (FF, BF) excreted less nitrogen than did cows in non-fungicide treatments (FC, BC); however, cows in fungicide treatments also consumed less nitrogen.

Key Words: foliar fungicide, corn silage, nitrogen

236 Nitrogen partitioning and microbial protein synthesis in lactating dairy cows with different residual feed intake. Y. Y. Xie*, Z. Z. Wu, D. M. Wang, and J. X. Liu, *Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China.*

Residual feed intake (RFI) is an inheritable indication of feed efficiency that is independent on level of production. Many factors may affect the variation of RFI, but the physiological and metabolic mechanisms underlying RFI are not fully elucidated. This study was conducted to investigate dietary nitrogen (N) partitioning and microbial protein synthesis in lactating dairy cows selected for different feed efficiency. Thirty multiparous Holstein dairy cows, with milk yield of 35.3 (±4.71 SD) kg/d, were used to derive RFI. Milk yields from all cows were recorded at each milking, and milk samples were collected and analyzed per week. Spot urine samples were taken on d 24–25 and d 49–50 before daily feeding. Urinary purine derivatives were used to indirectly estimate the microbial protein flow in the rumen. All the data were analyzed using PROC MIXED of SAS. After the RFI measurement period of 50 d, the 10 lowest RFI cows (−0.96 kg/d ± 0.34 SD) and 8 highest RFI cows (1.18 kg/d ± 0.28 SD) were selected. The lower RFI cows (more efficient) had 2.45 kg/d less dry matter intake (DMI) than the higher RFI cows ($P < 0.05$), but they produced similar energy-corrected milk. The ratios of milk to DMI (1.41 vs.1.24, $P < 0.01$) and energy-corrected milk to DMI (1.48 vs.1.36, $P < 0.01$) were higher in the lower RFI cows than those in the higher RFI cows. Milk urea nitrogen was lower in the lower RFI cows than in the higher RFI cows (12.7 vs. 13.9 mg/dL, $P < 0.05$). Compared with higher RFI animals, the lower RFI cows had a higher partition of feed N to milk N (29.7 vs. 26.5%, $P < 0.05$). Numerically, though not significantly, higher ratios of microbial protein to rumen degradable protein and metabolizable protein to crude protein intake were obtained in the lower RFI cows compared with higher RFI cows, eventually resulting in higher proportion of milk protein to the dietary CP. In summary, all processes from intake to milk protein secretion, including N partition, synthesis of microbial protein, and utilization of metabolizable protein, contribute to the variance in the RFI.

Key Words: residual feed intake, nitrogen partitioning, lactating cow

237 Effects of rumen-protected methionine fed during a heat stress challenge on physiological and production parameters of lactating Holstein cows. R. T. Pate*[1], D. Luchini[2], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Adisseo, Alpharetta, GA.*

Milk yield, content and composition are altered by heat stress (HS), however, rumen-protected methionine feeding may ameliorate the effects of HS. Thirty-two multiparous, lactating Holstein cows [DIM (184 ± 59); body surface area (5.84 ± 0.34m^2)] were randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with rumen-protected methionine (RPM; Smartamine M; Adisseo Inc., Antony, France; 0.105% DM of TMR as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases per period. In phase 1 (9d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. In phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets (THI = 89 ± 3). Group 2 (n = 16) remained in TN (THI = 61 ± 4) but was pair-fed (PFTN) to HS counterparts. After a 21d washout period, the study was repeated (period 2) and the environmental treatments were inverted relative to treatments from phase 2 of period 1, while dietary treatments remained the same. Cows were milked 3 × per d and samples were taken on d 1, 5, and 9 of each phase. Vaginal temperature was measured every 10 min, and respiration rate recorded once daily. Paired difference values were calculated for each cow for each period based on the difference between phase 1 baseline values and phase 2 values for each variable. Statistical analysis was performed on paired difference values using MIXED procedure of SAS. Cows in HS had greater ($P < 0.001$ and $P < 0.001$, respectively) increase in vaginal temperature and respiration rate (+0.2°C and +13.7 breaths/min respectively) compared with cows in PFTN (0.0°C and −1.6 breaths/min, respectively). Cows in PFTN had greater ($P = 0.001$ and $P < 0.001$, respectively) decrease in DMI and milk yield (−3.9 kg/d and −2.6 kg/d, respectively) compared with cows in HS (−3.2 kg/d and −0.9 kg/d, respectively). Cows in CON had greater ($P = 0.04$) decrease in milk protein proportion (%) for PFTN (−0.10%) and HS (−0.06%) compared with cows in RPM for PFTN (0.00%) and HS (−0.02%). In conclusion, HS altered physiological and production parameters, while RPM aided milk content and composition during HS.

Key Words: heat stress, methionine, pair-fed

238 Effects of dietary nitrogen levels on the expression of urea transporter mRNA in liver and rumen epithelium of 5 months old Holstein calves. X. X. Gong*, J. Huang, K. Zhan, M. Lin, and G. Q. Zhao, *Institute of Animal Culture Collection and Application, College of Animal Science and Technology, Yangzhou University, Yangzhou, JiangSu, China.*

The objective of this study was to evaluate the effect of different levels of dietary crude protein (CP) on mRNA expressions of urea transporter B (UT-B) and aquaporins (AQP) and nitrogen metabolism in liver and rumen epithelium of 5-mo old Holstein calves. Ten intact Holstein calves (bull, 172.80 ± 27.73 kg BW) were randomly assigned to 2 treatments (n

= 5 calves/treatment): 1. complete nutritional palletized feeds containing 21.02% of CP (HP); 2. complete nutritional palletized feeds containing 15.05% of CP (LP). All 10 bull calves were slaughtered at the end of 35-d trial period. Blood samples were collected at 0 h, 1 h, 3 h and 5 h after feeding at the day before slaughter for analyzing hematological parameters. After slaughter, rumen contents were obtained for ammonia nitrogen and urea nitrogen analysis. Meanwhile, liver and rumen epithelial tissue was collected immediately and the mRNA expression of UT-B and AQP was detected by quantitative PCR. Data were analyzed by the *t*-test with statistical significance indicated by $P < 0.05$ (SPSS 19.0). Our results showed that the concentrations of rumen ammonia nitrogen ($P = 0.001$) and urea nitrogen ($P = 0.001$) were both significantly higher in HP group compared with LP group. The concentrations of blood urea nitrogen (BUN) were higher in HP group at 1 h ($P = 0.005$), 3 h ($P = 0.012$) and 5 h ($P = 0.016$) after feeding. In comparison with LP group, the relative mRNA expression of AQP-3 in liver of HP group was significantly increased ($P = 0.011$), but there was no significant difference in the relative mRNA expression of UT-B ($P = 0.602$) and AQP-10 ($P = 0.135$). Similarly, there was no significant difference in the relative mRNA expression of UT-B ($P = 0.862$) and AQP-3 ($P = 0.706$) in rumen epithelium between 2 groups. In conclusion, high dietary CP level increased the mRNA expression of AQP-3 in liver of bull calves, which might indicate that AQP-3 may be an important transporter of hepatic nitrogen metabolism and therefore it is candidate to be further studied in hepatic nitrogen metabolism experiments.

Key Words: Holstein calf, urea transporter, aquaporin

239 Effects of dietary starch level and rumen-protected AA on milk production and plasma AA concentration in dairy cows.

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Metabolizable protein (MP)-deficient diets have been shown to reduce N excretion of dairy cows. However, such diets may limit synthesis of milk and milk protein possibly due to deficiencies in EAA, particularly Met, Lys, and His (MLH), insufficient energy supply or both. We aimed to investigate the interactions between rumen-protected (RP) MLH and starch level on performance of dairy cows fed MP-deficient diets. Sixteen multiparous Holstein cows (138 ± 46 DIM, 46 ± 6 kg/d milk) were used in a replicated 4 × 4 Latin square with 2 × 2 factorial arrangement of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for sample collection. Treatments included high-starch (HS), HS + RPMLH, reduced starch (RS), and RS + RPMLH. The basal diets consisted (DM basis) of 35.7% corn silage, 14.7% haylage, and 49.6% concentrate. Dietary energy level varied by replacing 30% ground corn with 20% beet pulp and 10% soyhulls. Dietary NE_L, starch, and CP averaged 1.68 Mcal/kg, 34.4% and 16.0% for HS diets, and 1.59 Mcal/kg, 12.3% and 16.4% for RS diets, respectively. Smartamine M, Aji-Pro L, and an Ajinomoto prototype His product were supplemented to meet digestible MLH requirements. Data were analyzed with the MIXED procedure of SAS. Compared with RS diets, feeding HS diets increased yields of milk (37.9 vs. 40.1 kg/d) and milk true protein (1.07 vs. 1.16 kg/d) and decreased DMI (25.4 vs. 24.7 kg/d). Feed and milk N efficiency were greater in cows fed HS vs. RS diets. Concentration of milk true protein increased, while that of milk fat decreased with HS vs. RS diets. Both MUN and PUN were lowered for HS vs. RS diets. Supplementation of RPMLH improved milk true protein concentration. Starch level by RPMLH interactions were observed for plasma Arg and Lys, with elevated values in cows fed RS diets. Increased dietary energy reduced the plasma concentrations of all EAA except Met and Thr. Plasma Met and His were increased by RPMLH. In conclusion, enhanced dietary energy by replacing fibrous byproducts with ground corn improved yields of milk and milk protein through better use of dietary N and elevated mammary uptake of EAA.

Key Words: dairy cow, energy, amino acid

240 Effects of dietary inclusion of hydroponic barley grass on the performance of dairy cows when fed two protein levels.

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The objective of the study was to examine the effect of the dietary inclusion of hydroponic barley grass (HBG) on the performance of mid-lactation dairy cows when fed 2 different protein levels. Forty-eight Holstein dairy cows that were, 119 ± 14.8 d in milk and yielding 39.9 ± 4.7 kg/d milk were randomly allocated to one of 4 treatment groups (n = 12) in a 2 × 2 factorial arrangement. Cows were fed a control diet without HBG (HC; 16.0% CP), a low protein diet without HBG (LC; 14.5% CP), a control diet with HBG (HB; 16.0% CP), or a low protein diet with HBG (LB; 14.5% CP). The HBG replaced 2.25 kg DM of alfalfa hay in treatments HB and LB. Cows were fed the diets as a TMR twice daily using a Calan Broadbent Individual Animal Feeding System. The experimental period consisted of 1 wk for adaptation to the diet and 8 wks for sampling. Individual intake was recorded daily and feed samples collected 3 time a week for subsequent analysis of their nutrient content. Milk yield was recorded daily with samples collected weekly from 3 consecutive milkings and analyzed for composition. Data were analyzed using the PROC MIXED of SAS. Protein level, HBG, and the 2-way interaction between HBG and protein level were the fix effects in the model. The HBG and dietary protein level did not affect DMI, milk yield or energy corrected milk yield ($P > 0.05$), with mean values of 24.6, 36.5 and 36.5 kg/d respectively. Similarly, milk composition was not affected by the inclusion of HBG or dietary protein level ($P > 0.05$), and there no effect of treatment on feed conversion efficiency or N utilization ($P > 0.05$). However, there was a trend for an interaction ($P = 0.05$) between HBG and dietary protein level, with cows fed HBG and 16.0% CP having the lowest cost of production at 1.74 yuan/kg milk.

Key Words: hydroponic barley grass, dairy cow, N utilization

241 Sources of variation in milk yield, milk fat, and protein responses to exogenous feed enzymes.

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We characterized responses to an enzyme treatment commenced during the pre-calving period using pens in 3 large dairy herds. It was hypothesized that variation in intake of dietary feed components (g/d) sampled weekly would influence milk yield, fat and protein responses of milking pens; that there would be differences in responses between pens fed rations treated with a mix of fibrolytic enzymes or not and that dairy of origin would influence responses. Time-series cross-correlation analysis by pen was used with those results being pooled by meta-analytical methods to produce effect size (ES) estimates. The study provides unique insights to responses to enzyme treatment with feed and milk production responses being assessed over at least 3, and often, 6 mo. Differences in response ($P < 0.05$) between treatment and control pens occurred for soluble protein (ES = 0.249) on the same wk, ADICP (ES = 0.293) and lignin (ES = 0.237) both 1 wk before with milk protein %, and for ADICP (ES = 0.276) and lignin (ES = 0.246) 1 wk before with milk protein yield. These differences are consistent with treatment improving the digestibility of feed, particularly the protein and fiber fractions. Differences in production responses to intake of feed components among dairies were most frequently observed for the protein and fiber associated components. There were many more significant differences among dairies, than for between treatments. Differences between dairies were only observed once between the Californian dairies (Dairies 2 and 3), but >20 times for the South Dakota dairy (Dairy 1). The ES of differences between dairies were larger, on an absolute basis, than those between treatments and explained more than 7% of the variance (ES >0.5 or < −0.5) in production outcomes, in some cases. The greater ES likely reflect a greater difference in response to dietary component intakes among dairies than the difference in response achieved by enzyme intervention. The dairy that responded most to treatment had an estimated metabolizable protein (MP) excess resulting from diet, while the least responsive dairy had an estimated MP deficit and was the highest producing.

Key Words: fibrolytic enzyme

242 L-Glutamine improves weight gain and starter intake in Holstein heifer calves weaned early from a high volume of milk.

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Weaning is a stressful event associated with lowered growth rates during weaning and potentially post-weaning. Early weaning, particularly from a high volume of milk could exacerbate those growth depressions as the calves would take a longer time to achieve a desired starter intake. This study was conducted to examine the effects of L-glutamine supplementation (2.0% of total DM intake) during weaning on starter intake, growth, and health of Holstein heifer calves weaned early from a high volume of milk. Thirty-six Holstein heifer calves of 28 d of age, were assigned to 3 treatments (n = 12), 1) a later weaning age of 49 d (LW), 2) an early weaning age of 35 d (EW), and 3) the early weaning age supplemented with L-glutamine (EW+Gln). Calves of EW+Gln group received L-glutamine in milk 1 wk before and 1 wk after weaning has begun by reducing milk allowance from 9.0 to 3.0 L/d. Calves were completely weaned once they achieved a 1.0 kg/d starter intake. Body weight, hip height, hip width (HW) and body length (BL) were measured weekly. Blood (jugular) was drawn and analyzed for serum haptoglobin (HPT) concentration during the first week of weaning. Treatment effects were analyzed using linear mixed-effect models including fixed effects of treatment, and parity of the dam, and random effect of calf. Calves of EW+Gln group had greater average daily gain (ADG, 0.2 kg/d, $P = 0.011$) during the first week of weaning as opposed to EWs. Moreover, EW+Gln took fewer number of days to achieve a starter intake of 1.0 kg/d (15 vs. 17 d, $P < 0.01$), and thus weaned completely at a younger age than EW. Besides, EW+Gln had numerically lower (1.04 vs. 1.78 g/L, $P = 0.225$) serum HPT concentrations during weaning, and greater HW ($P = 0.014$) and BL ($P = 0.054$) post-weaning (70 d). Meanwhile, LW were weaned completely at 59 d of age and had BW, ADG, and starter intake similar to that of the early-weaned calves post-weaning (70 d). It could be possible to wean calves from a high milk volume as early as 5 wk of age without compromising growth performance and starter intake post-weaning. Supplementation of L-glutamine could help calves withstand weaning stress successfully as reflected by improved ADG and starter intake during weaning.

Key Words: dairy calf, L-glutamine, weaning

243 Effects of dietary addition of *N*-carbamoylglutamate on milk compositions in mid-lactating dairy cows.

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N-Carbamoylglutamate (NCG) is a structural analog of *N*-acetylglutamate, which is an allosteric activator of the essential auxiliary factor ammoniacal phosphate synthetase in the endogenous synthesis of Arg. In addition to its stability in the rumen, NCG is considered as a potential Arg enhancer for ruminants. This study was conducted to investigate the effect of NCG on milk production and composition in mid-lactating Holstein dairy cows. Sixty multiparous cows with a mean body weight of 669 kg (±71 SD) and day-in-milk of 176 (±55 SD) were blocked based on parity and milk production and randomly assigned into 4 treatments: basal diet supplemented with 0, 10, 20 or 40 g NCG per day per cow. The experiment was conducted according to a random block design with 14-wk periods, with the first 2 wk for an adaptation. Milk yield and composition were recorded weekly, while dry matter intake and plasma variables were determined every 2 wk. The data were analyzed using PROC MIXED of SAS. Addition of NCG had no effect on the dry matter intake and milk yield of the cows ($P > 0.05$). Milk fat content and yield increased linearly with NCG addition ($P < 0.01$). The contents of milk protein and total solid also increased linearly in the cows fed NCG ($P < 0.05$), whereas the yield of protein was not affected by the treatments ($P > 0.05$). Conversely, dietary addition of NCG increased the plasma nitric oxide content in a quadratic manner ($P < 0.01$). Moreover, addition of NCG linearly increased the plasma Arg content ($P < 0.01$). Overall, the results indicate that dietary NCG addition increased the milk protein and fat contents, which improved the milk quality of lactating dairy cows.

Key Words: *N*-carbamoylglutamate, milk quality, dairy cow

244 Effect of *N*-acetyl-L-methionine supplementation on lactation performance and plasma variables in mid-lactating dairy cows.

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N-Acetyl-L-methionine (NALM) is a Met derivative with lower cost, but it is unclear if it is effective as Met supplement for lactating dairy cows. The current study was conducted to investigate the effect of NALM supplementation on lactation performance and plasma variables in mid-lactating dairy cows. Forty-eight multiparous Holstein lactating cows with parity of 2.87 (±0.96 SD), and day in milk of 167.6 (±12.76 SD) were blocked into 12 groups and randomly assigned to one of 4 treatments: 0, 15, 30, or 60 g/d of NALM per cow to supplement the basal diet containing 2.0% Met (MP basis). The NALM was rumen protected, and supplementation of 10 g NALM is equivalent to 4.62 g Met. The experiment was conducted over a 13-week period, with the first week as adaptation. Milk production was recorded and milk samples were collected weekly; and dry matter intake was recorded biweekly. Blood samples were collected from the coccygeal vein at 3 h after the morning feeding on d 7 of wk 6 and wk 12. The data were analyzed using PROC MIXED of SAS, with covariance type AR (1) for repeated measures analysis. The yield of milk ($P = 0.04$), and milk lactose ($P = 0.03$) was increased quadratically, and energy-corrected milk yield ($P = 0.06$) tended to increase with increasing NALM supplementation in a quadratic manner. Dry matter intake, milk protein yield, milk fat yield, and contents of milk composition (protein, fat, lactose, milk urea nitrogen) were not affected by NALM supplementation. Plasma Met concentration ($P = 0.04$) increased quadratically, and proline ($P = 0.08$), total nonessential AA ($P = 0.05$) and total AA concentrations ($P < 0.01$) were significantly higher in 30 g/d group compared with those in the control group. Adding NALM tended to increase plasma concentrations of total protein ($P = 0.06$, quadratic) and globulin ($P = 0.05$, quadratic), but tended to decrease plasma urea nitrogen concentration ($P = 0.08$) in a quadratic manner. Meanwhile, plasma malonaldehyde concentration linearly decreased ($P = 0.05$) with increased doses of NALM. The results from this study demonstrate the positive effects of NALM on the lactation performance and oxidative stress of lactating dairy cows.

Key Words: lactation, dairy cow, *N*-acetyl-L-methionine

Ruminant Nutrition Platform Session: Probiotics, Prebiotics, and Postbiotics: Gut Health and Beyond

245 Pre- and probiotics, your cows, and their microbial balance. R. S. Hampton, J. M. Lourenco, and T. R. Callaway*, *Department of Animal and Dairy Science, University of Georgia, Athens, GA.*

The rumen and gastrointestinal tract of cattle are populated by a dense and diverse microbial population. The complex microbial ecosystems play an important role in ruminant nutrition. We have long known that ruminal bacteria, protozoa, and fungi convert feedstuffs to volatile fatty acids and microbial crude protein which are utilized by the cow. Increasing evidence has accumulated that the microbial population is intimately involved with gut health, gut integrity, and the proper function of the immune system. The microbial population of the gut produces acids and other compounds that contribute to the development of conditions such as sub acute ruminal (or cecal) acidosis (SARA) which can lead to leaky gut syndrome, laminitis, and systemic inflammation in the host animal. If we can modulate the gastrointestinal microbial population, we can affect gut integrity and alter the immune system in ways that reduce inflammation, enhance gut health, improve nutrient uptake, and reduce carriage of opportunistic foodborne pathogenic bacteria. Some of the most effective methods to modify the microbiome involves the use of direct-fed microbials (DFM), which includes probiotics and prebiotics. Prebiotics are nutrients that are not utilized by the host animal but are degraded by microbes, whereas probiotics are live or dead microbial products or the end products of their fermentation. Feeding DFM products can have profound effects on the gut microbial population, because they create changes in specific microbial ecological factors that affect survival of microbes and the end products of the gastrointestinal fermentation. Shifts in the VFA proportions can profoundly affect the growth of cattle, milk production, gut health, and energy available for gestation, lactation, or growth. By shifting the composition of the microbial population, we can change the microbial impacts felt by the animal and thus. Including pre- and probiotic products in dairy rations can improve gut health and integrity, immune responses, animal health, and food safety of dairy cattle.

Key Words: microbial ecology, direct-fed microbial (DFM), ruminant microbiology

246 The strategic use of microbial-based solutions in calf production. C. Villot[1], D. L. Renaud[2], E. Chevaux[3,4], F. Chaucheyras-Durand[3], and M. A. Steele*[5], [1]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada,* [3]*Lallemand SAS, Blagnac, France,* [4]*UMR MEDIS, INRA-Université Clermont Auvergne, Clermont-Ferrand, France,* [5]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Calves are born with a naïve immune system and undergo many stressful events in early life contributing to a higher risk of disease, especially disorders of the gastrointestinal tract. Antibiotics are often used as a first intervention in calves with diarrhea, however, these treatments are not appropriate for most cases given that the majority of diarrhea is caused by viral and protozoal pathogens. The rise of antimicrobial resistance has highlighted the need for antimicrobial alternatives leading to an emerging research field investigating nutritional supplementation of microbial-based products to improve gastrointestinal health in calves. In the past 5 years it has been shown that gut microbial colonization during early life plays a role in immune and metabolic development of calves. Thus, calf production could benefit from the ability to control gut microbiota through supplementation of microbial-based solutions at strategic points. So far studies have shown that microbial based products can impact microbial diversity, gut integrity, and natural defenses that modulate the pro and anti-inflammatory response locally in the gut. Evidence is also mounting that a variety of probiotics and prebiotics namely yeast products, have been effective in improving calf health and performance. These beneficial results have been more recently reported with non-*Saccharomyces* yeasts, such as *Yarrowia lipolytica*, and have specifically shown promise. Additional research has found that maternal supplementation of microbial based solutions, in particular probiotics with pre or post biotics, can encourage early maturation of the adaptive and innate immune system of offspring. It is important to note that microbial-based solutions will not replace proper management on-farm, but can complement the practices to maximize the probability for efficacy. Also, the concentration, the time period and the specificity of microbial based solutions introduced in the diet are essential to consider for achieving the best results on-farm. In review, there is a positive future in the field of microbial-based product supplementation for young calves and more strategic use of specific microbial-based products should be at the forefront of research and application.

Key Words: microbial-based solution, calf production, gastrointestinal health

247 Effects of in-feed enzymes on milk production and constituents, reproduction, and health in dairy cows. H. Golder*[1], H. Rossow[2], and I. Lean[1], [1]*Scibus, Camden, NSW, Australia,* [2]*Veterinary Medical Teaching and Research Center, Tulare, CA.*

Our objectives were to characterize responses in the field to a mix of fibrolytic enzymes (enzyme) using large dairy herds and sufficient study power to evaluate milk production and reproductive responses to enzyme use that began pre-calving. We hypothesized that use of enzyme would increase milk yield, when provided to dairy cattle pre-calving and for approximately 200 d of lactation. There were 7,507 cows in 8 replicates and 16 pens on 3 US dairies. Eight pens were randomly allocated as control pens (received no enzyme), while another 8 received enzyme (750 mL/t of DM feed, with estimated 350,000 and 10,000 units/g of xylanase and cellulase activity, respectively). A mixed model was fitted to milk and bodyweight (BW) data with the fixed effects of treatment, parity, dairy, and herd test and all 2-, 3- and 4-way interactions. The data were repeated measures (herd test 1–5) within experimental units with the random effects of identity, nested within treatment, replicate, and dairy. Milk and energy corrected milk yield were increased by enzyme use by 0.73 and 0.75 kg/d, respectively across a 5-mo period ($P < 0.001$). Milk fat percent was not increased by enzyme use ($P = 0.914$), but milk fat yield was increased by 0.04 kg/d, compared with controls ($P < 0.001$). Milk protein yield increased 0.02 kg/d with treatment ($P < 0.001$) despite a reduction of 0.02 in milk protein percent ($P = 0.008$). Log SCC and BW were not influenced by treatment ($P = 0.888$ and 0.322, respectively). Dry matter intake (DMI) was numerically 0.2 kg/head per d higher for enzyme pens ($P = 0.160$). Most production responses to enzyme were influenced by dairy. Milk yield in treated cows compared with controls was significantly higher by 3.53 kg/d on

Dairy 2 and numerically higher by 0.55 and 0.18 kg/d on Dairies 1 and 3, respectively. Reproduction, health, and risk of removal or death were not influenced by treatment, apart from a reduced time to first breeding (hazard ratio = 1.266; $P = 0.001$). Production responses to enzyme varied with dairy from substantial to minor increases, but variation among dairies was not evident in differences in DMI nor BW among treated and control pens.

248 *Lactobacillus plantarum* as direct-fed microbial in high-producing dairy cow diets.

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The objective of this study was to evaluate the effects of *Lactobacillus plantarum* as direct fed microbials (DFM) in high-producing dairy cows' diets on ruminal fermentation and N metabolism in vitro. A dual-flow continuous culture system was used in a replicated 4 × 4 Latin square design. A basal diet was formulated to meet the requirements of a cow producing 45 kg of milk/d (16% CP and 28% starch). There were 4 experimental treatments: the basal diet without any DFM (CTRL); a mix of *Lactobacillus acidophilus* 1×10^9 cfu/g and *Propionibacterium freudenreichii* 2×10^9 cfu/g (MLP = 0.01% of diet DM); and 2 different inclusion levels of *L. plantarum* 1.35×10^9 cfu/g (L1 = 0.05% and L2 = 0.10% of diet DM). The DFM contained both carrier and microbes. The MLP treatment was used as an intermediate treatment containing bacteria that produce and utilize lactic acid, while L1 and L2 only produce lactic acid. The ^{15}N isotope was used as a microbial marker. Data were analyzed using linear and nonlinear mixed models; orthogonal contrasts were used to compare treatment means. The fermentation pH, lactate concentration, true digestibility of nutrients, and total VFA concentration were not different across treatments. All DFM treatments decreased NH_3-N concentration although no other major difference in N metabolism was observed. The L1 treatment tended to have a greater propionate molar proportion compared with L2. In sum, all DFM tested decreased ruminal NH_3-N in vitro, which suggests a better N utilization in the rumen.

Key Words: lactic acid bacteria, starch, nitrogen metabolism

Table 1 (Abstr. 248). Effects of lactic acid-producing durect-fed microbial (DFM) treatments on ruminal nitrogen metabolism in vitro

		DFM				*P*-value		
Item	CTRL	MLP	L1	L2		CTRL vs. DFM	MLP vs. L1/L2	L1 vs. L2
NH_3-N, mg/dL	15.4	14.1	14.3	14.7	0.46	0.05	0.53	0.51
N flows, g/d								
Total N	2.73	2.76	2.79	2.75	0.06	0.59	0.92	0.62
NAN	2.10	2.17	2.21	2.17	0.07	0.35	0.96	0.51
Bacterial-N	0.84	0.86	0.90	0.82	0.04	0.59	0.99	0.11
RDP-N	1.75	1.71	1.71	1.69	0.05	0.37	0.91	0.72
RUP-N	1.27	1.31	1.30	1.33	0.05	0.35	0.99	0.70
Eff. of N utilization, %	47.9	50.3	53.2	48.6	3.00	0.42	0.87	0.25
Bacterial efficiency, g/kg	14.0	14.7	15.3	13.8	0.81	0.51	0.87	0.17

249 Effect of an enzyme extract from *Aspergillus oryzae* and *Aspergillus niger* on milk production, blood metabolites, and nutrient digestibility in dairy cows.

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The objective of this experiment was to investigate the effect of an enzyme extract (ENZ) from *Aspergillus oryzae* and *Aspergillus niger* on lactational performance, blood metabolites, and nutrient digestibility in dairy cows. Forty-eight Holstein cows (days in milk, 139 ± 51.6 d; body weight, 621 ± 72.8 kg) were used in a 10-wk randomized complete block design experiment. Cows were blocked based on days in milk, milk yield, and parity following a 2-wk covariate period. Cows within a block were randomly assigned to one of the following treatments (24 cows/treatment): (1) control and (2) ENZ supplemented to the basal diet at 4.2 g/kg feed dry matter. Cows were housed in a free-stall barn equipped with Calan Feeding System for monitoring individual feed intake and fed ad libitum once daily. The basal diet consisted of 60% forage (corns silage and alfalfa haylage) and 40% concentrate feeds. ENZ was mixed with the total mixed ration daily, before feeding. Milk production and dry matter intake (DMI) were monitored daily. Milk samples for composition analysis were collected during wks 4, 6, and 8. Blood samples for analysis of glucose, nonesterified fatty acids, and β-hydroxy butyrate were collected during wk 6. Fecal samples for determining total-tract apparent digestibility of nutrients were also collected during wk 6. Data were analyzed using the MIXED procedure of SAS with treatment, week [for repeated measures variables using ar(1) covariance matrix], and interactions in the model. Block and treatment × block were random effects. Compared with the control, ENZ increased ($P = 0.04$) DMI (23.6 and 24.7 kg/d, respectively; SEM = 0.35) and milk yield (32.4 and 33.6 kg/d; SEM = 0.56) but had no effect on energy-corrected milk yield. ENZ also increased ($P < 0.01$) milk protein and lactose concentrations and yields and decreased ($P < 0.01$) somatic cell count. Treatment had no effect on blood metabolites and did not affect apparent total-tract digestibility of nutrients. In this experiment, dietary supplementation of ENZ increased DMI and milk and milk protein yields in dairy cows.

Key Words: enzyme, milk production, dairy cattle

250 The effects of Celmanax to dairy cows on pre- and postpartum health and productivity.

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The objective of this study was to evaluate the effect of feeding Celmanx (Cel) (enzymatically hydrolyzed yeast product) to 40 (20 each treatment group) dairy cows pre- (56 g/d for 21 d) and post- postpartum (28g/d for 60 d) versus a control group. Cows were not vaccinated at the time of dry-off with E Coli or Clostridium vaccines. Cows were individually fed via Calan headgates. Postpartum milk fat and protein % were greater ($P < 0.05$) for Cel cows: 4.03 vs 3.74, and 3.30 vs 3.11, respectively. Dry matter intake and ECM for Cel and control groups were: 25.7 and 25.2 kg/d; and 45.8 and 46.5 kg/d, respectively. Efficiency of milk production (ECM/DMI) was not different between Cel and control cows: 1.84 vs 1.90, respectively. The occurrence of subclinical ketosis was greater ($P < 0.05$) for the control group (9/20) compared with Cel group (3/20). Clostridium perfringens (C. perf), Type A was present in all but 7 out of 266 fecal samples. During the pre-partum period, the amount of C. perf present in feces was not different between treatments. During the postpartum period, C. perf score was greater ($P < 0.07$) in Con cows (1.91 ± 0.2) than in Cel cows (1.67 ± 0.2). *Escherichia coli* O157:H7 was present in 4 of the 360 fecal samples collected. Of the 4 positive samples, 3 were from 3 different control cows at −14, −7, and 5 DIM. The positive sample for *E. coli* O157:H7 from the Cel cow was at the first sampling, −21 DIM. Salmonella was present in 7 of the 360 fecal samples collected. Six of the 7 positive samples were taken from control cows. One control cow had Salmonella present in 3 consecutive samples, −7, 0, and 1 DIM. No treatment difference was observed for DIM at first ovulation ($P > 0.05$). Days in milk at first standing heat was significantly less ($P = 0.003$) for Cel cows (30 DIM) than for control cows (51 DIM). Incidence of milk fever, retained placenta, metritis, endometritis, mastitis, displaced abomasum, and ketosis were not different between groups. Data suggest a lesser concentration of fecal pathogens in cows supplemented with Cel.

Key Words: yeast, health, productivity

251 Evaluation of supplemental autolyzed yeast on production parameters of Holstein cows fed a high starch diet.

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The addition of autolyzed yeast (AY) has proven to have beneficial effects on high starch diets resulting to positive implications on production parameters. This study postulated that the addition of AY (*Saccharomyces cerevisiae*) supplementation in a high starch lactation diet would improve milk production. Fifteen rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (LS0; control), high starch diet without AY (HS0), high starch diet with either 15 g (HS15), 30 g (HS30), or 45 g (HS45) of AY supplementation. The period of 21 d was divided into the adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Cows were milked 3 times daily at 0400, 1200, and 1930 h. Milk weights were recorded at every milking during the measurement phase and milk samples were obtained at each milking on d 15 and 20 of each period. Data were analyzed using the MIXED procedure of SAS. Cows in HS0 had increased DMI (24.91 vs. 19.93 kg/d; $P < 0.0001$), BW (689 vs. 665 kg; $P = 0.003$), milk yield (34.51 vs. 30.50 kg/d; $P = 0.0006$), and ECM (34.39 vs. 31.27 kg/d; $P = 0.03$) compared with cows in LS0. Cows in HS0 had greater true protein (1.10 vs. 0.94 kg/d; $P = 0.0008$), casein (0.43 vs. 0.32 kg/d; $P = 0.002$), and lactose (1.63 vs. 1.41 kg/d; $P = 0.004$) yields compared with cows in LS0. In relation to the HS0 treatment, cows in LS0 had greater fat concentration (3.89 vs. 3.56%; $P = 0.007$), and MUN (14.37 vs. 13.56 mg/dL; $P = 0.09$). The LS0 treatment had greater FCM/DMI (1.62 vs. 1.32; $P = 0.0003$), ECM/DMI (1.64 vs. 1.32; $P < 0.0001$), and milk/DMI (1.56 vs. 1.38; $P = 0.008$) efficiencies when compared with cows in HS0. Cows in HS45 had increased DMI (24.91 vs. 25.59 kg/d; $P = 0.02$) than cows in HS0. Cows in HS15 tended to improve 3.5% FCM/DMI (1.42 vs. 1.32; $P = 0.09$), and ECM/DMI (1.41 vs. 1.32; $P = 0.07$) efficiencies when compared with cows in HS0. As expected, cows receiving a high starch diet had increased DMI, ECM, and milk yield than cows in the low starch diet. In conclusion, adding AY improved DMI and production efficiencies.

Key Words: yeast, efficiency, milk

Small Ruminant 1

252 Melatonin treatment during autumn did not impair the lactational performances of 2 breeds of dairy ewes in early lactation. A. Elhadi*, A. A. K. Salama, X. Such, and G. Caja, *Group of Research in Ruminants (G2R), Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain.*

Effects of melatonin implants during lactation were assessed in 72 dairy ewes of 2 breeds (Manchega, MN; n = 36, 72.4 ± 1.9 kg BW; Lacaune, LC; n = 36, 77.7 ± 2.3 kg BW) differing in milk yield and composition. Ewes were under intensive conditions, lambed in autumn and received a TMR ad libitum (forage:concentrate = 55:45). After the weaning of the lambs (d 28), they were penned in 12 balanced groups of 6 and randomly assigned to a 2 × 2 × 3 factorial design (treatment × breed × replicate) during early lactation (d 28 to 114). Treatments were: Control (CO, n = 36) and Melatonin (MEL, n = 36) that was subcutaneously implanted in the left ear-base (18 mg/ewe; Melovine, Ceva Animal Health, Barcelona, ES) at d 35 ± 1. Milk yield was recorded daily using automatic milk meters (MM25 SG, DeLaval, Tumba, SE) and ruminal bolus transponders (Datamars, Bedano, SW), whereas milk composition was measured biweekly. Data were analyzed by the PROC MIXED for repeated measurements of SAS (v.9.4, SAS Institute Inc., Cary, NC, USA) and LS means separated by PDIFF at $P < 0.05$. No MEL effects were detected on DM intake ($P = 0.12$) or milk yield ($P = 0.60$), although they were 51% and 87% greater ($P < 0.001$) in LC ewes when compared with MN, respectively. Milk composition varied by breed, the LC producing lower fat and protein contents than the MN ewes ($P < 0.001$), but no CO vs. MEL effects were detected on milk composition ($P = 0.25$ to 0.99) nor milk-fat standardized milk ($P = 0.73$). Nevertheless, numerical greater persistence coefficients of milk yield were estimated for the groups CO vs. MEL of both breeds (LC, –0.015 vs. –0.013 L/d; MN, –0.012 vs. –0.010 L/d, respectively), resulting in a decrease in milk yield of MEL by 7 and 3% in MN and LC ewes, respectively. On the other hand, BW and BCS did not vary by MEL treatment or by breed throughout the experiment. In conclusion, the use of exogenous MEL implants, together with the endogenous MEL secreted under decreasing photoperiod conditions, had no effects on the lactational performances of dairy sheep in early lactation, despite their milk yield and composition potential.

Key Words: dairy sheep, early lactation, melatonin

253 Inflammatory responses in the mammary gland after intrauterine infusion of lipopolysaccharide in goats. F. Purba*, T. Nii, Y. Yoshimura, and N. Isobe, *Graduate School of Biosphere Science, Hiroshima University, Higashihiroshima, Hiroshima, Japan.*

Mastitis is one of the most critical diseases of dairy herds, with around 15–35% of clinical cases that still have unknown etiology. We hypothesized that the transfer of endogenous bacterial component is one of the possible causes of mastitis. This study aimed to determine whether intrauterine-infused lipopolysaccharide (LPS) can be translocated into the mammary gland and induces the inflammatory responses. Eighteen goats were used in this experiment. Sixteen goats were divided into 2 groups: infused with 5 mL of saline with or without 100 μg of *E. coli* LPS into uterus, and 2 other goats were LPS-infused and used for mammary gland tissues collection. Milk and blood were collected at 0 - 144 h after infusion to measure the blood leukocyte count (BLC), plasma LPS-binding protein (LBP), milk yield, milk somatic cell count (SCC), and pro- and anti-inflammatory cytokines (IL-1β, TNFα, IL-8, IL-10 in plasma and IL-1β, TNFα, IL-6, IL-8 in milk). The enzyme immunoassay was conducted to measure the cytokines and LBP. Mammary gland tissues were collected in the parenchyma area before and at 24 h after LPS infusion by biopsy, and the localization of LPS was observed by immunohistochemistry. Statistical analysis was completed using SAS software (version 9.4). Data were assessed for normality using the UNIVARIATE procedure and the basal values were analyzed by ANOVA. The repeated-measures analysis was used to analyze continuous data using the MIXED procedure. This model included treatment, time, the interactions between treatment and time, and the basal values as a covariate. Statistical significance of the contrast analysis was defined as $P < 0.05$. The mean of BLC ($P = 0.053$), plasma LBP ($P = 0.058$) and TNFα ($P = 0.061$) tended to be affected by the interaction between treatment and time, while in milk, the mean concentration of IL-1β ($P = 0.004$) and IL-6 ($P = 0.017$) were significantly elevated compared with the control group. The LPS was not detected in the deep area of the mammary gland before infusion, but in the connective tissues and interepithelial spaces of alveoli of the mammary gland at 24 h after LPS infusion. We conclude that intrauterine-infused LPS can be translocated to the mammary gland and induced inflammatory responses.

Key Words: lipopolysaccharide, mastitis, goat

254 Effects of calcium nitrate on dry matter intake, milk yield, milk composition, and ruminal parameters in dairy goats. K. V. de Almeida*[1,2], J. A. C. Osorio[1], F. E. de Marchi[1], T. Durman[1], J. F. Cabral[1], K. L. Guimarães[1], M. R. Sippert[1], J. C. S. Lourenço[1], C. R. Alcalde[1], R. C. de Araújo[3], and G. T. de Santos[1], [1]*Universidade Estadual de Maringá, Maringa, PR, Brazil,* [2]*University of Florida, Gainesville, FL, Brazil,* [3]*GRASP EW Nutrition, Curitiba, PR, Brazil.*

The objective of this experiment was to evaluate the effect of calcium nitrate (CN) on dry matter intake (DMI), milk yield, milk composition, and ruminal parameters in dairy goats. The CN can be used as an alternative source for NPN supplementation, but its effects on dairy goats are not well established. The hypothesis was that CN could be fed to lactating goats without affecting rumen fermentation parameters or milk production. Twelve Saanen goats (98.5 ± 13.1 d in milk; 53.5 ± 3.3 kg -body weight; mean ± standard deviation) were enrolled in a replicated 3 × 3 Latin square (21-d period; 14-d adaptation). Goats were fed ad libitum with a TMR composed of 45% corn silage and 55% concentrate on DM basis. Diets were isocaloric and isonitrogenous with 15.5% of crude protein and 10.5% of rumen degradable protein. Treatments were: T1 - basal diet without CN; T2 - 1.0% CN (66.9% NO_3^-) on DM basis; T3 - 2% of calcium nitrate on DM basis. Milk samples were collected on d 15–16 of each period and analyzed for milk components by Bentley 2000 infrared analyzer. The blood was collected 3 h after the feeding and analyzed for methemoglobin. Ruminal fluid was collected 2 and 8 h after the feeding, the pH and volatile fatty acids were determined, the latter by gas chromatography. Data were analyzed by ANOVA with mixed models using the Mixed procedure in SAS. Diets with up to 2% of calcium nitrate did not affect DMI, milk yield, milk composition, methemoglobin, or ruminal parameters. The mean values of DMI, milk yield, and milk contents of fat, protein, lactose and MUN were 1.86 ± 0.01 kg/d, 2.10 ± 0.04 kg/d, 2.7 ± 0.01%, 3.20 ± 0.03%, 4.8 ± 0.01% and 22.8 ± 0.01 mg/dL, respectively. Ruminal parameters also did not differ between treatments and showed the following means: 7.02 ± 0.09 for pH, 49.05 ± 5.6 nmol/mL of total volatile fatty acids, 31 ± 4.17 nmol/mL of acetate, 24.53 ± 8.97 nmol/mL of propionate, 6.32 ± 0.76 nmol/mL of butyrate and 3.06 ± 0.36 of acetate:proprionate ratio. Calcium nitrate can be used up to 2% on DM basis without affecting milk production, milk composition, or ruminal fermentation parameters.

Key Words: milk component, small ruminants, volatile fatty acid

255 Changes in key blood metabolites and insulin in late-pregnant prolific Afec-Assaf ewes drenched with several doses and mixtures of propylene glycol and glycerol.

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In a previous study, we found that the effect of drenching late-pregnant prolific ewes with propylene glycol (PG; 106 mL) or glycerol (GL; 108 mL) was different; while PG was anti-ketogenic, the effect of GL was mainly glucogenic. In the present study, the effect of different doses of PG and GL was examined in late-pregnant ewes (~132 d pregnant) bearing 2–4 fetuses. Thirty ewes were divided according to BHBA blood levels, expected litter size, BW and BCS into 5 groups (6 ewes each) and were drenched with: 1) Control - 55 mL water; 2) PG100 - 106 mL PG; 3) GL100 - 108 mL GL (80%); 4) PG50 - 53 mL PG; 5) GL50 - 54 mL GL (80%). Blood samples were taken 60 and 30 min before, and every hour post-drenching (PD) for 13 h. Concentrations of glucose, BHBA, NEFA, lactate, glycerol and insulin were determined. Data were analyzed using the PROC MIXED procedure of SAS. According to the response pattern, data were analyzed in 2 time-periods PD: 1) 1- 6 h; 2) 7 - 13 h. During period 1, glucose and insulin concentrations were higher in GL100 than in other groups ($P < 0.05$); PG50 was more effective in reducing the BHBA concentrations than PG100 with no differences in NEFA concentrations. Lactate concentrations were similar between PG100 and PG50, but higher than other groups ($P < 0.02$). Further, we tested the effects of mixtures of both substances in a similar design and analysis. Eighteen ewes were divided into 3 groups, and were drenched with: 1) Control - 55 mL of water; 2) MIX100 - 53 mL PG + 54 mL GL (80%); 3) MIX50 - 26.5 mL PG + 27 mL GL (80%). No differences were observed in glucose, BHBA, NEFA, glycerol and insulin concentrations between groups in both periods; however, lactate concentrations were higher in the MIX100 group at period 1 ($P < 0.05$). In conclusion, in a few parameters, lower doses of both substances seemed to be more effective than higher doses. In addition, mixtures of PG and GL were not effective in achieving the anti-ketogenic and glucogenic effects simultaneously. The results of this study showed that further research is required to establish proper doses and composition of these substances.

Key Words: sheep, propylene glycol, glycerol

256 Characterization of plasmatic oxidative and metabolic profile in Italian goat breeds.

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Characterization of local breeds in terms of physiology and production is crucial to propose strategies for their preservation. Blood from Italian local breeds Garganica (GA), Girgentanta (GI), Jonica (JO), Rossa Mediterranea (RM), Maltese (MA) and Saanen (SA) was sampled throughout a complete lactation (28 wk) to characterize their metabolic and oxidative plasmatic profile when reared under the same experimental conditions. A total of 57 goats (9–10 does/breed) were enrolled in the study, and individual blood samples (n = 784) were collected every 2–3 wk. A mixed model with repeated measures was used to analyze the data considering breed, week of lactation and their interaction as fixed effects, and the animal and the residual as random. Metabolic plasmatic profile revealed that ($P < 0.05$): the greatest NEFA (mmol/L) value was observed in SA (0.63 ± 0.01); triglycerides (mmol/L) were greater in MA, GA and GI (35.1 ± 0.65) than in RM (31.8 ± 0.65); glucose (mmol/L) was greater in GA and JO (65.2 ± 0.65) than in GI, MA and SA (61.9 ± 0.62); total protein (g/L) was greater in GI and JO (7.18 ± 0.03) than in GA (7.04 ± 0.03); creatinine (mg/dL) was greater in RM (0.86 ± 0.01) than in MA (0.78 ± 0.01); the lowest uric acid (mg/dL) value was observed in JO (0.38 ± 0.01); ALT (U/L) was greater in GI (11.7 ± 0.21) than in MA (10.0 ± 0.20); AST (U/L) was greater in GI and RM (261 ± 4.8) than in GA and JO (196 ± 4.6); and ALP (U/L) was greater in MA (239 ± 1.1) than in GA, JO and SA (124 ± 1.1). The oxidative plasmatic profile showed that ($P < 0.05$): the greatest TBARS (nmol/L) and FRAP (mg AAeq/mL) value was in JO (0.97 ± 0.02) and GI (73.4 ± 1.67), respectively, while the other breeds showed no differences among them; IDROP (μmol/mL) was greater in MA (6.56 ± 0.08) than in RM (6.19 ± 0.09); carbonylated proteins (μmol/mL) were greater in GA, MA, SA and RM (111 ± 1.0) than in JO (105 ± 1.0); and SOD (U/mL) was greater in GA (114 ± 0.9) than in GI, JO, MA and RM (109 ± 1.0). This is the first characterization of the metabolic and oxidative profile of GA, GI, JO, RM and MA goat breeds. Our results revealed considerable differences between the breeds. The authors thank the Centro di Zootecnia e Acquacoltura (Italy) and the Associazione Italiana Allevatori (Italy).

Key Words: autochthonous, goat, lactation

257 Effects of sodium bicarbonate and chromium propionate supplementation on growth performance, blood and rumen indices of Beetal bucks under heat stress conditions.

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Objectives of current experiment were to determine the effects of sodium bicarbonate (SBC) and chromium propionate (Cr) supplementation on intake, growth performance, feed sorting, rumen pH, and blood indices under hot and humid conditions. Twenty-eight Beetal bucks were randomly assigned to 4 treatments (n = 7 bucks/treatment): Control (C) without supplementation, sodium bicarbonate (SBC); at 1.5% of DM, chromium propionate (Cr); at 1.5 mg chromium/animal/d), and (SBC+Cr) diet containing SBC at 1.5% of DM + Cr at 1.5 mg chromium/animal/d. Total duration of experiment was 8 wk. Animals were housed individually, fed on iso-nitrogenous TMR (30% oat silage and 70% concentrate) to ensure 10% daily refusal, and given free access to water. Temperature and humidity values were recorded thrice daily at 0800, 1400 and 2000 h. Feed sorting, body weights and body measurements were conducted weekly. Rumen samples (n = 4 bucks/treatment) were collected on fortnightly basis using oral tube to determine rumen pH. Weekly measures including ADG, DMI, feed sorting, rumen pH and blood metabolites were analyzed using Mixed Model of SAS. Data of live BW, structural measurements, and FE were analyzed using one way ANOVA and declared significant at $P < 0.05$. During entire experiment, mean daily THI (85.3 ± 1.94) remained above the threshold level of THI (72–75) for ruminants. Mean daily DMI was higher ($P < 0.05$) in the SBC and SBC+Cr (1227, 1258 g/d) compared with the C and Cr

(928, 962 g/d). Additionally, ADG was higher in the SBC+Cr ($P < 0.05$) compared with the C and Cr (185 vs. 124 and 136 g/d). Feed sorting behavior, body measurements, blood metabolites and feed efficiency were not affected ($P > 0.05$) by the treatments. However, rumen pH was higher in the SBC and SBC+Cr ($P < 0.05$) than in the C and Cr treatments. In conclusion, combination of SBC at 1.5% of DM and Cr at 1.5 mg/animal/d improved DMI and ADG without any negative impact on rumen and blood indices.

Key Words: heat stress, growth, Beetal buck

Teaching/Undergraduate and Graduate Education Symposium: Strategies for Assessing Student Learning

258 Do grades reflect student learning? K. Machtmes*, *Patton College of Education, Ohio University, Athens, OH.*

Student learning has always been assessed by grades but what do grades really measure? Do grades measure if students can apply their knowledge or if they can transfer their learning from one other scholarly environment to another? Instructors may not be able to eliminate the need to assign grades, but transforming the assignment is crucial. It is critical to teach students to be lifelong learners capable of transferring knowledge and skills from one condition to another. This session will focus on the development of projects that embrace experiential learning and enhance transferability of learning.

259 Are "learning styles" real and do they impact learning assessment? T. Kitchel*, *The Ohio State University, Columbus, OH.*

For decades, there has been much discussion over the role of learning styles in the teaching and learning process. Critics of the learning styles movement would say it's stereotyping learning and learners in a way that is not helpful for student learning. Champions would say the area of learning styles helps teachers understand their students better and therefore assists in the process of tailoring teaching to student needs. But, what does science say and what does practice support? In this session, we will unpack learning styles and its role in the teaching and learning process. In particular, we will talk about different ways learning styles have been operationalized, ways learning styles have been espoused to be use, and the connection, if any, to student learning. And, we will answer the question, if not learning styles, then what do we use?

Key Words: teaching, learning, assessment

260 Why do we assess? D. Guberman*, *Purdue University, West Lafayette, IN.*

Most of us rely upon a multi-interval grading schema (A, B, C, etc.), but there is little consensus regarding what these symbols mean. Three common views include: a measure of student learning and growth, a representation of student achievement, and a comparison of achievement between students. Currently, our grading systems are opaque, transcripts give no indication of a particular class's grading philosophy, and few instructors openly discuss their philosophies with their students. In this presentation, I seek to spark a discussion about this core artifact of student achievement and assessment, focusing on 3 points rooted in existing scholarship: (1) Research on teaching and learning has highlighted the importance of transparency for student success. While these discussions have often focused on the level of individual assignments, I hypothesize that the same is true at the level of courses and grades. I ask that we self-reflect on what we want to communicate with grades and how we can share this message with our students. (2) Current grading systems can cause harm, as evidenced in research on stereotype threat, social belonging, and growth mindsets. Thus, these practices contribute to achievement and completion gaps that disproportionately impact underrepresented students, first-generation students, and students from lower socioeconomic backgrounds. (3) There are alternatives to current grading systems, such as competency-based systems and developmental feedback systems. I offer concrete ideas for how we can structure entire classes without grades, as well as how we can create "ungraded" structures within current systems. Current grading schemes are symbolic of the banking system of education, in which instructors deposit knowledge into students, students regurgitate that knowledge on demand, and the relationship ends when the students are sorted into an order based on their regurgitational prowess. By moving to other systems, we find opportunities to embrace higher education as more than feeding facts and skills. Education becomes a practice of freedom, which will leave students empowered to make meaningful decisions in complex situations throughout their lives, while contributing productively to a democratic society.

Key Words: pedagogy, assessment, inclusion

261 Methods of assessing learning utilized by university faculty in animal and dairy science programs. A. J. Carpenter*[1], E. L. Karcher[2], and C. C. Williams[3], [1]*Department of Animal Biosciences, University of Guelph, Ridgetown, ON, Canada,* [2]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [3]*School of Animal Sciences, Louisiana State University, Baton Rouge, LA.*

Educators have a wide variety of assessment styles available to determine whether students are achieving the stated learning outcomes for a course, and the assessment chosen by an educator may be determined by several factors, including the educator's teaching style, the course content, the course level, and the size of the course. An electronic survey was distributed to members of the American Dairy Science Association to collect feedback from dairy and animal science educators in post-secondary education on the methods that they use for assessing student learning. Questions were asked to determine level of education and experience, teaching load, course expectations, methods of assessment, and attitudes toward using technology for assessing student learning. Educators may benefit from hearing about the various assessment strategies used by their peers and learn new ways of evaluating their students' knowledge.

Key Words: learning, education, assessment

Animal Behavior and Well-Being 1

T1 Evaluation of a commercial accelerometer for remote monitoring of lying and standing events in dairy calves in Puerto Rico.

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Formerly, cattle behavior studies were performed by direct visual observations or video analysis, which require considerable resources and time. Recently, accelerometers have been successfully used for remote monitoring of behavior in dairy calves. However, this validation was made under temperate weather conditions, and to the authors understanding no similar data exist for the tropics where heat stress affects cattle behavior. Thus, the y-axis values recorded by HOBO Pendant G accelerometers tied to the right rear leg (perpendicular to the floor) in confined Holstein calves (n = 5; 53 ± 20 d old) in Puerto Rico were evaluated as indicators of lying (L) and standing (S). Data were recorded at 1-s intervals from 0700 to 0900h (n = 23,408) on February 7, 2019. The L and S events were visually recorded as gold standards for validation. Transitional movements between L and S were excluded. To determine the best way to classify the obtained data, its overall mean and standard deviation (SD) during the visual L and S events were calculated and used to create a series of respective accelerometer categories (L or S) in the ranges between 0.4, 0.6, 0.8, 1, 1.2, 1.4, 1.6, 1.8, 2, 2.2, 2.4 or 2.6 SD bellow to above the mean. Proc FREQ (SAS) was used to determine the sensitivity (likelihood that a visually recorded event was also recorded by the accelerometer), visual-accelerometer misclassifications (L-S or S-L) and non-classified observations in each created category. All categories successfully identified over 99% of the recorded L and S events, with less than 0.04 and 0.16% of L-S and S-L misclassifications, respectively. However, the amount of non-classifications increased as the category range decreased (0.47 to 55% from 2.6 to 0.4 SD, respectively). The category that maximized the sensitivity for the L (99.89%) and S (99.98%) events, while maintaining low levels of the L-S (0.02%) and S-L (0.11%) misclassifications and non-classified values (1.23%) was the 2 SD bellow and above the mean. The evaluated accelerometers may be useful tools for remote monitoring of L and S behavior in dairy calves in the tropics.

Key Words: behavior, accelerometer, Holstein calf

T2 Deworming influences feeding and lying behavior in Holstein cows.

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Deworming is a common practice at dairy farms. Limited data are available to indicate the influence of deworming on behavior of dairy cows. The objective of current study was to assess the influence of deworming on feeding and lying behavior of Holstein cows. Twenty Holstein cows of second parity having 25.8 ± 4.8 kg/d milk and 215 ± 21 DIM (mean ± sd) were simultaneously enrolled from dairy herd at University of Veterinary and Animal Sciences, Lahore Pakistan. The data loggers (Nedap CowControl: Nedap N.V., The Netherlands) were attached to neck and leg of enrolled animals to record feeding and lying behavior, respectively. The observation period lasted for 4 d and categorized as day before (d −1), day of (d 0), d 1, and d 2 of deworming. The behavioral responses were analyzed using Proc mixed procedure of SAS with planned contrasts where d −1 was taken as control. Total feeding bouts tended to increase on d 1 after deworming (13.1 vs. 13.8 number/d; d −1 vs. d 1; SE = 0.46; $P = 0.150$) and became similar on d 2. Interestingly, total feeding time was similar for d −1, d 0, and d 1 of deworming but it was significantly lower for d2 (487.9 vs. 442.3 min/d; d −1 vs. d 2; SE = 18.2; $P = 0.025$). Total lying bouts tended to increase on and after deworming (8.2 vs. 8.6, 8.5, and 8.6 number/d; d −1 vs. d 0, d 1, and d 2; SE = 0.26; $P = 0.090$, d −1 vs. d 2). However, total lying time was similar on and after one day of deworming but it significantly increased on d 2 of deworming (620.5 vs. 678.6 min/d; d −1 vs. d 2; SE = 0.46; $P = 0.019$). These results indicated that deworming practice did not significantly influence feeding and lying behavior on and one day after deworming. The unpredictable behavioral response on d 2 of deworming could be confounded due to environment or farm management.

Key Words: dairy cow, deworming, lying behavior

T3 Effect of heifers rearing in early age on their behavior during first lactation.

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Aim of the study was tested hypotheses that the cow's behavior is affected by the method of rearing from the second week of life to weaning. Fifty-one Holstein cows were reared in 3 groups from d 7: A, pen with the milk drinking feeder, n = 19; B, hutch, bucket with nipple, n = 19; C, pen with nursing cows, n = 13. After weaning at 8 wks, all heifers were kept in group pens, after parturition in free-stall housing. The learning ability (8 × 14 m closed maze) was tested in mo 4 of first lactation. Cows solved 6 tasks during 3 days. Tasks 1 and 2 required a left-side solution, tasks 3 and 4 a right-side solution, and 5 and 6 a central solution. Each task had 2 runs. The time of standing in maze and speed of traversing the maze were recorded. At 7th month of first lactation, during 3 d (12 tests) the animals were subjected to test in an aversion alley (16.3 m long, 1.86 m wide). There was applied to a positive treatment on d 1 (brushing), a negative treatment on d 2 and 3 (single noise 128 dB.s^{-1} and electric shock 1.5 kV.s^{-1}). The time of run across the alley was recorded. The data were analyzed using a GLM ANOVA. Group A was the fastest in the first 4 tasks, group B ran through the maze the fastest in the fifth and sixth tasks. The average time of traversing the maze for 6 tasks was the highest in C (170 ± 122 s) and the lowest in A group (108 ± 97 s; $P < 0.05$). Differences ($P < 0.05$) at time of standing in maze and a speed of traversing the maze ($P < 0.05$) were found between groups C and A. We did not find out any significant differences in aversive behavior among groups. After negative treatment on the 2nd day the group B needed longer time to across the aversion alley than groups A and C (67 ± 41 s, 54 ± 31 s, 56 ± 44 s). On third day, the time to across the alley increased in all groups (98 ± 78 s, 98 ± 69 s, 90 ± 88 s). The results show that the method used to rear heifers may have a significant impact on their maze behavior; however, the rearing conditions had little long-term effect on the aversive behavior. This study was possible through projects APVV 15-0060 and QK1910438.

Key Words: dairy cattle, rearing, behavior

T4 Standing behavior during transition is associated with sole lesions at peak lactation.

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The laxity of the supportive structures of the claw increase during the transition period increasing the risk of mechanical damage. The aim of this prospective longitudinal study was to evaluate if standing behavior during the transition period related to sole lesions on commercial dairy farms. A convenience sample of 8 farms was visited every other week, and animals without previous severe claw horn lesions and remaining sound until calving were included. Standing behavior was measured with data loggers attached to a rear leg, and standing time and duration of the longest standing bout were determined for each cow and day. Standing behavior was further summarized into: 1) before (d −14 to −2), 2) around (d −1 to 1), and 3) after (d 2 to 14) calving. Average standing time and average longest standing bout were determined for each cow and period. Behavior was recorded for 262, 253 and 256 cows in the 3 periods, respectively. For 250 cows we had standing data both before and after calving. Trimming was performed 8 to12 wk postpartum; claw lesion data were summarized per cow, and the most serious injury of each lesion type was noted. Sole lesions were found in 31 cows. Mixed-effect logistic regression models with farm as random effect were used to analyze the risk of developing sole lesions, using animals without claw lesion as baseline. Separate models were fitted for the 2 standing behaviors, and for the periods before, around, and after calving. Change in standing behavior from before to after calving was also analyzed. BCS at wk 0, BCS loss, milk yield, parity, and DIM at trimming were included as covariates. No association was found between sole lesions and standing behavior before or around calving. Longer standing time (OR 1.4; 95% CI 1.1–1.9), and standing bouts (OR 1.6; 95% CI 1.1–2.2) postpartum increased the odds of sole lesions, as did an increase in standing bout duration from before to after calving (OR 1.5; 95% CI 1.1–2.2). In conclusion, cows with prolonged standing directly after calving were more likely to develop sole lesions.

Key Words: hoof pathology, longitudinal study, standing bout

T5 Detecting welfare status in a milk sample: Effects of tie-rail placement on milk composition by Fourier transform infrared spectroscopy.

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Animal welfare issues are detected on-farm using animal-based indicators such as body injuries requiring a visit by a trained assessor. The overall goal of this study is to develop precision markers that could be routinely recorded to detect cows experiencing lower level of welfare. A specific objective is to demonstrate that Fourier transform infrared (FTIR) spectroscopy can detect changes in milk composition related to cows subjected to housing treatments whose level of welfare is characterized by a given level of movement restriction at the stall. A total of 48 cows were assigned to 4 tie-rail configurations. Tie-rail heights were 122, 122, 112 and 102 cm and forward positions were 18, 36, 18 and 36 cm for treatments T1, T2, T3 and T4, respectively. Cows were assigned to 6 blocks to account for parity, days in milk and barn location effects. The trial lasted 10 weeks. Milk mid-infrared spectra were collected on weekly basis. Different preprocess treatments were applied and regions 3061–2803 cm^{-1}, 1797–1681 cm^{-1} and 1612–925 cm^{-1} were retained for analysis. Long-term average spectra were calculated for each cow using spectra collected in wk 8–10 of treatment. Principal component analysis was applied to spectral averages and scores of principal components (PCs) were tested for treatment effect by multivariate ANOVA and mixed modeling. Normalized first derivative long-term averaged spectra revealed a significant treatment effect on PC7 ($P = 0.01$). Tukey's means multiple comparisons of treatment scores for PC7 revealed that T3 was significantly different from T1 ($P = 0.02$), T2 ($P = 0.04$) and T4 ($P = 0.04$). Loading spectrum of PC7 revealed intense peaks that could be assigned to biomarkers related to negative energy balance, such as citrate and acetone. This observation suggests that T3 might have been restrictive for cows to access feed. T3 was the only treatment resulting in an increase in neck injuries on 2 locations of the cows' neck corroborating findings from spectral analysis. Milk FTIR spectroscopy showed promising results in detecting welfare status in dairy cows.

Key Words: milk Fourier transform infrared (FTIR) spectroscopy, animal welfare, biomarker

T6 Youth preferences for dairy calf housing options.

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Public preference of farming systems is a critical component of social sustainability. Recent research has been conducted on public attitudes to various aspects of dairy farming. However, youth perspectives in particular have been underexplored. The objective of this study was to understand preferences of dairy calf housing options among youth. Participants 5 – 17 years of age (n = 463) were invited to complete a 21-item survey at the Minnesota State Fair (St. Paul, MN) in summer 2018. The survey was administered via Qualtrics using iPads and, in addition to collecting demographics, presented 3 images of calf housing options (individual, pair, or group) and asked participants to select their preferred option. Most participants (median age = 11 yr) were female (61%), urban residents (59%), owned a pet (90%), and did not have prior experience handling agricultural animals (63%) but had visited a farm in the past (82%). Data were analyzed using PROC GLIMMIX of SAS, and multinomial models were built with housing option as the dependent variable. Overall, group housing was overwhelmingly preferred (mean ± SE; 81.1 ± 3.0%), followed by pair (10.4 ± 2.5%) and individual housing (8.5 ± 1.8%). Housing preference was not associated with age, gender, pet ownership, or prior visits to a farm. However, rural youth more frequently preferred individual housing compared with urban youth (14.0 ± 4.5% vs. 5.0 ± 1.3%, respectively, $P < 0.05$). These findings suggest that youth from urban backgrounds may be less accepting of individual housing systems for dairy calves and that group housing is preferred by majority of survey participants. Further qualitative analysis will be used to identify reasons underlying youth preferences of calf housing options.

Key Words: youth public perceptions

T7 Effects of disbudding on feeding behavior, lying time, and use of a shelter in group-housed calves.

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Disbudding results in behavioral changes indicating a response to pain. We examined the activity, feeding behavior, and shelter use of calves in the 12 h following hot-iron disbudding. Holstein bull and heifer calves (n = 32; 4 calves/pen) were group-housed (7.4 m^2/calf) at 16 ± 2 d of age (age range <7 d). Calves were provided milk replacer ad libitum via an automated milk feeder for 6 weeks and gradually weaned over 9 d. All pens had a 3-sided, open top shelter (1.2 × 1.2 m) made out of corrugated plastic. Calves were randomly assigned within pen to be disbudded on different days; half of the calves in each pen were disbudded during wk 5 and the rest were disbudded during wk 6. All calves in the pen were handled in both weeks. Calves received local anesthetic and analgesic before disbudding. Behavior of all calves was recorded continuously from video for 12 h in both weeks, following either disbudding or handling only (control), to characterize lying time and time spent using the shelter (>50% of body inside shelter). Milk intake and feeder visit frequency and duration were summarized for the same 12 h observation period. Data were summarized by week, and analyzed in a general liner model to test effects of treatment (disbudding or control), week, and order of exposure to treatment. Milk intake (8.85 L; SE = 0.45; $P = 0.26$), visit duration (27.13 min; SE = 2.43; $P = 0.62$), and visit frequency (4.49 visits/12 h; SE = 0.25; $P = 0.23$) were not affected by disbudding. Similarly, the frequency (12.4 bouts/12 h; SE = 0.68; $P = 0.35$) and duration of lying time (483.4 min/12 h; SE = 918.45; $P = 0.97$) did not differ. Following disbudding, calves entered the shelter more frequently (9.1 vs. 6.4 visits/12 h; SE = 0.81; $P = 0.022$) but the total duration of time in the shelter was similar (162.9 vs. 87.5 min/12 h; disbudded vs. control; SE = 0.27; $P = 0.44$). Use of the shelter also changed between weeks, with calves spending more time overall in the shelter during the second observation week (303.3 vs. 48.0 min; SE = 0.27; $P = 0.03$). These results suggest that the use of a shelter changes around disbudding, and further research is needed to evaluate how added environmental complexity may influence or predict animal welfare.

Key Words: dairy calf, disbudding, behavior

T8 Effects of showers in the parlor on reducing heat stress.

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Heat stress in dairy cattle negatively affects welfare and production. Holding pen and feedline sprinklers are effective cooling strategies, but have the potential to spray the ground if cows are not present. A novel cooling strategy is to use individual showers in the parlor, allowing controlled water application on each cow. Our objective was to quantify the cooling effectiveness of soaking dairy cattle during milking in the parlor. Eight pens (n = 8) of lactating Holstein cows were enrolled over 4 periods. Two pens/period were tested during 1 milking/d with 3 d unsprayed (control) and 3 d sprayed (1 min, 10.9 ± 2.3 L, mean ± SD). Fifteen focal cows/pen were evaluated 30 s before and after treatment application for respiration rate (RR) and body temperature (BT), measured from continuous video recordings and vaginally indwelling data loggers (1-min intervals), respectively. Microclimate (temperature and relative humidity) of the parlor was measured at 5-min intervals and converted to temperature-humidity index (THI; range 65.3–81.3). Linear mixed models were used to evaluate treatment and THI effects and their interaction. Cows mostly maintained homeothermy: BT did not differ between treatments before or after the treatment period (38.5 ± 0.04°C, mean ± SE; regardless of treatment or time point; $P = 0.65$). BT had a positive linear relationship with THI ($P < 0.001$): for every 10-unit THI change, BT increased by 0.2°C, and starting values > 38.6°C were observed when THI > 72. Elevated RR is an early heat-stress indicator. Beforehand, RR did not differ between treatments (control vs. spray: 56.7 ± 3.7 vs. 57.7 ± 3.7 breaths/min; $P = 0.64$). RR had a positive linear relationship with THI ($P < 0.001$): for every 10-unit THI increase, RR increased by 24 breaths/min, and starting values > 60 breaths/min were observed when THI > 70. The spray effectively reduced RR (control vs. spray: 57.0 ± 3.3 vs. 47.4 ± 3.3 breaths/min; $P = 0.004$) and also decreased the effects of THI on RR (treatment × THI interaction; $P < 0.001$): for every 10-unit THI change, RR increased by 23 vs. 18 breaths/min in the control vs. spray treatments, respectively. Individual showers in the parlor were effective at reducing RR.

Key Words: water spray, showers, cooling

Animal Health 2

T9 Effect of pulsed alternating wavelengths on melatonin, cortisol, and serotonin concentrations in Holstein heifers.

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Manipulating light exposure at strategic points in the life cycle of cattle could be a non-invasive technique to improve performance, health, and well-being. Pulsed alternating wavelengths system (PAWS; Xiant Technologies, CO) is a recently developed technology based on delivering specific light wavelengths and patterns. The objective of this small-scale study was to compare the hormonal (melatonin and cortisol) and neurotransmitter (serotonin) levels in PAWS exposed vs. unexposed control calves. Holstein heifer calves (3 d old) were housed individually in polyethylene hutches with a front yard of 2.25 m^2 with sand bedding and assigned into 1 of 2 treatments: (1) control (CON; n = 4); and (2) PAWS exposed (PAWS; n = 4). Hutches in the PAWS group had interior lamps affixed to the hutch roof and were constantly on. All the study calves had free access to the enclosed front yard. Calves were fed and managed according to farm management program. Blood samples were collected for determination of serum melatonin and serotonin concentration at 0600 h, 1200 h, 1800 h, and 2400 h on d 0 (enrollment), d 2, d 4, and d 14. Hair was sampled for cortisol determination on d 0, d 14, d 40, and d 60. No group differences for cortisol concentration were determined. Table 1 presents mean (SE) melatonin and serotonin serum concentrations for the overall monitoring period, by day, and by day at 2400 h, which was the time of maximum exposure to the treatment. Data from this initial small-scale study indicate a significant effect for PAWS on melatonin concentration. A larger study is needed to further explore these associations.

Key Words: calf, light, hormone

T10 Effects of a nutritional and handling stress challenge in Parmigiano Reggiano lactating dairy cows.

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Cows producing milk for Parmigiano Reggiano (PR) cheese are prohibited from consuming fermented feeds. Such diets contain finely chopped dry forages and high fermentable carbohydrates. The aim of this study was to: (1) characterize changes in rumen environment, immunity and production in high producing PR Holstein cows subjected to a nutritional and handling stress challenge and, (2) evaluate the effect of feeding OmniGen-AF© (OG, Phibro Animal Health, Teaneck NJ) on immune cell responses to an induced acidosis. Nutritional stress was induced by altering the forage to concentrate (F:C) ratio of the base diet from 46:54 F:C (28% starch) to 25:75 F:C (35% starch), and handling stress initiated by moving cows from free stalls to tie stalls. Cows (n = 24) were blocked by DIM, milk yield and parity and randomly assigned at trial start to control (CON, no OG, n = 12) or treatment (TRT, OG fed at 55 g/h/d, n = 12) group. The study consisted of 3 dietary periods: adaptation (AP: base diet, free stalls, 28d), challenge (CP: acidosis diet, tie stalls, 28d) and recovery (RP: base diet, free stalls, 28d). During the whole trial, rumination time (RT), reticulo-rumen pH, milk yield, milk compositions, blood components and stress markers were recorded. Data were analyzed with MIXED procedure (JMP 14). Period, treatment and interactions were considered as fixed effects, and cow as random. Feeding OG produced the best outcomes in terms of daily pH during CP and RP (6.07, 6.10; respectively, $P = 0.05$) compared with CON and showed a tendency of less daily minutes of pH <5.8 (249 *vs* 341 m/d; $P = 0.08$, CP). TRT showed a numerically higher milk fat % throughout CP than CON (3.07 *vs* 2.87). OG fed cows tended to have higher leukocytes and neutrophils counts during CP than CON. BHOB level was higher for CON in CP (+0.23mmol/L, $P < 0.01$) than TRT. FRAP resulted lower in OG during P ($P < 0.05$) after 3 and 14d (−28.98 and −24.73 µmol/L, $P < 0.05$ and $P < 0.10$). Results from this study demonstrated that a combination of stressors could impact the rumen environment and that OG can play a possible positive role to attenuate systemic inflammation in cows experiencing rumen acidosis.

T11 Assessment of the DQ-ovalbumin method, and comparison of neutrophil function assays in dairy cows.

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Table 1 (Abstr. T9).

	Melatonin (mean SE; pg/mL)			Serotonin (mean SE; ng/mL)		
Time	CON	PAWS	*P*-value	CON	PAWS	*P*-value
Overall	5.47 ± 1.86	11.6 ± 1.85	0.02	1,644 ± 91.5	1,462 ± 91.6	0.16
Day average						
d 0	2.66 ± 2.08	9.98 ± 2.08	0.02	1,406 ± 118	1,268 ± 119	0.42
d 2	4.58 ± 2.27	10.3 ± 2.27	0.08	1,926 ± 196	1,850 ± 196	0.78
d 4	4.99 ± 2.36	9.80 ± 2.36	0.16	1,369 ± 192	1,279 ± 192	0.74
d 14	9.10 ± 6.80	17.0 ± 6.80	0.41	1,870 ± 172	1,458 ± 172	0.10
Sample at 2400 h						
d 0	4.56 ± 7.66	22.6 ± 7.66	0.14	1,781 ± 311	1,458 ± 311	0.49
d 2	5.13 ± 7.13	23.7 ± 7.13	0.11	2,700 ± 405	2,543 ± 405	0.79
d 4	10.1 ± 9.06	20.8 ± 9.07	0.43	2,169 ± 470	1,758 ± 470	0.55
d 14	4.45 ± 9.62	15.4 ± 8.33	0.43	1,803 ± 320	1,925 ± 277	0.78

Traditional polymorphonuclear (PMN) leukocyte (neutrophil) function assays of oxidative burst (OB) and phagocytosis (PC) are widely used to evaluate innate immunity in the transition period of dairy cows. OB is evaluated by measuring PMN median fluorescence intensity (MFI) after in vitro stimulation. PC is measured by engulfment of fluorescent beads by PMN. DQ^{TM} ovalbumin (DQ-OVA) labels a pH-insensitive compound that fluoresces upon proteolytic degradation after endocytosis. DQ-OVA might, therefore, be informative about an additional pathway of pathogen handling by PMN. This study evaluated the use of the DQ-OVA assay for the assessment of PMN function, and the associations among OB, PC, and DQ-OVA outcomes in PMN isolated from blood of dairy cows between 5 and 21 d postpartum. The PMN function outcomes were assessed with mixed linear regression models. For the validation assay (9 cows in 3 replicates), PMN cocultured with DQ-OVA were stimulated with IFN-γ or inhibited with cytochalasin D and compared with control non-stimulated PMN. Stimulated and inhibited PMN had greater (970 ± 160 units, $P = 0.4$) and lesser (593 ± 55 units, $P = 0.03$) MFI relative to non-stimulated PMN (791 ± 154 units), respectively, indicating that DQ-OVA reflected enhanced or reduced endocytic and proteolytic function. From 160 samples from 40 cows, the only significant association among PMN assays was between DQ-OVA MFI and PC (Pearson r = 0.16; $P = 0.04$). When values of MFI were categorized according to the first, second and third, or fourth quartiles, agreement beyond chance (κ statistic) was moderate: κ = 0.38 for DQ-OVA and OB, κ = 0.43 for DQ-OVA and PC, and κ = 0.43 for OB and PC. The DQ-OVA assay may complement traditional PMN functional assays since it provides additional information regarding antigen processing, but it is not a substitute for either.

Key Words: transition period, innate immunity

T12 Biomarkers associated with granulocyte oxidative burst in uterus of cows in early lactation.

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Migration of functional neutrophils into the uterus following parturition is thought to be key to uterine health and fertility in early lactation dairy cattle, but the activity of uterine neutrophils has rarely been investigated. Data from 2 studies on peripartum dairy cows were used to assess relationships between measures of immune function and uterine granulocyte oxidative burst. Whole blood from multiparous Holstein cows (n = 39) on d 10 and 38 relative to calving was collected for flow cytometry and complete blood count analysis. On the same days, endometrial samples were collected by cytobrush technique. Cytobrushes were rolled onto a slide which was stained for polymorphonuclear cells (PMN; 300 cells counted) and then immersed in culture media. Whole blood and uterine brush samples were prepared for flow cytometry to measure oxidative burst of granulocytes after phorbol myristate acetate stimulation. Labeled antibodies (CH138a and CD45) and dihydrorhodamine were used to identify granulocytes and quantify oxidative burst, respectively. Granulocyte response (GR) was calculated as stimulated – basal oxidative burst. Statistical models evaluated associations with predictors, their interactions with day, and the random effect of study. Outliers were identified and removed using conditional residuals. Uterine GR was not associated with blood GR on d 10 ($P = 0.60$) but was positively associated with blood GR on d 38 ($r^2 = 0.41$, $P = 0.05$). Similarly, on d 38, blood leukocytes/μL ($r^2 = 0.51$, $P = 0.01$) and even band neutrophils/μL ($r^2 = 0.67$, $P = 0.03$) were positively associated with uterine GR, though not on d 10. Regardless of time point, the proportion of PMN in cytobrush samples was positively associated with uterine GR ($r^2 = 0.55$, $P < 0.001$). Blood markers of immune status predicted uterine GR only after the transition period, but greater uterine PMN populations are predictive of greater oxidative burst potential of uterine granulocytes across time points.

Key Words: immunity, neutrophil, transition cow

T13 OmniGen-AF alters metabolic profile and blood cell populations after 28 days of supplementation in LPS-challenged heifers.

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Previous studies in dairy cattle fed OmniGen-AF (OG, Phibro Animal Health, Teaneck, NJ) show OG modulates innate immunity and blood metabolites after 45 to 60 d with continuous feeding. Interestingly, in beef cattle a shorter interval between measured changes in immune signaling following the onset of OG feeding have been observed. However, this work has been limited to identifying cellular signals in healthy, unchallenged beef heifers. This study aimed to investigate immune and metabolic responses in beef and dairy heifers challenged with LPS after 28 d of feeding OG. Angus (n = 14) and Holstein (n = 15) heifers were assigned based on BW (within breed) to 2 dietary treatments: TMR top dressed with OG (OG, 4g/45.36 kg BW, beef n = 7, dairy = 8) or placebo (CON, 4g/45.36 kg BW beef n = 7, dairy n = 7). All heifers were fed their respective diets continuously and on d 28 were challenged with an IV LPS bolus (0.125 μg/kg BW). Complete blood counts and serum metabolites, were analyzed at different hours relative to LPS bolus (hour), period during which heifers were still on their respective dietary treatments. Data were analyzed using the PROC Mixed procedure of SAS with hour, treatment, breed, and all interactions evaluated as fixed effects. Treatment interactions with hour were not significant ($P > 0.05$). No differences in neutrophil counts, serum cortisol, or serum insulin were detected between treatments or its interaction with breed ($P > 0.05$). Heifers fed OG had increased monocyte counts and greater neutrophil:lymphocyte ratio compared with CON ($P < 0.01$). In beef heifers, OG reduced lymphocyte counts and serum NEFA but increased eosinophil counts and serum glucose compared with CON (treatment × breed interaction, $P < 0.01$). In dairy heifers, OG increased RBC counts compared with CON (treatment × breed interaction, $P = 0.03$). In conclusion, continuously feeding OG for 28 d altered blood cell populations and metabolism in beef and dairy heifers in response to LPS although beef heifers were more metabolically responsive. These data suggest cattle fed OG for as short as 28 d may be better suited to cope with stressors.

Key Words: OmniGen, LPS, metabolism

T14 Effects of a new preventive strategy with acetylsalicylic acid on metabolic status and inflammation in dairy cows after calving.

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Physiologically, dairy cows experience inflammation during calving; however, an exacerbated inflammatory response in the early postpartum can negatively impair cow health and performance during lactation. It has been suggested that anti-inflammatory therapy may decrease inflammation after calving, reducing these negative effects. The objective of this study was to assess the effects of a new preventive strategy with acetylsalicylic acid (ASA) on BCS, BHB, and inflammation (haptoglobin; HP) in postpartum dairy cows. Dairy cows (n = 246) from one dairy farm located in central Pennsylvania were included in this study. Cows were blocked by parity and randomly assigned to 2 groups: 1) ASP (n = 121): within ~12 h after parturition cows received 2 treatments with ASA (200 mg/kg; 4 boluses) 24 h apart; or 2) UNT (n = 125): remained untreated. Blood samples were collected at 7 ± 3 and 14 ± 3 d in milk (DIM) to measure BHB and HP concentrations. BCS was visually assessed by one experienced veterinarian at 7 ± 3, 14 ± 3 and 50 ± 10 DIM. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized block design. There was no difference in BCS at 7 ± 3, 14 ± 3 and 50 ± 10 DIM between groups. Although the proportion of cows with subclinical ketosis did not differ between groups at 14 ± 3 DIM (ASP = 10 ± 3.72%; UNT = 16 ± 4.96%), cows treated with ASA had lower serum BHB concentration at 14 ± 3 DIM (ASP = 0.99 ± 0.08 mmol/L; UNT = 1.18 ± 0.08 mmol/L) compared with UNT cows. There was no difference in HP concentrations at 7 ± 3 DIM between study groups. The results from this study suggest that treatment with ASA after calving may improve metabolic status during early lactation, which in turn may enhance welfare and health in dairy cows.

Key Words: dairy cattle, acetylsalicylic acid, inflammation

T15 Growth and health of recently transported dairy heifers fed a novel antibody to interleukin-10.

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The objective of this study was to determine heifer health and growth after transportation and acclimation to a new environment when fed a novel egg yolk antibody to interleukin-10. One hundred sixty recently transported Holstein heifers (13.7 ± 0.8 wk old and 125.9 ± 13.0 kg BW) were randomly allocated to pens of 8 heifers with pen considered the experimental unit. Every 4 pens of 8 heifers was considered a block with a total of 5 blocks in randomized complete block design. Each pen within a block was randomly assigned to 1 of 4 treatments and remained on the study for 70 d. Treatments consisted of 1) a positive control with heifers fed sodium monensin at 160 mg per heifer daily (ION), 2) a negative control without any medicated feed (NEG), 3) heifers fed egg yolks containing an antibody to interleukin-10 derived from chickens immunized against interleukin 10 (aIL-10) (1100 ug of antibody per heifer daily for 2 weeks after arrival), and 4) an egg control with egg yolks obtained from sham-immunized chickens (EC). Average daily gain, frame measures, feed efficiency, DMI, and natural log of coccidial oocysts were analyzed using PROC MIXED a procedure in SAS 9.4. Fecal floats were analyzed with the FREQ procedure for positive or negative for coccidial oocysts using CHI-SQUARE analysis. No differences in intake or growth measures were observed across treatments ($P > 0.18$) with a mean daily gain of 0.95 kg/d. A difference in feed efficiency across treatments was seen ($P = 0.05$) with ION having greater efficiency than EC ($P < 0.01$), and aIL-10 had a tendency to have greater efficient than EC ($P = 0.06$). Heifers fed ION had reduced incidence and coccidia counts than the other treatments at wk 4 after arrival ($P < 0.01$), however at wk 8 all treatments had similar incidence and counts ($P > 0.26$). All heifers were negative for coccidia at arrival with minimal infections until 4 weeks after arrival. Overall, aIL-10 had minimal effects possibly due to lack of coccidia infections during aIL-10 feeding.

Key Words: coccidiosis, bovine respiratory disease, interleukin-10

T16 Early life fecal microbiota transplantation affects systemic and polymorphonuclear leukocytes mRNA biomarkers of inflammation and liver function in neonatal dairy calves.

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The objective of this study was to evaluate the effects of early life fecal microbiota transplantation (FMT) on health and performance of neonatal dairy calves. The adult donor was selected based on health and production records as well as fecal samples testing negative for infectious pathogens. Sixteen healthy newborn Holstein calves (n = 8/trt) were used in a completely randomized design and housed in individual hutches from birth to 7 wk of age. Calves were fed 2.8 L/d of antibiotic-free milk replacer 2×/d during wk 1 to 5, 1×/d on wk 6, and weaned at wk 7. Antibiotic-free starter and water were fed ad libitum. Calves were assigned to either a baseline nutritional program (CON) or 1×/d inoculations with 25 g of fecal donor material (FMT) mixed in the milk replacer from 8 to 12 d of age. Blood samples were collected weekly for immunometabolic profiling and polymorphonuclear leukocytes (PMNL) isolation for gene expression analysis. Data were analyzed using the MIXED procedure of SAS, where treatment, wk, and their interaction were the fixed effects in the model, and calf the random effect. Orthogonal contrasts were used to determine linear and quadratic effects over time. There was a trend ($P = 0.09$) for greater BW (50.8 vs 52.7 ± 0.7 kg) in FMT calves. A TRT × Wk ($P = 0.02$) was observed in haptoglobin, that was reflected in a positive quadratic effect ($P = 0.04$) in FMT calves but not in CON. Similarly, a TRT × Wk ($P = 0.07$) in IL1B resulted in a positive quadratic effect ($P = 0.07$) over time in FMT, and a linear increase ($P = 0.07$) in CON. A trend for a TRT × Wk ($P = 0.06$) was observed in the liver function biomarker paraoxonase, which resulted in greater ($P < 0.01$) paraoxonase in FMT calves than CON at 3 wk of age. The TRT × Wk ($P = 0.09$) in *NFKB1* mRNA expression in PMNL was associated with a greater ($P = 0.03$) expression in FMT calves at wk 1 (or 7 d) before the FMT was performed, but *NFKB1* expression was similar between FMT and CON at wk 2 and 3. These results suggest that early life FMT in neonatal calves have positive effects in their neonatal stage not only on growth performance but also in mediating the inflammatory response and liver function.

Key Words: calves, immunity, microbiota

T17 Differences in hematological values by sex and age of Holstein and Jersey calves.

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The demand for Jersey cattle has increased among dairy farms in Republic of Korea because Jersey cattle are known to consume less feed and produce milk with better fat and protein ratio than Holstein Friesian cattle. Because the dairy industry using Jersey cattle is at the beginning stage, little information about Jersey cattle has been investigated in Republic of Korea. The aim of this study was to compare the differences of hematological values by breed, sex, and age. A total of 62 calves (27 female Holstein, 25 male Holstein, 4 female Jersey, 6 male Jersey) were used when they were 1 d and 1 mo old. All blood samples were collected through jugular vein at the NIAS in Cheonan, Korea. Procyte Dx analyzer and IBM SPSS 21.0 softeware were used. At d 1, female Holstein calves had an average of 7.8 RBC (M/μL) with 42.4 MCV (fL) and 9.0 WBC (K/μL) with 68.1% neutrophil (NE), 29.4% lymphocyte (LY) whereas male Holstein calves had 6.9 RBC with 42.1 MCV and 10.2 WBC with 67.5% NE, 28.1% LY. After a month, hematological values of female Holstein calves changed to 7.3 RBC with 33.6 MCV and 9.7 WBC with 35.2% NE, 54.3% LY while those of male Holstein calves turned to 7.0 RBC with 33.0 MCV and 9.7 WBC with 37.4% NE, 51.0% LY. As for Jersey, erythrocyte and leukocyte of female calves had changed from 7.4 RBC with 38.8 MCV and 7.5 WBC with 48.3% NE, 49.6% LY to 7.9 RBC with 34.8 MCV and 9.5 WBC with 25.8% NE, 58.0% LY for a month since a day old. Male Jersey calves had 6.8 RBC with 39.6 MCV and 9.7 WBC with 63.1% NE, 35.1% LY at d 1. After a month, male Jersey had 6.1 RBC with 30.8 MCV and 9.3 WBC with 29.1% NE, 55.7% LY. Female calves likely had more population of erythrocyte than male calves in both breeds. Holstein calves had bigger erythrocytes than Jersey calves at d 1. But, there was no big difference between male and female calves. The volume of erythrocytes had been reduced from d 1 to 1 mo regardless of sex and breed. The percentage of neutrophil had decreased whereas that of lymphocyte had increased for a month. The number of Jersey calves was so small that those results might not be convincing. Further works are required to study hematological and immunological differences between Holstein and Jersey.

Key Words: Holstein, Jersey, hematological values

T18 Effects of vaccinating against brucellosis and clostridia simultaneously or not on the immune response of dairy calves.

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The objective of this research was to evaluate the effects of vaccinating simultaneously or not against brucellosis and clostridia on the immune response. Fifty female dairy calves were enrolled (38 Gyr and 12 5/8 Holstein-Gyr). At 120 d of age animals were distributed into the 3 treatments: B (n = 18) vaccine only against brucellosis (B19- 2 mL subcutaneously); C (n = 14) vaccine only against *Clostridium* spp. (inactive culture of *C. chauvoei* and toxoids from *C. botulinum* tipo C and D, *C. septicum*, *C. novyi*, *C. perfringens* type B, C and D and *C. sordellii*, 5 mL subcutaneously) and CB (n = 18) a combination of both vaccine protocols (2 mL and 5 mL subcutaneously, respectively). Animals from groups C and CB were revaccinated against clostridia 28 d after the first vaccination. Blood sampling was performed on time 0 and on the 28th day for evaluation of the vaccine response against brucellosis; and on time 0 and 14 d after the booster to evaluate the immune response of the vaccine against selected clostridia agents. Serum neutralization was conducted for titration of antibodies anti-toxin epsilon from *C. perfringens* type D and botulinum from *C. botulinum* type C. For the analysis of the response against *Brucella* a cellular proliferation assay was performed. The software R was used for statistical analysis of the data. Values of $P < 0.05$ were considered significant. The medium of antibody titration against epsilon toxin was 14.57 UI/mL on group C, higher than for animals in group CB (9.20 UI/mL; $P = 0{,}03$). Similarly, animals from group CB also presented medium titration (1.44 UI/mL) against toxin C inferior to those observed in group C (4,92 UI/mL; $P = 0{,}03$). No differences were observed in the percentage of total lymphocytes and total proliferated; proliferated lymphocytes CD4+ and CD4+; proliferated lymphocytes CD8+ and CD8+ on group B and CB. These results suggest that the vaccine against brucellosis interferes on the humoral immune response of the tested clostridia antigens when the vaccines are simultaneously administrated.

Key Words: antibody, cellular proliferation, serum neutralization

T19 Supplementing dairy calves with colostral immunoglobulins for 14 days reduces death loss and antibiotic usage.

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There is an ethical need to reduce on-farm antibiotic use by implementing disease prevention strategies. Supplementing calves with colostral immunoglobulins (IgGs) post-gut closure (after 24 h of age) is an often discussed, seldom researched approach that could achieve this goal. The study objectives were to evaluate the effects supplementing calves with a concentrated source of whey-based IgG, from birth to d 14 on antibiotic use, morbidity, and mortality. The IgG was from a commercially available colostrum replacer product (Premolac, Zinpro Corp., Eden Prairie, MN; PZ). Upon arrival at the calf raising facility, 1,037 newborn, Holstein calves were randomly assigned to 1 of 3 treatments of added IgG: 1) 0 g (CON), 2) 10 g (CR10; 23 g PZ), or 3) 20 g (CR20; 46 g PZ) per day. Treatments were added to milk replacer diets fed to individually housed calves, 2x/d for 14 d. Antibiotic use, morbidity, and mortality were monitored throughout the study. Weaning started on d 53 and calves were removed from hutches on d 69, on average. Multivariable logistic regression analysis showed that the odds to be treated with antibiotics for diarrhea was 32% less for CR20 calves than for CON calves ($P = 0.05$). Overall, the incidence of calves treated with antibiotics for diarrhea was reduced from 44.4 to 35.3% (CON and CR20 respectively; $P = 0.05$). Calves fed CR20 were 48% less likely to be treated with antibiotics, for any reason, compared with CON calves ($P < 0.05$). Overall antibiotic treatment rates were reduced from 92.4 to 86.2% (CON and CR20 respectively; $P < 0.05$). Feeding CR10 reduced antibiotic use compared with CON, but this difference was only numerical. Calves fed CON had a 6.6% mortality rate, compared with 2.0% for CR20 calves ($P < 0.01$). Although numerically lower, mortality in calves fed CR10 was similar to CON (5.4%; $P > 0.05$). Overall, calves fed CR20 had a 70% lower mortality incidence ($P < 0.01$) than calves fed CON. These findings indicate, feeding calves at least 20 g of IgG from a PZ for 14 d can reduce antibiotic use and significantly decrease morbidity and mortality incidence.

Key Words: colostrum replacer, immunoglobulin, mortality

T20 Mitigating environmental mastitis microbes with the novel use of paper mill byproducts.

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To identify potential bedding conditioners for prevention of mastitis in dairy cattle, we determined the minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of a set of technical lignins. A broth macrodilution assay using Mueller Hinton media modified to pH 6 was used to evaluate antimicrobial activity against strains of *Streptococcus uberis*, *Staphylococcus hyicus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* isolated from mastitic and metritic cows. Technical lignin treatments (TRT) evaluated were: 1) alkali Kraft lignin (AKL), 2) sodium lignosulfonate (NaL), 3) magnesium lignosulfonate (MgL), and 4) washed Kraft lignin (WKL). The MIC and MBC assays were carried out in duplicate in each of 3 independent runs. In this study, gram-positive bacteria (*S. hyicus* and *S. uberis*) were more susceptible to the antimicrobial activity of NaL compared with gram-negative bacteria, with *E. coli* being the most resistant (Table 1). AKL was the most effective lignin against *K. pneumoniae*, but its activity against all the other bacteria was limited compared with NaL.

Except for *K. pneumoniae*, MgL required a much higher concentration to kill bacteria relative to the dose that inhibits growth. In the case of NaL, this large difference was observed only against *K. pneumoniae*. Overall, NaL had the broadest antimicrobial spectrum against the bacteria tested and has potential to be a novel bedding conditioner that can help to control environmental mastitis pathogens in stalls.

Table 1 (Abstr. T20). MIC and MBC values (mg/mL ± SD) for technical lignins against bacteria isolated from mastitic and metritic cows

	Treatment			
Bacteria	NaL	MgL	AKL	WKL
E. coli				
MIC	27.5 ± 5.0	20.0 ± 0	>60	>60
MBC	30.0 ± 8.2	56.7 ± 5.77	NA[1]	NA
S. hyicus				
MIC	6.25 ± 2.5	25.0 ± 5.77	16.6 ± 11.5	>60
MBC	8.75 ± 0.6	30.0 ± 4.8	50.0 ± 17.3	NA
S. uberis				
MIC	5.8 ± 1.4	23.3 ± 5.77	10.0 ± 0	>60
MBC	5.8 ± 1.4	30.0 ± 0	>60	NA
P. aeruginosa				
MIC	16.6 ± 5.77	26.6 ± 11.5	>60	>60
MBC	20.0 ± 0	40.0 ± 0	NA	NA
K. pneumoniae				
MIC	8.33 ± 2.88	10.0 ± 0	2.5 ± 0	ND[2]
MBC	40.0 ± 0	10.0 ± 0	4.37 ± 1.25	56.7 ± 1.25

[1] Not available.

[2] Could not be determined visually.

Key Words: technical lignins, mastitis, antimicrobial

T21 Lactoperoxidase potential in the diagnosis of subclinical mastitis in cows via image processing.

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The present study evaluated subclinical mastitis through the enzymatic activity of lactoperoxidase in dairy cows by image analysis using 50 milk samples, where 34 samples were from Jersey breed cows, 8 were from Gir, and 8 Guzerá breed. SCC, qualitative and quantitative lactoperoxidase determination analyzes were performed. The samples submitted to the qualitative analysis were photographed with digital cameras and the regions of interest for analysis were cut out using the GIMP 2.8.18 free software as a square plot of 50 × 50 pixels. Data analysis was performed using the R 3.5.0 statistical platform. The principal components analysis (PCA) (*Chemometrics With*[6] and *factoextra*[7] packages) for the sample image data was developed using the resolved absorbance values for RGB channels (standardized via self-scaling). The sample evaluation system consisted of correlating the color presented by the sample with the enzyme concentration using the RGB (Red-Green-Blue) color system. The RGB channels increased in intensity as the lactoperoxidase concentration increased in the samples. The standard calibration curve showed correlation ($R^2 = 0.98$) that validated the applied methodology for enzymatic quantification. The adjusted data coefficient was 0.86 for SCC and 0.96 for enzymatic activity. This expressiveness in color is indicative of increased enzymatic activity. The exploratory analysis also showed that the breeds presented different behavior against the enzyme, revealing that the Jersey breed presented samples containing greater activity when compared with the Gir and Guzerá samples, concluding that lactoperoxidase analysis via images can serve as an evaluation parameter of mammary gland health.

Key Words: RGB system, somatic cell count, lactoperoxidase system

T22 Prevalence, antimicrobial susceptibility, and virulence genes of *Klebsiella* spp. from bovine mastitis in China.

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Klebsiella spp. are an important environmental pathogen of mastitis in dairy cows, which cause many severe clinical symptoms and great economical losses of dairy farms. The aim of the work was to identify the prevalence of *Klebsiella* spp. from bovine mastitis milk in China, antibiotic resistance phenotypes, and the main virulence genes. We investigated 497 milk samples collected from cow with clinical mastitis in dairy farms (located in 5 provinces) of China in 2017. The minimum inhibitory concentrations (MICs) of antimicrobial susceptibility testing were determined for *Klebsiella* spp. isolates, the assay was performed by BD Phoenix 100 and the results were interpreted by the guidance of Clinical and Laboratory Standards Institute (CLSI, 2016) as susceptible, intermediate, and resistant. The detection of resistance genes and virulence genes was performed by real-time polymerase chain reaction. In our study, 32 (6.4%) isolated from 497 samples were identified as *Klebsiella* spp. An analysis of antimicrobial susceptibility testing showed that the highest percentage of the isolates were resistant to Tetracycline (40.6%). None isolates were resistant to Piperacillin-Tazobactam, Levofloxacin, Fosfomycin w/G6P. An analysis of the prevalence of antimicrobial resistance genes revealed that Sul2 gene was the most prevalent among all the resistance genes (96.9%), followed by blaOXA-48 (75%). Further investigation of virulence genes performed by PCR found that all the strains harbored one or more of these virulence genes, the percentage of the isolates were positive for ureA was 100%. The present study demonstrated that the prevalence of resistance and virulence genes was high, and it could contribute a threat to the health of animals or even humans. The result of the study highlighted the need for improvements in antimicrobial therapy and infection control programs in Chinese dairy herds to reduce the prevalence of resistance of *Klebsiella* spp.

Key Words: antimicrobial resistance, virulence genes, bovine mastitis

T23 Biofilm production by *Streptococcus agalactiae* isolated from milk of cows with subclinical mastitis.

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Streptococcus agalactiae is a contagious pathogen in subclinical mastitis affecting dairy farms and causing significant economic losses. This microorganism is able to use different mechanisms to colonize bovine mammary gland and the persistence of infections may be associated with the ability to form biofilm. The aim of this study was to investigate the capsular type and correlated with the capacity to form biofilm in *S.*

agalactiae isolated from milk of cows with subclinical mastitis. Thus, we performed the PCR for genes encoding capsular typing (Ia/Ib-VIII) in 145 *S. agalactiae* strains. For biofilm assays, we used 96-well polystyrene microplates and the strains were incubated in Tryptone Soy Broth (TSB) at 37°C for 18h without shaking, under 5% CO2 atmosphere. In the current study, all strains were biofilm producers and 83 (57.2%) were Ia capsular type followed 62 (42.8%) type III. Quantitative biofilm evaluation found 85 (58.6%) strong, 42 (29%) moderate, and 18 (12.4%) weak biofilm producers. When evaluated the quantitative distribution among Ia and III capsular type, the results were similar being, Ia - 46 (31.7%) strong, 28 (19.3%) moderate and 9 (6.2%) weak. Considering type III, 39 (26.9%) strong, 14 (9.7%) moderate and 9 (6.2%) weak producers. In our study, we found 2 capsular types and no difference was observed in biofilm formation regarding them, showing that all strains may have the capacity to establish a mammary gland infection with biofilm, independent of capsular type.

Key Words: capsular type, biofilm

Dairy Foods: Microbiology 2

T24 Formulation of a selective medium for the isolation and enumeration of *Lactobacillus bulgaricus*. A. Oyeniran*[1], R. Gyawali[1], T. Zimmerman[1], A. Krastanov[2], and S. A. Ibrahim[1], [1]*North Carolina Agricultural and Technical State University, Greensboro, NC*, [2]*University of Food Technologies, Plovdiv, Bulgaria.*

Lactobacillus delbrueckii ssp. *bulgaricus,* an important species of lactic acid bacteria, is widely used in the dairy industry, particularly in yogurt production. Commonly used in mixed cultures and especially with *Streptococcus thermophilus*, *L. bulgaricus* is mandatory for traditional yogurt and also confers several health benefits that meet the prerequisites for probiotic bacteria. The current standard medium, de Man, Rogosa and Sharpe (MRS), is unreliable as a selective method for isolating *L. bulgarcius* species from different sources including dairy products. MRS can also present a false positive for *L. bulgaricus* in some dairy products due to the presence of high background colonies of similar species. Consequently, there is a need for a better, more reliable medium for the differential enumeration of *L. bulgaricus.* The objective of this study was thus to formulate an agar medium that is effective for the differential isolation and enumeration of *L. bulgaricus*. A modified reinforced clostridial medium (mRCM) was formulated by adding 0.025% $CaCl_2$, 0.01% uracil, 0.2% Tween 80, 0.5% fructose, 0.5% dextrose, 1% maltose and 0.25% pyruvate to RCM. The inclusion of 0.04% aniline blue dye in the mix influenced the improved morphology and differentiation of the *L. bulgaricus* colonies observed in the mixed yogurt culture. The cell recovery and bacterial counts for *L. bulgaricus* in the tested yogurt brands using mRCM-BLUE were significantly higher than those found in the standard MRS medium as mRCM-BLUE largely inhibited the growth of other bacterial species (*Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidus* and *Lactobacillus reuteri*) present in the yogurt. Our results thus suggest that mRCM-BLUE could be recommended as a selective agar medium for more accurate differentiation and enumeration of *L. bulgaricus* in dairy products.

Key Words: *Lactobacillus bulgaricus*, selective growth medium, MRS (de Man Rogosa and Sharpe)

T25 Evaluation of inhibition of *Listeria monocytogenes* by lactic acid bacteria in fresh cheese. M. R. Lawton*, S. M. Kozak, K. G. Jencarelli, and S. D. Alcaine, *Department of Food Science, Cornell University, Ithaca, NY.*

The growth of *Listeria monocytogenes* (*L. mono*) in ready-to-eat (RTE) foods is a large concern in the United States due to its ability to cause morbidity and mortality, especially in immunocompromised individuals. Control of *L. mono* in food products has previously been evaluated using antimicrobial compounds such as nisin, acids, and other natural compounds such as essential oils. Recently, consumers are moving toward a more natural life style and look for clean label ingredients. Lactic acid bacteria (LAB) have been investigated as protective cultures to control the growth of *L. mono* in RTE products such as meat. However, contamination of *L. mono* is a large concern in the dairy industry due to its ability to thrive in fresh style cheeses like queso fresco. Four lactic acid bacteria cultures selected for their performance in inhibiting *L. mono* growth previously in meat—*Lactobacillus curvatus*, *Lactobacillus sakei*, *Pediococcus acidilactici*, and *Leuconostoc carnosum*—were evaluated against *L. mono* in a fresh cheese matrix stored at 6 and 21°C over 21 d. Lab-scale queso fresco cheeses (4 g) containing 5–6 log cfu/g of each LAB culture individually, were surface inoculated with 4 log cfu/g or 2 log cfu/g of a 5 strain cocktail of *L. mono*. Samples were enumerated for *L. mono* on d 0, 1, 7, 14, and 21 or d 0, 1, 2, and 4. Overall, none of the treatments showed any significant difference ($P > 0.05$) from the control when analyzed using the method of least squares and Tukey's test for pairwise comparisons over time. After 21 d, cheeses reached *L. mono* concentrations of approximately 7–8 log cfu/g. The results from this study indicate that cultures previously shown to prevent or inhibit the growth of *L. mono* in meat matrices do not exhibit the same effects in fresh cheese.

Key Words: *Listeria*, biopreservation, cheese

T26 Identification, subtyping, and tracking of dairy spoilage-associated *Pseudomonas* by sequencing the *ileS* gene. S. J. Reichler*, S. I. Murphy, N. H. Martin, and M. Wiedmann, *Cornell University, Ithaca, NY.*

Pseudomonas threatens fluid milk and uncultured cheese quality by producing unwanted odors, flavors, and pigments. *Pseudomonas* likely persists in the dairy plant environment and enters products predominantly as an uncontrolled postpasteurization contaminant. Previous techniques for tracking *Pseudomonas* in dairy products and plant environments included ribotyping, pulsed-field gel electrophoresis, and RAPD PCR, but these techniques are complex and provide limited and possibly unreliable information on bacterial phylogeny and identity. 16S rDNA sequencing, often used to identify bacteria, lacks the resolution to differentiate between *Pseudomonas* strains and even species. Our goal was to identify a single housekeeping gene that could be analyzed in dairy spoilage-associated *Pseudomonas* for accurate species-level identification, subtyping, and tracking of persistent spoilage issues. Initially, 115 *Pseudomonas* isolates from pasteurized milk produced at 9 different plants were characterized by a 7-gene MLST scheme previously designed for *P. fluorescens*. This initial data set was used to select a single gene that could be used for simple and affordable *Pseudomonas* characterization. Based on the number of isolates successfully sequenced, the number of unique allelic types, nucleotide and sequence diversity measures, and measures of recombination, the gene encoding isoleucyl tRNA synthetase (*ileS*) was identified as the most appropriate. Using type strain *Pseudomonas* whole-genome sequences, we demonstrated that *ileS* agrees closely with whole-genome phylogenies and is therefore suitable for isolate identification. Analysis of 91 isolates with the same 16S sequence type from 8 different plants found 28 unique *ileS* allelic types, greatly improved resolution. Proteolysis and lipolysis screening of 143 isolates showed that *ileS* clades differed regarding these spoilage-related phenotypes. Overall, our data suggest that *ileS* sequencing provides a promising strategy for affordable initial characterization of *Pseudomonas* isolates, which will help the dairy industry in the identification, characterization, and tracking of *Pseudomonas* in their facilities and products.

Key Words: *Pseudomonas*, subtyping, tracking

T27 Evaluation of the efficacy of commercial protective cultures against mold in cottage cheese. G. Makki* and S. D. Alcaine, *Cornell University, Ithaca, NY.*

Fresh cheese is highly susceptible to spoilage thus a contributor to both retail and consumer food waste. Traditionally, sorbates and propionates are used to inhibit common mold and yeast spoilage organisms. However, consumer demand for clean label necessitates adopting alternative measures to preserve food. The study objective is to investigate bacterial cultures efficacy in delaying mold outgrowth in cottage cheese. Cottage curd and dressing were sourced from a manufacturer in New York. Dressing was inoculated with 3 different commercial protective cultures (of *Lactobacillus* spp. designated PC1, PC2, and PC3) following manufacturer recommended dosage. Curd was added to dressing and mixed. A positive control (PC) with no protective culture was included. Eleven species of mold spanning 5 genera (*Penicillium commune*, *P. citrinum*, *P. decumbens*, *P. roqueforti*, *P. chrysogenum*, *Mucor racemosus*, *M. genevensis*, *Aureobasidium pullulans*, *Aspergillus cibarius*, *Phoma dimorpha*, and *Trichoderma amazonicum*) were each spotted on the surface of separate cheese samples at a rate of 100 cfu/5 g of cheese. Samples were stored at refrigeration temperature (6 ± 2°C). Cheese was evaluated visually for mold growth at 0, 7, 14 and 21 d post-inoculation and photographed. Negative control showed no growth through 21d. *P. decumbens, A. cibarius,* and *T. amazonicum* did not show growth in treatments or control. For *A. pullulans* and *Mucor* strains, all treatments showed full mold growth by 14d. For *P. commune*, PC, PC2 and PC3 showed full mold growth by 14d, while PC1 showed only matt appearance indicative of partial growth by 21d. For *P. citrinum* and *P. dimorpha* mold growth was detected only in PC by 21d. PC1 delayed matt appearance for *P. roqueforti* at 14d. *P. chrysogenum* was inhibited by PC1 while PC, PC2 and PC3 showed full mold growth at 21d. The results suggest potential efficacy of PC1 in delaying outgrowth of *P. commune, P. chrysogenum, P. roqueforti, P. citrinum* and *P. dimorpha,* and of PC2 and PC3 against *P. citrinum* and *P. dimorpha* in cottage cheese compared with positive control. Overall, protectives culture should be evaluated against molds specific to cheese type and facility to ensure efficacy.

Key Words: cottage cheese, biopreservation, mold

T28 Purification and characterization of a metallophosphoesterase produced by *Pediococcus acidilactici* isolated from Gouda cheese with hydrolysis over phospholipids.

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Lipolysis occurs during ripening of dairy products as a result of esterase/lipase activity. Lactic acid bacteria (LAB) are considered to be weakly lipolytic bacteria in comparison to other species. However, in cheeses with extended ripening periods, lipolytic LAB may have potential advantages. *Pediococcus acidilactici* is a LAB frequently found in fermented dairy products, but without previous reports on the production of lipases. The aim of this project was purified, characterize and identify the esterase produced by *P. acidilactici* isolated from Gouda cheese and determine its phospholipid hydrolysis profile, with a focus on increased absorption of these in the human gut. To purify the lipolytic protein, zymography was performed in native conditions. The band that displayed activity was excised from the gel, concentrated by ultrafiltration and sent for sequence analysis by LC-MS/MS. Also, this fraction was subject to biochemical characterization as a function of: pH, temperature, ions, hydrolysis of different substrates, and activity over phospholipids. A single protein with lipolytic activity was detected by zymography, with a molecular weight of 86 kDa. The peptides found by LC-MS/MS indicate it is a putative metallophosphoesterase with a theoretical molecular weight of 45.5-kDa, suggesting that this protein is active as a homodimer. The pure protein showed an optimal activity from pH 8.0 to 9.0. The optimal temperature for activity was 37°C and it lost 15% of activity after incubation at 90°C for 1 h. This lipase showed activity over short chain fatty acids and exhibited high hydrolysis of PG (phosphotidylglycerol) and PI (phosphotidylinositol). It also hydrolyzed PS, PC and PE (phosphotidylserine, phosphotidylcholine, and phosphotidylethanolamine, respectively) but with less effect. This is the first lipase reported for *Pediococcus* genus isolated from a dairy product and shown to hydrolyze phospholipids, which may exert human health benefits through increased digestibility in intestinal cells

Key Words: metallophosphoesterase, *Pediococcus acidilactici*, phospholipids

T29 Impact of ultrasound processing on some milk-borne microorganisms and the components of camel milk.

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Inactivation of pathogenic bioluminescence bacteria *Escherichia coli* O157: H7 and *Salmonella* Typhimurium in camel milk was investigated using ultrasound processing (900 W, 20 kHz, 100% power level). In addition, the effect of ultrasound treatment on camel milk components was studied to detect the changes in fatty acids, lipid peroxides, and protein fractions of raw camel milk. The bacterial cells were added to pasteurized camel milk samples (70 mL) and transferred into a sterile aluminum container (30 mm by 120 mm with a 100-mL total capacity) and then subjected to continuous ultrasound processing for 15 min in an ice water bath using a 13-mm diameter probe. In vivo imaging system (IVIS) and the traditional plate count agar (PCA) method were used to evaluate the viability of bioluminescence *E. coli* O157: H7 and *Salmonella* Typhimurium. The continuous ultrasound processing of camel milk resulted in a 4.4-log reduction in bioluminescence *Salmonella* Typhimurium and a 7-log reduction in bioluminescence *E. coli* O157:H7. Relative to unsonicated raw camel milk, the *cis*-9,*trans*-11 conjugated linoleic acid (CLA) and *trans*-10,*cis*-12 CLA contents were not affected ($P > 0.05$) by the ultrasound processing. The TBAR values, a marker of lipid peroxidation, were also similar ($P > 0.05$) between the sonicated and unsonicated raw camel milk. The presence and identity of milk protein fractions (β-casein, α-casein, α-lactalbumin, serum albumin, and lactoferrin) were also monitored using SDS-PAGE and no significant ($P > 0.05$) differences were seen between the sonicated and unsonicated raw camel milk. In conclusion, the results of this study showed that ultrasound processing of camel milk was efficient to inactivate the pathogenic microorganisms without affecting camel milk components.

Key Words: camel milk, ultrasound processing, pathogenic bacteria

T30 Effect of thermosonication in a batch system on the survival of thermophilic spoilage bacteria in milk.

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Thermophilic bacteria and spores can survive pasteurization temperatures and reduce shelf life of milk. These bacteria produce enzymes that produce free fatty acids and peptides altering the quality of milk. Coupling ultrasound with pasteurization may aid in reducing the surviving microbes in fluid foods and enhancing the product quality and shelf life based on previous studies.This study determined the D-value of *Geobacillus stearothermophilus* (GS) and *Anoxybacillus flavithermus* (AF) in 2% fat milk and tryptic soy broth (TSB) medium when treated with thermosonication in a batch system. Sterile 2% fat milk and TSB were inoculated with GS and AF and aliquots were plated on TSA plates before and after control and thermosonication treatments for enumeration to calculate the D-values. Batch sonication was performed using QSonicaQ500 (Newtown, CT) using a 10 ml flow cell and a 3.2 mm sonication microtip at 72 and 73°C and treated for 5 to 120 s (5 mL sample volume). D-values for GS in TSB and 2% milk with heat only at 72°C were >60 s and at 73°C were 60.02 and 63.92 s, respectively. With thermosonication, the D-values in TSB were 25.16 and 26.49 s at 72 and 73°C. D-values for GS in milk at 72 and 73°C with thermosonication were 19.98 and 18.68 s, respectively. Similarly, for AF, D-values in TSB and 2% milk with heat only at 72°C were >60 s and at 73°C were 48.41 and 56.11 s, respectively. Thermosonication decreased the D-values of AF in TSB to 44.39 s at 72°C and 36.91 s at 73°C and in 2% milk to 38.54 s at 72°C and 35.11 s at 73°C. ANOVA revealed that thermosonication had a statistically significant effect on GS reduction in TSB and milk but temperature did not. In the case of AF, although an increase in temperature and application of thermosonication decreased the D-values, ANOVA revealed that neither temperature nor thermosonication had a significant effect on the reduction in D-values. Studies are being performed to see the effect of thermosonication on D-values of GS and AF spores. Treating milk with pasteurization along with thermosonication may potentially reduce the microbial load of thermophilic organisms and spores in milk leading to products with extended shelf lives.

Key Words: milk pasteurization, thermosonication, thermophilic bacteria

T31 Competitive exclusion study demonstrates *Bacillus subtilis* as a predominant constitutive microorganism of reverse osmosis membrane biofilms.

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Microbial attachment and colonization on separation membranes lead to biofilm formation. The constitutive microflora might compete and result in certain species emerging as predominant, especially within older biofilms. To understand the microbial interactions within biofilms, the emergence of predominance was studied in the current investigation. An 18-mo-old reverse osmosis membrane was procured from a whey processing plant. The membrane pieces (1 × 1 inch^2) were neutralized by dipping in Latheen broth. Their resuscitation was done in Tryptic Soy Broth (TSB) at 37°C, followed by plating on Tryptic Soy Agar (TSA) to recover the constitutive microflora. Distinct colonies of isolates were further identified using MALDI-TOF as *Bacillus subtilis*, *Bacillus licheniformis*, *Exiguobacterium aurantiacum*, *Acinetobacter radioresistens*, and 2 unidentified species of *Exiguobacterium* and *Bacillus*. Further, the competitive exclusion study helped to establish the predominance using a co-culturing technique. Fifteen combinations of 2 isolates each were prepared. For which, isolates were spiked in the ratio of 1:1 in TSB, incubated at 37°C for 24 h, followed by plating on TSA. The isolates on plates were distinguished based on colony morphology, Gram staining, and MALDI-TOF. For establishing the predominance, plate counts were compared using ANOVA. In all the coculture combinations, *B. subtilis* emerged as predominant with a mean log counts of 6.73 ± 0.23 cfu/ mL. It was interesting to note that another isolate, *B. licheniformis*, competed equally with *B. subtilis*, while not with others. The predominance of *B. subtilis* was further validated using the process of natural selection, where the broth with overnight-incubated membrane piece (with mixed species biofilm) was inoculated in fresh TSB and incubated for another cycle. Five such sequential transfers resulted in demonstrating the predominance of *B. subtilis* based on its population density.

Key Words: reverse osmosis membrane, constitutive microflora, competitive exclusion

T32 Recovery potential of heat-injured cells of *Listeria* under ice cream temperature abuse conditions versus simulated gastrointestinal fluids.

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Serving practices in nursing homes may result in temperature abuse of ice cream before consumption. Presence of even low numbers of injured cells may pose a risk, due to their potential to recover, especially to immunocompromised patients. This investigation was conducted to evaluate injured cell recovery under temperature abuse conditions and on exposure to gastrointestinal (GI) fluids. Based on our previous studies, ice cream mix samples (42% TS), spiked with 4.54 log cfu/gram of *Listeria innocua*, were lab pasteurized (69°C for 30 min). Heat-injured cells were recovered in BLEB, followed by isolation on MOX. The ice cream mix samples, containing injured cells, were followed through overnight aging (7°C), freezing (−4°C), and overnight hardening (−40°C) steps. To simulate serving practices, the samples were held for 12 h at 4.4°C, followed by 30 min holding at room temperature (22°C), identified as the first cycle of temperature abuse. In all, the samples were exposed to 3 such consecutive cycles. At the end of each cycle, direct plating was done on MOX to detect any intact cells. Parallelly, the ice cream samples, containing injured cells, were mixed (1:1) with simulated gastric fluids (pH 1.0 and 2.0) and were held at 37°C in a shaker incubator. Samples were drawn at 15, 30, and 60 min intervals. For studying the effect of sequential transit through a simulated intestinal fluid, 2 mL of gastric fluid + ice cream (1:1) from gastric fluid experiments were added to 50 mL of simulated intestinal fluid (pH 6.8) and held at 37°C, as above. Samples were drawn at 30, and 360 min intervals. To ascertain the recovery of any injured cells, samples were direct plated on MOX. Experiments were conducted in replicates of 3 and counts were compared for differences. The temperature abuse or GI fluid exposure studies did not result in any recovery of injured cells in the ice cream samples. However, it was also observed that the injured cells were not eliminated during exposure to gastric fluid. Further studies are necessary to understand the exact implications of these findings.

Key Words: *Listeria*, recovery, injured

T33 Comparison of nucleic acid dyes for flow cytometric enumeration of bacteria in cultured milk.

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This study was focused on choosing a nucleic acid fluorescent dye for use in enumerating dead and living bacteria in cultured milk. Ultra-high temperature sterile milk was inoculated with a mixed strain lactococcal starter culture and incubated overnight at 37°C. A negative control containing only dead cells was obtained by heating the cultured milk at 85°C for 12 min. An aliquot of milk was then diluted 500-fold in PBS buffer, and then one of 5 nucleic acid dyes was added (Sybr Green, Syto 9, Syto 24, carboxy fluorescein diacetate (cFDA) or Thiazole Orange) in combination with propidium iodide. Fluorescence from binding of the dyes in living bacterial cells was detected at emission wavelength of 488 nm. Fluorescence from PI that had permeated into dead bacterial cells and quenched fluorescence from the other dyes was detected at 640 nm. Fluorescence from cFDA only occurs upon intracellular enzymatic metabolism after its permeation into living cells. The non-heated cultured milk contained both living and dead cells as shown by their position on the cytograph. After heating the cultured milk, only dead cells were detected. Sybr green performed better in distinguising between live and dead populations for assessment of viability of bacterial cells in cultured milk. Flow cytometery measurements using Sybr green were more reproducible than when using the other dye stained samples. Between the Syto dyes, Syto 24 exhibited more consistent results in terms of bacterial enumeration. A problem with the method was that diluting the milk so as not to clog the flow cytometer makes the background noise more prominent compared with the fluorescent from the bacterial cells. Flow cytometric based enumeration is a promising tool yet requires additional studies.

Key Words: *Lactococcus*, flow cytometry

T34 Determination of biofilm material released from a laboratory-scale HTST heat exchanger during long processing times. B. Tattersall*[1], D. McMahon[1], A. Vollmer[1], and C. Oberg[2], [1]*Utah State University, Logan, UT,* [2]*Weber State University, Ogden, UT.*

During HTST heating of milk, some thermoduric bacteria, as well as bacterial spores, can survive. Such microbes can attach and form biofilms downstream from the heating section. During long run periods, some portions of the regeneration and cooling sections will be at a temperature that will permit spore germination and bacterial growth. This can have a detrimental effect on product quality as bacterial numbers in the biofilm increases, and bacteria and biofilm material are released into the milk. Our aim was to determine when increased bacterial load occurs during such long operation times. A lab-scale heat exchanger was fabricated consisting of heating and cooling sections, peristaltic pumps, with temperature recorded on inlet and outlet positions on the product and heating and cooling water. Raw milk was run in a single pass through the heat exchanger for 18 h on 5 separate occasions. Samples were collected every other hour in the first 6 h and then every hour for the remaining hours. Total plate counts of bacteria present in each sample were enumerated using standard methods agar incubated aerobically at 30°C for 48 h. In general, the bacterial counts remained at the baseline level ($<10^2$ cfu/mL) for the first 10 to 12 h. As the processing continued there was an increase in bacterial numbers. There was a fluctuation in bacterial numbers throughout the 18 h of operation, especially after 10 h when hourly measurements fluctuated between 10^3 and 10^1 cfu/mL. The fluctuations in bacterial numbers were attributed to biofilm material with associated bacteria being released from the heat exchanger plates on an intermittent basis. In conclusion, running a pasteurizer for extended times will lead to increased bacterial load in pasteurized milk which can lower product quality and cause contamination of any downstream processing systems. Further work is being performed on identifying the bacteria that were being released into milk and in which part of the cooling heat exchanging they are growing.

Key Words: biofilm, milk pasteurization, thermophilic spores

T35 Predicting catabolic pathways in *Lactobacillus wasatchensis* using metabolic modeling. S. Young[1], M. Domek[1], M. Culumber[1], D. McMahon[2], and C. Oberg*[1], [1]*Weber State University, Ogden, UT* [2]*Utah State University, Logan, UT.*

Lactobacillus wasatchensis, an obligate heterofermentative nonstarter lactic acid bacteria can cause late gas production and splits and cracks in Cheddar cheese. Our goal was to identify potential sources of 6-carbon sugar compounds that may be present in cheese and cause release of CO_2 when converted to a 5-carbon sugar which is utilized by *Lb. wasatchensis* for energy production. Previous studies did not explain late gas production in cheese when no galactose was present. Potential relevant metabolic pathways were determined based upon the genome of *Lb. wasatchensis* WDC04. The genome sequence was exported from the NCBI GenBank database, then metabolic modeling was performed using Knowledgebase Predictive Biology software to map the genes present for various metabolic pathways. Based upon data output from a flux balance analysis, it was confirmed that *Lb. wasatchensis* contains a complete pentose phosphate pathway (PPP), while pathways for glycolysis, tricarboxylic acid, and galactose metabolism were incomplete. To confirm these predictions and to look for alternative carbon metabolic pathways, 5 strains of *Lb. wasatchensis* (CGL02, DH3, LD13, SH05, WDC04) were tested in carbohydrate restricted MRS (CR-MRS) broth in micro-well plates supplemented with 7% oxyrase and 0.5% of either ribose, lactose, galactose, or N-acetylglucosamine (NAG). Growth occurred with ribose but was negligible when lactose, galactose, or NAG were the only carbohydrates present. The metabolic modeling also predicted additional carbohydrates that might be utilized by *Lb. wasatchensis* including gluconate which is the oxidized form of glucose. Gluconate contains 6 carbons and *Lb. wasatchensis* contains the genes for it to be converted to ribose-5-P using phosphogluconate dehydrogenase by a decarboxylating step, producing CO_2 in a similar way to galactose, when used as an energy source for the PPP. When inoculated into CR-MRS containing 0.5% sodium gluconate, 4 of the *Lb. wasatchensis* strains grew, confirming utilization of gluconate. Presence of gluconate in cheese thus becomes another risk factor for unwanted gas production and formation of splits and cracks in cheese.

Key Words: late gas defect, gluconate, galactose

T36 Growth and gas formation by *Lactobacillus wasatchensis* WDC04 when ribose:galactose ratios were varied. I. Green*[1], D. McMahon[1], and C. Oberg[2], [1]*Utah State University, Logan, UT,* [2]*Weber State University, Ogden, UT.*

Lactobacillus wasatchensis WDC04 causes late gas formation in Cheddar cheese by releasing CO_2 from a 6-carbon sugar when its preferred carbohydrate source, ribose, has been depleted. Growth curves were generated for WDC04 at pH 6.5, 5.2, and pH 5.2 with 3% NaCl added, using MRS-carbohydrate restricted (MRS-CR) medium containing 1% ribose. Lowering the pH and adding salt to simulate cheese conditions decreased the growth rate of WDC04. The ratio of ribose to galactose (R:G) was then varied. Aliquots of WDC04 were inoculated ($n = 3$) into pH 6 MRS-CR media containing 1% carbohydrate with R:G of 10:90, 20:80, 30:70, 40:60, 50:50 and 100:0, so 3 tubes for each R:G ratio initially contained 10^1, 10^3, 10^5 or 10^7cfu/mL. Each tube contained a Durham tube for gas detection and the tubes were incubated at 30°C for 7 d. Gas was observed after 2 d in tubes with 30:70 R:G or higher, with as little as a 10^3 cfu/mL inoculum. No gas was detected in 10:90 or 100:0 R:G tubes. As the ribose concentration was increased, gas appeared sooner for each inoculum level. Gas production appears dependent on (1) having ribose available to allow growth of WDC04 to high numbers followed by (2) having residual galactose that when used by WDC04 for energy production generates sufficient CO_2 to saturate the medium with excess gas being captured in the Durham tube. When grown at pH 5.2 with 3% NaCl, the growth rate of WDC04 was so reduced that no gas formation was observed even after 28 d. Interestingly, under these cheese-like conditions, when the control 100:0 R:G tubes reached a maximum growth to $\sim 10^9$ cfu/mL, with further incubation there was a 2 log decrease in cell concentration (as shown by a drop in optical density). It then appeared that WDC04 was utilizing ribose released from dead lysed cells to go through a second growth phase. Risk factors for late gassing in cheese still appear dependent on the number of gas-forming lactobacilli present in the cheese, their ability to grow to high numbers using ribose from lysed starter culture cells, and being able to scavenge 6-carbon sugars present in the cheese after growth based on ribose has occurred.

Key Words: late gas defect, lactobacilli

T37 Influence of salt concentration on diacetyl production by *Lactococcus* and *Leuconostoc* in broth and cheese.

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Diacetyl is formed by fermentation of citrate by *Lactococcus lactis* ssp. *lactis* biovar diacetylactis and *Leuconostoc* adjunct cultures during manufacture and aging of some cheeses giving them a buttery flavor. Several lactic acid bacteria isolates were screened for their ability to ferment citrate to diacetyl in M17 lactose broth supplemented with 0.5% citrate, with 6 strains growing and producing diacetyl up to 5% salt. Two of the *Lc. lactis* ssp. *lactis* biovar diacetylactis strains (LD2 and SD5) were then studied further (in duplicate) in M17 broth with salt concentrations up to 9%. Diacetyl concentration was measured colorimetrically using the Vasavada and White method involving reaction with naphthol and creatine, then measuring absorbance at 540 nm. After 48 h incubation at 30°C, LD2 produced from 6.1 to 9.6 µg/mL of diacetyl with no significant difference up to 5% salt, with decreased amounts (only 1 to 2 µg/mL) at 6% to 8% NaCl. For SD5, the highest level (19.9 µg/mL) was produced with 1% salt, slowly decreasing to 10.2 µg/mL at 5% salt, and then dropping to 5.4 to 5.6 µg/mL at 6 to 8% salt. A similar pattern was observed when a mixture of LD2 and SD5 was used. Cheese was then made using 113 kg of milk (in duplicate) using a *Lactococcus lactis* starter culture with a combination of SD5 and LD2 added as adjunct cultures. Whey was drained at pH 6.45 and then the curd dry stirred until reaching pH 5.6, divided into 3 portions and then adding 15, 20 or 30 g/kg of salt. The cheese was then pressed and stored at 6°C. Salting promoted whey expulsion and also retarded further acidification of the curd. For the low, medium and high salt levels, cheese pH was 5.27, 5.38 and 5.42, average moisture was 42.2, 41.5 and 40.8%, and average salt content was 1.0, 1.5 and 1.7%, respectively. Average salt-in-moisture contents were 2.4, 3.5 and 3.9%, respectively. After 2 weeks of storage, all of the cheeses contained 6 to 7 µg/g of diacetyl. In conclusion, use of *Lc. lactis* ssp. *lactis* biovar diacetylactis strains that are relatively salt tolerant for diacetyl production in cheese made using a stirred curd does not appear limited because of the direct mixing of curd with salt rather than using a brining method.

Key Words: diacetyl, salt, cheese

Dairy Foods: Milk Quality

T38 Relationships between Pb, As, Cr, and Cd in soil and water in agricultural and industrial areas with heavy metals contents from individual cow milks. X. W. Zhou[1,2], H. Soyeurt[2], N. Zheng[1], C. Y. Su[1], and J. Q. Wang*[1], [1]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*TERRA Research and Teaching Centre, Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium.*

Various industrial activities lead to environment pollution with heavy metals. Heavy metals enter the food chain of dairy cows especially through feed and water. This study investigated the relationships between milk heavy metals' contents in individual cows with that in soil and water in industrial and agricultural areas. Sixty milk samples (10 per farm) were collected from the udder during milking. Six underground water and 6 soil samples were collected at the same farm that milk sampling. Levels of Pb, As, Cr and Cd in milk and water samples were measured by ICP-MS. Lead, Cr and Cd in soil were measured by AAS, and As was detected by AFS. Heavy metal contents in milk from agricultural and industrial areas were compared using Kruskal-Wallis tests. Spearman correlations were calculated between the studied metal contents in milk with that in water and soil. Ranges of Pb, As, Cr and Cd in milk were 0.025–10.45, 0.002–1.53, 0.017–5.01 and 0.006–0.27 µg/L. Mean levels of Pb, As, Cr and Cd were 0.07, 3.58, 1.80, 0.01 µg/L in water and 17.57, 7.91, 39.93, 0.15 mg/kg in soil, respectively. Contents of Pb and Cd in milk from agricultural area were significantly lower ($P < 0.01$) than that from industrial area. Significant ($P < 0.01$) higher As residue was observed in milk from agricultural area. No difference showed for Cd. Levels of As ($r = 0.37$) in milk and water were positively correlated. Those results suggested that As could be partially introduced into milk by cows' drinking water. A moderate positive correlation was found for Cr ($r = 0.60$) and Cd ($r = 0.66$) between milk and soil; and a negative value was observed in water for Cr ($r = -0.60$) and Cd ($r = -0.75$). Therefore, the contents of Cr and Cd in milk can be related to cows' feed that produced in Cr and Cd polluted soil. Lead ($r = -0.37$) levels in milk showed a negative correlation with Pb in soil, this leading no firm conclusions about the origin of Pb contamination in milk. The obtained results indicated that Pb, As, Cr and Cd in milk have complex source; water and soil in the farm had a partial contribution based on the obtained correlation amplitude.

Key Words: milk, individual cows, heavy metals

T39 A mycotoxin deactivator improves milk coagulation properties in dairy cows challenged with *Fusarium* mycotoxins deoxynivalenol and fumonisins in TMR. A. Gallo[1], P. Bani[1], T. Bertuzzi[1], B. Doupovec[2], J. Faas*[2], D. Schatzmayr[1], and E. Trevisi[2], [1]*Department of Animal Sciences, Food and Nutrition (DIANA), Faculty of Agriculture, Food and Environmental Science, Università Cattolica del Sacro Cuore, Piacenza, Italy,* [2]*BIOMIN Research Center, Tulln, Austria.*

Mycotoxins, secondary metabolites of toxigenic fungi, affect animal and human health worldwide. Two mycotoxins commonly found in dairy rations are the *Fusarium* mycotoxins deoxynivalenol (DON) and fumonisins (FUM). Under field conditions, it has been shown previously that milk of dairy cows exposed to mycotoxins had a significantly lower curd quality and curd firmness in comparison to cows not exposed to mycotoxins. The aim of this trial was to investigate the effect of DON and FUM in the feed on milk quality and rennet coagulation properties of dairy cows. A feeding trial was conducted using 12 Holstein cows in a randomized block design. Cows received either (1) a control diet (CTR), (2) a TRM contaminated with *Fusarium* mycotoxins (0.4 mg/kg DON and 1.1 mg/kg FUM) (MTX), or (3) the contaminated TRM supplemented with a mycotoxin deactivator (MD) (35 g/animal/day) (MTX+MD). The contamination level was lower than the recommended maximum values for dairy feed in Europe (5 mg/kg DON and 50 mg/kg FUM) and represent contamination levels that can be commonly detected in dairy rations. Each of the 3 experimental periods consisted of a 3-week treatment period followed by a 2-week clearance period. Individual milk samples were taken once a week for determination of coagulation properties (curd firming time k20 in minutes and curd firmness a30 in mm). The GLM (General Linear Model) procedure of SAS (SAS 9.4 TS, 2018) was used and LSMeans were compared post-hoc. The treatments had no significant effect on casein content, titrable acidity and clotting time of the milk. The mycotoxin diet showed however significant negative effects on curd firmness (a30) as well as curd firming time (k20) and in both parameters the addition of MD alleviated these negative effects (a30: CTR: 30.44 mm; MTX: 25.06 mm; MTX+MD: 32.44 mm; $P < 0.05$, k20: CTR: 8.07 min; MTX: 13.0 min; MTX+MD: 9.71 min; $P = 0.05$). In conclusion, dietary concentrations of FUM and DON commonly detected in dairy rations had a negative effect on milk rennet coagulation properties. The MD counteracted these negative effects.

Key Words: mycotoxin, milk coagulation properties, mycotoxin deactivator

T40 Effect of temperature variation on raw whole milk density and its impact on milk payment system for Irish dairy Industry. P. Parmar*[1], J. T. Tobin[1], J. Grant[3], J. A. O'Mahony[2], and L. Shalloo[1], [1]*Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland,* [2]*University College Cork, Cork, Ireland,* [3]*Teagasc Food Research Centre, Ashtown, Dublin, Ireland.*

Variation in temperature induces variance in milk density and estimation of fat content and subsequently affects the milk payment since fat content is an integral part of multiple component pricing systems. Thus, the objective of this study was to determine the effect in whole milk density due to variations in temperature. Whole milk samples were collected from morning milking of 32 individual dairy cows of national average genetic merit once every 2 weeks over a period of 6 weeks from the Teagasc research farm, in Kilworth, Co. Cork, Ireland. A total of 93 samples were assessed on the rapid testing technique Dairyspec FT system (Make- Bentley) for milk compositional analysis. Density of milk was evaluated using 2 methods, a portable density meter DMA 35 (a standard industrial method for quick results) and desktop version DMA 4500M (a standard lab testing method for higher accuracy levels), and also to compare and determine accurate density factor dependent upon processing temperature for weight-volume calculations. Statistical analysis using ANOVA showed a significant difference in means of densities (F, 78.866 > F-crit., 3.947 and $P < 0.01$) measured at different temperatures. The results were then analyzed using PROC GLM procedure, SAS software to develop a quadratic model and identify the relationship (linear or curved) between temperature and density. The output indicated a significant nonlinear relationship ($P = 0.0008$) with the model equation defining the curvature and density-temperature relationship ($r^2 = 0.659$) as Density = $1.033 + 0.0000632 \times \text{temp} - 0.0000114 \times \text{temp}^2$. There was an inverse correlation between whole milk density and temperature (i.e., as temperature increased, milk density decreased).

Mean density calculated at 5°C was 1.0332 g/cm^3 with corresponding figures of 1.0328, 1.0315 and 1.0300 g/cm^3 at 10, 15 and 20°C respectively. This implies that the volume of milk and subsequent total milk solids content estimated at lower temperature (5°C) will be higher than the values estimated at a higher temperature (20°C)

Key Words: whole milk, density, temperature

T41 Evaluation of influences of mycotoxin-contaminated diets on milk profiles and metabolites in dairy cows. Q. Wang[1,2], Y. D. Zhang[1,2], N. Zheng[1,2], S. G. Zhao[1,2], and J. Q. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Mycotoxins are key factor in safeties of food and feed. However, most previous studies about dairy ruminants have focused on the biochemical parameters or milk production. Little is known about the milk micro-profiles, such as fatty acids and amino acids, under mycotoxin exposure. With taking advantage of the well-established chromatography and NMR (nuclear magnetic resonance) analyses, milk micro-profiles and metabolic changes of dairy cow were measured after mycotoxins exposure. The results showed that milk composition of dairy cows are unchanged, but milk aflatoxin M_1 concentration changed along with the mycotoxins addition or clearance. Dairy cows fed cottonseed contaminated with mycotoxins had a significant reduction in unsaturated long-chain FAs in milk, but there was no reduction in the content of short- and medium-chain fatty acids. Interestingly, contaminated cottonseed did not reduce the content of amino acids in milk. Twelve metabolites were showed significant difference among the 3 groups. These metabolites are mainly involved in the metabolic pathways of phenylalanine, tyrosine and tryptophan biosynthesis, phenylalanine metabolism and valine, leucine and isoleucine biosynthesis. This may indicate that amino acid metabolism is affected by mycotoxins intakes. In conclusion, these results suggest that it is important not only to study milk composition, but also to pay attention to micro-indicators (FAs and amino acids) in the study of the harm of mycotoxins to dairy cows.

Key Words: mycotoxin, milk, fatty acid

T42 Use of principal component analysis for revealing and understanding differences in milk fatty acid profile in different ruminant species. F. Correddu[1], A. Cesarani[1], G. Gaspa[2], A. Pauciullo[2], G. Cosenza[3], A. Nudda[1], N. Macciotta*[1], and G. Pulina[1], [1]*Dipartimento di Agraria, University of Sassari, Sassari, Italy,* [2]*Dipartimento di Scienze Agrarie Alimentari e Forestali, University of Torino, Grugliasco, Italy,* [3]*Dipartimento di Agraria, University of Napoli, Portici, Italy.*

Fatty acid (FA) profile is a crucial factor in defining milk properties and quality. Increasing attention is placed on those FA having a positive role on human health, such as polyunsaturated FA (PUFA), oleic acid, vaccenic acid and conjugated linoleic acid (CLA). In the present work, the FA profile obtained from gas-chromatographic analysis of 703 individual milk samples (289 cows, 214 buffaloes and 200 ewes), were used to evaluate the suitability of principal component (PC) analysis (PCA) to investigate differences in milk FA profile among these species. Thirty-six FA were analyzed by PCA. The first and second PC (PC1 and PC2) accounted for 51% of the total variability (30%, 21%, for PC1, PC2, respectively). Elements of eigenvector of PC1 were positively associated with few FA, including C16:0 (0.50), the most abundant FA in milk of the 3 species (~29.3% of FA). Among those negatively correlated with PC1, some relevant FA for human health were retrieved: C18:1t11 (−0.75), CLAc9t11 (−0.79), C18:3n-3 (−0.76), EPA (−0.81) and DPA (−0.89). Negative correlations with PC2 were observed, in general, for medium chain FA (e.g., C15:0, C16:0), whereas long chain FA (e.g., C18:1 isomers, C18:2n-6 and other PUFA) exhibited positive values. Score values of PC1 and PC2 allowed the description of the relationship among animals based exclusively on the milk FA profile, revealing a clear discrimination of the 3 species: PC1 discriminated ewes milk from that of cows and buffaloes, whereas, PC2 was able to discriminate cows from buffaloes, with ewes showing intermediate values. In general, the use of PCA evidenced as a great part of variance of FA profile of ruminants (PC1) is related to the distinction between small or large ruminants, also evidencing higher amount of beneficial FA for ewes. An important part of variance (PC2) indicates as ewes milk FA has intermediate characteristics compared with cow and buffalo milk.

Key Words: fatty acids, ewe, cow

T43 The effect of casein genetic variants and diet composition on Holstein milk proteome. M. I. Rivelli*, J. E. Wessels, A. L. Roca, and F. C. Cardoso, *University of Illinois, Urbana, IL.*

Bovine milk casein (CN) account for about 80% of the total proteins in milk. Genes encoding bovine CN are in chromosome 6. The most common alleles in dairy cattle are A1 and A2, being the former one a genetic variation of A2 that happened thousands of years ago and affected European cattle origins. Variants A1 and A2 apparently occurs at the same allele frequencies in Holstein cows. Dairy milk protein profile can be influenced by many factors as breed, lactation stage, mastitis, and diet composition. The way these variants affect milk protein composition is off special interest due to their effect on dairy products processability and functionality, and their effect on human health. A database from 13 experiments completed at the University of Illinois (Urbana-Champaign) from 2016 to 2018 was developed. A total of 142 cows (117 multiparous and 25 primiparous) was included in the analyses. Cows β-CN genetic evaluation (i.e.; A1_A1, A1_A2; and A2_A2) for 128 cows was performed (Clarifide. Zoetis, Kalamazoo, MI). Treatments were as follow: cows A1_A1, cows A2_A2, and cows A1_A2. Parity was dichotomized as cows starting first lactation in one group (LAG1), cows starting second or third lactation in a second group (LAG2), and cows in the fourth-or-greater lactation in a fourth group (LAG3). Data were analyzed using the MIXED procedure of SAS, using 2 orthogonal contrasts. Contrast 1 (CONT1): A1_A1 compared with A2_A2 and contrast 2 (CONT2): A1_A1 compared with the average of A2_A2 and A1_A2. Milk yield was greater for cows A1_A1 than cows A2_A2 and A1_A2 (35.63 vs 34.24 ± 0.63 kg/d; $P = 0.03$, CONT2). There were no milk protein yield differences among treatments ($P > 0.1$, CONT1 and CONT2). There were no milk casein as a percentage of protein differences among treatments ($P > 0.1$, CONT1 and CONT2). Milk lactose yield was greater for cows A1_A1 than cows A2_A2 and A1_A2 (1.69 vs 1.61 ± 0.04 kg/d; $P = 0.02$, CONT2). Cows A1_A1 tended to have greater milk lactose yield than cows A2_A2 (1.69 vs 1.62 ± 0.04 kg/d; $P = 0.06$, CONT1). In conclusion, cows homozygotes A1_A1 had similar milk protein yield and milk casein yield than homozygotes A2_A2 and heterozygotes A1_A2. Cow homozygotes A1_A1 had the greatest milk yield and lactose yield.

Key Words: casein, milk protein, A2

T44 Effect of sonication combined with heat to improve the microbial quality of milk.

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Thermophilic bacteria and spores along can survive milk pasteurization and reduce its shelf life. Milk quality is altered by enzymes produced by surviving microbes in milk. Coupling thermosonication with pasteurization may reduce the microbial load and enhance the product shelf life. This study evaluated the effect of flow through thermosonication using a plate heat exchanger and sonicator source (Heischler UIP500hd, Ringwood, NJ) on the survival of thermophilic and indigenous organisms in milk. For the thermophilic study, sterile 2% fat milk was inoculated with *Geobacillus stearothermophilus* (GS) and for indigenous microflora, raw milk was used (initial microbial count for both were 10^4 to 10^5 cfu/mL). Milk inoculated with GS and raw milk were treated with either heat (control) or heat and thermosonication (treatment) at 72°C at settings 1, 2, and 3 and settings 1 and 2, respectively that resulted in different heat (15.8, 13.2, and 12.1 s) and thermosonication (18.8, 12.8. and 8.1 s) times. Heat treatment before thermosonication of GS inoculated milk resulted in significant log reductions (*P*-values: 0.04, 0.04, 0.0005) of 0.45 ± 0.04, 0.26 ± 0.07, and 0.15 ± 0.01, at setting 1, 2, and 3, respectively; for heat treatment after thermosonication, significant log reductions (*P*-values: 0.02, 0.03, 0.0004) were seen at setting 1, 2, and 3 (0.54 ± 0.08, 0.35 ± 0.03, and 0.08 ± 0.01). Heat treatment reduced the cfu/mL of GS (log reduction ranging between 0.018 and 0.368 at all settings); but the reduction was not significant (*P*-values >0.05). The position of the sonicator did not have a significant effect (*P*-value = 0.95) on GS reductions. For raw milk, heat alone resulted in significantly lower log reductions (*P*-values: 0.005 and 0.02) (1.43 ± 0.03 and 0.69 ± 0.05) as compared with heat along with sonication (1.97 ± 0.14 and 1.14 ± 0.15) for setting 1 and 2, respectively. The shelf life was 5 weeks for setting 1 and 3 weeks for setting 2. Sonication along with heat treatment resulted in significantly higher log reductions for inoculated thermophilic bacteria and inherent microflora in milk as compared with heat alone. Use of pasteurization along with thermosonication may potentially reduce the microbial load of in milk leading to products with extended shelf life.

Key Words: milk pasteurization, shelf life, thermosonication

T45 Jersey cattle milk-derived exosomes: Isolation and characterization.

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Milk-derived exosomes contains protein and RNA, which mediates the communication between cells information exchange and materials transfer. Objectives were to isolate and characterize exosomes in Jersey cattle milk. Milk samples were collected from 12 mid-lactation Jersey cows and 12 Holstein cows as comparison. We separately use 30 mL milk at a time to isolate. Milk fat was removed by centrifugation twice at 1,200 × *g*, 4°C,10 min. Milk protein and cell debris were also removed by centrifugation at 21,500 × *g*, 4°C for 30 min and 1 h, respectively. Supernatant (whey) was sequentially filtered through 0.45-µm and 0.22-µm filters. The whey samples were ultracentrifuged twice at 100,000 × *g*, 4°C for 90 min to isolate exosome. Exosomes obtained in the pelleted were resuspended in 100 µL PBS. Exosomes samples were diluted in PBS. Ten-microliter exosome samples were applied to 100 mesh copper grids and precipitated for 2 min, respectively. Excess material was removed by blotting and samples were negatively stained twice with 10 µL of a 2% uranyl acetate solution (wt/vol; Electron Microscopy Services). The grids were air-dried and exosomes were visualized using a transmission electron microscope at 100 kV (Hitachi H-7500, Hitachi, Japan). The particle size distribution and zeta potential were determined with a Zeta VIEW S/N 17–310 instrument (Particle metrix, Germany) at 4°C. The exosomes were diluted 6,000 times with ultrapure water to do nanoparticle tracking analysis. Exosomes marker proteins were detected by in-gel enzymatic hydrolysis. Transmission electron microscope images of exosomes isolated from Jersey cattle milk had a diameter in the range of 30–150 nm. Peak intensities for isolated exosomes were observed at 114.6 nm from nanoparticle tracking analysis. Two high abundance proteins, HSP70 and CD63, were identified in Jersey cattle milk-derived exosomes by In-gel enzymatic hydrolysis. These preliminary results indicate that exosomes from Jersey cattle milk has similar morphology to exosomes isolated with Holstein cow. The functional properties (e.g., miRNA, protein) in isolated exosomes merits further investigations.

Key Words: exosome, ultracentrifuged, transmission electron microscope

Dairy Foods: Probiotics, Bioactives, and Health

T46 Effect of milk fat globule membrane phospholipids in the adherence of probiotic lactic acid bacteria—Modelling interactions in the human gut. J. Ortega-Anaya*, D. Rocha-Mendoza, I. Garcia-Cano, and R. Jimenez-Flores, *The Ohio State University, Columbus, OH.*

The assortment of polar lipids that constitute the milk fat globule membrane (MFGM) affects infant gastrointestinal development and one of the possible mechanism might be via bacteria-lipid interaction that in turn affect the gut microbiome. However, there is scarce information about this topic. In our research group, we have proven that different lactic acid bacteria (LAB) bind and utilize different mixtures of MFGM phospholipids. Based on this information, we hypothesized that probiotic LAB modify their adherence properties when MFGM phospholipids are present during cell growth. To test this, we performed surface binding studies of bacterial cells grown in a defined medium (control), and with added MFGM phospholipids (0.5% wt/vol). We used a quartz crystal microbalance with dissipation (QCM-D) approach to measure binding kinetics. Our results show that the addition of MFGM during bacterial growth greatly alters the adherence properties to gold surfaces and more importantly, to intestinal cells as well. We calculated the affinity of the bacterial cells, via the total mass adsorbed, and were able to determine biochemical parameters such as the affinity constants (K_d) and found that *L. casei* and *L. reuteri* grown in MFGM increased significantly their basal binding affinity whereas the opposite effect was observed in cells of *P. acidilactici*, *L. plantarum* and *L. delbrueckii*. Interestingly, it was observed that regardless of the basal adherence properties, bacterial cells grown in MFGM, except for *L. plantarum*, displayed an unusual affinity toward intestinal cells characterized by such a strong binding affinity, that they are detached from the gold scaffold surface. Further experiments are needed to understand this interaction but based in our results, it is evident that in the presence of MFGM phospholipids, probiotic cells are subjected to metabolic changes or conditioning to surface attachment, which modify their binding properties having an impact in the gastrointestinal environment.

Key Words: adherence studies, LAB and milk fat globule membrane (MFGM), intestinal cells

T47 Effects of supplementation of citrulline and *Lactobacillus helveticus* ASCC 511 on the intestinal epithelial cell integrity. S. W. Ho and N. Shah*, *The University of Hong Kong, Hong Kong.*

Citrulline is an amino acid and a precursor of arginine that is believed to have the same beneficial effects as arginine. *Lactobacillus helveticus* ASCC 511 (LH511) utilizes arginine to produce extra energy for cell growth via arginine deiminase (ADI) pathway. Supplementation of citrulline and LH511 is considered to provide both benefits. The effects of LH511+Cit-2mM were examined on IPEC-J2 cell line and were used to determine anti-adhesion effects against pathogenic infection, the effect on cell integrity by measuring transepithelial electrical resistance (TEER) and that on tight junction (TJ) proteins (claudin-1, occludin and zonula occluden-1 (ZO-1)) expression by qPCR and Western blot analysis. The anti-apoptotic effect was also determined by a flow cytometric method. The adhesion level of LH511 on IPEC-J2 cells was enhanced when incubated with 2 mM citrulline. LH511+Cit-2mM exhibited the protective effect against the adhesion of enterohemorrhagic (EHEC) and enteroinvasive (EIEC) *Escherichia coli* (*E. coli*), and it also significantly improved TEER and stimulated TJ proteins expression. Treatment with LH511+Cit-2mM showed greater effects than arginine and citrulline alone. This study suggests that LH511 enriched with citrulline might be a potential supplement for enhancing the health of the intestine.

Key Words: citrulline, *Lactobacillus helveticus*, intestinal health

T48 Incorporation of bigels into yogurt to improve survival of probiotics. X. Zhuang*, S. Clark, and N. Acevedo, *Iowa State University, Ames, IA.*

The probiotic yogurt market is strong due to the potential health benefits that probiotics can provide to the host. However, many factors can cause the loss of probiotics viability, including processing conditions and the high acidity of yogurt. The objective of this study was to use bigel technology, a novel encapsulation system, to preserve viability of probiotics incorporated into yogurt. Bigels, composed of an oleogel emulsion (OGE) blended with a hydrogel (HG), were formulated. The OGE was prepared with 20% wt/wt oleogelators (5:5 soy lecithin: stearic acid), 10% wt/wt milk, and soybean oil as the continuous phase. The HG was composed of 25% wt/wt whey protein concentrate (WPC80) and 75% wt/wt deionized water. Probiotic bigels were prepared by homogenizing OGE and HG, followed by incorporation of *Lactobacillus acidophilus* and *Bifidobacterium lactis* suspended in milk. For Sundae-style yogurt, 18% wt/wt probiotic bigels were placed at the bottom of containers and covered with yogurt. For Swiss-style yogurt, 18% wt/wt probiotic bigels were mixed into containers with yogurt at a constant stir rate. Three controls were also included in the experimental design: yogurt without probiotics and bigel, yogurt with only probiotics (no bigel), and probiotic bigels from the Sundae-style yogurt. Probiotics viability at 4°C was monitored via plate counts for 6 weeks. The results showed that both the presence of phospholipids and the bigel structure enhanced *L. acidophilus* and *B. lactis* viability in yogurt. Throughout 42 d of storage, the presence of soy lecithin exhibited up to 2 log higher cfu/mL values than the control. Additionally, *B. lactis and L. acidophilus* survived for 7 d longer when inoculated in the structured system than control. The present study demonstrated that probiotics can be efficiently entrapped in bigel systems which extend their viability when incorporated in yogurt. This approach shows a promising future for its application to improve efficacy of probiotics in commercial yogurt production.

Key Words: bigel, probiotics, phospholipids

T49 Growth and short-chain fatty acid production by potential probiotic lactobacilli. J. Renye*, A. Hotchkiss, and A. White, *Dairy and Functional Foods Research Unit, ERRC, ARS, USDA, Wyndmoor, PA.*

Prebiotics are nondigestible food ingredients selectively used by beneficial bacteria within the colon to improve host health. Inulin and fructo-oligosaccharides (FOS) are well-studied prebiotics that can be metabolized by strains of lactobacilli and bifidobacteria; and are associated with improved digestive health in humans due to the production of short-chain fatty acids (SCFA). Prebiotics have also been shown to improve the growth, survival and bioactivities of probiotics, which has led to the development of synbiotics, where pre- and probiotics are delivered together to optimize their beneficial activities. In this study, we screened 87 strains of lactobacilli for their ability to grow with inulin (Synergy 1) or FOS (P95) provided as the sole source of fermentable carbohydrates. Growth in modified MRS broth (no glucose) containing 1% inulin or FOS (m/v) was monitored for 24 h in a Cytation 5 multi-mode plate reader (BioTek). Nine lactobacillus strains fermented both prebiotics, reaching an optical density (OD600) ≥ 1.2, including *L. casei* (strains: LC3, 441 and ATCC 4646); *L. helveticus* (strains: 1842 and 1929); *L. lactis* FARR; *L. paracasei* ssp. *paracasei* 4564; *L. acidophilus* 1426; and *L. reuteri* 1428. *Bifidobacterium breve* 2141 was also screened and fermented both prebiotics reaching an OD >1.6. High-performance liquid chromatography was used to identify SCFAs in cell free supernatants (CFS) from 20 cultures that reached an OD ≥0.5. For the 9 lactobacillus strains above, the concentration of lactic acid was between 175 and 206 m*M*, and *L. helveticus* 1929 produced the highest concentration of acetic acid (~19 m*M*). In the presence of FOS, the highest concentrations of propionic (3.9–6.2 m*M*) and butyric acids (0.9–1.2 m*M*) were detected in CFS from *L. reuteri* 1428, *L. paracasei* ssp. *paracasei* 4564 and *L. plantarum* 23115. With inulin, *L. acidophilus* 1426 and *L. delbrueckii* ssp. *lactis* 735 produced the highest concentrations of propionic acid (4.2 m*M*); and *L. acidophilus* 1426, *L. paracasei* ssp. *paracasei* 4564 and *L. plantarum* 23115 produced the most butyric acid (1.0 m*M*). Results from this study are essential to identify lactobacillus strains suitable for the development of synbiotics utilizing FOS or inulin as prebiotics components.

Key Words: prebiotic, *Lactobacillus*, probiotic

T50 Preparation of γ-aminobutyric acid-enriched fermented compound beverage by *Lactobacillus plantarum* J26. K. Zhuang[1], H. Li[1], Z. Zhang[1], X. Feng[1], S. Fu[1], T. Li[1], Y. Jiang[1,3], H. Zheng*[2], and C. Man[1], [1]*Key Laboratory of Dairy Science, Ministry of Education, College of Food Science, Northeast Agricultural University, Harbin, China,* [2]*California Polytechnic State University, San Luis Obispo, CA,* [3]*Yangda Kangyuan Dairy Company Limited, Yangzhou, China.*

Whey and corn oligopeptides are by-products from cheeses and cornstarch manufacturing processes respectively. Both of these by-products are highly nutritious. It was found in our previous research that some lactic acid bacteria (LAB) can utilize L-glutamate in corn oligopeptides to produce γ-aminobutyric acid (GABA). Moreover, research have shown blood-pressure-lowering effect of GABA-enriched dairy foods. Therefore, the objective of this study is to develop a GABA-enriched fermented compound beverage made up of whey and corn oligopeptides. Four species of LAB, including *Lactobacillus plantarum*, *Lactobacillus bulgaricus*, *Streptococcus thermophilus* and *Lactobacillus acidophilus*, were screened as single starter or mixed as compound starter. The quantity of substrates were characterized with viable counts and yield of GABA as evaluation indicators. Results showed that the optimal starter was *L. plantarum* J26 and the optimal substrates were 8% whey powder and 4% corn oligopeptides. In addition, the optimization of initial pH value, fermentation temperature and time were carried out using Box-Behnken response surface methodology. The optimal conditions of fermentation were 68 h fermentation at 32°C with an initial pH 5.4. The content of GABA reached 180.4 mg L^{-1} in the beverage which had significant difference ($P \leq 0.05$) compared with beverage fermented by other starters under same conditions, and it was far above the GABA content of unfermented beverage (12.0 mg L^{-1}). Furthermore, the viable counts of *L. plantarum* J26 were beyond 8.0 log cfu mL^{-1}. At last, the yield of GABA and viable counts were 155.2 mg L^{-1} and 7.6 log cfu mL^{-1} which declined slightly ($P > 0.05$) during storing at 4°C for 21 d. Sensory evaluation was done after the fermentation and during the whole storage. In general, results showed that the beverage received an equal or higher degree of overall hedonic sensory score comparing with commercial compound milk beverages.

Key Words: γ-aminobutyric acid (GABA), fermented compound beverage, corn oligopeptides

T51 Lactoferrin induces the synthesis of vitamin B6 and protects human umbilical vein endothelial cell (HUVEC) functions by activating PDXP and the PI3K/AKT/ERK1/2 pathway. Y. Wang[1,2], H. Li[1,2], H. Yang[1,2], J. Wang*[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

As a nutritional active protein in foods, multiple studies of the biological activities of lactoferrin had been proved, including antioxidant, antiviral, anti-inflammatory, antitumor, antibiosis and antiparasitic effects, while the mechanism related to its protection of cardiovascular system remained elusive. In the present work, the effect of lactoferrin on the viability of HUVECs (human umbilical vein endothelial cells) was detected to select the proper doses, the transcriptomics detection and data analysis were performed to screen out the special genes and the related pathway. Meanwhile, the regulation of lactoferrin in the functional factors thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) was detected. Then, SiRNA fragment of the selected gene pyridoxal phosphatase (PDXP) was transfected into HUVECs to validate its role in protecting HUVECs function. Results showed that lactoferrin inhibited expression of TXA2 and activated expression of PGI2, as well as activated expression of PDXP, which significantly upregulated the synthesis of vitamin B_6 and the PI3K/AKT/ERK1/2 pathway. For the first time, we revealed that lactoferrin could induce the synthesis of vitamin B_6 and protect HUVECs function through activating PDXP gene and the related pathway.

Key Words: lactoferrin, vitamin B_6, human umbilical vein endothelial cells (HUVEC)

T52 Investigation and comparison of the anti-tumor effects of lactoferrin, α-lactalbumin, and β-lactoglobulin in A549, HT29, HepG2, and MDA231-LM2 models. H. Li[1,2], P. Li[1,2], H. Yang[1,2], Y. Wang[1,2], G. Huang[1,2], J. Wang*[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

To investigate the anti-tumor activities of lactoferrin, α-lactalbumin, and β-lactoglobulin, 4 kinds of human tumor cells (lung tumor cell A549, intestinal epithelial tumor cell HT29, hepatocellular cell HepG2 and breast cancer cell MDA231-LM2) were exposed to 3 proteins, respectively. The effects on cell proliferation, migration, and apoptosis were detected in vitro, and nude mice bearing tumors were administered with 3 proteins in vivo. Results showed that 3 proteins (20 g/L) inhibited viability and migration, as well as induced apoptosis, in 4 tumor cells with different degrees (compared with the control, $P < 0.05$). In vivo, tumor weights in HT29 group (0.84 ± 0.22 g *v.s.* control 2.05 ± 0.49 g) and MDA231-LM2 group (1.11 ± 0.25 g *v.s.* control 2.49 ± 0.57 g) were significantly reduced by lactoferrin ($P < 0.05$); tumor weights in A549 group (1.07 ± 0.19 g *v.s.* control 3.11 ± 0.73

g) and HepG2 group (2.32 ± 0.46 g *v.s.* control 3.50 ± 0.74 g) were significantly reduced by α-lactalbumin ($P < 0.05$). Moreover, the roles of lactoferrin, α-lactalbumin, and β-lactoglobulin in regulating apoptotic proteins were validated. In summary, lactoferrin, α-lactalbumin, and β-lactoglobulin were proved to inhibit growth and development of A549, HT29, HepG2, MDA231-LM2 tumors in different degrees, via induction of cell apoptosis.

Key Words: lactoprotein, antitumor activity, tumor-bearing model

T53 Modulation of intestinal epithelial permeability and mucin mRNA (*MUC2*, *MUC5AC*, and *MUC5B*) expression and protein secretion in Caco-2/HT29-MTX co-cultures exposed to aflatoxin M$_1$, ochratoxin A, and zearalenone individually or collectively. C. Wu[1,2], N. Zheng[1,2], Y. Gao[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Aflatoxin M$_1$ (AFM1), ochratoxin A (OTA), and zearalenone (ZEA) are mycotoxins commonly found in milk. The combined effects of these mycotoxins on intestinal epithelial cells have not been reported. Herein, we investigated the combined effects of AFM1, OTA, and ZEA on intestinal integrity and define the underlying mechanisms(s) of their effects in Caco-2/HT29-MTX cocultures. Our results showed that the mixtures of AFM1+OTA, AFM1+ZEA, and AFM1+ZEA+OTA significantly decreased transepithelial resistance values and increased the paracellular flux of lucifer yellow and FITC-dextrans on Caco-2/HT29-MTX cells, which indicated an increased epithelial permeability. Although the expression levels of tight junction (TJ) proteins did not change significantly, immunofluorescence analysis and transmission electron microscopy revealed that mycotoxins altered TJ proteins morphology and disrupted their structures, namely, claudin-3, claudin-4, occludin, and zonula occludens-1. Also, the present study showed that mixtures of mycotoxins significantly modulated MUC5AC and MUC5B mRNA levels and protein secretion. This study demonstrated that the effects of mixtures of mycotoxins on intestinal barrier function were more significant than AFM1 alone. More importantly, the damage of intestinal integrity caused by mycotoxins was correlated with the change of the TJ proteins location and the decrease of mucin secretion. Mixtures of AFM1, OTA, and ZEA in food might pose a health risk to consumers, particularly in children, and toxin risks should be considered.

Key Words: Caco-2/HT29-MTX co-culture, tight junction, mucin

Dairy Foods: Processing 2

T54 Changes in the structure and stability of whey protein in milk as a marker for homogenization and pasteurization processing. P. Qi*[1], D. Ren[1,2], Y. Xiao[1,2], and P. Tomasula[1], [1]*Eastern Regional Research Center, Agricultural Research Service, USDA, Wyndmoor, PA,* [2]*Institute of Dairy Science, College of Animal Sciences, Zhejiang University, Hangzhou, Zhejiang, China,* [3]*Institute of Quality and Standard for Agro-products, Zhejiang Academy of Agricultural Sciences,, Hangzhou, Zhejiang, China.*

The effect of homogenization alone (H-) or in combination with high temperature, short time (HTST) pasteurization or UHT processing on the whey fraction of milk was investigated using highly sensitive spectroscopic techniques. In pilot plant trials, 1-L quantities of raw whole milk (3.25% fat) were homogenized in a 2-stage homogenizer at 60°C (6.9 MPa/10.3 MPa) and, along with skim milk (s-), were subjected to HTST pasteurization (72°C for 15s) or UHT processing (135°C for 2s). Other whole milk samples were processed without homogenization and used as is, or HTST only. The processed skim and whole milk samples were centrifuged to remove any residual fat and then acidified to pH 4.6 to fractionate the whey. The dialyzed whey fractions were investigated using the circular dichroism (CD), Fourier transform infrared (FTIR), and Trp intrinsic fluorescence spectroscopic techniques. Results demonstrated that homogenization combined with UHT brought about not only changes in protein composition but also a significant loss in secondary structure, particularly in the amounts of antiparallel β-sheet and α-helix, and diminished tertiary structural contact. These structural perturbations could lead to changes in the functional properties and nutritional values. On the other hand, homogenization (H-) and followed by HTST (H-HTST) treatments caused neither chemical change nor secondary structural reduction. The disruption was evident in the tertiary structural environment and the stability of the whey protein due to the homogenization of whole milk as revealed by the analyses from the near-UV CD and the intrinsic Trp fluorescence. The following order for tertiary structural stability was derived based on these studies: raw whole > HTST, H-, H-HTST > s-HTST and s-UHT > H-UHT. The methodology established in this study can be used to gain insight into the behavior of milk proteins when processed. It also provides a new empirical and comparative approach for analyzing and assessing the impact of processing schemes on the nutrition and quality of milk and dairy products without the need of extended separation and purification, which can be both time-consuming and disruptive to protein structures.

Key Words: whey protein, structure, stability

T55 Application of biochip array technology for the fast (under 19 minutes) multi-contaminant screening of milk samples using the fully automated Evidence MultiSTAT analyser. J. Mahoney, K. Crossey, J. Porter, L. Sibanda*, M. Rodríguez, R. McConnell, and S. FitzGerald, *Randox Food Diagnostics, Crumlin, Co. Antrim, UK.*

Biochip array technology allows the simultaneous detection of multiple analytes from a single undivided sample. Simultaneous immunoassays define discrete test regions on the biochip surface, which is also the vessel for the immunoreactions. This technology enables the comprehensive detection of veterinary drugs in milk, including all legislated antibiotics at or below the relevant regulatory requirements, which is important for consumer protection. The Infiniplex for Milk (IPM) biochip array, based on biochip array technology, allows the simultaneous screening of approximately 130 contaminants from a single undivided milk sample. This study reports the new application of IPM for the fast (under 19 min) multi-contaminant screening of milk samples using the fully automated biochip analyzer Evidence MultiSTAT. Simultaneous competitive chemiluminescent immunoassays, defining discrete test regions on the biochip surface, were employed and applied to the Evidence MultiSTAT analyzer. This system processes a self-contained cartridge with all the components required for the assays and has the capacity to assess 2 biochips in under 19 min. Raw milk samples (250 µL) was added directly to the biochips. The results are qualitative. Up to 130 authorized and unauthorized contaminants were detected (including antimicrobials, anti-inflammatories, anti-parasitics, corticosteroids, growth promoters and mycotoxins). The decision levels (concentration of contaminants resulting in > 95% of positive results) ranged from 0.04ppb (aflatoxin M1) to 150ppb (melamine). Assessment of natural incurred raw milk samples showed correlation > 90% with LC-MS/MS. Biochip array technology is applicable to the fast (under 19 min) multi-contaminant screening of milk samples when applied to the Evidence MultiSTAT analyzer. This offers a reliable analytical application to facilitate the comprehensive monitoring of contaminants in raw milk.

Key Words: biochip array, milk, contaminants

T56 Effect of temperature on IgM-milk fat globule-mediated agglutination. S. F. Hansen*, L. B. Larsen, and L. Wiking, *Department of Food Science, Aarhus University, Foulum, Denmark.*

A functioning agglutination mechanism is paramount to the quality of non-homogenized milks, because the process of agglutination causes firm cream layers in bovine milk. Agglutination is believed to occur due to interactions between the surface of milk fat globules and immunoglobulins, yet direct observations of the phenomenon have until now not been achieved. The presented research demonstrates how the process of agglutination can be visualized using confocal laser scanning microscopy, rhodamine red and a fluorescent immunoglobulin M (IgM) antibody. The effect on agglutination of storage temperature and pasteurization temperature was studies. Storage at 5°C resulted in clearly detectable agglutination, which was reduced at 15°C. Increasing storage temperature to 20 or 37°C cancelled any detectable interaction between milk fat globules and IgM. Thus the existence of cold agglutination was documented. Progressively higher inactivation of IgM and, hence, reduction of agglutination was observed, when pasteurisation (20 s) was increased from 69°C to 71°C and further to 73°C. Furthermore, 2-dimensional gel electrophoresis revealed a redistribution of Ig-related proteins in milk fat globule membrane isolates in response to changes in storage temperature. Poly-immunoglobulin G receptor was detected in milk fat globule preparations stored at cold (4°C) conditions, but was absent after storage at higher temperature (25°C). The findings provide valuable information about the right pasteurization temperature for dairy producers of non-homogenized milk.

Key Words: agglutinin, creaming, milk fat globule membrane

T57 Effect of pilot-scale ultrasonication on the physical, coagulation, and microbial properties of raw milk. D. L. Van Hekken, J. Renye, A. J. Bucci, and P. M. Tomasula*, *Dairy and Functional Foods Research Unit, USDA, Agricultural Research Service, Wyndmoor, PA.*

The ability of ultrasound waves to alter the properties of milk may provide opportunities to improve or develop new dairy foods yet scaling-up to continuous systems has been challenging. In this study, raw milk (3% fat) was processed through an innovative continuous, low-frequency, high-intensity sonication unit (16/20 kHz, 1.36 kW/pass) at different inlet temperatures (42 and 54°C), flow rates (0.15, 30, and 0.45 L/min), and passes (up to 7 passes to obtain ultrasound exposures of 14 to 18 min) to determine the processing effects on fat droplet size, coagulation properties, microstructure of milk and chymosin-set gels, and bacterial counts. Particle size analysis and confocal microscopy showed that largest fat droplets (2.26 ± 0.13 μm) found in raw milk were selectively reduced in size with a concomitant increase in the number of submicron droplets (0.37 ± 0.06 μm), which occurred sooner when exposed to shorter bursts of ultrasonication (0.45 L/min flow rates) at 54°C. Ultrasound processing with milk at 42°C resulted in faster gelling times and firmer curds at 30 min; however, extended processing at 54°C reduced curd firmness and lengthened coagulation time. This showed that ultrasonication altered protein-protein and protein-lipid interactions, thus the strength of the enzyme-set curds. Scanning electron microscopy revealed a denser curd matrix with less continuous and more irregular shaped and clustered strands, while transmission electron microscopy showed submicron lipid droplets embedded within the protein strands of the curd matrix. Processing at 54°C with flow rates of 0.30 and 0.45 L/min also reduced the total aerobic bacterial count by more than 1 log cfu/mL; and the number of psychrophiles below the limit of detection (10 cfu/mL). The findings show that this continuous ultrasound system, which is more conducive to commercial scale-up, will modify the physical and functional properties of milk under the parameters used in this study and may be a potential new tool for dairy food processing.

Key Words: raw milk, ultrasonication, fat droplet size

T58 Contribution of pressure-driven membrane separation operations to dairy processing eco-efficiency. S. Benoit*, C. Bouchard, and Y. Pouliot, *Université Laval, Quebec, QC, Canada.*

Pressure-driven membrane separation processes (MSP) are widely used in dairy processing. It is well known that MSP can improve economic profitability of dairy manufacturing by increasing cheese yields or by valorization of by-products such as whey and permeates. However, the contribution of MSP to the environmental impacts of dairy products is not clearly set out. Eco-efficiency is a concept that links both the economic and the environmental dimensions of a system. The objective of this study was to assess the contribution of MSP to the eco-efficiency of Cheddar cheese production in the Quebec province using a validated process simulation software. Three scenarios were compared: a conventional scenario (without MSP), a scenario integrating ultrafiltration (UF) operations at the standardization stage of cheese milk (5.93% proteins, wt/wt), and one final scenario integrating reverse-osmosis and UF operations in the by-product valorization processes (WPC 80 and permeate powder). By including mass balance, life cycle assessment, and net margin calculations in the process simulation, the study allowed for calculations of 4 eco-efficiency indicators. Each indicator expressed the net margin obtained per one environmental damage unit. The environmental damages considered were related to climate change, ecosystem quality, non-renewable resources, and human health. Results showed that even if the introduction of UF at the cheese milk standardization stage allowed for significantly improved productivity (+84%), it did not allow for improvement of the eco-efficiency of the process (8 to 16% less eco-efficient). However, introduction of MSP in the by-product valorization processes were highly beneficial to eco-efficiency since they allowed for reduction of non-renewable energy consumptions (reduced by more than 30% at drying stage) and increase in co-products values (addition of 2 to 5 percentage points to the net margin rate). This led to an overall improvement of the dairy process eco-efficiency of 3 to 24%. The process simulation software, validated using industrial data, allowed for exploration of possibilities and comparison of scenarios without the need for trials.

Key Words: eco-efficiency, membrane, environmental impact

T59 Application of zinc and calcium acetate to precipitate milk fat globule membrane components from a dairy by-product. N. Price*, T. Fei, S. Clark, and T. Wang, *Iowa State University, Ames, IA.*

There has been great interest in developing isolated dairy lipid fractions that are rich in phospholipids (PLs) due to their health benefits and functional properties. Dairy by-products that contain elevated content of PLs and milk fat globule membrane (MFGM) proteins, which are currently being discarded, can be an excellent source for these isolates. The β stream (a by-product of skim milk and anhydrous milk fat production) is a perfect candidate since it contains a higher concentration of PLs and MFGM proteins than many other dairy by-products. In this study, we investigated an economically feasible processing method to obtain the valuable components from the β stream. Various zinc acetate and calcium acetate concentrations (0–200 mM), heat treatments (30 and 60°C), and pH adjustments (5.0–8.0) were investigated to determine how effectively PLs and proteins were precipitated into a pellet. With a subsequent extraction of lipids from the pellet using ethanol (90%, 70°C), a PLs-enriched lipid fraction and good quality dairy proteins were obtained. The optimum precipitation conditions for the β stream were zinc acetate (25 mM) at pH of 7.5 and 30°C, and calcium acetate (100 mM) at pH of 7.5 and 60°C. With the ethanol extraction, PLs recovery of 97.7% from the zinc acetate precipitate and 94.9% from calcium acetate precipitate were achieved.

Key Words: dairy lipid, phospholipid, by-product

T60 Reclaiming water in dairy plants by reverse osmosis: Impact of type of fluid on overall process efficiency. A. Bouyer, J. Chamberland*, S. Benoit, A. Doyen, and Y. Pouliot, *STELA Dairy Research Center, Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Quebec City, QC, Canada.*

Reverse osmosis (RO) is increasingly used to concentrate dairy fluids and generate water that can be used for different purposes in a dairy plant. However, the composition of the dairy fluid used, namely total solids and protein contents, have a direct impact on the performance of RO membrane and on the permeate composition and quality. The objective of the present study was to compare the performance of RO membranes for reclaiming water from skim milk, cheese whey or ultrafiltration (UF) permeate from milk. A 2-step approach using a regular RO thin-film composite polyamide membrane element for the first water extraction, and a high-density RO membrane element for polishing the RO permeate was used. Filtrations were performed in triplicate at pilot scale (60 L batches) using 2.09-m^2 membrane elements (Parker-Hanifin Corp., CA, USA), at operating pressures between 1.38 and 3.45 MPa and up to maximal water recovery for both membranes. The energy consumption by the filtration system was monitored during experiments. The quality of reclaimed water was also characterized in terms of mineral content (Ca, Mg, Na, K, P and Cl) and conductivity. Process efficiency was assessed by determining the energy requirement to generate water reclaimed from the 3 dairy fluids (kWh/kg of water), and production costs. In the first stage, water recovery was 47, 55, and 60% for whey, skim milk, and UF permeate, respectively. The energy consumption was also lowest for UF-permeate (99 ± 8.0 kWh/kg water reclaimed). In the second stage, the polishing of water reclaimed from the 3 fluids showed similar results with a recovery of 83–86% and an energy consumption of 74 ± 3 kWh/kg water extracted. The conductivity (<25 mS/cm) and mineral content (<1 ppm) of polished water were also low. Overall, reclaiming water from UF-permeate led to a better process efficiency (higher water recovery and lower energy requirement for the first RO step) resulting directly from its low total solids and protein contents. Our results suggest that an appropriate selection of dairy fluid for reclaiming water can improve process efficiency, lead to savings in water reclaiming costs and reduce the dairy plants overall environmental footprint.

Key Words: efficiency, dairy fluids, reverse osmosis

T61 High-protein yogurt from milk concentrates—Impact of final pH and the application of power ultrasound on texture properties. A. Körzendörfer*[1] and J. Hinrichs[1], [1]*University of Hohenheim, Stuttgart, Germany,* [2]*University of Hohenheim, Stuttgart, Germany.*

High-protein fermented milk products such as Greek yogurt are conventionally produced by concentrating the coagulated milk after fermentation. This process generates acid whey that is undesired due to environmental concerns. An alternative approach to avoid acid whey is to concentrate the milk before fermentation by filtration or adding protein powders. However, resultant gels are firm so that stirring in the tank and further processing is difficult. It is also challenging to produce a smooth texture. In this study, yogurts from concentrate were manufactured and the effect of the final pH after fermentation on the physical properties was investigated. Additionally, the potential of power ultrasound (US) as a post-processing tool was examined. Skim milk was fortified with milk protein powder to 10% protein, heated (85°C, 30 min), and fermented with starter culture at 43°C. Fermentations were stopped at pH 5.0, 4.8, and 4.6, respectively, by breaking up the gel with a perforated disc and immersing the containers in iced water. Yogurts were sheared the next day through a nozzle (d = 3 mm) at a flow rate of 40 mL/s. Half of the samples were further treated with an US sonotrode for 10 s at a frequency of 20 kHz. Several physical properties were then analyzed (rheology, texture, particle size distribution, WHC). Additionally, the development of the gel firmness during acidification was monitored with a rheometer. Every 0.1 pH, a vane geometry was rotated in the milk/gel and the torque was recorded. The torque measured at pH 5.0, 4.8, and 4.6 was 4.2 ± 0.2, 4.8 ± 0.3, and 7.2 ± 0.3 Nm, respectively. The final pH after fermentation affected multiple physical properties of the final product. Yogurts fermented until pH 4.6 exhibited the highest apparent viscosity (2.18 ± 0.06 Pa·s). In contrast, when the acidification was already stopped at pH 4.8 and 5.0, the apparent viscosity was decreased by 36 and 40%, respectively. Moreover, the application of US as a post-process step further decreased the visual coarseness and viscosity, making it a beneficial tool to design high-protein products that meet ease of consumption.

Key Words: Greek yogurt, gelation

T62 Microencapsulation of probiotic organisms within a conjugated whey protein hydrolysate matrix. S. Minj*[1,2] and S. Anand[1,2], [1]*Midwest Dairy Foods Research Center, Brookings, SD,* [2]*Dairy and Food Science Department, South Dakota State University, Brookings, SD.*

Dairy foods enriched with probiotics are receiving considerable attention as modulators of the gut microbiota and host health. Hence, with increasing demand for probiotic foods, it is essential to develop novel products with added value in terms of enhanced health benefits and functionality. In addition, such products can benefit all types of consumer groups. In this study, conjugated whey protein hydrolysate (WPH10), which was identified in our previous study to have higher bioactive attributes, was used as an encapsulant to entrap probiotic cultures; *Bifidobacterium animalis* ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356, through spray drying process. Each culture was propagated at 37°C for 72 h, under anaerobic conditions, and the cell pellets were suspended in PBS to achieve 10^{10} cfu/mL. These cell suspensions were mixed in the ratio of 1:1 and inoculated into the conjugated WPH10 solution. The solution was spray dried in 2L batches using a Niro drier with an inlet and outlet temperature of 200°C and 90 ± 5°C, respectively. The final dried product was assessed for moisture content, functionality (solubility and wettability) and cell viability using standard plating procedure. All experiments were conducted in triplicates and one-way ANOVA was applied to differentiate the mean values. In addition, the microcapsules were observed under scanning electron microscope (SEM) to examine the structural conformity. Following spray drying, moisture content in the final product was 0.7 ± 0.14%. The solubility and wettability were considerably higher (91.5 ± 0.4% and 49 ± 2min respectively) as compared with WPH10 alone (77.28 ± 0.4% and 61 ± 2 min respectively). The mean probiotic counts (mixed population) before and after spray drying were 10.37 log cfu/mL and 8.50 log cfu/g, respectively. Under SEM, the outer structure of the capsules presented a round continuous surface with formation of irregular concavities. The conjugated whey protein hydrolysate is thus demonstrated as a carrier for probiotic bacteria. Such a product having even improved functionality, can offer opportunities as an ingredient for developing novel health formulations.

Key Words: encapsulation, conjugation

T63 Feasibility of soluble soybean polysaccharide for improving the drying ability and powder properties of Greek yogurt acid whey. V. Sunkesula* and L. E. Metzger, *Midwest Dairy Foods Research Center, South Dakota State University, Brookings, SD.*

The composition of Greek yogurt acid whey (GYW) is considerably different from that of cheese whey making it difficult to process into powder ingredients. The high lactic acid and mineral content in GYW delays lactose crystallization, which causes stickiness during spray drying and caking during storage of the powder. Previous researches have shown that soluble soybean polysaccharide (SSPS) can enhance lactose crystallization in aqueous lactose solutions as well in concentrated permeate. However, the effect of SSPS on the crystallization of lactose in concentrated GYW has not been evaluated. The objective of this study was to determine the feasibility of using SSPS to improve lactose crystallinity and drying characteristics of GYW powder. GYW obtained from a Greek yogurt was vacuum concentrated to 56% total solids (30% Lactose) and crystallized using a laboratory-scale crystallization tank. During crystallization, the concentrated GYW at 70°C was fast cooled to 30°C followed by slow cooling to 18°C (rate, −0.05°C / min) under constant stirring. Both the control and treatment solutions were seeded with lactose crystals (0.027g/100g of solution) and 0.1% SSPS was added to the treatment solution. After crystallization the concentrate was spray dried using a pilot scale NIRO dryer. GYW powder yield with 0.1% SSPS addition was observed to be less sticky on the dryer surface. The crystallized lactose content in treatment (77.46%) was also significantly higher than control (66.56%). The hygroscopicity at 43% relative humidity and caking properties of treated GYW powder

(14.4%) were significantly ($P < 0.05$) better than the control (12.4%). However, the glass transition temperature of the control (46.2°C) and treatment (51.1°C) powders were not significantly different ($P > 0.05$). The findings of this study indicate that SSPS can enhance lactose crystallization in concentrated GYW during crystallization, reduce the sticking of the powder in the dryer and improve the drying characteristics such as hygroscopicity and caking of the GYW powder.

Key Words: Greek yogurt acid whey, soluble soybean polysaccharide (SSPS), spray drying

Extension Education 1

T64 Improving culling decision using lifetime cost-benefit analysis: An interactive dairy management tool to assess herd profitability.

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A cost-benefit assessment can be a valuable tool in dairy management to assess an animal's lifetime contribution to herd profitability and make timely decisions regarding cow replacement. We developed a user-friendly interactive decision-making support tool to visualize the impact of costs and revenues on herd and cow level. The interactive tool was built with the Shiny add-on package in R. The dashboard consists of side-by-side panels with a built-in drop-down list for costs (feed costs, costs of breeding services, costs of health events, additional costs due to health events and extra breeding services) and revenues (milk value, margin over feed costs, margin over all costs) cumulated on lifetime basis. Herd-level data allow users to compare herd performance to that of the rest of the population. Integrated benchmarking tools facilitate the comparison with specific cohorts, such as region, management system, and calving year. In addition, cow-level data allow monitoring individual cow performance and contribution to herd profitability. A comparison across lactations between the top and bottom 25% herds in terms of cumulative milk yield indicated a 4.5-fold larger cumulative milk value. Nonetheless, we observed 5.4-fold greater cumulative disease costs and 4.2-fold greater cumulative additional costs due to health events and extra breeding services. A lifetime cost-benefit assessment has the potential to inform producers on making informed culling decisions by considering cumulative costs and revenues. Visualizing the magnitude of the impact of cumulative costs, in particular that of health and reproduction costs, on lifetime profitability should stimulate dairy producers to consider cumulative events in their decision-making process in keeping the most profitable cows in their herd, and to keep detailed on-farm records affecting lifetime profit. Our dashboard tool adds to the dairy extension deliverables through a user-friendly interface that allows producers, veterinarians, and advisory services nationwide to make timely decisions to improve herd profitability.

Key Words: decision support tool, dashboard, longevity

T65 A standardized method for characterizing ventilation in freestall dairy facilities.

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Two primary considerations for dairy barn ventilation are barn-level air exchange and the cow-level microclimate. Few studies and on-farm assessments address ventilation beyond limited description of fan presence and type of ventilation system. Our aim was to develop a standard method to characterize microenvironments and barn-level ventilation performance (for mechanical systems) to help dairy producers troubleshoot their ventilation systems. Forty-two barns (9, 5, and 28 cross-, tunnel-, and naturally ventilated, respectively) were evaluated in WI, MI, GA, TN, and TX to develop a novel airspeed mapping procedure. For barn-level ventilation performance, we measured barn and inlet dimensions, static pressure differential, temperature (T) and relative humidity (RH) at inlet and outlet, and fan number and models. To characterize microclimates at cow standing and resting heights (1.5 and 0.5 m, respectively), we measured T, RH, and airspeeds for 3 min/location. In mechanically ventilated barns, microclimate measurements to characterize the resting area and feed lane were taken in a representative half of the barn, divided along the direction of air flow; in naturally ventilated barns, these were taken in 1 pen per circulation fan configuration at every other stall for at least 2 fans/row. For each producer, we generated a report including barn description, ventilation performance, graphs of inlet and outlet T and RH over time, pen maps overlaid with microclimate measurements, an interpretive summary, and action items for potential improvements. In general, mechanically ventilated barns were consistent with industry standards, but 80% of barns had inconsistent airflow distribution resulting in some stalls with insufficient airspeeds (<1 m/s) at resting height. In naturally ventilated barns, 54 ± 30% of measured stalls had insufficient airspeeds at resting height, largely due to improper fan angle. 63% of all farms reported yearly fan maintenance. Our ventilation characterization method and novel airspeed maps provided dairy producers with customized feedback to improve their ventilation system performance.

Key Words: fans, microclimate, airspeed

T66 Assessing dairy employees' health status in South Dakota: Vision care.

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A dairy farm typically involves many day-to-day activities, use of machinery and equipment, and most importantly milking. Mastitis, a common disease in dairy cows, ultimately affects profitability of the dairy. Mastitis care includes following written protocols, signs detection, and safe medication practices that requires the farmworker to have optimal vision. We are observing elevated rates of vision impairment while providing milking school farm trainings. If not corrected, the vision impairment may interfere with the milking procedures and/or driving machinery. The aim of this study was to detect possible impaired vision issues within dairy farm employees and raise eye health awareness. Dairy farm employees (n = 88 on 4 farms) were tested with the Spot Vision Screener (Welch Allyn Inc., Skaneateles Falls, NY). The screener is a handheld non-invasive device that quickly and easily detects vision issues on people of all ages. The device instantly displays a full detailed report of pupillary diameter, ocular alignment, binocular refraction, and referral recommendation. The referral recommendation is 1) "All measurements in range" or 2) "Complete eye exam recommended." Participants using eyeglasses or contact lenses were included to ensure their prescriptions were within normal range. Descriptive statistics were calculated using SAS 9.4 by PROC FREQ and MEANS. The average age of those enrolled was 28 ± 1.6 and 34 ± 1.4 years old for female and male, respectively. Eighty percent were male, and 93% were Hispanic. One-fourth needed further eye examination and 40% (n = 35) had never visited an eye care professional either due to cost or language barriers. From the participants that needed an eye exam, 60% were milkers. If vision is impaired, the cow's wellbeing and quality of milk might be at risk due to the milker's vision challenges. Preliminary evidence using the screener suggests that future vision care programs should be developed

for farm workers, particularly for the milker subgroup. Study supported by HICAHS (Colorado State University).

Key Words: dairy farm, milker, vision problems

T67 Assessing dairy employees' health status in South Dakota: Eating habits and general health care.

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Dairy farm workers' eating habits may be compromised by their daily 12-h working shift. The intensive schedule demands high physical exertion with limited time for healthy choices, which include eating and general health care. The aim of this study was to assess South Dakota dairy farm employees' general health status including nutrition and health care (number of visits to the physician). A survey written in Spanish was conducted in person (n = 70 workers on 3 farms) assessing various topics and details related to employees' daily routine tasks, eating habits and general health status. Descriptive analysis was carried out using SPSS 25.0. The mean age was 28 ± 1.7 and 34 ± 1.6 for female and male, respectively. Most were Hispanics (96%) and males (76%). The large majority were Mexican (46%) and Guatemalan (44%) workers. Over half (53%) of workers were overweight or obese (mean BMI = 25.6 ± 4.2). Workers living in the United States 4 years or less had BMI = 25 whereas BMI was higher (>28) as years in the United States increased. One-third reported sleeping between 4 to 6 h/d and 46% reported eating in restaurants at least twice a week. The majority (80%) do not have health insurance, 53% have not seen a physician in the last 3 years, and 65% have not seen a dentist in the last 6 mo. Reasons for not receiving medical care included medical cost, lack of information, and language barriers. The only physical activity the workers practice is their job duties. They usually opt for healthier choices when arriving in the United States; however, as years increase, their habits change for either convenient fast food or pre-packaged food. Due to survey results, an educational workshop provided recommendations on improving general health care. The topics included healthier nutrition, awareness of cardiovascular diseases and oral health risk factors relating to eating habits. Personal health care might be influenced by individual values, culture, motivation, and economic opportunities. Strategic workshops designed to promote health education and healthy eating habits for farm workers are needed in their native language. Study supported by HICAHS (Colorado State University).

Key Words: dairy farm, farm workers, eating habits

T68 Survey about the use of allopathic treatments and sources of information for organic livestock farms in France.

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European Union law on organic production is the Regulation (EU)2018/848 of May 30th 2018. There are no official reports published about the use of allopathic treatments and conventional bedding materials in organic livestock in Europe. Thus, an online survey (36 questions, 6 sections) across European countries has been conducted from October 2018 to February 2019. The questionnaire was translated into several languages following Brislin's model. In France, 1,065 potential organic farmers were contacted by e-mail up to 3 times; 3 farmers' associations also disseminated the link among their members. Of the 155 responses received, 135 from certified organic producers were available for the analysis. Sex proportion (men:women) was 60:40, mostly between 31 and 50 years old (83/135). In general, the questionnaire was completed by the farm manager (80.2%) and farms were small (≤3 workers; 90.2%). Respondents mainly reared 1 (63.7%) or 2 (22.2%) animal species. Beef (38.5%), dairy cattle (27.4%) and sheep (18.5%) were most frequent. Last year, 82/130 farmers applied 1 (80.5%) or more treatments per animal. The selection between allopathic and alternative treatments depended on the health problem. Between 15.4% (skin problems) and 34.6% (lameness) of the farmers still relied on conventional treatments instead of phytotherapy, homeopathy or probiotics; and between 6.5% (reproductive issues) and 35.3% (mastitis) used those alternatives as well as conventional treatments. Other farmers (66.4%) and veterinarians (46.3%) were the main information sources for the use of those alternatives. Straw is still the most used bedding material (91.1%). This preliminary analysis suggested the need for further research on alternatives to the use of allopathic treatments and straw for bedding in organic livestock, and that farmers are the key factor for the dissemination/implementation of the results. This project received funding from the European Union's Horizon 2020 research and innovation program under grant agreement No [774340-Organic-PLUS].

Key Words: survey, animal health, production and management

Forages and Pastures 1

T69 Comparisons of fiber digestibility for triticale forages at two different sample sizes using the Ankom Daisy Incubator II System. W. Coblentz*[1] and M. Akins[2], [1]*US Dairy Forage Research Center, Marshfield, WI,* [2]*University of Wisconsin, Madison, WI.*

Accurate and precise determinations of in vitro NDF digestibility (NDFD) are critical to proper evaluation of forage nutritive value, and essential for proper diet formulation for dairy cows. The objective of this research was to compare NDFD values determined with the Ankom Daisy Incubator II System (Ankom) using 2 sample sizes (0.25 or 0.50 g); a further goal was to compare these results with those obtained from a commercial laboratory using traditional methodology without any restraint of samples during digestion. All determinations of NDFD were conducted with NDF determined with heat-stable amylase and sodium sulfite, and corrected for residual ash (*as*NDF*om*). Triticale forages (n = 48) were incubated in triplicate for 12, 24, 30, 48, 144, or 240 h by Ankom methods. For the 30-h incubation, a regression of values obtained from the 0.25-g sample size on the larger, 0.50-g samples was explained by a linear model ($Y = 1.206x - 1.1$; $R^2 = 0.933$), in which the slope differed from unity ($P < 0.001$), but the intercept did not differ from 0 ($P = 0.661$). After a 48-h incubation, a linear model ($Y = 1.014x + 7.1$; $R^2 = 0.964$) indicated that the slope did not differ from unity ($P = 0.631$), but the intercept was greater than 0 ($P < 0.001$). A linear regression ($Y = 1.040x - 1.8$; $R^2 = 0.861$) of NDFD using the 0.25-g sample size with Ankom methods on NDFD values obtained from the commercial laboratory indicated the slope and intercept did not differ ($P \geq 0.521$), from unity and 0, respectively. A similar relationship was obtained from the 48-h incubation ($Y = 1.021x - 3.4$; $R^2 = 0.866$). Relationships between Ankom and traditional methodologies were poorer for the 0.50-g sample size, particularly for the 30-h incubation, where the slope (0.824) was less than unity ($P = 0.002$). Generally, NDFD was greater by Ankom methods with the 0.25-g sample size, especially with short incubation times, and agreement with results obtained by traditional methods was improved relative to those obtained using a larger, 0.50-g sample sealed within fiber bags.

Key Words: in vitro incubation, NDF digestibility, sample size

T70 Milk performance of dairy cows as affected by the inclusion of corn silage or corn shredlage in a total mixed ration. A. Bach*[1,2], G. Elcoso[3], I. Joulie[4], and E. Chevaux[4], [1]*ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain,* [2]*Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Spain,* [3]*Blanca from the Pyrenees, Hostalets de Tost, Spain,* [4]*Lallemand Animal Nutrition, Toulouse, France.*

Corn silage is the most common forage used to feed dairy cows with inclusion rates typically around 20–40% of the diet DM. In recent years, the use of corn shredlage has been proposed as a substitute for corn silage. Corn shredlage is produced by a method that involves shredding the corn plant into unusually long sections and crushing the corn kernels. The objective of this study was to provide additional data on the effect of feeding shredlage (SDL) vs corn silage (CS) on milking performance and rumen microbial ecosystem. A total of 212,000 kg of whole plant were harvested on the same day an ensiled in 2 adjacent bunker silos of ~100,000 kg each. One silo was processed using a theoretical LOC of 26 mm (SDL) and other was harvested using a 16-mm LOC (CS). Both corn plants were treated at the rate of 100 mL/ton with a commercial inoculant (Lasil eFresh, Lallemand, France) to supply 150,000 cfu of *Lactobacillus hilgardii* and 150,000 cfu of *L. buchneri* per gram of fresh material. Sixty lactating Holstein cows (648 ± 66.6 kg of BW; 44.4 ± 9.9 kg/d of milk yield; 155 ± 75 DIM) were split in 2 groups and fed the same TMR (15.2% CP, 30.8% NDF on a DM basis) containing either 32.7% CS or 32.7% SDL, on a DM basis, for 21 weeks. Individual feed intake and milk production and composition was monitored daily. Also, at 50 d of study, a rumen sample was obtained from every cow, and DNA extracted and submitted to high-throughput sequencing to evaluate potential changes in rumen microbiota. Data were analyzed using a mixed-effects model which accounted for the fixed effects of treatment, week of study, and their 2-way interaction, plus the random effect of cow. Cows on SDL had a greater ($P < 0.05$) DMI toward the end of the study, but milk yield and composition were not affected by dietary treatments. As result, feed efficiency was greater ($P < 0.05$) in cows fed CS than in those fed SDL toward the end study. There were no changes in the relative abundances of the different microbial populations in the rumen between both groups of cows. It is concluded that SDL increases DMI of cows, but this increase is not followed by improvements in production.

Key Words: efficiency, preservation, processing

T71 Effects of environment, genotype, maturity, and plant height on in vitro fiber digestibility of corn vegetative tissues. G. Ferreira*, S. Thomas, and C. L. Teets, *Department of Dairy Science, Virginia Tech, Blacksburg, VA.*

The objective of this study was to evaluate the effects of environment (i.e., growing conditions) and genotype on fiber digestibility of corn plant tissues. This study was performed on a 200-cow dairy farm located in Virginia. In 2 cornfields (i.e., blocks), one conventional (CONV) and one brown midrib (BMR) corn hybrid was planted in plots (4.5 m × 30 m). Two environments were targeted; a limiting environment (POOR) that consisted of planting 100,000 seeds/ha (i.e., high population) and fertilizing with 163 kg N/ha (i.e., low fertilization), and a non-limiting environment (GOOD) that consisted of planting 60,000 seeds/ha (i.e., low population) and fertilizing with 214 kg/ha (i.e., high fertilization). At tasseling (EARLY) and early-dent (LATE) stages of maturity, 5 plants from each plot were cut by hand, and stems, leaf-sheaths, and leaf-blades from phytomers 9 (LOWER) and 16 (UPPER) were dissected and frozen for analysis. Tissues were analyzed for in vitro apparent dry matter digestibility (IVDMD) and for in vitro neutral detergent fiber digestibility (IVNDFD). All variables were analyzed with Proc MIXED of SAS, and the statistical model included the fixed effects of environment, genotype, maturity, phytomer, and all their interactions and the random effects of field (i.e., block), and the interactions of field by environment by hybrid and field by environment by hybrid by maturity. IVDMD was greater for BMR than for CONV (75.7 vs. 61.8%; $P < 0.01$) and for EARLY than for LATE (77.7 vs. 59.8%, $P < 0.01$) but was not affected by environment (68.8%; $P > 0.19$). Within the plant, IVDMD was greater for UPPER than for LOWER phytomers (77.4 vs. 60.2%; $P < 0.01$). IVNDFD was greater for BMR than for CONV (68.1 vs. 54.0%, $P < 0.01$) and for EARLY than for LATE (72.3 vs. 49.8%; $P < 0.01$) but was not affected by environment (61.1%; $P > 0.65$). Within the plant, IVNDFD was greater in UPPER than for LOWER phytomers (70.2 vs. 51.9%; $P < 0.01$). In conclusion, while genotype, maturity, and tissue height within the plant had substantial effects on IVDMD and IVNDFD, environment had no effects on IVDMD and IVNDFD.

Key Words: corn, fiber digestibility, environment

T72 Bacterial microbiota of rehydrated corn and sorghum grain silages.

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Monitoring the changes of bacterial communities during ensiling enables to understand and manipulate the fermentative process. In this context, it was explored the succession of bacterial populations and evaluated the impacts caused by microbial inoculants on bacterial community of rehydrated corn and sorghum grain silages by 16S rRNA Illumina MiSeq sequencing. A 2 × 3 × 6 factorial scheme was used, with 2 grains (corn -CG and sorghum-SG), 6 fermentation periods (0, 3, 7, 21, 90 and 360 d) and 3 inoculants: CTRL: non-inoculated; Inoc1: *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (Lalsil Milho, Lallemand Animal Nutrition) and Inoc2: *Lactobacillus buchneri* (Lalsil AS, Lallemand Animal Nutrition), with 3 replicates in a completely randomized design. Grains were rehydrated with water to moisture content at 30%, inoculated and packed into plastic film bags (25.4 cm × 35.56 cm). The predominance (>80%) of *Proteobacteria* was observed in both grains at the beginning of the fermentation (d 0) except in CG-CTRL and CG-Inoc1 silages, which had 51% of *Proteobacteria* and 61% of *Actinobacteria*, respectively. In all CG silages *Firmicutes* philum predominated (>84%) from 3 to 90 d after fermentation. As early as 3 d of fermentation, it was observed 85% of *Firmicutes* in SG-Inoc1, which unlike CG silages, the predominance of this phylum was extended up to 360 d. The *Bacilli* and *Gammaproteobacteria* classes were the main representatives of the *Firmicute* and *Proteobacteria* phyla, respectively. In CS-CTRL the fermentation from the 3rd day was predominantly by *Weissella* spp. with gradual replacement by the *Lactobacillus* genus. Bacteria present in Inoc1 induced the predominance (>80%) of *Lactobacillus* from 3 to 90 d of fermentation. The SG samples had lower initial diversity which was a reflection of the predominance (51–80%) of the *Pantoea* genus, mainly in SS-Inoc1 silages (80%). Species of *Lactobacillus* and *Weissella* are the main genera involved in the fermentation of rehydrated silages of corn and sorghum grains. The inoculant containing *L. plantarum* and *P. acidipropionici* resulted in a sharply growth of *Lactobacillus* and greater stability of the bacterial community during long period of storage in both grain silages. Supported by CNPq, CAPES, and INCT-CA

Key Words: 16S rRNA, *Lactobacillus*

T73 Fungal microbiota of rehydrated corn and sorghum grain silages.

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It was evaluated the impacts caused by microbial inoculants on the fungal population of rehydrated corn and sorghum grain silage by Internal transcribed spacer region (ITS) rRNA Illumina MiSeq sequencing. A 2 × 3 × 6 factorial scheme was used, with 2 grains (corn, CG; sorghum, SG), 6 fermentation periods (0, 3, 7, 21, 90 and 360 d) and 3 inoculants: CTRL, non-inoculated; Inoc1, *Lactobacillus plantarum* and *Propionibacterium acidipropionici* (Lalsil Milho, Lallemand Animal Nutrition) and Inoc2, *Lactobacillus buchneri* (Lalsil AS, Lallemand Animal Nutrition), with 3 replicates in a completely randomized design. The phyla *Ascomycota* was predominantly found in both grains silages followed by small abundance of *Basidiomycota* and *Mucoromycota. Aspergillus* spp. represented 51–89% of the initial population of CG samples. In CG-CTRL silages as early as 3 d it was observed a sharp growth of *Wickerhamomyces* (60%) yeasts which extended up 21 d of fermentation. After, *Aspergillus* returned to dominate from the 90 d until the end of the fermentation. Similar response was observed in CG-Inoc2 silages. Bacteria present in Inoc1 resulted in fermentation with the predominance of *Aspergillus* genus up to 90 d. However, at 360 d, 86% of the genera were represented exclusively by unidentified yeasts belonging to the order *Saccharomycetales*. Greater participation of different genera were observed in initial samples of SG than CG. *Alternaria* accounted for 30–60% of the initial population of the SG which were replaced by *Wickerhamomyces anomalus* in the intermediate periods of fermentation. *Aspergillu*s spp. were predominant in rehydrated corn grain fermentation while *W. anomalus* was the major fungal in rehydrated sorghum grain silages. The addition of microbial inoculants did not have an influencial effect on fungal population of rehydrated sorghum grain silages, while the mix of *L. plantarum* and *P. acidipropionici* controled the growth of *Wickerhamomyces* yeast until 90 d of fermentation in rehydrated corn grain silages. Supported by CNPq, CAPES, and INCT-CA

Key Words: ITS rRNA, *Aspergillus*, *Wickerhamomyces anomalus*

T74 Effect of particle size on ruminal in situ dry matter and starch disappearance of sorghum kernels.

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The objective of this study was to evaluate particle size distribution and ruminal in situ DM and starch disappearance of sorghum kernels cut in varied particle sizes. Unfermented kernels from 25 sorghum hybrids were harvested at various silage maturities and combined into 3 400g composites. Each composite was assigned to 1 of 3 treatments: whole kernel [1P], 2 [2P] pieces, or 4 [4P] pieces. Kernels were manually cut to achieve treatments using X-Acto knives. After cutting, kernels were dried in a forced air oven at 60°C for 48 h. Thereafter, geometric mean particle size (GMPS), surface area, and particle size distribution were determined using a Ro-Tap Shaker with 9 sieves with nominal apertures of 6.70, 4.75, 3.35, 2.36, 1.70, 1.48, 0.59 0.30 mm and pan. Subsequently, a ruminal in situ disappearance trial was performed to determine ruminal DM and starch disappearance with 3 lactating Holstein cows. Incubation times were 0, 6, 12, 48, and 120 h and treatments were triplicated within each cow. Polyester bags containing 5.00 ± 0.02 g of DM, were utilized to measure fractions A, B, and C, and to estimate the fractional disappearance rate (K_d) of fraction B and the effective ruminal disappearance (ERD). Data were analyzed using PROC MIXED of SAS with the fixed effect of treatment and the random effect of cow. Measurements of GMPS were 2152.0, 1695.5, and 1277.5 µm for 1P, 2P, and 4P, respectively. Fractions A ($P < 0.01$), B ($P < 0.01$), and K_d ($P < 0.01$) of DM increased with a reduction in particle size, whereas fraction C ($P < 0.01$) decreased. Likewise, greater fraction A ($P < 0.02$), B ($P < 0.01$), and K_d ($P < 0.02$) of starch were observed with a reduction in particle size. Moreover, reductions in particle size enhanced ERD of DM ($P < 0.01$; 3.12, 17.8, and 23.9% DM for 1P, 2P, 4P, respectively) and starch ($P < 0.01$; 15.2, 22.6, 39.7% of starch). These results underscored that more extensive processing of sorghum kernels improves ruminal DM and starch disappearance through increased surface area for microbial attachment and fermentation.

Key Words: starch, disappearance, particle size

T75 Effect of hybrid and planting density on yield and nutritive value of whole plant sorghum forage. C. L. McCary*[1], C. Heinzen Jr.[1], L. Brown[2], E. M. Paula[1], D. M. Taysom[3], and L. F. Ferraretto[1], [1]*University of Florida, Gainesville, FL,* [2]*Advanta Seeds, Irving, TX,* [3]*Dairyland Laboratories Inc., Arcadia, WI.*

The objective of this study was to evaluate the yield and nutritive value of 2 sorghum hybrids (Advanta Seed Alta AF7401 [H1] and Alta XF372 [H2]) planted at densities of 148,260 (D1), 197,680 (D2), 247,105 (D3), 296,526 (D4) and 345,947 (D5) seeds/hectare on yield and nutritive value of whole-plant sorghum forage. The treatments (2 hybrids [H] x 5 plant densities [PD]) were randomly distributed in 4 replicate plots. Plots consisted of 4 rows spaced at 0.762 m apart, at a length of 6.096 m per row. Plots were harvested targeting 31% dry matter (DM). At harvesting, the 3 outermost plants in the center 2 rows were discarded and all remaining plants in a 3-m linear distance were collected, chopped and weighed to calculate yield within the respective row. Subsamples were collected and dried in a forced air oven at 60°C for 48 h for DM determination. Dry samples were then ground to pass through a 4 mm sieve and sent to Dairyland Labs (Arcadia, WI) for nutrient analysis and predicted ruminal in vitro NDF digestibility at 30h (NDFD) using a near infrared spectrometer. Estimated milk production per unit of forage (kg of milk/Mg of forage) or per hectare (kg of milk/ha) were based on the predictive equations of Milk2006. Data were analyzed using PROC GLM of SAS in a complete randomized design with a 5 × 2 factorial arrangement with fixed effects of hybrid, planting density and their interaction. A single H × PD interaction was observed in lignin ($P \leq 0.01$), otherwise only main effects will be discussed. Greater concentration of aNDF ($P < 0.01$) and NDFD ($P < 0.01$), but lower starch concentration ($P < 0.01$) was observed for H1. Additionally, H2 had greater yield of DM ($P < 0.01$), and milk production estimates per unit of forage ($P \leq 0.03$), and hectare ($P < 0.01$). As PD increased, the concentration of aNDF ($P \leq 0.01$) increased as well. Greatest milk estimates (kg of milk/Mg of forage; P value ≤0.01) was observed at D1 and reduced gradually with increasing PD. These results emphasize the effect of hybrid and planting density on yield and nutritional value in whole plant sorghum forage.

Key Words: plant population, yield, nutritive value

T76 Aerobic stability of total mixed ration with added microbial growth inhibitors. J. P. Santos[1], V. C. Souza[1], E. F. Barbosa[1], R. B. Silva[2], C. L. S. Avila[1], R. A. N. Pereira[3,2], D. N. Lobato[4], and M. N. Pereira*[1,2], [1]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [2]*Better Nature Research Center, Ijaci, MG, Brazil,* [3]*Empresa de Pesquisa Agropecuaria de Minas Gerais, Lavras, MG, Brazil,* [4]*Alltech do Brasil, Araucaria, PR, Brazil.*

Aerobically stable diets can increase DMI of cattle. We evaluated the effect of 3 products (Alltech, Brazil) at 2 iso-propionic acid dosages on the aerobic stability of a 48.8% silage based TMR (50.5% DM, pH 5.52). Treatments were: Control (CTL), Mold-Zap 55 (55% propionic acid, 12% NH_4OH) at 0.5 (MZ55-L) and 1 (MZ55-H) L/ton, Mold-Zap Aquativa (25% propionic acid, 3% NH_4OH, and acids citric, sorbic, formic, lactic, and ascorbic) at 1.1 (MZAQ-L) and 2.2 (MZAQ-H) kg/ton, and Mold-Zap Aquativa Sorbato (35% propionic acid, 3% NH_4OH, 15% potassium sorbate, and acids citric and sorbic) at 0.8 (MZSO-L) and 1.6 (MZSO-H) kg/ton. Water (CTL) or products diluted in water (30 mL) were mixed to 5 kg of TMR and placed into six 30-L buckets/treatment. A bucket with each treatment was placed on one of 6 shelves in a controlled environment room (29.1 ± 1.7°C) for 48 h. The temperature of the TMR on each bucket and of the environment on each shelf were measured at 30-min intervals. Microbial counts (log cfu/g) were performed at 24 h. The statistical model contained the effects of shelf and treatment. Data obtained over time was analyzed as repeated measures. Contrasts compared CTL with other treatments. The maximum temperature (46.4°C) and the moment of maximum (1505 min) did not differ ($P \geq 0.15$). The aerobic stability (moment of temperature of TMR + 2°C than environment) was increased by MZSO-H relative to CTL (830 vs. 710 min. $P < 0.01$) and trends ($P \leq 0.09$) were detected for MZAQ-H (775 min) and MZSO-L (770 min). The mean temperature from 0 to 24 h was reduced ($P \leq 0.05$) by MZ55-L (32.8°C), MZAQ-H (32.1°C), MZSO-L (32.4°C), and MZSO-H (31.5°C) relative to CTL (34.8°C), and trends ($P = 0.06$) were observed for MZ55-H (32.8°C) and MZAQ-L (32.9°C). The temperature from 24 to 48 h (44.0°C) did not differ ($P \geq 0.36$). MZAQ-H and MZSO-L reduced ($P \leq 0.03$) spore forming bacteria. Aerobic bacteria did not differ ($P \geq 0.34$). Yeast was reduced ($P \leq 0.01$) by MZ55-L, MZAQ-L, and MZSO-H and tended to be reduced ($P = 0.06$) by MZ55-H, MZAQ-H, and MZSO-L. Filamentous fungi were reduced ($P < 0.01$) by all treatments. The microbial growth inhibitors reduced TMR temperature from 0 to 24 h, yeast, and filamentous fungi. Product MZSO at the highest dosage was the most effective.

Key Words: propionic acid, sorbate, aerobic stability

T77 Efficacy of chemical additives on microbial growth and aerobic stability of total mixed ration. J. P. Santos[1], V. C. Souza[1], E. F. Barbosa[1], R. B. Silva[2], R. A. N. Pereira[3,2], C. L. S. Avila[1], L. F. Greco[4], and M. N. Pereira*[1,2], [1]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [2]*Better Nature Research Center, Ijaci, MG, Brazil,* [3]*Empresa de Pesquisa Agropecuaria de Minas Gerais, Lavras, MG, Brazil,* [4]*Kemin do Brasil, Indaiatuba, SP, Brazil.*

The aerobic stability of the diet can affect feeding management and DMI of cattle. We evaluated the effect of 2 commercial products (Kemin, Brazil) at 3 dosages on the aerobic stability of a corn silage based TMR (50.5% MS, pH 5.52). Treatments were: Control (CTL), calcium propionate (CP. Shield Base Feedgrade Dry, 100% calcium propionate) at 1 (CP1), 1.5 (CP1.5), or 2 (CP2) kg/ton, or a liquid blend of microbial growth inhibitors (FC. Fresh Cut Plus Líquido. Acids acetic, benzoic, and propionic, ammonium hydroxide, polysorbate) at 1 (FC1), 1.5 (FC1.5), or 2 (FC2) L/ton. Water (CTL) or products diluted in water (30 mL) were mixed to 5 kg of TMR and placed into 6 buckets/treatment. A bucket with each treatment was placed on one of 6 shelves in a controlled environment room (29.1 ± 1.7°C) for 48 h. The temperatures of the TMR on each bucket and of the environment on each shelf were measured at 30-min intervals. Microbial counts (log cfu/g) were performed at 24 h. The statistical model contained the effects of shelf and treatment. Data obtained over time was analyzed as repeated measures. Contrasts compared CTL with other treatments. Treatments FC1.5 (1820 min) and FC2 (1825 min) delayed ($P \leq 0.05$) the moment of maximum TMR temperature relative to CTL (1745 min). Maximum temperature (46.7°C) and aerobic stability (723 min for temperature of TMR + 2°C than environment) did not differ ($P \geq 0.28$). Treatments FC1.5 (32.4°C) and FC2 (32.2°C) reduced the 0–24 h mean TMR temperature relative to CTL (34.8°C). Additives did not affect ($P \geq 0.63$) the 24–48 h mean temperature (44.4°C). Counts of aerobic bacteria and spore forming bacteria were reduced ($P \leq 0.05$) by CP2 (4.77 and 4.11) and FC1.5 (3.86 and 3.27) and tended ($P \leq 0.07$) to be reduced by FC1 (4.98 and 4.24) relative to CTL (6.85 and 5.74). Yeast count tended ($P = 0.09$) to be reduced by FC1 (0.98 vs 2.71). Filamentous fungi count was 3.35 for CTL and was reduced ($P \leq 0.05$) by CP2 (1.59), FC1 (1.37), FC1.5 (1.07), and FC2 (1.73) and tended ($P = 0.06$) to be reduced by CP1.5

(1.85). Product FC at the highest dosages reduced the 0–24 h TMR temperature and was more inhibitory of microbial growth than product CP.

Key Words: propionic acid, aerobic stability, total mixed ration

T78 Effect of multiple fermentation times on NDF digestion kinetics in alfalfa hay.

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Objectives of the experiment were to evaluate the interactions between undigested ash-free NDF (uNDFom) measurements and parameters of 2 and 3-pool models, and determine if precision of long-term fermentations (>72h) justifies their measurement. On a DM basis, the 35 alfalfa samples ranged from 21.4 to 54.7% amylase-treated ash-free NDFom. In vitro uNDFom was measured after 6, 12, 18, 24, 72, 120 and 240 h of fermentation. The mean aNDFom at 0 h was 36.8%. The mean and duplicate variation was 28.1 ± 1.57, 23.8 ± 0.95, 22.6 ± 1.18, 20.9 ± 0.82, 19.2 ± 0.98, 18.9 ± 0.70 and 19.0 ± 0.56% of DM at 6, 12, 18, 24, 72, 120 and 240 h, respectively. Using Solver in MS Excel, individual uNDF for each sample was fitted to a 2-pool model of digestion: uNDFom(t) = pdNDF*exp(-kd*(time-lag)) + iNDF2; where pdNDF = potentially digestible NDF, kd = fractional digestion rate, lag = discrete time before digestion begins and iNDF2 = indigestible NDF for a 2-pool model at time = infinity. When uNDFom at 0 to 72 h (uNDF0–72) were used, average kinetic parameters were: 0.30 h, 0.129/h, 47.4% of NDFom and 52.6% of NDFom for lag, kd, pdNDF and iNDF2, respectively. When uNDFom measured for 0 to 240 h (uNDF0–240) were used, average kinetic parameters were: 0.04 h, 0.113/h, 48.6% of NDFom and 51.4% of NDFom for lag, kd, pdNDF and iNDF2, respectively. In this study, 90% of pdNDF in alfalfa digested in 20–22h suggesting that fermentations > 24h may underestimate kd. Attempts to derive parameters for a 3-pool model were unsuccessful with the exception of indigestible NDF at time = infinity (iNDF3). Typically, fast kd were >1.0/h and fractions of fast and slow pools were variable. Duplicate variation for uNDFom was small, but even with low variability it was difficult to detect differences among uNDF72, uNDF120 and uNDF240. Adding uNDF120 and uNDF240 measurements to estimate kinetic parameters resulted in small changes in kd and iNDF for a 2-pool model of NDF digestion and did not affect RumNDFD [= pdNDF%NDF*kd/(kd+0.03)]. The cost:benefit of fermentations longer than 72 h are not justified for a 2-pool model, and more measurements between 24 and 120 h are needed to parameterize a 3-pool model.

Key Words: fractional digestion rate (kd), digestion rate, undigested NDF

T79 The difference of chemical composition and NDF digestibility of corn stalk treated with different fungus.

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The objective of this paper was to evaluate chemical composition and NDF digestibility of corn stalk treated with fungi strain, *Lentinula edodes* (L), *Pleurotus eryngii* (P) or mixing strain (L+P), respectively. Corn stalk were randomly assigned to 3 treatments and incubated in plastic culture bottle. Samples were obtained on wk 0, 1, 3, 6, 9 for nutritive value and NDF digestibility determination using the near-infrared (NIR) method. All data were analyzed using MIXED procedure in SAS (version 9.4) with fungus, Time (W), and their interaction as fixed effects. The results showed that: (1) CP, ADF and lignin were differed between samples with different fungi strain treatment process ($P < 0.05$). Corn stalk samples with fungal incubation time were significantly different in Moisture, pH, CP, ADICP, NDICP, ADF, lignin and WSC ($P < 0.01$). The interaction of fungi strain treatment and fermentation treatment had significant effect on the content of lignin ($P < 0.01$). (2) A fungus treatment × incubation time interaction also occurred for NDFD30, NDFD120, NDFD240, uNDFom30 and uNDFom240, respectively ($P < 0.01$). L+P group had the lowest NDFD30, NDFD120, NDFD240 content than the other 2 treatment. Therefore, it was concluded that *Lentinula edodes* and *Pleurotus eryngii* had a great potential to decrease the content of lignin and enhance NDF digestibility of corn stalk, but *Lentinula edodes* or *Pleurotus eryngii* treatment effect was better than they mixed treatment result.

Key Words: corn stalk, fungal incubation, lignin

T80 European grass silage in vitro rumen undigestible NDF measures at 30-, 120- and 240-hour comparisons between two laboratories.

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Forage in vitro rumen undigested NDF, corrected for ash (uNDFom, % of DM) is thought related to gut fill and dry matter intake in dairy cattle, however assay repeatability between laboratories, is unknown for long-term digestion periods. The objective here was to evaluate uNDFom repeatability for extended time points between 2 forage analysis laboratories. European grass silages were selected based upon NIR predictions to range in aNDF and uNDFom. Samples (n = 27) were dried, ground (1mm), mixed and split for analysis at both Rock River Laboratory (RRL) and Cornell University (CU) forage analysis laboratory for in vitro rumen uNDFom at 30, 120 and 240 h. Cornell University utilized the Raffrenato et al. (2018) technique and RRL used a modification of this technique, including Goeser and Combs (2009) rumen fluid preparation, re-inoculation for 240h time period (at 120h), and using an Ahlstrom 141 glass fiber filter (3.1-µm pore size) to filter samples post digestion and refluxing. Samples were digested in triplicate, within in vitro run, at each time point, and in 2 runs at CU and 1 run at RRL. The single run was chosen at RRL to mirror commercial feed analysis procedures. Following sample analyses, uNDFom was related back to laboratory, time point, and the interaction using the Fit Model function in JMP v14.0. Laboratory and time point were treated as fixed effects. The interaction was not significant and was removed. Following linear modeling, uNDFom was related to time ($P < 0.0001$) but not laboratory ($P > 0.4$). Model adjusted R-square was 0.23 and RMSE was 6.9. The least squares means (lsmean) for European grass silage uNDFom at each digestion time point (% of DM) were 18.9, 11.0, and 10.3 for 30, 120 and 240h, respectively, across both labs. The lsmean uNDFom for each laboratory were 13.8 and 13.0 for CU and RRL, respectively. Based on these observations for selected European grass silages, uNDF measures appear repeatable between 2 different laboratories. However further comparisons, with alternative forage and feed types, are warranted to better understand repeatability for long-term incubated samples.

Key Words: undigestible fiber, laboratory analysis

T81 The effects of *Lactobacillus diolivorans* on fermentation characteristics and aerobic stability of corn silage.

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Table 1 (Abstr. T79). Chemical composition and NDF digestibility of different fungi strain treated corn stalk from 0 to 9 weeks

	Treatment				*P*-value		
Item	L	P	L+P	SEM	Trt	Week	Trt × Week
Moisture	88.77	88.72	89.28	0.223	0.18	<0.01	0.27
pH	4.75	4.68	4.69	0.031	0.25	<0.01	0.28
CP	4.21	5.00	4.74	0.296	0.02	<0.01	0.11
ADICP	1.02	1.07	1.09	0.032	0.19	0.01	0.12
NDICP	0.88	0.91	0.95	0.038	0.35	0.01	0.15
ADF	47.97	45.92	46.17	0.638	0.01	0.01	0.68
Lignin	4.26	4.33	5.25	0.143	<0.01	<0.01	<0.01
WSC	4.56	4.65	4.74	0.183	0.78	<0.01	0.32
NDFD30	69.17	69.64	64.31	0.664	<0.01	<0.01	<0.01
NDFD120	83.68	83.17	78.79	0.583	<0.01	<0.01	<0.01
NDFD240	85.56	85.41	80.69	0.719	<0.01	<0.01	<0.01
uNDFom30	23.72	23.42	26.83	0.341	<0.01	<0.01	<0.01
uNDFom240	11.68	10.74	14.90	0.531	<0.01	<0.01	<0.01

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The objective of this study was to evaluate the effectiveness of *Lactobacillus diolivorans* 1k202752 (LD, Provita Supplements Inc., Mendota Heights, MN) on the fermentation and aerobic stability of corn silage. Whole plant corn (38% DM) was untreated (UN) or treated with LD (theoretical application rate of 250,000 cfu/g of fresh forage). Four individually replicated silos were packed (208 kg of DM/m^3) for each treatment into 7.5 L silos and opened after 3, 14, 30, 49, and 90 d of ensiling. Silos opened on d 14, 30, and 49 were subjected to air stress for 24 h: on d 7 for 14 d silos, on d 7 and 23 for 30 d silos, and on d 28 and d 42 for 49 d silos. Air-stressed silos had 3 1.60-cm holes plugged with stoppers and silicone glue, 2 located on the bottom of the bucket at opposing ends, and one on the lid of the bucket that were opened during times of air stress. 90 d silos had no air stress. Silages were analyzed for numbers of yeasts, fermentation end products, and aerobic stability (AS, h before a 2°C increase above baseline after exposure to air at 21°C). An additional set of silos at 90 d was subjected to aerobic stability measurements at 29°C. Data were analyzed using the Fit Model in JMP (SAS Institute Inc., Cary, NC) as a 2 × 5 factorial arrangement of treatments with the main effects of treatment, day, and their interaction. Silage with LD was lower ($P < 0.001$) in yeasts than UN after 30 (4.13 vs. 5.80 log cfu/g), 49 (3.60 vs. 6.21), and 90 d (3.35 vs. 4.33). Silage with LD had higher ($P < 0.001$) AS than UN at 49 (200 vs. 20 h) and 90 d (262 vs. 68 h). After 90 d of ensiling, UN differed ($P < 0.001$) from LD for lactic acid (4.95 vs. 2.83%), acetic acid (1.55 vs. 3.47%), 1,2 propanediol (0.10 vs. 0.27%), 1-propanol (0.00 vs. 0.52%), and propionic acid (0.17 vs. 0.54%). Exposure to the higher temperature for 90 d silage reduced ($P < 0.005$) AS for UN silage (68 h at 21°C vs. 37 h at 29°C) but not for LD (262 h for both temperatures). Addition of LD markedly improved the aerobic stability of corn silage even with air stress and at hot temperatures of spoilage.

Key Words: *Lactobacillus diolivorans*, aerobic stability, silage

T82 Comparison of growth and relationship with genomic body size for dairy heifers managed in confinement or on pasture. C. Hribar*[1], J. C. Cavadini[2], and M. S. Akins[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*University of Wisconsin Marshfield Research Station, Marshfield, WI.*

Genomic estimates of performance of dairy replacements are becoming more common on farm. Our objective was to evaluate the relationship of genomic body size estimates with heifer growth for heifers managed in confinement or on pasture. To evaluate this, heifers (n = 32) were managed in a confinement pen-based bedded pack barn (n = 16 heifers) or on pasture in a rotational grazing system (n = 16 heifers) for each grazing season with 2 grazing seasons completed (total of 32 heifers in each management setting). Heifers were blocked by weight (light or heavy) and randomly assigned to a management setting. The pasture contained a mixture of red and white clover, meadow fescue, festulolium, and ryegrass. Heifers on pasture were moved twice a week to a new paddock. A mineral/vitamin mix was provided daily, but no supplemental concentrate was provided. Body measurements were taken at the start and end of the grazing season for both confinement and pastured heifers. Heifers in confinement were limit-fed a total mixed ration to obtain growth rates of 0.8 to 1 kg/d. Genomic estimates of body size were obtained from a herd database with tissue samples previously submitted as a pre-weaned calf. Forage height measurements were taken with a rising plate meter. Available forage was then determined using 3 calibration locations taken weekly on each paddock. Forage availability was 1991 kg/ha in 2017 and 2736 kg/ha in 2018. Data for comparison of management system were analyzed using Proc Mixed (SAS v9.4) as a randomized complete block design with year as a repeated measures. Heifers grazing pasture had greater growth than those in confinement during both years (0.93 vs. 0.76 kg/d; $P = 0.02$). Hip height gains and body condition change during the season were similar ($P > 0.34$) for grazing (13.4 cm and 0.41 units) and confinement (12.7 cm and 0.23 units) heifers. Heifers raised in a managed intensive grazing system can have similar or even improved daily gains compared with heifers raised in confinement.

Key Words: heifer grazing, confinement, genomics

T83 Use of nylon bag technique in fistulated cattle to assess forage nutrient profiles. M. A. Snider*, S. E. Ziegler, H. M. Darby, and S. L. Greenwood, *University of Vermont, Burlington, VT.*

Orchard grass is a common forage grown on New England organic dairies. However, other forages may provide more energy and protein while using the same land area. By gaining a better understanding of the nutrient profile of various forages, feeding plans and botanical profiles of paddocks could be modified to improve cattle productivity. The objective of this experiment was to determine the nutrient profile and digestibility of 5 warm- (sudan grass (SD), millet (ML)) and cool- season (orchard grass (OG), white clover (WC), and meadow fescue (MF)) forages commonly grown in New England. Grasses were harvested pre-heading and legumes pre-bloom stage. Samples were oven-dried and ground using a Wiley mill (4-mm screen). Twenty nylon bags containing 7g DM of forage were inserted into each of 4 lactating ruminally fistulated Holstein cows. Samples were run in technical duplicates within cow. Nylon bags were assessed at 10 incubation periods (0, 1, 2, 3, 4, 6, 7.5, 19.5, 48, and 72 h). Recovered bags were washed, dried, and pooled within incubation period within cow for wet chemistry analysis of DM, CP, ADF, NDF, WSC, and starch. Data were analyzed using PROC MIXED and GLIMMIX of SAS (v9.4) with the effects and contrasts of species, time, and species by time. Species by time interactions were present for starch, DM, CP, ADF, NDF, and WSC. White clover had a greater CP content than other forages from h 0 to 48, but at 72 h the CP content of MF and WC were not different from each other. Compared with the other forages, at 72 h, OG had the lowest CP content ($P < 0.05$). The WSC of all forages decreased as time progressed and were all equivalent at the 72 h incubation time ($P < 0.05$). However, WC had greater amounts of WSC at h 2 and h 7.5. These results indicate that WC had the highest nutrient profile, but that warm- and cool- season grasses were comparable. Differences in the patterns of ruminal forage degradation and nutrient availability between the grasses and WC samples suggests that using a mixture of these forages may be a practical and feasible strategy to increase the nutritive value of a forage-based diet on organic dairy farms in New England.

Key Words: organic dairy, white clover, in situ

T84 Effect of different levels of waste dates on the chemical composition and quality of alfalfa silage.

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The goal of this study was to evaluate the effects of alfalfa silage with different types and levels of waste dates on the chemical composition and quality of alfalfa silage. Alfalfa hay with 65% dry matter was chopped in 3 cm size and ensiled using 3 different varieties of waste dates (*Berin*, *Ghybani*, *Amobahri*) in different levels 0, 6, 12 and 18%. Silages were prepared in 10 kg weight with 6 replication for every treatment into plastic bags. Chemical composition, pH, ammonia nitrogen, volatile fatty acids, air-stability and appearance properties were measured. Data were analyzed as factorial design 3 × 4 by the MIXED procedure of SAS ($P < 0.05$). Results showed that DM and OM (%) of silages in all levels waste date increased ($P < 0.05$) and CP (%) significantly decreased ($P < 0.05$) than control. PH of alfalfa silages with different levels and varieties of waste dates (range of 4.13 to 4.3) was significantly decreased ($P < 0.05$) than control (4.69). Ammonia nitrogen concentration (mg/dl) of alfalfa silage including of all varieties waste dates decreased significantly ($P < 0.05$) compared with control. Air-stability of alfalfa silage including of waste date increased significantly ($P < 0.05$) compared with control, and level of 18% waste date was shown the highest ($P < 0.05$) air-stability between different levels of waste dates. Acetic and propionic acid increased significantly ($P < 0.05$) and butyric acid decreased significantly ($P < 0.05$) in alfalfa silage including of 18% waste dates. Appearance evaluation was shown that calculated score of smell, structure and color of alfalfa silages with 12 and 18% of all varieties of waste dates was 20 (very good rank), alfalfa silage with 6% of all varieties of waste dates was 18 (good rank) and alfalfa silage without the addition of waste dates was 16 (acceptable rank). The fleing point in the alfalfa silage with waste dates was range 90.23 to 105.16, whereas in alfalfa silage without the addition of waste dates was 65.99. This study confirmed that using of 12 and 18% of waste dates when ensiling of alfalfa can improve the quality of alfalfa silage, but there was no meaningful difference between 12 and 18% levels.

Key Words: alfalfa silage, nutritive quality, waste dates.

T85 Effects of foliar fungicide application on floury and brown midrib corn varieties for whole-plant corn silage on silage temperature and fermentation parameters.

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With nearly half of a lactating cow diet composed of whole-plant corn silage (WPCS), it is important for the forage to be of high quality to achieve maximum milk production. Temperature can be an indication of WPCS quality. Quality can also be reflected in fermentation products. The purpose of this experiment was to determine silage temperature and quality of floury and brown midrib (BMR) WPCS varieties treated with foliar fungicide (FUN). A complete randomized design was used with 4 treatments: floury WPCS with FUN application (FF), floury WPCS without FUN application (FC), BMR WPCS with FUN application (BF), and BMR WPCS without FUN application (BC). FUN (pyraclostrobin, $C_{19}H_{18}ClN_3O_4$ + metconazole, $C_{17}H_{22}ClN_3O$; Headline AMP, BASF, Florham Park, NJ) application occurred at vegetative tassel (VT). Upon harvest, WPCS was inoculated (Silo-King; Agri-King, Fulton, IL) and stored in Ag Bags (Ag Bag Systems, St. Nazianz, WI) for 295 d before opening. Temperature of WPCS treatments was recorded every 10 min for 48 h using HOBO temperature loggers (Maxim Integrated, San Jose, CA). WPCS from each treatment was placed into 3 buckets with 3 loggers each. Ambient temperature was recorded using an empty bucket with 3 loggers. Fermentation profile of WPCS samples was analyzed at a commercial laboratory (Dairy One Forage Lab, Ithaca, NY). Data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Floury treatments had a lower temperature (23.13 ± 0.03°C), compared with BMR treatments (23.73 ± 0.03°C; $P < 0.01$) over 48 h. DM concentration was greater for floury treatments (30.33 ± 0.47%) compared with BMR treatments (29.24 ± 0.47%; $P = 0.05$). Lactic acid concentration was greater for BMR treatments (3.09 ± 0.28%) compared with floury treatments (2.41 ± 0.28%; $P < 0.01$). In conclusion, increased temperature and DM concentration indicate decreased quality for BMR treatments; however, greater lactic acid concentrations indicate better fermentation compared with floury treatments. The application of FUN neither hindered nor improved silage temperature or fermentation products.

Key Words: foliar fungicide, corn silage, aerobic stability

T86 An evaluation on the effect of a microbial inoculant on the fermentation of alfalfa silage ensiled at two dry matters.

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The objective of this research was to evaluate the effect of a microbial inoculant (Land O'Lakes) on the fermentation of alfalfa silage ensiled at 2 levels of DM. Alfalfa from the same field was wilted to 38 (mid-DM, mDM) and 46% (high DM, hDM) DM. Forages were untreated (CN) or treated with *Pediococcus acidilactici, Enterococcus faecium* and *Lactobacillus plantarum* with a final application rate of 165,000 cfu of lactic acid bacteria (LAB)/g of fresh forage weight (T1X), or the same inoculant with a final application rate of 330,000 cfu of LAB/g of fresh forage weight (T2X). After treatment, about 1 kg of forage was packed into 5 individually replicated nylon-polyethylene vacuum barrier bags for each treatment. Bags were evacuated of air and heat sealed. All silos were allowed to ferment at 21 ± 0.5°C for 3, 30, and 60 d. Data were analyzed using the Fit Model in JMP (SAS Institute Inc., Cary, NC) as a 3 ´ 3 factorial arrangement of treatments with the main effects of treatment (3), days of ensiling (3), and their interaction. Fresh alfalfa had a pH of 6.24 before ensiling. The pH of mDM silages treated with inoculants was lower ($P < 0.01$) than CN after 3, 30 and 60 d. Specifically, the pH at 3 d, for CN, T1X and T2X was 5.51, 5.17, and 5.11, respectively. In contrast, inoculation had no effect on pH drop 3 d in hDM silage and T2X treatment was lower ($P < 0.01$) in pH than both CN and T1X after 30 and 60 d. Changes in lactic acid closely reflected changes in pH. Acetic acid was lower ($P < 0.01$) in inoculated silages at 30 and 60 d compared with CN in mDM silages but were similar among treatments in hDM silages. Compared with CN inoculation had no effects on soluble protein (% of CP), NH3-N, and 24 h in vitro NDF-D, but concentration of total CP was higher ($P < 0.01$) in T1X (18.19%) and T2X (18.12%) than CN (17.94%). The inoculants used in this study stimulated the fermentation equally in mid-DM alfalfa silage, but a higher application rate was needed to show improvement in high-DM silage.

Key Words: alfalfa silage, inoculant

T87 Effect of corn planting population on phosphorus concentration and extraction in the forage (study 3).

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The objective of this study was to evaluate the effects of corn planting population on dry matter (DM) yield, plant phosphorus (P) concentration, and P removal from the soil. The study was performed on a 200-cow dairy farm. In 2 cornfields and during 2 growing seasons, 2 corn hybrids were planted in plots at a theoretical seeding rate of 60,000, 80,000, and 100,000 seeds/ha (LOW, MID, and HIGH, respectively). Each seeding rate had 4 replicates within each cornfield and year. Plots were three 30.5-m long rows separated by 76 cm. Pre- and post-planting fertilization included 112 and 77 kg N/ha, respectively. The resulting populations were 63,000, 72,500, and 86,000 plants/ha for LOW, MID, and HIGH, respectively. Harvesting occurred at early-dent and 1/2 milk-line stages of maturity during years 1 and 2, respectively. At harvesting, 10 plants from each plot were cut by hand (15 cm above ground), weighed, chopped, mixed, and analyzed for DM and P concentrations. Data were analyzed as a completely randomized design. The model included the fixed effects of year, field, hybrid, planting density, and all their interactions. Due to their interaction ($P < 0.01$), increasing corn plant density increased DM yield in year 2 (21.5, 22.7, and 23.7 Mg DM/ha for LOW, MID, and HIGH, respectively) but not in year 1 (19.2, 17.8, and 19.1 Mg DM/ha for LOW, MID, and HIGH, respectively). Due to their interaction ($P < 0.02$), the concentration of P in the forage differed among planting populations during year 1 (0.248, 0.225, 0.226% P for LOW, MID, and HIGH, respectively) but not during year 2 (0.285% P). Due to their interaction ($P < 0.01$), increasing corn plant density increased P output in year 2 (61.0, 64.6, and 68.3 kg P/ha for LOW, MID, and HIGH, respectively) but not in year 1 (47.8, 40.4, and 43.3 kg P/ha for LOW, MID, and HIGH, respectively). In conclusion, high corn planting population can increase DM yields and P removals, although this response is likely dependent on harvesting time. Although harvesting time was confounded with year, our data suggest that greater DM yields and P removals can be obtained when harvesting occurs at more advanced stages of maturity.

Key Words: corn, plant population, phosphorus

T88 Effects of feeding two different types of sorghum-sudangrass silage based diets on nutrient intake and digestibility and growth of Holstein dairy heifers.

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Pregnant dairy heifers can overconsume energy when fed diets with lower fiber levels causing higher intakes. Use of high fiber forages can help control intake and gains. The objective of the study was to determine the potential to use sorghum-sudangrass (SS) to control DM and nutrient intakes and weight gains. This study evaluated the nutrient intakes and growth of dairy heifers offered an alfalfa silage/corn silage diet (CON; 14.3% CP, 61.1% TDN, 47.9% NDF) compared with diets containing 1 of 2 types of SS silages [conventional (CSS) or photoperiod-sensitive (PSS)]. Both diets were similar in nutrient composition, with approximately 13% CP, 60 to 61% TDN, and 55% NDF. Seventy-two Holstein heifers (16 to 18 mo old at study initiation) were blocked by initial body weight (light, 422 ± 12.8 kg; medium, 455 ± 14.8 kg; heavy, 489 ± 16.7 kg) with 3 pens assigned to each weight block (8 heifers/pen; 24 heifers/block). The 3 diets were randomly allocated to the pens within each block and offered for 12 weeks. Data were analyzed as a randomized complete block design using Proc Mixed of SAS v9.4. Heifers offered CON had greater DM (10.9 kg DM/d), protein (1.51 kg CP/d), and energy intakes (6.68 kg TDN/d; $P < 0.01$) compared with both SS silage based diets (9.14 kg DM, 1.16 kg CP, 5.54 kg TDN/d) due to the greater NDF concentration of the SS diets. With lower DM and nutrient intakes, average daily gain was in the recommended range of gains (0.8 to 1 kg/day for Holstein heifers) for heifers offered the SS silage based diets (mean of 0.92 kg/d for both SS diets vs. 1.11 kg/d for CON; $P = 0.02$)). Sorting behaviors for heifers offered either SS diet were more aggressive against long particles and preference for medium and short particles compared with CON ($P < 0.01$); however, heifers sorted large particles from PSS more aggressively than those from CSS ($P < 0.05$). Based on this study, SS silage based diets can control the DM and energy intakes for heifers and maintain optimum growth rates, with harvesting at a shorter chop length likely helping to alleviate sorting issues.

Key Words: dairy heifer, sorghum silage, growth

Growth and Development: Colostrum and Transition Milk

T89 Evaluation of passive transfer of calves receiving maternal colostrum or colostrum replacer.

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Failure of passive immunity transfer (FPIT) remains a major problem in dairy production. Therefore, commercially available colostrum replacers have emerged to mitigate this deficiency. Literature suggests that consumption of levels of Ig, beyond that needed to prevent FPIT, may improve calf health and performance. Fifty calves were blocked according to sex, birth weight and date of birth and were distributed among different colostrum feeding protocols: 2MC: 2L of maternal colostrum (MC); 4MC: 4L of MC; 2MC1CR: 2L of MC + one dose of colostrum replacer SCCL (100g IgG) all given at birth; 2CR: 2 doses of colostrum replacer (CR) SCCL (200 g IgG) given at birth; 3CR: 2 doses of CR SCCL (200 g IgG) given at birth + one dose of CR SCCL (100 g IgG) given between 6 and 8 h after birth. Calves received starter (24,6% of CP; 5,22% of CF; 13,89% of NDF and 46,57% of NFC as-fed) and water free-choice and were fed 6L/d of milk replacer (22,44% of CP, 16,2% of CF and 14% of solids) until 56d of age. The apparent efficiency absorption (AEA) was calculated using the following equation: AEA IgG (g) = {[serum IgG g/L 24h − serum IgG g/L birth] × birth weight kg × 0.09}/IgG intake (g), where: 0.09 = plasma volume of 9% of BW at birth. Protocols affected Ig intake and AEA ($P < 0.01$), but did not influence serum IgG or total serum protein at 48h ($P > 0.05$). Increasing Ig intake with higher volumes of MC or CR decreased AEA. There were no differences on performance or health among the different colostrum feeding protocols. The ADG (kg) was also not affected colostrum protocols (2MC = 0.29; 4MC = 0.24; 2Mc1CR = 0.25; 2CR = 0.25 and 3CR = 0.21; $P > 0.05$). However, calves from all protocols had low ADG, which can be explained by the milk replacer composition and the history of *Cryptosporidium* infection in the herd. The CR fed in the study may be an alternative to MC. However, while all treatments resulted in excellent levels of passive transfer the potential benefits of increasing the amounts of colostrum (>200 g IgG) with either MC or CR were not perceived in this short-term study.

Key Words: health, IgG, newborn

T90 Performance and diarrhea occurrence of suckling calves supplemented with colostrum replacer.

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The importance of colostrum for the survival of neonatal calves and its consequences on their productive and reproductive life has been known for many years. The objective of this study was to evaluate the effects of a colostrum replacer (CR) supplementation in the first 5 d of life on performance and health of dairy calves. Sixty-six 1-d-old female Holstein calves from one herd were assigned to 1 of 2 groups (n = 33 in each group) and received 4 L of colostrum within 4 h after birth. Blood samples were collected from all calves 24 h after the first colostrum intake and used to ensure adequate immunity (serum IgG >10.0 g/L). Calves assigned to the control group received 6 L of milk 3 times daily without colostrum inclusion. A milk replacer (22% protein: 19% fat) was used to increase to 18% total solids in pasteurized milk for the basal liquid diet. The treatment group received 60 g (20/20/20 g) of supplemental CR powder dissolved in the high solids 6 L of milk from d 1 to 5 of life. From d 6, all calves received the same quantity of milk (18% solids) and ad libitum calf starter. After 30 d calves received 4 L twice a day until weaning with 60 d of life. Calves were evaluated daily for disease symptoms and weighed at 30 and 60 d. BW and ADG were analyzed using a mixed model with repeated weight measures by calf, treatment and their interactions as fixed effects with calf birth weight as a covariable; calf within treatment was considered random effect. There was no interaction treatment × period. Independent of periods, calves CR supplemented were 5.1 kg heavier ($P < 0.01$) and gained 0.08 kg/d more than control (0.86 vs 0.78 kg/d, respectively) at weaning. Even the number of cases of diarrhea almost doubled in non-supplemented calves (13/7), diarrhea occurrence was not significantly different among calves from the treatment and control groups ($P = 0.11$). Diarrhea was negatively correlated with BW at d 30 and d 60 ($P < 0.05$), and ADG at d30 and d60 ($P < 0.03$). Initial serum IgG level had no correlation with any of the studied variables confirming adequate transfer of passive immunity. Calves fed high solids milk supplemented with CR in the first 5 d of life increased BW and ADG before weaning.

Key Words: calf weight, immunoglobulin G, weaning

T91 Effect of feeding transition milk on growth and health of dairy calves.

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Transition milk (TM, defined as milk from the 2nd through 4th milkings after calving) supplies additional fat, protein and immunoglobulins to the calf compared with traditional milk replacer. Our objective was to determine if feeding TM on d 2 through 4 of life increases growth rate and overall health of calves. Starting on d 1 of life, Holstein heifer calves on a commercial farm were fed 1 of 3 diets (n = 35/diet): milk replacer (MR), transition milk (TM), and a 1:1 by weight mix of milk replacer and colostrum replacer (MR+CR, positive control). Transition milk was harvested from Holstein cows on the farm, pooled and pasteurized at 161°F for 15 s. Nutrient composition of TM was 3.79% fat, 6.10% protein, and 14% solids. MR and MR+CR were administered at 14 and 15% solids respectively. Over a 4-mo period from June through September, newborn calves were blocked by age and assigned to 1 of 3 treatments. All calves received colostrum replacer for the first 2 feedings after birth. Subsequently, calves were fed 1.9 L of MR, TM or MR+CR 3 times per day for 3 d. After treatments were complete at 4 d of age, calves were fed and managed similarly. Body weights, blood samples and daily health scores (scale of 0 to 3) were collected through weaning at 56 d of age. All but one calf achieved successful passive transfer of immunity with serum IgG values over 10.0 mg/ml. Daily BW gain for the first 3 wk of life was 0.41, 0.49, and 0.45 kg/d for MR, TM, and MR+CR groups respectively; thus, calves fed TM and MR+CR gained 0.06 kg/d more than those fed MR ($P = 0.06$) with no difference for MR+CR compared with TM. From birth through weaning, calves fed TM and MR+CR calves tended to gain 2.5 kg more total BW than those fed MR (34.3, 33.9, and 31.6 kg, respectively; $P = 0.06$). When comparing MR with TM and MR+CR, treatment did not alter health scores for ears (0.11, 0.14, and 0.12 MR, TM, and MR+CR, respectively; $P = 0.55$), eyes (0.03, 0.007, 0.019; $P = 0.15$), and feces (0.30, 0.37, 0.35; $P = 34$). In conclusion, feeding transition milk for 3 d after first colostrum increased growth rate of calves throughout the preweaning period and produced similar effects on growth and health as milk replacer supplemented with colostrum replacer.

Key Words: transition milk, calf

T92 Prenatal choline supplementation programs the metabolome of the fetus after birth.

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Supplementation of rumen-protected choline (RPC; ReaShure, Balchem Corp., New Hampton, NY) during late-pregnancy in Holstein cows improves development of the offspring's immune system and growth. Here we evaluated if RPC concurrently altered the systemic metabolome. Twenty-four Holstein heifers born to cows fed a basal diet [1.59 Mcal/kg DM, 15.8% CP, 2.9% methionine (% MP) and a lysine to methionine ratio of 2.6] without (control) or with RPC (last 21 d of gestation at a rate of 60 g/d) were used. Immediately after birth whole blood samples were taken and stored at −20°C. Global metabolomics profiling was performed on a Thermo Q-Exactive Oribtrap mass spectrometer with Dionex UHPLC and autosampler. All samples were analyzed in positive and negative heated electrospray ionization with a mass resolution of 35,000 at m/z 200 as separate injections. Separation was achieved on an ACE 18-pfp 100 × 2.1 mm, 2-µm column with mobile phase A as 0.1% formic acid in water and mobile phase B as acetonitrile. This is a polar embedded stationary phase that provides comprehensive coverage, but does have some limitation is the coverage of very polar species. The flow rate was 350 µL/min with a column temperature of 25°C. A total of 7745 molecular features were detected of which 356 peaks with putative identification represent 305 unique metabolites, including amino acids, benzoic acids, lipid molecules, carbohydrates, purines, pyrimidines, vitamins, and other intermediate and secondary metabolites. Statistical analysis was performed using the Mixed procedure of SAS for molecular features with putative identification. There were 12 unique metabolites altered by RPC ($P < 0.05$). In particular glycodeoxycholic acid, lactate, 5′deoxyadenosine, and L-arginine were reduced by RPC, whereas cytidine, α-linolenic acid, cytosine, 1-aminocyclopropane-1-carboxylate, glutathione disulfide, pipecolate, threonine/homoserine, and L-proline increased by RPC. Further data analyses are needed to unravel the specific consequences of these changes. Overall, maternal supplementation with RPC during late-gestation changes the offspring's systemic metabolome, which is likely to be involved in the positive effect of prenatal RPC supplementation on neonatal heifer growth and immune responses.

Key Words: choline, calf, metabolome

T93 Plasma concentrations of biotin, folic acid, and vitamin B_{12} of calves fed with colostrum from cows receiving those vitamins before calving.

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In mature ruminants, B vitamins are produced by rumen microbes but the newborn calf relies on placental and colostral transfer of the vitamins to cover its requirements. The study was conducted to evaluate biotin (B8), folates (B9) and vitamin B_{12} (B12) concentrations in colostrum of cows receiving vitamin supplements and in plasma of their calves fed with their colostrum. Dietary B8 supplement (0 or 20 mg/d) and dietary B9 supplement (0 or 2.6 g/d) + weekly intramuscular injections of B12 (0 or 10 mg) were used in a 2 × 2 factorial arrangement. Cows (n = 34) received one of the 4 treatments from −26 (SD 8) to 0 relative to the parturition. Cows were blood sampled once the week before calving. Colostrum was harvested at first milking from all cows. Calves were fed 2.4 (SE 0.3) kg of their dam colostrum with a nipple bottle within 3 (SD 2) h of birth. Any calf born as twin or not receiving their dam colostrum were excluded; 23 calves were blood sampled and weighed 25 (SD 4) h after birth (B8-B9B12- n = 4; B8+B9B12- n = 7; B8-B9B12+ n = 6; B8+B9B12+ n = 6). Data (log-transformed when needed) were analyzed using Proc MIXED of SAS. In dams, supplementary B vitamins increased their respective plasma concentrations ($P \leq 0.01$). Colostral and calf plasma IgG concentrations did not differ among treatments ($P \geq 0.14$). The B8 supplement increased colostrum B8 from 35 to 298 ± 23 ng/mL and calf plasma B8 from 1.0 (95% confidence interval (CI):0.8–1.2) to 8.1 (CI:6.7–9.7) ng/mL ($P < 0.01$). Supplementary B9B12 increased colostrum B9, from 673 to 1,094 ± 52 ng/mL, colostrum B12, from 29 to 58 ± 3 ng/mL, calf plasma B9 from 16 to 30 ± 2 ng/mL ($P < 0.01$) and tended to increase ($P = 0.09$) calf plasma B12 from 0.8 (CI:0.5–1.1) to 1.2 (CI:0.9–1.7) ng/mL ($P < 0.01$). Calves born from dams receiving the B9B12 supplement were heavier (50 vs. 44 ± 1 kg; $P < 0.01$); B8 supplement did not affect calf weight ($P \geq 0.6$). Further studies are needed to evaluate if the increase in calf birth weight when a B9B12 supplement was given to cows in late gestation could be due to an epigenetic effect as reported for other species.

Key Words: biotin, folic acid, cobalamin

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T94 Effect of fatty acid profile shifts on bovine primary hepatocyte gluconeogenic and oxidative gene expression.

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During the peripartum period, dairy cows diagnosed with hyperketonemia experience both an increase in circulating FA, and a change in circulating FA profile, compared with non-hyperketonemic cows. Supplementation of dietary lipids can also result in subtle shifts in the circulating FA profile. As FA are known regulators of hepatic genes, these shifts could differentially influence gene expression. The objective was to determine the expression of gluconeogenic and oxidative genes in primary hepatocytes when exposed to an in vivo relevant FA profile and that profile enriched with additional C16:0, C18:0, or C18:1. Primary hepatocytes were isolated from 4 Holstein bull calves (<7 d) and cultured for 24 h. Treatments applied to cells for 24 h were no FA (1% BSA); 0.75 mM FA cocktail (3% C14:0, 27% C16:0, 23% C18:0, 31% C18:1, 8% C18:2, and 8% C18:3; to mimic the serum FA profile of dairy cattle at calving); 0.90 mM FA cocktail; 0.75 mM FA cocktail + 0.15 mM C16:0; 0.75 mM FA cocktail + 0.15 mM C18:0; and 0.75 FA cocktail mM + 0.15 mM C18:1. After harvest in TRIzol, samples were stored at −80°C until RNA extraction, purification, reverse transcription, and quantitative real time PCR. Expression of genes of interest (carnitine palmitoyltransferase 1A, pyruvate carboxylase, cytosolic and mitochondrial phosphoenolpyruvate carboxykinase [*PEPCKc* and *PEPCKm*], and glucose-6-phosphatase) was calculated relative to the geometric mean of 2 reference genes chosen by geNorm (ribosomal protein L32 and GAPDH). Data were analyzed using Proc Mixed (SAS 9.4) with the fixed effect of treatment and calf in the random statement. The addition of FA compared with no FA increased the expression of carnitine palmitoyl transferase 1A (2.22 vs. 3.96 ± 1.59 arbitrary units [AU]; $P = 0.05$) and *PEPCKc* (0.51 vs. 1.03 ± 0.28 AU; $P = 0.03$). Enrichment with individual FA did not affect the expression of the genes tested when compared with the 0.90 mM FA cocktail treatment ($P \geq 0.40$). These results suggest additional shifts in circulating FA profile within a biological range have minimal additional effects on hepatic gluconeogenic and oxidative gene expression.

Key Words: oxidation, pyruvate carboxylase, liver

T95 Coordinated responses of hepatic lipid-associated proteins in cows with high or low liver lipid content peripartum.

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Lipid associated proteins may allow for dynamic storage or utilization of liver triglyceride (lvTG). The objective of this study was to examine the coordinated response of liver lipases during the transition to lactation. Multiparous cows, representing a subset of a larger nutritional study (n = 40), were retrospectively grouped by maximum lvTG into a high (>15% DM; n = 6) or low (<15% DM; n = 10) group with no effect ($P = 1.0$) of original dietary treatment. Liver biopsies were collected at −28, −14, +1, +14, and +28 DRTC and LvTG was quantified. Protein abundance of abhydrolase domain containing 5 (ABHD5), hormone sensitive lipase (HSL), perilipin 1 (PLIN), patatin-like phospholipase domain containing 2 and 3 (PNPLA2, PNPLA3) was determined by Western blot analysis. Data were transformed as $\log_{10}$(abundance + 1), to achieve normal residuals. Data were analyzed for main effects of lvTG group, DRTC, and lvTG group x DRTC, and random effect of cow(lvTG group), dietary treatment, and repeated measures within cow using PROC GLIMMIX (SAS 9.4). Main effects were considered significant at $P < 0.05$ or marginal at $P < 0.1$. Means were separated by Tukey's adjustment when interactions were $P < 0.05$. Abundance of ABHD5 tended to be greater ($P = 0.09$) at +28 compared with +1 DRTC and PNPLA2 was greater ($P < 0.03$) at +28 compared with −14 DRTC. An interaction of lvTG group × DRTC was detected ($P < 0.05$) for HSL and PLIN, but means could not be separated by Tukey's post hoc analysis. There was an interaction ($P < 0.01$) of lvTG group × DRTC on the proportion of phosphorylated PLIN, relative to total PLIN (%PPLIN), with reduced ($P < 0.03$) abundance at +14 and +28 compared with prepartum time points in high lvTG cows. Additionally, %PPLIN tended to be greater ($P = 0.07$) in low lvTG cows, compared with high lvTG cows, at +28 DRTC. These data indicate that some hepatic proteins, namely ABHD5 and PNPLA2, are increased postpartum regardless of lvTG group while abundance of PLIN and HSL may reflect lvTG content. Shifts in the %PPLIN coincide with changes in lvTG and should be further examined for potential to mediate remobilization of stored lvTG.

Key Words: lipolysis, transition cow, fatty liver

T96 Actions of recombinant bovine somatotropin revisited: Characterization of the plasma metabolome and lipidome.

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Recombinant bovine somatotropin (rbST) restores homeorhetic mechanisms to support lactation; however, the effects of rbST on the bovine metabolome and lipidome are undefined. Therefore, 8 multiparous lactating Holstein cows (195 ± 34 DIM) were enrolled in a 2 × 2 replicated Latin square design with 14 d periods. Cows received a single injection of rbST (Posilac; Elanco Animal Health, Indianapolis, IN; 0.062 mg/kg BW) or no injection (CON) at period start. On d 8, 9 and 10, an epinephrine challenge (EC; 1.6 µg/kg BW), insulin tolerance test (ITT; 0.1 IU/kg BW), and liver biopsy were performed, respectively. Plasma glucose and total fatty acids (FA) were measured. Plasma was also processed for metabolomics or lipidomics using mass spec. Univariate and multivariate ANOVA were performed. Omic data were generalized log-transformed. Somatotropin elevated milk yields compared with CON ($P < 0.01$) despite lower DMI ($P < 0.05$). Milk fat yield and percent, and milk protein and lactose yields were higher in rbST cows ($P < 0.01$). Plasma total FA levels were increased with rbST (peaking at d 8; $P < 0.01$). At the start of the EC, rbST cows had 75% higher circulating total FA compared with CON (478 vs 119 µmol/L; $P < 0.01$) which increased post epinephrine (866 vs. 218 µmol/L for rbST and CON by 15 min, respectively; $P < 0.01$). Epinephrine responsiveness (change from baseline), and insulin-stimulated plasma glucose clearance and reductions in total FA were not modified by treatment. Plasma levels of oxoproline, glutamic acid, threonine, and fatty acids and their derivatives (e.g., linoleic acid, arachidonic acid, ethyl myristate, and eicosatrienoic acid) were higher in rbST cows ($P < 0.05$). The plasma lipidome was dynamically modified with treatment (218 out of 385 features; $P < 0.05$). Treatment with rbST decreased plasma mono-, di-, and triacylglycerols levels (e.g., MG-18:0, DG-18:0/16:0, and TG-16:0/14:0/16:1; $P < 0.05$). Similarly, many phosphatidylcholines and sphingomyelins were significantly lower in rbST cows by d 8 ($P < 0.05$). We conclude that rbST modifies the bovine plasma metabolome and lipidome with increased milk production.

Key Words: metabolome, lipidome, somatotropin

T97 Body condition score in late pregnancy is associated with abundance of hepatic microRNA involved in energy metabolism.

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The main objective was to evaluate associations between body condition score (BCS) in late-pregnancy, plasma biomarkers, and hepatic abundance of microRNA linked to energy metabolism during in silico and in vivo analyses. At 4 weeks before calving, 26 cows were divided into 2 groups based on BCS, BCS ≥3.50 (n = 13; HiBCS) and BCS ≤ 3.25 (n = 13; LoBCS). Dry matter intake (DMI) from −4 wk of pregnancy through 30 d in milk (DIM) and milk production during the first 30 DIM were recorded. Blood was sampled at −30, −10, 7, 15, and 30 DIM. Liver biopsies were performed at −15, 7, and 30 DIM for analysis of miR-369 5p, miR-186, and miR-200b abundance via RT-PCR after normalization with 3 internal controls. Pathway analysis using the dynamic impact approach revealed these miRNAs play a role in relation to energy metabolism by means of fatty acid metabolism, oxidative phosphorylation, gluconeogenesis, PPAR signaling, and insulin signaling. Data were subjected to repeated measures ANOVA in SAS using PROC MIXED. Main effects were BCS, time, and their interaction, while cow was the random effect. Although daily DMI did not differ prepartum, HiBCS cows averaged 1.54 kg/d ($P \leq 0.05$) more DMI/d postpartum. Overall milk production was 5.34 kg/d greater ($P = 0.02$) in HiBCS compared with LoBCS. Cows in HiBCS also had greater ($P = 0.03$) overall concentrations of fatty acids, myeloperoxidase ($P = 0.03$), and β-hydroxybutyrate (BHB) ($P = 0.06$) compared with LoBCS. In contrast, LoBCS had greater plasma concentrations of alkaline phosphatase ($P \leq 0.01$), tocopherol ($P = 0.03$), and carotene ($P = 0.03$). miR-186 was the most abundant of the target miRNA evaluated, but had no detectable changes in relation to BCS or time. Abundance of miR-369 5p was lower ($P = 0.01$) overall in cows with HiBCS. In contrast, abundance of miR-200b had a BCS × time effect ($P = 0.03$) due to a marked upregulation between −15 and 7 d followed by increased abundance at 30 d in cows with LoBCS. Overall, the responses in miR-369 5p and miR-200b underscore a potentially important physiological role during the periparturient period as it relates to BCS.

Key Words: BCS, microRNA (miRNA), transition period

T98 Effects of butyrate supplementation on blood glucagon-like peptide-2 concentration and gastrointestinal function in lactating dairy cows fed diets differing in starch content.

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The objective of this study was to evaluate effects of butyrate supplementation on blood glucagon-like peptide-2 (GLP-2) concentration, nutrient digestibility and responses to SARA challenge of cows fed diets differing in starch content. Eight Holstein cows were blocked by parity and assigned to one of 4 × 4 Latin squares balanced for carryover effects with a 2 × 2 factorial arrangement of treatments. Treatments were dietary starch content [low starch = 20% (LS) vs. high starch = 29% (HS)] and butyrate supplementation (butyrate vs. control) with 21-d periods. Butyrate was provided as Gustor BP70 WS (NOREL, S.A., Madrid, Spain), containing 70% sodium butyrate and 30% fatty acid mixture, at 2% of dietary DM, and control premix contained 70% wheat bran and 30% fatty acid mixture. Feeds, orts, and fecal samples were collected from d 17 to 19 to determine apparent total-tract nutrient digestibility. Blood samples were collected on d 19. Cows were feed-restricted at 60% of daily intake on d 20, and SARA challenge was conducted by providing 0.6%BW of steam flake corn grain in addition to each treatment diet on d 21, and blood and ruminal fluid samples were collected. Data were analyzed using Fit model procedure of JMP. The model included fixed effects of dietary starch content, butyrate supplement, their interaction, period, and square, and random effects of cow nested in squares. Cows fed butyrate increased serum BHB concentration ($P < 0.01$), tended to increase plasma GLP-2 concentration ($P = 0.06$), and increased DM digestibility ($P < 0.05$) compared with control. During SARA challenge, rumen endotoxin concentration was higher for cows fed HS + butyrate compared with cows fed the other diets ($P < 0.05$), but cows fed butyrate tended to decrease plasma/rumen endotoxin ratio than control ($P = 0.08$). Serum haptoglobin concentration was not affected by treatment. These results indicate that butyrate supplementation may increase plasma GLP-2 concentration and total-tract DM digestibility, and keep plasma endotoxin concentration low relative to its ruminal concentration.

Key Words: butyrate, glucagon-like peptide-2 (GLP-2), gut function

T99 Characterization of metabolic and oxidative status in Italian Mediterranean water buffalos during the peripartum period.

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During the transition from pregnancy to lactation, markers of oxidative stress in blood commonly increase and peak in the first weeks of lactation in dairy cows. Our objective was to characterize the oxidative status in water buffalos, in context with indicators for metabolic stress during that phase. Blood samples were collected weekly from 10 buffalo cows (lactation number 4.6 ± 1.6; daily milk yield 9.0 ± 1.9 kg; means ± SD) from 6 weeks (wk) ante partum (ap) until 8 wk postpartum (pp). Beside nonesterified fatty acids (NEFA) and β-hydroxybutyrate (BHB), the following variables were determined in serum using photometric assays: derivatives of reactive oxygen metabolites (dROM), ferric reducing ability (FRAP), thiobarbituric acid reactive substances (*TBARS*), and advanced oxidation protein products (AOPP). Linear mixed models with time (wk) as repeated effect considering the nested periods ap and pp and cow as random effect were used to evaluate the time courses using SPSS. The dROM values declined with time ($P = 0.02$) from peak values in wk 2 and 1 ap toward lowest values from wk 3 to 8 pp. FRAP was not affected by time. The oxidative stress index (OSi), i.e., the calculated ratio (dROM/FRAP), also decreased with time showing greater values ap than pp ($P = 0.001$). The TBARS values did not change with time, whereas AOPP concentrations were greater ap than pp (<0.001); BHB concentrations were not affected by time. Greater NEFA values were observed ap than pp ($P < 0.001$). Both BHB and NEFA values remained below the thresholds applied for dairy cows to define subclinical or clinical ketosis, thus indicating that the buffaloes studied herein were not under particular metabolic stress. The greater

OSi values before calving resulted from increased concentrations of pro-oxidants rather than from decreased anti-oxidants. The profiles of indicators for oxidative status reported in the literature for dairy cows indicate that oxidative stress occurs in early lactation; in contrast, the most oxidative stress in water buffaloes was observed in late pregnancy rather than lactation in our study.

Key Words: oxidative stress, water buffalo, transition period

T100 Gene expression of hepatic lipid metabolism in primiparous dairy cows with different feeding strategies during early lactation.

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The study objective was to evaluate the effect of grazing in early lactation on changes in triglyceride content and expression of genes related to lipid metabolism in the liver of dairy cows. Primiparous Holstein cows (n = 18, 528 ± 40 kg BW and 3.2 ± 0.2 BCS; fall calving) were used in a randomized block design and assigned, at calving, to 2 nutritional treatments during the first 65 d postpartum (DPP) of lactation: [i] TMR ad libitum (58% forage, 42% concentrate; TMR) or [ii] grazing of *Medicago sativa* (6-h am grazing in 3-d strips; pasture allowance = 20 kgDM/d) plus TMR (70% of ad libitum TMR; PAS). All cows consumed at each milking, 2.0 kgDM/d of a grain-soybean meal concentrate. Plasma and liver biopsies were collected pre and postpartum (−7 and +42 DPP) to measure plasma NEFA concentrations and hepatic triglyceride (TGA) content and mRNA abundance of genes related to lipid metabolism (SYBR-Green real time RT-PCR). Data were analyzed as repeated measures with a mixed model that included DPP and treatment within DPP as fixed effects. Milk energy output was greater ($P = 0.04$) for TMR than PAS cows (21.6 vs. 20.0 ± 0.41 Mcal/d) while cow BW did not differ between nutritional treatments. Expression of *ACADVL* and *PPARG* mRNA were not affected by nutritional treatments. However, *CPT1A* mRNA was greater in TMR than PAS cows (2.63 vs.1.99 ± 0.2; $P = 0.04$), consistent with the greater plasma NEFA concentration in former cows during postpartum (0.63 vs. 0.42 ± 0.03 mmol/L; $P < 0.01$). In addition, although liver TAG content did not differ between TMR and PAS cows (14 vs. 11 ± 2%), hepatic TAG and plasma NEFA concentrations were negatively correlated only in TMR cows ($r = -095$, $P = 0.01$). Results indicated that TMR cows adapted their hepatic metabolism to increase fatty acid oxidation parallel to the increased energy demands of their greater milk production.

Key Words: lipid metabolism, hepatic gene expression, grazing

T101 Rumen-protected choline acts directly in the liver regulating expression of genes involved in reduction of fatty liver in dairy cattle.

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Supplementation of rumen-protected choline (RPC; ReaShure, Balchem Corp., New Hampton, NY) reduced liver fat accumulation in feed restricted pregnant, nonlactating dairy cows. In these same cows, we evaluated if RPC concurrently altered hepatic gene expression. Pregnant, nonlactating multiparous Holstein cows (n = 77) were enrolled at 64 ± 10 d before expected calving date. Dietary treatments were 0, 30, 60, 90, and 120 g/d of ReaShure. Experimental periods were 14 d with 5 d of ad libitum intake (AL), and 9 d of feed restriction to consume 31% of caloric requirement (FR) to induce negative nutrient balance. Methionine was supplemented during the FR period to maintain the same daily intake of the AL period. Liver tissue was collected on d 5 and 14. Transcript abundance was determined by RT-qPCR using the Fluidigm assay for 93 target genes involved in assembly and secretion of VLDL, CDP-pathway, gluconeogenesis, inflammation, oxidative stress, transcription factors, metabolism of lipid, lipoprotein, and phosphatidylcholine; and 3 reference genes. The ΔCt values were calculated relative to the geometric mean of the reference genes and used for statistical analyses using the Mixed procedure of SAS. Birth weight of the calves and number of days prepartum at enrollment were used as covariates. Expression of genes was affected ($P < 0.05$) by RPC during both periods. After cows developed fatty liver (d14) there were 20 differentially expressed genes (DEG), whereas there wer 13 DEG on d5. Changes in expression of genes due to RPC suggest increase in phosphatidylcholine metabolism, and reduction of TAG synthesis, among others. In conclusion, RPC regulates expression of genes in the liver, more extensively under fatty liver conditions to reduce TAG contents. Results demonstrate that regulation of hepatic gene expression is one mechanism by which RPC supplementation leads to the optimal performance of transition cows.

Key Words: choline, fatty liver, gene expression

T102 Physiologic responses to induced subclinical hypocalcemia in different lactational stages of Holstein dairy cows.

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Poor regulation and maintenance of circulating calcium is of concern in dairy cows, particularly during the peri-parturient period as cows adapt to increased demands of colostrum and milk production. When regulation of circulating calcium fails, hypocalcemia ensues, with repercussions of this disease negatively affecting cow health throughout early lactation. The objective of this study was to characterize physiologic responses of a subclinical hypocalcemic challenge in lactating and nonlactating dairy cows. Using a randomized complete block design, 12 dry, non-pregnant multiparous Holstein cows and 12 early lactation (5–20 DIM) multiparous Holstein cows received either (1) a continuous 24-h intravenous solution of 0.9% saline or (2) 5% ethylene glycol tetraacetic acid (EGTA) in 0.9% saline (n = 6 lactating, n = 6 nonlactating/treatment) with the aim of maintaining blood ionized calcium (iCa) less than 1.0 mM. Blood samples were collected immediately before infusion, hourly during infusion, and 4, 8, 12, 24, 48, and 72 h post-infusion to monitor blood iCa concentrations. Groups were compared using a mixed model ANOVA with time included as a repeated measure. Infusion of EGTA effectively decreased circulating iCa concentrations in both lactating and dry, non-pregnant cows compared with saline infusion (0.90 ± 0.01 mM, 0.84 ± 0.01 mM vs 1.25 ± 0.01 mM, 1.23 ± 0.01 mM; $P < 0.0001$). Lactating-EGTA infused cows had higher iCa during the infusion period compared with dry, non-pregnant EGTA cows ($P = 0.003$). Lactating-EGTA cows had higher iCa concentrations than all other treatments (1.36 ± 0.026 mM; $P < 0.05$) 24 h post-infusion. Additionally, dry, non-pregnant, EGTA cows required less EGTA to maintain subclinical hypocalcemia compared with lactating EGTA cows ($P < 0.0001$). This data suggests that homeostatic response to perturbation of calcium metabolism differs among early lactation and dry non-pregnant cows. Additionally, this demonstrates the early lactation dairy cow is capable of adapting to calcium challenges more rapidly and has improved feedback mechanisms to maintain circulating calcium concentrations than a dry, non-pregnant cow.

Key Words: calcium, hypocalcemia

T103 Hepatic pyruvate carboxylase expression at parturition differed in cows that subsequently developed hyperketonemia.

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Hepatic gene expression during the transition period is dynamic and responsive to changes in hormones, nutrients, and fatty acids (FA). Concentration and profile of FA differs postpartum between cows diagnosed with hyperketonemia (HYK) or not (nonHYK). The objectives were to determine hepatic gene expression and the relationship between FA profile and gene expression in HYK and nonHYK cows. Cows were enrolled 28 d before calving. Plasma samples were collected at −3, 1, 3, 7, 9, 11 and 14 d and liver biopsies at 1, 14 and 28 d. Postpartum plasma samples were analyzed for BHB and nonesterified FA. Fatty acid profile (−3, 1, 14 d) was determined via acid methylation and GC. Cows were classified as HYK (BHB $\geq$ 1.2 mM postpartum; average onset ± SD, 9 ± 5 d; n = 13) or nonHYK (BHB < 1.2mM; n = 15). Liver was analyzed for FA profile and expression of pyruvate carboxylase (*PC*) and cytosolic phosphoenolpyruvate carboxykinase (*PEPCKc*) quantified by real time PCR, analyzed by standard curve method, and normalized to ribosomal protein L32 (6 reference genes explored with geNORM). Data were analyzed in SAS 9.4 using PROC MIXED containing the fixed effects of time [repeated cow(HYK)], HYK, and time × HYK. Time × HYK affected *PC* ($P = 0.01$) with HYK cows having decreased *PC* on d1 (0.94 vs. 0.33 ± 0.08 arbitrary units [AU]; $P < 0.01$) but not d14 (0.18 vs. 0.13 ± 0.08 AU; $P = 0.74$) or 28 (0.28 vs. 0.08 ± 0.08 AU; $P = 0.12$). Abundance of *PEPCKc* was not affected by HYK ($P = 0.28$) or time × HYK ($P = 0.87$). This resulted in decreased *PC:PEPCKc* on d 1 ($P = 0.01$), but not d 14 or 28 ($P > 0.10$), suggesting decreased complete oxidative capacity at parturition in cows that later develop HYK. The decreased *PC* in HYK cows at d 1 was not correlated with differences in nonesterified FA concentration or FA profile ($P > 0.10$). These data support that there are differences in PC:PEPCKc at calving in cows with subsequent HYK; however, shifts in FA profile do not appear to be related to downregulation of PC at d 1 in cows that subsequently develop HYK. Ultimately, the decreased PC:PEPCKc at parturition may contribute to onset of HYK and the regulatory mechanism should be further examined.

Key Words: liver, transition cow, ketosis

T104 Using path analysis to explore short-term herbage intake of dairy cows under grazing temperate pasture.

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Under grazing conditions, daily intake can be estimated as the product of intake rate and grazing time but interactions with cow's pre-grazing energy state may modify this relationship. A path analysis approach was used to explore relationships between ingestive behavior and cow's pre-grazing metabolic variables affecting voluntary herbage intake of the first grazing bout in a morning session (VHI) in grazing dairy cows. Data of short-term ingestive behavior and pre-grazing metabolic variables was collected of 18 dairy cows (9 multiparous and 9 primiparous, days in milk = 73 ± 7; BW = 521 ± 32 kg BW; BCS = 2.75 ± 25; milk yield = 26 ± 3 kg) that grazed vegetative oat pasture (8 h of access to pasture from 8:30 to 16:30 h; pasture allowance = 30 kg DM/day; DM = 14%, CP = 23%, NDF = 46%, dry basis) and received supplementation after pm milking (6 kg DM/day of TMR of 70:30 forage to concentrate ratio, as-fed basis) from a short grazing experiment (20 d). Data were analyzed in SAS using the CALIS procedure. The direct and indirect relationships between bite rate (BR) and length of the first grazing bout (LFG, min), incisor arcade breadth (AB, cm) and pre-grazing serum glucose, insulin, glucagon, NEFA and BHB concentrations, with VHI (expressed as DM per 100 kg of metabolic weight) were estimated. The path analysis showed that direct effect of LFG was higher than AB (0.84 *vs* 0.34, $P < 0.05$), and BR did not correlate (0.026, $P > 0.05$) with VHI. In addition, pre-grazing NEFA and BHB concentrations correlates negative and positively to VHI (−0.56, $P < 0.005$; 0.71, $P < 0.005$), respectively, as indirect effects, and, pre-grazing NEFA and BHB concentrations correlates negative and positively (−0.74, $P < 0.005$; 0.71, $P < 0.005$), respectively, with LFG, as direct effects. The AB also correlates negatively to LFG but with lower standardized path correlation coefficient (−0.32, $P < 0.05$) than pre-grazing NEFA and BHB concentrations. Results predicted that the duration of the first grazing bout and pre-grazing NEFA and BHB concentrations are the main explanatory variables that affect the short-term herbage intake of dairy cows under temperate pastures.

Key Words: grazing bout, metabolic variable, Holstein cow

T105 Intravenous lipopolysaccharide infusion modifies the bovine metabolome and lipidome.

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Endotoxemia is a feature of steatohepatitis and sepsis. To define bovine metabolism in response to lipopolysaccharide (LPS) during a fatty acid (FA) insult, 10 multiparous Holstein mid-lactation cows were treated with a single i.v. bolus of saline (control; n = 5) or endotoxin (LPS *E. coli* O55:B5 at 0.375 µg/kg of BW; n = 5). Immediately post saline or LPS administration, all cows were i.v. infused a triglyceride (TG) emulsion (Intralipid 20% at 200 mL/h; Frasenius Kabi) for 16 h while fasted. Plasma was collected at h 0, 4, 8, 12, and 16, relative to bolus administration, start of TG infusion, and fasting. Liver was biopsied before (d −5) and after (h 16) these conditions. Plasma and liver metabolites were extracted for untargeted metabolomics or lipidomics using mass spec. Data were generalized log-transformed, auto-scaled, and analyzed using multivariate analyses. For metabolomics, metabolite identification was based on a mzCloud mass spectral score >80%. Mass spec detected 511 compounds. Although plasma FA (e.g., 18:2) levels increased with time ($P < 0.05$), only 50 of 122 hepatic TG (e.g., TG 16:0/18:2/18:2) increased by h 16 ($P < 0.05$). Whereas 25 of 122 hepatic TG decreased ($P < 0.05$). In liver, TG infusion with fasting increased 7 of 8 ceramides ($P < 0.05$). Plasma salicylic and 2-hydroxyhippuric acids, and leucine, tyrosine, and threonine levels decreased by h 16 of FA insult ($P < 0.05$). Pronounced reductions in plasma lysophosphatidylcholine (LPC) levels developed post LPS (17 of 18; e.g., LPC-16:0; $P < 0.05$). Similar reductions in plasma LPC:phosphatidylcholine ratios were observed ($P < 0.05$). Endotoxin increased plasma pyruvic and lactic acids, and microbial-derived phenylacetylglutamine ($P < 0.05$). Also, LPS decreased plasma citric and 5-aminovaleric acids, and leucine and ornithine levels ($P < 0.05$). In non-ruminants, LPC is an immune modulator and counteracts endotoxin-induced inflammation. Additionally, endotoxin promotes aerobic glycolysis and intestinal permeability, and inhibits intestinal leucine uptake. We conclude that LPS modifies the metabolome and lipidome of the lactating cow experiencing elevated circulating FA.

Key Words: dairy cow, endotoxin, metabolome

T106 Lipopolysaccharide induces lipolysis and reduces insulin sensitivity in subcutaneous adipose tissue from transition dairy cows.

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Adipose tissue (AT) inflammation and excessive lipolysis predispose transition cows to metabolic disorders. In human and rodent AT, lipopolysaccharide (LPS) has been shown to trigger inflammatory responses and lipolysis and reduce insulin sensitivity (IS). The effect of LPS on lipolysis and IS in AT of dairy cows during the transition period has not been determined. We hypothesized that LPS triggers lipolysis and reduces IS in AT of transition dairy cows. Subcutaneous AT (SCAT) explants were collected from 12 Holstein dairy cows at −14 d prepartum and +6 d and +12 d after calving. Explants were incubated in the presence of LPS (CON = 0 µg/mL medium or LPS = 20 µg/mL medium). The effect of LPS on stimulated lipolysis was determined using isoproterenol (ISO = 1uM) and LPS plus isoproterenol (LPSISO) The impact of LPS on the anti-lipolytic responses induced by insulin at high (1µL/L, LPS-IH) and low (0.2µL/L, LPS-IL) concentrations was determined by comparing it to the effect of insulin on lipolysis during ISO stimulation (ISO-IH; ISO-IL). Lipolysis was determined by quantification of glycerol release. Statistical analyses were performed using a mixed effect linear model. Compared with CON, LPS increased glycerol release from SCAT by 73 ± 18% across all time points ($P < 0.001$) and tended ($P = 0.09$) to be affected by time relative to parturition with higher release of glycerol at −14 d (87 ± 2%) compared with +6 d (70 ± 2%) and +12 d (63 ± 2%). LPSISO increased the lipolytic response by 40 ± 17% compared with ISO ($P < 0.05$) and 255 ± 37% compared with CON ($P < 0.001$). Compared with ISO-IH, LPS-IH reduced the antilipolytic effect of insulin by 9 ± 2% ($P < 0.05$). No differences were observed between ISO-IL and LPS-IL. Our results demonstrate that LPS reduces IS and triggers lipolysis in SCAT. LPS also potentiates SCAT lipolytic response to adrenergic agonists. Collectively our results suggest that in diseases where plasma levels of LPS are increased, the lipolytic response of AT may be exacerbated through activation of lipolytic pathways and inhibition of the anti-lipolityc effects of insulin by LPS.

Key Words: LPS, lipolysis, adipose tissue

T107 Effects of rumen-protected methionine fed to lactating Holstein cows during a heat stress challenge on mammary mechanistic target of rapamycin (mTOR) signaling. D. N. Coleman*[1], M. Vailati-Riboni[1], R. T. Pate[1], D. Luchini[2], F. C. Cardoso[1], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Adisseo, Alpharetta, GA.*

The objective was to investigate the effects of supplementing rumen-protected methionine (RPM) during a heat stress (HS) challenge on abundance and phosphorylation of mTOR (mechanistic target of rapamycin)-related signaling proteins in the mammary gland. Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were housed in tie stalls and randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc.; 0.105% DM as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases per period. In phase 1 (9d), all cows were in thermoneutral conditions (TN) and fed ad libitum. During phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets while group 2 (n = 16) remained in TN but were pair-fed to HS counterparts to control for DMI decreases associated with HS. After a washout period (21d), the study was repeated (period 2). Environmental treatments were inverted in period 2 (sequence), while dietary treatments remained the same. Mammary tissue was harvested via biopsy at the end of both periods and a subset of cows (12/treatment) were used for protein analysis. Data were analyzed using PROC MIXED in SAS with the effects of diet environment and their interaction, and period and sequence to account for the crossover design. Compared with TN cows, HS cows had greater vaginal temperatures ($P < 0.001$) and respiration rates ($P < 0.001$). No significant environment by diet interactions or sequence effects ($P > 0.10$) were observed for the proteins measured. The abundance of phosphorylated mTOR (p-mTOR) was greater with RPM supplementation ($P = 0.04$) and tended to be greater with HS ($P = 0.08$). No differences were observed in the abundance of AKT or phosphorylated AKT ($P > 0.10$). Additionally, CON cows had a greater decrease in milk protein (%) during phase 2 (difference from phase 1) compared with RPM cows ($P = 0.04$). Overall, preliminary evaluation suggests that RPM supplementation during a HS challenge could alter mTOR activation which may support greater milk protein synthesis.

Key Words: amino acid, lactation, mammary gland

T108 Innate immune response of late-lactation dairy cows is affected to a greater extent by heat stress than rumen-protected methionine. M. Vailati-Riboni*[1], D. Coleman[1], R. T. Pate[1], D. Luchini[2], F. C. Cardoso[1], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Adisseo, Alpharetta, GA.*

Heat stress (HS) has been shown to reduce immune functions, however, feeding rumen-protected methionine (RPM), a known immunostimulant, may mitigate its effects. Thirty-two multiparous, lactating Holstein cows (DIM 184 ± 59) were randomly assigned to 1 of 2 environmental groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc.); 0.105% DM of TMR as top dress) or without (CON)] in a crossover design, with 2 periods and 2 phases per period. In phase 1 (9d), cows were in thermoneutral conditions (TN; 16.0 ± 2.5°C, 71.4 ± 7.5% humidity, THI = 60 ± 3) and fed ad libitum. In phase 2 (9d, 16.8 ± 2.5°C, 66.8 ± 8.0% humidity, THI = 61 ± 4), group 1 (n = 16) was exposed to HS using electric heat blankets (THI = 89 ± 3). Group 2 (n = 16) remained in TN but was pair-fed to HS counterparts. After a 21d washout period, the study was repeated (period 2). Environmental, but not dietary treatments were inverted (sequence effect). Blood was sampled 6d into period 2 and incubated with PI-labeled *E. coli*. Cells were stained to segregate neutrophils (CH138A) and monocytes (CD14) via flowcytometry. DHR was used as indicator of oxidative burst. Data were analyzed using PROC MIXED in SAS, with diet, environment, and their interaction as main effect, as well as period and sequence to account for the crossover design. Cow was used as random effect. HS increased ($P < 0.001$) rectal temperature and respiration rate (+0.3°C, +13.7 breaths/min) compared with CON. RPM did not affect ($P > 0.05$) functionality of both cell type. HS, instead, decreased neutrophil ($P = 0.01$, 72 vs 64%) and monocyte ($P = 0.001$, 27 vs 19%) oxidative burst and monocyte phagocytosis capacity ($P = 0.05$, 10 vs 8%), while it only tended to decrease ($P = 0.11$, 9 vs 7%) neutrophil phagocytosis. A sequence effect was detected for both monocyte ($P = 0.01$, 19 vs 27%) and neutrophil ($P = 0.03$, 64 vs 73%) oxidative burst, with overall lower response in animals subjected to HS in period 1. Results highlight the negative effect of HS on dairy cow innate immune function, including a probable carry over chronic effect. RPM was ineffective in counteracting the detrimental effect of HS during late-lactation, probably due to the short feeding period before the imposed stress.

Key Words: methionine, heat stress, immune function

T109 Physiological responses to heat stress of dry dairy cows receiving a negative DCAD diet during the entire dry period. T. F. Fabris*, J. Laporta, A. L. Skibiel, B. Dado-Senn, and G. E. Dahl, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

The advance of genetics is continually leading cattle to more efficient utilization of nutrients and improved performance, but also to more metabolic disorders. Environmental changes, i.e., higher ambient temperature and humidity contribute to changes in metabolic requirements. A completely randomized design was used to evaluate the effect of heat stress and cooling while feeding an −11 mEq/kg of dietary cation anion difference (DCAD) diet to characterize thermoregulatory and hematological changes of cows in the dry period (DP). Cows were dried-off ~46 d before parturition and randomly assigned to cooling (shade, fans and soakers; CL) or heat stress (shade; HT). In the DP, vaginal temperature (VT, °C; every 10 min), respiration rate (RR; breaths per min) and temperature humidity index (THI) were recorded to evaluate heat strain. On one day (THI ≥78.8 in CL and HT) blood samples were collected at 0600, 1200, 1800 and 2400 from cows (HT = 27.5 ± 12.8 d dry; CL = 24.3 ± 14.8 d dry; n = 12/group) to assess electrolytes, hematology, and blood gases. The model included the fixed effect of prepartum environment (HT vs. CL) in a completely randomized design with cow as random effect analyzed using repeated measures. Urine pH was recorded weekly and was similar for HT and CL cows ($P = 0.36$). Cows under HT had higher VT and RR ($P < 0.01$), lower blood pH (HT = 7.41 ± 0.003, CL = 7.43 ± 0.003, $P < 0.05$) and had higher iCa at 0600 (HT = 1.30 ± 0.02, CL = 1.25 ± 0.01, $P < 0.05$) relative to CL. There was a time effect, whereby iCa was lower at 1200 but higher at 2400, regardless of treatment ($P < 0.01$). CL cows had higher blood glucose at 1200 and 2400 relative to HT cows (66.6 ± 1.08 vs. 63.4 ± 1.06, $P < 0.05$; 68.3 vs. 64.1 ± 1.08 $P < 0.01$, respectively). There was also a time effect on K concentration, whereby K was lower at 1200 but higher at 0600, regardless of treatment ($P < 0.05$). HT cows had higher PCO2 relative to CL cows ($P < 0.05$). Comparing HT vs CL, no differences were found for Na, Hct, Hgb, HCO_3, pO_2, SO_2 and tCO_2. Thus, we observed different physiological responses of cows receiving the same level of negative DCAD, but under different environmental conditions.

Key Words: electrolytes, hematology

Production, Management, and the Environment 2

T110 Measurement of the carbon footprint in dairy production systems in Chile. P. Toro-Mujica*[1,2], L. Robles[3], and D. Enriquez-Hidalgo[2], [1]*Instituto de Ciencias Agronómicas y Veterinarias, Universidad de O'Higgins, San Fernando, Chile,* [2]*Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile,* [3]*Departamento de Nutrición Animal, Universidad Autónoma del Estado de México, Toluca, México.*

Animal production has been identified as an important source of greenhouse gases emission. The dairy sector contributes to around 2.7% of total anthropogenic emissions. Dairy production systems in Chile are heterogeneous as they are influenced by farm location and structure, markets, environmental conditions, and producers' preferences. Thus, the aim of this study was to estimate the carbon footprint (CF) of 4 dairy farms representative of Chilean dairy production systems using the Life Cycle Assessment approach. IPCC Tier 2 and the cradle-to-farm-gate methodologies were used. Emission sources include enteric fermentation, manure, cropland used in feed production, fuel used to produce feed, electricity, fertilizer, and pesticides. Carbon balance in the soil was not included. The first was an intensive dairy farm where cows remained confined in a compost barn system, with 233 Holstein, Jersey, Montbéliarde crossbreed cows, with an average weight of 600 kg, a production of 14153 kg of FPCM (Fat and Protein Corrected Milk)/cow/yr and average use of supplementary feed of 16.7 kg/cow/d. In this farm, the CF was of 1.5 kg of $CO_{2\text{-eq}}$/kg FPCM. The second farm was a grass-based system, with 579 American Holstein with European Holstein cows with an average weight of 500 kg, a production of 6210 kg of FPCM/cow/yr, and average use of supplementary feed of 7.2 kg/cow/d, presented a CF of 0.6 kg of $CO_{2\text{-eq}}$/kg FPCM. The third farm was a grass-based system with 495 Jersey cows with a weight of 430 kg, with a production of 5836 FPCM/cow/yr, and use of supplementary feed of 5.3 kg/cow/d, registering a CF of 0.7 kg of $CO_{2\text{-eq}}$/kg FPCM. The fourth dairy was a grass-based using a milking robot with 371 Jersey cows, with an average weight of 430 kg, a production per cow of 4731 kg of FPCM/cow/yr, and use of supplementary feed of 3.8 kg/cow/d that result in a CF of 1 kg of $CO_{2\text{-eq}}$/kg FPCM. The differences in emissions between systems were mainly due to the intensity of use of inputs such as supplementary feeding, fertilizer, and fuel. The higher milk productivity obtained on the intensive dairy farm was not enough to balance out the low CF of the grass-based farms evaluated.

Key Words: carbon footprint, dairy, milking

T111 Effect of the supplementation level with concentrate on milk and methane production in crossbreed tropical dairy cows grazing tropical pastures. L. E. Robles-Jimenez[1], A. Xochitemol[2], M. Benaaouda[1], L. Corona[2], E. Castillo[2], O. A. Castelan-Ortega[1], and M. Gonzalez-Ronquillo*[1], [1]*Universidad Autonoma del Estado de Mexico, Toluca, Mexico, Mexico,* [2]*Universidad Nacional Autónoma de México, Ciudad de México, Mexico.*

The objective of this study was to evaluate the level of supplementation with concentrate in dairy cows grazing native pastures in milk production, and estimate methane (CH_4) and nitrous oxide (N_2O) emissions. The study included 12 crossbred F_1 dairy cows (1/2 *Bos taurus* 1/2 *Bos indicus*) over 60 d of lactation. Cattle grazed tropical grasses (*Paspalum* spp., *Axonopus* spp., *Brachiaria* spp. and *Cynodon neumfluensis*) and supplemented with 0, 0.150, 0.300 and 0.450 kg of concentrate (kg DM / kg daily milk production) in 3 periods of 15 d each one in a crossover design (all animals receive all treatments). N_2O emissions from excreta and urine were calculated (IPCC, 2006). The quantity of daily CH_4 production were calculated ($\text{kg}\cdot\text{head}^{-1}\cdot\text{day}^{-1}$) (IPCC, 2006). Data were analyzed with the SAS MIXED procedure and the means were subjected to a trend analysis using orthogonal polynomials. There were no differences ($P > 0.0001$) for live weight and milk yield production between treatments. The supplementation with concentrate in tropical dairy cows did not increase milk yield (Table 1) but increased CH_4 and N_2O ($P < 0.0001$) excretion per cow. The use of concentrates in crossbreed F1 dairy cows fed with native tropical pastures affects negatively the emission of greenhouse gases, producing more $CH_4$and N_2O per cow per day; however, cows supplemented present a higher concentrate intake, which means more gross energy intake and higher DMI, without increase milk yield production.

Key Words: greenhouse gases, methane, crossbreed

Table 1 (Abstr. T111). Dry matter intake (DMI), milk yield production, CH_4 and N_2O production in F1 crossbred dairy cows grazing, supplemented with different levels of concentrate (kg/kg daily milk production) in humid tropics

Variable	0.0	0.15	0.30	0.45	SEM	*P*-value
Live weight ($LW^{0.75}$)	108.5	107.6	109.1	109.9	0.39	0.7403
DMI, kg/d	12.78	12.57	12.35	13.43	0.12	0.2113
GE intake (MJ/d)	212.7^b	217.2^b	219.3^b	251.9^a	2.86	0.0001
Milk yield kg, FCM 3.5%	7.3	8.6	8.8	8.7	0.94	0.1575
CH_4 g/cow/d	390.5	398.7^b	402.5^b	462.5^a	4.09	0.0093
N_2O g/ cow/d	61.1^c	67.4^{bc}	69.3^b	82.6^a	1.23	0.0001

T112 Investigation of supplementing a methionine derivative, *N*-acetyl-L methionine, on lactational performance and economic returns of dairy cows during early to mid-lactation. J.-S. Eun*[1], F. X. Amaro[2], D. Vyas[2], S. H. Lee[1], and J. O. Moon[1], [1]*Institute of Integrated Technology, CJ CheilJedang, Suwon, South Korea,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

The purpose of the current study was to determine if supplementing a Met derivative, *N*-acetyl-L-Met (NALM; CJ CheilJedang, Seoul, South Korea) would affect lactational performance and economic returns of

Holstein dairy cows during early to mid-lactation. Sixty multiparous Holstein dairy cows in early lactation (27 ± 4.3 d-in-milk) were tested for 24 wk in a randomized complete block design. Animals were grouped based on actual milk yield and calving date. Dietary treatments included; 1) control (no NALM); 2) 15 g/d NALM (15NALM); 3) 30 g/d NALM (30NALM); and 4) 45 g/d NALM (45NALM). Diets were formulated to meet or exceed nutritional requirements of dairy cows producing at least 42 kg/d of milk and to insufficiently supply metabolizable Met (control). Data were analyzed using the MIXED procedure of SAS. Milk and its component prices used to calculate income-over feed cost (IOFC) were averaged for the entire trial. The IOFC for milk yield was calculated using the weighted average mailbox milk prices per hundredweight for reporting areas of all federal milk market. Feed prices were reflective of local prices. The IOFC was calculated based on the income generated from milk, and milk component yield relative to feed costs associated with actual dry matter intake. Intake of dry matter was similar across treatments, but numerically greater milk yield responses with 15NALM and 30NALM led to quadratic effects on feed efficiencies ($P < 0.07$). Likewise, the IOFC for milk yield was greatest with 30NALM (quadratic effect; $P = 0.05$), while it tended to be greater with NALM supplemented groups ($P = 0.10$) when compared with control. Quadratic trend was also observed for the IOFC based on milk component yield, as it was improved with 15NALM and 30NALM but tended to decrease with 45NALM compared with control ($P = 0.08$). Overall data in the present study imply that supplementing NALM at 30 g/d improved feed efficiencies, and these improvements contributed to sizable increases in IOFC based on milk (22.2%) and milk component (11.9%) yields. Therefore, NALM supplementation can be an option to improve economic returns of dairy farming.

Key Words: lactating dairy cow, *N*-acetyl-L-methionine (NALM), income-over feed cost

T113 Effect of cow stocking density and milk yield on milk production by square meter in compost bedding pack dairy farms.

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In compost bedding pack (CBP) dairy barns, milk yield (MY) and cow stocking density (CSD) are key economic indicators, but others such as milk yield per square meter (MYSM) could be also of interest. Our aim was to study the association among MY (kg/d), CSD (m^2/cow) and MYSM (kg/m^2). From January to December 2018, data from 2 CPB dairy barns (Córdoba, Argentina), one with concrete feed alleys (LD Dairy) and the other without (AT Dairy), was collected daily, Both CBP barns were tilled 2×/d using a tractor with deep tiller and rototiller but cows were milked 3×/d at AT and 2×/d at LD. The CSD was calculated as total barn area (AT = 3960 m^2 and LD = 3672 m^2) divided the number of lactating dairy cows (AT = 286 ± 14 and LD = 287 ± 13) by barn. The MYSM was estimated dividing MY by CSD. We ran Pearson correlations and regression analysis using Dummy variables between MYSM with MY and MYSM with CSD. MY and CSD for AT and LD averaged (±SE) 39.83 ± 5.42 and 30.87 ± 2.65 kg/d, and 13.88 ± 0.70 and 12.77 ± 0.56 m^2/cow, respectively. Therefore, MYSM averaged (±SE) 2.87 ± 0.40 and 2.42 ± 0.18 kg/m^2 for AT and LD, respectively. Pearson correlation between MSYM and MY was positive (r = 0.93 for AT and r = 0.86 for LD; $P < 0.01$). Linear regression analysis showed significant relationship ($R^2 = 0.89$; $P < 0.01$) between MYSM and MY (MYSM = 0.13 + 0.07 MY in AT; YMYSM = 0.58 + 0.06 MY in LD). Pearson correlation between MYSM and CSD was negative (r = −0.24 for AT and r = −0.05 for LD), but only significant for AT ($P < 0.01$). Linear regression analysis showed significant relationship ($R^2 = 0.38$; $P < 0.01$) between MYSM and CSD (MYSM = 4.80–0.14 CSD for AT; MYSM = 2.62–0.02 CSD for LD). Our data shows that MYSM is affected more by MY than by CSD.

Key Words: compost bedding pack, cow stocking density, milk per square meter

T114 Deviation between the formulated target and the real weight loaded into the mixer in dairy farms in Argentina.

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The number of dairies using software to monitor feeding management is increasing in Argentina. The aim of this study was to make a characterization of the deviations between the target weight (TW) and the real weight (RW) loaded into the mixer of the most frequently used ingredients in dairies using the software Nutrir+ in Argentina. We worked with more than 42,000 records from a total of 38 loaded ingredients in 40 dairies. We calculated the deviation from the target weight for each of the 5 most frequently used ingredients in diets for lactating cows. We expressed this result as an absolute difference (AD) between both values (AD = TW − RW; kg) and as a relative difference (RD) considering the formulated target weight (RD = (TW − RW)/TW x 100; %). We calculated the range of the median values of the RD for each farm on each ingredient. The results are shown in the table. One of the least accurate ingredients loaded was soybean meal as there were 25% of the loads that exceeded 13.8% of deviation from the target. This is important as soybean meal is one of the most expensive ingredients. On the other hand, corn silage is the most accurate ingredient loaded in relative terms, probably because it is included in a higher proportion of the diet. Alfalfa hay loads showed the highest RD. There is a great variation between farms on the deviation between formulated target and real weights, meaning there are opportunities to improve feeding accuracy.

Table 1 (Abstr. T114). Deviation from target weight for the most frequently used ingredients

Ingredient	N (farms)	Median absolute difference (IQR),[1] kg	Median relative difference (IQR),[2] %	Range of farm median relative difference, %
Corn silage	6338 (37)	30 (8–80)	1.9 (0.5–5.2)	1.0–21.2
Soybean meal	3578 (27)	10 (3–34)	4.5 (1.2–13.8)	0.2–21.2
Corn	3464 (26)	13 (3–40)	3.9 (1.0–11.4)	0.5–19.7
Alfalfa hay	3271 (27)	12 (3–32)	6 (2.0–16.5)	0.2–40.5
Water	3008 (21)	17 (4–43)	3.8 (0.9–11.7)	0.3–58.0

[1]Absolute difference calculated as target weight minus the real loaded weight.

[2]Relative difference calculated as absolute difference divided by the target weight.

Key Words: feeding management, deviation

T115 Effects of long-term postbiotic supplementation on dairy heifer calves: Performance and metabolic indicators.

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Seventy heifer calves were used to evaluate the effects of a postbiotic supplement on growth performance and blood metabolites from birth

to wk 18 at a commercial dairy farm in South Dakota. After birth calves were assigned to: 1) Control with no supplement (CON; n = 35) and 2) supplemented with 3 g/d Probisan in milk from birth to weaning, and 1.5 g/kg concentrate mixture after weaning (PRO; n = 35). All calves were fed 2.6 L per meal until wk 8 (weaning transition). A commercial starter was fed ad libitum from d 14 and hay and concentrate mixture after weaning. Body weight, hip width and intake were individually recorded (weekly, biweekly and daily; respectively). Blood was drawn biweekly from birth to measure plasma concentrations of glucose, BHB, and triglycerides. Data were analyzed as a completely randomized design with repeated measures using mixed models (SAS 9.4). No differences were detected ($P > 0.10$) between groups for average body weight (71.8 ± 0.9 kg), growth rate (785 ± 11 g/d), or hip width (20.1 ± 0.1 cm). Groups consumed similar ($P > 0.10$) amount of starter from wk 3 to 5 (226 ± 67 g/d), but from wk 6 to 10, PRO calves consumed less ($P = 0.043$) amounts (CON = 926 ± 51 and PRO = 804 ± 50 g/d). Glucose (103.5 ± 1.21 mg/dL), BHB (0.648 ± 0.035 mmol/L), and triglycerides (13.20 ± 0.71 mg/dL) did not vary ($P > 0.10$) between groups and no interaction between treatment and week. However, metabolites changed ($P < 0.001$) throughout time where glucose decreased from 110.4 to 93.7 mg/dL (wk 0 to 6 and 10 to 18, respectively), BHB increased from 0.448 to 1.015 mmol/L (wk 0 to 6 and 10 to 18, respectively), and triglycerides were highest at wk 0 (21.77 mg/dL) and lowest at wk 18 (7.46 mg/dL). These changes were in accordance with the milk consumption reduction and the development of rumen functions throughout time. In conclusion, despite the lower consumption of solid feeds intake from wk 6 to 10 by PRO calves, no differences were detected in growth performance. Blood metabolites were similar between groups and their pattern change over time indicated similar rumen development. Study supported by Pentabiol, Spain.

T116 Early lactation feeding behavior as a predictor of feed intake and lactation performance.

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Feed intake is frequently used in the dairy industry as an indicator of early lactation performance; however, it is difficult to assess individual dry matter intake (DMI) in group-housed cows. Therefore, measurable feeding behaviors in early lactation could be valuable in estimating DMI and predicting lactation outcomes. To investigate, feeding behavior collected during the first week of lactation from 5 studies at Kansas State University were compiled for analysis. Cows (n = 260) were housed in tie-stalls; feeding behaviors were recorded using individual feed bunks continuously measuring feed weight. Independent variables included meals (count/d), meal length (min/meal), and eating time (min/d). Additional data collected included wk 1 DMI (kg/day), parity, calving date, and the PTA for milk (PTAM). Variables were evaluated as either means or slopes over the 7-d period. Univariate analyses were used as a preliminary screen for predictors ($P < 0.20$) of DMI during wk 1 of lactation and 305MEM. Potential predictor variables were then included in a backward stepwise elimination model, along with associated quadratic and biologically relevant interaction terms, and the random effect of treatment nested within study. Criterion for model selection was minimal Bayesian Information Criterion. Feeding behavior in wk 1 was predictive of wk 1 DMI; meals/d, in addition to parity and PTAM (all $P \leq 0.006$), were positively associated with wk 1 DMI (model $r^2 = 0.29$). Feeding behavior in wk 1 also predicted 305MEM; positive associations of PTAM ($P < 0.001$) and d 1 eating time ($P = 0.009$), along with negative associations of d 1 meals ($P = 0.002$) and wk 1 eating time ($P = 0.01$) combined to explain 50% of the variation in 305MEM. These results indicate that feeding behaviors in wk 1 of lactation can be used to predict DMI of individual cows as well as 305MEM. In addition, PTAM was retained in all models, indicating that genetic propensity for milk production is positively associated with early lactation DMI.

Key Words: biomonitoring, transition period, forecasting

T117 Effects of storing temperature on dissipation of ceftiofur and antibiotic-resistance genes in dairy manure slurry.

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The objective of this study was to investigate the effects of storing temperature on dissipation of ceftiofur and antibiotic-resistance genes in dairy manure slurry. Feces and urine were totally collected from 5 lactating dairy cows with ceftiofur administration, and then mixed completely to achieve a homogeneous manure status. Manure slurry was generated by mixing manure and sterile water at 1:1. A 200 mL aliquot of slurry was transferred into 400 mL glass beakers and incubated at 10, 25, 35, or 55°C. Each temperature treatment had 4 replicates. The beakers were weighted daily, and sterilized water was added to compensate the weight loss due to water evaporation. Six g of slurry was sampled from each beaker on d 0, 1, 3, 7, 14, 28, 56, and 90 for ceftiofur concentration analysis. Slurry samples from d 0, 3, 7, 28 were used to determine gene expressions of 16S rRNA, CfxA, and TetW using the q-PCR. The q-PCR results were normalized using natural log-transformation. The temperature and incubation time effects were evaluated using a mixed statistical model which included temperature and time as fixed effects with glass beakers as repeated measurements. Significant temperature and time effects were observed for ceftiofur concentrations ($P < 0.0001$). With increased incubation temperature, the dissipation rate of ceftiofur was increased. Ceftiofur concentration was completed reduced at 55°C on d 14, while 76.2, 70.8, and 84.6% of ceftiofur was removed at 10, 25, and 35°C on d 90. The lowest relative abundance of CfxA and TetW were observed at 55°C on d 28, which reduced around 60% for CfxA and 50% for TetW relative to the initial abundance ($P < 0.05$). In conclusion, the thermophilic treatment is recommended for the dissipation of ceftiofur and antibiotic-resistance genes in dairy manure slurry.

Key Words: antibiotic-resistance genes, ceftiofur, dairy manure

T118 Early lactation management strategies and blood β-hydroxybutyrate concentration on pasture-based dairy farms in Colombia.

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Our objectives were to characterize dairy farms, milk yield, and nutritional strategies in 3 different regions of Colombia and determine associations with blood BHB concentrations in the first 42 DIM. Dairy herds (n = 55) in the Antioquia, Caldas, and Cundinamarca regions of Colombia were visited once from May through July 2018. A survey was administered to farm owners to collect management and nutrition information. Whole blood [BHB] was measured from dairy cows (n = 868) between 1 and 42 DIM using a point-of-care device. Associations between nutritional strategies and blood [BHB] were examined using mixed models. Prevalence of hyperketonemia was calculated as the number of samples with [BHB] ≥ 1.2 mmol/L divided by the total number samples. The estimated early lactation diet composition was 65.5% pasture, 31.8% commercial concentrates, and 2.7% other feed ingredients. The median (range) milk yield, [milk protein], and [milk fat], were 21.0 kg (13.1 to 36 kg), 3.2% (2.7 to 4.1%), and 3.5% (3.0 to

4.1%), respectively; mean (±SE) milk yield differed by region at 22.3 ± 0.12 kg, 20.8 ± 0.20 kg, and 20.0 ± 0.20 kg in Antioquia, Caldas, and Cundinamarca, respectively ($P < 0.001$). Median (range) blood [BHB] was 0.5 mmol/L (0.1 to 4.4 mmol/L), and mean blood [BHB] did not differ between regions ($P = 0.16$). Overall prevalence of hyperketonemia was 4.0%. Geographical location affected the prevalence of hyperketonemia at 2.4%, 4.0%, and 9.6% in Antioquia, Caldas, and Cundinamarca, respectively ($P < 0.001$), possibly due to production differences or unmeasured management variables. Neither commercial concentrates nor pasture as percent of estimated early lactation diet intake were associated with blood [BHB] ($P \geq 0.14$). Our results suggest that pasture-based dairies in Colombia have lower early lactation blood [BHB], hyperketonemia prevalence, and production yields compared with confined production systems in temperate zones; further epidemiological work is needed to establish the suitability of the commonly used hyperketonemia cut-point for pasture-based dairies in tropical regions.

Key Words: Colombia, β-hydroxybutyrate, pasture

T119 The effects of supplementing butafosfan plus cyanocobalamin and calcium formate in postparturient dairy cows.

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The objectives of the experiment were to evaluate the effects of oral Ca supplementation and injectable butafosfan plus cyanocobalamin on mineral and energy metabolism. Two hundred eight multiparous Holsteins were randomly assigned in a 2 × 2 factorial arrangement: Negative Control (NC = 52), which received 3 subcutaneous (SC) injections of 25 mL of saline solution at 0, 3, and 7 DIM; Oral Ca (OC = 52), which received orally 2 doses of 350 mL (50 g of Ca) as Ca formate at 0 and 1 DIM; Cyanocobalamin plus Butafosfan (CB = 52) which received 3 SC injections of 25 mL of cyanocobalamin (0.005 mg/mL) plus butafosfan (0.1 g/mL) at 0, 3, and 7 DIM; and Positive Control (PC = 52), which received Ca formate and cyanocobalamin plus butafosfan. Daily milk yield was recorded during the first 100 DIM. Milk was sampled and analyzed in the first 2 weeks postpartum. Blood was sampled at 3, 7 and 14 DIM to analyze β-hydroxybutyrate (BHB) and at 0, 12, 24, 36 and 72 h postpartum to analyze ionized Ca (iCa). Clinical evaluations on d 1, 3, 7 and 14 were performed to determine incidence of diseases. Data were analyzed using the MIXED and GLIMMIX procedures of SAS. Cows which orally received OC had lower ($P < 0.01$) milk yields than control ones (47.48 vs. 48.41 kg/d; SEM = 0.22), and cows supplemented with CB had higher ($P < 0.05$) yields than non-treated ones (48.34 vs. 47.55 kg/d; SEM = 0.26), with no ($P > 0.10$) interaction. Milk composition and BHB concentrations did no differ ($P > 0.10$) among treatments. Concentrations of iCa were higher at 12h ($P = 0.08$) and 36h ($P < 0.01$) in the OC treatment and at 36h ($P = 0.05$) in the CB treatment, with ($P = 0.05$) interaction at 36h. The incidence of subclinical ketosis was lower in cows treated with OC ($P = 0.03$) than non-OC treated cows. There was a lower incidence of mastitis in cows treated with CB ($P < 0.01$) than non-CB treated ones. Other disorders did not differ ($P > 0.10$) among treatments. Our results show that OC supplementation increased iCa concentrations and reduced incidence of ketosis, but they had lower milk yields. Cows that received CB postpartum had increased milk yield and reduced incidence of mastitis.

Key Words: hypocalcemia, ketosis

T120 Effect of measures of milk yield and dry period length on prediction of milk loss in the subsequent lactation.

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To optimize cows' dry period lengths, it is important to estimate milk loss in the subsequent lactation associated with non-optimal days dry (DD). It is unclear how different measures of milk yield in the current lactation in combination with DD affect predicted milk loss. Therefore, the objective of this study was to quantify the effect of DD combined with 2 measures of milk yield in the current lactation on milk loss. DHI milk test records of 824,809 Holstein cows from 4,766 herds with the last dry date in 2014 or 2015 were obtained from DRMS in Raleigh, NC. Three groups of adjacent lactations were constructed: lactation 1 and 2 (lac1–2), lactation 2 and 3 (lac2–3), and lactation 3 and greater (lac3+). Included cows had ≥ 6 milk tests including fat and protein observations in the current lactation; gestation length of ≥ 270 d; DD from 5 to 120 d. Five models with a fourth-degree polynomial function of DD and control covariates with combinations of cumulative ECM yield to the last test day (ECMLTD), daily ECM yield 14 d before the dry off date (ECM14), and random herd-season effects were developed to predict 305-d ECM yield in the subsequent lactation (ECM305) for each group. Milk loss was calculated as the predicted ECM305 given a certain DD minus the predicted ECM305 at 58 DD at which ECM305 of all cows in these data was maximized. Compared with the model including only a polynomial DD function, the model including ECMLTD had a 10% smaller RMSE, whereas including both measures of milk yield reduced RMSE by 11%. The inclusion of the 3-way interaction reduced RMSE only by 0.2% compared with the model with only the 2-way interactions. The full model was selected to illustrate predicted milk loss of ECM305 by group. The effect of DD shorter than 58 d on milk loss was greatest in lac1–2. A low level of ECM14 resulted in less milk loss in all groups when DD was short, whereas a high level resulted in greater milk loss. In conclusion, milk yield interacts with DD and affects milk loss in the subsequent lactation. Both ECMLTD and ECM14 help predict milk loss and are therefore useful for economic optimization of DD.

Key Words: dry period, milk loss, prediction

T121 Comparison of rumen microbiome structure in samples collected using an oro-esophageal probe, and solid, liquid and combined solid-liquid fractions collected using a rumen fistula in Holstein dairy cows.

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The use of oro-esophageal probes to collect rumen samples for sequencing and characterization of microbiome became a widely used procedure in the last few years. While the methodology represents a proxy for the rumen microbiota, remain unclear how diversity, richness, and structure of microbiome is affected by the different methods of collection and by the different fractions of rumen sample that can be obtained by a rumen fistula. Our objectives were to compare richness, diversity, and structure of rumen microbiome in samples collected using an oro-esophageal probe (oral), and solid, liquid and the mixed solid-liquid fractions collected using a rumen fistula in Holstein dairy cows. Rumen-fistulated, mid-lactation, multiparous Holstein dairy cows (n = 9) fed the same diet had samples collected using the oro-esophageal tube and the rumen fistula. The rumen fistula samples included liquid, solid, and mixed liquid-solid fractions. Genomic DNA was extracted, and the V3-V4 hypervariable region of the 16S rRNA gene was amplified and sequenced using the Illumina MiSeq platform. Data were processed using QIIME and analyzed using JMP. The Chao1 richness index did not differ ($P = 0.46$), while the solid and mixed liquid-solid sample had a greater Shannon diversity index ($P = 0.03$) when compared with oral and liquid samples. The most common phyla across all samples were *Bacteroidetes*, *Firmicutes*, *Proteobacteria*, *Spirochaetes*, *Euryarchaeota*, *Fibrobacteres*, *Actinobacteria*, and *Tenericutes*. Mean relative abundance (MRA) of *Fibrobacteres* was greater ($P = 0.02$) in solid and mixed liquid-solid samples when compared with liquid and oral samples. *Prevotella*, *Bacteroides*, *Succiniclasticum*, *Treponema*, *Ruminococcus*, *Butyrivibrio*, *Dysgomonas*, *Blautia*, and *Pedobacter* were the most prevalent genera. No differences in the 30 most prevalent genera MRA were detected among sampling types nor were there differences among rumen source and methods and samples in our discriminant analysis. In conclusion, solid and mixed-solid liquid samples had greater microbiome diversity and MRA for the phylum *Fibrobacteres* than oral and liquid samples.

Key Words: rumen microbiome, sampling method, dairy cow

T122 Probiotics supplementation for dairy calves: A systematic review. R. Branco Lopes* and N. Silva-del-Río, *Veterinary Medicine Teaching and Research Center, University of California-Davis, Tulare, CA.*

The objective of this study was to systematically review the current literature on probiotics for dairy calves. In October 2018, 5 electronic databases (CAB Abstracts, PubMed, Science Direct, Scopus and Web of Science) were used to retrieve manuscripts. The literature search strategy was defined based on population, intervention, and outcome. An a priori protocol was developed, including screening and data extraction forms. Descriptive statistics were performed on Microsoft Excel. A total of 2,177 publications were retrieved, 1,808 abstracts were screened and 90 manuscripts (corresponding to 97 trials) met the inclusion criteria. The earliest manuscript included was published in 1991, and most studies (n = 65) were performed between 2008 to 2018. Studies took place in 22 different countries, mostly in Asia. Ninety-three trials had an untreated group and 4 a positive control group (applying antimicrobial). *Lactobacillus* spp. (n = 37), *Bacillus* spp. (n = 10) and *Saccharomyces* spp. (n = 11) were the most evaluated genera; 69% of trials tested a single strain probiotic and 31% a multi-strain product. The average study sample size was 58.3 ± 113.8 dairy calves. Studies ranged from 6 to 770 calves; 52 studies used less than 30 calves. The age of calves enrolled on the trials varied from 1 to 180 d (10.6 ± 24.9). Trials enrolling newborns calves administered probiotics in colostrum. For older calves (>1d), probiotic administration was via milk replacer, milk, concentrate, syringe, drench, or esophageal tube. Holstein and its crossbreeds (n = 67) were the breeds most assessed; only 1 study used Jersey calves. The duration of probiotic supplementation varied from 1 to 364 d (64.2 ± 75.8); most studies evaluated preweaned calves (n = 80). Calves performance was consistently evaluated across trials; however, studies reporting health outcomes used multiple and non-standardized measurements. Substantial research has examined the effect of probiotics on performance and health of calves, but experimental design varied across studies. Future studies should adhere to guidelines for health measurements. Funding provided by CDFA–AUS project.

Key Words: calf, probiotic, systematic review

T123 Effects of long-term postbiotic supplementation on dairy heifer calves: Health status and wound healing after dehorning. M. Rovai*[1], L. Guifarro[1], and A. A. K. Salama[2], [1]*Dairy and Food Science Department, South Dakota State University, Brookings, SD,* [2]*Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain.*

The aim was to assess the health status of calves supplemented with a postbiotic (Probisan) from birth to wk 18. The Calf Health Scorer App (University of Wisconsin-Madison, Madison, WI) was used to monitor health status in control (CON; n = 35) and Probisan-supplemented calves (PRO; n = 35). The scoring was based on a scale of 0 to 3 for general health parameters (0 = normal; 3 = very abnormal), fecal consistency (0 = normal, 3 = watery feces), nasal discharge (0 = normal, 3 = mucopurulent discharged), and cough (0 = normal, 3 = repeated spontaneous coughs). At 43 ± 2 d, calves were dehorned and the wound status was evaluated 10 and 20 d later using a customized numerical score of 1 to 3, where 1 = no infection, 2 = inflammation and 3 = infection. The FREQ procedure and a logistic model of SAS 9.4 was used to analyze the data. Diarrhea (score ≥2; loose feces but stays on top of bedding) was the most frequent disease observed with 13 and 12 cases at 13 ± 1.9 and 11 ± 1.2 d old for CON and PRO groups, respectively. Only one calf from each group had repeated occasional spontaneous coughs (score 3) and received 4 d of treatment to prevent pneumonia. One CON calf that presented diarrhea also had ear infection at 3 mo of age. The average dehorning wound score was greater ($P < 0.05$) in CON than in PRO calves at d 10 (1.77 vs. 1.38; SEM = 0.13) and d 20 (1.51 vs. 1.18; SEM = 0.11) after the procedure. After 10 d of dehorning, almost 50% of the CON calves presented either wound inflammation or infection (20.0% score 2, and 28.6% score 3), while only 27% of PRO calves suffered wound healing problems (14.7% score 2, and 11.8% score 3). At d 20, PRO calves had lower percentages of score 2 (5.9 vs 14.3%) and score 3 (6.0 vs 17.1%) compared with CON calves. The lack of difference in medical issues at early age (d 11–13) would be because the postbiotic supplement needs longer time (few weeks) to exert its positive effects. The better wound healing after dehorning in PRO calves suggests improved response of postbiotic-supplemented calves to stressful conditions. Study supported by Pentabiol, Spain.

Key Words: calf, inflammation, postbiotics

T124 Production, economic viability, and risks associated to switching from drylots to compost barns. M. I. Marcondes*[1,2], W. H. Mariano[1], and A. De Vries[2], [1]*Federal University of Vicosa, Vicosa, MG, Brazil,* [2]*University of Florida, Gainesville, FL.*

The use of compost bedded barns (CB) has increased in tropical countries in the past 5 years, and studies associating production traits and economical outcomes are warranted. Our objectives were to evaluate productive traits, economic outcomes, and risks of loss from dairy farms that switched from drylots (DL) to CB. We collected data from 18 farms located in Brazil during 36 consecutive months. All farms started the study as DL, with average milk production per day of 1,815 L, and 6 farms switched to CB in the second year. The other 12 farms remained DL during the 3 years. Annual technical and economic indexes were collected and calculated. Additionally, a risk analysis was performed based on a 10-year historical series of milk prices. The results were analyzed using a regression model including year and herd as categorical variables (fixed), system and herd size as quantitative variables (fixed), and system × herd as random variable. Furthermore, a non-metric multidimensional scaling (NMDS) plot was used to evaluate producers' profiles. Milk fat, milk total solids, and somatic cell count did not change when farms switched from DL to CB. However, milk protein decreased in CB farms. The majority of variables related to milk

production were also not affected. Nevertheless, farms that switched to CB increased 13.3% milk production per cow (from 20.8 to 23.6 kg/d). Total operational costs were not affected by system and neither were expenses with concentrate, roughage, labor or medicines. Net margin per liter, operational profit, assets per liter, and assets returning rate did not change when farms switched from DL to CB. Net margin ($/L and $/cow), profit per cow and assets turnover rate were increased in CB farms. The risk was reduced by 38% in CB farms. Furthermore, the NMDS analysis indicated that producers that decided to switch to CB had already similarities in technical and economic indexes in the first year, before changing the system. This study demonstrates that CB may be promising for producers in tropical countries looking for a more productive and less risky system. It should be also noted that we did not observe improvements in animals' health as previously reported in the literature.

Key Words: compost bedded pack, economy, semi-confined

Ruminant Nutrition: Carbohydrates

T125 Nitrogen and energy balance of primiparous Holstein and Jersey cows fed 2 levels and 2 sources of forage neutral detergent fiber. M. E. Uddin*[1], O. I. Santana[1,2], and M. A. Wattiaux[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*INIFAP, Aguascalientes, México.*

Our objective was to determine the effects of dietary neutral detergent fiber (NDF) level and source on Energy (E) and nitrogen (N) balance of Holstein and Jersey. Mid-lactation Holstein (n = 4) and Jersey (n = 4) were used in a split-plot triplicated 4 × 4 Latin square with breed and diet as the main and subplots, respectively. Treatment factors (as 2 × 2 arrangement) were: forage NDF (FNDF) level [19 (LF) and 24% (HF) of dry matter (DM)] and FNDF source [alfalfa silage (AS):corn silage (CS) NDF at 70:30 and 30:70 ratio]. Diets had 17% crude protein, 23% starch and 4.38 Mcal/kg gross E (DM basis). Total collection was performed from d 24 to 27 in each 28-d period and methane (CH_4) was measured with GreenFeed. Model included breed, FNDF level and source, and all possible interactions as fixed terms; square within breed, and cow within breed and square were the random terms. Significance was declared at $P < 0.05$. Holstein had greater DM intake (DMI, 34%), outputs of fecal DM (37%) and urinary DM (20%), and fat-protein corrected milk (FPCM, 31%) than Jersey. LF-fed cows had 8% greater N intake but 17% lower urinary N loss resulting in 11% decreased manure N excretion than HF-fed cows. Nitrogen balance (N intake - N in milk and manure) was not affected by breed and FNDF source but was greater for LF than HF-fed cows (194 vs. 139 g/d). These unusually high values may have reflected cumulated errors and accumulation in various N pool in the body. Neither E intake nor E output variables (% of gross E intake) were affected by breed and FNDF level. CS-fed cows lost 15% less urinary E and 10% less CH_4 E, resulting in a slight (3%) but significantly greater metabolizable E than AS-fed cows. Yet, FPCM/DMI was 4% lower for CS than AS-fed cows and the former had 12% greater loss of non-maintenance heat E than the latter. Nitrogen excretion and balance were not affected by breed and FNDF source but LF-fed cows had lower urinary and manure N loss resulting in greater N balance than HF-fed cows. Breed and FNDF level affected none of the E variables but CS-fed cows had lower losses of urinary and CH_4 E than AS-fed cows.

Key Words: alfalfa silage, corn silage, methane

T126 Switchgrass (*Panicum virgatum*) as a replacement for wheat straw in high-starch lactating cow rations. R. L. Nagle*[1], B. R. Lemay[1], J. C. DeBruyn[2], A. Heeg[3], M. Thimmanagari[2], and A. J. Carpenter[4], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Ontario Ministry of Food, Agriculture, and Rural Affairs, Guelph, ON, Canada,* [3]*Ontario Ministry of Food, Agriculture, and Rural Affairs, Woodstock, ON, Canada,* [4]*Department of Animal Biosciences, University of Guelph, Ridgetown, ON, Canada.*

Milk fat depression in lactating dairy cows is a result of the downregulation of milk fat synthesis in the mammary gland when certain intermediates are produced as a result of low rumen pH during rumen biohydrogenation of unsaturated fatty acids. Anecdotes from dairy producers suggest that feeding switchgrass (*Panicum virgatum*) to lactating dairy cattle improves milk fat production, assumedly due to greater physical effect of the fiber in this forage. To test this hypothesis, mid-lactation Holstein dairy cows (n = 24) were randomly assigned to a high-starch diet containing switchgrass (SG; starch = 27.6 ± 2.45% of diet DM) or wheat straw (WS; starch = 29.6 ± 1.54% of diet DM). Treatments were administered in a switchback design with 2 periods consisting of 17 d of adaptation and 4 d of sampling. Data were analyzed in SAS using PROC GLIMMIX to model treatment and treatment × day interaction. Two cows were excluded from analysis for stealing feed. Cows on the WS diet had a tendency for higher ECM (kg/d, $P = 0.0519$). DMI was lower for cows on SG (24.6 ± 0.70 vs. 25.4 ± 0.71 kg/d; $P = 0.03$), as was milk yield (34.8 ± 1.40 vs 35.6 ± 1.40 kg/d; $P = 0.04$). However, there was no effect of diet on milk fat % (4.11 ± 0.121 vs 4.10 ± 0.122%; $P = 0.83$) and yield (1.42 ± 0.068 vs 1.46 ± 0.068 kg/d; $P = 0.20$), as well as protein % (3.37 ± 0.048 vs 3.38 ± 0.048%; $P = 0.85$). There was a tendency for lower protein yield when cows were on the SG diet (1.17 ± 0.044 vs 1.20 ± 0.045 kg/d; $P = 0.0522$). A significant diet × day interaction ($P \leq 0.03$) was detected for milk fat %, protein %, and protein yield, along with ECM (kg/d) per kg of daily DMI. Blood samples were analyzed for BHBA, NEFA, and glucose, but showed no statistically significant differences between treatment groups ($P \leq 0.18$). The switchgrass used in this trial was contaminated with head smut, and future research should focus on the effects of uncontaminated switchgrass. Overall, our results indicate that under current conditions, SG depressed DMI and milk yield.

Key Words: switchgrass, milk fat, high starch

T127 Effects of aflatoxin with or without binders on in vitro rumen fermentation dynamics. Y. Jiang*[1], I. M. Ogunade[2], K. G. Arriola[1], A. A. Pech-Cervantes[1], D. H. Kim[1], Y. L. Xue[3], X. Li[4], D. Vyas[1], and A. T. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Division of Food and Animal Science, Kentucky State University, Frankfort, KY,* [3]*Inner Mongolia Academy of Agriculture and Animal Husbandry Science, Hohhot, Inner Mongolia, China,* [4]*School of Agriculture and Biology, Shanghai Jiao Tong University, Shanghai, China.*

The effects of aflatoxin B_1 (AFB_1), with or without bentonite clay (CL) and *Saccharomyces cerevisiae* fermentation production (SCFP)-based aflatoxin binders on in vitro rumen fermentation of a dairy cow TMR was examined. The corn silage-based TMR (0.5 g, 17.3% CP and 1.67 Mcal/kg NE_L) was incubated in duplicate in a rumen fluid – buffer inoculum (1:2 ratio; 50 mL) containing the following treatments: 1) Control (nothing); 2) T (37.5 ng of AFB_1); 3) CL (T + 40 mg of CL); 4) CL+SCFP (CL + 7 mg of SCFP). The ruminal fluid was collected from 3 cannulated cows fed the same TMR 3 h after the morning feeding. Each treatment was incubated at 39°C for 4, 8, 16 and 24 h in each of 3 runs. Data were analyzed using Glimmix procedure of SAS. The model included fixed effect of treatment, random effects of cow, run and cow × run. Adding T reduced ($P < 0.05$) total volatile fatty acid (VFA) concentration after 4 and 8 h and molar proportion of propionic acid after 4 and 24 h of incubation, relative to control. Adding binders with T did not affect total VFA concentration after 4 and 8 h, but increased ($P < 0.05$) the measure after 16 h and tended to increase ($P = 0.10$) molar proportion of propionic acid after 24 h, compared with T. At 24 h, T had lower ($P = 0.04$) DMD and higher NH_3-N ($P = 0.04$) concentration compared with the control as well as lower ($P < 0.05$) pH compared with the binders. Thus, AFB_1, even at very low concentration (0.75 µg/L), had detrimental effects on the fermentation and DMD of

Table 1 (Abstr. T127). Effects of AFB_1 with or without binders on in vitro rumen fermentation dynamics

	Treatment					Contrast P-value		
	Con	T	CL	CL+SCFP	SEM	Con vs. T	T vs. binders	CL vs. CL+SCFP
Total VFA, mM								
4h	110	91.1	93.8	101	12.8	0.02	0.38	0.39
8h	128	104	106	98.5	9.21	0.01	0.80	0.35
16h	105	99.1	127	114	10.4	0.54	0.01	0.22
24 h	132	135	135	151	17.1	0.82	0.45	0.21
Fermentation parameters, 24 h								
pH	6.64	6.61	6.67	6.68	0.05	0.24	0.01	0.75
NH_3-N, mM/dL	11.8	12.4	11.8	12.4	0.80	0.01	0.15	0.03
DMD, %	57.2	53.2	52.7	51.6	2.96	0.04	0.52	0.57

the TMR. Adding binders to T did not prevent the negative effects of T within 8 h of incubation did so after 16 h or 24 h of incubation.

Key Words: rumen fermentation, aflatoxin, binder

T128 Effects of particle size on ruminal in situ dry matter and starch disappearance of processed corn. E. M. Paula*[1], T. Fernandes[1,2], G. S. Dias Junior[2,3], R. D. Shaver[3], and L. F. Ferraretto[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Departamento de Zootecnia, Universidade Federal de Lavras, Lavras, MG, Brazil,* [3]*Department of Dairy Science, University of Wisconsin, Madison, WI.*

Starch digestibility is affected by a starch-protein matrix which limits digestion by rumen microbes. Therefore, disrupting this protein matrix is of great interest to dairy nutritionists. This study aimed to evaluate a commercially available processed corn product either intact or ground at various particles size on ruminal in situ DM and starch disappearance. Corn processing involved proprietary heat and pressure treatments that sheared the prolamin structure. Experiment 1 evaluated ruminal DM disappearance of processed corn (0Z45; patent #9,446,094; Matrix Nutrition LLC) either intact, cracked, or ground to pass through a 0.5, 2, or 4 mm sieve. A dry ground corn sample (ground to pass a 0.5 mm sieve) was used as a negative control. Ruminal in situ incubations were performed at 3, 6, 12, and 24 h to determine ruminal DM disappearance. Experiment 2 evaluated the kinetics of starch disappearance. Processed corn was either unground (intact) or ground to pass a 2 mm sieve. Ruminal in situ incubations were performed at 0, 3, 48, and 120 h to determine fractions A, B, and C of starch and the effective ruminal starch disappearance (ERD). Statistical analyses were performed using PROC Glimmix of SAS. For ruminal DM disappearance (Experiment 1), model included treatment, time, and their interaction as fixed effects, and the random effect of cow. For Experiment 2, fractions A, B, C, kd, and ERD were analyzed with fixed effect of treatment and random effect of cow. Overall, processed corn had greater ($P < 0.05$) DM disappearance than dry ground corn at 3, 6, and 12 h of incubation, but not at 24 h ($P > 0.05$). In addition, this effect was exacerbated with grinding or cracking ($P < 0.05$). Processed corn ground at 2 mm increased ($P < 0.05$) fraction A (42.5 vs. 17.9, % of starch) and ERD (62.7 VS. 40.8% of starch) and decreased ($P = 0.001$) fraction B (57.5 vs. 82.0% of starch) compared with the intact processed corn. These results indicate greater DM disappearance for processed corn compared with dry ground corn. In addition, reduced particle size improved ruminal starch disappearance of processed corn.

Key Words: particle size, processed corn, starch disappearance

T129 Dietary starch level does not interact with parity and level of milk production in mid-lactation dairy cows fed isoenergetic diets. P. Piantoni*, C. J. Canale, B. D. Strang, and G. F. Schroeder, *Cargill Animal Nutrition Innovation Center, Elk River, MN.*

Eighteen multiparous and 15 primiparous lactating cows with a wide range of milk production (24 to 58 kg/d) were used in a crossover design experiment with a covariate period to evaluate the effect of isoenergetic diets with different levels of starch. Cows were randomly assigned to treatment sequence within parity and level of milk production. Dietary treatments were: high starch (HS) and low starch (LS; 31.8% and 23.9% starch of diet DM, respectively). Diets were formulated with similar levels of forages and energy by replacing dry ground corn by non-forage fiber sources and fat. During the 14-d covariate period, all cows received a common diet, which was a mixture of both treatment diets; milk yield data collected during the last 4 d of this period were used as covariate (cMY). Data were analyzed using the GLIMMIX procedure of SAS with fixed effects of treatment, period, parity, and cMY, and random effect of cow. Interactions evaluated were treatment by period, parity, and cMY and treatment by cMY by parity. In general, parity did not interact with treatments except for milk protein yield. Compared with LS, HS increased milk protein yield in multiparous cows of all production levels and in high producing primiparous cows, but not in low producing primiparous cows (interaction, $P = 0.07$). HS decreased MUN (13.5 vs. 15.5 mg/dL; SEM = 0.26) and increased nitrogen efficiency (26.9 vs. 23.5%; SEM = 0.59, both $P < 0.01$), but did not affect milk protein or fat contents. Overall, treatment diets did not affect yields of milk, energy-corrected milk, or milk fat yield. HS increased DMI and decreased feed efficiency in high producing cows, but not in low producing cows (interactions, both $P < 0.05$). Regardless of level of milk production and parity, HS increased DMI (25.9 vs. 25.4 kg/d; SEM = 0.50) and decreased total-tract NDF digestibility (31.7 vs. 39.0%; SEM = 0.73, both $P < 0.05$). Treatments did not affect body weight change over the 28-d periods. High starch isoenergetic diets increased intake and decreased NDF digestibility, but did not affect body weight change or yields of milk and energy-corrected milk in mid-lactation cows of different production levels and parities.

Key Words: starch level, stage of lactation, total-tract digestibility

T130 The effects of varying undigested NDF and physically effective NDF content of fresh cow rations on hepatic metabolism and gene expression in multiparous Holstein cows.

S. E. LaCount*, W. R. Butler, and T. R. Overton, *Cornell University, Ithaca, NY.*

The objective of this study was to determine the effect of varying undigested NDF at 240 h of in vitro fermentation (uNDF$_{240}$) and physically effective NDF (peNDF) content of fresh cow rations on hepatic metabolism and gene expression. Multiparous Holstein cows (n = 56) were fed a common prepartum ration beginning 28 d before expected parturition and assigned randomly at calving to one of 2 postpartum diets differing in content of uNDF$_{240}$ and peNDF. High fiber (HF; 35.3% NDF, 12.2% uNDF$_{240}$, 23.2% peNDF; n = 27) and low fiber (LF; 32.8% NDF, 9.5% uNDF$_{240}$, 21.6% peNDF; n = 29) treatment diets were formulated for equivalent metabolizable protein (110 g/kg DM) and starch (24.8% DM), with higher fiber levels achieved through the addition of straw. At 29 DIM, cows fed HF were switched to the LF diet and all cows were fed the LF diet through 42 DIM. Liver biopsies were obtained from a subset of 40 cows on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum, liver tissue was immediately frozen in liquid nitrogen. Liver samples were analyzed for glycogen and triglyceride content, and analyzed via RT-qPCR to evaluate mRNA expression of key metabolic enzymes. Data were analyzed by repeated measures ANOVA with random effect of cow within treatment and fixed effects of treatment, time, parity group, and all interactions. Cows fed the HF diet tended ($P < 0.15$) to have higher liver triglycerides at d 14 and lower overall liver glycogen than cows fed LF. Cows fed the LF diet tended ($P < 0.15$) to have lower gene expression of pyruvate carboxylase at d 14 and lower overall expression of carnitine palmitoyltransferase 1A compared with cows fed HF. Expression of 3-hydroxy-3-methylglutaryl-coA synthase 2 tended to differ ($P = 0.12$) by treatment and parity group such that 2nd lactation cows fed LF had lower expression than 3rd and greater lactation cows fed the LF diet, while cows fed HF had no difference due to parity. Cows fed the HF diet had altered liver metabolism in both glucose and fatty acid metabolic pathways, likely due to intake restrictions beginning in wk 2 postpartum for cows fed HF.

Key Words: transition cow, fiber, metabolism

T131 Effects of drying method and in vitro or in situ technique on starch digestibility.

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The objective was to compare the effects of drying method on starch digestibility in corn silage (CS) using in situ (IS) and in vitro (IV) techniques. Three CS were selected with soluble protein ranging from 34.6 to 64.1%CP and split into 2 fractions. One fraction was dried via microwave (M) and the other was dried in an oven (O). Both fractions were ground to 4mm and 0.5g was weighed into IV flasks or 5 × 5 cm Dacron bags in triplicate. Blended ruminal fluid from 3 steers fed a diet of 30% starch in DM was used in a modified Goering and Van Soest (1970) IV method to measure starch digestibility after 3, 7 and 12h. Dacron bags were soaked in warm water for 10 min, then placed into the rumen immediately before feeding. Bags were removed after 3, 7, and 12h of incubation, washed, and starch remaining was measured. The IV and IS measurements were performed on the same days using the same steers. There were no differences between O and M in DM ($P = 0.48$), aNDFom ($P = 0.64$), and starch ($P = 0.29$). Starch washout was higher for IS-O vs. IS-M (78.7 vs 9.05%, respectively, $P < 0.001$). After 3h, starch disappearance (SDP) for IS-O was higher than IS-M (81.0 vs. 65.4%, respectively, $P < 0.0001$) but lower for IV-O vs. IV-M (27.2 vs. 55.1%, respectively, $P < 0.0001$). By 7h, SDP for IS-O was lower than IS-M (84.9 vs. 88.3%, respectively, $P < 0.0001$) and IV-O was lower than IV-M (61.3 vs. 84.6%, respectively, $P < 0.0001$). At 12h, SDP for IS-O remained lower than IS-M (89.9 vs. 93.7%, respectively, $P < 0.0001$), and IV-O was lower than IV-M (83.6 vs. 92.7%, respectively, $P < 0.0001$). At 7h, range of SDP was wider for IV-O vs. IV-M (42.1 vs. 12.1 units, respectively) and for IS-O vs. IS-M (13.7 vs. 8.39 units, respectively). There was no difference in IV-O kd calculating it using one time point (7h) vs. 3 ($P = 0.86$), but it was different for IV-M ($P < 0.0001$). Calculating kd using 1 vs. 3 time points was different for both IS-O and IS-M ($P < 0.0001$). These data suggest that the oven-dried in vitro method at 7h is appropriate to measure starch digestibility because it has the widest range of values and no sample loss due to washout.

Key Words: microwave, oven, corn silage

T132 Following up the efficacy of bacterial inoculants from corn silage fermentation quality to performance of dairy cows.

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A vast number of studies have been conducted on using silage inoculants most of which have investigated their effects on silage quality without addressing performance of dairy cattle. The present experiment was designed with almost 900 tons of whole crop corn in 3 silage bags of 300 tons. Each bag was treated with one of the following: C (no additive), homo-fermentative bacteria (HF, including *L. plantarum*, *E. faecium* and *L. brevis*) and hetero-fermentative bacteria (HTF, including *L. buchneri*) that was sprayed to chopped corn forage during packing. One hundred ninety cows housed in 3 freestall pens and 18 cows housed in individual tie-stalls were randomly assigned to treatments and fed with a TMR containing one of the treated silages at 30% DM. Feeding lasted for 4 weeks. Results showed no significant effect of inoculants on DM, crude protein, NDF, ADF, pH, ammonia N%, water-soluble carbohydrates and particle size of corn silage ($P > 0.05$). Bacterial inoculants also resulted in no significant difference in aerobic stability of silages ($P > 0.05$). Cows in individual pens did not show different responses to silage inoculation compared with cows fed untreated silage in terms of DM intake, milk yield and composition, milk somatic cell counts and urea nitrogen ($P > 0.05$). However, in HF, milk lactose was lower than C ($P = 0.008$). Digestibility of DM, crude protein and NDF differed significantly with C being higher than HF and HTF ($P < 0.05$). Consistently, feeding the same TMRs to freestall-housed cows resulted in similar effects on milk production, and composition between treatments ($P > 0.05$). Results showed no effect of HF and HTF on silage quality and performance of cattle fed the treated silages.

Key Words: lactic acid bacteria, performance, silage additive

T133 Effects of butyrate supplementation on productivity of lactating dairy cows fed diets differing in starch content.

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The objective of this study was to evaluate effects of butyrate supplementation on DMI, milk production, and ruminal fermentation of lactating dairy cows fed diets differing in starch content. Eight Holstein cows in early lactation (58.6 ± 9.96 d in milk; mean ± SD), including 4 primiparous ruminally cannulated cows, were blocked by parity and assigned to one of 4 × 4 Latin squares balanced for carryover effects with a 2 × 2 factorial arrangement of treatments. Treatments were dietary starch content (20 vs. 29%) and butyrate supplementation (butyrate vs. control) with 21-d periods. Experimental diets contained 36 and 30% corn silage, 18 and 15% grass silage, 46 and 55% concentrates, respectively for low starch (LS) and high starch (HS) diets, on DM basis. Butyrate was provided as Gustor BP70 WS (NOREL, S.A., Madrid, Spain), containing 70% sodium butyrate and 30% fatty acid mixture, at 2% of dietary DM (providing butyrate at 1.1% of dietary DM), and control premix contained 70% wheat bran and 30% fatty acid mixture. All data were analyzed using Fit model procedure of JMP with a model including fixed effects of dietary starch content, butyrate supplementation, their interaction, period, and square, and random effects of cow nested in squares. Interaction effects between dietary starch content and butyrate supplementation were not observed for primary response variables, and rumen pH and milk yield were not affected by treatment. However, cows fed butyrate increased milk fat content (4.58 vs. 4.37%; $P = 0.04$), milk fat yield (1.51 vs. 1.42 kg/d; $P = 0.02$), tended to increase 4% FCM yield (35.9 vs. 34.3 kg/d; $P = 0.08$) and feed efficiency (4% FCM/DMI; 1.56 vs. 1.50; $P = 0.08$), and decreased MUN concentration (10.8 vs. 11.7 mg/dL; $P = 0.02$) compared with control. Cows fed HS diets tended to increase DMI (23.3 vs. 22.5 kg/d; $P = 0.06$), increased yields of milk protein (1.13 vs. 1.05 kg/d; $P < 0.01$) and SNF (3.02 vs. 2.85 kg/d; $P = 0.04$), and decreased MUN concentration (10.3 vs. 12.2 mg/dL; $P < 0.001$). These results indicate that butyrate supplementation may increase milk fat production while high starch diets increase milk protein production.

Key Words: butyrate, starch, milk production

T134 Effect of enzyme extracts from *Aspergillus oryzae* and *Aspergillus niger* on in situ neutral-detergent fiber degradability in dairy cows. M. T. Harper[1], S. E. Räisänen*[1], X. Chen[1], A. Melgar[1], J. Oh[1], D. M. Paulus Compart[2], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*PMI, Arden Hills, MN.*

The objective of this experiment was to investigate the effect of an enzyme extract (ENZ) from *Aspergillus oryzae* and *Aspergillus niger* on in situ neutral-detergent fiber (NDF) degradability of a total mixed ration (TMR) compared with an untreated control. ENZ was evaluated at 3 TMR DM levels: 40, 50 and 60%. The TMR had 29.4% NDF and consisted of (DM basis): 57% forage and 43% concentrate feeds. ENZ was mixed with the TMR at a rate of 0.4 g per kg DM (equivalent to 10 g/cow/d at 25 kg/d DM intake) and allowed to react at room temperature for periods of 0, 6, 12, 18, and 24 h, before the ruminal in situ incubation. Bags were incubated in duplicates in 4 ruminally-cannulated Holstein cows for 0, 3, 6, 18, 24, 48, and 72 h. Cows were fed a 57% forage/43% concentrate feeds TMR with 29.4% NDF (DM basis). Rate of NDF degradability (NDFD) was estimated using LN-transformed data and the NLIN procedure of SAS. Effective NDFD (ED) was estimated using 2%/h rate of passage and assuming 0% soluble and 100% potentially degradable NDF fractions. Data were analyzed using the MIXED procedure of SAS including the fixed effects of treatment (ENZ), TMR DM, ENZ pre-incubation time, and interactions. TMR DM and pre-incubation time data were analyzed using orthogonal polynomial contrasts. Rate of NDFD decreased linearly ($P \leq 0.04$) with increasing TMR DM (3.58, 2.61, and 2.78%/h, respectively; SEM = 0.28) and with increasing ENZ pre-incubation time (from 3.32 to 1.89%/h; SEM = 0.46). Compared with the untreated control, ENZ increased ($P = 0.01$) the rate of TMR NDFD (2.58 vs. 3.40%/h; SEM = 0.23). ED tended to decrease linearly ($P = 0.07$) with increasing TMR DM content and decreased quadratically ($P = 0.02$) with increasing ENZ pre-incubation time. ENZ increased ($P = 0.02$) ED compared with the control (49.8 vs. 44.0%; SEM = 1.58). There were no ENZ × TMR DM and ENZ × pre-incubation time interactions for rate of NDFD and ED ($P \geq 0.61$). In this in situ experiment, enzyme treatment increased ruminal rate and effective degradability of TMR NFD.

Key Words: enzyme, neutral-detergent fiber, rumen degradability

Ruminant Nutrition: Digestion and Metabolism

T135 Estimating daily energy flows in freestall-housed dairy cattle with automated data collection. P. M. Kedzierski*[1], G. C. Reyes[1], A. van der Hout[2], S. Mellors[1], L. E. Wright[1], D. J. Seymour[1], V. R. Osborne[1], and J. P. Cant[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Wageningen University and Research, Wageningen, the Netherlands.*

Remote, automated data collection may assist in the goal of precision feeding to reduce variation between individual animals. The objective of this study was to evaluate variation associated with integration of 3 different systems, each estimating different parts of the nutritive energy balance in dairy cows. Twenty-nine Holstein cows, 9 primiparous and 20 multiparous, ranging from 22 to 472 DIM were housed in a freestall pen and subjected to automatic data collection for a period of one month. Milk yield in a parlor was recorded at 0530 and 1730 h daily. Milk samples were collected 4 times weekly and analyzed for fat, protein and lactose content. Body weight and BCS were recorded twice daily using an automated scale and 3D imaging camera (DeLaval, Tumba, Sweden) as cows exited the parlor. Intake of a partial mixed ration based on corn and alfalfa silages was recorded continuously using Insentec feeders (Hokofarm, Marknesse, the Netherlands). Respiratory emissions of CO_2 and consumption of O_2 were recorded with a GreenFeed system (C-Lock Inc., Rapid City, USA), programmed to offer individual cows up to 440 g per visit of a pelleted protein supplement up to 12 times daily. Cows visited the GreenFeed 4.06 ± 1.82 (mean ± SD) times per day, with an average duration of 6.23 ± 1.05 min, for a total of 21.1 ± 9.9 min/d of recorded gas exchange. Diet and milk energy flows were estimated according to NRC (2001). Heat production was estimated from respiratory gas exchange. Variation in observations due to fixed effects of cow and day was estimated by 2-way ANOVA using PROC GLM of SAS (SAS software version 9.4; SAS Institute Inc., Cary, NC). NEL intake was 39.1 ± 5.4 Mcal/d, milk NEL output was 28.1 ± 7.1 Mcal/d, and heat production was 35.7 ± 3.8 Mcal/d. Root mean square errors due to cow and day, respectively, were 57.9 and 25.4% of the mean for NEL intake, 129.2 and 18.6% of the mean for milk NEL output, and 39.2 and 18.7% of the mean for heat production. Variation within cow was less than that between cows. Heat production was the least variable of the 3 energy flows. The integrated systems offer the potential to quantify energy balance in free-moving dairy cows.

Key Words: dairy cows, dietary energy, heat production

T136 Evaluation of two pH strips on urine from multiparous Holstein cows fed a partially acidified diet prepartum. L. K. Fehlberg* and F. C. Cardoso, *University of Illinois, Urbana, IL.*

Feeding a partially acidified diet prepartum (negative DCAD) can induce metabolic acidosis, thereby decreasing urine pH. The objective of this study was to determine the efficacy of 2 methods to measure urine pH and the effect of urine pH on prepartum dry matter intake (DMI) and milk yield of the first week postpartum. Thirty multiparous, multiparous Holstein cows were provided a DCAD diet (DCAD = −118 mEq/kg) beginning at 28 d relative to predicted calving (DRC) until calving. Urine pH was collected by manual massage at 5 time points (TP1, TP2, TP3, TP4 and TP5). Time point 1 was −30 DRC (±4.2; n = 23) before cows consuming the DCAD diet, TP2 was −23 DRC (±3.9; n = 30), TP3 was −16 DRC (±4.0; n = 30), TP4 was −9 DRC (±3.9; n = 30), and TP5 was −4 DRC (±3.0; n = 24). Cows were blocked (n = 5) by calving month. Urine pH was measured using Fisher pH sticks (pHF; Thermo Fischer Scientific, Waltham, MA), pHion balance test strip (pHI; pHion Balance, Scottsdale, AZ), or a portable pH meter (pHP; Accumet AP115, Thermo Fisher Scientific, Waltham, MA). Cows were categorized as HIGH (urine pH greater than the median) or LOW (urine pH lower than median) for each TP. Individual DMI and milk yield were obtained daily. Cows were milked 2×/d. Statistical analyses were performed using the MIXED and REG procedures of SAS (SAS Institute Inc., Cary, NC). Milk yield and DMI were not different between cows in LOW or HIGH using the pHP at any time point ($P > 0.10$). Dry matter intake tended ($P = 0.06$) to be greater in cows classified as HIGH (15.12 kg) compared with LOW (12.79 kg) at TP 1 when pHF and pHI were utilized but not pHP. Milk yield and DMI in cows were not different at TP 2, 3, 4, and 5 when urine was measured using pHF and pHI ($P > 0.10$). When all TP were pooled (n = 156), the pHP and pHF were positively correlated ($P < 0.01$; $R^2 = 0.95$; pHP = 0.88pHF+0.94). There was also a positive correlation between pHP and pHI ($P < 0.01$; $R^2 = 0.94$; pHP = 0.88pHI+0.98). In conclusion, pHF and pHI seems to be accurate methods of urine pH measurement. Additionally, the DCAD diet did not affect milk yield or DMI.

Key Words: dietary cation-anion difference, pH, urine

T137 Evaluation of the effects of feeding solvent-extracted carinata meal to dairy cows on lactation performance. R. D. Lawrence* and J. L. Anderson, *South Dakota State University, Brookings, SD.*

The objective of this study was to determine the effects of feeding solvent-extracted carinata meal, as a new potential protein source, to lactating cows on feed intake, milk production and milk composition. Twenty Holstein cows (12 primiparous and 8 multiparous) at 83.3 ± 0.05 DIM were used in a 12-wk randomized complete block design study. Treatments included: 1) control diet with 10% (DM basis) canola meal (CON) and 2) 10% carinata meal (CRM). Canola meal was used in the control diet for comparison because it is also a Brassica oilseed. Forage inclusion of the diet was similar (18% alfalfa hay, 33% corn silage), and most ingredients in the grain mix were similar, but the soybean meal and soyhulls varied slightly between treatments to make diets isocaloric and isonitrogenous. Both diets were fed as TMR using Calan gates. Cows were milked 2×/d. Body condition scores (BCS) and body weights (BW) were measured on 2 d during wk 0 and every 2 wk throughout. Milk samples were taken at each milking on the same days. Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significance was declared at $P < 0.05$. Dry matter intakes (25.0, and 25.3 kg/d, for CON and CRM, respectively; SEM = 0.72) were similar ($P > 0.05$) between treatments. Treatments had similar BW (675 and 670 kg; SEM = 5.5) and BCS (3.00, and 3.04; SEM = 0.04). Milk production (37.0, and 36.0 kg/d; SEM = 1.63), milk protein yield (1.25, and 1.23 kg/d; SEM = 0.06), and lactose yield (1.25, and 1.23 kg/d; SEM = 0.06) were similar between treatments but had treatment by wk interactions ($P < 0.05$). Feed efficiency (1.55, and 1.46; SEM = 0.08) was similar. The MUN (9.10, and 8.70 mg/dL; SEM = 0.30), SCC (56.50, and 82.00 10^5/mL; SEM = 0.59) and milk fat yield (0.93, and 0.88 kg/d; SEM = 0.10) was not different. Overall results indicate that 10% CRM can be fed to lactating dairy cows without detrimentally affecting milk production and composition. Based on this study, carinata meal is a high quality protein source for dairy cows and may be used in replacement of canola meal to maintain lactation performance.

Key Words: carinata meal, dairy cow, milk production

T138 Effect of fresh unsalable grocery waste substituted for corn grain on production in lactating dairy cows.

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Up to 40% of human food is wasted in the USA and food is the largest component of landfill waste. Unsalable fresh fruit and vegetables and bakery products at grocery stores provides a convenient point for collection and recycling into animal feed. Our hypothesis was that fresh grocery waste (Fruit Plus, Organix Recycling) would maintain milk production when substituted for ground corn in diets of lactating dairy cows. Forty-five multiparous Holstein cows were blocked by milk production and assigned to either control or Fruit Plus fed at 7.5% or 15% of diet DM in a randomized block design. Treatments were fed for 40 d and milk samples were collected weekly. Fruit Plus product was collected in a storage container at grocery stores and contained various unsaleable fruits, vegetables, and bakery products and was delivered to the farm approximately every 4 d. The product was stored on a concrete pad and average pH was 3.5 and was never greater than 4.5 through the entire experiment. Data were analyzed as a randomized block design in PROC MIXED with repeated measures. The model included the random effect of cow within treatment and the fixed effect of treatment, time and their interaction and the pretrial parameter value as a covariant. Subject was cow within treatment and repeated was time. There was a treatment by time interaction for DMI ($P < 0.001$) as 15% Fruit Plus decreased intake for the first 14 d, but increased intake after 28 d. There was no effect of treatment or interaction of treatment and time on milk yield or milk fat percent or yield. There was a treatment by time interaction on milk protein percent as 15% Fruit Plus decreased milk protein after d 21 (3.15 vs 3.01%). However, there was no effect of treatment on milk protein yield. Milk urea nitrogen was increased by 15% Fruit Plus (12.5 vs 15.7 mg/dL; $P < 0.01$). In conclusion, fresh grocery waste product can maintain milk yield at up to 15% of the diet when substituted for ground corn and provides an opportunity to increase efficiency of the food system.

Key Words: grocery, byproduct, alternate feeds

T139 The rumen degradability of palm date seeds when treated with sodium hydroxide and ultrasound processing.

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The effect of chemical treatment and ultrasound processing (sonication) time on the ruminal degradability of palm date seeds (PDS) were evaluated. The PDS were either incubated with 4% NaOH at 50°C for 6 h (T1) or subjected to sonication(900 W, 20 kHz, 100% power level) for 5 (T2), 10 (T3), 20 (T4), and 30 (T5) min in a metal reactor containing 4% NaOH solution. The treated PDS were then recovered, freeze-dried and grounded. A 3 g sample of the untreated PDS (control) or treated PDS (T1 to T5) were placed in Dacron bags and then incubated in the rumen of a cannulated Holstein dairy cow fed a high forage-based diet (80:20; forage: concentrate). After 24 h of incubation, the Dacron bags were removed from the rumen, washed several times with cold water and then analyzed for DM, NDF, and ash to estimate ruminal degradability. Data were analyzed using one-way ANOVA model of JMP. The NDF_{om} and OM degradability for control PDS averaged 24.7 and 22.8%, respectively and both increased ($P < 0.01$) to 36.3 and 32.1%, respectively, with the T1 treatment. The NDF_{om} degradability of PDS also increased ($P < 0.01$) with sonication averaging 44.2, 46.5, 49.3 and 53.5% for the T2, T3, T4, and T5 treatments, respectively and was highest ($P < 0.01$) with the T4 and T5 treatments. Similarly, the OM degradability of PDS was also increased ($P < 0.01$) with sonication averaging 49.7, 48.9, 45.9, and 46.3% for the T2, T3, T4, and T5 treatments, respectively, and they were all higher ($P < 0.01$) than the control and T1 treatments. In conclusion, our results showed that treating PDS with 4% NaOH increased their ruminal degradability and subjecting PDS to sonication further improved their degradability in the rumen.

Table 1 (Abstr. T139). The effect of treatments of the palm date seeds ruminal degradability

	Control	T1	T2	T3	T4	T5	MSE
NDF_{om}, %	23.5^{d}	33.6^{c}	44.2^{b}	46.5^{b}	49.3^{ab}	53.5^{a}	2.65
OM, %	23.3^{c}	29.5^{b}	45.9^{a}	46.3^{a}	49.0^{a}	49.7^{a}	2.03

$^{a–d}$Means with different letters within the same row are significantly different ($P < 0.05$).

Key Words: palm date seeds, chemical treatment, sonication

T140 Liver composition of dairy cows in two contrasting feeding strategies.

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The liver plays a key role regulating fatty acid metabolism and gluconeogenesis and failure to adapt during lactation may lead to decreased milk yield and pathologies in dairy cows. Thus, our aim was to study the effect of pasture grazing on changes in liver composition during lactation. Multiparous Holstein cows (n = 24, 664 ± 65 kgBW, 3.0 ± 0.4 BCS, spring calving) were assigned in a randomized block design to (G0) a total mixed ration (TMR) fed ad libitum (70% forage: 30% concentrate) or (G1) grazing plus supplementation from 0 to 180 d postpartum (DPP). The G1 cows grazed *Festuca arundinacea* or *Medicago sativa* in 2 (18 h) or 1 (6 h) session depending on heat stress (30 or 20 kgDM/cow/day) and were supplemented with 5.4 kgDM of a commercial concentrate or offered TMR (50% of G0 offer). From 180 to 250 DPP, all cows grazed *Festuca arundinacea* (10h; 30 kgDM/cow/day) and were offered TMR (50% of G0 offer). Liver biopsies and blood samples were collected at −14, 35, 60, 100, 180 and 250 DPP and protein, free glucose, glycogen and triglyceride concentrations were determined. Data were analyzed with a mixed model using repeated measures. Liver protein peaked ($P < 0.001$) at 100 DPP for both treatments. While free liver glucose increased ($P < 0.001$) only at 35 DPP, hepatic glycogen decreased ($P < 0.001$) in early lactation (35 DPP), increased during mid-lactation and dropped again at 250 DPP. Hepatic glycogen was higher for G0 than G1 cows (1.66 vs. 1.31 ± 0.07% m/m, $P < 0.01$). Glycogen mobilization at 35 DPP could have led to increased free glucose since the correlation was negative (r = −0.4, $P < 0.01$). Liver triglyceride concentrations tended to be affected ($P = 0.06$) by the interaction between treatment and DPP increasing only at 35 DPP for G1 (10.2 vs. 6.3 ± 0.7%, G1 vs. G0 respectively, $P < 0.05$) and remaining unchanged during the rest of lactation for both treatments. Plasma insulin correlated positively with glycogen (r = 0.3, $P < 0.01$) and tended to correlate negatively with triglyceride (r = −0.2, $P = 0.08$), confirming the role of this hormone in lipid and glucose metabolism. Our results suggest that liver composition is modified during lactation and nutrition may affect energy reserves.

Key Words: liver, dairy cow, grazing

T141 Effects of increasing exogenous carbohydrases levels on intake, milk yield and composition, and production efficiency of dairy cows.

J. Marques[1], L. Ghizzi[1], G. Silva[1], M. Dias[1], A. Nunes[1], L. Sakamoto[1], L. Fernandes[1], T. Silva[1], L. Gheller[1], N. Scognamiglio[1], C. Cortinhas*[2], T. Acedo[2], and F. Rennó[1], [1]*University of São Paulo, Pirassununga, São Paulo, Brazil,* [2]*DSM Nutritional Products, São Paulo, São Paulo, Brazil.*

Our objective in this study was to evaluate the effects of feeding increasing exogenous carbohydrases levels on dry matter intake, milk yield and composition, and production efficiency of lactating dairy cows. Eight rumen cannulated Holstein cows [27.8 ± 5.3 (mean ± SD) kg of milk yield per day, 182 ± 58.6 d in milk, 590 ± 85.9 kg of body weight, 2.78 ± 0.160 of body condition score] were blocked by parity and milk yield, divided in 2 replicated Latin squares 4 × 4 and randomly assigned to the following treatments: 1) Control, basal diet with no enzyme; 2) Carb 1 (carbohydrases): diet with 10 g of a blend of 2 enzymes (xylanase, Ronozyme WX; β-glucanase, Ronozyme VP, DSM Nutritional Products, Basel, Switzerland); 3) Carb 2: diet with 20 g inclusion of a blend of 2 enzymes (xylanase, Ronozyme WX; β-glucanase, Ronozyme VP); 4) Carb 3: diet with 30 g inclusion of a blend of 2 enzymes (xylanase, Ronozyme WX; β-glucanase, Ronozyme VP). Experimental diets were formulated according to NRC (2001), with a 50:50 roughage to concentrate ratio and corn silage as unique roughage source. Each experimental period included 14 adaptation days and 7 sampling days. Individual dry matter intake and milk production were recorded daily; milk samples were collected for 3 consecutive days, during the morning and afternoon milking. Data were analyzed using the MIXED procedure of SAS Inst (2001), using linear and quadratic contrasts to evaluate dose response of enzyme inclusion and control vs. enzyme contrast. Carbohydrase inclusion increased ($P = 0.027$) milk fat yield (1.00 vs. 1.06 kg/d), while tended to increase ($P \leq 0.071$) 3.5% fat-corrected milk yield (28.1 vs. 29.3 kg/d) and milk fat percentage (3.70 vs. 3.86) in contrast to control. Furthermore, carbohydrase tended to linearly increased ($P \leq 0.089$) 3.5% fat and energy-corrected milk production efficiency. There were no effects on feed intake, milk yield, milk protein and lactose content, and milk production efficiency. Therefore, exogenous carbohydrase inclusion increases milk fat yield and brings benefits to dairy cows performance.

Key Words: additive, milk fat, performance

T142 Addition of grape marc to dairy sheep diet improves milk yield and affects oxidative status of animals.

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The aim of this study was to evaluate the effect of diets containing dried grape marc and tomato pomace on productive performance, and milk and blood oxidative status. Twenty-four mid lactating Sarda ewes were used. One group of ewes received a total mixed ration as a control diet (CTR group) and other the same total mixed ration supplemented with 100 g/d of grape marc (GM group) or 100 g/d of tomato pomace (TP group) for 8 weeks. Daily intake, milk production, milk composition and blood parameters were affected by the inclusion of byproducts in the diet. Ewes supplemented with byproducts showed lower DM intake compared with CTR. The ewes fed GM produced more milk, protein and fat in milk than did the CTR group. The addition of TP did not affect production traits in comparison to CTR group. The blood parameters were within the physiological range for sheep in all groups, evidencing good health conditions of ewes throughout the experiment. GM group evidenced a higher antioxidant capacity measured either ferric reducing antioxidant power (FRAP) and including 2,2-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) in blood in comparison with the CTR. In conclusion, the GM at the inclusion level of 100 g/day, turned out to be an appropriate byproduct to improve milk yield and maintain animals health status. Research funded by Mignini-Petrini S.p.a. (Perugia, Italy) and Animal New Technology (ANT s.r.l.).

Key Words: dairy sheep, milk production, hematolocical parameters

T143 Switchgrass (*Panicum virgatum*) as a replacement for wheat straw in lactating dairy cow rations.

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Switchgrass (*Panicum virgatum*) is a native tall grass which has been researched as a novel species in the biomass industry, but also as a promising crop in the area of livestock feed. This study aimed to determine if switchgrass can be used as a replacement for wheat straw in lactating dairy cow rations in a crossover design. Holstein cows (n = 24, DIM = 129 ± 16.0) were randomly assigned a TMR containing equal amounts of either wheat straw (CON) or switchgrass (SGR) over 2 periods consisting of 17 d of adaptation and 4 d of sampling, where milk, blood, and feed samples were collected. Statistical analysis was conducted by a repeated measures analysis using PROC GLIMMIX of SAS with the fixed effect of treatment, day, and treatment × day, and the random effect of cow in the model for milk production, components and blood metabolites. DMI was consistent between groups (SGR = 25.1 ± 0.74, CON = 24.2 ± 0.74 kg/d; $P = 0.17$), but cows on the CON diet tended to produce greater daily milk yield (SGR = 34.2 ± 1.25, CON = 36.3 ± 1.24 kg/d; $P = 0.07$). Milk yield (kg) per kg of DMI was not significant between treatments but tended to be better for cows given the CON diet (SGR = 1.4 ± 0.12, CON = 1.6 ± 0.12; $P = 0.09$). Blood sample analysis for glucose, BHBA, and NEFA showed no differences between diets ($P \geq 0.32$). Fat yield between diets was consistent (SGR = 1.46 ± 0.064, CON = 1.54 ± 0.064 kg/d; $P \leq 0.01$), while protein was different (SGR = 1.09 ± 0.045, CON = 1.19 ± 0.045 kg/d; $P = 0.03$). Analysis of feed samples indicated no significant differences between the 2 diets in terms of NDF or NE_l (SGR = 33.5 ± 0.74, CON = 34.8 ± 0.74; $P = 0.26$; and SGR = 1.6 ± 0.01, CON = 1.6 ± 0.01% diet DM, $P = 0.29$, respectively); however, CP tended to be different between the diets (SGR = 7.5 ± 0.31, CON = 6.6 ± 0.31% diet DM; $P = 0.06$). The switchgrass crop used in this trial was infected with head smut; however, these results suggest that replacement of wheat straw with switchgrass at an inclusion of 0.5 kg DM/d does not affect the metabolic health or fat production of Holstein dairy cows. Future trials should be repeated with different switchgrass crops to determine the effect of uninfected switchgrass under similar conditions.

Key Words: switchgrass, milk production, DMI

T144 Effects of metritis treatment strategies on metabolites and rumination and activity of dairy cows. V. R. Merenda*[1], D. Lezier[1], A. Odetti[1], C. C. Figueiredo[1], C. A. Risco[1], and R. C. Chebel[1,2], [1]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Objectives of the study were to compare the effects of 2 metritis treatments on metabolite concentrations and rumination/activity before and after diagnosis. Cows were examined at 4, 6, 8, 10 and 12 DIM for metritis (fetid, watery, red/brown uterine discharge). On the day of enrollment (d 0), cows were paired by parity and severity of metritis [metritis vs. puerperal metritis (rectal temperature ≥ 39.5°C)] and assigned randomly to Ampicillin (AMP) or Ceftiofur Crystalline Free Acid (CCFA) treatments. Cows enrolled in the AMP (n = 307) treatment were moved to a hospital pen where they were treated once daily for 5 d and were moved back to their original pen 72 h after the last treatment (d 7). Cows enrolled in the CCFA (n = 306) treatment remained in their original pen and received 2 doses of CCFA, 72 h apart. Contemporary healthy cows (POS = 636) were paired by parity with metritic cows and were enrolled in the study. A subgroup of cows (AMP = 95, CCFA = 106, POS = 122) had blood sampled on d 0, 1, 2, 6, 7, 8, and 14 after enrollment for determination of glucose, nonesterified fatty acid (NEFA), and BHB concentrations. Automated devices that record rumination/activity were fitted on a subgroup of animals (AMP = 102, CCFA = 90, POS = 416) 21 d before expected calving date. Cows treated with AMP had greater glucose concentration (67.8 ± 5.1 vs. 64.4 ± 5.1 mg/dL; $P < 0.01$) and reduced BHB concentration (750.8 ± 38.5 vs. 919.7 ± 36.8 μmol/L; $P < 0.01$) from d 1 to 8 than cows treated with CCFA. Concentration of NEFA from d 1 to 8 did not ($P = 0.85$) differ between treatments. Treatment ($P = 0.74$) and the interaction between treatment and day relative to enrollment ($P = 0.15$) did not affect rumination. The interaction between treatment and day relative to enrollment affected ($P < 0.01$) activity because on d 7 (458.2 ± 4.6, 432.3 ± 4.0 arbitrary units) and 8 (445.0 ± 4.7, 428.5 ± 5.0 arbitrary units) post-enrollment cows treated with AMP had greater activity than cows treated with CCFA. In conclusion, treatment of metritic cows with AMP vs. CCFA mildly altered metabolic parameters and activity, but their biological significance are likely negligible.

Key Words: metritis therapy, metabolites, behavior

T145 Energy utilization in lactating Jersey cows consuming a mixture of distillers dried grains with solubles and straw in replacement of alfalfa hay. A. L. Knoell*[1], J. V. Judy[1], H. C. Wilson[1], K. J. Herrick[2], S. C. Fernando[1], and P. J. Kononoff[1], [1]*University of Nebraska-Lincoln, Lincoln, NE,* [2]*POET Nutrition LLC, Sioux Falls, SD.*

In some areas of the world irrigation is needed to grow alfalfa and the use of crop residues and feed coproducts in dairy rations may represent opportunities to reduce the dairy industry's use of water. A study using indirect calorimetry and 12 multiparous lactating Jersey cows (BW = 447.5 ± 43.7 kg; DIM = 71 ± 11 d, mean ± SD) was conducted to determine the effect of feeding a mixture of straw and distillers dried grains with solubles (DDGS) in replacement of alfalfa hay on milk production and energy utilization. A replicated 4 × 4 Latin square design was used to evaluate the replacement of alfalfa hay with a coproduct mixture (CoP) containing approximately 1/3 wheat straw and 2/3 DDGS. The experimental treatments were as follows (proportions on a DM basis): a control diet (CON) containing 18.2% of alfalfa hay, a low coproduct diet (LCP) that contained 8.1% of CoP, a medium coproduct diet (MCP) that contained 16.3% of CoP, and a high coproduct diet (HCP) that contained 24.3% of CoP. No differences ($P \geq 0.307$) were observed for dry matter intake, milk, and protein yield averaging (mean ± SEM) 19.5 kg ± 0.60, 29.6 kg ± 0.91, and 1.05 kg ± 0.04, respectively. Increasing the proportion of CoP tended to result in a quadratic ($P = 0.06$; SEM = 1.06) effect in energy corrected milk which increased from 37.6 to 39.0 in CON and MCP but then decreased to 36.6 in HCP. Increasing the proportion of CoP linearly (0% to 24% total DM) decreased free water intake ($P = 0.02$; 17.2% reduction) and methane production ($P < 0.01$; 19.7% reduction). The concentration of gross energy in the diet increased ($P < 0.01$) linearly with the inclusion of CoP with the concentration of digestible energy ($P = 0.09$) and metabolizable energy tending to increase ($P = 0.07$) linearly with the inclusion of CoP. The concentration of net energy balance was not observed to be affected by treatment. Results of this study indicate that alfalfa hay with a mixture of straw and DDGS can maintain milk production and dry matter intake but the replacement with the CoP mixture may result in some differences in energy utilization mostly driven by effects on methane production.

Key Words: coproducts, energy utilization, indirect calorimetry

T146 Differences in mammary gland uptake of post-absorptive energy metabolites may contribute to residual feed intake variation. M. J. Martin*, S. J. Erb, E. T. Ronk, S. J. Bertics, M. R. Moede, K. A. Weigel, and H. M. White, *University of Wisconsin Madison, Madison, WI.*

Feed efficiency has become a strong driver for dairy cattle selection, but the underlying physiological sources of between-cow variation are unknown. One possible contributor could be differences in post-absorptive nutrient utilization. The objective of this study was to compare arterial-venous differences (AVdiff) of circulating energy metabolites in cows with high and low residual feed intake (RFI). Mid-lactation primi- and multiparous (PP, MP) Holstein cows (n = 61/replicate) were housed in a freestall pen with Insentec feeders (2 replications; 45d). Residual feed intake was calculated (PROC MIXED, SAS 9.4) by regressing energy intake on milk energy output(parity), median DIM(parity), metabolic BW(parity), and ΔBW(parity). Plasma samples were collected concurrently from the tail vessel (TV) and the subcutaneous abdominal vein (MV). Plasma was analyzed for glucose (glc), nonesterified fatty acids (NEFA), BHB, and triglycerides (TG). Cows within the top and bottom third (PP n = 17/group; MP n = 23/group) were deemed high RFI (least feed efficient) and low RFI (most feed efficient). The effect of RFI group on metabolite concentration and AVdiff was determined using PROC MIXED accounting for the fixed effect of group, parity, and group × parity, and the random effect of replicate. Concentration of TV NEFA ($P = 0.09$) and TG ($P = 0.013$) were greater in low RFI cows. AVdiff of NEFA (0.016 vs −0.001 ± 0.022 mM) and TG (6.0 vs 4.8 ± 0.4 mg/dL) were also greater ($P < 0.05$) for lowRFI cows. Glucose (16.9 vs 12.9 ± 1.8 mg/dL) and TG (6.1 vs 4.7 ± 0.4 mg/dL) AVdiff were greater ($P < 0.02$) for MP cows. In MV, concentration of glc, TG, and BHB were greater ($P < 0.02$) in PP animals. Interestingly, the percent of TV glc and TG sequestered by the mammary gland was greater ($P < 0.01$) in MP than PP cows. The observed parity differences in mammary uptake of metabolites suggests altered post-absorptive metabolism in PP and MP animals. Differences in mammary gland uptake of NEFA and TG between low and high RFI groups could contribute to individual cow variation in feed efficiency.

Key Words: feed efficiency, nonesterified fatty acids, triglyceride

T147 Methyl donor supplementation strategy in fresh cows. A. Van De Kerchove[1], A. Delaquis[2], F. Mueller[3], T. Steen[4], J. Guyader[5],

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Betaine and choline were compared as supplementation strategies in fresh cow diets. Sixty multiparous cows at 14 d in milk were housed and randomly assigned to a treatment in a randomized design (4 cows per pen) across 15 pens. Supplements were control (CON), Reashure choline (CHOL, 55 g/cow/d), or betaine (BET, 25 g/cow/d) and were fed for 14 weeks. Cows were milked 3 times per day with daily individual milk yield averaged by week. Dry matter intake (DMI) per pen was averaged by week as well as individual milk composition was averaged by week (2 d average). Individual bodyweight and condition score occurred at initiation, d 28, d 70, and d 98 in lactation. Plasma concentrations of albumin, aspartate amino transferase, urea nitrogen (PUN), nonesterified fatty acids (NEFA), BHB, and haptoglobin were determined at d 0, d 28, and d 77. Proc GLIMMIX procedure of SAS with repeated measures over time was utilized to analyze the data. Differences were noted at $P < 0.05$ and trends at $P < 0.15$. The BET treatment reduced pen DMI compared with CON cows (25.35 vs 26.18 kg/d; $P < 0.01$) which led to higher milk fat (3.48 vs. 3.72%; $P < 0.001$) and lower milk protein percentage (2.89 vs. 2.83%; $P < 0.02$). These differences led to a similar ECM but greater 3.5 FCM yield over CON cows (53.84 vs. 55.42 kg/d; $P < 0.001$) for the BET treatment. Cows fed BET had greater 3.5 FCM (2.25 versus 2.10) and ECM (2.19 vs. 2.08) efficiencies ($P < 0.001$) over CON cows. The CHOL treatment increased milk yield (55.76 vs. 53.59 kg/d; $P < 0.001$), ECM yield (55.52 vs. 54.13 kg/d; $P < 0.001$), and elevated gross protein efficiency (35.27 vs. 34.18%) over BET. No differences were noted in initial or final body weight or condition as well as days to peak or peak milk yield. Choline lowered PUN levels (13.59 vs. 12.21 mg/dL) compared with CON and BET fed cows while BET increased NEFA (0.53 vs. 0.39 mEq/L) and BHB (8.91 vs. 7.03 mg/dL) levels compared with CON and CHOL fed cows. Choline appears to function via enhanced metabolism while betaine exerts an intake effect. More research is required to understand how to optimally feed betaine as well as to maximize choline inclusion.

Key Words: ketosis, fresh cow, betaine

T148 Effect of adding water to a high-straw dry cow diet on the intake and behavior of Holstein dairy cows.

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The objective of this study was to determine the effect of adding water to a high-straw dry cow diet on intake and behavior of dairy cows. Multiparous Holstein cows (n = 40) were enrolled at dry off (~45 d before expected calving) and assigned to 1 of 2 dietary treatments, a high-straw (35% wheat straw on DM basis; 11.6% CP, 1.35 Mcal/kg NE_L) dry cow diet with: 1) no water (CON; n = 20; DM = 53.6%) or 2) water added to decrease the DM by ~10% (WAT; n = 20; DM = 45.4%). At calving all cows were fed the same lactating TMR (45.2% DM, 14.0% CP, 1.64 Mcal/kg NE_L) and followed for 28 d. DMI, feeding behavior, and rumination activity were recorded automatically. TMR and ort samples were collected 2×/wk to determine differences in sorting. A particle separator was used to separate feed samples into 4 fractions: long (>19 mm), medium (<19 mm, >8 mm), short (<8 mm, >4 mm), and fine (<4 mm) particles. Feed sorting was calculated as actual intake of each particle fraction expressed as a % of predicted intake. Data were analyzed in mixed-effect linear models, treating day as a repeated measure. DMI (CON = 13.8 ± 0.3, WAT = 14.2 ± 0.2 kg/d) and rumination (CON = 534.4 ± 9.1, WAT = 516.6 ± 8.1 min/d) did not differ ($P \geq 0.17$) between treatments. WAT cows tended to spend less time feeding (173.5 ± 11.7 vs 205.1 ± 12.3 min/d; $P = 0.07$) and have shorter meals (51.9 ± 2.8 vs 60.7 ± 3.0 min/meal; $P = 0.04$) compared with CON cows, although the frequency of meals did not differ between treatments (CON = 5.5 ± 0.27, LFI = 5.8 ± 0.24; $P = 0.52$). WAT cows consumed their feed faster (0.09 ± 0.001 vs 0.08 ± 0.001 kg DM/min; $P = 0.05$) compared with CON cows. Regardless of treatment, cows sorted against the longest ration particles, with cows on the CON sorting more against these (81.8 ± 2.3 vs 95.6 ± 2.4%; $P = 0.002$). Treatment did not influence how cows sorted for or against the other particle fractions. Post-calving DMI and feeding behavior were not affected by dry diet treatment. The results suggest that decreasing the moisture content of high-straw dry cow diets, by adding water, may reduce sorting against the longest ration particles, which may help promote consistency in targeted nutrients consumed during the dry period.

Key Words: dry cow diet, feeding behavior, sorting

T149 Effect of wheat straw chop length in high-straw dry cow diets on intake, behavior, and health of dairy cows across the transition period.

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The objective of this study was to determine the effect of wheat straw chop length in a high-straw dry cow diet on intake, feed sorting, and health of dairy cows. Holstein cows (n = 40) were enrolled at dry off (~45 d before expected calving) and assigned to 1 of 2 treatments, a high-straw (29% wheat straw on DM basis; 13.2% CP, 1.5 Mcal/kg NE_L) dry cow diet with straw chopped with a: 1) 10.16cm screen (Long; n = 20), or 2) 2.54cm screen (Short; n = 20). At calving all cows were fed the same lactating TMR (16.0% CP, 1.64 Mcal/kg NE_L) and followed for 28 d. DMI was recorded automatically, reticulorumen pH was recorded using wireless telemetric probes, and blood BHB was recorded cow-side. TMR and ort samples were collected 2x/wk to determine differences in sorting. Feed samples were separated into 4 particle fractions: long (>19 mm), medium (<19 mm, >8 mm), short (<8 mm, >4 mm), and fine (<4 mm) particles. Feed sorting was calculated as actual intake of each particle fraction expressed as a % of predicted intake. Data were analyzed in mixed-effect linear models, treating day as a repeated measure. To model the day-to-day changes in DMI and pH around calving, an analysis of covariance was conducted. Short cows had greater DMI (15.6 vs 15.0 kg/d; SE = 0.16; $P = 0.02$) in the dry period, while Long cows experienced a more rapid drop ($P < 0.05$) in DMI in the week before calving. Regardless of treatment, during the dry period, cows sorted against the long and in favor of the short particles; cows on the Long treatment sorted to a greater extent than cows on the Short treatment (80.2 vs 88.4%; SE = 2.0; $P \leq 0.01$). Across treatments, cows sorted in favor of the long particles (105.9 ± 2.7%; $P = 0.01$) and against the fine particles (91.8 ± 1.8%; $P = 0.01$) during wk 1 post-calving. There were no differences in rumen pH between treatments in the dry and lactating period ($P \geq 0.6$), although Long cows tended to have a greater decline in rumen pH in wk 1 post-calving ($P = 0.07$). Long cows had higher BHB in the wk 3 post-calving (1.3 ± 0.11 vs 0.8 ± 0.10 mmol/L; $P = 0.05$). The results suggest that reducing the chop length of straw in dry cow diets may improve pre-calving intake, reduce feed sorting, and promote greater health across the transition period.

Key Words: dry cow diet, sorting, health

T150 Using National Research Council equations to estimate respiratory carbon dioxide output in dairy cattle.

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Lactating dairy cows partition absorbed nutrients to milk and body components and catabolism. Nutrient catabolism results in O_2 consumption and CO_2 production which can be measured remotely and automatically in individual free-moving cows. To manipulate the partitioning of nutrients into milk vs. other sinks, a model that estimates each of the major nutrient flows would be useful. The objective of the present study was to modify the National Research Council (2001) model to estimate respiratory CO_2 output by lactating dairy cows. The predictions were compared against observations collected from 29 Holstein cows (22 to 444 DIM; 9 primiparous and 20 multiparous) housed in a freestall pen for a 29-d period, where daily DMI, milk yield and composition, BW, BCS, and respiratory O_2, CO_2 and CH_4 exchanges were monitored automatically. NRC (2001) equations were used to estimate digestible carbohydrate, fat and protein supplies from observed DMI and ration composition. Rates of incorporation of glucose into lactose, acetate into milk fat, fatty acids into milk fat, and amino acids into milk protein were estimated from observed milk and component yields. Incorporation of acetate into body fat and amino acids into body protein was estimated from the surplus of NEL between intake and production + maintenance. The balance of absorbed glucose, fat and amino acid was assumed to be catabolized, resulting in O_2 consumption and CO_2 production according to established stoichiometry. Net catabolism of glucose to acetate was assumed to occur in both the GI tract and the cow body, where observed CH_4 production determined the former rate. Average daily CO_2 production by cows on trial was 14567 ± 1700 g/d and predicted CO_2 output was 20619 ± 3234 g/d with a root mean square prediction error (MSPE) of 6729 g/d. Decomposition of MSPE indicated that 80.9% was due to mean bias indicating an overestimation of CO_2 production; 0.2% of the MSPE was due to slope bias and 18.9% was attributed to random disturbance. The modified NRC model offers a means to describe nutrient partitioning that can be applied to optimize nutrient supplementation on dairy farms.

Key Words: carbon balance, nutrient partitioning, precision feeding

T151 Effects of feeding blend pellet products (based on pulse screenings, feed additive, carinata meal, and canola meal) on lactational performance in high-producing dairy cows.

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The objectives of this study were to examine the effect of feeding newly developed blend pelleted products (BPP) based on carinata meal (BPPCR) or canola meal (BPPCN) in combination with pulse screenings (low grade of pulse seeds) and lignosulfonate on lactation performance and ruminal degradation kinetics in high-producing dairy cows. In this study, 9 mid lactating (3 canulated + 6 non-cannulated) Holstein cows (body weight: 679 ± 124 kg; days on milk = 96 ± 22; average parities = 3) were randomly assigned to one of the following 3 dietary treatments: T1 = control diet (Con; common barley-based diet in western Canada); T2 = basal diet supplemented with 12%DM BPPCR (carinata meal 71.4% + pea screenings 23.8% + lignosulfonate 4.8%DM), and T3 = basal diet supplemented with 13%DM BPPCN (canola meal 71.4% + pea screenings 23.8% + lignosulfonate 4.8%DM) in triplicate 3 × 3 Latin square design. Each experimental period lasted for 21 d with14 d for adaptation and 7 d sampling. The data were analyzed using the Mixed model procedure in SAS 9.4 with the analysis triple LSD model including treatment, square, period, and cows effects. The treatment differences were compared using Tukey method. The results showed that there were no differences ($P > 0.05$) between treatments in milk yield (averaging 47.4 kg/d; SEM = 1.56) and fat corrected milk (averaging 46.3 kg/d, SEM = 1.60). There was no effect ($P > 0.05$) of dietary treatments on milk composition or milk component yield. The feed efficiency expressed as fat corrected milk / DMI was not affected ($P > 0.05$) by the treatments (averaging 1.8; SEM = 0.05). There was no effect ($P > 0.05$) of the blend pelleted products on the ruminal degradation kinetics of DM and CP. The Implication of this study is that the blend pelleted products based on carinata meal as a new co-product from bio-fuel processing industry is equal to the other pelleted products based on canola meal as a protein source for dairy cattle without affecting performance and rumen degradation kinetics of feed in high producing dairy cows.

Key Words: blend pellet products with carinata or canola meal, lactating dairy cow, pulse screenings

Ruminant Nutrition: Feed Additives 2

T152 *Bacillus subtilis* and *Bacillus licheniformis* used as probiotics to enhance lactation performance and milk branched-chain fatty acids in dairy cows. J. Lamontagne*[1], D. Rico[1,2], R. Gervais[1], and P. Chouinard[1], [1]*Université Laval, Québec, QC, Canada,* [2]*Centre de recherche en sciences animales de Deschambault, Deschambault, QC, Canada.*

Branched-chain fatty acids (BCFA) are generating interest for their benefits on human health particularly for their prebiotic effect on newborn gastrointestinal tract. Those fatty acids are synthesized by ruminal bacteria and incorporated into milk, the latter being the main source of BCFA in the North American diet. Some *Bacillus* genus species are used in animal production as direct-fed microbials and are known to synthesize BCFA to form their cell walls. Those probiotics have been shown to increase milk production and composition in small and large ruminants. To confirm that probiotics from the *Bacillus* genus can increase cow performance and affect milk fatty acid composition, 6 multiparous cows fitted with rumen cannula were used in a randomized replicated crossover design. Cows either received 200 g/d of whey powder as a control or 200 g/d of Bioplus 2B (Chr. Hansen, Milwaukee, WI), a commercial direct-fed microbial of *Bacillus subtilis* and *Bacillus licheniformis*, representing a daily dose of 6.4×10^{11} cfu using whey powder as carrier. The 2 experimental periods lasted 14 d and a 7-d washout period was observed after each one. Samples were collected at d 0, 13 and 14 of each period. Milk production, composition and fatty acid profile as well as ruminal parameters and microbiota were evaluated. Data from d 0 were used as covariate. Treatments did not affect productivity and milk composition. The use of Bioplus 2B increased the relative concentration of *anteiso* 13:0 and *anteiso* 15:0 ($P < 0.05$) and tended to increase total concentration of BCFA ($P < 0.10$) compared with control. Treatments did not affect ruminal pH, ammonia nitrogen and concentrations of acetate, propionate and butyrate. However, Bioplus 2B increased concentration of isovalerate ($P < 0.05$) and tended to increase the concentration of isobutyrate ($P < 0.10$). This trial indicates that milk fatty acid profile is sensitive to ruminal microbiota modifications. Probiotics of the *Bacillus* genus could be used as part of a BCFA enhancing protocol to increased those fatty acids in dairy products.

Key Words: direct-fed microbial, branched-chain fatty acid, milk fat

T153 A postbiotic from *Aspergillus oryzae* decreased the inflammation response of lactating dairy cows exposed to heat stress. J. Kaufman*[1], H. Bailey[1], P. De Toledo Shimoda[1], F. Bargo[2,3], I. Ipharraguerre[4], G. Pighetti[1], and A. Ríus[1], [1]*University of Tennessee, Knoxville, TN,* [2]*BioZyme Inc., St. Joseph, MO,* [3]*Universidad de Buenos Aires, Buenos Aires, Argentina,* [4]*University of Kiel, Kiel, Germany.*

The objective of this study was to evaluate the effect of a postbiotic additive from *Aspergillus oryzae* (AO) on the inflammation response in lactating dairy cows exposed to heat stress. Thirty-six Holstein cows (105 ± 23 SD days in milk, 714 ± 23 kg body weight) were used in a completely randomized design and randomly assigned to 1 of 3 treatments for 36 d: 0 g/d (control; CTL), 3 g/d (low), and 6 g/d (medium) of the AO postbiotic (Biozyme Inc., St. Joseph, MO). A 41% forage and 59% concentrate TMR (18.1% CP, 33.0% NDF, 1.61 Mcal/kg NEL) was individually fed twice daily, and AO was top-dressed twice daily. Cows experienced warm climate during June and July 2018. Cows were provided with heat abatement of fans and misters from d 1 to 10 (period 1) and without heat abatement from d 11 to 36 (period 2). Acute phase proteins [haptoglobin, serum amyloid A, and lipopolysaccharide (LPS) binding protein] were determined from plasma collected on d 11 and 35. On d 36, 2 whole blood samples were collected for an ex-vivo challenge with or without LPS (5 µg/µL). The expression of IL-1β, IL-6, and TNF-α was measured using qPCR. Three analyses were conducted on cytokine expression: 1) no LPS stimulation, 2) LPS stimulation, and 3) ratio of LPS to no LPS stimulation. Mild heat stress was attained in period 1, whereas heat stress intensity was increased in period 2 (temperature-humidity index = 74.6 ± 2.4 and 77.3 ± 4.2 [mean ± SD]). In period 1, AO quadratically decreased ($P = 0.03$) LPS binding protein concentrations in plasma by 25.7%. In period 2, AO quadratically decreased ($P = 0.01$) serum amyloid A concentrations in plasma by 65.6% and tended to quadratically decrease ($P \leq 0.10$) haptoglobin and LPS binding protein concentrations by 35.4 and 23.3%, respectively. From the ex vivo LPS challenge, AO linearly decreased ($P = 0.02$) the IL-6 expression ratio (LPS to no LPS stimulation) by 65.6%. In summary, AO decreased markers of inflammation and cytokine production in cows exposed to heat stress. These findings may be associated with improvements of health in cows exposed to heat-stress.

Key Words: heat stress, inflammation, prebiotic

T154 A commercially available yeast culture alters VFA production in a ruminal in vitro fermentation system. S. Armstrong*, S. Bascom, and D. McLean, *Phibro Animal Health Corporation, Teaneck, NJ.*

The objective of this study was to evaluate the effect of a commercial yeast culture, [Cellerate Culture Classic Plus (CC+), Phibro Animal Health Corp.] on volatile fatty acid (VFA) production, in vitro dry matter digestibility (IVDMD), and kinetics of gas production using an in vitro rumen fermentation system and lactating cow TMR. Bottle (250 mL) was considered the experimental unit; each bottle contained TMR (1.4 g) and treatment in 100 mL of buffered rumen fluid. Treatments were: CNTL (no additive) and CC+ (14 mg /bottle). All treatments were incubated in triplicate for 24 h at 39°C and constant agitation (60 rpm). Bottles were capped with units to capture temperature and pressure every 15 min (RF1, Ankom Technology, Macedon, NY). At the end of incubation, final pH and temperature measurements were taken; a sample of rumen fluid was frozen for analysis of VFA. Triplicate 50-mL centrifuge tubes were incubated in parallel with larger bottles to asses IVDMD; tubes contained 0.5 g TMR, 5 mg CC+ and 32 mL buffered rumen fluid. Three replicate incubation days were performed, data were analyzed using PROC GLIMMIX of SAS; significance was defined as $P \leq 0.05$ and tendencies as $0.05 < P \leq 0.10$. Treatment did not affect gas production kinetics, final pH, final temperature, IVDMD, isobutyric, isovaleric, or valeric acid (mmol/L) concentrations, or VFA production analyzed on a proportional basis. Including CC+ (vs CNTL) increased total acetic acid (70.09 vs 68.12 ± 4.01 mmol/L; $P = 0.02$), butyric acid (9.51 vs 9.32 ± 0.56 mmol/L; $P = 0.04$) and total VFA concentrations (105.38 vs 102.69 ± 7.12 mmol/L; $P = 0.02$). Our results indicate adding yeast culture to a lactating cow diet may increase VFA production without altering kinetics of gas production, pH or temperature in an in vitro system which may modulate ruminal fermentation.

Key Words: yeast culture, volatile fatty acid, rumen

T155 Supplementation of dairy cows with a blend of direct-fed microbes: Performance and digestion. L. N. Resende[1], R. B. Silva[2], R. A. N. Pereira[3,2], and M. N. Pereira*[1,2], [1]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [2]*Better Nature Research Center, Ijaci,*

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Probiotics (PBT) may improve diet digestibility and lactation performance of dairy cows. This experiment evaluated the effect of a liquid supplement containing a blend of viable lactic acid bacteria, spore forming bacteria, and yeast (*S. cerevisiae*) cultured in consortium (Global Saúde, Brazil/SCD Probiotics, USA) on digestion, intake, and lactation performance of dairy cows. Twenty-six Holstein cows (185 ± 141 DIM) were individually fed a standard TMR for 14 d and treatments control or PBT (3.5 mL/kg of TMR DM) for 56 d, in a covariate adjusted randomized block design with repeated measures over time. The PBT increased DMI (23.5 vs 22.5 kg/d. $P < 0.01$, SEM 0.37) and tended to increase the yields of milk (30.0 vs 29.4 kg/d. $P = 0.06$, SEM 0.50) and lactose (+ 60 g/d. $P = 0.09$). Energy-corrected milk to DMI ratio was reduced by PBT (1.25 vs 1.31. $P = 0.02$, SEM 0.033). Milk solids concentration and yield did not differ ($P \geq 0.28$). Cows fed PBT tended to have higher BCS (3.02 vs 2.91. $P = 0.08$, SEM 0.039), but BW did not differ (641 kg. $P = 0.27$). The PBT tended to reduce the total-tract digestibility of the non-fibrous OM (81.2 vs 84.1%. $P = 0.08$, SEM 1.11) whereas starch (90.7%) and NDF (48.4%) digestibilities did not differ ($P \geq 0.20$). Molar proportion of butyrate in ruminal fluid tended to be reduced by PBT (9.4 vs 10.4%, $P = 0.06$, SEM 0.40) as well as total protozoa concentration ($P = 0.10$). The acetate to propionate ratio (3.1. $P = 0.89$) and the ruminal microbial yield estimated by the daily urinary allantoin excretion ($P = 0.21$) did not differ. The PBT reduced the proportion of daily intake in the morning (35.1 vs 40.4%. $P = 0.02$, SEM 1.52) and increased the proportion in the afternoon (45.9 vs 41.9%. $P = 0.03$, 1.23). Rumination and ingestion behaviors did not differ ($P \geq 0.16$). The PBT reduced blood urea-N at 0900 h ($P = 0.01$) and tended to reduce at 1630 h ($P = 0.06$) and 2300 h ($P = 0.09$), but did not affect milk urea-N (18.6 mg/dL, $P = 0.47$, SEM 0.44). The PBT induced a greater increase in DMI than in milk yield, decreasing feed efficiency, and had effects on digestibility and ruminal fermentation profile.

Key Words: probiotic, direct-fed microbes, yeast

T156 Supplementation of dairy cows with a blend of direct-fed microbes: Thermoregulation and immunity.

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Probiotics (PBT) may have systemic effects on dairy cows. This experiment evaluated the effect of a liquid supplement containing a blend of viable lactic acid bacteria, spore forming bacteria, and yeast (*S. cerevisiae*) cultured in consortium (Global Saúde, Brazil/SCD Probiotics, USA) on body temperature and immune response of dairy cows. Twenty-six Holstein cows (185 ± 141 DIM) were individually fed a standard TMR for 14 d and treatments control (CTL) or PBT (3.5 mL/kg of TMR DM) for 56 d, in a covariate adjusted randomized block design with repeated measures over time. Immunity in response to a virus/leptospirosis vaccine on d 36 (D0) was evaluated. Rectal temperatures were increased by PBT at 0900 h (38.3 vs 38.1°C. $P < 0.01$, SEM 0.05), 1530 h (38.8 vs 38.5°C. $P = 0.01$, SEM 0.03), and 2200 h (38.5 vs 38.2°C. $P = 0.02$, SEM 0.07). The frequency of cows with rectal temperature >39.2°C at 1530 h was 18.2% on PBT and 6.3% on CTL ($P < 0.01$). Skin temperature tended to be increased by PBT at 0900 h (34.1 vs 33.8°C. $P = 0.10$, SEM 0.13) and was increased at 2200 h (35.1 vs 34.7°C. $P = 0.02$, SEM 0.12). Sweating rates in wk 4 (90.4 vs 66.9 g/m^2/h. $P = 0.05$, SEM 7.33) and 5 (103.9 vs 72.1 g/m^2/h. $P = 0.01$, SEM 10.34) were increased by PBT. Time spent laying down was reduced by PBT (742 vs 813 min/d. $P = 0.04$, SEM 29.3). Respiratory rate did not differ ($P \geq 0.34$). Interleukin 4 in serum tended to be reduced by PBT on d 0 (779 vs 859 pg/mL. $P = 0.10$, SEM 38.2) and did not differ on d 7 and 14 relative to vaccination ($P \geq 0.26$). Interleukin 12 was reduced by PBT on d 7 (611 vs 709 pg/mL. $P = 0.02$, SEM 47.4), but did not differ on d 0 and d 14 ($P \geq 0.27$). Interferon gamma and Interleukin 10 did not differ ($P \geq 0.20$). At d 0, the concentration of monocytes as a proportion of leukocytes tended to be increased by PBT (3.8 vs 5.7%. $P = 0.10$, SEM 0.83). Milk SCC was increased by PBT (49,500 vs 67,000 cells/mL. $P = 0.03$, SEM 6,250). The increase in DMI and milk yield induced by PBT was associated with increased body temperature and sweating rate at similar respiration rate, alteration in immunity markers, and increased SCC of cows with low SCC (<100.000 cells/mL), suggesting increased immunity of the mammary gland.

Key Words: probiotic, direct-fed microbes, yeast

T157 Evaluation of supplemental autolyzed yeast on ruminal pH, fecal pH, and VFA response from Holstein cows fed a high starch diet.

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High starch diets are known to reduce rumen pH, and cause shifts in the VFA profile, resulting in potential health problems. The aim of this study was to investigate if the addition of autolyzed yeast (AY; *Saccharomyces cerevisiae*) supplementation in a high starch lactation diet would improve rumen pH, fecal pH, and shifts in VFA response. Fifteen rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (LS0; control), high starch diet without AY (HS0), high starch diet with either 15 g (HS15), 30 g (HS30), or 45 g (HS45) of AY supplementation. The period of 21 d was divided into the adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Rumen fluid was collected via rumen cannula on d 15 and 16 in relation to feeding at 1400 h (time point 0). Rumen samples were extracted at 0, 4, 8, 12, 16, 20, and 24 h relative to feeding. Cows in HS0 experienced lower ruminal pH (6.10 vs. 6.38; $P < 0.0001$), nadir pH (5.53 vs. 5.74; $P < 0.0001$), and fecal pH (6.71 vs. 6.95; $P = 0.042$) compared with LS0. The addition of AY increased rumen pH ($P = 0.04$), and nadir pH ($P = 0.009$), compared with HS0 with no effect on fecal pH. Supplementing AY reduced individual VFA proportions of acetate, isobutyrate, and isovalerate ($P = 0.02$;$P = 0.0004$ and $P = 0.002$, respectively) when compared with cows in HS0. Total VFA proportion was greatest (136.71mmol/L; $P = 0.0005$) in cows fed HS0 compared with LS0. Total VFA proportions were greater for propionate (23.87 vs. 20.75%; $P < 0.0001$) and valerate (1.50 vs.1.35%; $P = 0.0001$) for cows in HS0 than LS0. Supplementing AY positively increased total propionate proportion ($P = 0.002$) and negatively decreased total acetate *(*$P < 0.0001$), isobutyrate, ($P = 0.0003$), and isovalerate ($P = 0.01$) when compared with HS0. Total VFA acetate (65.51 vs. 62.41%; $P < 0.0001$), isobutyrate (0.85 vs. 0.78%; $P = 0.0001$), and isovalerate (0.63 vs. 0.60%; $P = 0.02$) were greater in LS0 treatment compared with HS0. In conclusion, these results confirm the addition of AY aids in increased rumen pH values and shifts in VFA response.

Key Words: rumen pH, VFA, yeast

T158 Evaluation of supplemental autolyzed yeast on the presence of inflammatory biomarkers from Holstein cows fed a high

starch diet. S. E. Knollinger*[1], B. Miller[2], I. Mueller[3], and F. C. Cardoso[1], [1]*University of Illinois, Urbana, IL,* [2]*BIOMIN America Inc., Overland Park, KS,* [3]*BIOMIN Holding GmbH, Getzersdorf, Austria.*

The aim of this study was to investigate an AY (*Saccharomyces cerevisiae*) supplemented in high starch lactation diets and its effect on blood metabolites, in particular inflammatory biomarkers. Fifteen rumen-cannulated Holstein cows were assigned to 1 of 5 treatments in a replicated 5 × 5 Latin square design balanced to measure carryover effects. Treatments were: low starch diet without AY (LS0; control), high starch diet without AY (HS0), high starch diet with either 15 g (HS15), 30 g (HS30), or 45 g (HS45) of AY supplementation. The period of 21 d was divided into the adaptation phase (d 1 to 14) and a measurement phase (d 15 to 21). Blood was sampled from the coccygeal artery or vein at 0600 h on d 15, 18, and 21($n = 3$) of each period from each cow. Data collected were analyzed using the MIXED procedure of SAS. Orthogonal contrasts were used. Contrasts; CONT1 = LS0 compared with HS0; CONT2 = HS0 compared with the average of the 3 AY treatments (HS0, HS15, HS45) and linear and quadratic treatment effects (HS0, HS15, HS30, and HS45). NEFA (93.69 vs. 82.71 ± 4.08 μEq/L; $P = 0.04$) was greater in cows fed LS0; insulin (0.93 vs. 0.74 ± 0.10 μg/L; $P = 0.04$) concentration was greater in cows fed HS0. Plasma gamma-glutamyl transferase (GGT; 26.09 vs. 24.94 ± 3.57 U/L; $P = 0.05$), and mineral phosphorus (5.86 vs. 5.38 ± 0.15 mg/dL; $P = 0.005$) concentrations were greater in HS0 compared with LS0. However, the LS0 diet had greater total protein (7.60 vs. 7.43 ± 0.42 g/dL; $P = 0.03$), and bilirubin (0.14 vs. 0.12 ± 0.008 mg/dL; $P = 0.03$) when compared with cows fed HS0. Supplementing AY to cows had greater plasma total protein *(*$P = 0.008$*)*, globulin *(*$P = 0.008$*)*, serum amyloid A (SAA; $P = 0.02$), but lower albumin:globulin ($P = 0.03$), and tendency for lower superoxide dismutase activity (SOD; $P = 0.07$; CONT2). Linear treatments effects for plasma BHB (0.52 ± 0.03 mmol/L; $P = 0.08$) was higher at HS15. In conclusion, cows in HS0 had lower insulin, total bilirubin and protein compared with LS0 but greater concentrations of albumin:globulin, and tendency for SOD compared with treatments with added AY (CONT2).

Key Words: high starch, yeast, inflammation

T159 Efficacy of exogenous amylases at increasing in vitro dry matter digestibility of dent corn.

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The objective was to examine the efficacy of 8 amylases at increasing in vitro dry matter digestibility (IVDMD), ruminal fluid pH, ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFA) concentration resulting from fermentation of dent corn (DC; 4-mm, ground; 0.5 g per F57 ANKOM bag). The 8 amylases (5Trga, 15C3281, 22Amylex, 23LAT, 27TrGA, 29PZ, 35C4687, 36C8955) differed in activity and were applied at doses of 0 (Control; CON) 0.25, 0.50, and 1.0 mg/g DM of DC and compared with a positive (Flaked corn; FC) and negative (Flint corn) control. Three ruminally-cannulated lactating dairy cows were used as rumen fluid donors and the substrate was in buffered ruminal fluid at 39°C in quadruplicate in 3 independent runs. Gas production was measured at 2, 5, and 7 h, while IVDMD and pH were measured at 7 h of incubation. Data were analyzed using the GLIMMIX procedure of SAS and differences were considered significant at $P \leq 0.05$. Treatments and sampling time (for gas production) were used as fixed effects while run was considered a random factor. Enzymes 22Amylex and 36C8955 increased IVDMD relative to CON regardless of the dose used. However, 5Trga, 27TrGA and 23LAT increased IVDMD at the 1.0 mg/g DM dose, while 29PZ did at doses of 0.50 and 1.0 mg/g DM. Only 22Amylex increased IVDMD relative to FC at the 0.50 and 1.0 mg/g DM doses. Also, 22Amylex increased gas production at the 1.0 mg/g DM dose after 5 and 7 h of incubation, and decreased molar proportion of acetate at the 0.50 and 1.0 mg/g DM doses, relative to CON. The molar proportion of butyrate was greater with 5Trga and 22Amylex at 0.50 and 1.0 mg/g DM doses, respectively, while isobutyrate was greater for 5Trga, 23LAT, and 27TrGA at the 1.0, 0.50, and 0.25 mg/g DM doses, respectively, compared with CON. No treatment differences were observed for concentrations of NH_3-N, total VFA, propionate and acetate:propionate ratio. Amylases 22Amylex, 36C8955, 29PZ, 5Trga, 27TrGA and 23LAT increased IVDMD and their efficacy should be validated with in vivo studies.

Key Words: dent corn, amylase, in vitro digestibility

T160 Effect of withdrawing OmniGen-AF from lactating cow diet on performance and peripheral blood mononuclear cells proliferation.

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Supplementation of OmniGen-AF (OG) improves innate immunity and affects stress hormone concentrations in blood. However, it is not known if immune benefits persist in lactating cows after OG is removed from the diet. The aim was to evaluate the effect of withdrawing OG from the diet on performance, stress hormone concentrations, and peripheral blood mononuclear cells (PBMC) proliferation of mid-lactation dairy cows. Multiparous Holstein cows ($n = 32$), blocked by parity and days in milk (109 ± 39 d), were randomly assigned to 2 dietary treatments within each block: TMR top dressed with OG (56 g/d) or placebo (CON, 56 g/d). Cows were housed in the same free-stall pen, and fed individually 1×/d through Calan gates. All cows were fed the same diet containing OG before the onset of treatments, for at least 60 d. Cows were milked 3×/d and milk yield was recorded at each milking. Milk composition was analyzed weekly. Body weight and condition score were measured weekly. Blood samples were collected at −1, 1, 3, 5, and 7 wk relative to the onset of treatments for cortisol and prolactin (PRL) analyses, and isolation of PBMC. PBMC were cultured with hydrocortisone, PRL, concanavalin A (ConA), lipopolysaccharides (LPS), or their combinations for 72 h ex vivo to determine proliferative responses. Data were analyzed using PROC MIXED of SAS. Withdrawing OG from the diet of mid-lactation dairy cows with no physical signs of disease and not subjected to an in vivo immune challenge did not affect ($P > 0.1$) milk yield or composition, DMI, body weight, and blood concentrations of cortisol and PRL. Compared with CON cows, OG cows maintained greater body condition score ($P = 0.04$). Relative to CON cows, PBMC isolated from OG cows had greater proliferative rate when stimulated by LPS ($P = 0.03$), but not by ConA ex vivo. There were no interactions ($P > 0.1$) between dietary treatments and addition of hormones on PBMC proliferation. In conclusion, withdrawing OG from the diet of mid-lactation cows impaired proliferative responses of PBMC stimulated by LPS, suggesting OG supplementation enhances immune response.

Key Words: OmniGen-AF, lactating cow, PBMC proliferation

T161 Effects of an additive designed to improve nutrient digestibility on production by lactating Holstein cows.

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Improvement of digestibility releases more of the nutrients in total mixed rations (TMR) and so increases profitability. The commercial product Optipartum C+ (OC; AB Agri Ltd., Peterborough, UK) is a proprietary blend of enzymes and essential oils, which may increase nutrient digestibility and therefore increase milk production. The purpose of this experiment was to determine the effects of feeding OC without or with supplemental fat (Energy Booster 100, Milk Specialties Global, Eden Prairie, MN) on DMI and production by lactating cows. Primiparous (n = 8) and multiparous (n = 16) Holstein cows [DIM = 170 ± 48.3 d; BW = 665 ± 82.9 kg] were used in 4 × 4 Latin square design with 35-d periods. The 4 treatments were ground corn carrier (CON), 350 g of OC, 350 g of fat (EB), and a combination of 175 g of OC and 175 g of fat (50/50). All treatments were provided as a top-dress on the daily TMR and were a total of 453.6 g/d with the remainder of the weight made up by ground corn. Statistical analysis was conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Dry matter intake was greater for EB (22.07 ± 0.45 kg/d) compared with OC (20.40 ± 0.45 kg/d; $P < 0.01$) and CON (21.12 ± 0.45 kg/d; $P = 0.04$). Milk production was greater for 50/50 (32.58 ± 0.64 kg/d) compared with CON (30.53 ± 0.64 kg/d; $P < 0.01$) and greater for EB (31.74 ± 0.64 kg/d) compared with OC (29.95 ± 0.64 kg/d; $P < 0.01$). Fat yield was greater for 50/50 (1.13 ± 0.04 kg/d) compared with CON (1.05 ± 0.04 kg/d; $P < 0.01$), OC (1.05 ± 0.04 kg/d; $P < 0.01$), and EB (1.07 ± 0.04 kg/d; $P = 0.03$). Energy-corrected milk (ECM) was greater for 50/50 (31.85 ± 0.59 kg/d) compared with CON (29.88 ± 0.59 kg/d; $P < 0.01$). Efficiency (ECM/DMI) was greater for 50/50 (1.50 ± 0.03 kg/kg) compared with EB (1.41 ± 0.03 kg/kg; $P < 0.01$). In conclusion, providing a combination of EB and OC resulted in greater yield of ECM and efficiency of production.

Key Words: fat supplement, feed enzyme, dairy cow

T162 Sodium acetate and sodium bicarbonate increase milk fat yield through different mechanisms.

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Sodium acetate (NaAcet) treatment increases milk fat yield when ruminally infused or mixed in a TMR. Sodium acetate increases acetate supply, but also increases dietary cation anion difference (DCAD), which can also increase milk fat yield. Our objective was to determine if the effect of NaAcet on milk fat production is due to increasing acetate supply or DCAD. The study included 12 multiparous cows in a replicated 3x3 Latin square balanced for carryover effects with 14 d periods. Treatments were control TMR (17% CP, 30% NDF, 26% starch, and 5% EE on DM basis), NaAcet providing 590 g/d of acetate, and sodium bicarbonate ($NaHCO_3$) providing an equal amount of sodium as the acetate treatment. All treatments were mixed in the control TMR and fed ad libitum once a day and intake was recorded daily. Milk production was recorded daily with an integrated milk meter and milk components and milk fatty acid profile were determined the last 3 d of each period. Blood and fecal samples were collected every 9 h during the last 3 d of each period. Plasma acetate was determined by GC/MS. Production data were analyzed in JMP Pro 13 using a model that included the random effect of cow and period and fixed effect of treatment and a protected LSD mean separation. Plasma metabolites were analyzed with proc mixed in SAS 9.4 with repeated measures. Milk yield was not different between treatments, but NaAcet and $NaHCO_3$ increased dry matter intake by 6.6 and 7%, respectively ($P = 0.02$). Milk fat yield was increased 134 g by NaAcet and 118 g by $NaHCO_3$ ($P < 0.01$). Sodium acetate increased milk fat yield predominantly by increasing the yield of de novo and mixed source fatty acids, while $NaHCO_3$ increased the yield of preformed and de novo fatty acids. Sodium acetate increased plasma acetate and decreased plasma glucose concentration during the afternoon and early evening, which coincides with the higher intake period of the day. In conclusion, NaAcet and $NaHCO_3$ both increase milk fat production, but NaAcet achieves this through increased mammary gland de novo lipogenesis.

Key Words: de novo fat, acetate, dietary cation-anion difference (DCAD)

T163 Effect of daily feeding of a direct-fed microbial to dairy cows during midlactation on production performance and milk composition.

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Feeding direct-fed microbials (DFM) to lactating dairy cattle has been demonstrated to improve milk production efficiency and alter milk composition. The objectives of this experiment were to evaluate the effects of feeding a DFM on milk production, feed intake, and milk composition of midlactation dairy cows. Forty-eight multiparous Holstein cows were assigned randomly to 1 of 2 treatments: 1) a basal control diet (CON) top-dressed with 100 g of ground corn or 2) a basal control diet top-dressed with 96 g of ground corn and 4 g of a lactate-producing direct-fed microbial (DFM; 10-G; Life Products, Inc., Norfolk, NE). All cows had ad libitum access to feed (offered at 0800 and 1600 h) via individual feeding gates and were milked 3 times daily (0800, 1400, and 2000 h). Milk production and feed intake were recorded daily whereas BW, BCS, and milk components were determined weekly over the experimental period (14 weeks). Data were analyzed by using repeated measures in the MIXED procedure of SAS. Cows supplemented with DFM had similar milk yield compared with CON cows (40.8 and 41.5 ± 1.80 kg/d, respectively $P = 0.76$). Likewise, DMI was similar for both treatments and averaged 26.2 ± 0.58 kg/d ($P = 0.77$). Milk urea nitrogen tended to decrease in DFM cows compared with CON cows (12.84 vs 13.44 ± 0.28 mg/dL, respectively; $P = 0.13$). The trend for lower MUN was accompanied by a concomitant numerical increase in milk protein percentage in DFM cows compared with CON cows (3.24 vs 3.18 ± 0.05%, respectively; $P = 0.37$) with no differences in milk fat concentration (average 3.82 ± 0.12% for both treatments; $P = 0.88$), thus resulting in similar yield of energy corrected milk (43.73 ± 1.84 kg/d; $P = 0.88$). Interestingly, SCC was reduced ($P = 0.03$) from 278,800 to 103,900 ± 53,700 cells/mL when cows were supplemented with DFM. Similarly, $\log_{10}$-transformed SCC from cows consuming DFM tended to decrease compared with CON cows ($P = 0.11$). Supplementing DFM to lactating Holstein cows appears to improve milk nitrogen use efficiency and decrease SCC while maintaining DMI and milk production.

Key Words: probiotic, rumen health, feed additives

T164 Effects of garlic extract and citrus flavonoid feed additive on dairy cow performance.

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Feed additives that claim to reduce enteric methane exist. However, these feed additives have not been evaluated on how they affect dairy cattle performance. Therefore, the objective of this study was to evaluate the effects of a garlic extract and citrus flavonoid feed additive on the performance of dairy cows. Multiparous crossbred dairy cattle (n = 48) were housed at the Southwest Regional Dairy Center in Stephenville, TX. All cows were housed in a sand-bedded freestall pen equipped with a Calan Broadbent feeding system (American Calan, Inc., Northwood, NH) to allow for individual feedings. The control diet (CON) was the basal total mixed ration. The treatment diet (TRT) had the feed additive top-dressed daily as 15 g of a pelleted supplement. Both diets were offered at 110% of the previous day's consumption. Orts were collected before daily feeding and weighed for determination of ad libitum consumption. All cows were adapted to the diets and facilities for 14 d followed by a 77-d data collection period. Cows were milked 3 times daily and milk yield was averaged daily. Milk samples were collected at each milking one day each week and were averaged daily. Data were analyzed as a randomized complete block design, with pen serving as the random blocking factor. The MIXED procedure of SAS (SAS Institute Inc., Cary, NC,) was used to evaluate the fixed effects of treatment with day as a repeated measurement on the subject of cow. No statistical differences were observed in daily intake ($P = 0.63$) or milk yield ($P = 0.33$) at 58.70 and 58.00 and 46.10 and 44.00 kg/day between CON and TRT, respectively. No significant differences existed ($P > 0.05$) between fat percent at 4.10 and 4.20, protein percent at 3.30 and 3.30, urea percent at 11.20 and 11.40, acetone percent at 0.04 and 0.05, lactose percent at 4.80 and 4.80, and solids nonfat percent at 8.90 and 9.10 for CON and TRT, respectively. The data suggests that the addition of the garlic extract and citrus flavonoid feed additive does not affect dairy cow performance.

Key Words: feed additive, garlic extract, citrus flavonoid

T165 Effects of additives based on phytogens, yeast products, and direct-fed microbials in milk replacers and starters for Holstein calves.

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The objective of this study was to determine if feed additives in non-medicated milk replacer (MR) and starter could improve health and growth of Holstein calves through 13 wk of age. The experiment was a randomized complete block design with 30 male calves per treatment, purchased when < 3 d old from a commercial farm. Treatments were: control MR and starter with no additives (CON); a commercially available phytogen and yeast extract (Surmount, PMI Nutrition, Arden Hills, MN) in MR and a blend of yeast products and phytogenics (Victant, PMI Nutritional Additives) in starter (CTP); and the same MR with starter (CEP) containing an experimental blend of direct-fed microbials and phytogens (Calf EXP Pak, PMI Nutrition). Intakes of MR, starter, and water were recorded daily. Measurements of BW, body length (BL), withers height (WH), hip height (HH), and hip width (HW) were recorded weekly. Calves were weaned on d 49 and moved from individual hutches to group housing by treatment group on d 56. Calves remained in groups until d 91. Growth and intake data were analyzed using the MIXED procedure and health data were analyzed using GLIMMIX and FREQ procedures in SAS. Intakes of MR and starter did not differ through d 56 ($P > 0.17$), but group starter intake was greater ($P < 0.02$) for CTP than CEP or CON. During the pre-weaning period BW was greater ($P < 0.04$) for CTP, which continued to d 56 and after ($P < 0.04$). Mean BW at d 91 were 100.9, 112.6, and 107.2 kg for CON, CTP, and CEP. Differences in BW were reflected in similar differences in BL, HW, and HH. The number of times calves were medicated was different among the groups ($P < 0.01$), with CTP calves being medicated the least (63 times), compared with CON and CEP (144 and 145 times, respectively). Instances of fecal scores > 2 (4-point scale) were lower ($P = 0.04$) for CTP (366 instances) compared with CON and CEP (463 and 441 instances, respectively). Nasal discharge scores showed a similar pattern among treatments. In conclusion, both feed additive groups showed greater growth than CON, but only CTP improved measures of health.

Key Words: phytogen, yeast product, calf

Ruminant Nutrition: Protein and Amino Acid Nutrition 2

T166 Effects of methionine coated with vegetable fat on performance and feed intake of mid-lactation dairy cows fed a low-protein diet. J. M. Ruiz-Rodriguez[1], M. Puyalto[2], J. J. Mallo[2], G. Elcoso[3], and A. Bach*[4,5], [1]*Universidad Politécnica de Madrid, Madrid, Spain,* [2]*Norel S.A., Madrid, Spain,* [3]*Blanca from the Pyrenees, Hostalets de Tost, Spain,* [4]*Department of Ruminant Production, IRTA, Barcelona, Spain,* [5]*Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

The aim of the study was to evaluate the effects of supplementing Met coated with hydrogenated palm fatty acid distillates (PFAD) on feed intake and milk yield of dairy cows. Fifty Holstein cows (146 ± 39 DIM, 30.9 ± 5.00 kg/d; 44 multiparous; 6 primiparous) were split in 2 groups (n = 25) and exposed for 58 d to 2 treatments following a complete randomized design. Treatments consisted of supplementation with 30 g/d of hydrogenated PFAD plus 20 g/d of DL-Met, where Met was provided in free form (CTRL), or 50 g/d of coated Met (39% Met; thus providing 20 g of Met/d) by the aforementioned fat (MET), commercially available as Bymet by Norel S.A. Cows were fed a TMR (13.2% CP, 30.1% NDF, 1.63 Mcal of NEl/kg; DM basis) twice daily. On a daily basis, lying time, milk yield, and milk fat and protein contents were determined individually. Individual feed intake was daily monitored using electronic feed bins as cows were freestall housed. As treatments were applied at the animal level, the experimental unit was the animal. Data were summarized by week of study and animal, and analyzed using a mixed-effects model for repeated measures. Dry matter intake was greater ($P = 0.01$) in MET (23.8 ± 0.42 kg/d) than in CTRL (22.3 ± 0.42 kg/d). Also, milk fat content and milk fat yield were affected ($P < 0.001$) an interaction between treatment and week, with MET cows producing greater amounts of milk fat and greater content of milk fat after the third week of study. Similarly, yield of ECM was also affected by an interaction between treatment and week ($P = 0.02$), and progressively increased in MET cows along the study. It is concluded that MET coated with hydrogenated PFAD has the potential to improve DMI of cows in mid-lactation fed low protein diets, and as a result milk fat content, milk fat yield, and yield of ECM may increase after about 3 weeks from the beginning of supplementation.

Key Words: methionine, palm fatty acid distillates (PFAD), milk

T167 Effects of methionine sources on rumen fermentation and biohydrogenation of linoleic acid in vitro. J. E. Copelin*[1], P. A. Dieter[1], J. L. Firkins[2], and C. Lee[1], [1]*Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

We examined the effects of different forms of methionine on rumen fermentation and biohydrogenation in a condition of feeding polyunsaturated fatty acids. An in vitro batch culture was conducted with the following dietary treatments: a typical diet (50:50 of forage to concentrate on a DM basis; CON), CON with addition of 3.0% linoleic acid (DM basis; LA), LA with 0.1% of D/L-methionine (MET), 0.1% of a methionine analog (HMTBa; Rhodimet, Adisseo Inc.). The biohydrogenation of linoleic acid at 2, 4, 8, and 24 h and pH, ammonia, and volatile fatty acids (VFA) at 24 h of incubation were determined. Data were analyzed using the MIXED procedure of SAS where incubation was random effect and treatment, time, and their interaction were fixed effect. At 24-h incubation, pH and ammonia were not affected by treatments. Total VFA concentration was not affected by treatments. However, LA decreased acetate and butyrate and increased ($P = 0.02$) propionate as proportion of total VFA. Compared with LA, HMTBa increased acetate and decreased ($P = 0.02$) propionate, but no difference in VFA composition between LA and MET were found. Dry matter digestibility was decreased ($P < 0.05$) for LA vs. CON without a difference among LA, MET, and HMTBa. Digestibility of NDF was highest ($P < 0.05$) for CON followed by MET, HMTBa, and then LA. At 2, 4, and 24 h of incubation, no or minimal differences in concentrations of linoleic acid and biohydrogenation intermediates were observed among LA, MET, and HMTBa. However, at 8 h of incubation, MET and HMTBa tended to increase ($P = 0.06$) 18:1 t11 and increased ($P = 0.01$) 18:2 c9t11 compared with LA. However, 18:1 t10 and 18:2 t10c12 were not affected by MET and HMTBa. In conclusion, linoleic acid at 3% of substrate DM depressed feed digestibility and altered acetate and propionate production. However, MET and HMTBa alleviated the negative effect of LA on fiber digestibility and HMTBa produced more acetate and less propionate compared with LA, which did not occur for MET. Changes in the biohydrogenation of linoleic acid by MET and HMTBa were small but altered biohydrogenation pathways.

Key Words: biohydrogenation, methionine, in vitro

T168 Associations of early lactation rumen-protected methionine supplementation with herd level health and production performance in the northeastern United States. K. R. Gallagher*[1], A. L. Kerwin[1], J. N. Tikofsky[2], M. M. McCarthy[3], and T. R. Overton[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Purina Animal Nutrition, Gray Summit, MO,* [3]*Adisseo USA Inc., Alpharetta, GA.*

Twelve Northeastern US herds were enrolled in a prospective cohort study with the objective of identifying associations of early lactation rumen-protected Met supplementation with herd level health, production performance, and management. Farms were enrolled as either experiment (EXP; n = 7 herds; feeding >11.25 g/d of rumen-protected Met postpartum; Smartamine M; Adisseo, Antony, France) or control (CON; n = 5 herds; postpartum diet containing ≤11.25 g/d of supplemental Met). Data were analyzed in SAS v. 9.4 using the MIXED, MEANS, and FREQ procedures, and means ± SD are reported throughout. In the formulated postpartum diet, EXP herds had higher levels of Met (1.04 ± 0.03 vs. 0.94 ± 0.05 g Met/Mcal ME; $P < 0.01$ and 2.52 ± 0.06 vs. 2.32 ± 0.11 Met as % MP; $P < 0.01$). Postpartum pooled plasma Met concentrations (12 to 15 cows/herd) were greater in EXP herds compared with CON (4.05 ± 0.50 vs. 3.32 ± 0.13 µg/mL; $P = 0.01$). Compared with CON, EXP had greater average milk yield at 4 wk postpartum (WK4M; 48.8 ± 1.4 vs. 43.4 ± 4.5 kg; $P = 0.02$), combined fat and protein yield (2.81 ± 0.15 vs. 2.47 ± 0.26 kg; $P = 0.04$) and 305-d mature equivalent milk (13,473 ± 349 vs. 12,122 ± 413 kg; $P = 0.03$). Female calf dead on arrival rate (DOA) was lower in EXP compared with CON (4.6 ± 1.1 vs. 8.8 ± 2.5%; $P < 0.01$), and EXP herds had a numerically lower 60 d cull rate than CON (8.8 ± 4.4 vs. 11.5 ± 5.3%; $P = 0.37$). Postpartum feed pushup frequency was greater for EXP herds than CON (86% vs. 0% pushup ≥ 6x/d; $P = 0.03$; n = 10). After standardizing ingredient cost and milk price, diet cost estimates based on WK4M tended to be lower for EXP (5.03 ± 0.38 vs. 5.76 ± 0.87 $/45.4 kg of milk; $P = 0.09$). Overall, postpartum supplementation with rumen-protected Met was associated

Table 1 (Abstr. T169).

	Treatment					*P*-value		
	LEAA−	LEAA+	HEAA−	HEAA+	SEM	AA	E	AA × E
DMI, kg/d	21.1	21.1	21.4	21.0	0.4	0.12	0.71	0.18
MPY, kg/d	828	916	927	962	39	0.02	0.01	0.22
MY, g/d	28.2	29.6	30.1	31.2	1.3	0.07	0.02	0.82
MPC, g/kg	29.3	31.1	30.9	30.9	0.9	0.08	0.13	0.08
MFY, g/d	1,289	1,317	1,363	1,410	55	0.07	<0.01	0.59
Lactose, g/kg	52.6	49.2	50.1	50.8	1.4	0.21	0.66	0.08
MPef, %	75.0	80.6	72.6	76.3	2.0	<0.01	0.03	0.43

with higher levels of plasma Met, greater 305-d mature equivalent milk yield, WK4M, and combined fat and protein yield at the herd level.

Key Words: methionine, transition cow, herd-level management

T169 Methionine, lysine, and histidine supplementation at low and high net-energy supply in dairy cows. C. Omphalius[1,3], H. Lapierre[2], L. Bahloul*[3], and S. Lemosquet[1], [1]*PEGASE, INRA, Agrocampus Ouest, Saint Gilles, France,* [2]*Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada,* [3]*Adisseo France S.A.S., Antony, France.*

The responses in milk yield (MY) and composition to a supplementation in Met, Lys and His at 2 levels of NE_L were investigated. Four Holstein cows received 2 dietary levels of NE_L (LE: 30.9 vs. HE: 36.0 Mcal/d) and duodenal infusion of 2 AA mixtures, arranged in factorial and distributed according to a 4 × 4 Latin square, with 3-wk periods. Diets were composed of corn silage and concentrates (60:40). The 2 AA mixtures, both providing 92 ± 2 g/d of PDI (MP in INRA 2018), contained (g/d) Lys (28), Met (9), His (9) and NEAA in AA+ or only NEAA in AA−. Diets plus infusions provided 2.4 vs. 2.0, 7.2 vs. 5.8, 2.4 vs. 2.0% of PDI of Met, Lys and His respectively in AA+ vs. AA- compared with INRA (2018) requirements at 2.4, 7.0 and 2.4% PDI, for Met, Lys and His, respectively. Data were analyzed using the MIXED procedure of SAS with treatment and period as fixed effects and cow as random. Treatments did not affect DMI. The CP content of LE and HE diets were 147 vs. 145 g/kg DM, but current estimated MP supply increased by 101 g/d between LE and HE diets. Analyses of rumen N degradability will confirm the MP supply from LE and HE diets and associated MP efficiency (MPef) variations in response to NE_L. Increasing NE_L supply increased milk protein yield (MPY: 73 g/d) through an increased MY (1.8 kg/d) and increased milk fat yield (MFY: 84 g/d). Better balancing AA increased MPY (61 g/d) through a tendency to increased MY at the 2 NE_L levels and increased true milk protein content (MPC) at LE only (AA × E interaction), leading to an overall increase in MPef from 73.8 to 78.5%. Milk fat yield (38 g/d) tended to increase in response to AA+ vs. AA-. Milk lactose content tended to be lower in LEAA- than in the other treatments (AA × E). In conclusion, better balancing Met, Lys and His improved MPef at both levels of NE_L and has other effects on MY and composition.

Key Words: amino acid, efficiency, dairy cow

T170 Rumen-protected methionine supplementation on blood metabolites of transition dairy cows. G. F. M. Leão*[1], S. K. Santos[2], E. J. Askel[1], T. C. Michelotti[1], G. Negro[1], F. Lopes[3], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brazil,* [3]*Adisseo South America, São Paulo, SP, Brazil.*

Recent data have shown several benefits of methionine supplementation during the transition period to health parameters of dairy cattle. The objective of this study was to investigate the effects of rumen-protected methionine (RPMet) during the transition on blood metabolites of dairy cows in a commercial herd. One hundred and 66 Holsteins, 58 nulliparous and 108 multiparous cows, were blocked by parity and expected calving date and randomly allocated in 2 treatments groups: Control and RPMet. Cows were individually fed and the RPMet group received a top-dressed methionine supplementation (Smartamine; Adisseo Inc.) during 21 d prepartum (8 g/cow/d) and 16 d postpartum (15 g/cow/d), maintaining a lysine/methionine ratio of 2.81/1. After 16 d postpartum all animals received methionine (15 g/cow/d). Blood samples were collected from the coccygeal vein before morning feeding on −14, −7, +1, +7 and +14 d relative to calving date. Data were analyzed using the MIXED procedure of SAS with a model containing the effects of block, treatment, time, and treatment × time interaction as fixed effects and cow within treatment as a random effect. Metabolites did not differ ($P > 0.10$) between groups. Nevertheless, there was a trend of RPMet animals to present lower values of AST ($P = 0.08$) and higher values of total calcium ($P = 0.05$) on +1 d, and albumin ($P = 0.06$) on +14 d. In summary, results suggest that RPMet supplementation had small effects on blood metabolites of dairy cows in a commercial herd.

Table 1 (Abstr. T170).

Metabolites	Control	RPMet	SEM	*P*-value
Albumin (g/dL)	3.25	3.29	0.02	0.34
Alanine aminotransferase (U/L)	15.25	14.99	0.47	0.69
Aspartate aminotransferase (U/L)	83.70	74.90	4.17	0.14
BHB(mmol/L)	0.46	0.44	0.01	0.43
Bilirubin (mg/dL)	0.20	0.19	0.01	0.82
Calcium (mg/dL)	8.15	8.19	0.04	0.52
Cholesterol (mg/dL)	73.73	75.76	1.85	0.44
Globulin (g/dL)	3.51	3.54	0.06	0.78
Glucose (mg/dL)	64.70	64.58	1.10	0.94
Nonesterified fatty acids (mmol/L)	0.75	0.73	0.03	0.72
Paraoxonase (U/mL)	102.5	104.1	1.96	0.56
Total protein (g/dL)	6.96	6.99	0.18	0.92
Triglycerides (mg/dL)	11.52	11.59	0.27	0.86
Urea nitrogen (mg/dL)	29.07	30.05	0.66	0.30

Key Words: amino acid, health, metabolic profile

T171 Rumen-protected methionine supplementation on milk yield and its composition, morbidity and culling of transition dairy cows. G. F. M. Leão*[1], S. K. Santos[2], E. J. Askel[1], T. C. Michelotti[1], G. Negro[1], F. Lopes[3], and R. Almeida[1], [1]*Universidade Federal do Paraná, Curitiba, PR, Brazil,* [2]*Pontifícia Universidade Católica do Paraná, Curitiba, PR, Brazil,* [3]*Adisseo South America, São Paulo, SP, Brazil.*

The goal of this study was to investigate the effects of RPMet supplementation during transition on milk yield and composition, diseases' prevalence and culling. One hundred and 66 Holstein animals, 58 nulliparous and 108 multiparous cows, were blocked by parity and expected calving date and randomly allocated in 2 treatments: Control and RPMet. Cows were individually fed and the RPMet group received a top-dressed methionine product (Smartamine, Adisseo Inc.) during 21 d prepartum (8 g/cow/d) and 16 d postpartum (15 g/cow/d), maintaining a lysine/methionine ratio of 2.81/1. After 16 d postpartum all animals received RPMet (15 g/cow/d). Daily milk yield (kg) was recorded as an average of the first 60 DIM. Milk samples were collected in the first and second lactation weeks to determine its composition. Mortality and morbidity, considered as the occurrence of one or more of the following diseases: retained placenta, ketosis, mastitis, hypocalcemia, metritis, and pneumonia, were recorded during the first 60 DIM. Milk yield and its composition were analyzed using MIXED procedure, with a model containing the effects of block, treatment, time, and treatment × time interaction as fixed effects and cow within treatment as a random effect, while binary data for morbidity and culling were analyzed using GLIMMIX procedure of SAS. Milk yield did not differ ($P = 0.87$; SEM = 1.14) between treatments, as well as energy-corrected milk ($P = 0.47$; SEM = 0.89). There was a trend of RPMet cows to produce higher ($P = 0.07$; SEM = 0.12) milk fat content, 4.40 vs. 4.07%, respectively. Rumen-protected methionine animals did not show higher milk protein (3.46 vs. 3.38%; SEM = 0.04; $P = 0.20$) or casein (2.73 vs. 2.65%; SEM = 0.04; $P = 0.19$) contents. Other milk components were similar ($P > 0.10$) between treatments. In addition, morbidity showed similar ($P = 0.87$; EPM = 0.05) rates, while culling was much higher for control animals than for RPMet cows (12.05% vs. 2.41%; SEM = 0.02; $P = 0.03$). Overall, results suggest that RPMet can improve milk fat content and decrease involuntary culling of early lactation dairy cows.

Key Words: diseases, milk composition, periparturient cows

T172 Relative metabolizable methionine content of rumen-protected products using the seleno-methionine technique. W. P. Weiss[1], A. W. Tebbe*[1], K. Estes[2], and C. Zimmerman[2], [1]*Ohio Agricultural Research and Development Center, The Ohio State University, Wooster, OH,* [2]*Balchem Corporation, New Hampton, NY.*

Estimating metabolizable Met (MP-Met) in diets and supplements is difficult and expensive; however, the seleno-Met technique can estimate relative supply of MP-Met easily and inexpensively. The technique is based on the proportionality of a change in supply of dietary MP-Met to the change in milk Se-Met concentration (Weiss and St-Pierre, 2009). Our objective was to further evaluate the Se-Met procedure and determine the MP-Met supplied by a RP Met prototype (BRPM, 72% Met, Balchem Corp., New Hampton, NY) and a commercially available RP product (Mepron, 85% Met, Evonik, Germany). Holstein cows (15) averaging 31 kg/d milk were fed a basal diet (0.3 mg/kg Se from Se-yeast) for 10 d. Then in 5 replicated 3 × 3 Latin squares with 10 d periods, cows were fed the basal diet plus 1 of 3 treatments: 1) Mepron fed at 19 g of Met/d; 2) BRPM fed at 19 g of Met/d (BRPM-1X) and 3) BRPM fed at 38 g of Met/d (BRPM-2X). After the last treatment period, cows were fed the basal diet for 9 d. Milk was sampled from the final 4 milkings of each period (basal and treatment), composited by cow-period, and assayed for N and Se. The relative dilution of milk Met when treatments were fed was calculated as the ratio of basal Se/N to treatment Se/N (Table 1). Data were analyzed using Proc Mixed (SAS) with the random effects of square and cow within square and the fixed effects of treatment and period. Feeding Mepron or BRPM-1X increased MP-Met by 13% compared with basal. BRPM-2X increased MP-Met by 25%, which was significantly greater than Mepron or BRPM-1X. On a gram of Met basis, results were identical for the 1X and 2X BRPM treatments. The identical results for the 2 BRPM treatments lend validity to a method that is a practical and useful tool to evaluate met supplements.

Table 1 (Abstr. T172). Relative change in MP-Met with different supplements

Treatment	Milk Se/N, µg/g	Ratio[1]
Basal	1.12	—
Mepron	0.99^{a}	1.129^{a}
BRPM-1X	0.99^{a}	1.128^{a}
BRPM-2X	0.89^{b}	1.254^{b}
SEM	0.020	0.008

^{a,b}Values with differing superscripts differ ($P < 0.05$).

[1] Calculated as [(Milk Se/Milk N)$_{\text{Basal}}$]/[(Milk Se/Milk N)$_{\text{Treatment}}$] and equals the change in Met entry rate relative to the basal diet.

Key Words: metabolizable methionine, rumen protected

T173 Rumen-protected choline or methionine affects the choline metabolite profile of plasma and milk and alters expression of genes associated with choline and lipid metabolism in periparturient cows. S. B. Potts*, K. M. Brady, C. M. Scholte, K. M. Moyes, and R. A. Erdman, *University of Maryland, College Park, MD.*

To better understand the beneficial effects of rumen-protected choline (RPC), rumen-protected Met (RPM), and their interaction, our objective was to determine if these nutrients modify the choline metabolite profile of plasma and milk or influence hepatic expression of genes associated with choline, Met, and lipid metabolism in periparturient cows. Cows (25 primigravid, 29 multigravid) were blocked by expected calving date and parity and assigned to one of 4 treatments: CON (no RPC or RPM); CHO (60 g/d RPC); MET (12 g/d RPM prepartum; 18 g/d RPM postpartum);

or CHO + MET. Treatments were applied daily as a top-dress from 19 ± 2 d prepartum through 35 d in milk (DIM). Blood and milk samples were collected at 7 and 14 DIM for analysis of choline metabolites, including 16 species of phosphatidylcholine (PC). Liver collected at 7 DIM from multiparous cows was used for gene expression analysis. Blood and milk data were analyzed using a mixed model that included fixed effects of choline, Met, and day (7 or 14 DIM), and random effects of cow and block. Pretreatment blood metabolite concentrations were included as covariates. RPC reduced milk betaine yield in multiparous cows ($P = 0.05$) and tended to increase it in primiparous cows ($P = 0.09$), but these changes were driven by changes in milk yield. RPC and RPM increased yields of 4 and 3 PC species by an average of 101 ($P \leq 0.02$) and 81% ($P \leq 0.03$), respectively, for multiparous cows. RPM also reduced plasma free choline concentration by 10% ($P = 0.03$), while RPC tended to increase glycerolphosphocholine concentration by 58% in multiparous cows ($P = 0.07$). When fed without RPM, RPC tended to increase hepatic expression of betaine-homocysteine methyltransferase (CHO x MET: $P = 0.09$) and phosphate cytidylyltransferase 1 choline α (CHO x MET: $P = 0.10$). Additionally, RPC tended to decrease expression of 3-hydroxy-3-methylglutaryl-CoA synthase 2 ($P = 0.10$) and peroxisome proliferator activated receptor α ($P = 0.08$). Our results indicate that both Met and choline have different effects on choline metabolism in periparturient cows and that these effects vary between primi- and multiparous cows.

Key Words: methionine, choline metabolite, gene expression

T174 Effects of rumen-protected methionine fed to lactating Holstein cows during a heat stress challenge on blood biomarkers harvested at 2 time points post-feeding.

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Heat stress (HS) causes alterations in blood biomarkers, however, limited research is available on the effects of rumen-protected methionine (RPM) feeding or sample harvesting time on blood biomarkers during HS. Thirty-two multiparous, lactating Holstein cows [DIM (184 ± 59)] were randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., Antony, France; 0.105% DM of TMR as top dress) or TMR without RPM (CON)] in a crossover design. The study was divided into 2 periods with 2 phases per period. During phase 1 (9d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets. Group 2 (n = 16) remained in TN (THI = 61 ± 4) but was pair-fed (PFTN) to HS counterparts. After a 21-d washout period, the study was repeated (period 2). Environmental treatments were inverted relative phase 2 in period 1, while the dietary treatments remained the same. Plasma samples were taken 4 and 8 h post-feeding on d 1, 3, 6, and 9 of each phase, and phase 1 samples from each period were pooled relative to h collected. Paired difference values were calculated for each cow for each period based on the difference between phase 1 (baseline) values and phase 2 values for each variable at each time point (4 or 8 h). Statistical analysis was performed on paired difference values using MIXED procedure of SAS. At 4 h, cows in RPM tended to have greater ($P = 0.10$) increase in plasma urea nitrogen (PUN) concentration for PFTN (+1.26 mg/dL) and HS (+1.08 mg/dL) compared with cows in CON for PFTN (+0.68 mg/dL) and HS (+0.15 mg/dL). At 4 h, PUN concentration change was not different between cows in PFTN and HS (+0.97 vs. +0.62 mg/dL, respectively; $P = 0.31$). However, at 8 h, cows in PFTN had greater decrease in PUN compared with cows in HS (−0.66 mg/dL vs. −0.09 mg/dL, respectively; $P = 0.04$). At 8 h, cows in PFTN had a greater increase in NEFA concentration compared with cows in HS (+9.61 vs. +0.77 µEg/L; $P = 0.04$). In conclusion, HS altered blood biomarker concentration compared with PFTN. Additionally, blood sample timing effected biomarker concentration.

Key Words: heat stress, methionine, blood urea N

T175 Effects of supplementing a lysine derivative at varying doses on lactational performance of dairy cows.

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The objective of this study was to evaluate the effects of increasing doses of a lysine derivative *N*-acetyl-L-Lys (NALL, CJ CheilJedang, Seoul, South Korea), on the performance of lactating dairy cows fed corn silage-based diets. Sixteen multiparous lactating Holstein dairy cows (parity = 2 to 3; 60–70 d-in-milk) were used in a 4 × 4 Latin square design with 21-d experimental periods. Diets were formulated using NDS Professional (R.U.M.&N, Reggio Emilia, Italy), a software based on the Cornell Net Carbohydrate and Protein System equations (CNCPS v.6.5) to supply adequate metabolizable energy but inadequate metabolizable Lys (−7 g/d) for dairy cows producing 42 kg/d of milk with 3.8% milk fat, and 3.2% milk protein. Cows were blocked by DIM and assigned to one of 4 treatments: 1) 0 g/d NALL (control); 2) 40 g/d NALL (40NALL); 3) 80 g/d NALL (80NALL); and 4) 120 g/d NALL (120NALL). The fixed effects of treatment and period and random effects of cows nested within square were tested using the GLIMMIX procedure of SAS. Intake of dry matter linearly increased ($P = 0.04$) with increasing NALL supplementation and was for 80NALL (24.3 kg/d). Milk yield increased ($P = 0.02$) with 80NALL and 120NALL (45.6 kg/d) compared with the control and 40NALL (43.2 kg/d). Milk fat yield was unaffected, whereas milk protein yield linearly increased ($P = 0.05$) with increasing NALL supplementation. Milk lactose yield tended to increase linearly ($P = 0.07$) with increasing NALL supplementation. Concentration of MUN was the greatest with 120NALL (quadratic trend; $P = 0.02$). In contrast, there were no effects of NALL supplementation on concentrations of blood glucose, BHB, total ruminal VFA concentration, ammonia N or molar proportion of VFA. Nonesterified fatty acid concentration tended to be lower ($P = 0.08$) for 40NALL and 80NALL. Concentrations of plasma Lys were similar across all treatments ($P = 0.15$). In conclusion, NALL supplemented at 80 g/d improved feed intake and milk yield but not ruminal fermentation.

Key Words: dairy cow, milk production, *N*-acetyl-L-lysine

T176 Evaluating methionine bioavailability of rumen protected technologies using the plasma amino acid dose-response technique without using ruminally cannulated animals.

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The plasma free AA dose-response technique is used to determine AA bioavailability of commercially available rumen-protected (RP-AA) products. The technique assesses AA bioavailability compared with a positive control AA infusion, but ruminally cannulated cows are needed. Our objective was to measure the Met bioavailability of a RP-Met supplement relative to a reference RP-Met supplement already documented in terms of its bioavailability using the plasma free AA dose-response technique. Six multiparous Holstein cows fed a Met deficient diet were

Table 1 (Abstr. T176). Intake, lactation performance and plasma AA concentrations for cows fed RP-Met supplements

Item	Control	SMM	EBM	SEM	*P*-value
DMI, kg/d	25.3	25.7	25.0	0.80	0.78
Milk yield, kg/d	38.1	39.0	37.8	1.37	0.78
Milk protein content, %	3.02^{b}	3.12^{a}	3.01^{b}	0.03	0.04
Methionine, μM	19.5^{b}	46.4^{a}	21.3^{b}	1.84	<0.001
TSAA, μM	81.3^{b}	120.1^{a}	85.0^{b}	3.75	<0.001
Slope for TSAA, %TAA – TSAA	—	0.06908^{a}	0.01436^{b}	0.0015	<0.001

^{a,b}Means within rows differ at $P < 0.05$.

used in a replicated 3 × 3 Latin square with 7-d experimental periods. The 3 treatments were: 1) a negative control (CON); 2) 24 g/d Met from Smartamine M (SMM; Adisseo; Met bioavailability = 80%); and 3) 24 g/d Met from EB-Met (EBM; Milk Specialties Global). Intake, milk yield and milk components were collected the last 3 d per period. Blood was collected from the tail vein the last 3 d per period 2, 4, 6 and 8 h after the morning feeding (0500 h). Data were analyzed using the PROC MIXED and REG procedures of SAS. Significance was declared at $P \leq 0.05$. Milk yield and DMI were unaffected by treatment. Milk protein content was increased for cows fed SMM relative to CON and EBM. Plasma Met and total sulfur AA (TSAA) concentrations (μ*M*) were greatest for cows fed SMM compared with CON or EBM. Slopes for RP-Met supplements expressed as %TAA−TSAA was greater for SMM compared with EBM. The resulting bioavailability of EBM was 20.8% [0.01436/0.06908] relative to SMM. Evaluating bioavailability of RP-AA supplements can be done relative to a reference RP-AA supplement with a known bioavailability to eliminate the need for ruminally-cannulated cows.

Key Words: bioavailability, methionine

T177 Effects of rumen protected lysine and histidine on performance and energy and nitrogen partitioning in high-hydrolyzed-feather-meal diets fed to Jersey cows.

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The objectives of this study were to determine the effects of supplementing rumen protected (RP) Lys and His individually or in combination in a diet containing hydrolyzed feather meal (HFM) on milk production and composition and energy and N partitioning in dairy cows. Multiparous Jersey cows (n = 12; 91 ± 18 d in milk) were used in a triplicated 4 × 4 Latin square with 4 periods of 28 d (24-d adaptation and 4-d collection). Throughout the experiment, all cows were fed the same TMR (17.1% crude protein, 1.64 Mcal/kg of net energy of lactation) with HFM inclusion at 5% of diet DM. Cows were grouped by dry matter intake and milk yield and cows within a group were randomly assigned to one of 4 treatments: no RP Lys or RP His; RP Lys only [70 g/d of Ajipro-L (approximately 23 g/d of digestible Lys); Ajinomoto Co., Inc., Tokyo, Japan]; RP His only [32 g/d of experimental product (approximately 8 g/d of digestible His); Balchem Corp., New Hampton, NY]; or both RP Lys and His. Data were analyzed with a model that included the fixed effect of Lys, His, and 2-way interaction and the random effect of period, square, cow within square, and error. For milk production and components, no Lys effect or interactions were observed ($P > 0.14$). Supplementing RP His increased ($P < 0.10$) milk yield (22.5 vs. 21.6 ± 2.04 kg/d) and milk protein yield (0.801 vs. 0.772 ± 0.051 kg/d). Across treatments, dry matter intake (18.5 ± 0.83 kg/d), energy supply (48.8 ± 2.21 Mcal of metabolizable energy/d) and N partitioning were not observed to differ ($P > 0.10$). With RP His, plasma concentration of His increased ($P = 0.06$; 21.8 vs. 18.7 ± 2.95 μM). Plasma Lys concentration increased when RP Lys was supplemented without RP His (77.7 vs. 66.0 ± 4.69 μM), but decreased when RP Lys was supplemented with RP His (71.4 vs. 75.0 ± 4.69 μM; interaction $P = 0.04$). Our results suggest that His may be a potentially limiting AA in high-HFM diets and that supplementation of RP His may increase milk and milk protein production. No response to RP Lys suggesting that Lys was not limiting.

Key Words: lysine, histidine, hydrolyzed feather meal

T178 Stability of rumen-protected lysine in rumen and TMR with different moisture contents.

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This study aimed to evaluate the lysine releasing rate (LR %) from 3 rumen-protected lysine (RPL) products upon mixing with TMR diets having various moisture contents, and to determine the in situ rumen stability of these PRL products. Three PRL products commercially available in Japan (A: AjiPro-L; B: LysiGEM; C: Feedtech Bypass Lysine) were used for this assessment. L-Lys contents of product A, B and C were 39.1, 53.1 and 46.8%, respectively. Each RPL (2 g) was mixed with 200 g of corn silage-based TMRs having 3 levels of moisture contents (low, L: 17.6%; medium, M: 35.6%; high, H: 61.1%). The treated TMRs were stored in plastic bags (3 replicates) at 20°C for 0, 1, 3, 6, 12, and 24 h. TMRs without RPL were used as control. At each time point, the solubilized free Lys in the treated TMR was extracted with 1 L of water. After filtration of the extracted solutions, free Lys content in the extract was analyzed, and LR % observed for the mixed TMR was calculated. Additionally, 2 ruminally fistulated lactating cows were used to determine the in situ rumen stability. The 3 RPLs were placed separately in Nylon bags (1 g/ bag: 3 replicates, 5 × 7 cm, pore size 50 μm) and incubated in the rumen for 0 (the washed control), 6, 12, and 24 h, respectively. After drying the incubated bags and extracting Lys from residues left in bags through acid hydrolysis, Lys content was analyzed by UPLC. Data were analyzed by Mixed model ANOVA of SAS. Fixed effects in the model were the RPL product, time, and their interactions. The results showed that all RPLs exhibited an increase in the LR % over storage time for M- and H-TMRs but not for L-TMR (*P*

< 0.01). However, RPLs by time interaction was observed ($P < 0.01$), the LR % was highest in the product C, followed by B, and then A (67.0%, 17.0%, and 3.0% at 24 h for M, and 62.0%, 24.0%, and 4.0% at 24 h for H, respectively). The in situ ruminal protection of all RPLs was highest in the product A, followed by B, and then C (87.3%, 51.5%, and 0.2% at 24 h, respectively; RPLs by time interaction, $P < 0.01$). Our results indicate that it is important to investigate the RPL stability not only in the rumen but also during incorporation in the high moisture diets.

Key Words: rumen-protected Lys, in situ bioavailability

T179 Evaluation of rumen-protected lysine product on performance of lactating dairy cows.

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The objective of this study was to determine the effects of rumen-protected Lys (RP-Lys) supplementation in dairy cows on feed intake, AA in blood, and milk production and milk composition. Thirty-six Holstein dairy cows, averaging 123 (±32) DIM, 653 (±67) kg body weight, and 38.3 (±5.0) kg/d of milk production were housed in individual tie stalls and fed one of 3 diets as a TMR. Treatments (TRT) were a control diet (CON) with no added RP-Lys, a diet containing 0.3% of RP-Lys (0.3RP-Lys), and a diet containing 0.6% of RP-Lys (0.6RP-Lys). Dry matter intake (DMI) and milk yield were measured daily and milk composition, BW and BCS were measured weekly. Data were analyzed using the MIXED procedure of SAS with the fixed effects of TRT and wk with random effects of cow within TRT. Means were compared using Tukey's multiple comparison and were considered to be different when $P < 0.05$ and tended to differ when $0.05 \leq P \leq 0.1$. There were no differences in DMI (26.2 ± 3.3 kg DM, $P = 0.97$), milk yield (37.8 ± 5.5 kg/d; $P = 0.41$) and milk composition between TRT (% Protein 3.02 ± 0.22, $P = 0.30$; % Fat 3.38 ± 0.69, $P = 0.70$). There was no effect of TRT on total essential AA (EAA) in plasma (916, 893, 862 ± 44.5 µM, for CON, 0.3RP-Lys, and 0.6RP-Lys, respectively). Plasma His was reduced in a linear fashion by Lys feeding ($P < 0.05$, with 45.68, 41.28, 32.80 ± 3.661 µM, for CON, 0.3RP-Lys and 0.6RP-Lys, respectively). Plasma Lys and Met, as a % of EAA, increased linearly ($P < 0.05$) with RP-Lys supplementation. There was a tendency ($0.05 \leq P \leq 0.1$) for cows fed 0.6RPLys to have higher plasma Lys as % EAA than cows fed CON (5.72, 5.94, and 6.46 ± 0.242% EAA for CON, 0.3RP-Lys and 0.6RP-Lys respectively). In conclusion, cows supplemented with 0.6RP-Lys had similar DM intake and milk production despite elevated plasma Lys. The data indicate post-ruminal delivery of RP lysine. The decrease in plasma His suggests the inability of the cows used in this study to respond with changes in milk production linked to AA limitations other than additional Lys. Support for VMRM was from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil(CAPES).

Key Words: rumen-protected lysine, plasma lysine

T180 In situ rumen degradability and in vitro intestinal digestibility of rumen-protected methyl donors and lysine.

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Rumen degradation and intestinal digestibility of rumen-protected (RP) methyl donor and lysine products vary. Our objectives were to evaluate the in situ rumen degradability and in vitro intestinal digestibility of 4 RP products containing choline chloride, DL-methionine, betaine, and/or L-lysine in a triglyceride matrix. Four rumen-cannulated multiparous Holstein cows (175 ± 4 DIM; 34 ± 6 kg of milk/d) were used to test commercially microencapsulate products (Vetagro S.p.A., Reggio Emilia, Italy) which contain 25% choline chloride (RPC; Ruprocol), 55% DL-methionine (RPM; Timet), 33% L-lysine (RPL; ReLys) and 22% methionine, 10% choline chloride, 3% betaine, < 1% riboflavin and vitamin B12 (RPMD; MecoVit). The difference in composition was composed of hydrogenated palm fat. In paired duplicates, 5 g of RP product were placed in separate nylon bags and rumen-incubated for 0, 4, 8, 16, and 20 h. Bags were inserted in reverse order, removed collectively after incubation and washed in cold tap water. Intestinal digestibility was in vitro estimated using 16 h samples and an enzyme cocktail containing lipase (28 U/mL). All in situ and in vitro samples were dried and composited for N analysis using block digestion and steam distillation with automatic titration. The statistical model included the random effect of cow and replicates, and the fixed effects of product, time and their interaction. The percentage of N that passed the rumen varied across product ($P < 0.001$) and time ($P < 0.001$), and interactions were detected ($P < 0.05$). Percentage of rumen-undegraded N following in situ incubation is shown in Table 1. In vitro intestinal digestibility was 54.8 ± 3.48%, 78.1 ± 3.26%, 64.6% ± 2.89, and 74.9 ± 3.41% for RPC, RPM, RPL and RMD, respectively. Rumen protection for RPM, RPL, and RPMD was high, relative to RPC. Intestinal digestibility was high for all products tested.

Table 1 (Abstr. T180). Least squares means of rumen-undegraded nitrogen following in situ incubation

	Undegraded N, %				
Product	4 h	8 h	16 h	20 h	SEM
RPC	68.8	54.3	40.2	31.5	2.33
RPM	91.2	81.1	74.3	70.4	3.17
RPL	89.4	85.6	75.6	68.9	2.33
RPMD	76.2	64.8	51.7	49.1	3.13

Key Words: lysine, methyl donor, rumen protection

T181 Evaluation of rumen-protected lysine prototypes on performance of lactating Holstein cows.

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Supplementing dairy diets with rumen-protected lysine (RPL) could help to lower N emissions and improve productive efficiency. The objective of this study was to determine the short-term effects of targeted RPL and post-ruminal Lys supplementation to dairy cows on protein and AA in blood; and production of milk and milk components. A total of 18 multiparous Holstein cows [BW (mean ± SD) = 687 ± 68 kg; DIM = 151 ± 57] were assigned to 1 of 3 treatments in a replicated 3 × 3 Latin Square design balanced to measure carryover effects. Total length of the experiment was 73 d including a 10 d diet adaptation period before the start of the trial. Experimental periods were 21 d in length with each divided by adaptation phase (d 1 to 14) and sample phase for statistical inferences (d 15 to 20). Treatments were as follows: cows fed a basal TMR + 150g of dried ground molasses (CON); basal TMR+ 150g of dried ground molasses + a commercially available rumen-protected lysine source (AjiPro-L, Ajinomoto Heartland Inc., Tokyo, Japan) (AJP, positive control); and basal TMR + 150g of dried ground molasses + a rumen-protected lysine prototype source (NPL, prototype B, NutraPass 50, ADM Animal Nutrition, Quincy, IL). Protected lysine sources were each included at a rate of 0.51% [w:w] of the DM. Data were analyzed using the MIXED procedure of SAS, using 2 contrasts. Contrast 1 (CONT1): CON compared with NPL and contrast 2 (CONT2): AJP compared with NPL. There were no differences among treatments for either contrast (CONT1 or CONT2) for DMI, BW, DMI as a percent of BW, and BCS ($P > 0.05$). Milk yield was greater for cows fed NPL (35.98 ± 0.92 kg) than cows fed AJP (34.55 ± 0.93 kg; $P = 0.03$, CONT2). Milk protein yield was greater for cows fed NPL (1.11 ± 0.03kg) than cows fed AJP (1.06 ± 0.03kg; $P = 0.02$; CONT2). Milk casein yield was greater for cows fed NPL (0.42 ± 0.02kg) than cows fed AJP (0.34 ± 0.02kg, $P = 0.01$; CONT2). In conclusion, cows fed NPL performed similarly than cows fed AJP and had increased milk yield and protein yield.

Key Words: rumen-protected lysine, MUN, milk protein

T182 Dietary starch level and rumen-protected amino acids: Effects on CH_4 emissions and heat production in lactating dairy cows. Y. Zang*[1], L. H. P. Silva[1], Y. Geng[2], M. Ghelichkhan[1], N. L. Whitehouse[1], M. Miura[3], and A. F. Brito[1], [1]*University of New Hampshire, Durham, NH,* [2]*Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*Ajinomoto Co. Inc., Kawasaki-shi, Japan.*

Addition of fermentable energy and rumen-protected (RP) AA to metabolizable protein (MP)-deficient diets has been shown to improve milk yield and N efficiency. However, research on the interactions between RPAA and dietary starch level on energy utilization is lacking. We aimed to investigate the impact of RP Met, Lys, and His (MLH) and dietary starch concentration on CH_4 emission and heat production (HP) in dairy cows fed MP-deficient diets. Sixteen multiparous Holsteins cows (138 ± 46 DIM, 46 ± 6 kg/d milk) were used in a replicated 4 × 4 Latin square with 2 × 2 factorial arrangements of treatments. Each period lasted 21 d with 14 d for diet adaptation and 7 d for sample collection. Treatments were high-starch (HS), HS + RPMLH, reduced starch (RS), and RS + RPMLH. The basal diets consisted (DM basis) of 35.7% corn silage, 14.7% haylage, and 49.6% concentrate. Dietary starch level varied by replacing 30% ground corn with 20% beet pulp and 10% soyhulls. Dietary NE_L, starch, and CP averaged 1.68 Mcal/kg, 34.4%, and 16.0% for HS diets, and 1.59 Mcal/kg, 12.3%, and 16.4% for RS diets, respectively. Smartamine M, Aji-Pro L, and an Ajinomoto prototype His product were fed to meet digestible MLH requirements. The GreenFeed system was used to measure gaseous fluxes, with HP estimated using the Nienaber and Maddy (1985) equation: $HP (Mcal/d) = [16.18 \times O_2 (L/d) + 5.02 \times CO_2 (L/d) - 2.17 \times CH_4 (L/d)]/4.183/1{,}000$. Data were analyzed with the MIXED procedure of SAS. Daily CH_4 production (434 vs. 545 g/d; 605 vs. 760 L/d), yield (17.7 vs. 21.6 g/kg of DMI), and intensity (10.7 vs. 13.6 g/kg of ECM) were lower with feeding HS vs. RS diets, respectively. Consequently, cows fed HS diets had reduced CH_4 energy losses than those fed RS diets (5.72 vs. 7.19 Mcal/d). Moreover, O_2 consumption (mean = 8,340 L/d), CO_2 emission (mean = 6,043 L/d), and HP (mean = 39.2 Mcal/d) did not differ significantly across diets. No effect of RPMLH supplementation on energy utilization was observed. Collectively, elevated energy supply by substituting fibrous byproducts with ground corn decreased energy losses as CH_4 without changing HP.

Key Words: dairy cow, energy, amino acid

T183 Use of the plasma free amino acid dose-response technique to quantify bioavailability of rumen-protected histidine. N. Whitehouse*[1], B. Veilleux[1], Y. Zang[1], A. Brito[1], and M. Miura[2], [1]*University of New Hampshire, Durham, NH,* [2]*Ajinomoto Co. Inc., Kawasaki-shi, Japan.*

Rumen-protected (RP) Met, Lys, and His have been added to diets for optimizing milk protein synthesis and reduce N excretion to the environment. Therefore, it is critical to accurately estimate the relative bioavailability (RBV) of RPAA supplements. The plasma free AA dose-response technique has been used to determine RB of RP-Met and RP-Lys, but not RP-His. Our objective was to determine the RBV of a prototype RP-His supplement (Ajinomoto Co., Inc., Japan) using the plasma AA dose-response method. Six multiparous Holstein cows fitted with ruminal cannulas were used in a 6 × 6 Latin square design with 7 d experimental periods. The basal diet consisted (DM basis) of 31% corn silage, 20.4% haylage, and 48.6% concentrate. The diet was 16.4% CP, 177 g/d MP, and 3.5 Mcal/d NE_L. Treatments were abomasal infusions of His at 0, 8, 16 and 24 g/d, and 20.2 and 33.6 g/d of RP-His (42% His in the prototype). Blood samples were collected at 2, 4, 6, and 8 h after the morning feeding in the last 3 d and composited by day. Plasma AA was quantified using ultra performance liquid chromatography tandem mass spectrometry. Data were analyzed with the MIXED and PROC REG procedures of SAS. Both DMI and milk yield were not affected by treatments. Plasma His concentration linearly increased from 49.1 to 79.0 μM ($P < 0.0001$) in response to incremental amounts of His infused in the abomasum. Feeding RP-His also elevated the concentration of plasma His linearly (49.1 to 66.8 μM, $P < 0.0001$). Plasma carnosine concentration showed linear responses to both abomasal infusion of His (14.4 to 16.3 μM, $P = 0.017$) and RP-His supplementation (14.4 to 16.2 μM, $P = 0.043$). Treatments did not significantly change the concentrations of other plasma AA. The slopes from regressing plasma concentration of His (%TAA minus His) on incremental amounts of abomasally infused His or RP-His averaged 0.0463 and 0.0225, respectively. The RBV of the prototype RP-His tested averaged 48.6 ± 3.24 (0.0225 divided by 0.0463 times 100). Our data suggest that approximately 48% of His in RP-His was absorbed in the small intestine when using the plasma free AA dose-response technique.

Key Words: relative bioavailability, dairy cow, histidine

T184 Lactation performance of dairy cows fed increasing amounts of microencapsulated methionine. L. King*[1], E. Branstad[1], C. McCarthy[1], B. Dooley[1], M. O'Neil[1], D. Cooke[3], E. Grilli[2,3], and H. A. Ramirez-Ramirez[1], [1]*Iowa State University, Ames, IA,* [2]*DIMEVET, University of Bologna, Bologna, Italy,* [3]*Vetagro Inc., Chicago, IL.*

Amino acid requirements of high-producing dairy cows may be greater than what dietary and microbial crude protein can provide. Therefore, study objectives were to determine lactation performance of dairy cows fed rumen-protected methionine (MET; Timet, VETAGRO, Italy). Individually fed, multiparous Holstein cows (n = 48, 127 ± 41 DIM and 671 ± 8 kg BW) were used in a replicated 4 × 4 Latin square design with 28-d periods. Dietary treatments consisted of a basal TMR deficient in metabolizable MET (−10 g/cow/day): 1) Control (CON) with no supplement; 2) low methionine (LM) diet with 11 g/cow/d MET; 3) medium methionine (MM) containing 19.25 g/cow/d MET; and 4) high methionine (HM) diet plus 27.5 g/cow/d MET. Milk yield and feed intake data from the last 7 d of each period were used for analyses; BW, BCS and milk components were determined on d 27 and 28 of each period. Statistical analysis was performed using the MIXED procedures of SAS with square, period within square, and treatment as fixed effects and cow within square as a random effect. Linear, quadratic, and cubic effects were also tested. No evidence of quadric or cubic effects was observed for any of the response variables. Dry matter intake was similar ($P = 0.64$) across treatments whereas milk production increased linearly from 38.0 kg/d for CON to 39.7 ± 0.87 kg/d for HM. Milk fat concentration was similar across treatments ($P = 0.83$) averaging 3.69 ± 0.88% while milk protein concentration tended to increase linearly ($P = 0.11$) from 3.17 ± 0.04% for CON to 3.21 ± 0.04% with high MET supplementation. Overall, supplementation with MET resulted in greater ($P \leq 0.01$) yield of milk protein and fat. Consequently, yield of energy-corrected milk (ECM) increased linearly, namely, cows consuming the CON diet produced 38.3 ± 1.05 kg ECM/d whereas MET supplementation resulted in 41.4, 40.9 and 41.7 ± 1.05 kg ECM/d, for LM, MM and HM, respectively. Concentration of MUN averaged 13.5 ± 0.23 mg/dL across treatments ($P = 0.58$). These data suggest that the MET supplement increased the supply of metabolizable methionine resulting in increased yield of milk and milk components.

Key Words: limiting amino acid, nitrogen efficiency, rumen protected

T185 Assessment of dairy cow performance fed soybean meal or canola meal through a meta-analysis.

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Performance of dairy cows fed soybean (SBM) meal in comparison with canola meal (CM) remains controversial. The objective of this study was to evaluate dry matter intake (DMI), milk yield, and milk composition of cows fed diets containing either SBM or CM. Data from 10 published papers (1998–2019) were evaluated through a meta-analysis performed using fixed or random effect models in R. The degree of heterogeneity was measured with I^2 statistics. Publication bias was determined with funnel plots and Egger's regression test. Other sources of heterogeneity of response were analyzed through a mixed-effect meta-regression. Estimated effect size was calculated for DMI, milk yield, and milk composition. The meta-analysis indicated no evidence of publication bias for any of the variables tested. Cows fed diets with SBM produced 0.41 kg/d less milk than cows on diets with CM. However, DMI, energy corrected-milk (ECM), milk composition, and feed efficiency (FE) were not affected by meal protein source. Meta-regression indicated that the inclusion of forage and grain in the diets affected milk yield. Overall, there was no difference in performance and efficiency between cows fed CM- or SBM-based diets.

Key Words: meta-analysis, canola or soybean meal, milk yield

T186 Slow-release urea (Optigen) does not elevate ammonia levels and leads to lower lactic acid accumulation when compared to free urea in an in vitro rumen model.

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The objective of this study was to compare the effects of slow-release urea (SRU) or free urea on rumen fermentation using an in vitro model. Low energy (20:80, wheat: grass silage) and high-energy rations (60:40, wheat: grass silage) were treated with 3 different nitrogen sources: soybean meal (SBM), urea and a SRU product (Optigen Alltech Inc., KY). SBM replaced 20, 60 or 100 mg of the 1000 mg diets. The dose of urea or SRU was calculated to provide the same amount of nitrogen as the SBM treatments, with the basic diet substituted with 2.3, 6.9 and 11.5 mg for the urea treatments and with 2.6, 7.8, 13.1 mg for the SRU treatments. This design resulted in 20 treatments with 5 replicates each. The components of each treatment were weighed in serum bottles which were flushed with CO_2 and sealed, after which 38 mL of anaerobic, reduced, buffer solution (+38°C) was introduced under oxygen free CO_2 flow. Simulation began when 2 mL of freshly strained rumen fluid was added and continued for 24 h. Ammonia, lactic acid and total gas production were measured. Two-tailed *t*-tests were used to analyze differences between treatments and controls and between the SRU and urea treatments. The addition of urea significantly increased ($P < 0.001$) ammonia concentrations in low energy diets after 9 and 12 h, while the level of ammonia did not differ between SRU and control treatments. For high-energy diets urea led to modest increases in ammonia ($P \leq 0.05$) after 9 h, with no differences observed between SRU, SBM and control treatments. In terms of lactic acid accumulation, SRU resulted in a 10–39% ($P < 0.1$) lower accumulation of lactic acid than the urea for the low energy diets, and 21–33% lower ($P \leq 0.01$) accumulation for the high-energy diets. Total gas production for the high-energy diets increased significantly ($P < 0.001$) for both urea and SRU treatments in a dose-dependent manner while the corresponding amount of SBM had no effect. These findings indicate that the use of SRU results in lower ammonia and lactic acid accumulation when compared with free urea.

Key Words: rumen, urea, non-protein nitrogen

Table 1 (Abstr. T185). Estimated effect size derived from meta-analysis in dairy cows fed diets containing SBM or CM

Outcome	Cows (n) SBM	Cows (n) CM	Weighted mean difference for SBM – CM [95% CI]	Effect size [95% CI]	I^2 (%)	*P*-value for effect size	Begg's test
DMI (kg/d)	81	81	−0.32 [−0.77, 0.13]	−0.18 [−0.49, 0.13]	0.0	0.16	0.75
Milk yield (kg/d)	81	81	−0.41 [−0.73, −0.09]	−0.41 [−0.73, −0.09]	0.0	0.01	0.24
ECM (kg/d)	81	81	0.25 [−0.72, 0.22]	−0.20 [−0.51, 0.11]	0.0	0.21	0.52
Fat (%)	81	81	0.14 [−0.32, 0.60]	0.09 [−0.23, 0.40]	0.0	0.59	0.36
Fat yield (kg/d)	81	81	0.009 [−0.44, 0.46]	0.0002 [−0.31, 0.31]	0.0	0.97	0.80
Protein (%)	81	81	−0.003 [−0.43, 0.42]	0.002 [−0.31, 0.31]	0.0	0.99	0.98
Protein yield (kg/d)	81	81	−0.02 [−0.45, 0.41]	−0.01 [−0.32, 0.30]	0.0	0.93	0.19
Lactose (%)	81	81	0.04 [−0.37, 0.45]	0.02 [−0.29, 0.33]	0.0	0.85	0.26
Lactose yield (kg/d)	81	81	−0.01 [−0.40, 0.38]	−0.01 [−0.32, 0.30]	0.0	0.95	0.57
FE (ECM/DMI)	81	81	0.02 [−0.07, 0.10]	0.03 [−0.28, 0.34]	0.0	0.73	0.86

T187 Nutritive value of mechanically pressed cottonseed cake compared with soybean meal for lactating dairy cows.

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Forty-eight lactating Holstein cows were used in a randomized block trial to evaluate the nutritive value of mechanically pressed cottonseed cake (CSC) compared with solvent extracted (SBM) or expeller soybean meal (ESM). The CSC was produced by dry extruding whole cottonseed at 121 to 149°C for 12–20 s. The resulting CSC contains approximately 33% CP with 40% RDP and 60% RUP (DM basis). All cows were fed a control diet during the first 3 wk and data collected were used as a covariate in the statistical analysis. At the end of the preliminary period, cows were fed experimental diets for the following 8 wk. Treatments included: 1) control diet supplemented with SBM and ESM (CONT); 2) CSC substituted for SBM (CSBM); and 3) CSC substituted for ESM (CESM). No differences ($P > 0.10$) were observed in DMI (27.2, 28.1, and 28.1 kg/d), milk yield (33.0, 32.8, 33.4 kg/d), milk fat (3.96, 3.96, and 4.00%), lactose (4.67, 4.67, and 4.69%), solids-not-fat (8.62, 8.52, and 8.57%) or ECM (35.1, 34.7, and 35.7 kg/d) among CONT, CSBM, or CESM, respectively. Milk protein percentage was lower ($P = 0.0418$) for CSBM compared with CONT but not different to CESM (2.96, 2.84, and 2.90% for CONT, CSBM and CESM, respectively). Milk urea nitrogen concentrations were lower ($P = 0.0003$) for CSBM compared with CONT and CESM: 8.56, 7.58, and 9.27 mg/dL for CONT, CSBM, and CESM, respectively. No difference ($P > 0.10$) were observed in BW change (31.7, 32.5, and 28.1 kg for CONT, CSBM, and CESB, respectively). The lower MUN observed for CSBM suggest that substituting the CSC for SBM may have limited degradable nitrogen. The results of this trial indicate that the CSC can be substituted for soybean meal or expeller soybean meal without affecting intake, milk yield or composition.

Key Words: mechanically pressed cottonseed cake, soybean meal, expeller soybean meal

T188 Camelina cake in dairy cow diets: Effects on production and milk composition.

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Increasing the nutritional quality of fat in dairy products is a rising consumers claim motivated by the potential benefits of long chain n-3 fatty acids and low n-6/n-3 ratio on human health. Camelina is a sustainable crop and a good source of n-3 fatty acids. The goal of the trial was to study the effects of camelina cake inclusion in the diet of milking dairy cows on production and milk composition. Twenty-two primiparous and multiparous Holstein Friesian dairy cows housed at the Experimental Station (Centro Zootecnico Didattico Sperimentale) of the University of Milan in Lodi were divided in two homogeneous groups for milk production, parity and DIM (24.62 kg, 1.95, 160d). Diets consisted of a TMR corn silage-based, supplemented with 800g/h/d of camelina cake (CAME) or an isonitrogenous and isoenergetic soybean-based premix (CTR). Performances were recorded weekly. Milk composition were assessed weekly on fresh samples. Milk samples for milk fatty acid compositional analysis were collected on day 0, 21, and 42 of the trial. Data were analyzed by MIXED procedure by SAS. No differences were detected for milk production, feed intake, live body weight and body condition score. CAME group had a reduced milk fat percentage (4.27 vs. 4.72%; $P < 0.05$) compared to CTR but not a reduced production of fat (1.11 vs. 1.17 kg). Saturated milk fatty acids were also reduced in CAME compared with CTR (2.8 vs. 3.14%; $P < 0.05$). Linolenic acid and rumen biohydrogenation intermediates were increased in CAME whereas stearic acid decreased. Camelina cake could be useful to improve nutrition value of milk, but its impact on rumen metabolism and possible interferences on biohydrogenation steps need further attention.

Key Words: camelina cake, dairy cow, milk fat composition

T189 Insulin potentiates the effect of individual amino acids on mechanistic target of rapamycin complex 1 (mTORC1) signaling in mammary epithelial cells.

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The mechanistic target of rapamycin complex 1 (mTORC1) integrates endocrine and nutritional signals to modulates metabolic functions. The objective of this study was to investigate the role of insulin in essential AA regulation of mTORC1 signaling in mammary epithelial cells. Serum starved MAC-T cells were incubated for 1 h in media containing 5.6 mM glucose, 10 mM sodium acetate, and 1.25 mM non-essential AAs, and lacking EAAs. To confirm the effect of individual AAs on mTORC1 activity, cells were treated with 100 nM insulin and each essential AA at 0, 1×, or 3× the reference plasma level of total essential AA in a lactating dairy cow, considering the profile observed in milk proteins (i.e., for 1× 55 µM Arg, 55 µM His, 130 µM Ile, 210 µM Leu, 150 µM Lys, 50 µM Met, 90 µM Phe, 100 µM Thr, 20 µM Trp, 160 µM Val). The ratio of phosphorylated to total protein for the mTORC1 substrates and mRNA translation regulators S6 kinase 1 (P-S6K1 T389) and translation initiation factor 4E-binding protein 1 (P-4E-BP1 S65) were determined by Western blotting. Essential AAs that stimulated mTORC1 activity were studied in individual 4 by 3 factorial studies with each AA at 0, 0.5×, 1×, and 3× plasma concentration (as above), and insulin at 0, 10, or 100 nM . Data were analyzed with the lm function in R. Only the EAA Arg, Ile, Leu and Met significantly increased S6K1 and 4E-BP1 phosphorylation in a quadratic fashion (x and x^2 $P < 0.05$). For these 4 AAs, insulin significantly ($P < 0.05$) affected polynomial parameters of S6K1 and 4E-BP1 phosphorylation, decreasing with the decrease in insulin concentration. At the lowest concentration of insulin, linear and quadratic parameters for any of the 4 AA effects on S6K1 and 4E-BP1 were different than zero ($P > 0.05$). Our results challenge the conventional representation of the relationship between individual AAs and energy, at least for their effect on the regulatory network of milk protein translation (i.e., mTORC1 signaling). These results also entertain the idea of energy beyond calories, in which insulinemic and non-insulinemic energy sources could play contrasting roles in production outcomes.

Key Words: essential amino acid, insulin, mechanistic target of rapamycin complex 1 (mTORC1)

Small Ruminant 1

T190 Relationship between somatic cell count variation and mastitis in dairy goats of northwest Argentina. V. Suarez and G. Martinez*, *INTA, Salta, Argentina.*

The aim was to study the variations of somatic cell counts (SCC) of goat milk according to pathological and physiological variables. Aseptic samples of foremilk were collected every 20 d from both udder halves (UH) during entire lactation from 87 goats, resulting in a total of 929 samples. The infectious status was assessed by bacteriological cultures, and the SCC (cells/mL) by a Fossomatic counter. Chi-squared, ANOVA, and regression analysis (positive cultures transformed into dummy variable) were carried out to analyze the relationship between variables. Prevalence of intramammary infection (IMI) was 14.2% of all UH samples examined. The prevalent mastitis agents were coagulase-negative staphylococci (CNS, 10.6%) and *Staphylococcus aureus* (3.6%). Pathogens increased ($P < 0.001$) the SCC. The average values for SCC of uninfected UH, CNS and *S. aureus* are shown in Table 1. Uninfected SCC averages for the first (≤90 d), second (91–180 d) and third (>180 d) lactation stages were $674 \pm 1087 \times 10^3$, $995 \pm 1300 \times 10^3$ and $1637 \pm 2424 \times 10^3$, respectively. Uninfected UH of goats with more than 3 lactations (n = 295 samples) had more ($P = 0.004$) SCC average ($1075 \pm 1752 \times 10^3$) than those of 1 or 2 lactations (n = 501 samples; $792 \pm 664 \times 10^3$). The SCC regression with IMI and days in lactation were $R^2 = 0.19$ ($P < 0.001$) and $R^2 = 0.10$ ($P < 0.001$), respectively. The SCC averages of negative UH were 1946×10^3, 1004×10^3, 647×10^3, and 335×10^3 for daily milk yields less than 0.5 L, between 0.51 and 1.0 L, between 1.01 and 2.0 L, and more than 2.01 L, respectively. Although subclinical mastitis was the principal factor for SCC increases, these results showed that there are other non-infectious factors (lactation stage, parity number and milk yield) that cause variation in SCC, and must be considered when monitoring udder health and milk quality of dairy goats.

Key Words: dairy goat, somatic cell count, pathogen.

T191 Productive performance of dairy goats supplemented with two different iodine sources. G. M. Martinez* and V. Suarez, *Instituto Nacional de Tecnología Agropecuaria, Salta, Argentina.*

In the northwestern of Argentina it is very common to detect goats with iodine deficiency disorders. Information about the effects of iodine supplementation on milk yield in dairy goats is rather scarce. Therefore, the aim of this work was to evaluate the effects of the supplementation with 2 different iodine sources on dairy goat's productive performance. Twenty multiparous Saanen dairy goats were randomly assigned to 2 treatments: potassium iodide (PI) and organic iodine long acting (OI). Doses were suggested by the manufacturer: PI: 3 mg/goat and OI: 200 mg/goat. In both treatments, iodine was administered subcutaneously 45 d prepartum. For the PI group, additional dose was administered 45 d postpartum. During the entire trial period each group was fed alfalfa hay ad libitum and 0.5 kg corn/d per goat. Data were collected at 15, 30, 60 and 90 d postpartum. Milk yield was determinate by Waikato lactometers and milk samples for composition were collected during milking and analyzed by Milko-Scan FT 600. Data were analyzed with linear mixed model according to a repeated measures design by Infostat program. Milk yield and milk composition are reported in Table 1. Iodine source did not affect any of the studied parameters, except milk fat content that increased in OI goats. Based on the data obtained in the present trial and the fact that all parameters evaluated were within the standard values suggested for goat milk, it is possible to conclude that there are no differences between both iodine sources in dairy goats.

Table 1 (Abstr. T191). Milk yield and milk composition from goats supplemented with potassium iodide (PI) or organic iodine (OI)

	Treatment			*P* <		
Item	PI	OI	SE	Iodine source	Sampling day	Interaction
Milk yield, L/d	2.59	2.24	0.20	NS	0.001	0.001
Fat, %	3.55	4.08	0.13	0.008	0.001	NS
Protein, %	3.04	3.09	0.05	NS	0.001	NS
Lactose, %	4.90	4.84	0.07	NS	0.001	NS
Solids-not-fat, %	8.76	8.76	0.10	NS	0.001	NS
pH	6.83	6.82	0.02	NS	0.001	NS
Acidity, °D	12.04	11.66	0.48	NS	0.001	NS

Key Words: dairy goat, iodine source, productive performance

T192 Performance of Saanen kid fed different diets and slaughtered at different ages. G. Martinez*, E. Alfaro, and R. Alfaro, *Instituto Nacional de Tecnología Agropecuaria, Salta, Argentina.*

In Argentinian goat production systems, kids are weaned and sold between 45 and 60 d of age and weight between 10 to 12 kg. The aim of the present study was to determine average daily gain (ADG), and slaughter and carcass weights in Saanen kids slaughtered at 50, 60 and 70 d of age and fed ad libitum 3 diets: alfalfa hay (A), alfalfa hay + cracked grain (30:70, soya:corn) (AG) and only cracked grains(G) according to a completely randomized design. Twenty 7 Saanen kids were separated from their dams at birth and artificially colostrated. Throughout the experiment kids were fed with 1.5 l of goat milk twice a day. From the 2nd week of life, kids had free access to feed according to the assigned treatment. Kids were weighed once a week before milk supply. Immediately after slaughter, entire digestive apparatus was removed from the carcass. Data were analyzed with linear mixed

Table 1 (Abstr. T190). SCC values from uninfected UH (UI) and with subclinical infections due to SCN and *S. aureus*.

				95% CI		
Udder half health	n	Mean	SD	IL	SL	Median
UI	796	789,427	1,139,886	710,118	868,734	381,000
CNS	99	1,891,432	1,521,385	1,384,177	2,398,687	1,619,000
S.aureus	34	5,296,615	3,806,640	3,118,445	7,290,169	4,431,000

model according to a repeated measures design by Infostat program. The Multiple comparisons were evaluated by LSD-Fisher test ($P = 0.05$). The weight at birth of the animals was similar between treatments (3.48 ± 0.51 kg, $P = 0.42$). ADG according to the diet was higher for those animals that consumed alfalfa, with or without grain supplementation (A: 147 ± 13 g - AG: 152 ± 17 g) compared with the grain group (G: 124 ± 20 g) ($P < 0.01$). Slaughter age did not affect ($P = 0.96$) ADG. Age affected ($P < 0.01$) body weight at slaughter; kids slaughtered at 70 d showed higher slaughter weight (12.83 ± 0.38 kg) than those slaughtered at 60 (11.32 ± 0.33 kg) or 50 d (10.08 ± 0.38 kg). Interaction between diet and age was not detected ($P = 0.36$) for slaughter weight. Kids slaughtered at 70 d of age had heavier ($P < 0.05$) carcass weight (5.11 ± 0.31 kg) than those slaughtered at 60 (4.61 ± 0.65 kg) or 50 d (4.10 ± 0.35 kg). Carcass weight tended ($P = 0.06$) to be greater in kids fed AG diet (4.88 kg) compared with those fed G diet (4.36 kg). No interaction between diet and slaughter age was detected for carcass weight ($P = 0.78$). In conclusion, obtained results indicate that diet only affected average daily gain, whereas age at slaughter affected both slaughter weight and carcass weight.

Key Words: dairy kid, average daily gain, slaughter weight

T193 Effect of a novel selenium product on growth performance of lambs.

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The objective of this study was to investigate the bioavailability of a novel Se product (B-Traxim Se-11; Pancosma, Switzerland) and its effect on growth performance of lambs. Sixty lambs (Suffolk × Hampshire) were used in an 8-wk randomized complete block design experiment. Lambs were housed in 12 pens (5 lambs/pen) and pens were blocked based on lambs' initial body weight (average 35.7 kg; SD = 4.15 kg). Pens within a block were randomly assigned to one of the following treatments (3 pens/treatment): basal diet supplemented with (1) sodium selenite providing 0.15 mg Se/kg dietary DM (NaSe); (2) B-Traxim Se providing 0.08 mg Se/kg DM (BTLow); (3) B-Traxim Se providing 0.15 mg Se/kg DM (BTHigh); and (4) selenized yeast providing 0.15 mg Se/kg DM (YST). The basal diet included 40% grass hay (offered ad libitum) and 60% concentrate feeds and a mineral premix without Se. Body weight of the lambs was recorded weekly and blood samples were collected from the jugular vein twice, at wks 4 and 8 of the experiment. Data were analyzed using the MIXED procedure of SAS (repeated measures for the production data) with pen as the experimental unit; block and block × treatment were random effects. Average daily gain tended to be lower ($P = 0.06$) for BTLow, compared with NaSe, BTHigh, and YST (263, 286, 275, and 298 g/d, respectively; SEM = 9.3). Dry matter intake and feed-to-gain ratio were not affected by treatment ($P \geq 0.09$). Compared with NaSe and YST, Se concentration in blood plasma was decreased ($P = 0.007$) by BTLow and BTHigh (67.4, 70.4, 58.2, and 60.9 µg/L, respectively; SEM = 2.28). Se concentrations in feces were greater ($P < 0.001$; SEM = 21.7) for NaSe and BTHigh (498 and 489 µg/kg DM, respectively) than the groups supplemented with YST (356 µg/kg DM) and BTLow (309 µg/kg DM). Concentration of Se in urine was similar among treatments ($P = 0.37$; average 22.9 µg/L; SEM = 5.54). In this experiment, Se from B-Traxim had no effect on productive performance of lambs and, based on the plasma Se concentration and fecal excretion data, had similar or lower bioavailability than Se from sodium selenite and selenized yeast.

Key Words: selenium, growth performance, lamb

T194 Effects of selenium yeast supplementation on milk selenium concentration, milk performance, milk fatty acid profile, and antioxidant and immunity status in lactating dairy goats.

P. Wang*[1], J. Wu[1], G. Lin[2], A. Koontz[3], and J. Luo[1], [1]*College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi, China,* [2]*Alltech China, Beijing, China,* [3]*Center for Animal Nutrigenomics and Applied Animal Nutrition, Alltech Inc., Nicholasville, KY.*

The objective of this study was to evaluate the effects of increasing level of Se-enriched yeast on milk Se concentration, milk performance, and antioxidant and immunity status in lactating dairy goats. Four primiparous dairy goats (BW = 49.69 ± 3.17 kg) were assigned to 4 doses of Se-enriched yeast treatments in a 4 × 4 Latin Square design for 4 21-d periods. Se-enriched yeast was provided at 0 (Control), 0.23 (LSY), 0.46 (MSY) and 0.69 mg (HSY) per goat daily. Milk samples were collected on d 17–21 and blood samples were taken on d 21 of each period. Data were analyzed as a 4 × 4 Latin square design using the mixed procedure of SAS software. The statistical mixed model contained the fixed effects of treatment and period, and the random effect of the goat. Selenium concentration in milk increased with increasing Se-enriched yeast supplementation. Milk Se concentration for goats with 0.69 mg Se-enriched yeast supplementation was elevated by 2 times as compared with those with Control (0.027 mg/kg vs. 0.014 mg/kg; $P < 0.05$). No significant differences among treatments ($P > 0.05$) were observed for milk performance or milk fatty acid profile. Serum malondialdehyde concentration was linearly decreased (5.48, 4.75, 3.67 and 2.69 nmol/mL for control, LSY, MSY and HSY, respectively; $P = 0.006$), whereas glutathione peroxidase concentration was quadratically ($P < 0.0001$) and total antioxidative capacity concentration was linearly ($P = 0.001$) increased in response to incremental Se-enriched yeast supplementation. These results suggest that Se-enriched yeast supplementation improved antioxidant status of lactating dairy goats. In conclusion, increasing level of Se-enriched yeast supplementation elevated milk Se concentration, and improved antioxidant capacity in lactating dairy goats without negative influence on milk performance and immune status.

Key Words: selenium-enriched yeast, milk selenium concentration, dairy goat

T195 Response to an *Escherichia coli* LPS intramammary challenge of dairy ewes supplemented with barley β-glucans.

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Adult ewes of 2 breeds (Lacaune, n = 18, 77.4 ± 1.6 kg BW; Manchega, n = 18, 79.3 ± 1.2 kg BW) at late lactation (DIM, 210 ± 5) were used to assess the effects of barley β-glucans (BBG) supplementation on their immune response to a lipopolysaccharide (LPS) intramammary challenge. Ewes were fed alfalfa hay ad libitum and 350 g/d of barley grain cv Hispanic (3.8% BBG; Batlle, Lleida, ES) for 7 d. Thereafter, they were allocated into 3 groups to which the experimental treatments were applied for 14 d. Groups were: 1) Control (CO), fed as during adaptation (13.3 g BBG/d); 2) Oral (OR), fed with a new barley variety (cv Anapurnna, Batlle) of 10% BBG (35 g BBG/d); and, 3) Injected (IN), same as CO and intraperitoneally injected with a single dose of BBG

(140 mL of 1.4% BBG; 2 g BBG/ewe). After 9 d, ewes were submitted to an intramammary LPS challenge with 1 mL of an *E. coli* endotoxin (5 µg/mL, Sigma-Aldrich, St Louis, MI) in an udder-half and 1 mL of saline in the other. Rectal temperature, milk yield, milk composition and plasma interleukin (IL)-1α and IL-1β were monitored daily for 5 d. Data were treated by PROC MIXED for repeated measurements of SAS (v.9.4, SAS Institute Inc., Cary, NC) and LS means separated by PDIFF. No differences by treatment were detected in rectal temperature ($P = 0.13$), milk yield ($P = 0.29$) and IL-1α and IL-1β ($P = 0.31$ to 0.84). However, BBG tended ($P = 0.06$) to attenuate the lactose decrease in milk by LPS, the decrease being lower in the IN treatment vs. the others (3.80 vs. 3.57%, respectively). The SCC in milk increased due to the effect of LPS, but there were no differences by BBG. The IL-1α and IL-1β did not differ by BBG or time. However, their numerical values increased by time, agreeing with the increase in SCC and inflammation. Moreover, basal IL values were lower ($P < 0.01$) for IN when compared with the others (299 vs. 352 pg/mL and 79 vs. 152 pg/mL for IL-1α and IL-1β, respectively), indicating that BBG supplementation may increase the immune status and the protection against mastitis. In conclusion, the LPS challenge triggered a short-term immune response in BBG supplemented ewes, which was more effective when injected intraperitoneally. Supported by MINECO/FEDER (AGL2015–69435-C3–3-R).

Key Words: interleukin, β-glucan, mastitis

T196 Impact of culture positive samples on goat milk composition. G. Santana[1], K. Reis[2], M. Marques[1], L. Guifarro[3], M. Rovai[3], and L. da Costa*[4], [1]*Federal Rural University of Pernambuco, Recife, PE, Brazil,* [2]*University of São Paulo, São Paulo, SP, Brazil,* [3]*South Dakota State University, Brookings, SD,* [4]*The Ohio State University, Columbus, OH.*

The dairy goat industry is growing worldwide to meet the increased demand for milk and artisanal cheeses. Challenges to produce milk with quality exist due to the impact of microorganism causing intramammary infection (IMI). Milk culture stills a good alternative to monitor mammary gland health status. However, caution should be taken when interpreting the results in goats and its implications on milk characteristics. Thus, the aim of this study was to better understand the impact of an IMI on goat milk quality and composition. A total of 98 milk samples from 49 goats were used. Two simultaneous sterile samples of milk (15 mL) were collected from each half to perform microbiological culture and milk composition. Culture was performed streaking 10 µL of milk onto 5% sheep blood agar, incubated at 37°C for 24/48h and species identification made through MALDI-TOF. Milk composition (protein, fat, lactose, casein, and SCC) for each udder half was performed by a service provider (Eurofins DQCI– MN) using FTIR method. Statistical analysis (considering each half as statistical unit) for milk components were performed by *t*-test for unequal sample size to compare the means of positive and negative samples. The SCC was transformed to linear score. Milk culture revealed 73 negative samples and 25 positive samples as follow: *S. simulans* (11), *S. aureus* (4), *S. chromogenes* (3), *Bacillus* spp. (3), *S. epidermidis* (2), *S. caprae* (1), and *S. warneri* (1). For milk composition, culture negative samples contained 0.2% more ($P < 0.05$) casein, but 0.5% less ($P < 0.003$) fat and less ($P < 0.001$) SCC (637×10^3 cells/mL) when compared with culture positive samples. No differences were observed for lactose or protein. Higher levels of fat in subclinical mastitis milk can be explained by a greater permeability of mammary gland tissue to lipids present in blood during inflammatory response or also a concentration effect. Notably, goats with subclinical mastitis can have important decrease in milk quality leading to significant losses in milk yield which has a direct impact on dairy products.

Key Words: milk, composition, goat

ADSA Southern Branch Graduate Student Oral Competition

262 Effects of mean occupation rate on hourly automatic milking system use and total milk production in Holstein cows. A. Lee*[1], P. Krawczel[1], E. Ternman[2], L. Schneider[1], P. Løvendahl[2], and L. Munksgaard[2], [1]*University of Tennessee, Knoxville, TN,* [2]*Aarhus University, Tjele, Denmark.*

Automatic milking system (AMS) recommendations for stocking density are based on company guidelines rather than empirically derived results. Our objectives were to evaluate how: a) hourly AMS visits varied with occupation rate (total h the AMS was available, minus time spent on other activities) and b) fat- and protein-corrected milk (FPCM) varied with stocking density (daily number of cows milked by an AMS). A retrospective analysis of 2 AMS (Voluntary Milking System, DeLaval, Tumba, Sweden) was performed on data collected from 2004 to 2006 and 2012 to 2018 at the Danish Cattle Research Center (Foulum, Denmark). Hourly AMS visits were analyzed using an ANOVA (GLIMMIX Procedure, SAS 9.4, Cary, NC, USA). Fixed effects included days in milk (DIM), parity (primiparous and multiparous), occupation rate, h, and interaction of occupation rate and h, with yr and AMS by yr considered random effects. FPCM was assessed by linear mixed models using the MIXED procedure. Fixed effects of parity and week in milk were included in all models. We also tested the polynomial effects of stocking density at a quartic relationship. Random effects were defined as AMS, week, and week nested within year. Fixed effects remained in the model regardless of significance. Stocking density averaged 56.3 ± 4.3 and 55.9 ± 4.6 cows per AMS (range: 38 to 64). Parity, DIM, occupation rate, h, and occupation rate by h were associated with hourly AMS visits (F ≥44.7, $P < 0.001$, DF = 141862). As occupation rate increased, cows were most likely to visit the AMS at 8:00, 16:00, and 17:00. Hourly AMS use was lowest from 2:00 to 4:00 and was unrelated to occupation rate (t <1.14, $P \geq 0.22$). Mean FPCM per AMS was greater at 60 to 64 cows per AMS (2356.9 ± 82.0 kg) compared with <60 cows (2002.4 ± 97.9 kg; t ≥11.9; $P < 0.001$), suggesting that FPCM per AMS had plateaued. Stocking density affected hourly occupation rate and FPCM. Results from this study suggest a stocking density of 60 to 64 cows within an AMS, while considering optimal cleaning times, is key for maximizing productivity.

Key Words: automatic milking system, stocking density, fat- and protein-corrected milk

263 Simulating the cost to raise dairy heifers from birth to 60 days pre-calving under different post-weaning management and housing practices. A. C. Hawkins*, K. H. Burdine, D. M. Amaral-Phillips, and J. H. C. Costa, *University of Kentucky, Lexington, KY.*

Heifer rearing costs represent a substantial financial investment for a dairy operation. While costly, raising heifers are necessary for maintaining herd size and continuing genetic improvement. The objective of this project was to develop a stochastic simulation model for the total cost of raising a single heifer from birth to 60 d pre-calving under different post-weaning management styles. Situations modeled include: situation 1 (S1), confinement facilities and fed stored feeds including silage, situation 2 (S2), managed on a dry lot with shade and fed stored feeds including silage, situation 3 (S3), housed on pasture and supplemented as needed with grain and hay. Pre-weaning (group housed, 10 L/d milk replacer in automatic feeder) and breeding expenses (CIDR with visual heat detection) were constant for all situations. Post-weaning costs estimates for S1 and S2 included feed with storage and feeding losses, housing infrastructure and equipment, manure management, paid and unpaid labor, and veterinary expenses. Costs were calculated the same for S3 with the addition of land value and annual pasture management, reflective labor and manure costs. An economic simulation with 10,000 iterations was completed for each situation using @Risk (Palisade Corporation, Ithaca, NY). To account for variation expected between farms, pre-weaning mortality rate varied between 2 and 10% and age at first calving from 22 to 25 mo. Each simulation assumed 1000 heifers were raised from birth to 60 d pre-calving. Cost and biological responses were determined based on USDA market reports (2014–2018), published surveys, and literature. Average total costs were, S1: $1,899.49 ± $57.37, S2: $1,582.69 ± $42.25, S3: $1,324.16 ± $28.42. Feed was the largest contributing expense to each situation at 53, 63, and 57% of total cost, respectively. Labor accounted for 20% in S1, 24% in S2, and 19% in S3. Additional housing infrastructure accounted for most of the increase in cost between S1 and S2. Reducing facility and labor needs as well as feed costs are reflected in the lower total cost in S3.

Key Words: replacement animal, stochastic model, dairy economics

264 Polyhalite reduces urine pH in nonlactating cows without affecting dry matter intake. E. S. Richardson*[1], G. Ferreira[1], and R. J. Meakin[2], [1]*Department of Dairy Science, Virginia Tech, Blacksburg, VA,* [2]*Sirius Minerals, Scarborough, UK.*

Polyhalite is a natural mineral that could be fed as an acidogenic product to induce metabolic acidosis and prevent hypocalcemia in dairy cows after calving. The objectives were to determine if feeding polyhalite is an effective means of reducing urine pH and if feeding polyhalite negatively affects dry matter intake during the prepartum period. Twenty-four nonlactating and pregnant Holstein cows entering their second or greater lactation were fed one of 4 experimental close-up diets. The experimental diets included (DM basis) 48% corn silage, 25% grass hay, and 27% of 1 of 4 pelleted concentrates, which provided: 1) no acidogenic product (CON), 2) 200 g/day of polyhalite (LOW), 3) 400 g/day of polyhalite (HIGH), and 4) 250 g/day of calcium chloride (CHL). At 21 d before their expected calving date (ECD), cows were transferred from the far-off to the close-up group. While in the close-up group, cows were fed individually using a Calan gate feeding system. Dry matter intake was measured individually on a daily basis. On d −21, −14, −7, and 0 relative to ECD, urine samples were collected after vulva stimulation in the morning before feeding. Urine pH was determined immediately after collection using a portable pH meter. Data were analyzed with Proc MIXED of SAS. The statistical model included the fixed effects of diet, day, and diet by day and the random effects of block and block by diet. Both variables were tested using the autoregressive covariance structure for repeated measures (cow = subject). Urine pH differed among diets ($P < 0.01$). Cows consuming CON and LOW had the highest urine pH (8.06 and 8.02, respectively), and cows consuming HIGH and CHL had the lowest urine pH (6.35 and 6.70, respectively). Dry matter intake did not differ among diets (13.7 kg DM/d; $P > 0.83$) but decreased toward calving ($P < 0.01$). Dry matter intake toward calving decreased similarly for all diets ($P > 0.76$). In conclusion, feeding polyhalite is an effective means for inducing metabolic acidosis without reducing dry matter intake. Based on these results, polyhalite should be fed at a dose of 400 g or more per cow per day to reduce urine pH.

Key Words: polyhalite, urine pH, hypocalcemia

265 Evaluating anti-Müllerian hormone as a reproductive tool in dairy cows. K. Alward*[1], W. Graves[1], R. Palomares[2], A. Nelson[1], and J. Bohlen[1], [1]*University of Georgia Animal and Dairy Science, Athens, GA,* [2]*University of Georgia College of Veterinary Medicine, Athens, GA.*

Anti-Müllerian hormone (AMH) is produced by granulosa cells, found in early antral follicles on the ovary. Higher circulating AMH concentrations are indicative of a larger number of viable follicles present and published data shows that cows with low AMH have lower pregnancy risk and greater incidence of pregnancy loss between d 30 and 65 of gestation. These and other studies indicate that AMH, used as a marker of fertility, may provide use in determining breeding strategies for dairy cattle. The objective of this study was to examine whether categorization of animals based on AMH levels (HIGH or LOW) can allow for tailored breeding programs to increase first service pregnancy risk. Lactating, Holstein cows (n = 197) were enrolled at 45–60 d in milk (DIM) at which time blood was collected and analyzed for AMH (Ansh Labs, Webster, TX) and transrectal ultrasonography was performed to record antral follicle count (AFC), presence of corpora lutea (CL) and any reproductive tract anomalies. Animals were then randomly assigned to either an estrous detection (ED; n = 60) or a timed artificial insemination (TAI; n = 137) breeding protocol and first service conception risk was recorded. Animals were further categorized as HIGH (>200 pg/mL; n = 109) or LOW (<200 pg/mL; n = 88) AMH based on the profile of AMH distribution. AMH concentration was positively correlated with AFC ($P = 0.0002$). First-service conception risk was not affected by breeding protocol, AMH category, DIM or lactation number ($P > 0.05$). However, milk weight at breeding may have an impact on AMH level ($P = 0.0621$). With a normal distribution of AMH levels in the current research group, tailored breeding programs based on AMH categorization may only prove beneficial when the extreme outliers in the HIGH/LOW classifications are targeted.

Key Words: anti-Müllerian hormone, breeding strategies, fertility

Animal Behavior and Well-Being: Focus on Affective State

266 Behavioral approaches to assess and improve affective state of dairy cattle. E. K. Miller-Cushon*[1] and J. M. C. Van Os[2], [1]*University of Florida, Gainesville, FL,* [2]*University of Wisconsin, Madison, WI.*

A central issue in the study of animal welfare is how and when animals suffer. Understanding and alleviating poor welfare states, and providing resources that allow for positive experiences, may be considered an ethical imperative. Approaches to understanding affective state have often focused on behavioral changes associated with adverse or challenging events. For example, changes in time budgets (e.g., feeding, activity, play, and social behavior) have been observed in calves around disbudding and during illness. Body posture, movement of specific body parts, and pain-face or grimace scales are also increasingly studied. Preference and motivation tests can tell us how animals perceive and value different resources and opportunities for behavioral expression, which is informative for managing animals to improve affective state. For example, animals are motivated to access resources which may alleviate negative states, such as shade in hot conditions. This can be assessed through short-term tests or by observing behavior and resource use over longer periods in normal housing. Other short-term tests can offer insights into an animal's pervasive affective state (e.g., cognitive bias tests) or perception of stimuli as positive or negative (e.g., aversion races or conditioned place preference tests). Our ability to gain insight into an animal's subjective experience remains subject to some limitations. Behavioral observation and testing often provide insight into only the valence (positive vs. negative) of emotions, which is important for welfare assessment but may not inform how to improve animal welfare. Further, whereas responses associated with presumed negative states have been well studied, we have limited means to evaluate positive states. Monitoring individual responses and understanding the scope of individual wants and needs also remains a challenge. Nonetheless, increasing use of automated technology and continuing study in this area, including recognition of how behavior patterns may be reflective of personality and individual ability to cope and respond to changes, is shedding new light on our understanding of animal emotions.

Key Words: affective state, emotion, behavior

267 Hock injury healing through facility transitions on dairy cattle in Canada. A. M. Armstrong*[1], J. Schenkels[2], T. F. Duffield[1], D. B. Haley[1], and D. F. Kelton[1], [1]*University of Guelph, Guelph, ON, Canada,* [2]*Atlantic Veterinary College, Charlottetown, PEI, Canada.*

Hock injuries are prevalent across the dairy industry, with recent benchmarking in North America showing that cows managed in intensive systems are at a higher risk for injury development. It is unknown which hock injuries heal based on descriptions of severity and complexity, and how long the healing process takes. The objective of this observational study was to determine if hock injuries heal or improve over time through facility transitions into more 'cow friendly' housing environments, and how long this healing process takes. Fifteen commercial dairy farms were enrolled, 5 in Ontario and 10 in Nova Scotia based on housing type and transitions made on the farm. The transitions were to facilities with mattresses, sand, pack or pasture cow resting areas. Mattresses were considered the least 'hock friendly'. A total of 682 cows were assessed for the presence of hock injuries using the Canadian proAction Animal Care Assessment scoring system. Hock injuries with scores of 0 or 1 on the 4-point scale are deemed acceptable, and scores of 2–3 are unacceptable, and require corrective action. Cows were scored for 14-weeks in their new environment. The preliminary results are from the 10 farms, and 292 cows assessed in NS. Eighty-one cows that started their facility transition with a hock injury score that required corrective action met the inclusion criterion and were included in the survival analysis for time to healing. The analyses were completed using Stata/SE 15.0, with a significance level of $P = 0.10$ for the preliminary analysis. A Cox proportional hazards model was fit for hock injury healing with stall base as the predictor. Hock injuries healed 6.00 times faster on sand ($P = 0.001$, 95% CI: 2.04–17.7), 3.08 times faster on packs ($P = 0.096$, 95%CI: 0.82–11.6), and 5.81 times faster on pasture ($P = 0.001$, 95%CI: 2.08–16.6) compared with mattresses. Based on the preliminary results, hock injuries are more likely to heal, and heal faster when cows transition to more cow ideal housing conditions. These results will provide the dairy industry with information on what housing environments are best for resolution of injuries to occur, as well as a timeline for resolution.

Key Words: hock injury, healing, welfare

268 A survey of dairy cattle veterinarians' perspectives on timely management and euthanasia for common calf conditions. M. C. Cramer*[1], A. M. Dietsch[1], J. K. Shearer[2], K. L. Proudfoot[1], and M. D. Pairis-Garcia[1], [1]*The Ohio State University, Columbus, OH,* [2]*Iowa State University, Ames, IA.*

Despite high morbidity and mortality in dairy calves, few scientific guidelines exist for timely treatment and euthanasia. The objectives of this study were to determine dairy veterinarians' (n = 49) perspectives on euthanasia decisions in response to common calf conditions (n = 9) and to assess preferred timelines for euthanasia for each condition. We requested veterinarians complete an online survey using an email invitation sent to the American Association of Bovine Practitioners listserve. Responses to management decisions for calf conditions included: "euthanize immediately," "treat and monitor for signs of improvement," "cull/sell for beef," or "n/a." If veterinarians selected "treat and monitor," a follow-up question was asked: "how many days are you willing to give the animal to improve until you decide that euthanasia is the best option?" The proportion of respondents that selected each option was determined using PROC FREQ in SAS. The only condition that most veterinarians selected "euthanize immediately" was if the calf was identified as non-ambulatory (53%; 23/49). "Treat and monitor for signs of improvement" was the most common response for all other conditions: bloat (97%; 48/49), diarrhea (100%; 49/49), joint infection (95%; 36/49), severe lameness (79%; 30/49), navel infection (100%; 49/49), nervous system disorders (82%; 31/49), pneumonia (97%; 37/49), and traumatic injury (71%; 27/49). The number of days reported (median, range) until euthanasia was deemed the best option were as follows: bloat (n = 26; 3, 1–14), diarrhea (n = 26, 7, 2–14), joint infection (n = 1; 5), severe lameness (n = 17; 5, 1–10), nervous system disorders (n = 23; 3, 1–7), non-ambulatory (n = 16; 2.5, 1–7), pneumonia (n = 24; 5, 1–14), and traumatic injury (n = 17; 3, 1–7). Veterinarians generally agreed upon management decisions for all conditions. However, the wide range of responses for euthanasia timelines make it difficult to establish recommendations for the industry. Our next step to understand veterinarian decision-making is to determine what drives the wide variation in responses regarding euthanasia decision-making.

Key Words: health, welfare

269 Effect of stocking density and a barrier in a group close-up pen on the odds that dairy cows develop metritis after calving. K. Creutzinger*[1], H. Dann[2], L. Moraes[1], P. Krawczel[3], and K. Proudfoot[1], [1]*The Ohio State University, Columbus, OH,* [2]*William H. Miner Agricultural Research Institute, Chazy, NY,* [3]*University of Tennessee, Knoxville, TN.*

A high number of dairy cows succumb to disease after calving, and the risk of disease may be affected by the social environment before calving. The objective of this study was to determine the effect of stocking density and the provision of a barrier in group close-up pens on the likelihood that cows developed metritis after calving. A total of 319 Holstein dairy cows (primiparous = 113, multiparous = 206) that were part of a larger experiment were included in the study. Pens were dynamic as cows moved in 21 ± 3 d before their expected calving date and removed immediately after calving. At enrollment, cows were assigned randomly to one of 4 treatments using a 2 × 2 factorial arrangement including 1) high vs. low stocking density (9.7 to 12.9 m^2 vs. 19.4 to 25.8 m^2 lying space /cow), and 2) presence of a barrier (yes vs. no). The barrier was created using 2 road Jersey barriers and plywood (3.6 × 0.6 × 1.5 m). Pens were created using gates separating 4 areas within a large sawdust bedded pack and were replicated 4 times at 4 different periods so that all treatments were in all positions in the larger pack. Vaginal discharge was scored on 3, 7, 10, 14 d after calving with a Metricheck to diagnose subclinical and clinical metritis. Data describing metritis (healthy, subclinical and clinical) were analyzed with a multinomial proportional odds mixed model. Random effects for period, pen and pen × period × treatment were included in the model. Fixed effects describing the factorial arrangement of treatments as well as parity, calving assistance, and retained placenta were included in the model. Calving assistance (OR = 3.9; $P < 0.0001$) and retained placenta (OR = 6.4; $P < 0.0001$) significantly increased the odds of metritis severity. Stocking density (OR = 1.6; $P > 0.05$) and the presence of barrier (OR = 1.3; $P > 0.05$) did not affect the odds of developing metritis. No significant interactions were detected. Results suggest that moderate increases in stocking density and provision of a physical barrier in a group bedded-pack for close-up cows did not affect the odds of developing metritis after calving.

Key Words: stocking density, transition

270 Housing tie-stall dairy cows in deep-bedded loose-pens during the dry period has the potential to improve gait. E. Shepley* and E. Vasseur, *McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

Increasing locomotor activity can improve leg health and decrease the prevalence of lameness in dairy cows. The dry period offers an opportunity to provide alternative housing to tie-stall cow that can increase locomotor activity. Our objective was to determine if housing tie-stall dairy cows in a deep-bedded loose pen during the 8-week dry period affected gait and step activity. Twenty cows, paired by parity and calving date, were assigned at dry-off to a deep-bedded loose-pen (LP) or a tie-stall (TS). Step activity was measured by leg-mounted pedometers. Cows were walked 1x/wk on a test corridor and video recordings of gait were taken. Six aspects of gait were scored on a 0–5 scale (interval: 0.1): tracking up, joint flexion, back arch, asymmetric step, swing, and reluctance to bear weight. Overall gait was also scored using a 1–5 scale (interval: 0.5). Data for gait was analyzed based on the change in gait between the dry-off and calving. Daily step data were averaged per week of the dry period. Analyses were performed using a using a mixed model with treatment, week, and pair as fixed effects and cow nested within treatment and pair as a random effect for step data. The same model, omitting the fixed effect of week, was used for gait. There was no difference in step activity between LP and TS cows (842.1 ± 88.86 vs 799.5 ± 76.92 steps/d, LP vs TS, respectively; $P = 0.73$). Only joint flexion yielded a treatment difference with LP cows improving over time and TS cows worsening (−0.4 ± 0.15 vs +0.2 ± 0.16; $P < 0.05$). Although step activity was similar in both housing options, the increased space allowance in the LP treatment may have allowed for a larger range of motion for each steps, increasing the overall benefits to leg health. The denser lying surface in the LP may also have provided a cushioning effect when transitioning between rising and lying, improving joint health and, thus, joint flexion. Providing tie-stall cows with alternative housing during the dry period has the potential to help cows to recover their health in preparation for their next lactation.

Key Words: dry cow, gait, housing

271 Association between feeding behavior and wellness scores in Jersey dairy cows around calving. D. du Toit*, G. Esposito, J. H. C. van Zyl, and E. Raffrenato, *Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa.*

Triaxial accelerometers have been validated, to measure behavior, mainly in Holstein-Friesian (HF). Our objective was to investigate feeding behavior of transition Jersey cows and its interaction with wellness scores, using a commercial triaxial accelerometer. Jersey cows (n = 145) were fitted with the Silent Herdsman neck collar (Afimilk, Israel) which recorded eating and rumination time hourly from −21 to 21 DIM. Rumen fill, fecal, lameness, leg hygiene and body condition scores (BCS) were assigned every 2 d. Afimilk (Israel) provided feeding behavior data before algorithmic transformation. Pearson correlations evaluated associations between collar data and scores. Feeding behavior was also analyzed with the GLIMMIX procedure of SAS, with cow and DIM as random and repeated variables, respectively. Animals belonging to the specific score value, parity, DIM and the respective interactions were included as fixed factors. BCS was included as a continuous variable. Feeding and rumination times were positively correlated (0.34 to 0.55; $P < 0.01$) with fill score and BCS, and negatively correlated with leg hygiene and lameness scores (−0.32 to −0.12; $P < 0.01$). Parity did not affect daily rumination time, but older cows ruminated more ($P < 0.001$) around parturition. DIM was significant ($P < 0.0001$) resulting in cows reducing rumination time from 423 ± 52 min at DIM −21 to 197 ± 8 min at calving. Only after 20 d postpartum, cows ruminated at least 400 min/day. Higher lamenes scores were associated with reduced rumination ($P < 0.0001$), decreasing of 1 h from score 1 to 4. When including eating time as response variable, younger cows spent more time at the manger (599 ± 32 vs. 538 ± 30 min; $P < 0.0001$). DIM affected eating time, mainly due to a drastic reduction of eating time after calving, from 635 ± 33 min, of the −21 DIM, to 542 ± 10 min for the first 10 DIM. Lameness resulted in a drastic reduction of time spent eating from 583 min, for score 1, to 512 min, for score 4. These data proved to be different from what reported for HF suggesting that accelerometers might need to have Jersey cows-specific algorithms and that, relatively to the scores analyzed, at least locomotion will affect feeding behavior and may be detected early.

Key Words: accelerometer, precision farming, transition cow

272 Could the first time be the last time? Implications of the first incident of mastitis or lameness on total milk production in first-lactation cows. M. A. Puerto*[1], R. I. Cue[1], D. Warner[1,2], and E. Vasseur[1], [1]*McGill University, Ste-Anne De Bellevue, QC, Canada,* [2]*Valacta, Valacta Dairy Centre of Expertise, Ste-Anne De Bellevue, QC, Canada.*

Mastitis and lameness are 2 of the most prevalent diseases on dairy farms and increase culling risk while negatively affecting cow welfare and milk production. Identifying critical points on the lactation curve, where the first incidence of mastitis or lameness has the biggest impact on milk production, is a strategy to improve lifetime profitability and is a useful tool to help farmers make informed decisions. The aim of this study was to analyze the impact of the first incidence of mastitis or lameness on total milk production at different stages of lactation. Data were retrieved from Valacta's database from first parity Holstein cows that calved between 2000 and 2015. After editing to ensure complete health records, the final data set consisted of 14,025 health records from 103 herds, yielding 1770 first mastitis events and 1063 first lameness events, with the remainder of records representing cows with no recorded health event (Control). First event records were grouped into transitional (1–21DIM), early (22–100DIM), mid (101–200DIM) and late (201+DIM) lactation stages to account for the most critical points of the lactation curve. Mid and late lactation cows were stratified by cumulative milk yield before the mastitis or lameness event to account for production level. Data were analyzed in a mixed model with herd as the random effect. Control cows produced 588 ± 115.6, 412 ± 130.3 and 420 ± 143.6 ($P < 0.001$) kg/lactation more milk than cows with the first mastitis event in transition, early and mid stage of lactation, respectively. Control cows produced 1331 ± 206.4, 803 ± 142, and 2052 ± 36.6 ($P < 0.001$) kg milk/lactation more than cows with a lameness event in transition, early and mid stage of lactation, respectively. Cows that reported a first case of mastitis or lameness in the late period showed no difference with the control group. These results allowed identifying long-lasting effects of mastitis and lameness on the total milk production, and therefore, could be used by farmers to make informed culling decisions to maximize both herd profitability and cow longevity.

Key Words: lifetime profitability, cow longevity, decision making

273 Condition of cull dairy cows from farm to slaughter plant.

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Cull dairy cows are regularly removed from dairy herds and enter the marketing system which involves transport to auction markets and then to an abattoir. Many cull dairy cows are removed from the herds because of health reasons and their ability to withstand transportation may vary. Moreover, dairy producers lack feedback about potential transport delays and the condition of the cows throughout the transport continuum. The objectives of this study were (1) to follow cull cows from farm to abattoir, (2) to monitor changes in the cows' condition, and (3) to record transport delays. From May 2017 to March 2018, data were collected from 20 dairy farms, 2 auction markets and at 6 abattoirs in British Columbia, Alberta, and the USA. The dairy farms were visited regularly before cows were shipped and a researcher scored the animals for body condition (BCS; 5-point scale), lameness (5-point scale) and udder condition (3-point scale). Logistic mixed effects models were used to test the effect of transport on the animals' condition, with cow within farm as random effect. During the study, 1,171 cull cows were removed from participating farms and 714 of those animals were observed at one of the participating abattoirs. After leaving the farms, cows spent on average 82 h in the marketing system until being processed. Including delays at auctions or assembly yards, about 41% of cows were in transit for 4–5 d and 11% for 6–16 d. Regarding distance, 11% were transported 1,100 km from farm to abattoir. The percentage of thin cows increased from 8% at the farm of origin to 22% at the abattoir ($P < 0.001$). Lameness did not change, but transport increased the development of acute milk accumulation and udder inflammation from 8% at farm of origin to 41% at the abattoir ($P < 0.001$). At the auctions, 10% of the cows were thin (BCS ≤ 2), 7% were severely lame (lameness score ≥ 4), 13% had udder edema, and 6% had other injuries including abscesses (2.1% of cows), signs of diseases (2.0%), hobbles (0.9%), signs of pneumonia (0.3%), eye injury (0.2%), and lump jaw (0.1%). This information about delays to slaughter and changes in cow condition will now be communicated to producers and veterinarians.

Key Words: livestock market, transport

Animal Health: Joint Animal Health/Reproduction Symposium: Transition Cow Calcium Homeostasis—Health Effects of Hypocalcemia and Strategies for Prevention

274 Calcium transport mechanisms in different epithelia of ruminants. M. Wilkens*, *Institute of Physiology and Cell Biology, University of Veterinary Medicine, Hannover, Foundation, Hanover, Germany.*

If luminal Ca concentrations are high, Ca transport across epithelia occurs mainly via passive, paracellular mechanisms driven by an electrochemical gradient or solvent drag. Restricted supply as well as an increased demand induce an endocrine response, mainly mediated by calcitriol, resulting in a relatively greater contribution of a more efficient, active, transcellular Ca transport. In intestinal and renal epithelia, this is a 3-step process involving apical uptake via Ca channels (TRPV5, TRPV6), protein-bound diffusion through the cell (CaBPD28K, CaBPD9K) and active extrusion at the basolateral membrane (NCX1, PMCA1b). To further improve our strategies to prevent hypocalcemia, applied studies should be combined with fundamental research on the complex regulation of these transport processes, especially because ruminants show some particularities in comparison to monogastric animals. Although the small intestine expresses the same vitamin D-dependent Ca transport proteins (TRPV6, CaBPD9K, PMCA1b), flux rates determined using mucosal preparations from sheep and goats were demonstrated to be much smaller than those detected in samples from pigs or horses. Nevertheless, the expression of the above mentioned transport proteins is regulated and altered by dietary Ca, N restriction and lactation. The rumen has also been shown to actively absorb Ca in many in vitro and in vivo experiments. But the absence of any relevant amounts of TRPV6 or CaBPD9K, the unresponsiveness to dietary Ca restriction or calcitriol treatment and the pivotal role of luminal SCFA indicate an alternative, so far unknown mechanism. Interestingly, lactation and a diet negative in DCAD increased ruminal flux rates. Renal Ca excretion is generally low in ruminants and cannot be significantly diminished to compensate for challenges of Ca homeostasis. But when the animals are kept on a ration negative in DCAD renal Ca resorption is inhibited. Preliminary experiments demonstrated that the expression of TRPV5, CaBPD28K and NCX1 is not significantly altered under these conditions. This might explain why renal resorption is immediately restored when the ration is changed p.p. An adaptation on the functional level occurs faster than the stimulation via the genomic pathway.

Key Words: Ca absorption, vitamin D, dietary cation-anion difference (DCAD)

275 Implications of vitamin D physiology and nutrition in regulation of calcium in dairy cows. C. D. Nelson*, J. E. P. Santos, M. B. Poindexter, A. Vieira-Neto, and R. Zimpel, *University of Florida, Gainesville, FL.*

Attempts have been made to utilize vitamin D for prevention and treatment of hypocalcemia in postpartum dairy cows since the discovery that vitamin D was required for calcium homeostasis. A few initial experiments established that feeding 10,000 to 20,000 IU of vitamin D_3 was adequate for maintaining blood Ca concentrations in lactating dairy cows. Hypocalcemia, however, is not necessarily a vitamin D nutrition problem but, rather, the inability of Ca homeostatic mechanisms to account for irreversible mammary loss with the onset of lactation. The failure to maintain Ca homeostasis, in part, is due to inadequate synthesis of 1,25-dihydroxyvitamin D_3 from 25-hydroxyvitamin D_3 in response to parathyroid hormone and decreased sensitivity of tissues to 1,25-dihydroxyvitamin D_3. Feeding acidogenic diets prepartum to induce a compensated metabolic acidosis is a proven strategy to minimize postpartum hypocalcemia. A diet with negative DCAD increases concentrations of 1,25-dihydroxyvitamin D_3 in serum in response to parathyroid hormone and, perhaps more important, it increases Ca flux from intake and skeletal stores to urine which can be rapidly diverted to the mammary gland at the onset of lactation. Increasing supplemental vitamin D_3 above current practices (30,000 to 50,000 IU/d) does little to augment a prepartum diet with negative DCAD because cows seem to have a limited capacity to convert vitamin D_3 to 25-hydroxyvitamin D_3. However, dose titration experiments with supplemental vitamin D_3 in combination with a negative DCAD are needed. In contrast, feeding 25-hydroxyvitamin D_3 in combination with a diet with negative DCAD is a promising approach to improving Ca homeostasis and performance of transition cows on the basis of ouctomes from recent experiments. Parental administration of 1,25-dihydroxyvitamin D_3 at, or soon after, parturition also results in a sustained increase in serum Ca. In summary, vitamin D is required for Ca homeostasis, but vitamin D nutrition is not the sole solution to hypocalcemia. A prepartum diet with negative DCAD is effective at reducing the risk of hypocalcemia and may benefit from nutritional or therapeutic use of vitamin D metabolites.

Key Words: vitamin D, calcium, transition cow

276 Novel role for serotonin in calcium homeostasis and effects on transition health. L. Hernandez*[1], J. Laporta[2], S. Weaver[3], and M. Connelly[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*University of Florida, Gainesville, FL,* [3]*Mayo Clinic-Rochester, Rochester, MN.*

Serotonergic regulation of calcium (Ca) metabolism during the peripartal period has only recently been elucidated. The discovery of mammary gland-derived serotonin and its subsequent interaction with peripartal Ca is a relatively new concept, particularly in the bovine model. Much progress has been made understanding serotonin's role in regulating Ca status by utilizing the precursor to serotonin synthesis, 5-hydroxy-L-tryptophan (5HTP). A study performed in late-lactation dairy cows demonstrated that treatment with 5HTP decreased circulating total Ca concentrations and urine Ca concentrations, but increased milk Ca concentrations. This suggested that serotonin was potentially coordinating Ca flux between kidney, bone, gut, and milk. When peripartal multiparous cows were treated with 5HTP prepartum, total Ca concentrations increased postpartum compared with controls, and further improved when 5HTP was given in combination with a negative DCAD diet. Interestingly, cows treated with 5HTP had decreased circulating parathyroid hormone concentrations compared with controls and increased concentrations of urinary deoxypyridinoline, a bone resorption marker. Previous data in mice suggested serotonin induced transcriptional and translational changes in Ca transporters and pumps in the mammary gland. We confirmed these findings in the dairy cow, demonstrating that transcription of several pumps and transporters, along with parathyroid hormone related-protein were increased on d 8 of lactation in mammary glands of cows treated with 5HTP prepartum. In an attempt to determine if serotonin and Ca are working in a feedback loop to maintain blood Ca concentrations, we measured circulating serotonin in response to chelation of circulating Ca, in non-pregnant, nonlactating cows. We determined circulating serotonin concentrations differentially responded to the challenge, based on dietary Ca fed. Our data to this point indicates that serotonin and Ca are working in a feedback loop to regulate Ca homeostasis in the peripartal cow. We are currently working on dissecting pathways involved in the serotonin-Ca feedback loop and the contribution of the mammary gland to the regulation of this process.

Key Words: serotonin, calcium

277 Use of oral calcium for treatment of hypocalcemia and effects on health and production.

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Given the technological constraints of accurately measuring Ca and the negative consequences of hypocalcemia on cow health and farm economics, many dairy cows are supplemented with Ca immediately after calving. Administration of oral Ca, generally in bolus form containing 40 to 50 g of Ca, is a common supplementation strategy both for prevention and treatment of subclinical hypocalcemia, and several manufacturers have introduced oral Ca boluses containing differing combinations of rapidly and slowly absorbed Ca salts. The benefit of rapidly absorbed salts, such as Ca chloride, is that it is both highly bioavailable and acidifying (supporting mobilization of the cow's own Ca stores); however, it is irritating to oral mucous membranes and must be administered quickly. Slowly absorbed Ca salts (such as Ca propionate, Ca sulfate, and Ca carbonate) have either an equivalent efficacy and longer duration of action than quickly-absorbed Ca salts or are ineffective as an immediate Ca source due to their poor bioavailability. Depending on the product, label instructions require administration of 1 to 3 boluses at calving, with some products requiring a second bolus administered 12 h later. Although studies agree that oral Ca increases blood Ca concentration, the length of increase varies from 1 to 24 h, likely due to the dose and frequency of administration and the production capacity of the cows under study. It is interesting that this short-term change in blood Ca concentration can have effects on cow health and production. However, these effects are not always beneficial, and most studies do not recommend their use as a blanket treatment, especially in primiparous cows. Conversely, there is good evidence that oral Ca supplementation to older cows and those with a greater production potential is valuable. It is thus important to take the formulation of oral Ca boluses, the timing and frequency with which they are administered, and cow-level variables into account when using oral Ca for treatment or prevention of hypocalcemia in dairy cows. It is important that these supplements complement the progression of, but do not interfere with, Ca homeostasis.

Key Words: calcium, subclinical hypocalcemia, oral calcium

Breeding and Genetics: Health, Efficiency, Resiliency, and Other Novel Traits

278 Estimation of genetic parameters for young stock survival in Danish beef × dairy crossbred calves. R. B. Davis*[1], E. Norberg[3,2], and A. Fogh[1], [1]*SEGES, Aarhus N, Denmark,* [2]*Aarhus University, Tjele, Denmark,* [3]*NMBU (Norwegian University of Life Sciences),* Ås, Norway.

Calf mortality leads to economic losses for the farmer and is an animal welfare issue. Currently, only calf mortality within the first 24 h is accounted for in the Danish breeding goal for beef x dairy calves. However, survival throughout the rearing period is also of upmost importance. Therefore, the aim of this study was to estimate genetic parameters for young stock survival, to evaluate if it is feasible to implement such a trait. Data on 90,926 crossbred calves was extracted from the Danish Cattle Database and was provided by the Danish research center, SEGES. Two traits were defined, young stock survival from 1 to 30 d and 31–200 d after birth. The traits were analyzed with a univariate animal model using the AI-REML algorithm in the DMU package. The model contained a fixed effect of year × month of birth, herd, sex, breed combination, parity of the dam, transfer and a random effect of the calf and herd × year. The pedigree was traced back 5 generations, for both the sires and dams. Results showed low but significant heritabilities (0.045–0.075) for both survival traits. Breeding values were calculated using DMU4. Breed combinations with Belgium Blue cattle sires outperformed all other sire breeds. The lowest survival rates were found for breed combinations with Jersey dams or Blonde d'Aquitaine sires. Sufficient genetic variation between sires for young stock survival was found. The breeding values of the sires had an effect on young stock survival that ranged from −2.5 to 3.5% and −5.4 to 4.7% for survival from 1 to 30 d and 31–200 respectively. It is therefore feasible to implement young stock survival traits in a genetic evaluation for beef × dairy crossbred calves. This will increase the survival rate of the calves and hereby increase animal welfare and decrease economic loss for the farmers.

Key Words: young stock survival, beef × dairy, genetic parameter

279 Assessing the predictive value of facial biometrics for genomic health traits via a statistical learning approach. C. McVey*[1,2] and P. Pinedo[1], [1]*Colorado State University, Fort Collins, CO,* [2]*University of California Davis, Davis, CA.*

Among horse breeders, distinctive facial features have been anecdotally attributed to traits such as trainability and soundness. Genes controlling early hormonal development have been implicated but never formally proven to drive correlations between facial morphology, and health in human and animal literature. A pilot study demonstrated significant associations between health PTAs and facial biometric measurements among diary sires. In this study, side profile images were acquired from 573 genotyped Holstein cows of mixed parity, of which 344 also had genomic estimates for wellness traits from Clarifide Plus (Zoetis, Service LLC). Images were manually annotated with anatomical landmarks, and 60 validated biometric measures computed. Biometric values were then combined with genomic estimates of type traits in independently optimized statistical learning models to predict the 22 available genetic merit estimates for performance, fertility, and health. LASSO regression models, fit by k-fold cross validation using the glmnet package in R, revealed little improvement in R^2 values on the validation set with addition of image data, and yet a subset of biometrics was retained in over half the models. Further analysis using smoothing splines implemented in the mgcv package revealed evidence of nonlinearity in these relationships, with a notable number of significant associations to calving ease and stillbirth values. Finally, bagged regression trees optimized by k-fold cross validation on a custom grid in the gbm package showed little evidence of significant interaction effects and little improvement in R^2 on the validation set, though higher depth trees for retained placenta and metritis did show an improvement of 0.35 R^2 over type traits alone in their respective training models. Variable importance measures also revealed biometric values to be significant components across the range of models. While biometrics did not consistently improve predictions of genetic merit over type traits, their consistent inclusion in cross-validated models and low phenotypic correlation to type traits (mean 0.05, max 0.20) suggests they may provide novel useful information to dairy genetic evaluations as potential indicator traits.

Key Words: biometrics, facial inference

280 Genomic evaluation for cow and calf wellness traits in US Jersey cattle. D. Gonzalez-Pena*, N. Vukasinovic, J. Brooker, C. Przybyla, and S. DeNise, *Zoetis, Kalamazoo, MI.*

In recent years, the number of Jersey cows in the USA has been steadily increasing. To help producers maintain health and wellness of their Jersey animals, Zoetis has developed genomic predictions for wellness traits in Jersey cattle using producer-recorded data. The traits included mastitis (MAST), metritis (METR), retained placenta (RETP), displaced abomasum (DA), ketosis (KETO), lameness (LAME), and milk fever (MFV) in cows, and calf livability (DEAD), respiratory disease (RESP), and scours (DIAR) in calves. Phenotypic data on health events, pedigree, and genotypes were collected directly from producers upon obtaining their permission. Each trait was defined as a binary event, having a value of one if an animal has been recorded with a disorder, and zero otherwise. The number of phenotypic records ranged from 216K for DA to 629K for MAST and from 186K records for DIAR to 380K records for DEAD for cows and calves, respectively. The number of genotyped animals was 41,271. All traits were analyzed using a univariate threshold animal model. The model for cow wellness traits included fixed effect of parity and random effects of herd by year by season of calving, animal, and permanent environment. The model for calf wellness traits included fixed effect of year of birth by calving season by region and random effects of herd by year of birth and animal. A total of 45,163 SNPs were used in genomic analyses. Animals genotyped with low-density chips were imputed. All analyses were based on the single step genomic BLUP (ssGBLUP). Heritabilities ranged from 0.06 for DA to 0.12 for LAME. Predicted transmitting abilities (PTA) were expressed in percent points as deviations from the average estimated probability of a disorder in the base population. Reliabilities of genomic PTAs for young genotyped animals without recorded health events or progeny had average values between 32% (DA) and 51% (MAST). The results indicate that a direct evaluation of cow and calf wellness traits under a genomic threshold model is feasible and offers predictions with average reliabilities comparable to other lowly heritable traits for Jersey cattle.

Key Words: Jersey, genomic prediction, wellness traits

281 Extending genomic evaluations to direct health traits in Jerseys. L. Jensen*, K. L. Parker Gaddis, and H. D. Norman, *Council on Dairy Cattle Breeding, Bowie, MD.*

The Council on Dairy Cattle Breeding (Bowie, MD) initiated evaluations for 6 direct health traits in Holsteins in April 2018. Currently, there are no health evaluations for Jerseys, the second largest dairy population in the United States. An increasing number of health records are being submitted to the cooperator database for Jerseys, doubling in the past year. The objective of this research was to determine the feasibility of providing accurate genetic predictions of direct health traits for disease resistance in Jerseys. We retrieved producer-recorded health trait observations in Jerseys from the cooperator database including milk fever (MFEV), displaced abomasum (DA), ketosis (KETO), mastitis (MAST), metritis (METR), and retained placenta (RETP). After extensive editing, records ranged from 39,716 for KETO to 97,507 for MAST, resulting in 134,403 total phenotypic records from 79,334 animals with 35–45% of animals providing multiple observations. Data were from 213 herds with 88% of the records submitted in the past 5 years. Incidence rates were lowest for MFEV (1.3%), highest for MAST (10.2%), and comparable to those observed in the Holstein population. In all traits, excluding METR, incidence rate increased with each consecutive lactation. However, METR had the highest incidence rate in the first lactation. Traditional PTA were calculated for approximately 6 million Jerseys using the same linear animal model as applied in routine Holstein evaluations. Effects accounted for in the model included year-season, age-parity, herd-year, and permanent environment, in addition to a regression on inbreeding. Although traditional reliabilities reached 90% for some traits, the mean reliabilities of bulls with progeny were 12–22%. Genomic PTA were calculated using 79k markers for 310,232 Jerseys. Mean genomic reliabilities were higher and ranged from 29 to 35%, depending on the trait. This is approximately 15 points lower than the average for Holsteins, but as more health records are accumulated, Jersey reliabilities will increase. With these genetic predictions, Jersey producers will have a growing arsenal of tools for breed improvement.

Key Words: Jersey, genetic evaluation, health

282 Genetic evaluation of residual feed intake and feeding behavior patterns of growing Holstein heifers. J. R. Johnson*[1], G. E. Carstens[1], C. Heuer[2], and N. Deeb[2], [1]*Texas A&M University, College Station, TX,* [2]*STgenetics, Navasota, TX.*

The objectives of this study were to evaluate heritability estimates of residual feed intake (RFI), DMI, and feeding behavior traits in growing Holstein heifers (n = 609; Initial BW = 246 ± 43 kg), and to examine corresponding genetic correlations. DMI and feeding behavior traits were measured using a GrowSafe System for 70 to 100 d (15 trials) at the Ohio Heifer Center (STgenetics research farm) while heifers were fed a corn-silage based ration. RFI was computed within trial as the difference between actual and expected DMI from linear regression of DMI on mean $BW^{0.75}$ and ADG. Six feeding behavior traits were evaluated, including frequency and duration of bunk visit (BV) and meal events, head-down duration (HDD), and the ratio of BV events per meal event. Pedigree information consisting of 10 generations of 4,513 animals from 616 founder animals were used in the genetic evaluation. Heritability estimates were obtained for each trait using multivariate mixed models which included fixed effects of trial and age, and genetic correlations estimated by pairwise bivariate linear mixed models for any 2 traits. The heritability estimates for RFI, DMI and feed:gain were 0.15, 0.26 and 0.04, respectively, which were lower than the heritability estimates observed for the 6 feeding behavior traits that ranged from 0.30 to 0.50, and for ADG (0.54). As expected, RFI was highly correlated with DMI (0.52), but not ADG (0.10), although RFI was negatively correlated with final BW (−0.33). RFI was positively correlated with HDD (0.35), BV frequency (0.72), meal frequency (0.39) and duration (0.44), and the ratio of BV events per meal event (0.31), but not with BV duration (0.01). In general, the magnitude of the genetic correlations between RFI and feeding behavior traits were higher than those between DMI and feeding behavior traits, suggesting that feeding behavior traits were more predictive of RFI than DMI. The moderate to high genetic correlations between RFI and feeding behavior traits indicate that they may be useful indicator traits for predicting feed efficiency. Accordingly, future research should further evaluate the use of feeding behavior traits in multivariate genomic prediction models to improve the accuracy of RFI predictions.

Key Words: dairy cattle, residual feed intake (RFI), feeding behavior

283 Genetic parameters for dry matter intake, body weight, and energy corrected milk in dairy cattle. T. C. Seleguim Chud*[1], F. Miglior[1], D. J. Seymour[1,2], D. Hailemariam[3], C. Baes[1,4], and F. S. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Centre for Nutrition Modelling, University of Guelph, Guelph, ON, Canada,* [3]*Livestock Gentec, University of Alberta, Edmonton, AB, Canada,* [4]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Feed efficiency is an important aspect of dairy cattle productivity, and is a term used to describe the relative ability of a cow to convert feed into milk or milk components. Because feed intake is expensive to accurately measure on large populations, incorporation of feed efficiency traits in genetic selection programs has been difficult. Recently, several measures of feed efficiency have been discussed as potential traits for genetic analysis, and technological advances have allowed the collection of data on numerous animals. Data on related traits such as body weight or body condition score is also being measured. To produce breeding value estimates for feed efficiency, it is important to understand the genetic and phenotypic correlations between relevant traits. Furthermore, physiological aspects, such as the stage of lactation, must be considered.Peak production normally occurs between 6 to 8 weeks (42 to 56 DIM) after calving and coincides with the phase of negative energy balance in which cows use more energy than they can take in. When considering feed efficiency, it is important to split the lactation between negative and positive energy balance periods to ensure that the important physiological, metabolic and nutritional changes during the period after calving have no negative effects on cow health. In this study, 3,244 daily feed intake and milk parameter, and 1,024 weekly body weight records were collected on 204 Canadian first lactation cows from 2014 to 2018. These records were used to calculate dry matter intake (DMI) and energy corrected milk (ECM). Parameters (heritability estimates and both genetic and phenotypic correlations) were estimated for DMI, metabolic body weight (MBW) and ECM at 2 time-periods: a) 5–60 DIM, and b) 60–150 DIM using AIREML methodology. The results of this parameter estimation provide a basis for the development of breeding value estimation procedures and a subsequent selection index for feed efficiency.

Key Words: feed efficiency, heritability

284 Inclusion of herdmate data improves genomic prediction for milk production and feed efficiency traits within North American dairy herds. N. Schultz* and K. Weigel, *University of Wisconsin, Madison, WI.*

Genomic data are widely available in the dairy industry and provide a cost-effective means of predicting genetic merit to inform selection decisions and increase genetic gains. As more dairy farms adapt genomic selection practices, dairy producers will soon have genomic data available on all of the animals within their herds. This is a very rich, but currently underutilized, source of information. Herdmates provide an excellent indication of how a selection candidate's genetics will perform within a given herd, noting that herdmates often include close relatives that share a similar environment. The study objective was to evaluate the utility of incorporating herdmate data into genomic predictions in a data set comprised of 3303 Holsteins from one herd in Canada and 6 herds throughout the United States. Within-herd prediction accuracy was assessed for milk production and feed efficiency traits determined from genomic best linear unbiased prediction under 4 different scenarios. Scenario one did not include herdmates in the training population. Scenarios 2 through 4 included herdmates in the training population while scenarios 3 and 4 also included modeling of herd-specific marker effects. Leave-one-out cross validation was used to maximize the number of herdmates in the reference population in scenarios 2 through 4, while maintaining constant reference population size with scenario one. Results from the present study reveal the importance of incorporating herdmate data into genomic evaluations. Scenarios 2, 3, and 4 improved mean within-herd prediction accuracy across the 6 milk production and feed efficiency traits by 0.06 ± 0.01, 0.07 ± 0.01, and 0.08 ± 0.01, respectively, in comparison to scenario one which did not include herdmates in the training data. Herds with higher within-herd heritability and low genomic correlation with the remaining herds benefitted most from the inclusion of herdmate data.

Key Words: genomic prediction, genotype by environment interaction, dairy herd

285 The Efficient Dairy Genome Project: An overview. C. F. Baes*[1,2], A. Cánovas[1], E. E. Conner[4], E. Goddard[5], S. Wegmann[6], G. Hailu[7], V. Osborne[8], J. Pryce[9], E. Wall[2], Z. Wang[10], G. Kistemaker[11], P. Stothard[10], F. Miglior[1], and F. Schenkel[1], [1]*Centre for Genetic Improvement of Livestock, University of Guelph, Guelph, ON, Canada,* [2]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Bern, Switzerland,* [3]*Animal and Veterinary Sciences, Scotland's Rural College, Edinburgh, UK,* [4]*Animal Genomics and Improvement Laboratory, USDA-Agricultural Research Service, Beltsville, MD,* [5]*Resource Economics and Environmental Sociology, University of Alberta, Edmonton, AB, Canada,* [6]*Zuchtwertschätzung, Qualitas AG, Zug, Zug, Switzerland,* [7]*Department of Food, Agricultural and Resource Economics, University of Guelph, Guelph, ON, Canada,* [8]*Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [9]*Biosciences Research Division, Department of Economic Development and La Trobe University, Victoria, Melbourne, Australia,* [10]*Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [11]*Canadian Dairy Network, Guelph, ON, Canada.*

The development and application of novel genomic approaches within the dairy sector to address challenges and opportunities related to sustainable production is the goal of many national breeding organizations. The Efficient Dairy Genome Project is an international research initiative to improve feed efficiency and reduce methane emissions in dairy cattle using genomics. The aim is to deliver a worldwide database for feed efficiency and methane emission. This collation of data will allow for data exchange between partners, which is expected to greatly enlarge the reference population for the genomic analysis of feed efficiency and build a reference population for methane emissions. This project, which builds on partnerships with international collaborators, is currently well underway. Here we present specific objectives of the project, statistics of the current collated data, as well as updates on specific activities within the project, including establishment of a reference population for feed efficiency and methane traits, genomic analyses, whole-genome sequencing, as well as variant discovery and functional studies. Other aspects of the project include investigation into the societal value and acceptance of these new technologies as well as methods to implement genomic evaluations for feed efficiency and methane emissions into routine evaluations.

Key Words: efficiency, methane, genomics

286 Breeding for resilience in dairy cows using daily milk yield recording. M. Poppe*, H. Mulder, and R. Veerkamp, *Wageningen University & Research, Wageningen, the Netherlands.*

Cows are constantly subject to environmental challenges, such as pathogens and heat waves. The ability to cope with such perturbations is called resilience. The objective of this research was to study the use of variability in daily milk yield resulting from perturbations as an indicator of resilience in dairy cows using a genetic analysis. Daily milk yield of 198,754 first-parity cows, recorded by automatic milking systems, were studied. To avoid influence of the shape of the lactation curve on the variability, first the general trend in milk yield was removed by fitting lactation curves for each cow. Four different methods were investigated: moving average, moving median, Wilmink curve and quantile regression. Next, the variability of the residuals was quantified by taking the log-transformed variance (LnVar). A lower LnVar would indicate less fluctuations and thus a more resilient cow. Heritabilities of LnVar, genetic correlations between LnVar based on different curve fitting methods, and genetic correlations with milk yield level were estimated using univariate and bivariate analyses in ASReml. Also, genetic correlations between de-regressed breeding values for LnVar and de-regressed breeding values for functional traits were estimated. The genetic analysis showed that LnVar had a moderate heritability (0.20 to 0.24) and that the genetic correlations between LnVar based on the different curve fitting methods were > 0.94. Because of large genetic correlations with average milk yield level (0.75 to 0.79), the correlations with functional traits were converted to partial genetic correlations, corrected for the correlations with average milk yield. LnVar had negative partial genetic correlations with udder health (−0.22 to −0.33), ketosis resistance (−0.27 to −0.30), and longevity (−0.29 to −0.34). This confirms the expectation that a lower variability in milk yield indicates a better resilience. For all functional traits, LnVar based on quantile regression gave the largest correlation, although the differences were small. Concluding, variance in daily milk yield is a promising resilience indicator that can be used on a large scale to breed resilient cows.

Key Words: resilience, automatic milking system (AMS), health

287 The genetic relationship of production traits recorded during grazing and non-grazing seasons in US organic Holstein cows. L. Hardie*[1], I. Haagen[1], L. Han[1], B. Heins[2], D. Fitzsimmons[3], and C. Dechow[1], [1]*Penn State University, University Park, PA,* [2]*University of Minnesota, Minneapolis, MN,* [3]*Alfred State, Alfred, NY.*

The objective of this study was to characterize the genetic relationship of production traits in US organic Holstein cows during the grazing and non-grazing season. Eleven US organic dairy herds provided 18,894 testday milk yield records and up to 11,435 testday component records from 3,654 and 2,201 cows, respectively, during the grazing season (May through August) and 16,222 testday milk yield records and up to 11,373 testday component records from 3,117 and 2,386 cows, respectively, from December through March (non-grazing season). Means within the grazing season were 28.27 kg/d, 3.66%, 3.00%, 0.93 kg/d, and 0.76 kg/d for milk yield, fat %, protein %, fat yield, and protein yield, respectively. Means during the non-grazing season were 28.14 kg/d, 3.90%, 3.16%, 1.02 kg/d, and 0.83 kg/d for milk yield, fat %, protein %, fat yield, and protein yield, respectively. Variance components within and genetic correlations between the 2 seasons were established using repeated records, treating each season as a separate trait in 2-trait animal models. Fixed class effects of age group at parturition within lactation group, year of parturition within herd, and days in milk group within lactation group were included in all models along with random effects of testday within herd, animal, and permanent environment. The pedigree consisted of 22,900 animals. For all traits, residual variances were generally greater during the grazing season whereas additive genetic variances were greater in the non-grazing season. Parameter estimates for fat % displayed the largest difference between grazing and non-grazing seasons, respectively, for additive genetic variance (0.09 ± 0.014 and 0.13 ± 0.019), heritability (0.20 ± 0.02 and 0.30 ± 0.03) and repeatability (0.27 ± 0.01 and 0.43 ± 0.01). Genetic correlations between grazing and non-grazing seasons were 0.93 ± 0.05, 0.90 ± 0.04, 0.93 ± 0.04, 0.96 ± 0.05, 0.96 ± 0.04, for milk yield, fat %, protein %, fat yield, and protein yield, respectively. In conclusion, fat percent appears to be most influenced by season, though overall, the strong genetic correlations between grazing and non-grazing seasons suggest that the genetic basis of production does not strongly change in association with the grazing season.

Key Words: grazing, organic, genetics

288 Sire predicted transmitting ability for production and fertility traits in US organic Jersey cows. G. M. Pereira*[1], B. J. Heins[1], L. C. Hardie[2], and C. D. Dechow[2], [1]*University of Minnesota, West Central Research and Outreach Center, Morris, MN,* [2]*Pennsylvania State University, University Park, PA.*

The objective of this study was to determine the relationship between US organic Jersey cow performance and sire predicted transmitting ability (PTA) for production and fertility traits. Production and fertility data were provided by Dairy Record Management Services (Raleigh, NC), and sire PTA were from the December 2018 genetic evaluations from Council on Dairy Cattle Breeding (Bowie, MD). Data were from 5,461 cows sired by 814 A.I. bulls from 31 organic herds and spanned from 2012 to 2017. The mean 305-d mature equivalent (305ME) milk yield was 8,021 kg and mean combined fat and protein yield was 656 kg, mean SCS was 2.55, and mean days open was 136 d. Lactations greater than 5, herd-year-seasons with less than 5 records and herds with less than 40 cow records were removed. Daughter records with days open greater than 250 d were truncated to 250 d open. The PROC HPMIXED of SAS was used for statistical analysis, with the fixed effect of age group at calving within lactation group, and random effects of cow and herd-year-season. Daughter records of 305ME milk, fat, and protein production, SCS and days open were regressed on sire PTA for milk, fat, protein, SCS and daughter pregnancy rate, respectively. The regression coefficients on sire PTA for production were expected to be 1, and were 1.07 ± 0.06, 1.11 ± 0.07, 1.02 ± 0.07 ($P < 0.01$) for 305ME milk, fat, and protein, respectively, and 0.76 ± 0.12 ($P < 0.01$) for SCS. For days open, the regression coefficient on sire PTA was expected to be −4, and was −3.09 ± 0.39 d ($P < 0.01$) per unit PTA. The regression coefficients for production were similar to the expected coefficients, suggesting that US organic Jersey cows are expressing their genetic potential. The regression coefficient for days open was lower than the expected coefficient, suggesting a need for genetic improvement in fertility of US organic Jersey cows.

Key Words: Jersey, organic, sire predicted transmitting ability

Dairy Foods: Microbiology and Health

289 Bioengineered nisin derivatives to control *Listeria monocytogenes* in Queso Fresco. L. A. Ibarra-Sanchez*, W. Kong, T. Lu, and M. J. Miller, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Fresh Hispanic-style cheeses, such as Queso Fresco (QF), have often been implicated with outbreaks with *Listeria monocytogenes*. Nisin has been widely used in the food industry as a preservative due to its ability to inhibit *L. monocytogenes*; however, nisin has not been successful at limiting the growth of this pathogen in QF. The objective of this study was to evaluate the efficacy of bioengineered nisin derivatives against *L. monocytogenes* in QF. Nisin derivatives with a single amino acid substitution at residue 30 (I30X) or 32 (V32X) with positively charged amino acids (H, K, R) were produced in *Lactococcus lactis* MG1363, an engineered strain having the nisin biosynthetic pathway yet lacking *nisA*. Commercial nisin (nisin A) and nisin derivatives were evaluated to determine their minimum inhibitory concentration (MIC) and residual nisin after 24 h exposure to pH 7 + 22% milk fat at 37°C. The antilisterial activity of nisin A and nisin derivatives was tested in QF (250 µg nisin/g cheese), and untreated cheeses were included as a control. Cheese curds were inoculated with approximately 4 Log cfu/g of *L. monocytogenes* cocktail of 5 different foodborne outbreak-associated strains, and *L. monocytogenes* cells were enumerated by spread plating on PALCAM agar supplemented with ceftazidime, across 7 d of storage at 4°C. All experiments were repeated 3 times with samples prepared in duplicate. Evaluated nisin derivatives exhibited a reduced antilisterial activity in vitro (MIC = 25 µg/mL) compared with nisin A (MIC = 3.125 µg/mL). Residual nisin A was not detected after 24 h of exposure to pH 7 + 22% milk fat, on the contrary, all nisin derivatives evaluated showed residual nisin of 40–75%. After 7 d of cold storage, either nisin I30H and V32R resulted in fewer *L. monocytogenes* cells in QF with viable counts approximately 0.7 Log cfu/g less than control QF. Similar final *Listeria* counts were observed in QF added with nisin A and control cheeses. In conclusion, our results support that altering residue 30 or 32 of nisin might result in nisin derivatives with enhanced antilisterial activity in QF.

Key Words: Queso Fresco, *Listeria monocytogenes*, nisin derivatives

290 Manufacturing low-spore-count skim milk powder by controlling raw milk holding conditions—A pilot-scale trial. N. Awasti*[1], S. Anand[1], and G. Djira[2], [1]*Midwest Dairy Foods Research Center, Department of Dairy and Food Science, South Dakota State University, Brookings, SD,* [2]*Department of Mathematics and Statistics, South Dakota State University, Brookings, SD.*

Milk powder is one of the most traded dairy products globally, largely being used to manufacture commercially sterilized products. Skim milk powder is frequently contaminated with spores of *Bacillus* species and carried over from raw milk due to their ability to survive processing conditions. Our previous study proved that it is possible to use optimized raw milk holding conditions to keep their population low, which may help in making low spore count powder. This pilot scale trial was conducted to produce low spore count powder by controlling the raw milk holding conditions, to keep the spore populations low. *Bacillus licheniformis* being a predominant spore former in milk powders was used as an inoculant for the challenge study. Inoculated raw milk samples (batches of 1500 lbs each) were held at optimized storage conditions T1 (4°C for 24 h) and T2 (8°C for 72 h). The PMO based storage conditions (10°C for 4 h followed by 7°C for 72 h) were kept as a control, before producing skim milk powder. Samples were drawn at different stages pre- and post-storage, pasteurization (73°C for 15 s), evaporation, and spray drying (outlet and inlet temperature of 200 and 95°C, respectively), and analyzed for sporeformers using standard culturing methods. Spore counts were done by heating the samples at 80°C for 12 min, before plating on Brain Heart Infusion agar. All samples were analyzed in the replicates of 3, and means were compared using ANOVA. Treatment T1 and T2 log spore counts (1.79 ± 0.03 and 1.82 ± 0.04, respectively), were significantly lower than control (2.59 ± 0.05), after raw milk storage step. Similarly, sporeformers log counts for T1 (3.84 ± 0.02) were significantly lower than T2 (4.07 ± 0.08) and control (4.13 ± 0.04). Skim milk powders prepared using optimized storage conditions T1 showed significantly ($P < 0.05$) lower spore and sporeformer counts (0.58 ± 0.04 and 1.82 ± 0.05 log cfu/g), as compared with T2 (0.86 ± 0.16 and 1.90 ± 0.03) respectively, and control (1.03 ± 0.06 and 2.74 ± 0.03, respectively). This shows that skim milk powders with reduced counts can be prepared by just optimizing raw milk holding conditions.

Key Words: spore, drying, *Bacillus*

291 Transcriptional and proteomic analysis revealed a synergistic effect of aflatoxin M_1 and ochratoxin A mycotoxins on the intestinal epithelial integrity of differentiated human Caco-2 cells. Y. Gao[1,2], S. Li[1,2], J. Wang*[1,2], and N. Zheng[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality and Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China.*

Aflatoxin M_1 (AFM_1) is a common mycotoxin in dairy milk, and it is typically concurrently present with other mycotoxins that may represent a threat to food safety. However, knowledge of how AFM_1, alone or in combination with other mycotoxins, may affect human intestinal epithelial integrity remain to be established. We employed transcriptome and proteome analysis to reveal the molecular basis underlining the effect of exposure to AFM_1, ochratoxin A (OTA), or both on the differentiated Caco-2 cells. The genes with a fold change ≥ 2 and a false discovery rate (FDR) < 0.05 were considered as significant differentially expressed genes (DEGs). And only proteins with significant quantitative ratios between the 2 treatments ($P < 0.05$) and fold changes >1.2 or <0.83 were considered to be differentially regulated. The integrated transcriptome and proteome analysis of AFM_1 and OTA, alone or in combination, indicate the synergistic effect of the 2 mycotoxins in disrupting intestinal integrity. This effect was mechanistically linked to a broad range of pathways related to intestinal integrity enriched by downregulated genes and proteins, associated with focal adhesion, adheren junction, and gap junction pathways. Furthermore, the cross-omics analysis of mixed AFM_1 and OTA compared with OTA alone suggest that kinase family members, including myosin light-chain kinase, mitogen-activated protein kinases, and protein kinase C, are the potential key regulators in modulating intestinal epithelial integrity. These findings provide novel insight into the synergistic detrimental role of multiple mycotoxins in disrupting intestinal integrity and, therefore, identify potential targets to improve milk safety related to human health.

Key Words: toxin, intestinal integrity, synergistic effect

292 iTRAQ-based quantitative proteomic analysis of the global response in high EPS-producing *Streptococcus thermophilus* ASCC 1275 in the presence of different sugars. A. Padmanabhan*, Y. Tong, C. Lo, and N. Shah, *The University of Hong Kong, Hong Kong.*

Exopolysaccharides (EPS) produced by LAB improve functional properties of fermented food. In our previous studies, *Streptococcus thermophilus* ASCC 1275 (ST1275) was found to produce high amount of EPS. We studied the influence of sugars (glucose, sucrose, and lactose) on the global proteomics of ST1275 to find the differentially expressed proteins (DEP) during EPS production. M17 medium was supplemented with 1% of each of the 3 sugars and ST1275 was grown at 37°C. The samples for proteomics analysis were collected at the log phase (5h) and stationary phase (10h). Isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomic analysis was conducted to identify the global protein responses of ST1275 under the influence of the 3 sugars. ProteinPilot software was used to validate MS/MS-based isobaric tag peptide for protein identification. Overall, 215, 474, 119 proteins were upregulated in the M17-glucose, M17-sucrose, and M17-lactose media, respectively. M17-sucrose medium, which showed high EPS production at 10h when compared with M17-glucose and M17-lactose media, had a significant upregulation of proteins involved in EPS assembly, phosphoenolpyruvate (PEP) transport system, methionine and cysteine/arginine synthesis. Even though, in M17-lactose medium, at 5 h, there was a considerable upregulation of most of the proteins in nucleotide sugar synthesis and EPS assembly, the same level of expression was not observed at 10 h due to fast utilization of lactose in the medium and its unavailability thereafter. This study found that for high EPS production, the availability of sugars and the metabolic pathways that lead to EPS production should function in an organized manner.

Key Words: *Streptococcus thermophilus*, proteomics, exopolysaccharide

293 Novel antidiabetic bioactive peptides from camel milk protein hydrolysates. S. Maqsood*[1], P. Mudgil[1], G. Yuen[2], A. Nongonierma[3], and R. J. FitzGerald[3], [1]*United Arab Emirates University, Al Ain, United Arab Emirates,* [2]*University Sains Malaysia, Malaysia,* [3]*University of Limerick, Limerick, Ireland.*

Bioactive peptides present in milk proteins could potentially have beneficial effects on human health. Various camel milk protein hydrolysates were produced using different food-grade enzymes at various time of hydrolysis. In vitro inhibitory properties of *novel bioactive* peptides released from camel milk proteins against dipeptidyl peptidase-IV (DPP-IV), and pancreatic α-amylase (PA), were studied. Results revealed that upon hydrolysis by different enzymes, camel milk proteins displayed dramatic increase in inhibition of DPP-IV and only slight improvement in PA inhibition was noticed. Peptide sequencing revealed a total of 20 and 3 peptides for A9 and B9 respectively, obtained the score of 0.8 or more on peptide ranker and were categorized as potentially active peptides eligible to be DPP-IV inhibitory peptide. KDLWDDFKGL in A9 and MPSKPPLL in B9 were identified as most potent PA inhibitory peptide. The present study report for the first time PPA and PPL inhibitory and only second for DPP-IV inhibitory potential of protein hydrolysates from camel milk. Moreover, trypsin hydrolysed camel milk proteins released LLQLEAIR and LPVP, which had a DPP-IV half maximal inhibitory concentration (IC_{50}) of 83.6 ± 4.6 and 93.3 ± 3.6 μM ($P > 0.05$), respectively. DPP-IV inhibitory peptide sequences within camel milk protein hydrolysates differ from those found in a bovine milk protein hydrolysate. Camel milk proteins can provide novel DPP-IV inhibitory peptides, which may be used to regulate glycemia in humans.

Key Words: camel milk protein, bioactive peptide, dipeptidyl peptidase-IV (DPP-IV)

294 Development and characterization of novel bigel system. M. Bollom*, N. Acevedo, and S. Clark, *Iowa State University, Ames, IA.*

Oleogels, typically made with plant-based oils and oleogelators, have limited application in dairy foods. Incorporation of whey protein concentrate (WPC) into oleogels, to create bigels, may change that. Bigels are semi-solid biphasic systems where each phase, an oleogel and hydrogel, is structured to give superior physical, mechanical, and stability properties. The novel bigel developed here is composed of a WPC hydrogel and an oleogel emulsion (soybean oil, soy lecithin, stearic acid, and water). There are many advantages to using a bigel over an individual oleogel or hydrogel, such as higher dairy content, higher protein content (on trend), improved drug delivery, potential to delay oxidation (due to the physical structure hindering prooxidant mobility), and improved consumer acceptance. Since bigels are so new, little is known about their microstructure and mechanical properties. Therefore, the purpose of this study was to better understand bigel structure and mechanical properties. Garnering a better understanding of structure and mechanical properties will allow optimization of bigel encapsulation abilities and incorporation into dairy foods. To understand bigel structure, 3 main techniques were used: small angle X-ray scattering, rheology, and fluorescence microscopy. Different ratios of oleogel emulsion:hydrogel were tested (0:10, 3:7, 5:5, 7:3, and 10:0), as well as 2 hydrogel protein contents (15% and 25%) and 2 oleogel emulsion moisture contents (10% and 20%). Higher WPC content yielded greater firmness (because of a stronger hydrogel) and higher moisture in the oleogel emulsion resulted in a less firm bigel. Pure oleogels and hydrogels had greater strength than the bigels. We hypothesize that this is due to each gel structure preventing the other from forming a cohesive, continuous structure. This research has shown that the functionality of an oleogel can be improved, through the addition of a WPC hydrogel, for addition into dairy products, while still retaining key stability features of the oleogel.

Key Words: bigel, whey protein concentrate (WPC)

Growth and Development 1

296 Impact of increasing grass hay inclusion level on weaned dairy calf growth and metabolism. L. K. Mitchell* and A. J. Heinrichs, *Pennsylvania State University, University Park, PA.*

The objective was to determine effects of increasing grass hay (GH) inclusion level on weaned dairy calf growth and metabolism. Holstein calves (n = 45) were randomly assigned to 1 of 3 total mixed rations (TMR) with increasing GH (10%, 17.5%, or 25% on a dry matter (DM) basis; LGH, MGH, or HGH respectively). Calves were weaned at 6 wk of age, housed individually, and studied from 7 to 16 wk of age. Rations, consisting of texturized calf starter (20% crude protein) and chopped GH, were offered ad libitum as separate components from 7 to 9 wk of age. After 9 wk, feed was offered ad libitum as a TMR containing the assigned level of GH. Initial 9-wk body weight (BW) was 81.6 ± 9 kg. Intake and growth were measured weekly. Blood samples were collected at 9, 10, 12, 14, and 16 wk of age. Total fecal collection (12 calves) was conducted for 4 d at 11 and 15 wk of age. Feeds and feces were evaluated for DM, neutral detergent fiber, acid detergent fiber, and starch to estimate total-tract digestibility. The statistical model included initial 9-wk BW as a covariate, fixed effect of TMR, random effects of calf, week of study, and sex, and repeated effects of age. Linear and quadratic responses were tested using orthogonal polynomial contrasts. Final BW decreased linearly with increased GH ($P < 0.01$), but frame measurements did not vary between groups. Intake and weight gain were analyzed from 7 to 9 wk and 9 to 16 wk, representing pretreatment and treatment periods. There were no differences between groups from 7 to 9 wk. However, differences were found from 9 to 16 wk. Average daily gain, DM intake, and metabolizable energy intake all decreased linearly with increased GH ($P < 0.01$). Plasma β-hydroxybutyrate tended to decrease with increased GH ($P = 0.07$). There were no differences in DM or starch digestibility, but neutral detergent fiber and acid detergent fiber digestibility increased linearly with increased GH ($P < 0.01$ and $P = 0.06$ respectively). Levels of GH > 10% may reduce intake and growth before 16 wk.

Key Words: digestibility, grass hay inclusion, weaned calf

298 Effects of YANG (multi-strain yeast) supplementation on health and performance in male Holstein calves. D. Cavallini*[1], M. Pollesel[1], M. Gauthier[2], and M. Tassinari[1], [1]*DIMEVET, Dipartimento di Scienze Mediche Veterinarie, Università di Bologna, Bologna, Italy,* [2]*Lallemand SAS, Blagnac, France.*

The objective of this study was investigate the effects of a multi-strain yeast (*Saccharomyces cerevisiae* and *Cyberlindnera jadinii*) fraction product, YANG, on the health and performance of young male dairy calves (17 ± 3d). At a commercial farm, 158 veal calves were enrolled and divided in 2 groups: a control group (CTR) 57 animals (no supplementation) and a treated one (TRT) 101 animals (YANG supplementation), both balanced for live weight. TRT received YANG through the milk replacer: 5g/h/d during the first 30d, then 3g/h/d until d90. From d90 to 190 (slaughter) the diet was the same for both groups. For all animals, individual carcass weights, mortality and morbidity (veterinary treatments) were collected. Blood parameters (Hg, RDW, MCV; d30, 73, 115, 150) and electrophoresis for blood protein characterization (d47, 118) were analyzed in 23 randomly selected animals (12 CTR and 11 TRT). On sampled animals, body weights were measured (d0, 47, 82). No differences were observed on carcass weights at slaughter and carcass classification between the 2 groups. However, sampled animals' ADG was affected. ADG from d0 to 47 and 47 to 82 was 263g/d and 321g/d higher, in TRT than in CTR (0.47kg/d, $P = 0.01$; 1.01kg/d, $P < 0.01$ respectively). No difference was observed in mortality; however, health of calves was improved. Morbidity was reduced from 56% in CTR to 38% in TRT ($P = 0.02$), with a reduction of number of chronic animals (≥2 treatments per animal) by 50% ($P = 0.03$). Numerically, the reduction of veterinary treatments was mainly due to reduction of enteric treatments in TRT (−25.6%). TRT animals showed higher content of total proteins ($P < 0.01$), with higher percentage of γ-globulins ($P < 0.01$) and higher percentage of α1-globulins ($P < 0.01$). These analyses are in favor of a better activated immune system of veal calves in TRT. No differences were observed on RDW and MCV. No differences in Hg average concentration, but more homogeneity in TRT. This study demonstrates that the health of veal calves was improved during their whole lifecycle with the addition of YANG in the milk replacer during the first 90d. These results are in line with reduction in use of antimicrobial treatments in farms.

Lactation Biology Symposium: Refining the Old to Answer the New—Moving Approaches Forward to Study Mammary and Lactation Physiology

299 Determinants of milk production: Understanding population dynamics in the bovine mammary epithelium. A. V. Capuco*, *Animal Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD.*

The mammary gland undergoes distinct periods of growth, development and secretory activity. During a bovine lactation, a gradual decrease in number of mammary epithelial cells largely accounts for the decline in milk production with advancing lactation. The net decline in cell number (~50%) is due to apoptotic cell death, but is accompanied by cell renewal. Though the rate of cell proliferation is slow, by end of lactation most cells in the gland were formed after calving. Typically milking is terminated when cows are in the final 2 mo of pregnancy. This causes regenerative involution, wherein there is extensive cell replacement and mammary growth. We hypothesized that replacement of senescent secretory cells and progenitor cells during the dry period increases milk yield in the next lactation. Analysis of global gene expression revealed networks and canonical pathways during regenerative involution that: support cell turnover and mammary growth, that are consistent with oxidative stress, mitochondrial dysfunction and ER-stress, as well as processes that ameliorate those effects, immune responses consistent with influx of neutrophils, macrophages and lymphocytes, and processes that support mammary differentiation and lactogenesis. Data also suggest that replication of stem/progenitor cells occurs during the dry period. Relying on long-term retention of bromodeoxyuridine-labeled DNA, we identified putative bovine mammary stem cells. These label retaining epithelial cells (LREC) are in low abundance within mammary epithelium (<1%), are predominantly estrogen receptor-negative and localized in a basal or suprabasal layer of the epithelium. Analyses of gene expression in laser-microdissected LREC are consistent with the concept that LREC represent stem cells and progenitor cells, which differ in properties and location within the epithelial layer. We identified potential markers for these cells and have increased their number by infusing xanthosine through the teat canal of prepubertal heifers. Altering population dynamics of mammary stem/progenitor cells during the mammary cycle may be a means to increase efficiency of milk production.

300 Studying hormonal regulation of mammary gland homeostasis. N. D. Horseman*, *University of Cincinnati, Cincinnati, OH.*

Homeostasis during lactation is a special case in which the unit of homeostatic regulation is not a single organism, but rather a dyad comprising the mother and her offspring (the mother–infant dyad). This dyadic arrangement is not trivial. The familiar laboratory mouse model system includes a mother and litter of infants whose mass can easily exceed the mass of the mother. Some physiological variables, such as body temperature, can remain under conventional homeostatic control within the individual. But other variables such as milk secretion, appetite, and calcium metabolism must come under control of the mother–infant dyad. Prolactin is the primary systemic component of mammary homeostasis. Suckling inhibits dopamine, which induces reflex prolactin secretion in response to nursing. Other factors, such as oxytocin and endorphins increase prolactin output, which sustains enhanced prolactin throughout lactation. In addition to the systemic homeostasis, local homeostatic mechanisms control mammary gland functions. The sophistication of intramammary homeostatic mechanisms is perhaps best illustrated in kangaroos in which individual mammary glands produce different volumes and compositions of milk because they are being nursed by offspring of different ages. The millions of alveolar sacs that comprise the mammary glands are capable of independently regulating their milk outputs in response to local conditions. Most obviously, each alveolus must regulate its secretory output in response to the degree of filling. Serotonin was discovered as a primary regulator of mammary homeostasis. Serotonin synthesis is in the mammary epithelium is elevated during lactation, and increases during milk stasis. Two important functions have been attributed to the intramammary serotonin system. First, when alveolar spaces are filled with milk serotonin inhibits milk secretion and causes tight junction opening. Ultimately, this feedback system induces early phases of involution. Second, serotonin induces secretion of parathyroid hormone-related peptide (PTHrP), which is the primary regulator of calcium homeostasis for the mother–infant dyad. These 2 intramammary homeostatic responses are mediated by different receptors. Feedback inhibition of milk secretion is mediated by 5-HTR7, which is autoinhibited and requires sustained occupation to become activated. PTHrP secretion is mediated by 5-HTR2B receptors, which are activated at tonic levels of serotonin.

Key Words: serotonin synthesis, lactation, mammary homeostasis

301 Delayed response of xanthosine on goat mammary gland: Quantification of stem/progenitor cells, differentiation and proliferation markers, and milk production in next lactation. T. P. Kaur*[1], R. Verma[1], S. Choudhary[1], R. Udehiya[3], S. Kaswan[2], and R. K. Choudhary[1], [1]*School of Animal Biotechnology, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India,* [2]*Department of Animal Nutrition, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India,* [3]*Department of Veterinary Surgery and Radiology, College of Veterinary Science, Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India.*

The intramammary infusion of xanthosine (XS) has effects on mammary glands in altering stem/progenitor cell population and possibly on milk production. The aim of this study was to evaluate prolong response of XS on stem/progenitor cells, cell proliferation and differentiation and milk production in following lactation in goats. Six primiparous goats were assigned to the study. Twenty milliliters of 10 m*M* (2 × 3 d) XS was infused 5 d after parturition into one of the randomly allotted gland (TRT) immediately after morning (0900 h) and evening (2100 h) milking. The other gland of the same animal served as control (CON) with no infusion. Mammary tissues were harvested during the dry period (Mean + SE; 136.8 + 11.2 d) and processed for histology. In the next lactation, milk production was recorded until 17 weeks. Immunolocalization of alveolar cell differentiation markers (estrogen receptor α (ESR1), progesterone receptor (PR), mucin 1 MUC1), mammary stem cell markers (nuclear receptor subfamily 5 group A member 2 (NR5A2), aldehyde dehydrogenase 1 (ALDH1), fibronectin type III domain containing 3B (FNDC3B), cell proliferation (Ki67) and apoptosis marker (p53) were quantified in terms of number of immune-positive cells of the total cells counted. XS appeared to enhance expression of NR5A2 (7.7 + 0.9 vs. 4.9 + 0.7; Mann-Whitney U test; z = 0.02) and FNDCB (6.9 + 1.2 vs. 3.1 + 0.3; z = 0.004) in relation to CON glands. Expressions of other markers namely ALDH1, ESR1, PR, MUC1, Ki67 and p53 did not differ between TRT and CON glands. Milk yield (mean + SE) of

TRT glands were not different from the CON glands (3462.6 + 156.1 g/wk vs. 3761.3 + 205.8 g/wk; paired *t*-test $P = 0.28$). These results suggest XS may have a prolong effects on increasing stem/progenitor cell population during the dry period but has no response in milk production in the next lactation.

Key Words: xanthosine, milk production, stem cell marker

302 Deep tissue imaging of lobuloalveolar development in the mouse.

C. J. Watson*, *Department of Pathology, University of Cambridge, Cambridge, UK.*

The pregnancy/lactation/involution cycle of mammary gland development requires the rapid proliferation and subsequent differentiation of both luminal and basal epithelial cells that are presumed to arise from stem cells. To investigate this process, we utilized 2 neutral lineage tracing approaches coupled with new protocols for clearing of mammary tissue to allow deep 3-dimensional (3D) confocal imaging. By labeling stem cells at clonal density we were able to show that individual alveoli arise from unipotent basal and luminal stem cells and that more than one stem cell of each lineage is required to generate an entire alveolus. Furthermore, 3D imaging revealed the presence of multiple binucleate cells in the lactating gland suggesting failed cytokinesis. In addition, deep imaging with antibodies to smooth muscle actin and keratin 14 revealed the distinctive structure of basal cells in alveoli compared with ducts and allowed the collapse of alveoli during involution to be monitored. Surprisingly, we discovered an intimate and dynamic association of leukocytes with the epithelium and have analyzed these further by flow cytometry.

303 A comparative multi-species approach to improve our understanding of mammary gland biology.

G. R. Van de Walle*, *Baker Institute for Animal Health, Cornell University, Ithaca, NY.*

Mammary stem/progenitor cells (MaSC) in non-mouse, non-human species are considerably understudied. Interspecies variation in lactation strategies and mammary cancer incidence, combined with the role of MaSC in normal mammary gland function, warrants a comparative study of these cells from various mammalian species. Our laboratory has developed a method for the enrichment of MaSC from virtually any mammal. This method is antibody-independent, uses a small volume of fresh tissue, and consists on propagating freshly isolated mammary cells as mammospheres in suspension culture. We found that the ability to form mammospheres is conserved among the mammalian species we tested thus far, including domesticated and wild animals. Moreover, the cells derived from these mammospheres (which we term mammosphere-derived epithelial cells or MDEC) share several key properties: they express markers of stem-like mammary cells in culture, form at least 2 types of morphologically distinct clones in culture, and contain a population of mammosphere-reforming cells.

Recent studies have shown that the cellular secretome, comprised of secreted soluble factors and factors secreted in vesicles, plays an important role in various physiological processes such as cellular cross-talk and tissue regeneration. While studying the secretome of MDEC isolated from various domesticated mammals, several interesting observations were made. For example, we identified a novel form of intercellular communication between MDEC from canine and equine origin that plays an important role in self-renewal and relies on microvesicle-mediated Wnt/beta-catenin signaling. We also found that the secretome from bovine MDEC promotes angiogenesis, epithelial cell migration, and contains factors associated with defense and immunity; all of which are necessary for healing damaged mammary gland tissue. Finally, our group has initiated studies in which to compare the behavior of MDEC from mammary cancer-susceptible and -resistant species, both at baseline as well as in response to pro-tumorigenic stimuli, in order to gain new insight into the mechanisms of breast cancer susceptibility and resistance.

304 Milk omics: Modern tools to answer ancient questions.

D. G. Lemay*[1,2], [1]*USDA Western Human Nutrition Research Center, Davis, CA,* [2]*University of California-Davis, Davis, CA.*

Advances in high throughput biology have revolutionized the study of milk and mammary gland biology. This talk will review applications of genomics, epigenetics, transcriptomics, proteomics, and metagenomics in the field. The availability of mammalian genome sequences, particularly the bovine genome assembly, enabled multi-species comparisons of the genes that are expressed to produce milk. Across mammals, gene duplication and protein sequence variation both contributed to differences in milk composition. ChIP-Seq and transcriptomic data were then leveraged to understand epigenome-wide features involved in milk production. Identification of cis-regulatory elements in the bovine genome using RNA-Seq and ATAC-Seq technology is ongoing. Meanwhile, non-invasive technologies to study mammary biology in humans using RNA-Seq of milk fat RNA have been developed and validated. Whole transcriptome comparisons in a non-human primate model demonstrated that RNA from milk samples provides a more accurate representation of RNA from milk-producing cells than does RNA from whole mammary tissue. Advances in proteomics have expanded the milk proteome from dozens to thousands of unique proteins. The use of RNA-Seq to develop a comprehensive database of protein sequences has the potential to further expand the milk proteome to include isoforms of milk proteins. Finally, microbial metagenomics can be used to survey all microbes in a sample to investigate causal agents in mastitis, manage spoilage organisms, or track antibiotic resistance. Applications of these “omic” technologies in dairy science will be discussed.

Key Words: mammary biology, genomics, epigenetics

305 Dry period heat stress alters mammary protein expression throughout the subsequent lactation.

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Climate change adversely affects the dairy economy as high temperatures and humidity (i.e., heat stress) result in greater incidence of cattle disease and mortality and lower milk yield. Previous research by our group demonstrated consequences of dry period heat stress on cow health and milk production in the subsequent lactation. However, the molecular mechanisms through which dry period heat stress impairs lactation performance have yet to be fully elucidated. In this study, we evaluated the impacts of dry period heat stress on the mammary proteome across the subsequent lactation. During the dry period (~46 d), multiparous Holstein cows were housed in either shaded barns with fans and water soakers (cooled group [CL]; $n = 4$) or shaded barns without cooling (heat stressed group [HT]; $n = 4$) at the University of Florida Dairy Unit. All cows were cooled postpartum. Mammary biopsies were obtained at 14, 42, and 84 DIM. Proteomes were examined using iTRAQ technology at the UF Institute of Biotechnology Research by a Q Exactive Plus mass spectrometry system coupled to the NanoEasy nLC-1200. Data were searched against Uniprot *Bos taurus* database (45,234 contigs) using ProteinPilot v4.5. A meta-analysis from Student’s *t*-test was employed to test for protein expression differences between HT and CL at each time

point. Proteins were differentially expressed (DEPs) at a fold change of less than 0.7 and greater than 1.3 and a *P*-value of < 0.05. A total of 4,964 proteins were identified. 251 unique proteins were differentially expressed between HT and CL. There were more upregulated proteins in HT compared with CL and the most DEPs occurred at 84 DIM. DEPs are involved in functions such as the heat shock response (e.g., ST13, HSPB1), energy metabolism (e.g., NDUFA4, ATP5O), carbohydrate metabolism (e.g., MDH1, TALDO1), amino acid metabolism (e.g., IVD, ASRGL1), fatty acid biosynthesis (e.g., ACSF3, ACACA), lactose synthesis (e.g., B4GALT1), and translation (e.g., RPS2, EIF1AX). These processes are critical for milk synthesis and the response to stress and may explain, at least in part, the impaired lactation performance of HT cows.

Key Words: proteomics, mammary gland, iTRAQ

Milk Protein and Enzymes Symposium: Don't Have a Cow—Plant Proteins, Bovine Protein Expression, and Milk Humanization

306 The dairy matrix—Bioaccessibility of nutrients and physiological effects. S. L. Turgeon*, *STELA Dairy Research Centre and Institute of Nutrition and Functional Foods (INAF), Department of Food Science, Université Laval, Québec City, QC, Canada.*

Studies have linked food structure and texture to kinetics of nutrient delivery. Changes in some nutrient release rates, such as for protein and lipids, could induce different physiological effects (e.g., satiety effect, reduction of postprandial lipemia). Examples will be presented to showcase the contribution of dairy food structure, as found in yogurt and cheese, on nutrient release rates. The impact of the casein:whey protein ratio or fiber addition on digestion kinetics of protein, and subsequently, satiety, was examined in one study. A static in vitro digestion model was used on experimental yogurts differing in the casein-to-whey protein ratio (C:W) or dietary fiber content. Healthy men (20) consumed the 5 isocaloric/isoproteinemic yogurt snacks previously studied. Ad libitum food intake was monitored 2.5 h post-snack consumption. A human clinical trial revealed that yogurt formulation with increased whey protein content significantly reduced subsequent energy intake compared with the control, and increased satiety. This result was linked to a slower in vitro disintegration rate. Soluble protein release for yogurt increased in whey protein whereas no difference was observed for yogurts with fiber. A second study allowed for the discrimination of the effects of cheese attributes on lipid release and absorption. Nine commercial cheeses were digested in vitro and 2 were selected for an in vivo study. Healthy women/men (43) ingested 33 g of fat from cheddar, cream cheese or butter, incorporated in a standardized meal. Plasma concentrations of triglycerides (TG) were measured before the meal and at 2, 4, 6 and 8 h. At the end of the in vitro gastric digestion, cheddar and cream cheese showed different disintegration, attributed to the texture and manufacturing processes. The in vivo study revealed that cream cheese, but not cheddar, induced a more important increase in TG concentrations than the butter (Δ vs baseline: +44% vs +24%, $P = 0.002$) at 2 h. These studies demonstrate that the dairy food matrix per se modulates nutritional properties. Studies published on this topic will be included to put into perspective the role of the dairy food matrix on nutrient release, the physiological effects, and how this can be compared with other foods.

Key Words: nutrient delivery, dairy food, food structure

307 Functionality, bioactivity, and structure of bovine and plant proteins. H. Patel*, *US Dairy and Foods Consulting LLC, Plymouth, MN.*

It is well known that the sequence of amino acids determines the 3-dimensional structure of proteins, and that protein structure determines its function. The amount of essential amino acids present in the protein determines protein quality. In the recent years, there has been an emerging consumer trend toward plant-based foods and beverages, which is mainly driven by "vegan" and "flexitarian" lifestyles. It also offers more choice of food formats to consumers. Plant-based proteins differ significantly in their structure, functionality and bioavailability compared with bovine proteins due to differences in their amino acid profile, the sequence of amino acids, the way they are folded to form 3-dimensional structures and the amount of essential amino acids (EAA). The structure-function relationship of bovine proteins is well studied, whereas there is limited information available about plant proteins. The bovine proteins, particularly whey proteins, are considered as complete proteins because of their relatively high amount of EAA and branched chain amino acids, whereas most singular plant proteins are not complete proteins. Therefore, combining multiple plant proteins is necessary to get a complete protein. This presentation aims to discuss the key differences in the structure, functionality, and bioavailability of bovine versus plant proteins.

308 Sustainability of dairy product production on a protein basis. Y. Wang*, *Innovation Center for US Dairy, Rosemont, IL.*

Global agriculture faces the prospect of a changing climate and the challenge of feeding the fast-growing world's population. It has been estimated that we need to produce more food in the next 40 years than we have ever produced in human history given the projected population growth, the limited farmland and the competition with urbanization. Foods derived from plants and animals can both provide protein, but there are some differences nutritionally, environmentally and more. This paper will provide a life cycle perspective for dairy protein production to further engage dairy sustainability discussions. For years, US dairy farmers have been using technology and advanced management practices to increase efficiency and reduce environmental impact. The Innovation Center for US Dairy commissioned a series of life cycle assessment studies (Thoma et al., 2010, https://data.nal.usda.gov/dataset/data-greenhouse-gas-emissions-milk-production-and-consumption-united-states-cradle-grave-life-cycle-assessment-circa-2008; Henderson et al., 2012, University of Michigan and University of Arkansas) to understand the environmental impacts of dairy production, processing and transportation. For example, based on a 2010 study, the US dairy industry contributes only 2% of GHG, 5% of water withdrawal and 9% of US cropland usage. Dairy cattle rely primarily on forages, crop residues, and agri-food industry by-products that are not edible for humans, to produce a much more valuable food for humans. According to an FAO study, 86% of the global livestock feed intake in dry matter consists of feed materials that are not currently edible for humans (Mottet et al., 2017, Global Food Security 14:1–8). A similar study in the US [Tricarico, 2015, J. Dairy Sci. 98 (Suppl. 2):712] showed that 20% of dairy feed is human-edible by composition and only 0.9% of dairy feed is in competition with humans by demand. Furthermore, replacement of edible feed crops with human-inedible biomass in animal diets is a potential strategy that could reduce food-feed competition and mitigate the environmental impacts of livestock (Salami et al., 2019, Anim. Feed Sci. Technol. 251:37–55). For dairy products and the ingredients industry, it is important to understand the importance of environmental stewardship.

309 Humanization of bovine milk using modern genetic technologies. G. Laible*, *AgResearch, Ruakura Research Centre, Hamilton, New Zealand.*

The domestication of cattle has provided humans with almost unlimited access to bovine milk and processed dairy products that are important human foods.They provide an excellent source to meet human nutritional demands for proteins, lipids, carbohydrates, and minerals.Although regarded as a high-quality food source, cows' milk has evolved to deliver optimized nutrition to the suckling calf and not as human food.Hence, there remains great potential to improve all major components of cows' milk to further enhance benefits for human health and wellbeing tailored to the different stages of life and their specific nutritional demands. Compared with human milk, cows' milk has a higher protein content, greater casein to whey protein ratio, lower lactose levels and more saturated and less unsaturated fats. Efforts to change bovine milk by conventional selective breeding approaches were largely unsuccessful. Technological progress for efficiently introducing targeted and precise genetic changes provides new opportunities to directly change specific characteristics of bovine milk. This includes humanizing bovine milk for superior infant nutrition or altering bovine milk to accommodate the different nutritional requirements of adolescents, adults, and elderlies. Early proof-of-principle studies aimed at improving cows' milk by complementing it with additional antimicrobial human whey proteins used transgenic technology and were hampered by low acceptance. Since then technology has become much more refined and it is now possible to humanize cows' milk without leaving a technological footprint. If this new, so-called genome editing technology will be received with greater levels of acceptance is still uncertain. Much will depend on our ability to develop proof of concept models. This is particularly important because milk is a very complex biological fluid and the changes resulting from a specific component can potentially lead to more wide-ranging implications. Only with appropriate animal models will we be able to fully evaluate potential risks and clearly demonstrate health benefits for consumers that could be delivered by these enhanced food products. Then it is up to the consumer to decide whether these new food products provide sufficient additional health benefits to outweigh any remaining technological risks.

310 Taking a page out of nature: Catering to the future of proteins. B. Oommen*, *Perfect Day Inc., Berkeley, CA.*

Global demand for high-quality animal-sourced protein is expected to rise substantially over the next 2 decades as population grows and global household incomes rise. Simultaneously, climate experts are calling for a rapid transition away from animal-derived foods in an effort to curb greenhouse gas emissions from animal agriculture. Novel solutions are needed for producing quality protein with minimal environmental and climate impacts. Perfect Day has developed a means of producing dairy protein—nutritionally and chemically the same as conventional bovine dairy protein—via the fermentation of bioengineered microflora. This is the same technology widely employed by the food industry to produce ingredients such as vitamins, amino acids, flavors, enzymes, and rennet for cheese making. The first non-animal whey protein looks and behaves similar to its bovine counterpart. Similar processes could be used to produce a variety of other common food ingredients and materials that are sourced from animals today. On a longer time scale, the same approach could be used to manufacture fully designed novel proteins that could be superior in nutrition and functionality than what nature has provided thus far.

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311 Effect of protein absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites in dairy cows. I. Salfer*, C. Matamoros, R. Bomberger, and K. Harvatine, *The Pennsylvania State University, University Park, PA.*

Dairy cows have a daily rhythm of milk synthesis that appears to be driven by the molecular clock of the mammary gland and is modified by the time of feed availability. Protein metabolism is intimately linked to circadian rhythms in other species, but the effect of amino acids on the mammary clock in unknown. The objective was to determine the effects of the timing of protein absorption on the daily rhythms of milk synthesis in dairy cows. Nine cannulated lactating Holstein cows (128 ± 46 d postpartum; mean ± SD) were randomly assigned to 1 of 3 treatment sequences in a 3 × 3 Latin square. Treatments were abomasal infusions of 500 g/d of sodium caseinate either 24 h/d (CON) or for 8 h/d from 0900 to 1700 h (DAY) or from 2100 to 0500 (NGT). Treatment periods were 15 d with a 6 d washout. During the final 8 d of each period, cows were milked every 6 h. A 24-h rhythm was fit to time-course production data using cosine analysis and the amplitude and acrophase (time at peak) were determined. Daily milk and protein yield were decreased by NGT compared with CON, while fat yield was increased by DAY ($P < 0.05$). Daily fat and protein concentration were not affected by treatment. Milk yield failed to fit a 24 h rhythm in CON or DAY, but a rhythm was induced by NGT ($P = 0.03$). Neither fat yield nor protein yield fit a rhythm in either treatment. Fat concentration fit a daily rhythm in all treatments ($P < 0.05$), with the amplitude decreased 57% by DAY and 26% by NGT ($P < 0.05$). The rhythm of milk fat concentration was phase advanced ~2 h by DAY and phase delayed ~1 h by NGT ($P < 0.05$). Protein concentration fit a daily rhythm in CON and DAY but not NGT. The phase of protein concentration was delayed ~1.25 h by DAY and the amplitude was increased 2-fold relative to CON ($P < 0.05$). The time of protein infusion influenced daily rhythms of milk and milk protein synthesis. Night infusion abolished rhythms of protein concentration and induced rhythms of milk yield while day infusion increased the amplitude of protein concentration. The results of this study suggest a role of amino acids in entraining the molecular clock of the mammary gland.

Key Words: daily rhythm, milk synthesis, nutrient entrainment

312 Effects of circadian rhythm disruption during the dry period on tissue mobilization in multiparous dairy cattle. R. Klopp*[1], T. S. Steckler[1], A. Suarez-Trujillo[1], M. Grott[2], J. R. Townsend[3], K. Plaut[1], T. M. Casey[1], and J. P. Boerman[1], [1]*Department of Animal Sciences, Purdue University, West Lafayette, IN,* [2]*Animal Science Research and Education Center, Purdue University, West Lafayette, IN,* [3]*Department of Veterinary Clinical Sciences, Purdue University, West Lafayette, IN.*

During the transition period, dairy cattle go through intense physical and metabolic changes. The circadian timing system (CTS), functions to coordinate internal physiology including metabolic processes, therefore, disruption of the CTS, is associated with changes in metabolism. Our objective was to determine if disrupting the CTS from 35 d before expected calving (BEC) until calving affected muscle and adipose tissue mobilization in dairy cattle. In a completely randomized design, 32 multiparous cows were blocked by lactation number, disease incidence from previous lactation and assigned to 1 of 2 treatments, control (C) or phase-shift (PS). C were exposed to 16 h light:8 h dark. PS were exposed to the same amount of light and dark however, every 3 d there was a 6-h shift in the light-dark cycle. BW, BCS, back fat thickness, and muscle depth were measured on 35, 21, and 7 d BEC and 0, 10, 30 and 60 d postpartum (PP). Blood samples were collected on 35, 21, and 7 d BEC and 0, 2, 5, 9, 15, and 22 d PP and analyzed for NEFA, 3-methyl-histidine (3MH), and creatinine. Data were analyzed using the Mixed Procedure of SAS v.9.4 with the fixed effects of treatment, time point, and their interaction and the random effect of cow nested within treatment. There were no treatment differences for BW, BCS, or NEFA (all $P > 0.25$). There was a significant effect of time point on BCS, BW, and NEFA ($P < 0.0001$), with a mean loss of 97.6 kg of BW and 0.69 points of BCS from calving to 60 DIM. Cows mobilized 19.1% of their muscle depth and 50% of their subcutaneous body fat from 7 d BEC to 60 DIM. C cows had greater muscle depth ($P = 0.07$, 4.25 vs. 3.97 cm), also reflected in higher creatinine ($P < 0.0001$, 3,127 vs. 2,775 ng/mL), which is used to estimate muscle mass. However, 3MH, which is an indicator of muscle mobilization, was also greater ($P = 0.03$, 431 vs. 356 ng/mL). Disruption of the circadian rhythm during the dry period did not cause increased tissue mobilization and could potentially reduce the amount of muscle mobilization. Regardless of treatment, the extent of both muscle and adipose tissue mobilized during the transition period is substantial.

Key Words: tissue mobilization, multiparous, transition cow

313 Effect of fatty acid absorption on the daily rhythms of milk synthesis and plasma hormones and metabolites in dairy cows. I. Salfer* and K. Harvatine, *The Pennsylvania State University, University Park, PA.*

Dairy cows display daily rhythms of milk synthesis that are altered by the time of feed availability and appear to be driven by the circadian clock of the mammary gland. Fatty acids have been shown to entrain circadian rhythms in tissues such as the liver and adipose tissue in experimental models, but their role in the mammary gland is unknown. The objective was to determine the effects of the timing of fatty acid absorption on the daily rhythms of milk synthesis. Nine cannulated lactating Holstein cows (132 ± 90 d postpartum; mean ± SD) were randomly assigned to 1 of 3 treatment sequences in a 3 × 3 Latin square. Treatments were absomasal infusions (132 ± 90 d postpartum; mean ± SD) of 350 g/d of a high C18:1 oil either 24 h/d (CON) or for 8 h/d from 0900 to 1700 h (DAY) or from 2100 to 0500 (NGT). Treatment periods were 12 d with a 5 d washout. During the final 7 d of each period, cows were milked every 6 h to determine the daily patterns milk synthesis across the day. A 24-h rhythm was fit to time-course data using cosine analysis and the amplitude and acrophase (time at peak) were determined. Daily milk, fat, and protein yield and fat and protein concentration were not affected by treatment. Milk yield fit a 24 h rhythm in CON and DAY, and tended to fit a rhythm in NGT. The amplitude of the rhythm of milk yield was increased 29% in DAY compared with CON ($P < 0.05$), but NGT was not affected. Furthermore, DAY phase delayed daily rhythm of milk yield by 2 h compared with CON and NGT ($P < 0.05$). Fat and protein concentration exhibited a daily rhythms in CON and NGT ($P < 0.05$), but not DAY. The amplitude of the rhythm of fat concentration was increased 29% by NGT compared with CON. Fat yield tended to fit a 24 h rhythm in DAY ($P = 0.07$), but not CON or NGT. Moreover, protein yield fit a 24 h rhythm in all treatments and the amplitude of the rhythm was increased 30% by DAY and decreased 47% by NGT. Fatty acid infusion during the daytime modified the daily rhythms of milk synthesis by increasing the amplitude of milk yield and decreasing the amplitude of fat and protein concentration, whereas infusion at night had little effect.

Key Words: daily rhythm, milk synthesis, nutrient entrainment

314 Nitrogen metabolism and insulin signaling targets in adipose tissue of Holstein cows during the periparturient period differ by body condition score. Y. Liang*[1], E. Trevisi[2], and J. Loor[1], [1]*University of Illinois at Urbana-Champaign, Urbana, IL,* [2]*Università Cattolica del Sacro Cuore, Piacenza, Italy.*

The periparturient period is characterized by increased insulin resistance status in dairy cows. In non-ruminants, excessive fat deposition is associated with decreased insulin sensitivity and alterations in amino acid (AA) metabolism in adipose tissue. The hypothesis was that abundance of AA transporters, components of the urea cycle, and insulin signaling targets differs by prepartal body condition score (BCS) in dairy cows. Twenty 2 multiparous Holstein cows were divided by BCS before parturition (−30 d prepartum) into a BCS ≤ 3.25 (LoBCS, n = 11) or BCS ≥ 3.50 (HiBCS, n = 11) group. Adipose tissue obtained on d −15, 7, and 30 relative to calving date was used for RT-PCR analysis. Blood was obtained at −30, −15, 7, 10, and 30 d relative to calving date for energy balance biomarker analysis. The statistical model in SAS included the fixed effect of treatment, time and its interaction. There was no difference in prepartal DMI and milk yield between groups ($P > 0.05$). A treatment × time interaction was observed for postpartal DMI due to greater responses in LoBCS cows ($P < 0.05$). A treatment × time was observed for the plasma concentrations of fatty acids (NEFA) due to greater ($P < 0.05$) responses in HiBCS cows. Compared with LoBCS, HiBCS cows had greater ($P < 0.05$) plasma concentrations of glucose and hydroxybuyrate (BHBA). Among 6 AA transporters measured, HiBCS cows had greater overall ($P < 0.05$) mRNA abundance of high-affinity cationic *(SLC7A1)* and sodium-coupled AA transporters (*SLC38A1*). Abundance of arginino-succinate lyase (*ASL*) and arginase 1 (*ARG1*) ($P < 0.05$) also was greater overall in HiBCS cows, suggesting that adipose tissue in those cows had greater urea cycle activity. Abundance of protein kinase B1 (*AKT1*) was upregulated ($P < 0.05$) in HiBCS cows suggesting enhanced insulin signaling. Overall, the data indicate that BCS is associated with unique profiles of targets related to insulin sensitivity and nitrogen utilization in adipose tissue of dairy cows. The significance of these findings in the context of physiologic adaptations during the periparturient period merit further research.

Key Words: insulin resistance, amino acid transporter, transition cow

315 Plasma metabolome profiles in heifer calves at birth and during the preweaning period are altered by supply of methionine during late-pregnancy. A. Elolimy*[1,2], A. Alharthi[1,2], E. Abdel-Hamied[1,3], C. Parys[4], and J. Loor[1,2], [1]*Mammalian NutriPhysioGenomics, Department of Animal Sciences, University of Illinois, Urbana, IL* [2]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [3]*Animal Medicine Department, Beni-Suef University, Beni-Suef, Egypt,* [4]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

Maternal methionine supplementation in pregnant sows increases amino acid content (methionine, tryptophan, and valine) in plasma of piglets which likely helps support better growth performance during the preweaning period. However, the influence of maternal methionine in dairy cows on plasma metabolome of neonatal calves is unknown. The objective of the current study was to evaluate the impact of maternal methionine supply during late-pregnancy in dairy cows on plasma metabolome in neonatal calves, and its association with growth performance during the preweaning period. Heifer calves born to Holstein cows receiving either a control (CON) diet (n = 10) or CON plus rumen-protected methionine (MET; Evonik Nutrition & Care GmbH) (n = 10) during the last 28 d of pregnancy were selected. Plasma samples collected at d 0 (i.e., at birth before colostrum feeding), 2, and 42 of age were used for LC-MS untargeted metabolomics. Multivariate analysis of metabolome data was performed with MetaboAnalyst 4.0. No differences between groups were detected in daily starter intake ($P = 0.77$) during the preweaning period. However, MET heifers had greater body weight, hip height, wither height, body length, and average daily gain ($P < 0.05$). Plasma metabolomics revealed a clear separation between MET and CON heifers during the preweaning period. Among enriched metabolic pathways upregulated (≥2-fold) in MET heifers were several associated with amino acid metabolism (tryptophan, valine, tyrosine), thiamin metabolism, and peroxisomal oxidation. In contrast, downregulated pathways in MET calves (≥2-fold) were primarily associated with b-oxidation of fatty acid, TCA cycle flux and CoA synthesis. Overall, in the absence of differences in feed intake, the responses in calf plasma metabolome profiles as a result of enhanced maternal methionine supply suggest that those calves experienced alterations in metabolism. Whether the metabolome profiles reflect provision of extra nutrients and how those profiles are associated with greater body mass and growth performance remain to be establishe.

Key Words: methionine, calf, metabolomics

316 Effects of hindgut acidosis on metabolism, inflammation and production in dairy cows consuming a standard lactation diet. M. A. Abeyta*[1], E. A. Horst[1], E. J. Mayorga[1], B. M. Goetz[1], M. Al-Qaisi[1], C. S. McCarthy[1], M. R. O'Neil[1], B. C. Dooley[1], P. Piantoni[2], G. F. Schroeder[2], H. A. Ramirez-Ramirez[1], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Cargill Animal Nutrition Innovation Center, Elk River, MN.*

Impaired intestinal integrity caused by excessive hindgut starch fermentation is a potential source of peripheral inflammation in dairy cattle. Study objectives were to evaluate effects of hindgut acidosis in lactating dairy cows. Five rumen-cannulated Holstein (244 ± 49 DIM, 33 ± 3 kg milk/d) cows were enrolled in a study with 2 experimental periods (P) during which cows were fed a diet with 26% starch ad libitum. During P1 (4d), baseline data were collected. During P2 (7d), cows were abomasally infused with 4 kg/d of starch (1 kg pure corn starch + 1.25 L H2O/infusion at 0000, 0600, 1200, and 1800 h). Milk, feces, and blood samples were collected daily. Effects of time were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Compared with P1, starch infusion markedly reduced fecal pH (5.98 vs. 6.76; $P < 0.01$) and increased fecal starch content (3-fold; $P = 0.01$). Milk yield and DMI were reduced by starch infusion (4 and 5%, respectively; $P < 0.05$). Rectal temperature remained unchanged following infusions. Milk urea nitrogen decreased (10%; $P < 0.01$) relative to P1; however, starch infusions did not affect milk fat, protein or lactose content. Milk SCC increased 11-fold ($P = 0.05$) during P2. Circulating insulin and β-hydroxybutyrate increased (141 and 53%, respectively; $P < 0.01$), while plasma glucose tended to decrease (9%; $P = 0.06$) at 14h relative to the first infusion (RFI). Starch infusion increased blood urea nitrogen 2h RFI (22%) before it promptly decreased (12%; $P = 0.03$) for the remainder of P2. Additionally, P2 NEFA concentrations decreased (32%; $P < 0.01$) relative to P1. Monocytes decreased (38%; $P = 0.03$) at 2h RFI, but there were only minor increases in white blood cells, neutrophils, lymphocytes, eosinophils and basophils relative to P1 (10, 13, 7, 30, and 20%, respectively; $P < 0.05$). Circulating serum amyloid A and lipopolysaccharide binding protein concentrations were unaffected by starch infusion. Although abomasal starch infusion markedly reduced fecal pH and altered bioenergetics, it only had minor effects on metabolism, inflammation, and production in cows consuming a moderately high starch diet.

Key Words: gut permeability, gut health, endotoxin

317 Hepatic one-carbon metabolism, methionine cycle, and transsulfuration pathways are altered by prepartum adiposity and feed intake in peripartal grazing cows. M. Vailati-Riboni*[1], Y. Lian[1], D. Coleman[1], S. Meier[2], C. Burke[2], J. K. Kay[2], M. D. Mitchell[3], C. G. Walker[2], M. A. Crookenden[2], A. Heiser[4], J. R. Roche[2], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*DairyNZ, Hamilton, New Zealand,* [3]*University of Queensland, Queensland, Australia,* [4]*AgResearch, Palmerston North, New Zealand.*

Twenty 8 mid-lactation grazing dairy cows of mixed age and breed were randomly allocated to 1 of 4 treatment groups in a 2 × 2 factorial design: 2 prepartum body condition score (BCS) categories [4.0 (thin) and 5.0 (optimal); 10-point scale] and 2 levels of energy intake during the 3 wk preceding calving (75% and 125% of estimated requirements). Liver tissue was harvested by biopsy at −7, 7, and 28 d relative to calving. Samples were homogenized and used for ^{14}C assays to investigate betaine-homocysteine S-methyltransferase (BHMT), methionine synthase (MS), and cystathionine-β-synthase (CBS) activity. Metabolites relative concentration was analyzed using a targeted GC/MS profiling approach. Data were normalized with tissue protein concentration, and subjected to repeated measures ANOVA via PROC MIXED in SAS, with BCS, Feeding, and Time as fixed effects, and cow as random effect. All enzymes were affected by time, with BHMT activity peaking ($P < 0.05$) at 7 d, while CBS and MS activity decreased postpartum ($P < 0.05$). Overall, thin cows had greater ($P < 0.05$) MS activity, while cows fed 125% requirements had greater ($P < 0.05$) CBS activity. An interaction was detected for CBS activity, as thin cows fed 125% of requirements had greater ($P < 0.05$) overall activity. BCS 5 cows had overall greater betaine, glycine, acetylcholine, serine, and taurine concentrations. The same metabolites, plus choline, and N-N-dimethyl glycine were overall greater in liver of cows fed 75% of requirements. An interaction was detected for the aforementioned metabolites and also methionine, cystathionine, cysteinesulfinate, and hypotaurine due to overall greater levels in BCS 5 cows fed at 75% of requirements compared with other groups. Data indicate activity of enzymes involved in hepatic choline and methionine metabolism in pasture-based cows follow a different trend to those reported for high yielding cows in confinement. Furthermore, metabolomics underscores the need to restrict BCS 5 cows prepartum to ensure an optimal transition into lactation.

Key Words: BCS, prepartum nutrition, methionine cycle

318 Plasma branched-chain amino acids (BCAA) and mRNA abundance of 3 different BCAA transporters in adipose tissue, muscle and liver of dairy cows with high or normal body condition score. L. A. Webb*[1], H. Sadri[2], K. Schuh[1,3], S. Egert[4,5], P. Stehle[5], C. Koch[6], G. Dusel[3], and H. Sauerwein[1], [1]*Institute of Animal Science, Physiology and Hygiene Unit, University of Bonn, Bonn, Germany,* [2]*Department of Clinical Science, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran,* [3]*Department of Life Sciences and Engineering, Animal Nutrition and Hygiene Unit, University of Applied Sciences Bingen, Bingen, Germany,* [4]*Institute of Nutritional Medicine, University of Hohenheim, Stuttgart, Germany,* [5]*Department of Nutrition and Food Sciences, Nutritional Physiology, University of Bonn, Bonn, Germany,* [6]*Educational and Research Center for Animal Husbandry, Hofgut Neumühle, Münchweiler a.d. Alsenz, Germany.*

Branched-chain amino acid transporters (BCAAT) are crucial to sensing AA availability and regulating BCAA homeostasis, e.g., via interaction with the mechanistic target of rapamycin. Except for mammary gland and placenta, their abundance in metabolically active tissues in dairy cows is mostly unknown. Thus, we aimed to 1) compare the mRNA abundance of the most relevant BCAAT, i.e., solute carrier family 1 member 5 (*SLC1A5*), *SLC7A5*, and *SLC38A2* in muscle, liver and subcutaneous adipose tissue (AT) as well as the plasma BCAA profiles and 2) test whether high and normal BCS cows may differ in these variables. Thirty-eight multiparous Holstein cows, 15 wk ante partum (a.p.) were allocated to high (HBCS; BCS > 3.75) or normal BCS groups (NBCS; BCS < 3.5) receiving 7.2 or 6.8 MJ/kg DM, respectively, until dry-off to promote the difference. During the dry period and early lactation, cows were fed the same diets; differences in BCS ($P < 0.001$) were maintained. Blood and biopsies from *M.* semitendinosus, liver and AT (tail head) were sampled at d 49 a.p., 3, 21 and 84 postpartum (p.p.). Free BCAA were quantified by HPLC; mRNA abundance was assessed via qPCR. Data were analyzed as repeated measures with a Linear Mixed Model (SPSS). Concentrations of all BCAA changed with time ($P \leq 0.01$), most markedly in HBCS cows, with a nadir around calving. Neither individual nor total BCAA differed between groups. For Ile, a group × time interaction was found ($P = 0.03$): At d 3 p.p. HBCS cows tended to have lower Ile concentrations than NBCS cows ($P = 0.08$), vice versa at d 21 ($P = 0.02$). Abundance of BCAAT varied only with tissue and time ($P < 0.01$). The *SLC1A5* and *SLC7A5* mRNA were most abundant in AT; liver had 3 and 24% and muscle 11 and 37% of the AT values, respectively. In muscle, *SLC1A5* and *SLC7A5* mRNA peaked at d 21 p.p. Both AT and muscle had 4-fold greater *SLC38A2* abundance than liver, with irregular time courses. Our results indicate that AT could be a major site of BCAA uptake that is rather independent of BCS and time relative to calving in dairy cows.

Key Words: branched-chain AA (BCAA) transporters, tissue abundance, body condition

319 Abundance of hepatic patatin-like phospholipase domain-containing protein 3 protein was inversely related to peripartum hepatic triglyceride accumulation. R. S. Pralle*, H. T. Holdorf, C. R. Seely, R. Caputo Oliveira, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

The objectives of this study were to determine hepatic patatin-like phospholipase domain-containing protein 3 (PNPLA3) protein abundance peripartum and the relationship with hepatic triglyceride (TG) content. Multiparous cows were blocked by expected calving date and randomly assigned to a control (n = 13) or fatty liver induction (FLI; n = 12) treatment. Control cows were fed ad libitum peripartum, while FLI cows were offered a 6 kg cracked corn top-dress prepartum and feed restricted to 80% ad libitum intake at +14 d relative to calving (DRTC) until blood β-hydroxybutyrate ≥3.0 m*M*. Liver biopsies were collected at −28, −14, +1, +14, +28, +42, +56 DRTC. Quantification of hepatic PNPLA3 via semiquantitative Western blot was normalized to total sample protein. Statistical analysis was performed using the GLIMMIX procedure (SAS 9.4). Hepatic TG %DM and PNPLA3 abundance were $\log_{10}$ transformed. Models included fixed effects of treatment, DRTC, and treatment × DRTC, as well as random intercepts of cow, block, and repeated measures of cow across DRTC. The PNPLA3 model included the −28 DRTC abundance as a covariate. Hepatic PNPLA3 abundance was also regressed against hepatic TG. All FLI and 2 control cows had blood β-hydroxybutyrate ≥3.0 m*M*. Hepatic TG content did not differ between treatments ($P = 0.50$) but differed over time ($P < 0.001$). Prepartum hepatic TG was lower than postpartum; the postpartum maximum and nadir TG were at +14 and +56 DRTC ($P < 0.05$), respectively. No treatment difference was detected for hepatic PNPLA3 abundance ($P = 0.31$). A time effect was observed ($P < 0.001$) with increasing PNPLA3 from −14 to +56 DRTC. The greatest PNPLA3 abundance was observed during the postpartum decrease of hepatic TG. Hepatic PNPLA3 had an inverse relationship ($\beta = -0.31$; $P = 0.03$) when regressed against hepatic TG content. These results suggest hepatic PNPLA3 protein abundance was not altered by FLI but changed over time inversely compared with postpartum hepatic TG. Furthermore, regression analysis suggests that incremental increases in hepatic PNPLA3 may lessen TG accumulation peripartum.

Key Words: fatty liver, lipase, transition cow

Production, Management, and the Environment Symposium: Advancing Artificial Intelligence on Dairy Farms

320 Automated collection and processing of data in livestock farms. J. Koltes*, *Iowa State University, Ames, IA.*

Smart livestock farming will enable real-time animal management to maximize health and efficiency. The dairy industry has been generating "big data" for many years for use in genetic and management decision making. Thus, a large existing data chain exists to implement smart farming, which is being augmented by new automated, high-throughput data collection tools (e.g., sensors, milking systems, images). Automated data collection and processing cyberinfrastructure will be central in facilitating real-time predictive analytics to provide actionable tasks for producers. Challenges in creating these systems include: network connectivity, sensor range, data dimensionality, data ownership/ privacy concerns, and development of effective predictive analytic cyberinfrastructure. Processing (i.e., cleaning, denoising, normalizing) should ideally allow rapid data mining, analytics and integration. Given the high value of data, preservation (i.e., backup) will be critical to prevent information loss. Capturing provenance (e.g., edits/ updates) and metadata will be important for downstream analytics. Livestock data has a broad array of uses from on-farm decision making to research, thus a broad range of animal and computational scientists should be involved in developing informatics systems. In principle, all data would be FAIR (Findable, Accessible, Interoperable, and Reusable) to encourage new research and expanded application. Protecting data ownership and maintaining privacy is an important consideration as downstream processing will likely require centralized cloud computing resources. Development of databases and software that facilitate machine learning, artificial intelligence or other prediction methods would help in improving computational efficiency and reduce wait time for analytic information. Thoughtful development of cyber-physical systems for smart farming could facilitate a new era of big data animal science. For producers, affordability, accuracy and sensitivity of analytic tools will determine if efforts to create automated data collection and processing systems will be fruitful.

Key Words: cyberinfrastructure, precision livestock farming, sensors

321 Use of big data to monitor herd health. H. White*, *University of Wisconsin Madison, Madison, WI.*

The ability of early detection and treatment to ameliorate negative outcomes associated with health indicies have emphasized the value of early detection and treatment. Although this is recognized, the labor-intensiveness and costs associated with monitoring herd health are cumbersome for many farms. Increasing data streams available to farms presents opportunities to use data to better monitor cow and herd health. Within the transition to lactation period, data are presented in the form of milk production and composition, fourier-transform infrared spectroscopy (FTIR) analysis, cow management records, and genomics: all components are employed to monitor postpartum onset of hyperketonemia (HYK). First attempts to predict postpartum HYK from test-day milk and performance variables incorporated into multiple linear regression models had sufficient accuracy to monitor monthly herd prevalence; however, the sensitivity (SEN) and specificity (SPC) lacked for individual cow diagnostics. Subsequent artificial neural network prediction models employing FTIR data and milk composition variables achieved 83 and 81% SEN and SPC. While this fails to reach the diagnostic goals of 90%, it is achieved without individual cow blood samples, which may justify acceptance of lower performance. The caveat is that these models depend on milk analysis which is traditionally done every 4 weeks, only catching half of the fresh cows once. Benefits to farms are greatly improved if postpartum cows are tested weekly. This also allows for close monitoring of SCC and may open the door for other herd health monitoring tools to be employed. Future improvements in these models may be achievable by tailoring models to reflect risk factors for each farm or by maximizing SEN at the expense of SPC, especially in HYK and other disorders when the cost of treatment is less than mistreating. Incorporation of genome-wide association studies identifying SNPs associated with risk for metabolic disorders may allow for at-risk cows to be identified and flagged with lower thresholds on prediction models. Alignment of data sources, and integration of existing models, may provide valuable real-time management tools to producers.

Key Words: ketosis, hyperketonemia, transition cow

322 Real-time continuous decision-making using big data. V. Cabrera*, J. Barrientos, L. Fadul, and H. Delgado, *University of Wisconsin-Madison, Madison, WI.*

We are developing a real-time, data-integrated, data-driven, continuous decision-making engine, a Dairy Brain. We illustrate the concept with 3 practical applications. (1) Precision feeding: all lactating cows (N~1,898 ± 115) in a farm are housed in 14 same-sized pens and fed 7 diets. Cows are allocated to each pen only according to DIM and lactation number. Every week employees move cows in and out of pens (N~256) following lists manager generates from printouts of disjointed data. A continuous, more systematic cows' allocation to pens according to nutritional requirements is possible by automatically aggregating data streams from management, feed, DHI, and parlor records. Algorithms can provide more precise diets to more homogeneous clusters of cows allocated to pens. Preliminary results indicate the farm could increase net margin by $67,834/yr when providing pen-based more precise diets. (2) Early risk of clinical mastitis (CM): first-lactation cows under risk of developing CM can be identified by analyzing integrated data from their genomic traits scores such as Genomic Total Performance Index (GTPI), management, and laboratory results. The risk can be reassessed at every DHI report or GTPI score variation. This integrated continuous algorithm allows to identify high-risk CM cows even when their SCC is low (<200,000 cells/mL); for example, cows with GTPI > 2,000 report the lowest SCC during the entire lactation. However, animals within this category and SCC > 115,000 cells/mL should be carefully monitored because they might already be infected. (3) Predicting CM: Cows at risk of CM show higher milk conductivity and milk production than healthy cows. Continuous, integrated data could be used to permanently predict the onset of CM. The difference on milk production and conductivity between milkings analyzed with gradient boosting machine learning algorithms can predict CM 5 milkings earlier with a sensitivity of 0.82, a specificity of 0.58, and a prediction accuracy of 0.81. Farmers can count with a monitoring tool to flag cows at risk of contracting CM and follow up them closely in a preventive way. More data for training the machine learning algorithms and integration of other data streams such as genetics, sensors, and diet changes, will improve the prediction accuracy.

Key Words: decision support tools, simulation, optimization

323 Optimized decisions using big data analytics in dairy farms. M. Ferris*, A. Christensen, and S. Wangen, *University of Wisconsin, Madison, WI.*

Management decisions can be informed by real-time data streams to not only improve the economics of the farm but also positively benefit the overall health of a dairy herd or the larger environment. Decision support tools can provide data management services and analytics to exploit data streams from farm and other economic, health and agricultural sources. We will describe a decision support tool that couples an optimization model to underlying cow, herd and economic data with an easy to interpret user interface. This interface allows the user to operate the optimization model and understand the impacts of different decisions. Specific examples related to ketosis and to culling will be described, along with some discussion of security of information and control of uncertainties in mathematical modeling approaches. The proposed analytical capabilities will utilize several tools from the data mining, simulation, machine learning, and optimization disciplines. Broadly, these tools will form the basis of the Dairy Brain decision support system, which will provide management suggestions to farmers that might affect a single animal or they could broadly affect a farm's overall business portfolio (i.e., large capital expenditures, outsourcing opportunities, and interactions with entities that have regulatory control). All of the analytical tools are operationalized in a way that allows them to accept near real-time data updates from the farm to our agricultural data hub that gathers and disseminates multiple data streams relevant to dairy operations.

Key Words: optimization, data science, decision support

Reproduction 2

324 Interaction of estrous expression and progesterone on the impact of GnRH administration at the time of AI on pregnancy and ovulation rates.

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Cows with reduced estrous expression have compromised fertility. This study aimed to determine if the administration of GnRH at AI affects ovulation, progesterone and fertility of cows expressing low estrous behavior. Cows were enrolled at estrus from 3 farms (n = 1629 AI events; Farm A: 757, Farm B: 305, Farm C: 567) and randomly assigned to receive GnRH at AI. Estrous expression was monitored by leg-mounted activity monitors and quantified as the maximum activity occurring at estrus; using the farm median, estrous expression was categorized as high or low. On Farm C, cows were assessed at alert and 7d post alert for ovulation (n = 707) using ultrasonography. On Farm B, ovulation was assessed using plasma samples analyzed for progesterone (P4). A subset of animals were assessed for P4 at estrus and at 7d (n = 526) and grouped using the median. Pregnancy/AI (P/AI) was confirmed at 31 ± 3d post-AI. Outcome variables were tested using GLIMMIX of SAS with estrus as the experimental unit and cow within farm as a random effect. An interaction of treatment and estrous expression on P/AI was found. Without GnRH, cows with higher estrous expression had greater P/AI than those with lower expression, whereas GnRH administration increased P/AI for cows with low estrous expression but not those with high expression (GnRH-High: 46.8 ± 2.5, GnRH-Low: 40.0 ± 2.7; No GnRH-High: 42.3 ± 2.5, No GnRH-Low: 30.8 ± 2.7%; $P < 0.01$). Cows with high expression had greater P4 7d post-alert ($P = 0.04$), but not at estrus. There was an interaction of treatment with P4 at alert on P/AI, cows with lower P4 receiving GnRH had greater P/AI than all other groups (GnRH-Low P4: 58.0 ± 4.5, GnRH-High P4: 39.4 ± 4.5; No GnRH-Low P4: 39.9 ± 4.7, No GnRH-High P4: 40.0 ± 4.7%; $P = 0.01$); this was consistent by estrous expression. Ovulation was not impacted by P4 at estrus but there was an interaction of estrous expression and treatment, where cows with high estrous expression receiving GnRH had greater ovulation rates than all other groups (GnRH-High: 92.0 ± 1.8, GnRH-Low: 85.1 ± 2.0; No GnRH-High: 87.6 ± 1.9, No GnRH-Low: 87.6 ± 2.0%; $P = 0.05$). In conclusion, fertility of cows with low estrous expression may be increased using GnRH at the time of AI; however, the mechanism behind this relationship is unclear.

Key Words: estrus, GnRH

325 Efficacy of intravaginal administration of prostaglandin $F_{2\alpha}$ for induction of luteal regression in lactating dairy cows.

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Our objectives were to test the efficacy of intravaginal (IVG) administration of $PGF_{2\alpha}$ (PGF) to induce corpus luteum (CL) regression, compare circulating progesterone (P4) profiles in cows receiving IVG versus intramuscular treatment with PGF, and evaluate reproductive outcomes. Lactating Holstein cows were synchronized using a Double-Ovsynch (DO) protocol (GnRH-7d-PGF-3d-GnRH-7d-GnRH-7d-PGF-56h-GnRH-16h-AI) to receive timed AI (TAI) at 67 ± 3 DIM. Seven days after the first GnRH treatment (time 0), cows with at least one visible CL ≥ 15 mm were blocked by parity and randomly assigned to a control (CON; n = 201) or treatment group (TRT; n = 201). Cows in CON received 25 mg of PGF (Dinoprost tromethamine) intramuscular. Cows in TRT received 2 25 mg doses of PGF 12 h apart delivered through a catheter in the cranial portion of the vagina. Blood samples were collected at 0, 12, 48 and 72 h after treatment. Ovulation to the first GnRH of DO was determined through transrectal ultrasonography. Only cows with P4 ≥ 1 ng/mL (functional CL) at time 0 (CON = 169; TRT = 179) were included in the analyses. Binomial and quantitative data were analyzed by logistic regression and ANOVA with repeated measures, respectively. Concentrations of P4 and the proportion of cows with a new CL at time 0 did not differ ($P > 0.10$). Overall, the proportion of cows with CL regression using 1 ng/mL ($P = 0.48$; CON = 87.0%, TRT = 84.4%) or 0.5 ng/mL ($P = 0.98$; CON = 81.1%, TRT = 81.0%) of P4 as cutoff did not differ. Concentrations of P4 were affected by treatment ($P = 0.01$), time ($P < 0.001$) and the treatment by time interaction ($P < 0.001$). Cows in TRT had greater ($P < 0.001$) mean P4 at 12 h than cows in CON. Mean P4 did not differ ($P > 0.10$) at 48 or 72 h after treatment. The proportion of cows with estrus recorded within 3 d of treatment ($P > 0.10$; CON = 47.0%; TRT = 51.1%), ovulation risk after treatment (CON = 88.0%; TRT = 84.1%) and pregnancy per AI after TAI (CON = 49.7%; TRT = 56.3%) did not differ. We concluded that 2 intravaginal doses of 25 mg of PGF 12 h apart were as effective as a single 25 mg intramuscular dose of PGF for inducing luteal regression in lactating dairy cattle. Funded by USDA-NIFA under NYC1007421 and NYC 1014955.

Key Words: intravaginal, luteal regression, dairy cow

326 Effects of GnRH and hCG administration during early diestrus on estrous cycle length, expression of estrus, and fertility in lactating dairy cows.

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Our objective was to determine the effects of a single treatment of human chorionic gonadotropin (hCG) or GnRH during early diestrus on estrous cycle length, expression of estrus and fertility in lactating dairy cows. Lactating Holstein (n = 365) and Jersey (n = 212) cows detected in estrus by an automated activity monitoring (AAM) system between 27 and 50 DIM were randomly assigned to receive 1 of 3 treatments d 5 to 7 of the estrous cycle: hCG (3.300 IU i.m.; n = 200), GnRH (86 µg gonadorelin i.m.; n = 195), or control (no treatment; CON; n = 182). Ovaries were scanned with ultrasound in a subgroup of cows (n = 236) on the day of treatment and 3–4 d later to determine ovulation. Estrus was detected following treatment by an AAM during 32 d after pre-treatment estrus, and peak activity index was recorded. A random subgroup of cows observed in estrus received first AI 51 to 80 DIM (n = 342). Pregnancy diagnoses were performed by ultrasound at 37 ± 3 d post-AI. Data were analyzed by ANOVA and logistic regression using proc MIXED and GLIMMIX of SAS. The model included fixed effects of treatment, parity and the interaction of treatment × parity. Cows treated with hCG had higher ($P < 0.05$) ovulation rate than GnRH (85 vs. 73%). The proportion of primiparous cows returning to estrus was greater ($P < 0.05$) for hCG (93%) and tended to be greater ($P = 0.06$) for GnRH (93%) than CON (83%). However, hCG (73%) and GnRH (76%) decreased ($P < 0.05$) the percentage of multiparous cows returning to estrus than CON (87%). Cows treated with hCG had longer ($P < 0.01$) estrous cycle length (23.9 d) than CON (22.2 d) and GnRH (22.2

d). Percentage of cows with high peak activity (index ≥ 80) did not differ between groups (CON: 93.4%, GnRH: 96.2%, and hCG: 93.7%). Primiparous cows pregnancy per AI (P/AI) was greater ($P = 0.05$) and tended ($P = 0.09$) to be greater for hCG (53.1%) than CON (33.3%) and GnRH (36.0%), respectively. Treatment did not affect ($P > 0.10$) P/AI in multiparous cows (CON: 38.5%; GnRH: 38.1%; hCG: 32.8%). In summary, inducing the formation of an accessory corpus luteum on d 5 to 7 of the estrous cycle with hCG increased the likelihood of primiparous cows exhibiting estrus within 32 d and fertility of primiparous cows bred following estrus compared with controls.

Key Words: human chorionic gonadotropin (hCG), estrus, fertility

327 Effect of progesterone before an Ovsynch-CIDR protocol on fertility in dairy cows with low body condition score. D. Scandolo[1], P. Melendez*[2], M. Bilbao[3], S. Perez Wallace[4], and J. Bartolome[3], [1]*Faculty of Veterinary Sciences, National University of Litoral, Santa Fe, Argentina,* [2]*College of Veterinary Medicine, University of Georgia, Tifton, GA,* [3]*Faculty of Veterinary Sciences, National University of La Pampa, La Pampa, Argentina,* [4]*Zoetis, Argentina, Buenos Aires, Argentina.*

The aim was to assess the effect of progesterone before an Ovsynch-Controlled intravaginal drug releasing device (CIDR; 1.9 g P4) protocol on fertility in dairy cows with low body condition score (BCS; ≤ 2.75; scale 1–5). Lactating Holstein cows (n = 299) were assigned at the beginning of presynch to receive a 1.9 g CIDR on d −12 (Progesterone treatment, n = 148) or no treatment (Control, n = 151). On d −3, CIDR was removed and both treatments received 2 doses of 25 mg of $PGF_{2\alpha}$ (5 mL, i.m., Lutalyse) 12 h apart. At Day 0 (45 ± 3 DIM) all cows were enrolled in a synchronization protocol and treated with 10 µg of GnRH analog (2.5 mL, i.m., Receptal) and 1.9 g CIDR. On d 7, CIDR was removed and cows received 2 doses of 25 mg of PGF2α 12 h apart. Cows received a second dose of GnRH analog 56 h after first dose of PGF_{2a} and were TAI 16 h later (d 10). On d −12, parity, BCS, milk yield, DIM and stage of the estrous cycle were recorded. Ultrasonography of the ovaries was conducted on d −12 to determine the presence of a corpus luteum (CL). Pregnancy at AI (PAI) was diagnosed by ultrasonography at 32 d post AI. Logistic regression was used to evaluate the effect of treatment, parity, and presence of CL at d −12 and 2-way interactions on pregnancy. There was no difference ($P = 0.18$) in PAI between the progesterone treatment (33.1%) and control (30.5%). No interaction between treatment and parity was detected ($P = 0.65$). However, progesterone treatment before the CIDR-Ovsynch protocol increased ($P = 0.04$) PAI in cows without a CL on d −12 (32.2% vs. 18.7%) but not in cows with a CL (34.5% vs. 42.1%).

Key Words: BCS, progesterone, fertility

328 Impact of assisted reproduction techniques on subsequent reproductive performance of dairy heifers and cows. C. C. Figueiredo*, D. Z. Bisinotto, G. V. R. Brandão, S. G. Umana Sedo, and R. S. Bisinotto, *Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL USA.*

Use of assisted reproduction techniques (ART) such as ovum pickup and in vitro fertilization (IVF) and multiple ovulation for embryo transfer (ET) has increased in beef and dairy herds. Objectives of this retrospective cohort study were to evaluate the impact of ART on reproductive performance of dairy donors. Donor heifers (n = 683; 440 IVF and 243 ET) from a single herd in north Florida were enrolled with ART procedures were performed from January 2002 to July 2017. Up to 3 non-donors were paired to each donor based on birth date. Logistic regression was used to evaluate risk of culling. Cox proportional hazards regression models were used to evaluate age and DIM at first breeding, time to pregnancy, age at first calving in heifers and first lactation cows. Statistical models included the fixed effects of ART type, birth year, season of breeding and first calving, and 2-way interactions. Correction for multiple comparisons was performed using method of Tukey. Information on genomic predicted transmitting ability for production and fertility traits was not accounted by statistical models due to lack of original genomic predictions for older animals. First service hazard was smaller for ET heifers compared with non-donors (AHR = 0.59; 95% CI = 0.52–0.67) whereas no difference was observed between non-donors and IVF heifers. Age at first calving was 11 d and 9 d longer for IVF (AHR = 0.86; 95% CI = 0.77–0.95) and ET heifers (AHR = 0.83; 95% CI = 0.72–0.95) compared with non-donors. Risk of culling before first calving was smaller ($P < 0.001$) for heifers that underwent ART procedures compared with non-donors (IVF = 0.2; ET = 0.4; non-donors = 2.1%). First lactation cows that were ET donors as heifers had increased hazard of pregnancy ($P = 0.05$) compared with non-donors (AHR = 1.21; 95% CI = 1.00–1.46) whereas no difference was observed between IVF and non-donor heifers during their first lactation (AHR = 0.85; 95% CI = 0.70–1.03; $P = 0.11$). Use of ART in dairy heifers before first calving did not negatively affect the risk of culling or time to pregnancy during first lactation.

Key Words: uterine flushing, follicular aspiration, assisted reproduction

329 Lipidomics of uterine fluid in lactating cows during diestrus. E. Ticiani[1,2], J. F. W. Spricigo*[1], M. R. Carvalho[1], A. Moore[1], M. Bertolini[2], and E. S. Ribeiro[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Animal Sciences Research Program, Federal University of Rio Grande do Sul, Porto Alegre, RS, Brazil.*

Our objectives were to investigate the concentration and composition of fatty acids (FA) and oxylipids (OxL) in the uterine fluid (UF) of lactating cows at early, mid, and late diestrus. After synchronization of the estrous cycle, cows (n = 30) were blocked by parity and assigned randomly to receive transcervical uterine flushing on d 5 (UFD5), 10 (UFD10) or 15 (UFD15) of the estrous cycle. Flushing was performed by infusion of 30 mL of PBS into the uterine horn ipsilateral to the corpus luteum. The recovered flushing was centrifuged and stored at −80°C. Samples in which the recovered volume was >15 mL without visible blood contamination (8 samples/group) were used for analyses of FA and OxL composition by LC-MS/MS. Internal standards were used to estimate the concentration of specific lipids. Data were analyzed by ANOVA using the PROC GLIMMIX of SAS. Statistical models included the effects of day, parity, and their interaction. A total of 29 FA and 63 OXL were identified. Concentration of total FA and total OxL tended ($P < 0.10$) to be greater in UFD15 (1.7 ng/mL and 62 pg/mL, respectively) than in UFD5 (0.9 ng/mL and 31 pg/mL) and UFD10 (0.8 ng/mL and 26 pg/mL). In addition, concentration of polyunsaturated FA (PUFA) and monounsaturated FA (MUFA) were greater ($P < 0.05$) in UFD15 than in UFD5 and UFD10. Concentration of SFA and the proportion of PUFA, MUFA, and SFA were similar between groups. Concentration of n-6 FA was greater ($P < 0.05$) and concentration of n-3 FA tended to be greater ($P < 0.09$) in UFD15 than in UFD5 and UFD10. The ratio n-6:n-3 did not differ between groups and averaged 3.7. Concentration of series-2 prostaglandins (S2PG), DGLA-OxL, and n-3-OxL were all greater ($P < 0.05$) in UFD15 than in UFD5 and UFD10. Proportionally, HODE OxL was reduced ($P < 0.05$) while S2PG, DGLA-OxL, and n-3-OxL were increased ($P < 0.05$) in UFD15 compared with UFD5, which were both similar to UFD10. In conclusion, multiple FA and OxL accumulate in the uterine lumen during diestrus, changing the abundance and profile of lipids in the UF. These changes are likely caused by rising concentrations of progesterone during diestrus and are relevant for early conceptus development and survival.

Key Words: uterus, fatty acids, oxylipids

330 Development of a research model to investigate pregnancy-derived endocrine signals in the ovary during maternal recognition of pregnancy in heifers.

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Our objectives were to investigate the potential presence and nature of pregnancy-derived endocrine signals in the ovaries during maternal recognition of pregnancy (MRP) in heifers. Pubertal heifers (n = 66) had the estrous cycle and ovulation synchronized with 5-d CIDR Cosynch protocol. On the d of the last GnRH (d 0), 3 in each 5 heifers were selected randomly to be inseminated (AI), while the remaining formed a nonbred nonpregnant group (NP). On d 11, all follicles ≥4 mm were ablated using ovum pickup (OPU) equipment to induce recruitment of new follicles. On d 16.5, presence and size of ovarian structures were evaluated. Heifers with a corpus luteum (CL) >18 mm had the dominant follicle (DF) aspirated by OPU. The aspirated DF was classified as ipsilateral (IP) or contralateral (CO) to the ovary bearing the CL. After centrifugation of the recovered follicular fluid (FF), supernatant and granulosa cells (GC) pellet were stored separately at −80°C for analyses of FF composition and GC gene expression. On d 28, pregnant (P) heifers in the AI group were identified by ultrasonography, and those with a negative diagnosis were excluded. The experimental design was a 2 × 2 factorial based on pregnancy status (P or NP) and DF (IP or CO) on d 16.5. Data were analyzed by ANOVA using the GLIMMIX procedure of SAS, and included the effects of the main factors and their interaction. The number of heifers retained for analyses in each factorial combination were PIP = 12, NPIP = 14, PCO = 11, and NPCO = 12. The CL diameter tended ($P = 0.06$) to be greater in P than NP (22.8 vs. 21.5 mm). The DF diameter and volume were similar between groups and averaged 12.3 mm and 987 µL. The proportion of heifers with successful FF collection and the volume recovered were similar between groups and averaged 84% and 897 µL. Relative gene expression of *ISG15* in GC was 3.4-fold greater ($P = 0.03$) in P than NP. This difference was mainly explained by the higher expression in PIP (NPIP = 1.0; NPCO = 1.6; PCO = 3.2; PIP = 5.5), suggesting a local communication involving IFNT signaling between P uterine horn and ovary during MRP. Proteomics and lipidomics of FF will determine the presence and nature of specific endocrine signals in the ovary.

Key Words: maternal recognition of pregnancy (MRP), pregnancy, ovary

331 Galectin gene expression in the elongating bovine conceptus.

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Pregnancy loss in cattle is greatest in the first 30 d of gestation. Near Day 15 of gestation, bovine conceptuses will elongate and express galectin-1 (LGALS1), an immunosuppressive glycan binding protein believed to be essential for implantation. It is unclear, however, if other members of the bovine galectin family, including *LGALS3*, *LGALS4*, *LGALS7*, *LGALS8*, *LGALS9* and a galectin 13-like gene (*LGALS13L*), are also expressed by the elongating conceptus. Therefore, galectin expression in elongating, Day 16, bovine conceptuses was examined by RT-qPCR. Considering galectins may also be expressed in other ruminant conceptuses, galectin expression was also measured in elongating ovine conceptuses. Angus heifer and Texel ewe uteri were collected 16 and 14 d after insemination, respectively, and flushed with PBS to collect elongating bovine (n = 4) and ovine (n = 3) conceptuses. The conceptuses were individually snap frozen and stored at −80°C until RT-qPCR. Bovine and ovine conceptus gene expression data were normalized to control genes *GAPDH* and *YWHAZ*. A general linear model (GLM) in SAS was used to test for differences between log-transformed relative expression data. The α-level was set at 0.05 and a Tukey's test was included in the analysis. Data are presented as non-transformed means ± standard deviation of the mean. In bovine conceptuses, expression of *LGALS3* (24.47 ± 6.41) and *LGALS1* (21.80 ± 2.07) was similar yet greater than all other galectins measured ($\alpha = 0.05$). Expression of *LGALS8* (1.73 ± 0.27) was 12-fold less than *LGALS1*; however, *LGALS8* expression was greater than *LGALS9* (0.77 ± 0.77), *LGALS13L* (0.30 ± 0.10), *LGALS4* (0.08 ± 0.01) and *LGALS7* (0.02 ± 0.01) ($\alpha = 0.05$). In sheep conceptuses, expression of *LGALS3* (4.30 ± 0.78), *LGALS1* (1.90 ± 0.25) and *LGALS8* (1.68 ± 0.25) was similar. Other than *LGALS9*, which was undetectable, *LGALS4* (0.20 ± 0.10) had the lowest level of expression of all galectins measured ($\alpha = 0.05$). In conclusion, elongating bovine and ovine conceptuses express various members of the galectin gene family. The galectins may have important functions during early pregnancy in ruminants.

Key Words: embryonic loss, implantation, elongation

Ruminant Nutrition 3: Vitamins and Minerals

332 Dietary starch level alters blood indicators of copper status in lactating Holstein-Friesian dairy cows when fed without or with additional sulfur and molybdenum. J. H. McCaughern*, A. M. Mackenzie, and L. A. Sinclair, *Harper Adams University, Newport, UK.*

The objective of our study was to assess the effect of dietary starch level on reticulorumen pH and determine the subsequent effects on blood indicators of copper (Cu) status when fed without or with additional sulfur (S) and molybdenum (Mo). Sixty Holstein-Friesian dairy cows (33 ± 2.5 DIM) yielding 41 (±0.9) kg/d were blocked and randomly allocated to 1 of 4 dietary treatments in a 2 × 2 factorial design experiment for a duration of 98 d. The treatment diets contained 15 mg Cu/kg DM, were isonitrogenous and isoenergetic, and had a grass silage-to-corn silage ratio of 1:1 on a DM basis. Dietary starch levels were 225 g/kg DM (HS) or 148 g/kg DM (LS), and were either unsupplemented (−) or supplemented (+) with 1.2 g of S/kg DM and 4.1 mg Mo/kg DM. Reticulorumen pH was measured using pH boluses inserted in 6 cows per treatment, and blood samples were collected at wk 0, 1, 2, 4, 6, 10 and 14 of the study. Performance and blood parameters were analyzed as a 2 × 2 factorial design using a repeated measures ANOVA, with main effects of starch level (S), Cu antagonists (A) and their interaction (S × A) using Genstat (v18). Mean reticular pH was 0.2 higher in cows receiving LS diets. The addition of dietary antagonists had no effect ($P > 0.05$) on milk yield but reduced ($P < 0.001$) DMI by 1.9 kg/d. We observed no effect ($P > 0.05$) of dietary treatment on plasma Cu, iron, or zinc with mean values of 15.3, 42.1, and 14.4 µmol/L respectively. In contrast, we found that ceruloplasmin activity was 3.9 mg/dL higher ($P < 0.05$) in cows fed HS diets, and there was an interaction ($P < 0.05$) on plasma Mo, whereby cows fed additional S and Mo had a 0.25 µmol/L higher concentration in cows fed HS+ compared with LS+. Our results demonstrate altered Cu-antagonist metabolism resulting from feeding different starch levels that altered rumen pH, and highlight the need to take into consideration the pH modulatory potential of diets when selecting appropriate Cu supplementation strategies for lactating dairy cows.

Key Words: copper, dairy cow

Table 1 (Abstr. 327). Pregnant cows, adjusted odds ratio (AOR), and 95% CI for treatments adjusted by parity, CL at d −12 and interactions

Variable	Pregnancy per AI, % (no/no.)	AOR	95% CI	*P*-value
Treatment				0.18
Control	30.5 (46/151)	—	—	
Progesterone	33.1 (49/148)	1.84	0.75–4.50	
Parity				0.82
Primiparous	33.3 (41/123)	—	—	
Multiparous	30.7 (54/176)	0.92	0.45–1.87	
CL at d −12				0.75
No	26.1 (43/165)	—	—	
Yes	38.8 (52/134)	1.12	0.55–2.28	
CL at d −12 by treatment				0.04
No/Control	18.7 (14/75)	—	—	
No/Progesterone	32.2 (29/90)	2.07	0.99–4.29	
Yes/Control	42.1 (32/76)	3.16	1.51–6.62	
Yes/Progesterone	34.5 (20/58)	2.29	1.03–5.07	

333 Effects of zinc hydroxychloride on production and metabolism during 5 d of feed restriction. E. A. Horst*[1], E. J. Mayorga[1], M. Al-Qaisi[1], S. Rodriguez-Jimenez[1], B. M. Goetz[1], M. A. Abeyta[1], R. S. Fry[2], S. K. Kvidera[2], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Micronutrients LLC, Indianapolis, IN.*

Objectives were to evaluate effects of supplemental zinc hydroxychloride (HYD; Micronutrients, Indianapolis, IN) on production parameters and metabolism during feed restriction (FR) in midlactation Holstein cows. Twenty-four cows were randomly assigned to 1 of 4 treatments: 1) ad libitum-fed and control diet (ALCON; 75 ppm zinc [Zn] from Zn sulfate; n = 6), 2) ad libitum-fed and HYD diet (ALHYD; 75 ppm Zn from HYD; n = 6), 3) 40% of ad libitum feed intake and control diet (AL40CON; n = 6), or 4) 40% of ad libitum feed intake and HYD diet (AL40HYD; n = 6). Before study initiation, cows were fed their respective diets for 21d. The trial consisted of 2 experimental periods (P) during which cows continued to receive their respective dietary treatments. Period 1 (5d) served as the baseline for P2 (5d), during which cows were fed ad libitum or restricted to 40% of P1 feed intake. Effects of treatment, day, and their interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Feed restriction decreased body weight (45 kg) and markedly reduced EBAL compared with controls (3.01 vs. −14.42 Mcal/d, respectively; $P < 0.01$). DMI of AL40 cows decreased (60%) during P2 relative to AL cows ($P < 0.01$). Milk yield in AL40 cows progressively decreased throughout the 5 d of FR relative to ad libitum-fed cows (30%; $P < 0.01$). Milk yield and DMI tended to be increased in HYD supplemented cows across both P1 and P2 (12 and 7% relative to CON, respectively; $P \leq 0.13$). There was limited treatment effect on milk composition. Relative to AL cows, FR cows had decreased circulating glucose, insulin, and glucagon (10, 68, and 31%, respectively; $P \leq 0.05$). FR increased circulating NEFA relative to AL cows (5-fold; $P < 0.01$). BHB concentrations were unaffected by FR, but were increased in HYD supplemented cows relative to CON (33%; $P = 0.06$). Circulating BUN initially increased then gradually decreased in AL40 cows during P2, while concentrations remained unaffected in AL cows ($P < 0.01$). In summary, HYD supplementation tended to increase DMI and milk yield during both periods, tended to increase BHB during P2, but did not influence the other parameters during an intense FR.

Key Words: feed restriction, zinc

334 The effect of supplementing dairy cows with vitamins based on the BASF annual industry vitamin supplementation survey on productive and reproductive parameters. M. Coelho*, R. Jones, and F. Parks, *BASF Corp., Florham Park, NJ.*

As the dairy industry strives for efficiency, the cell metabolic rate increases, and the neutrophil function decreases. In the last 15 years, there has been a 28% increase in milk production (33.9 to 39.5 kg/cow/d), a 12% decline in total-tract feed passage rate (9.72 to 8.52 h), and a 1% decline in DM digestibility (66.02 to 65.12). A study was conducted to determine the response of dairy cows to different levels of vitamin supplementation based on the 2018 BASF Industry vitamin supplementation survey (Coelho, 2018). A total of 835 Holstein cows were used in a randomized complete block design with cow as the experimental unit, treatment as the fixed effect, and block as the random effect (167 cows × 5 treatments). Cows were blocked by age, reproductive and productive performance, and were fed corn/corn silage diets

Table 1 (Abstr. 334). Lactation for 658-kg cow BW

Vitamin[1]	Unit/hd/d	NRC	Low 25%	AVG	High 25%	High 10%	High 5%
A	IU	72,531	90,000	174,375	258,750	297,563	327,319
D_3	IU	0	30,000	38,779	45,000	51,750	56,925
E	IU	435	1,000	1,750	2,012	2,561	3,157
Riboflavin	mg	0	0	500	1,000	1,150	1,265
Niacin	mg	0	0	3,000	6,000	6,900	7,590
Choline	mg	0	0	10,000	15,000	17,250	18,975
Thiamine	mg	0	0	0	120	138	152
Pyridoxine	mg	0	0	0	66	76	83
Biotin	mg	0	0	0	180	207	228
B_{12}	mg	0	0	0	135	155	171
Pantothenic acid	mg	0	0	0	240	276	304
Folic	mg	0	0	0	201	231	254

[1] B vitamins were coated.

formulated to meet or exceed 2001 NRC recommendations. Treatments were T1 = 2001 NRC, T2 = low 25%, T3 = AVG, T4 = high 25% and T5 = high 5% vitamin supplementation (Table 1). Vitamin supplementation significantly increased several productive and reproductive parameters. Bulk tank somatic cell count (T1 = 356, T2 = 194, T3 = 172, T4 = 161 and T5 = 146 cells/mL, $P = 0.03$, respectively; days dry (68, 60, 58, 56 and 56 d, $P = 0.04$, respectively); incidence of clinical mastitis (35.2, 19.7, 17.6, 12.0 and 11.7%, $P = 0.03$, respectively); conception rate of 1st service (32.1, 49.3, 52.7, 58.4 and 58.9%, $P = 0.03$, respectively); conception rate, all services (31.2, 45.7, 48.2, 53.1 and 53.1%, $P = 0.02$, respectively); heat detection rate (45.2, 63.5, 67.8, 72.1 and 73.0%, $P = 0.03$, respectively); calving interval (14.5, 13.6, 13.4, 13.1 and 13.0 mo, $P = 0.04$, respectively; peak milk, first lactation (37.2, 41.3, 42.0, 43.1 and 43.6 kg, $P = 0.02$, respectively) and peak milk, third lactation (50.8, 56.1, 59.1, 59.9 and 60.7 kg, $P = 0.02$, respectively). In conclusion, vitamin supplementation in low 25%, AVG, high 25% and high 5% vitamin supplementation significantly ($P < 0.05$) increased productive and reproductive dairy cow parameters versus the 2001 NRC, AVG versus low 25%, high 25% versus AVG and high 5% versus high 25% supplementation.

Key Words: dairy cow, vitamin supplementation, performance

335 Effect of nanoparticle copper oxide fed alone or in combination with dietary antagonists on mineral status and performance of dairy cows. A. Williams*[1], A. M. Mackenzie[1], J. J. Bennison[2], and L. S. Sinclair[1], [1]*Harper Adams University, Newport, UK,* [2]*Agrimin Ltd., Kirmington, UK.*

Apparent absorption of copper (Cu) in ruminants is low, with between 0.02 and 0.07 g/g Cu from sources such as Cu oxide (CuO) being absorbed resulting in high levels of excretion. Improving bioavailability of Cu could reduce the dose required to maintain Cu status, particularly in the presence of dietary antagonists to Cu absorption such as sulfur (S) and molybdenum (Mo). One way in which bioavailability may be improved is by supplying Cu as nanoparticles. The objective of our study was to establish the bioavailability of nanoparticle CuO compared with conventional CuO in dairy cows without or in combination with antagonists (S and Mo) in the diet. Fifty-six multiparous Holstein-Friesian cows that were 48-d (SD ± 17.4) post calving and yielding 40.6 (SD ± 6.9) kg milk/d were used in a 2 × 2 factorial design. The 4 treatment groups were N−; nano CuO; N+; nano CuO and antagonists; C−; conventional CuO and C+; conventional CuO and antagonists. The diets contained approximately 17 mg Cu/kg DM, diets with antagonists contained an additional 6 mg Mo/kg and 1 g S/kg DM compared with diets without added antagonists. Blood samples were taken at wk 0, 2, 4, 6, 10 and 16 and liver biopsy samples at wk 0 and 16. Data were analyzed as a 2 × 2 factorial design using wk 0 data as a covariate where appropriate using Genstat 18. There was no effect of dietary treatment on dry matter intake, live weight, body condition score or milk yield. Plasma Cu and Fe concentrations were not affected by dietary treatment whereas plasma Mo concentration was increased ($P < 0.001$) in cows receiving dietary antagonists. Dietary antagonists decreased liver Cu concentration in cows fed conventional CuO (C+) but in cows fed dietary antagonists and nano CuO (N+) liver Cu concentration was increased ($P < 0.05$). In cows fed diets without antagonists liver Cu concentration was increased when supplemented with either conventional CuO (C−) or nano CuO (N−). In conclusion, nano CuO has a greater bioavailability than conventional CuO in the presence of Cu antagonists, and supplying CuO as a nanoparticle had no effect on animal performance.

Key Words: nanoparticle, copper, antagonist

336 Impact of B-complex vitamins on dairy cow productive and reproductive performance. M. Coelho*, R. Jones, and F. Parks, *BASF Corp., Florham Park, NJ.*

In the last 15 years there has been a +28% increase in milk production (33.9 to 39.5 kg/cow/d), a 12% decrease in total-tract feed passage rate (9.72 to 8.52 h) and a 1% decline in DM digestibility (66.02 to 65.12). This study examined the dairy cow performance by feeding B-complex vitamins in addition to vitamins A, D_3 and E. A total of 935 Holstein cows were used in a randomized complete block design with cow as the experimental unit, treatment as the fixed effect, and block as the random effect (187 cows × 5 treatments). Cows were blocked by age, reproductive and productive performance and were fed corn/corn silage diets formulated to meet or exceed 2001 NRC recommendations. Treatments were T1 = vitamins A/D_3/E, T2 = A/D_3/E/riboflavin, T3 = A/D_3/E/riboflavin/niacin, T4 = A/D_3/E/riboflavin/niacin/choline and T5 = A/D_3/E/B complex (Table 1). Bulk SCC (T1 = 170.3, T2 = 167.1, T3 = 163.5, T4 = 164.1 and T5 = 162.2 cell/mL, $P = 0.03$, respectively); days dry (57.0, 56.9, 56.2, 55.3, and 55.6 d, $P = 0.04$, respectively); incidence of clini-

Table 1 (Abstr. 336). Vitamins for 658-kg cow BW

Vitamin[1]	Unit/hd/d	Dry	Fresh	Lactation
A	IU	151,800	279,330	258,750
D3	IU	35,000	60,750	45,000
E	IU	3,000	4,000	2,561
Riboflavin	mg	500	1,000	1,000
Niacin	mg	3,000	6,000	6,000
Choline	mg	7,000	15,000	15,000
Thiamine	mg	0	240	120
Pyridoxine	mg	0	135	66
Biotin	mg	0	243	180
B12	mg	0	225	135
Pantothenic acid	mg	0	330	240
Folic	mg	0	255	201

[1] B vitamins were coated.

cal mastitis (16.4, 16.2, 16.0, 15.7, and 15.8%, $P = 0.03$, respectively); conception rate of first service (53.2, 53.8, 54.7, 55.1 and 56.0%, %, $P = 0.03$, respectively); conception rate, all services (49.1, 50.6, 51.2, 51.7 and 52.3%, $P = 0.02$, respectively); peak milk, first lactation (41.5, 40.9, 42.5, 43.4 and 44.3 kg, $P = 0.02$, respectively), peak milk, third lactation (59.7, 60.2, 63.4, 64.7 and 65.6 kg, $P = 0.02$, respectively); milk fat (3.86, 3.85, 3.52, 3.51 and 3.53%, $P = 0.04$, respectively) and milk protein (3.20, 3.24, 3.32, 3.39 and 3.40, $P = 0.03$, respectively). In conclusion, riboflavin, riboflavin/niacin, riboflavin/niacin/choline and all B-complex vitamins significantly increased productive and reproductive dairy cow parameters.

Key Words: dairy, performance, B vitamins

337 Survey on the impact of diet composition on folate and vitamin B12 plasma concentrations of cows across the United States and Canada.

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The aim of this survey was to study if diet composition could have an impact on plasma folate (B9) and vitamin B12 (B12) concentrations of Holstein cows across the USA and Canada. A total of 22 American (cows = 427) and 24 Canadian (cows = 476) herds participated. Cows were divided into 3 categories: 0–55, 56–120 and 121–200 d in milk (DIM) and according to parity (first, second and third and more). Each dietary ingredient was sampled for wet chemistry analysis. Nutrient composition of the diets was used in statistical analyses. Principal component (PC) analysis was performed on nutrient components of the diet to reduce the number of interdependent variables. Three PC explaining 79% of the variation were retained for analysis as independent variables. Proc MIXED of SAS was used to analyze data (log-transformed). Plasma B9 increased from 12.3 to 14.8 ng/mL between 0 and 120 DIM and reached a plateau afterward. It was greater by 10% for multiparous than for primiparous cows ($P < 0.01$). Between 56 and 200 DIM, plasma B12 was greater by 21% than before 55 DIM ($P < 0.01$). Plasma B12 increased as parity number increased (First: 172, 95% confidence interval (CI): 161–184, Second: 185, 95% CI: 173–198 and Third and more: 198, 95% CI: 185–211 pg/mL, $P < 0.01$). Plasma B9 and B12 were respectively less and greater for American cows (B9: 13.6, 95% CI: 12.9–14.4 ng/mL and B12: 206, 95% CI: 192–221 pg/mL) than for Canadian cows (B9: 14.7, 95% CI: 14.0–15.3 ng/mL and B12: 170, 95% CI: 159–182 pg/mL; $P \leq 0.04$). Plasma B9 and B12 were associated with the same PC (PC1; $P \leq 0.02$). Plasma B9 was negatively related to fiber (ex. neutral detergent fiber) and crude protein, and positively with nonfiber carbohydrate ($P = 0.01$) of the diet whereas the opposite was observed for plasma B12 ($P = 0.02$). Dietary fat (PC2), and Co (PC3) were not related to plasma B9 and B12. These models explained 40 and 42% (pseudo-R^2) of the variability of plasma B9 and B12, respectively. In summary, both plasma B9 and B12 concentrations were affected by parity, DIM, and nutrient components of the ration, mainly related to fiber and carbohydrate.

Key Words: cobalamin, folic acid, vitamin status

338 Evaluation of source of trace minerals and corn silage on lactational performance and total-tract nutrient digestibility of Holstein cows.

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The objective of this study was to evaluate the effect of source of trace minerals and corn silage on lactation performance and total-tract digestibility (TTD) of nutrients of Holstein cows. Fourteen cows averaging 82 (SE = 3) days in milk were used in a replicated 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments with 28-d periods. The diets consisted (dry basis) of 55% conventional (CON) or brown midrib-3 (BM3) corn silage, 2% straw, and 43% grain mix with either sulfate (STM) or hydroxy (HTM; IntelliBond, Micronutrients USA) source of copper, zinc, and manganese trace minerals. The targeted levels of copper, zinc, and manganese were 194, 1657, and 687 mg/d, respectively. The dietary treatments were: 1) CON-STM, 2) CON-HTM, 3) BM3-STM, and 4) BM3-HTM. Cows were housed in tie stalls, fed TMR 1×/d, and milked 3×/d. Dry matter intake (DMI) and milk yield were measured daily from d 18 to 28. Milk composition was measured on d 25 and 26 and TTD was determined on d 21 to 23. Behavior was observed on d 23 to 25. Data were summarized by period and analyzed as a replicated Latin square design with fixed model effects for corn silage, trace mineral, corn silage and trace mineral interaction, period within replicate, and replicate using the MIXED procedure of SAS (version 9.4). Cow within replicate was a random effect. Cows fed the BM3 diets had greater ($P \leq 0.05$) DMI (28.1 vs. 27.5 kg/d, SE = 0.5), milk yield (47.0 vs. 44.7 kg/d, SE = 1.1), and TTD of dry matter (72.8 vs. 71.1% of DM, SE = 0.6) and organic matter (OM; 74.1 vs. 72.3% of DM, SE = 0.5) and ruminated less (496 vs. 524 min/d, SE = 16, $P = 0.01$) than cows fed the CON diets. Cows fed the HTM diets had greater ($P = 0.01$) DMI (28.1 vs. 27.5 kg/d, SE = 0.5) and a tendency ($P = 0.10$) for higher TTD of neutral detergent fiber (aNDFom; (56.8 vs. 54.9% of DM, SE = 0.9)) than cows fed the STM diets. Corn silage with higher NDF digestibility affected DMI, milk yield, and TTD of DM and OM. Source of trace minerals can influence DMI and TTD of aNDFom, and should be taken into consideration when formulating diets for high producing dairy cows.

Key Words: trace mineral, corn silage, total-tract digestibility

339 The effect of substantial decreases on vitamin A supplementation for 6 and 36 months on dairy cow productive and reproductive performance.

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Dairy cow vitamin A (A), D3 (D3) and E (E) supplementation increased 45, 36, and 107%, respectively, in the last 18 years, while milk produc-

tion/cow increased by 30%. The objective of this trial was to evaluate the effect of vitamin A supplementation reduction on productive and repproductive performance. A total of 380 Holstein cows were used in a randomized complete block design with cow as the experimental unit, treatment as the fixed effect, and block as the random effect (190 cows × 2 treatments). Cows was pre-tested for the level of A and E required to maintain the neutrophil function level (using neutrophil phagocytosis test measured by phagocytosis assay, Phagotest^{R}), which was 2,125 IU E and 185,236 IU A/cow/day (adjusted to 658 kg cow BW). Treatments were positive control (PC) supplemented with 2,125 IU E and 185,236 IU A/cow/day to maintain neutrophil function level (dry and lactating) and 1.5× for fresh cows (277,854 IU/H/day) and a negative control (NC) group with 60% reduction of A (75,000 IU/H/day) for dry and milking cows, and 1.5× for fresh cows (112,500 IU/H/day), while maintaining the E at the same level 2,125 IU/H/day for dry and lactating cows and 3,000 IU/H/day for fresh cows. These levels of supplementation were maintained for 36 mo. Cows were blocked by age and milk production/cow/day and fed corn/corn silage diets formulated to meet or exceed 2001 NRC recommendations, except for A and E. The first 6-mo showed no significant difference between the 2 treatments. Neutrophil phagocytosis % (98 and 99, $P = 0.50$, respectively); days open (115 and 117, $P = 0.45$, respectively) and number of lactations/cow (4.2 and 4.1, $P = 0.25$, respectively). After 36 mo, the NC showed a 24% significant decrease in neutrophil phagocytosis (99, 75%, $P = 0.03$, respectively); a 26% significant increase in days open (112 and 154, $P = 0.03$, respectively); and a 28% significant decrease in number of lactations/cow (4.3 and 3.1, $P = 0.02$, respectively). In conclusion, vitamin A may be substantially reduced for several months. However, long-term vitamin A reduction leads to significant reductions in productive and reproductive performance.

Key Words: dairy, performance, vitamin A

340 Effects of dose and source of vitamin D on mineral homeostasis and performance in transition dairy cows. M. B. Poindexter*[1], A. Vieira-Neto[1], A. Husnain[1], R. Zimpel[1], A. Faccenda[2,1], A. Sanches de Avila[2,1], A. Silva[1], P. Celi[3], C. Cortinhas[3], J. E. P. Santos[1], and C. D. Nelson[1], [1]*University of Florida, Gainesville, FL,* [2]*State University of Western Paraná, Cascavel, Paraná, Brazil,* [3]*DSM Nutritional Products, Columbia, MD.*

Objectives of the experiment were to determine the effects of supplementing 2 doses of either vitamin D_3 (VitD) or 25-hydroxyvitamin D_3 (25D) on peripartum mineral metabolism and lactation performance. One hundred and 33 parous and 44 nulliparous pregnant Holstein cows were enrolled in the experiment. Cows were blocked by parity and previous lactation milk yield or genetic merit and then assigned randomly to receive a daily dietary top-dressed supplement containing 1 mg VitD (1mgD), 1 mg 25D (1mg25D), 3 mg VitD (3mgD), or 3 mg 25D (3mg25D) from 250 d in gestation until parturition. The prepartum diet was approximately −130 mEq/kg in dietary cation anion difference. Data were analyzed by ANOVA with mixed models using the Mixed procedure of SAS. Cows receiving the 25D treatments had greater ($P = 0.01$) concentrations of P in serum prepartum and a tendency for greater ($P = 0.07$) concentrations of P postpartum. Cows receiving the 25D treatments had greater ($P = 0.04$) concentrations of total Ca in serum postpartum. Cows receiving the 3mg25D treatment produced more milk ($P < 0.01$) in the first 42 d in milk compared with other treatments (Table 1). Prepartum DM intake, postpartum disease incidence, colostrum production, and concentrations of Ca and Mg in colostrum and milk did not differ among treatments. Cows receiving 25D were in a better mineral status postpartum and performed better compared with cows receiving just VitD.

Table 1 (Abstr. 340). Effect of dose and source of vitamin D on mineral status and performance

Item	1mgD	3mgD	1mg25D	3mg25D	SE
Prepartum					
Ca, m*M*	2.34	2.32	2.34	2.35	0.02
Mg, m*M*	0.85	0.84	0.83	0.83	0.02
P, m*M**	1.89	1.87	1.96	2.05	0.03
Postpartum					
Ca, m*M**	2.13	2.11	2.15	2.18	0.02
Mg, m*M*	0.88	0.85	0.87	0.84	0.02
P, m*M*	1.72	1.7	1.75	1.8	0.04
Colostrum yield, kg	4.3	3.9	5.4	4.5	0.55
Milk yield, kg/d¥	35.3	32.4	33.4	36.6	1.3

*Source ($P < 0.05$), §Level ($P < 0.05$), ¥Level × Source ($P < 0.05$).

Key Words: vitamin D, calcium, transition

341 Effect of cobalt source and folic acid on lactation performance of dairy cows. K. E. Ritz*[1], C. L. Engel[1], M. T. Socha[1], C. S. Kending[1], P. Stark[1], and T. B. Bennett[2], [1]*Zinpro Corporation, Eden Prairie, MN,* [2]*University of Wisconsin-School of Veterinary Medicine, Madison, WI.*

Cobalt source and folic acid (FA) may affect vitamin B_{12} (B_{12}) synthesis and milk production. A study was conducted to determine effects of Co source (Zinpro Corp.) and metabolizable folic acid on milk production and serum vitamins. Lactating cows on 7 dairies were fed standard lactation diets and received 3 treatment (Trt) premixes in a switchback design (4 60 d periods: 30 d adaptation, 30 d data collection). Trace minerals (TM) Zn, Mn, Cu (40, 20, 3.5 ppm, respectively) were from Availa (AV) or ProPath (PP) amino acid complexes (Zinpro Corp.). Treatments were: 1) CON: AV TM, 1 ppm Co from Co glucoheptonate (COPRO) and 0 ppm FA; 2) ACF: AV TM, 1 ppm Co from Co pectin (CoPectin) and 3 ppm FA; 3) PPD (ProPath Dairy, Zinpro Corp.): PP TM 1 ppm Co from CoPectin and 3 ppm FA. Data were analyzed using Proc MIXED of SAS. Experimental unit was herd (milk data), or cow (transition cow index (TCI), serum). Effects were Trt, temperature humidity index, days since start (fixed), and herd (random). Milk yield was greatest ($P < 0.01$) for PPD (40.2 kg), intermediate for ACF (39.6 g) and lowest for cows fed CON (38.8 kg). ECM was greater ($P < 0.05$) for PPD (42.2 kg) cows than CON or ACF (41.8 or 41.6 kg). Milk fat percent decreased ($P < 0.01$) across Trt (3.99, 3.87, and 3.81% for CON, ACF and PPD, respectively). Milk fat yield was similar across Trt (1.54, 1.54, and 1.53 kg/d for CON, ACF, and PPD respectively). Milk protein % was greater ($P < 0.01$) in cows fed PPD and CON (3.19%) than ACF (3.16%). Milk true protein yield increased ($P < 0.01$) across Trt with PPD > ACF > CON (1.28 > 1.25 > 1.23 kg/d, respectively). The SCC was variable ($P < 0.05$) across Trt at 115, 107 and 154 (1000/mL) for CON, ACF and PPD, respectively. The TCI was greatest ($P < 0.01$) for PPD (7.0 kg/d) compared with ACF and CON (2.7 and 1.4 kg/d). Serum FA was similar among Trt (13.2, 13.8, and 13.1 ng/mL for CON, ACF and PPD respectively). Serum B_{12} was greatest ($P < 0.05$) in PPD (184 pg/mL) compared with CON and ACF (162 and 157 pg/mL). Compared with CON and ACF, feeding PPD increased milk, ECM, and milk protein yield, improved TCI, and increased serum B_{12} status in lactating cows on commercial dairies.

Key Words: cobalt, folic acid, lactating cow

342 The effect of vitamin antioxidant status and neutrophil function on dairy cow productive and reproductive performance.

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As the dairy industry strives for efficiency, the cell metabolic rate increases, and the neutrophil function decreases. In the last 15 years, there has been a 28% increase in milk production (33.9 to 39.5 kg/cow/d), a 12% decline in total-tract feed passage rate (9.72 to 8.52 h) and a 1% decline in DM digestibility (66.02 to 65.12). This study examined the neutrophil function, productive and reproductive performance at 4 levels of cow vitamin supplementation (Low, AVG, high 25% and high 5% antioxidant/electron transport vitamin supplementation of vitamin A (A), vitamin E (E) and riboflavin (Ribo), measured as bacteria phagocytosis by neutrophils using a phagocytosis assay, Phagotest. The phagocytosis test was standardized to 100% phagocytosis, which indicates full neutrophil activity and no depression effect. A total of 756 Holstein cows were used in a randomized complete block design with cow as the experimental unit, treatment as the fixed effect, and block as the random effect (189 cows × 4 treatments). Cows were blocked by age, reproductive and productive performance, and were fed corn/corn silage diets formulated to meet or exceed current NRC recommendations. Treatments consisted of antioxidant vitamin supplementation (T1 = 80,000 IU A, 963 IU E and 0 mg Ribo, T2 = 120,000 IU A, 1444 IU E and 30 mf Ribo, T3 = 160,000 IU A, 1925 IU E and 60 mg Ribo, T4 = 224,000 IU A, 2650 IU E and 80 mg Ribo/H/D, respectively (adjusted to a 658 kg cow BW). Neutrophil phagocytosis (T1 = 65,T2 = 82, T3 = 95 and T4 = 100%, $P = 0.04$, respectively); bulk SCC (356, 285, 225 and 180 cell/mL, $P = 0.03$, respectively); incidence of clinical mastitis (35%, 20%, 14% and 10%, $P = 0.02$, respectively); number of days open (154, 132, 119 and 112, $P = 0.03$, respectively) and days to first service (97, 82 75 and 68, $P = 0.03$, respectively). Goodness-of-fit tests determined cow neutrophil and performance responses to vitamin supplementation. In conclusion, there was an excellent correlation between vitamin A, E and Riboflavin supplementation with neutrophil function ($R^2 = 0.94$), bulk somatic cell count ($R^2 = -0.89$), incidence of clinical mastitis ($R^2 = -0.93$) and number of days open ($R^2 = -0.91$).

Key Words: dairy cow, vitamin antioxidant, neutrophil

ADSA Southern Branch Symposium: Dairy Cattle Longevity Revisited—Trends, Economics, and Opportunities

343 Why revisit dairy cattle longevity? A. De Vries*[1], K. Daniels[2], J. Fain Bohlen[5], G. Ferreira[2], A. Rius[4], and S. Ward[3], [1]*University of Florida, Gainesville, FL,* [2]*Virginia Tech, Blacksburg, VA,* [3]*North Carolina State University, Raleigh, NC,* [4]*University of Tennessee, Knoxville, TN,* [5]*University of Georgia, Athens, GA.*

Dairy cattle longevity averages approximately 3 years after first calving, despite improvements in genetics and cow comfort over the past several decades. The natural life span of cattle is about 20 yr. In some parts of Western agriculture, there is a strong push to extend dairy cattle longevity. Our objectives are to first explore economic and environmental incentives for increasing average dairy cattle longevity and then discuss deterrents to increasing longevity. The vast majority of culling decisions, and by extension of dairy cattle longevity, are driven by economic decision-making. Younger herds capitalize on genetic progress but have fewer efficient mature cows and greater replacement costs. There is also a growing interest in the use of beef semen in dairy cattle, which does not add to the supply of dairy heifers. High premiums for crossbred calves suggest a greater marginal value of extending dairy cattle longevity. Extending dairy cattle longevity might also decrease the environmental footprint of milk production. Historically, models focused on optimizing culling decisions for individual cows, while assuming an unlimited supply of heifers, found optimal cull rates of approximately 30%. This suggests a slightly longer longevity than is currently observed. However, cow performance has changed and the average findings of these models may no longer be optimal. Some consider a short longevity a result of unavoidable forced culling and a welfare issue. Management and housing may affect longevity by improving health care and cow comfort. On the other hand, this short longevity is also driven by an abundance of replacement dairy heifers now available through the use of sexed semen and good reproduction. The dairy community should reevaluate how many dairy heifers are needed, which animals should be the dams, and what to do with animals that do not need to supply replacements. Genetic selection, cow comfort, and health care will increase the ability of cattle to avoid culling for forced reasons. These topics are interdependent and will drive future changes in dairy cattle longevity.

Key Words: longevity, culling, productive life

344 The effect of management and facilities on cow culling rates. N. Cook*, *University of Wisconsin-Madison, Madison, WI.*

An individual dairy cow's productive life in the herd is determined by her health and the need for dairy producers to optimize the production of each individual within the herd for a duration determined by replacement availability. Within this system, several aspects of management and facility design impact the health and productivity of the cow. Comfort is maximized by optimizing resting behavior in freestall facilities to achieve approximately 12 h/d of lying time, through stall design and bedding management, by taking measures to cool the cow during periods of heat stress and by allowing sufficient time for rest. Recommendations exist to size the stall relative to the size of the cows using them and utilize deep loose bedding, particularly sand. Loose-bedded packs provide an alternative housing option. The management of heat stress involves implementing a program which provides fast moving air in each lying area (target 200–400 ft/min, 1–2 m/s), sufficient exhaust capacity to force heat, moisture and noxious gases from the barn (target 40–50 air changes per hour and 1,500 ft^3/min, 2,550 m^3/h per cow), designs which ensure that the system works as well in the winter as it does in the summer, and the strategic use of water to mist the air or soak the cow at the appropriate times to assist with cooling in the warmest climates. The time available for lying is influenced by pen sizes relative to parlor throughput or robot milker availability, and by management interactions such as health screening cows in headlocks at the feed bunk for prolonged periods. Resting behavior is also significantly affected by lameness. Sufficient space for access to feed and the design of the feed bunk appear particularly critical during the transition period and there is a need to minimize regrouping stress during this important period. Cows must move easily between feeding, drinking, milking and resting areas of the farm, making alley dimensions and flooring surfaces a critical element of design. Enormous strides have been taken to improve cow comfort over the last decade by developing facility design recommendations that merge structural requirements with the needs of the cow.

Key Words: comfort, rest, transition

345 Replacement heifers: How many, what kind, and how should we manage it all? M. W. Overton*, *Elanco Animal Health, Greenfield, IN.*

Historically, most producers raised every heifer born. However, advancements in transition and reproductive management, coupled with widespread use of sex-sorted semen in dairy heifers and cows, have led to an oversupply of dairy replacement heifers in the US In addition to be a large negative cash flow, raising costs are significantly higher than replacement values, resulting in large economic losses, assuming market values fail to recover quickly. Consequently, producers are asking "How many heifers do I need?" and "What do I do with the excess?" Future herd turnover is the driver of replacement needs. To calculate herd turnover, divide the total number of cows (milking and dry) that leave the herd (sold or dead) over 12 mo by the average population at risk (milking and dry) for that same time. Assuming a stable herd size, cows removed due to mortality, disease, infertility, low milk production, or sold for dairy purposes should be replaced immediately with fresh heifers to keep facilities full and more completely dilute fixed costs. Historic farm-level herd turnover risks are a logical starting point for predicting replacement needs but cannot account for unanticipated health, fertility problems, or economic opportunities to improve the herd. Herd turnover is costly, but limiting the number of replacement heifers raised may lead to large future lost opportunity costs due to an inability or unwillingness to replace a less productive cow at the appropriate time. Aside from the anticipated herd turnover, other considerations, beginning at the time of conception, include sire choice, abortion risk, stillbirth risk, heifer mortality risk, heifer reproductive efficiency, heifer abortion risk, and heifer growth rate. Producers are now breeding some of their dairy animals to beef sires and culling excess heifers at various ages, depending on the anticipated market values. Criteria for culling include genetic potential, growth performance, and health history. Proper and timely culling decisions can reduce the cash flow drain, lower future economic loss, and increase the net value of heifers entering the milking herd.

Key Words: replacement heifer, herd turnover, culling

346 Impact of genomic selection and sexed semen on the economics of longevity. D. Kendall*, C. Heuer, C. Sun, and J. Deeb, *ST Genetics, Navasota, TX.*

The impact of genomics combined with Advanced Reproductive Techniques including the improved performance of sexed semen has revolutionized the rate of improvement in dairy cattle genetics and milk production in the last 10 years. According to CDCB, the rate of improvement in production traits doubled in the first 5 years of genomic selection compared with the previous 5 years. Further management traits such as PL have doubled the rate of improvement while DPR has increased by 10-fold (0.17 to 1.74) with genomic selection during the same period as PL. Combining genomic selection with IVF embryo production from heifers as young as 6 mo and improved utilization of early cell production from bulls has decreased the generation interval in the elite genetic population from 7 years for sires of bulls and 5.1 years for dams of bulls to approximately 26 mo for both. As Dechow and Rogers note (2018, J. Dairy Sci. 101:4312–4316), the effect of this shortened generation interval and genetic improvement will migrate rapidly to the commercial population. Sex-sorted semen used on the highest genetic merit animals in a herd has been modeled by STG (Kendall, Heuer, Sun, Deeb) to lower the generation interval for commercial cows to 2.5 years. This rate of genetic improvement combined with a shortened generation interval changes the economics of longevity. This presentation will review the combination of genomics, sexed semen and beef on dairy which will contribute to a longer productive life for the commercial dairy cows

Key Words: genomics, longevity, economics

Animal Health 2: Immunity

347 Transcriptomic analysis of circulating leukocytes in early postpartum dairy cows with and without uterine infection.

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It is proposed that transcriptomic mechanisms may be associated with suboptimal immune responses and disease in early postpartum dairy cattle. The influence of transcriptomic changes has yet to be defined, but an investigation into differential gene expression offers an opportunity to illustrate their potential impact. The aim of this study was to utilize RNA-sequencing data to demonstrate specific transcriptomic changes within peripheral immune cells during the early postpartum period in dairy cows with and without uterine infection along with associated physiologic changes. This study was conducted on a high-producing conventional dairy with 5,000 Holstein cows. Cows in the first 14 DIM were assessed for evidence of abnormal uterine discharge. Eleven cows were enrolled as diseased cases with postpartum physiologic dysfunction based on the presence of fetid uterine discharge along with hematologic derangements indicative of a response to an infectious or altered metabolic state. Nine healthy matched cohorts were enrolled based on comparable DIM, lactation number, and no evidence of uterine or systemic infection. Blood was collected on the day of enrollment and then weekly for a total of 3 samples per cow from which RNA was extracted for downstream sequencing in all 3 samples per cow. The transcriptomic impact of early postpartum physiologic dysfunction was evaluated using feature selection with the machine learning platforms Boruta-Python and SciKit-Learn. Initial results show a set of approximately 20 genes whose expression was found to be associated with disease in post-parturient dairy cattle (Boruta P-value < 0.05; outperforms 100% of shadow features). Using functional enrichment analysis, based on gene ontology annotations (Fisher's P-value < 0.01), these genes are related to biological processes involved in immune cell and receptor function, tissue repair, and cell signaling. These findings provide promising candidate genes for describing uterine health and identifying postpartum dysfunction. Improved understanding of transcriptomic disease mechanisms may lead to targeted interventions and allow producers a more refined means of providing optimal care.

Key Words: transcriptomics, RNA-sequencing, dairy

348 Effect of a non-specific immune stimulant on white blood cell counts around parturition.

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The objective of this study was to assess the effect of a commercial immune stimulant (Amplimune, NovaVive Inc., Napanee, Canada) on the peripartum dynamics of circulant neutrophils (NEU), lymphocytes (LYM), and monocytes (MON). Holstein cows (n = 34) were randomly assigned into 1 of 2 treatments (Trt): (1) Amplimune (AMP; n = 16), receiving a 5 mL subcutaneous (s.c.) dose of Amplimune at enrollment (7 ± 3 d before calving) and at calving (1 ± 1 DIM); and (2) Control (Con; n = 18), receiving 5 mL s.c. of saline solution at the same time points. White cell counts were log-transformed and examined using repeated measures analysis (PROC MIXED), with models including Trt, parity, occurrence of metritis (MET), sampling point (Time; 1 = enrollment; 2 = 2 d after enrollment; 3 = calving; 4 = 2 DIM; 5 = 7 DIM; and 6 = 14 DIM) and the interaction Trt × Time. LSM (95%CI) for NEU, LYM, and MON at different sampling points and by Trt are presented in Table 1. The effect of the interaction Trt × Time was not significant for NEU (P = 0.28), LYM (P = 0.84), and MON (P = 0.49). In conclusion, no treatment effect was determined for NEU, LYM and MON counts.

Key Words: immune stimulant, metritis, white blood cells

Table 1 (Abstr. 348). Means and confidence intervals of circulant cell counts (Log cell/mL)

	Sampling time						*P*-value		
	1	2	3	4	5	6	Trt	Time	Trt × Time
NEU									
Con	3.57 (3.31–3.82)	3.58 (3.33–3.83)	3.28 (3.02–3.53)	3.47 (3.22–3.73)	3.38 (3.12–3.63)	3.43 (3.17–3.68)	0.65	<0.001	0.28
Trt	3.60 (3.39–3.81)	3.6 (3.39–3.81)	3.49 (3.28–3.7)	3.40 (3.20–3.60)	3.35 (3.14–3.56)	3.45 (3.24–3.67)			
LYM									
Con	3.84 (3.63–4.1)	3.82 (3.61–4.00)	3.78 (3.56–4.00)	3.78 (3.56–4.00)	3.76 (3.55–3.98)	3.77 (3.56–4.00)	0.4	0.02	0.84
Trt	3.70 (3.52–3.9)	3.67 (3.48–3.85)	3.64 (3.45–3.82)	3.65 (3.46–3.83)	3.63 (3.45–3.82)	3.61 (3.42–3.8)			
MON									
Con	2.70 (2.52–2.89)	2.68 (2.49–2.86)	2.83 (2.64–3.02)	3.02 (2.83–3.2)	2.85 (3.66–3.04)	2.76 (2.57–2.94)	0.33	<0.001	0.50
Trt	2.69 (2.54–2.85)	2.78 (2.62–2.93)	2.79 (2.64–2.95)	3.03 (2.87–3.18)	2.83 (2.67–2.98)	2.71 (2.55–2.86)			

349 Evaluation of disease occurrence and production parameters of dairy cows treated with pegbovigrastim.

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The aim of this study was to evaluate the effects of pegbovigrastim injection (Imrestor, Elanco Animal Health, Greenfield, IN) on production parameters and postpartum disease occurrence (retained placenta, metritis, and displaced abomasum, and clinical mastitis (CM)). The study was conducted on a commercial Mississippi dairy from August 21, 2017, to March 31, 2018. Study cows (n = 270) were blocked by parity group (multiparous or primiparous) and randomly assigned to control (CON, n = 144) or treatment (IMR, n = 126). Ten ± 2 d before expected calving and again at calving, treated cows received 2.7 mL of Imrestor and CON cows received 2.7 mL of 0.9% saline. Milk yield (MY), fat, protein, lactose, solids nonfat percent, SCC, and body condition, hygiene, and lameness were evaluated weekly. Animals were evaluated for metritis twice weekly through rectal temperature (RT), palpation, and uterine discharge evaluation until 30 DIM. Other postpartum diseases were recorded by farm personnel. Milk composition and MY were analyzed as repeated measures in time with block, treatment, calving month, and lactation week included in mixed models. The LOGISTIC procedure was used to evaluate disease occurrence. Variables entered a model if $P \leq 0.1$ when screened individually. Variables with $P \leq 0.15$ were kept in the final model. Milk yield, fat, protein, lactose, SNF, and log SCC were not significantly affected by treatment ($P = 0.62$, 0.57, 0.24, 0.47, 0.34, and 0.49, respectively). The CM occurrence did not differ between treatments ($P = 0.54$) but only 17 cases were detected. The metritis logistic model contained treatment, block, and RT. Every 1°C temperature increase was associated with 2.57 times greater odds of developing metritis. Treated cows were 2.45 times more likely to develop metritis compared with control cows ($P < 0.01$). Cows given pegbovigrastim injections had increased odds of developing metritis, but no negative effects on milk production and composition were observed.

Key Words: Imrestor, mastitis, metritis

350 Using chitosan microparticles to treat metritis in lactating dairy cows.

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The objective was to evaluate the efficacy of intrauterine administration of chitosan microparticles (CM) for the treatment of metritis in dairy cows. Secondary objectives were to evaluate the effect of CM treatment on milk yield, reproductive performance, and survival. Holstein cows (n = 826) with metritis from 3 dairies located in northern FL were blocked by parity (primiparous or multiparous) and, within each block, randomly assigned to one of 3 treatments: CM (n = 276): intrauterine infusion of 24 g of CM dissolved in 40 mL of sterile distilled water at the time of diagnosis (D0), D2 and D4; Ceftiofur (CEF; n = 275): subcutaneous injection of 6.6 mg/kg of ceftiofur crystalline-free acid (Excede, Zoetis) in the base of the ear at D0 and D3; Control (CON; n = 275): no intrauterine or subcutaneous treatment. A group of healthy (HTH) cows was used for comparison. Data were analyzed by generalized linear mixed models. Cure rate 12 d after treatment was greater for CEF than for CM and CON (79.6 vs. 61.2 vs. 64.7%). The cure rate was similar between CM and CON. Culling in the first 60 d postpartum (DPP) was greater for CM than CEF and CON (19.2 vs. 8.4 vs. 10.2%), which were all greater than HTH (4.3%). Culling in the first 60 DPP was similar between CEF and CON. After adjusting for concentrations at enrollment, treatment did not affect plasma concentrations of NEFA, BHB or haptoglobin. Milk yield in the first 60 DPP differed among treatments, and it was 37.3, 38.3, 39.8 and 42.4 kg/d for CM, CON, EXD, and HTH, respectively. Also, milk yield in the first 10 mo differed among treatments, and it was 35.5 ± 0.5, 35.8 ± 0.4, 36.5 ± 0.4, and 37.6 ± 0.3 kg/day for CM, CON, EXD, and HTH, respectively. The hazard of pregnancy up to 300 DIM was greater for EXD than CM and CON, which were all lesser than HTH. Median time to pregnancy was 149, 137, 131, and 113 DIM for CM, CON, EXD, and HTH, respectively. In summary, CM did not affect cure rate or hazard of pregnancy and was detrimental to milk yield and culling compared with CON. EXD increased cure rate, milk yield and hazard of pregnancy compared with CON.

Key Words: metritis, chitosan microparticles, ceftiofur

351 Effect of chitosan microparticles on the uterine microbiome of dairy cows with metritis.

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The objective was to evaluate the shift in uterine microbiota after treating cows with metritis with chitosan microparticles (CM). A subset of cows (n = 89) were randomly sampled from a larger study (n = 826) where cows had been randomly assigned to 1 of 3 treatments: CM = intrauterine infusion of 24 g of CM dissolved in 40 mL of sterile distilled water at the time of diagnosis (D0), D2 and D4; Ceftiofur = subcutaneous injection of 6.6 mg/kg of ceftiofur crystalline-free acid (Excede, Zoetis) in the base of the ear at D0 and D3; Control = no intrauterine or subcutaneous treatment. A group of healthy (HTH) cows matched by days in milk at metritis diagnosis was added for comparison. Uterine swabs were collected from CM (n = 21), CEF (n = 25), CON (n = 23), and HTH (n = 20) on D0, D3, D6, D9, and D12. Swabs were used for genomic DNA extraction and sequencing of the V4 hypervariable region of the 16S rRNA gene on the Illumina MiSeq platform. Principal coordinate analysis paired with permutation ANOVA at the phylum and genus level showed that the uterine microbiome was similar among all metritic groups on D0, but different ($P \leq 0.001$) from HTH cows. Additionally, the microbiome of CEF cows shifted more rapidly toward HTH cows, with CM cows being the slowest to shift toward HTH and CON being intermediate. Comparison of relative abundance at the phylum level showed that metritic cows had greater ($P \leq 0.05$) abundance of Bacteroidetes and Fusobacteria, and lesser abundance of Proteobacteria and Tenericutes on D0 than HTH cows. CM tended ($P \leq 0.10$) to have a greater overall abundance of Bacteroidetes compared with CEF, and greater abundance of Fusobacteria on D3 compared with CEF and CON. In summary, CM slowed the progression toward a healthy uterine microbiome, whereas CEF hastened the progression toward a healthy uterine microbiome.

Key Words: metritis treatment, microbiome, dairy cow

352 Intracellular zinc bioavailability in bovine mammary epithelial cells is modulated by lipopolysaccharide: A fluorescence resonance energy transfer approach.

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Zinc is an important trace element which is involved in many intracellular pathways including cell growth, differentiation, and inflammation. Therefore, we evaluated the effect of the intracellular bioavailability of Zn in bovine mammary epithelial cells (MacT) in response to an LPS

(*Escherichia coli* O111:B4, Sigma-Aldrich) challenge. To test this, MacT cells were seeded 24h before transfection at 30,000 cells/well in a 96-well plate. Additional cells were seeded at 300,000 cells/well in a 6-well plate for gene expression analysis. The plasmid used in this study was the peZinCh-NB (Addgene) designed to detect Zn through fluorescence resonance energy transfer (FRET) technology. Cells were transfected with the transfection reagent Lipofectamine 3000 at 0.3 µL/well and at 150 ng/well of plasmid in a reduced serum medium deprived of FBS. Transfected cells were treated with 50 µ*M* concentration of Zn, and challenged at non-clinical (0 µg of LPS/mL) and clinical levels (250 µg of LPS/mL) for 2 h at 0, 6, and 12 h after the treatment. An inverted fluorescent microscope for live imagining equipped with scanning stage, and an environment-controlled chamber at 37°C and 5.0% of CO_2 was used to take 4 pictures/well at 4× magnification. Quantification of Zn and cell viability were assessed using the CellProfiler software. Data were analyzed using the PROC MIXED of SAS. Overall intracellular availability of Zn was not affected by LPS in MacT cells incubated with 50 µ*M* Zn at 0 and 12 h post-treatment. However, at 6 h post Zn treatment, there was a significant increase ($P < 0.01$) in intracellular bioavailability of Zn compared with the control over time during LPS challenge. Within 1 h post LPS challenge, the Zn intracellular bioavailability was greater ($P = 0.05$) in the challenged cells than the control. Similarly, at 1.5 and 2 h post LPS challenge, the Zn intracellular bioavailability was greater ($P < 0.001$) in the challenged cells than the control. These preliminary data indicate that FRET technology is sensitive enough to detect changes in intracellular Zn content mediate through an LPS model. To expand on these effects, gene expression analysis will be performed.

Key Words: zinc, bovine cells, lipopolysaccharide (LPS)

353 Needle-free vaccination of cattle against blue tongue virus (BTV) serotype 8.

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Needle-free vaccination reduces the risk of accidental transmission of infectious agents and induces higher antibody titers after vaccination compared with common needle vaccination. The aim of the study was to compare antibody titers and local responses at injection sites after needle-free and common needle vaccination in cattle using BTV8 vaccine. Thirty nonlactating, non-pregnant cows were assigned to 3 groups of 10 cows each and vaccinated twice in 4-week intervals in the left and right neck with an inactivated blue tongue 8 virus vaccine (BTV8, Bluevac8, CZ Veterinaria, Spain). The vaccine was either administered transcutaneously by AcuShot needle-free injector (battery-driven power cylinder technology; www.acushot.ca; group NF) or subcutaneously (sc) with needle (group N). Cows of the control (C) group received sc needle injections of sterile saline. Before and after vaccinations blood samples for determination of antibody titers (ELISA) were obtained weekly. Body temperature and signs of general condition were evaluated daily and skin thickness and pressure sensitivity at injection sites every other day until 4 weeks after second vaccination. Results were evaluated in a mixed model (fixed effects: group, time, random effect: cow) and contingency tables by Fisher's exact test (SAS 9.3 statistical package). No sample was positive for BTV8 antibodies in group C. Twenty-eight d after first vaccination 5 cows revealed seroconversion after needle-free and 3 after needle vaccination. Four weeks after second vaccination all cows were seropositive for BTV8. Mean antibody titers were not significantly different between groups NF and N. While after first and second vaccination in average skin thickness doubled and skin was mildly pressure sensitive in group NF almost no changes in skin thickness and pressure sensitivity were detected at injection sites in group N ($P < 0.05$). Swelling and skin sensitivity disappeared completely within 4 weeks after vaccination. No cow presented fever or disturbed general condition. In conclusion needle-free vaccination induced seroconversion in all BTV8 vaccinated cows. Temporary local reactions at injection sites were mild but significantly more prominent after needle-free than after needle vaccination.

Key Words: needle-free vaccination, cattle

354 A cohort study on the carcass weight loss associated with bovine leukemia virus infection in dairy production in Hokkaido, Japan.

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Bovine leukemia virus (BLV) is widely prevalent in Japanese dairy farms. To understand the burden of BLV infections in Japan, it is important to estimate the economic loss. The objective of our study was to assess the loss of carcass weight (CW) for dairy culled cows due to BLV infection. In total, 226 Holstein culled cows from 12 commercial dairy farms participating in the routine BLV infection monitoring scheme in the Nemuro and Kushiro regions of Hokkaido, Japan were studied. Information on age and the last delivery day was collected. BLV infection status was categorized according to the proviral load (PVL) which was measured using 2 different but well correlated tests according to the farms. Those cows whose PVL was not detected in the regular blood test just before import slaughterhouse were categorized into non-infected culled cows. Those cows whose PVL was under 2,500 copies/50 ng DNA or 60,000 copies/10^5 Cell by continuous 2 times blood tests just before sending to slaughterhouse was categorized as low PVL (LPVL), while those with PVL higher than 2,500 copies/50 ng DNA or 60,000 copies/10^5 Cell were high PVL (HPVL). CW information and postmortem examination results of the culled cows were provided by meat companies and meat inspection centers, respectively. A Generalized Linear Mixed Model (GLMM) framework with Gamma errors were used to estimate CW, age, lactation stage, status of BLV, and their interaction term as explanatory variables and owner was random effect. Additionally, GLMM with Poisson errors were applied to reckon the abnormal finding of the postmortem examination (AFPE), status of BLV was explanatory variable, and owner was random effect. GLMM with Gamma errors were practiced calculation CW, AFPE was explanatory variable, and owner was random effect. The mean CW of HPVL culled cows was 38.52 kg smaller than that of non-infected ($P = 0.039$). As the number of AFPE increased by 1, mean CW decreased 14.96 kg ($P < 0.001$). The mean number of AFPE of HPVL culled cows was significantly larger by 1.01 than non-infected cows (1.60, $P = 0.018$). In summary, BLV infection causes decrease in CW, and this may be due to the pathology of internal organs in persistent high viremia cows.

Key Words: bovine leukemia virus (BLV), carcass weight, postmortem examination

Joint Animal Health/Growth and Development Platform Session: Factors that Influence Calf Health, Including Fetal Programming

355 Late-gestation maternal factors affecting dairy calves' health and development. A. Abuelo*, *Department of Large Animal Clinical Sciences, Michigan State University, East Lansing, MI.*

Efficient production of heifers is fundamental to the productivity and sustainability of dairy farms. However, high pre-weaning morbidity and mortality rates are frequently reported worldwide, imposing substantial welfare and economic implications. A major contributing factor to disease susceptibility in the neonatal stage is the inability of calves to mount an effective immune response. Several stresses imposed on the late gestation cow can compromise intrauterine conditions during critical periods of the offspring's development. These downstream carry-over effects cannot only adversely affect calf growth, but also impair immune capabilities that can lead to increased disease susceptibility. For example, pre-weaning metabolic function and growth are associated with future milk production. Thus, late-gestation carry-over effects span into the lactating stage of the heifers. This presentation will review the effects of maternal stress during late-gestation on the offspring's growth, productivity, metabolism, and immune function. In addition, strategies focusing on maternal interventions to improve neonatal health will be discussed. A better understanding of the intrauterine conditions affecting calf health and development may facilitate the design of management practices that could improve neonatal development and future cow productivity.

Key Words: developmental programming, immunity, calf health

356 Effects of serum protein concentrations on selected health measures within the first 90 days of life in Holstein dairy calf. B. J. Tverdy*[1], C. Y. Tsai[1], W. J. Price[2], and P. Rezamand[1], [1]*Department of Animal and Veterinary Science, University of Idaho, Moscow, ID,* [2]*Statistical Programs, College of Agricultural and Life Sciences, University of Idaho, Moscow, ID.*

An objective of this study was to determine the effect of passive transfer status on morbidity and mortality in neonatal Holstein dairy calves (n = 1,631). Calves, from dairy farms in the western United States, were assigned an individual electronic identification and entered into Feedlot Health Management Services proprietary software system, iFHMS (Preston, ID). Cause-specific morbidity and mortality was recorded on an individual calf basis daily from entry to exiting or death. A 5 mL tube of whole blood was collected from each animal at 48 ± 6h post-arrival. Whole blood was centrifuged at 2000g for 10 min and serum was stored at −22°C until analyzed. Serum total protein (TP) was measured using a digital refractometer (Weaver et al., 2000). Calves were categorized based on proposed USDA serum TP guidelines into poor (TP <5.1 g/dL, n = 159, mean ± SD 4.68 ± 0.31 g/dL), fair (5.1 < TP = 5.7 g/dL, n = 399, 5.45 ± 0.19 g/dL), good (5.8 = TP = 6.1 g/dL, n = 322, 5.96 ± 0.11 g/dL) and excellent (TP >6.1 g/dL, n = 751, 6.90 ± 0.59 g/dL). Data were analyzed using generalized linear mixed model (Proc Glimmix) in SAS with significance declared at $P = 0.05$. Results showed that there was significant difference between poor and excellent in total respiratory disease treatments as well as the total gastrointestinal disease treatments ($P < 0.001$ for both). Gastrointestinal and respiratory diseases were defined and diagnosed subjectively on an individual animal basis. A calf treated for respiratory disease may exhibit crust on the nose, mucus in the nostrils, raspy breathing, and failing to drink. In cases of gastrointestinal disease, a calf may exhibit scours, lethargy, and dehydration. In addition, there were differences in the ear disease treatments in relation to serum total protein status (poor vs excellent and fair vs excellent; $P < 0.01$). Higher morbidity was demonstrated in calves with lower serum TP values measured within the first few weeks of life, suggesting that other factors affecting immunity and overall health, such as lipid soluble vitamins, may be involved, which warrants further investigation.

Key Words: passive transfer, serum total protein, lipid-soluble vitamins

357 Validation of an automated cell counter to determine leukocyte differential counts in neonatal Holstein calves. T. E. von Konigslow*, D. L. Renaud, T. F. Duffield, V. Higginson, and D. F. Kelton, *University of Guelph, Guelph, ON, Canada.*

Risk factors and biomarkers show promise for identifying high risk calves entering rearing facilities. Rapid automated leukocyte differential cell counts may be a good addition to augment or refine calf risk identification protocols. The objective of this study was to validate an automated leukocyte cell counter, the QScout BLD test (Advanced Animal Diagnostic, Morrisville, NC), in its ability to determine leukocyte differential cell counts in neonatal Holstein calves. From June to July 2018, blood samples collected in EDTA anticoagulant from 235 calves upon arrival at a veal research facility in Ontario, Canada, were evaluated by both the QScout BLD test and by manual leukocyte differential counts done by microscopy. These tests were compared using Lin's concordance correlation coefficient (−1 (perfect discordance) < ρ < 1 (perfect concordance)) and revealed very good agreement between tests for neutrophil counts $\rho = 0.83$ ($P < 0.001$); fair agreement for lymphocyte counts $\rho = 0.32$ ($P < 0.001$); slight agreement for monocyte counts $\rho = 0.14$ ($P < 0.001$); and, slight agreement for eosinophil counts $\rho = 0.20$ ($P < 0.026$). Test results were further examined to determine if they differed in their classification of samples as being above, within, or below reported 95% reference intervals for neonatal Holstein calves. Classification between tests resulted in very good agreement with only 4.2% and 5.8% disagreement in classification for neutrophils and lymphocytes, respectively. Moderate agreement for monocytes was observed with 23.3% classified differently; and, poor agreement was observed for eosinophils with 70.3% classified differently. Further study is required to determine the role of the leukocyte profile in risk assessment of neonatal calves. However, rapid leukocyte differentials that can be measured on farm present a promising addition for potential use in selective antimicrobial therapy protocols with the purpose of reducing antimicrobial use in veal and dairy beef facilities at arrival.

Key Words: calf, leukocyte, differential

358 Colostrum supplementation with omega-3 fatty acids alters plasma fatty acid profile and inflammatory mediators in newborn calves during the first week of life. J. Opgenorth*, L. M. Sordillo, A. L. Lock, J. C. Gandy, and M. J. VandeHaar, *Michigan State University, East Lansing, MI.*

Unresolved inflammation and oxidative stress (OS) can lead to decreased calf vitality. Omega-3 fatty acids (n-3 FA) produce cell mediators during metabolism that function to resolve and repair inflammatory and oxidative damage. We hypothesized n-3 FA enriched oils supplemented up to

120 mL to colostrum would linearly decrease OS, increase n-3 byproducts of FA metabolism, and increase plasma n-3 FA during the first week of life. Twenty-four Holstein calves were randomly assigned to receive 0 (Con), 30, 60, or 120 mL of a 1:1 mix of fish and flax oils (FFtrt 30, 60, 120) supplemented in colostrum. All calves received 3 L colostrum within 6 h of birth. Blood was sampled before colostrum feeding and on 1, 2, 4, 7, and 14 d of age to assess plasma FA, phospholipid FA, oxidant status, and oxylipid concentrations. Plasma FA and oxylipids were determined with liquid chromatography-mass spectrometry, and phospholipid FA were determined with gas-liquid chromatography. Health indicators were observed daily. Data were analyzed with a mixed procedure in SAS version 9.4 including treatment, sex, and day as fixed effects and calf and block as random effects. FFtrt 30, 60, and 120 increased n-3 free FA concentrations by 23 to 90% in the first week of life ($P < 0.01$). Compared with control, FFtrt linearly increased n-3 FA of plasma phospholipids (Con: 4.3, FFtrt 30: 4.7, FFtrt 60: 5.7, FFtrt 120: 6.2 g/100g ; $P < 0.01$) and some n-3 FA derived oxylipids such as 14,15-dihydroxy-eicosa-tetraenoic acid (14,15-Di-HETE) ($P < 0.01$) and 19,20-dihydroxy-docosapentaenoic acid (19,20-Di-HDPA) ($P = 0.01$), but did not change oxidant status ($P = 0.35$). Treatments did not alter calf health or growth ($P > 0.22$). All variables returned to control values by d 14. In conclusion, a colostrum supplement of n-3 FA administered in volumes of 30, 60, and 120 mL linearly increased plasma concentrations of n-3 FA and n-3 FA metabolites, but did not alter overall oxidant status.

Key Words: omega-3, oxidative stress, oxylipids

359 Extracellular vesicles modulate pro-inflammatory signaling in bovine macrophages.

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Exosomes are extracellular vesicles that are released into circulation to facilitate communication between cells. These vesicles transport a variety of cargo, including cytokines, bioactive lipids, and regulatory RNA, that can modulate immune function. Immune suppression exhibited by dairy cows during early lactation may involve exosome-mediated communication between immune cells. We sought to determine whether fatty acids that are elevated in circulation of dairy cows during early lactation can alter exosome-mediated inflammatory signaling. Specifically, we studied the ability of bovine exosomes to alter immune responses of primary bovine monocyte-derived macrophages (MDM). Circulating monocytes were isolated from 6 healthy mid-lactation Holstein cows. Cells were incubated for 7–10 d to allow for differentiation into MDM. Cells were treated with either fatty-acid-free bovine serum albumin (BSA; control) or palmitic acid (PA; 100 μ*M*) plus BSA carrier for 6 h before exosomes were isolated from culture media. Untreated MDM were incubated for 12 h with either no treatment or exosomes from PA or BSA treatment, equalized by protein concentration of the isolated exosomes. Cells were then incubated for 6 h with or without LPS (100 ng/mL) before media was harvested for cytokine analysis. Treatment with exosomes from either control or PA-exposed MDM increased TNFα concentrations independently of LPS treatment ($P < 0.001$ compared with untreated cells), and in fact, PA exosome treatment resulted in greater TNFα concentrations than LPS treatment ($P = 0.03$). Surprisingly, PA exosomes also attenuated the TNFα response to LPS compared with control exosomes ($P = 0.04$). These results suggest that PA-treated exosomes caused an increase in basal inflammatory state of MDM but made them refractory to further inflammatory stimuli. Alterations in circulating metabolites may have both direct and indirect effects on inflammatory signaling, and further investigation of exosome signaling may contribute to our understanding of immune function during times of stress.

Key Words: exosome, immune function, transition cow

360 Deep RNA-Seq reveals genetics and nutritional regulation of miRNomes in mammary gland of lactating Holstein and Montbéliarde cows.

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The mammary gland (MG) is a complex secretory organ synthetizing milk, the production and composition of which vary under genetic and nutrition regulations. The mechanisms underlying the variations are not totally understood. MicroRNAs (miRNAs, small non-coding RNAs) regulate the expression of genes involved in many cellular processes, including in MG. The objective was to identify the effects of feed restriction and dairy breed on bovine MG miRNomes. Five Holstein and 6 Montbéliarde multiparous cows in midlactation (165 ± 21 DIM) underwent 6 d of feed restriction (meeting 50% of NE_L requirements calculated before the challenge). Milk, fat, protein and lactose yield were measured before and during restriction. MiRNomes were analyzed by RNA-Seq using Illumina HiSeq 4000 from MG biopsies performed 1 d before (control) and on d 6 of feed restriction. Statistical analyses for milk production and composition and for miRNAs were performed using mixed models of SAS and the DESeq2 package of R, respectively. Significance was considered at $P_{adj} = 0.05$. As expected, milk, fat, protein, and lactose yields were lower in Montbéliarde than in Holstein cows and decreased by feed restriction. RNA-Seq analyses revealed 623 distinctly expressed miRNAs, among which 596 are known and 27 predicted. Breed influenced the expression of 19 miRNAs during the control period. The restriction modified the expression of 33 miRNAs in MG of Holstein cows, but only 2 miRNAs tended ($p_{adj} = 0.10$) to change in MG of Montbéliarde cows. Three miRNAs (miR-25, miR-2898, and miR-500) were commons between those modified by restriction and by breed. MiR-25 presented a high expression in lactating MG (over 4000 counts) and is known to repress triacylglycerol synthesis and lipid accumulation in mammary epithelial cells. The expression of miR-25 was higher whereas milk fat were lower in Montbéliarde and after restriction. In conclusion, we showed genetics and nutrition regulation of MG miRNomes, which suggest a potential role of miRNAs MG function and may be related to milk production and composition.

Key Words: microRNA, mammary gland, energy balance

361 Genome-wide association study in colostrum reveals QTL for natural antibodies in Swedish dairy cattle.

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Colostrum with sufficient antibodies is essential for the newborn calf, as it requires this passive immunity to survive until weaning. High variation in the amount of colostrum antibodies in Swedish dairy cows has been reported, with a large proportion having low antibody levels. Natural antibodies (NAb) are produced without any antigenic stimulation and target self-antigens and pathogen-associated molecular patterns

(PAMPs). Our objective was to estimate genetic parameters and detect quantitative trait loci (QTL) for 2 NAb isotypes (IgG, IgM) in colostrum binding keyhole limpet hemocyanin (KLH) and muramyl dipeptide (MDP). Three experimental farms were included in the study, 1719 colostrum samples from 1313 cows between 1 to 6 parities, calving from January 2015 to April 2017 were collected. 70% of the animals were Swedish Red and 30% Swedish Holstein. Antibodies were measured from colostrum using indirect ELISAs. To estimate genetic parameters, a linear mixed model with repeated measures (different calvings from the same cow) was run using ASReml 4, correcting for cow parity number, time from calving to colostrum sampling and breed, including herd-year-season of calving and sample storage plate as random effects. An imputed 50K SNP array from a LD 7K array was used for the Genome-wide association study (GWAS), running the same model but including the SNP genotype as a fixed effect. Heritabilities for colostrum NAbs ranged from 0.15 to 0.27, with a permanent environment effect for IgG isotypes accounting for 30% of the variance and for IgM ranging from 15 to 19%. Genetic correlations between IgG and IgM ranged from 0.1 to 0.4. The GWAS revealed one QTL on BTA3 for IgM (KLH and MDP), the latter comprised of 7 SNPs ($-\log_{10}(P) = 4.4$), 2 significant and 5 suggestive, ranging from 80 to 105 Mbp and another QTL on BTA7 for IgG (KLH and MDP) consisting of 3 SNPs ($-\log_{10}(P) = 4.1$), from 85 to 113 Mbp. Our results suggest that natural antibodies can potentially provide an effective tool to improve colostrum quality using genetic selection.

Key Words: colostrum, natural antibodies, genome-wide association study (GWAS)

Breeding and Genetics: Breeding Strategies and Male Fertility

362 Realized genetic selection differentials in Canadian Ayrshire dairy cattle herds. B. A. Hagan*[1], J. Moro-Mendez[2], and R. I. Cue[1], [1]*Animal Science Department, McGill University, Ste-Anne-de-Bellevue, QC, Canada,* [2]*Dairy consultant, Montreal, QC, Canada.*

Estimated breeding values for Lifetime Performance Index (LPI), production traits, mammary system (MS), somatic cell score (SCS) and herd life (HL) from Canadian Ayrshire bulls and cows born from 1950 and 1960 respectively, were used to estimate and study realized genetic selection differentials (GSDs). The objectives of the study were to define and determine the progress that has been achieved (realized GSDs) along the sire of bull (SB), dam of bull (DB), sire of cow (SC) and dam of cow (DC) pathways of genetic improvement and the variabilities among herds in realized GSD. The mean annual realized GSDs of LPI between 1980 and 2015 were 101.7, 322.9, 83.0 and −7.2 points for SB, DB, SC and DC respectively. There were, however, little to no selection for LPI in the DC path. The mean realized GSDs per year of conception of offspring (YOC) for the DB were higher than the other pathways and increasing for LPI, 305-d milk yield (MY), 305-d fat yield (FY) and 305-d protein yield (PY). For mammary system, there were increasing realized GSD in the DB and SC paths, but an irregular pattern of selection in the SB path. There were no clear trends in realized GSDs of the SB, DB and SC paths for HL between 1980 and 2015 while favorable and decreasing trends were observed for SCS in the 3 most influential paths of selection. Year of conception significantly influenced realized GSD of AI bulls, but not replacement cows in the Ayrshire population. Housing system (HS), agricultural region and the interaction between them and with YOC were largely not important in realized GSDs of AI sires and replacement cows. Variations in realized GSD due to herd and herd × YOC were significant and contributed between 0.6 and 5.5% and 4.5 to 30.2% respectively of the total variation in realized GSD for sires of bulls. There were also significant variations due to herd and herd × YOC for the DC ranging between 6.3 and 13.8% and between 0 and 1.1% respectively. This study demonstrates considerable variations among Ayrshire cattle herds in their selection of economically important traits.

Key Words: Ayrshire, realized genetic selection differential, dairy

363 Genetic update of lost Holstein male lineages. C. D. Dechow*[1], J. Ziegler[2], C. G. Sattler[2], H. Wei[3], and H. Blackburn[4], [1]*Pennsylvania State University, University Park, PA,* [2]*Select Sires Inc., Plain City, OH,* [3]*Trans Ova Genetics, Sioux Center, IA,* [4]*National Animal Germplasm Program, Fort Collins, CO.*

Only 2 Holstein male lineages remain since the introduction of artificial insemination (AI), suggesting there is limited Y-chromosome variation. Two additional male lines were present at the beginning of the AI era with genetic merit that was comparable to the founders of the current Y-chromosome lineages. The objective of this research was to resurrect and modernize those 2 lost lineages. Semen from ZIMMERMAN ALSTAR PILOT (born in 1954) and a ROSAFE CALIBAN (born in 1953) son from the University of Minnesota control line experiment (CUTHBERT) was available from the National Animal Germplasm Program (NAGP) repository. In vitro produced embryos sired by CUTHBERT were generated at Trans Ova Genetics and implanted as fresh embryos at the Penn State dairy herd (n = 12) with 3 bulls and 3 heifers born in March 2017. PILOT semen had poor motility (3%), but embryos were successfully produced with 15 fresh embryos resulting in 4 Pilot sired bulls and 5 Pilot sired females born in November 2017. Five bulls (2 Caliban sons and 3 Pilot sons) were transferred to Select Sires Inc. for semen collection with Lifetime Net Merit (NM$) values ranging from −$82 (5th percentile) to +$130 (14th percentile) and Lifetime Grazing Merit ranging from −$5 to +$233. All 5 had $NM that exceeded parent average and increased from sire to son 3.4 genetic standard deviations because the bulls were mated to modern elite females and because they have low genomic future inbreeding levels. This research demonstrates that semen stored near the beginning of the AI era is viable and of sufficient quality to facilitate a rapid genetic update of rare or lost lineages. Lower relationship to the commercial population may lead to genetic merit increases that exceed previously published predictions, supporting a broader use of samples maintained in a germplasm repository. Semen from the 5 bulls is commercially available and has been added to the NAGP repository to facilitate research and further efforts to restore lost Y-chromosome lineages. This project will also serve as an evaluation of methods to reintroduce lost genetic diversity during an era of rapid inbreeding.

Key Words: genetic diversity, male lineage, Y chromosome

364 Effect of genomic selection on rate of inbreeding and effective population size in North American Holstein and Jersey dairy cattle populations. B. Makanjuola*[1], F. Miglior[1,2], M. Sargolzaei[1,3], C. Maltecca[1,4], F. Schenkel[1], and C. Baes[1,5], [1]*Centre for Genomic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Ontario Genomics, Toronto, ON, Canada,* [3]*HiggsGene Solutions Inc., Guelph, ON, Canada,* [4]*Department of Animal Science and Genetics Program, North Carolina State University, Raleigh, NC,* [5]*Institute of Genetics, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Genetic diversity in livestock populations is a significant contributor to the sustainability of animal production, and also, it allows animal production to become more responsive to environmental changes and market demands. The loss of genetic diversity poses a potential plateau in production and may also result in loss of fitness or viability in animal production. In this study, we investigated the rate of inbreeding (ΔF) and effective population size (Ne) as important quantitative indicators of genetic diversity and evaluated the effect of the recent implementation of genomic selection on the loss of genetic diversity in North American Holstein and Jersey. Pedigree and genotype data of 214,566 and 95,732 Holstein and Jersey animals, respectively, born between 1990 and 2018 inclusively were provided by the Canadian Dairy Network (CDN). Estimated average pedigree inbreeding coefficient was 7.74 and 7.20% for Holsteins and Jerseys, respectively. The corresponding values for the genomic inbreeding coefficient was 13.61 for Holsteins and 21.16% for Jerseys. Generation interval for the whole 29-year time period averaged approximately 5 years for all selection pathways combined. The ΔF per generation was estimated to be 1.03, 1.64% for Holstein animals and 0.54 and 0.42% for Jersey animals using pedigree and genotype data, respectively. The estimated effective population size ranged from 30 to 48 animals for Holsteins and 93 to 119 animals for Jerseys. The current state of genetic diversity following the implementation of genomic selection calls for measures and means of controlling the rate of inbreeding, which will help to manage and maintain farm animal genetic resources.

Keywords: genetic diversity, rate of inbreeding, effective population size

365 A web-based mating program for global optimization on commercial dairy herds. C. Sun, S. Westberry*, D. Kendall, and D. Castellani, *STgenetics, Navasota, TX.*

Genomic selection has become a standard tool in dairy cattle breeding to help to accelerate genetic gain, and the majority of dairy inseminations are to young genomic bulls. Higher rates of inbreeding under genomic selection means that new methods of control at a population, breeding company, and herd level are becoming increasingly important. Chromosomal Mating (CM) is a web-based mating program that was developed by ST-Genetics to accommodate these challenges. Using linear programming (LP) methods, CM was designed to control inbreeding and lethal haplotypes and maximize predicted producing values (PPV) of future offspring through the optimization of a linear objective function (the PPV of the offspring) subjecting to the above constraints. CM uses the genetic merit of an animal after removing the adjustment of expected future inbreeding from the official PTA. Within the framework of CM, the inbreeding penalty on PPV is based on economical values rather than setting a threshold value that cannot be exceeded. CM has 3 main platforms: genomic mating, pedigree mating, and corrective mating. Genomic relationship files provided by CDCB (includes 5731 bulls and > 1.3 million females) are employed for genomic mating, while females with missing genomic relationships automatically receive pedigree-based mating by tracing back the pedigree as many generations as possible for the calculation of pedigree relationship matrices. For corrective mating, a mobile application was also developed to score type traits of females in the field, and the data are instantly uploaded to database. Our database holds more than 5 million animals' pedigree and all the bulls' information; female data can be uploaded through a web interface by the user. The computation is very efficient and the user receives results within a few minutes. LP was much better on simultaneously maximizing total PPV and reducing inbreeding, and results indicated that the average progeny PPV increased 17% and inbreeding decreased 30% for Holstein relative to random mating.

Key Words: mating program, genomic selection, inbreeding

366 Effects of recent and ancient inbreeding on performance of Dutch Holstein Friesian dairy cattle. H. P. Doekes*[1,2], R. F. Veerkamp[1], P. Bijma[1], S. J. Hiemstra[2], G. de Jong[3], and J. J. Windig[1,2], [1]*Animal Breeding and Genomics, Wageningen University & Research, Wageningen, the Netherlands,* [2]*Centre for Genetic Resources the Netherlands, Wageningen University & Research, Wageningen, the Netherlands,* [3]*Cooperation CRV, Arnhem, the Netherlands.*

Inbreeding decreases animal performance (inbreeding depression), but not all inbreeding is expected to be equally harmful. Inbreeding on recent ancestors is expected to be more harmful than inbreeding on more ancient ancestors, because of purging. Purging is the removal of deleterious recessive alleles over time by selection. We investigated inbreeding depression in Dutch Holstein Friesian cattle, expecting to find stronger effects of recent inbreeding compared with ancient inbreeding. The effect of inbreeding on yield, fertility and udder health traits was determined with linear mixed models using 38,792 first-parity cows. Pedigree data were used to compute traditional inbreeding (F_{PED}) and 75k genotype data were used to identify regions of homozygosity (ROH) and compute ROH-based inbreeding (F_{ROH}). Inbreeding depression was apparent, e.g., a 1% increase in F_{ROH} was associated with a decrease in 305-d milk yield of 36.3 kg (SE = 2.4), an increase in calving interval of 0.48 d (SE = 0.15) and an increase in mean somatic cell score in d 150 to 400 of 0.86 units (SE = 0.28). Distinguishing recent from ancient inbreeding gave mixed results. For example, only very long ROHs (indicating more recent inbreeding) significantly increased calving interval, whereas both long and short ROHs decreased protein yield. Across all traits, standard errors were larger for inbreeding that was more ancient. When F_{PED} was split into new and ancestral components, based on whether alleles were identical by descent for the first time or not, there was clear evidence of purging. For example, a 1% increase in new inbreeding was associated with a 2.2 kg (SE = 0.4) decrease in 305-d protein yield, compared with a 0.9 kg (SE = 0.8) increase for ancestral inbreeding. The mixed results we obtained may be partly due to difficulties in determining ancient inbreeding. Distant ancestors are less well registered, and short ROHs may be less reliable than long ROHs. Furthermore, selection history is complex and purging may have acted on some, but not on all alleles. Results suggest that, despite the presence of purging, both recent and ancient inbreeding contribute to inbreeding depression and should be considered in management strategies.

Key Words: inbreeding depression, purging, dairy cattle

367 Crossbreeding affects the production performance of dairy cows exposed to a range of temperature and humidity in a pasture-based system. J. Graham*[1], S. Biffani[2], and F. Tiezzi[1], [1]*Department of Animal Science, North Carolina State University, Raleigh, NC,* [2]*Istituto di Biologia e Biotecnologia Agraria, Milan, Italy.*

The aim of this study was to evaluate the response in production traits of different crossbred combinations exposed to increasing temperature and humidity. Data included 15,790 test-day records from purebred and crossbred cows (n = 713) located at the North Carolina Department of Agriculture Research Station (Goldsboro, NC) from 1996 to 2018. Traits analyzed were: milk yield (MY), fat percentage (F%), protein percentage (P%), somatic cell score (SCS), fat kilogram (Fkg), protein kilogram (Pkg), total somatic cell score (TotSCS) and fat to protein ratio (F:P). The 8 traits were analyzed by fitting a mixed model and a random regression model, with heterogeneous residual variances as defined by the environmental descriptor. Fixed effects in the mixed model included stage of lactation, parity, environmental descriptor (E), breed group (BG) and the interaction between E and BG (E×BG). Random effects included test date and animal. BG consisted of 8 different Holstein (H) and Jersey (J) combinations: H×H, J×J, H×J, J×H, J×JH, H×JH, J×HJ, H×HJ. Environmental descriptors were evenly distributed into 5 classes (class 5 being the most challenging). E×BG estimates were used to determine reranking of breed groups when exposed to more challenging environments. BG that reranked between extreme classes of E were: H×H vs H×J for MY, H×JH vs H×J for Pkg. Fixed effects in the random regression model consisted of stage of lactation, parity and zero, first and second-order Legendre polynomials on E nested into the random effects of breed (with breed relationship matrix), additive genetic (with pedigree relationship matrix) and permanent environmental. The random effect of breed had larger variance explained than additive genetic for Pkg (intercept) and F%, Fkg, Pkg and F:P (slope). For the same traits, intercept and slope of the breed random effect showed similar amounts of variance explained, indicating a sizable breed by environment interaction. Mean heritability estimates over values of E were ~0.2 for MY, F%, P%, and ~0.1 for all others. Heritability decreased over values of E for the traits F%, P%, Fkg, Pkg, F:P, and increased for all others.

368 Consequences of crossbreeding, genotyping, and use of sexed semen and beef semen on genetic merit and profitability in Swedish dairy herds. J. Clasen*[1], M. Kargo[2,5], S. Østergaard[3], W. Fikse[4], L. Rydhmer[1], and E. Strandberg[1], [1]*Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*Department of Molecular Biology and Genetics, Science and*

Technology, Aarhus University, Foulum, Denmark, [3]Department of Animal Science, Science and Technology, Aarhus University, Foulum, Denmark, [4]Växa Sverige, Uppsala, Sweden, [5]SEGES, Danish Agriculture & Food Council, Skejby, Denmark.

Crossbreeding, genotyping, sexed semen and beef semen can improve herd economy in different ways. The economic and genetic consequences of combining them are complex and need to be studied further. We have investigated 22 different scenarios of average Swedish herds having only purebred Holstein or managing systematic terminal crossbreeding. The crossbreeding system implied having Holstein purebreds and terminal F1 Swedish Red x Holstein crossbreds within the same herd. The scenarios had different combinations of strategic use of sexed semen with or without genotyping of purebred heifers to select females to produce future replacement heifers based on genomic breeding values. Use of beef semen was included in all scenarios to produce calves for slaughter and limit the surplus of replacement heifers. In the crossbreeding system, all dairy crossbred females were serviced with beef semen, i.e., they had no influence in breeding. The scenarios were simulated using a combination of 2 simulation models from Aarhus University, Denmark: SimHerd Crossbred that simulates herd dynamics including heterosis effects but not additive genetic effects, and ADAM that simulates the additive genetic herd level within scenario. Crossbreeding had positive effects on the herd profit compared with purebreeding, due to heterosis and increased income from beef x dairy slaughter calves. Interestingly, when large amounts of sexed semen were used, the purebred cows in the crossbreeding scenarios achieved a larger genetic merit compared with the purebreeding scenarios. Furthermore, genotyping and sexed semen had clear positive effects on the genetic merit. Use of sexed semen allowed for more use of beef semen, which had positive effects on the herd profit. Crossbreeding reduced the number of purebred heifer calves to be genotyped, which reduced the annual costs of genotyping. In general, any combination of the breeding tools were economically beneficial, compared with not using any of them. The most favorable scenario on herd profit included crossbreeding, sexed semen in 50% of heifers and in 25% of cows, and genotyping of all purebred heifers.

Key Words: crossbreeding, genotyping, herd economy

369 Genomic prediction of male fertility in Jersey dairy cattle.

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Service sire has been recognized as an important factor affecting herd fertility in dairy cattle. Recent studies have reported accurate predictions for Holstein bull fertility using genomic data. This study was conducted to evaluate the feasibility of genomic prediction of sire conception rate (SCR) in US Jersey bulls using alternative predictive models. Data set consisted of 1.5k Jersey bulls with SCR records and 95k SNP spanning the entire genome. Both linear and Gaussian kernel-based models were used either fitting all SNP or subsets of markers with presumed functional roles, such as SNP significantly associated with SCR or SNP located within or close to known genes. The prediction of Jersey SCR records using a multi-breed reference population including the entire US Holstein SCR data set (11.5k bulls) was also investigated. Model predictive ability was evaluated using 5-fold cross-validation with 10 replicates. The entire SNP set exhibited predictive correlations around 0.30. Interestingly, either SNP marginally associated with SCR or genic SNP achieved higher predictive abilities than their counterparts using random sets of SNP. Among alternative SNP subsets, Gaussian kernel models fitting significant SNP achieved the best performance with increases in predictive correlation up to 7% compared with the standard whole-genome approach. Notably, the use of a multi-breed reference population allowed to achieve predictive correlations up to 0.315, gaining 8% in accuracy compared with the standard model fitting a pure Jersey reference set. Overall, our findings indicate that genomic prediction of Jersey bull fertility is feasible. The use of Gaussian kernels fitting markers with relevant roles and the inclusion of Holstein records in the training set seem to be promising alternatives to the standard whole-genome approach. These results have the potential to help the dairy industry improve Jersey sire fertility through accurate genome-guided decisions. Future research should investigate the benefits of using an across-country Jersey reference population.

Key Words: biologically informed model, kernel-based prediction, sire conception rate

370 Investigation of genetic variation in global DNA methylation in bull semen and its relationship with semen quality and fertility parameters.

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As an important mediator of gene expression, epigenetic modifications, such as DNA methylation, affect transcription and cause variation among phenotypes. DNA methylation has received much attention in studies of male fertility in human and livestock. In bulls, although epigenetic modifications have been found to significantly influence semen quality, the mechanisms and patterns underlying these modifications are not fully understood. Therefore, the objectives of the study were to determine the effects of global methylation on sperm quality parameters and to investigate genetic variability in semen DNA methylation among sire families (n = 27) and paternal lines (n = 4). Semen samples and semen quality parameters were obtained from bulls (n = 402) selected based on pedigree and relevance of phenotypic information. From each sample, DNA extraction was performed and global methylation was measured on 50–200 ng of DNA using the MethylFlash Methylated DNA Quantification Kit (Colorimetric) with absorbance readings measured at 450 nm. A linear mixed model was utilized to predict traits related to sperm morphology, motility, and viability, using global sperm methylation and sires as fixed effects. Additionally, variance components and heritability (h^2) were estimated for global methylation of overall sire families and paternal lines respectively. Mean methylation of the sampled bulls ranged from 0.383 to 1.994%-5mC, and from 0.982 to 1.204%-5mC across sire families and paternal lines respectively. For the analyzed semen parameters, methylation was a significant effect for motility and normal spermatozoa ($P < 0.10$), while sire was a significant effect for viability ($P < 0.10$). Estimated variance for sire family and paternal line was 0.065 and 0.018 respectively. Heritability estimates for global methylation were 0.179 for sire family variance and 0.053 for paternal line variance. The current study has established the first association between overall methylation and semen quality parameters, contributing to the selection of a new criterion for fertility in dairy bulls.

Key Words: DNA methylation, semen quality, genetic variation

371 Dissecting the genetic role of sex chromosomes on dairy bull fertility.

H. A. Pacheco*[1], F. M. Rezende[1,2], and F. Peñagaricano[1], [1]*University of Florida, Gainesville, FL,* [2]*Universidade Federal de Uberlândia, Uberlândia, Minas Gerais, Brazil.*

Improving reproductive performance remains a major goal in dairy cattle. Service sire has been recently recognized as an important factor affecting herd fertility. Most genome-wide mapping studies for bull fertility have focused on genetic markers only on autosomal chromosomes. Little is known, however, about the genetic role of sex chromosomes on dairy bull fertility. As such, the main objective of this study was to dissect the genetic contribution of chromosomes X and Y (BTAX and BTAY) on sire conception rate (SCR) in US Holstein bulls. The analysis included a total of 5.1k Holstein bulls with SCR records and genotypes for roughly 3.3k SNPs located on the pseudoautosomal region (PAR), 49.7k BTAX-specific SNPs, and 1.2k BTAY-specific SNPs. The effect of each SNP on SCR was evaluated using an efficient 2-step mixed model-based approach. Three regions located on BTAX ($-\log_{10}P$ = 6.7) and one region located on PAR ($-\log_{10}P = 4.9$) showed significant associations with service sire fertility. Interestingly, these regions harbor candidate genes, such as TBL1X and PIH1D3, that are directly implicated in spermatogenesis and testis development. On the other hand, BTAY showed very low genetic variability, and none of the segregating markers was associated with SCR. This study provides evidence of the importance of both PAR and BTAX-specific regions on male fertility in dairy cattle. These findings may help to improve conception rates in dairy herds through accurate genome-guided decisions on bull fertility.

Key Words: chromosome X, pseudo-autosomal region, sire conception rate

372 Whole-genome homozygosity mapping reveals candidate regions affecting male fertility in US Holstein cattle. J. P. Nani* and F. Peñagaricano, *University of Florida, Gainesville, FL.*

Balancing rapid genetic progress and maintenance of adequate genetic diversity has become one of the major challenges of the dairy industry worldwide. The loss of genetic diversity can be monitored using the inbreeding coefficient. Inbreeding increases the proportion of loci that are homozygous throughout the genome, some of which causes homozygosity of recessive alleles that negatively affect an animal's performance. This phenomenon is known as inbreeding depression and tends to be most pronounced on fitness-related traits, such as male fertility. Traditionally, inbreeding has been monitored using pedigree information, or more recently, genomic data. Alternatively, the loss of genetic diversity can be quantified using runs of homozygosity (ROH), i.e., contiguous lengths of homozygous genotypes observed in individual chromosomes. The objective of this study was to evaluate the association between ROH and sire conception rate (SCR). The ROH were evaluated using about 300k genetic markers in 11,790 US Holstein bulls. Interestingly, the sum, mean, and maximum length of ROH were negatively associated with bull fertility. The association analysis between ROH and sire fertility was performed comparing 300 high-fertility vs. 300 low-fertility bulls. Both the average and sum of ROH length were significantly higher in the low-fertility group. The enrichment of overlapping ROH regions in low-fertility bulls was assessed using a Fisher's exact test. Eight different ROH regions were significantly enriched in low-fertility bulls. Notably, these regions harbor many genes that are only expressed in testis or are reported in the literature to be responsible for male infertility. The results of this study can help not only to manage inbreeding in genomic selection programs by designing custom mating schemes, but also to better understand the mechanisms underlying male fertility.

Key Words: inbreeding, runs of homozygosity, sire conception rate

Dairy Foods Symposium: Recent Advances in Dairy Food Safety Research

373 Overview of National Dairy Council Listeria Research Consortium, industry needs identification, and funding of research gaps. T. Stubbs*, *National Dairy Council, Rosemont, IL.*

The Innovation Center for US Dairy (IC) is a non-profit organization led by the National Dairy Council with a board comprising CEOs of dairy co-ops, processors, trade organizations, and dairy farmers. The Food Safety committee of the IC leverages the knowledge of over 75 volunteers to identify ways to improve food safety industrywide through best practice sharing, training workshops, publishing best practice guides, and funding research through the Listeria Research Consortium. In addition to funding research projects, the consortium team monitors scientific developments, convenes expert panels, and regularly gathers industry input on pathogen controls gaps and needed tools. Since its creation in 2015, the consortium has funded 10 multi-year research projects at universities.

Key Words: *Listeria*, research, dairy

374 Protective cultures: Applications to control spoilage organisms and pathogens in high-risk cheese. G. Makki, M. Lawton, S. Kozak-Weaver, and S. Alcaine*, *Cornell University, Ithaca, NY.*

Current estimates suggest that dairy accounts for nearly 20% of all food loss at food service, retail, and the home. A myriad of factors plays into dairy loss, but microbial spoilage is a significant one. The contamination by pathogenic bacteria of dairy products, leading to large food recalls and subsequent food destruction, is another important though less predictable contributor to food loss. The concurrent consumer push for sustainability and clean ingredient labels is a challenge for dairy producers and calls for the application of new tools. These tools are particularly important for fresh cheeses where high moisture content makes these products more susceptible to microbial contaminants. Protective cultures, i.e., bacterial strains with ability to inhibit the outgrowth of undesirable microorganisms, embody tools with the potential to satisfy consumer and producer demands. Protective cultures are currently applied to some food products, but questions remain as to their efficacy. Our lab has begun to explore some of these questions in regards to fresh cheeses. In one study, we evaluated whether commercial protective cultures, currently utilized to control *Listeria monocytogenes* in fermented meats, would be effective in controlling *L. monocytogenes* in the high-risk cheese queso fresco. In our second study, we evaluated the breadth of inhibition of a set of protective cultures against an array of dairy relevant yeast and molds in both queso fresco and cottage cheese. Our results suggest that protective cultures can be effective tools for the inhibition of undesirable microorganisms. However, all protective cultures are not equal, with each having unique patterns of inhibition. Furthermore, the efficacy of a single protective culture is not necessarily consistent across product types. The takeaway for producers is that yes, protective cultures can be valuable clean label tools for microbial control, but they must be chosen with an understanding of the contaminants that are likely to occur in your facility, and if multiple product types are made, each may require a specific protective culture. Preliminary evaluation is a key step in effectively leveraging these microbial tools to protect dairy products.

Key Words: bioprotection, spoilage, pathogen

375 Functionalized mesh materials for *Listeria* control in dairy applications. S. M. C. Ritchie*, M. Costa-Teixeira, M. P. Richier, J. A. Colburn, W. D. Baker, T. A. Hendrich, S. G. Brooks, and R. M. Summers, *University of Alabama, Tuscaloosa, AL.*

Pathogenic bacteria are ubiquitous in dairy systems. They are mostly eliminated by pasteurization, though improper treatment and recontamination can occur during downstream processing. Spiral elements are used widely in downstream processing of milk, and offer an excellent opportunity for mitigation of pathogens. Specifically, the feed spacer in spiral elements has been functionalized to provide anti-microbial activity. The surface may be further modified to target specific pathogenic bacteria. The purpose of this paper is to discuss development of these materials targeted for control of *Listeria monocytogenes* (LM) with applications in dairy processing, including milk and brine systems. The feed spacer mesh has been modified to include anti-microbial groups. Polypropylene (PP) feed spacer has been modified in several steps to obtain several types of quaternary amine (QA) groups. The QA groups are on polymer grafts attached to the PP spacer. Polymerization of the base polymer was achieved by photo-polymerization, and functional groups were added post-polymerization. We have characterized the polymerization kinetics as a function of time, monomer concentration, and initiator concentration. We have also demonstrated anti-microbial activity against gram-positive bacteria. We have also observed significant bacteria adsorption, which can be used to remove bacteria from large volume brine solutions without membrane filtration.

Key Words: dairy, safety, antimicrobial

376 Controlling *Listeria monocytogenes* in soft cheese with high voltage atmospheric cold plasma (HVACP) treatment. K. M. Keener* and Z. Wan, *Iowa State University, $Ames, IA.*

Queso Fresco (QF), a type of Hispanic-style soft and fresh cheese, is a popular food in Latin-American diet. Because of its high moisture content, near neutral pH, and moderate salt content, QF provides an optimal substrate for the growth of spoilage and pathogenic microorganisms. Currently, there are no effective commercial technologies to reduce microorganisms in soft cheeses such as QF. High voltage atmospheric cold plasma (HVACP) is a novel, non-thermal technology, which can be used to treat packaged food products and achieve significant reduction of pathogenic and spoilage microorganisms without compromising products' qualities. In this study, various gases, such as dry air and MA50 (50% CO_2, 50% N_2), were evaluated for inactivation of *Listeria innocua* (LI), a non-pathogenic surrogate for *Listeria monocytogenes*, in QF by HVACP treatment. Survival LI after HVACP treatments were enumerated on *Listeria* selective agar and by thin agar layer method for injured cell enumeration. Quality effects were analyzed for lipid oxidation, pH, color, texture and moisture content. Plasma characterization was done using optical emission spectrometry. The results have shown that a maximal of 4.9 $\log_{10}$ cfu/g LI reduction was achieved after dry air HVACP treatment. A slight increase in lipid oxidation was observed in QF samples after HVACP treatment in dry air. Negligible changes were observed in color, texture, moisture content and pH after HVACP treatments. Moreover, after direct HVACP treatments in dry air, LI population decreased with longer storage at 4°C. After 28 d, 1.4 $\log_{10}$ cfu/g LI population was found on treated QF while 7.4 $\log_{10}$ cfu/g was observed in control samples. Lastly, HVACP treatment in dry air reduced and inhibited the growth of background microorganisms. After 28 d of storage at 4°C, less than 2.0 $\log_{10}$ cfu/g background microorganisms was observed in treated QF, while more than 6.0 $\log_{10}$ cfu/g was recorded in control samples. The results demonstrate the efficacy of HVACP treatment for LI inactivation in QF and the potential of HVACP technology for non-thermal processing of delicate dairy products.

Key Words: atmospheric cold plasma, *Listeria innocua*, Queso Fresco cheese

377 Effects of acid type, fermentates, and culture selection on the safety of high moisture cheeses.

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High-moisture, low-acid cheeses, e.g., soft, Hispanic-style cheese, have been shown to support growth of *Listeria monocytogenes* during refrigerated storage. Previous studies have suggested that both acid type and pH are important to inhibit growth. Furthermore, cultured milk or sugars can be added as clean label ingredients to inhibit microbial growth. A third strategy is to utilize protective cultures to directly generate antimicrobial compounds that could be found in fermentates. For the first objective, model cheeses were prepared with direct acidification using 4 acid types (citric, lactic, acetic, propionic), 4 pH values (5.25, 5.50, 5.75, 6.00), 2 moisture levels (50, 56%), and single salt level (1.25%). Batches were inoculated with *L. monocytogenes* and stored at 4°C for 8 weeks. All citric acid cheeses (pH > 5.25) supported *L. monocytogenes* growth within 1–2 weeks. Lactic acid model cheeses supported *L. monocytogenes* growth at pH ≥ 5.50 within 1–4 weeks, but pH 5.25 prevented growth for 8 weeks. Acetic and propionic acid inhibited growth at pH < 5.75 for the duration of the study. Objective 2 tested 3 commercial fermentates (0.5, 1.0%) to enhance the inhibition in lactic acid cheese (pH 6.0, 56% moisture, 1.25% salt). All 3 fermentates delayed growth compared with the control, but only the cultured sugar-vinegar blend (containing acetic acid) inhibited growth for 8 weeks. Objective 3 tested 3 commercial protective cultures as adjuncts; none of the 3 cultures prevented listerial growth compared with the control for longer than 1 week at 4°C. The protective cultures were not effective at 4°C, whereas previous studies have shown that adjunct cultures are effective when cheese is temperature abused. These differences are likely due to the low rate of metabolism of the commercial cultures compared with *L. monocytogenes* at 4°C. These data confirm that acetic acid has greater inhibitory properties than lactic acid, and that modifying pH or the addition of a cultured sugar-vinegar blend will significantly influence *L. monocytogenes* growth. Additional study needs to be completed to identify protective cultures with the ability to produce antimicrobial components in soft cheeses stored at refrigeration temperatures.

Key Words: *Listeria*, high-moisture cheese, acid type

378 Interventions to control *Listeria monocytogenes* as a surface contaminant on high-moisture cheese.

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Despite efforts to control *Listeria monocytogenes* in dairy processing environments, contamination and subsequent outbreaks of listeriosis continue to occur. Among cheeses, listeriosis outbreaks have been disproportionality attributed to environmental contamination of soft, high-moisture products (e.g., Queso Fresco) in recent years. The ability of *L. monocytogenes* to grow on these products during refrigerated storage necessitates strategies to prevent contamination, reduce pathogen numbers, and limit growth during storage. This presentation will review recent research on approaches to control *L. monocytogenes* as surface contaminants on high moisture cheese including modified atmosphere packaging, protective cultures and other antimicrobials used alone and in combinations as dip and coating applications. We will conclude with a focus on the specific applications of hydrogen peroxide and address future research needs.

Key Words: cheese, *Listeria*, interventions

Dairy Foods: Chemistry

379 Calibration samples and calibration adjustment for mid-infrared milk fatty acid analysis.

D. Barbano*, *Cornell University, Ithaca, NY.*

The objective of this work was to develop a method to produce reference milks for calibration of mid-infrared (MIR) milk analyzers for milk fatty acid (FA) analysis and validate between laboratory agreement. Recently, MIR PLS models for de novo, mixed origin, preformed milk FA, FA chain length (CL) and FA unsaturation (double bonds/FA), milk estimated blood NEFA have been developed for Delta MIR milk analyzers for use to manage dairy cow feeding and health. Today, 9 commercial instruments are running milk FA analysis in North America using the PLS dairy herd management FA models developed at Cornell and calibrated with reference milk produced at Cornell. The 14-sample calibration set is made once every 4 weeks. Each sample has a reference value in g/100 g of milk for each major milk component and each milk FA, or group of FA measured. Values for each FA, or group of FA, divided by the sum of all FA measured by MIR (not by MIR measured fat test) to provide relative proportions of each that is independent of MIR milk fat concentration. This and the design of the PLS model makes the relative milk FA estimate independent of variation in fat concentration. Nine MIR Delta Instruments were calibrated for de novo, mixed, and preformed FA using the 14-sample calibration sample set 2 weeks before analysis of a set of 8 individual farm validation milks. The 8 validation milks ranged in fat content from 3.4 to 4.8 g/100 g milk in total fat content. For denovo, mixed origin, and preformed FA, FA chain length, and FA unsaturation the mean, mean difference (MD) of instrument minus GLC reference and standard deviation of the difference (SDD) were Mean 0.887, 1.214, 1.550, 14.745, and 0.298, MD −0.016, 0.080, −0.054, −0.06, and 0.00 and 0.028, 0.053, 0.058, 0.033, and 0.010, respectively, on the validation milks. Key instrument factors influence MIR milk fatty acid determination are variation in instrument homogenizer performance and moisture in the optical light path within the instrument.

Key Words: milk fatty acids, herd management, mid-infrared

380 Multi-laboratory performance evaluation of a spectrophotometric enzymatic method for measurement of milk urea nitrogen.

D. Barbano*, C. Coon, and M. Portnoy, *Cornell University, Ithaca, NY.*

The objective was to standardize an enzymatic method for measurement of milk urea nitrogen (MUN) and determine the within and between laboratory performance of the method by conducting an interlaboratory study. In the first step of the method, urea + water are converted to ammonia and CO_2. In the presence of glutamate dehydrogenase (GIDH) and reduced NADPH, ammonia (as ammonium ions: NH_4^+) reacts with 2-oxoglutarate to form L-glutamic acid and $NADP^+$. The amount of $NADP^+$ formed is stoichiometric with the amount of ammonia. For each mole of urea, 2 moles of NADPH are consumed. This is measured by the decrease in light absorbance at 340 nm. In November and December 2018 and January 2019, 9 labs tested 14 different milks in duplicate each month. Data were analyzed to remove statistical outliers using the Cochran and single and double Grubbs outlier tests. For November, December, and January 38, 8, and 6 statistical outliers, respectively, were removed before calculation of method performance statistics. With time and experience of using the method the number of statistical outliers has decreased. Within (S_r) and between (S_R) laboratory method performance statistics for the MUN reference method in November and December 2018 and January 2019 were S_r = 0.076, 0.083, and 0.069, S_R = 0.138, 0.121, and 0.104, RSD_r = 0.465, 0.502, and 0.395, RSD_R = 0.843, 0.736, and 0.595, r-value = 0.213, 0.231, and 0.193, R-value 0.385, 0.339, and 0.291, respectively. With time and experience the between laboratory performance of the method is improving. An official interlaboratory study of the method will be conducted to support Association of Official Analytical Chemists approval of the method for use as a reference method for calibration of infrared milk analyzers.

Key Words: enzymatic method, milk urea nitrogen, interlaboratory performance

381 Mid-infrared analysis of reconstituted milk powders.

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Our objective was to develop a method to analyze reconstituted milk powders and estimate the composition (fat, protein. lactose, and solids) of the original milk powder using mid-infrared (MIR). Nonfat dry milk (NFDM) powders (about 10 g) were dissolved in 90 g of lab grade (LG) water at 40°C and held for 30 min. The reconstituted powders (14) were cooled in ice and then held overnight at 4°C for rehydration. The reconstituted powders were analyzed for solids by forced air oven drying, fat by ether extraction, protein by Kjeldahl on a TN basis, and anhydrous lactose by an enzymatic method. The reference chemistry results on the liquids were used to calculate the composition of the original milk powders. The MIR milk analyzer was calibrated to display the powder composition on weight of component per weight of powder directly from the MIR milk analyzer. Traditional MIR filter models used for routine milk analysis, not partial least squares (PLS) models were used. A goal was to simplify calibration, make the calibration more global across instruments, allow the use of standard liquid milk samples for calibration of MIR analyzers in different factories, and avoid the need for development and maintenance of PLS models as is normally done with near IR NFDM powder analysis. The mean reference chemistry composition of the NFDM powders was moisture (mean 3.71%, min 2.17% and max 6.33%), fat (mean 0.60%, min 0.52% and max 0.81%), TN protein (mean 35.63%, min 33.48% and max 37.02%), and anhydrous lactose (mean 49.49%, min 47.56% and max 50.66%). The standard deviation of the difference (SDD) and standard error of prediction (SEP) between reference chemistry and instrument on the powders were fat (0.020% SDD, 0.025% SEP on a mean of 3.71%), TN protein (0.11% SDD and 0.11 SEP on a mean of 35.63%), lactose (0.13% SDD and 0.13% SEP on a mean of 49.64%), and moisture (0.27% SDD and 0.26% SEP on a mean of 3.71%). In the future, we will attempt to develop a MIR method to directly measure undenatured whey protein for thermal history classification of milk powders.

Key Words: mid-infrared, nonfat dry milk (NFDM), components

382 Infrared milk analyzers: Calibration samples for milk urea nitrogen.

M. Portnoy* and D. Barbano, *Cornell University, Ithaca, NY.*

Our objective was to add an orthogonal dimension of milk urea nitrogen (MUN) concentrations to an existing orthogonal (for fat, true protein, and anhydrous lactose) milk calibration sample set used by the USDA Federal Milk Marketing orders. MUN data is used to improve protein feeding efficiency, nitrogen utilization, and reproductive efficiency.

However, even though multivariate statistical tools are available for prediction of MUN through MIR analysis, calibration samples with a wide, well-distributed range of MUN concentrations (from 12 to 25 mg/100 g milk) to adjust slope and intercept to local conditions are needed. The calibration sample set was produced using a combination of pasteurization, gravity separation, and ultrafiltration to make milk-based ingredients, plus pure urea, pure lactose monohydrate and water to formulate the orthogonal design 14-sample set. Once the calibration milks including MUN were successfully developed and produced once every 4 wk, they were sent to 9 laboratories for determination of MUN using a chemical reference method (spectrophotometric enzyme-based assay expressed as mg/100 g milk) and were used to calibrate their MIR instruments starting in October of 2018. The MUN reference method had excellent performance with relative standard deviations (RSD) for within and between laboratories of <1%. Within (S_r) and between (S_R) laboratory method performance statistics for the MUN reference method in January 2019 were $S_r = 0.069$, $S_R = 0.104$, $RSD_r = 0.395$, $RSD_R = 0.595$, r-value = 0.193, R-value 0.291. MIR instruments use partial least squares (PLS) models to predict MUN from the MIR spectra for each milk. Different PLS models from one manufacturer to another may perform differently. Predicted MIR data for MUN produced by analysis of the orthogonal milk calibration sample set can be used to run a diagnostic evaluation of how well a MUN prediction model compensates for background variation in fat, true protein, and anhydrous lactose from milk to milk.

Key Words: mid-infrared, calibration, MUN

383 Evaluation of an automatic fat analysis system for determination of fat in dairy products. H. Jiang*, J. Romero, and S. Govindasamy-Lucey, *Wisconsin Center for Dairy Research, University of Wisconsin-Madison, Madison, WI.*

The official standard (reference) methods used for testing fat in dairy products involves acid hydrolysis and organic solvent extraction steps. These methods are labor intensive and time consuming; a simpler and rapid testing system would be desirable. This study aimed to validate an automatic fully enclosed fat analysis system (Hydrotherm-Soxtherm) for the analysis of fat content of dairy products by comparison to a standard method (modified Röse-Gottlieb). The automatic system consists of acid hydrolysis (Hydrotherm), which is based on Weibull-Berntrop gravimetric method (ISO 8262–3:205), and fat extraction by soxhlet method (Soxtherm). A single laboratory validation (SLV) was performed; linearity, precision, limit of detection (LOD), and limit of quantification (LOQ) were determined. As ultrafiltered (UF) permeate stream contains (almost) no fat, it was used as a blank sample to calculate LOD and LOQ. Fat quantification for fluids were performed by creating multi-point calibration with increasing fat contents (0.5–35%) by mixing UF permeate with cream samples. Trueness (how close a result is to the true value) was determined on milk samples (n = 5) and commercial cheeses (n = 48) containing fat contents ranging from 0.5 to 48%. A double-blind test was performed on 18 varieties of commercial cheese on 4 different days. Acceptability of this automatic system with respect to precision was determined by calculating Horwitz Ratios ($HorRat_R$). The LOD and LOQ values were determined to be 0.312g and 0.7839g per 100g, respectively. Linearity ($R^2 \geq 0.99$) was observed in the multi-point calibration method. Fat data obtained by the automated system were in very close agreement with the standard method for the commercial cheeses; there was no significant difference ($P > 0.05$) between the results obtained by the 2 methods, showing that the validated method is a suitable alternative. The $HorRat_R$ were found to be 0.3–1.3 with an average of 0.9, which is within the acceptable range for SLV. The automated method was less labor intensive and easier to use for routine standard fat analysis of dairy products.

Key Words: single lab validation, automatic fat analysis system, dairy products

384 Physicochemical properties of skim milk powder dispersions acidified by gluconic acid, hydrochloric acid, and citric acid. I. Choi* and Q. Zhong, *The University of Tennessee, Knoxville, TN.*

Skim milk powder (SMP) is an affordable option for acidic protein beverages. This application can be achieved with technologies allowing translucent dispersions with enhanced stability. The objective of the present study was to characterize SMP dispersions after dissociating casein micelles by acidification using gluconic, hydrochloric, or citric acid and subsequent heating. SMP was hydrated at 5% wt/vol in deionized water overnight, followed by acidification to pH 3.0 with gluconic, hydrochloric, or citric acid and subsequent heating at 90°C for 2 min. Samples were characterized for turbidity, hydrodynamic diameter, free calcium (Ca) ion concentration using an ion selective electrode, total Ca and phosphorous (P) concentrations using inductively coupled plasma optical emission spectrometry, zeta-potential, and transmission electron microscopy (TEM). Before heating, the SMP dispersion acidified with gluconic acid showed lower turbidity (394 NTU) than those acidified with hydrochloric (1491 NTU) or citric (1405 NTU) acid. However, this variation was significantly reduced after heating, showing 248, 431, and 599 NTU for the gluconic, hydrochloric, and citric acid treatments, respectively. The hydrodynamic diameter of dispersion decreased from 285 to 178 nm after acidification with gluconic acid and to 111 nm after heating, and no precipitation occurred after 2 weeks at 21°C. Ca and P concentrations in the serum of dispersions increased after acidification and heating. However, there was no linear correlation between the increased Ca and P concentrations and the reduced turbidity. The concentration of free Ca ions also increased from 112 mg/L before acidification to 342, 439, and 552 mg/L after acidification with gluconic, citric, and hydrochloric acid, respectively, and heating. The complex formation between Ca ions and chelating gluconate and citrate resulted in the lower measured Ca ion concentration than the HCl treatment. Heating did not affect zeta-potential of acidified SMP dispersions. Lastly, altered structure of casein micelle was confirmed by TEM. Calcium chelating acids may be used to produce novel acidic protein drinks based on SMP.

Key Words: casein micelle, colloidal calcium phosphate, skim milk powder

385 Effect of encapsulation on processing and storage stability of Vitamin D_2 in milk. G. Singh[1], P. K. Singh*[1,2], and H. Singh[2], [1]*GADVASU, Ludhiana, India,* [2]*Riddet Institute, Massey University, Palmerston North, New Zealand.*

Vitamin D is one of the fat-soluble vitamins, important for human nutrition. However, its low polarity and unstability are the main bottleneck in its food applications. It is generally considered that encapsulation improves the stability through protection from the adverse environmental conditions. The objective of present study was to quantify the processing and storage stability of encapsulated vitamin D_2 in milk systems. Vitamin D_2 fortified ghee (clarified butter fat) was taken as control sample which was compared with whey protein isolate (WPI) and Sodium caseinate (SC) stabilized nano-emulsions. All samples were stored under refrigerated condition at 4 ± 2°C temperature in capped glass vials. After 30 d storage period, concentration of vitamin D_2 in ghee reduced from

50.2 ± 1.1ppm to 21.9 ± 0.5ppm (56.37%) while during same period in WPI- and SC- stabilized nano-emulsions this concentration reduced from 37.66 ± 1.5 ppm to 22.97 ± 0.5 ppm (39%) and 35.81 ± 0.7 ppm to 24.00 ± 0.1 ppm (33.97%), respectively. Effect of encapsulation was further studied in liquid milk systems to assess its impact on the processing and storage stability of vitamin D_2. Fresh skim milk was fortified with WPI- and SC-stabilized dried emulsions and subjected to pasteurization and sterilization. Pasteurization leads to 39.36 and 37.28% loss of vitamin D_2 in milk fortified with WPI- and SC-stabilized dried emulsions, respectively. During 7 d storage of pasteurized milk, vitamin D_2 concentration reduced by 54.95 and 48.02%, respectively, for milk fortified with WPI- and SC-stabilized dried emulsions. Sterilization leads to 17.32 and 3.35% loss of Vitamin D_2 in milk fortified with WPI- and SC-stabilized dried emulsions, respectively. During sterilization though the processing loss of vitamin D_2 was low in milk fortified with casein proteins coated particles as compared with whey proteins coated particles however, during storage period of 90 d WPI was found to provide better stability as compared with sodium caseinate. It is concluded that encapsulation does give protection however, despite encapsulation processing and storage losses of vitamin D_2 in fortified milk was found to be significantly high.

Key Words: encapsulation, processing, stability

386 Gentle isolation of milk fat globule membrane material by microfiltration of raw milk. S. F. Hansen*[1], L. B. Larsen[1], L. Wiking[1], J. T. Rasmussen[2], S. A. Hogan[3], and J. Tobin[3], [1]*Department of Food Science, Aarhus University, Foulum, Denmark,* [2]*Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark,* [3]*Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

The milk fat globule membrane (MFGM) is a milk fraction with many potential applications, including addition to infant formulas, where it is suggested to facilitate neural development of the neonate. Severe heat treatment and mechanical processing is known to affect proteins and polar lipids associated with MFGM, with potential detrimental effects on its bioactivity and functionality. The present study describes a more gentle MFGM isolation method based on microfiltration of minimally processed milk, with perspectives for industrial implementation. Efficient separation of fat globules from milk proteins was achieved using polymeric 0.65 µm membranes with a significantly reduced casein and β-lactoglobulin content in the final MFGM isolate, when carried out before heat treatment (80, 85 or 88°C, 15s). Conversely, post-filtration pasteurization resulted in β-lactoglobulin contamination of MFGM isolate and a reduced content of the indigenous MFGM protein PAS 6/7. Heat- and homogenization-induced associations of whey proteins and caseins with the MFGM isolates consisted of both covalent (disulphide) and non-covalent bondings (hydrophobic interactions). Ceramic membranes (1.4 µm, lab scale) out-performed 0.8 µm membranes in terms of flux, fat and protein separation efficiency as well as purity of the MFGM protein composition. At pilot scale, 1.4 µm pore size ceramic membranes resulted in an MFGM isolate containing 7% polar lipids and 30% protein, with a low content of non-MFGM proteins. Applying a mild pasteurization (72°C, 15s) before or after filtration, in this way, did not affect MFGM composition significantly and underlined the importance of choosing pasteurization temperature and time combinations with care to ensure minimal changes to the MFGM isolates. The results point toward an isolation method to obtain less damaged and more pure MFGM material, with the potential of valorising protein-rich permeate streams.

Key Words: microfiltration, milk fat globule membrane, dairy processing

Physiology and Endocrinology 3

387 The effect of tea tree oil on bovine mammary epithelial cells and polymorphonuclear leukocytes function. T. Y. Yang*, K. Zhan, X. X. Gong, G. Q. Gao, and M. Lin, *Institute of Animal Culture Collection and Application, College of Animal Science and Technology, Yangzhou University, Yangzhou, JiangSu, China.*

Tea tree oil (TTO) plays an important role in antibacterial activity and anti-inflammatory properties. However, effect of TTO on the growth of *Staphylococcus aureus*, bovine mammary epithelial cells (BMECs) and polymorphonuclear leukocytes (PMNL) function remains not reported. Therefore, the aim of study was to investigate the effects of TTO on the growth of *S. aureus* and expression of genes involved in inflammatory response in BMECs and PMNL. Statistical analysis was performed using the SPSS 16.0 software. $P < 0.05$ was considered significant. Our data demonstrated that addition of 0.025 and 0.05% TTO enhanced ($P < 0.05$) the viability of BMECs in culture, whereas 0.1% TTO inhibited ($P < 0.05$) the viability of BMECs. The BMECs treated by 0.0125, 0.025, and 0.05% TTO exposed to *S. aureus* promoted ($P < 0.05$) the viability of BMECs exposed to *S. aureus*. Meanwhile, the growth of *S. aureus* was significantly inhibited ($P < 0.001$) after treatment with TTO after 4 h. In addition, the rate of *S. aureus* invasion into BMECs was also attenuated ($P < 0.05$) by 0.05% of TTO compared with controls. The morphology of *S. aureus* has a profound change by the treatment of 0.05% TTO using scanning electron microscope compared with controls. The adding of 0.05% TTO decreased ($P < 0.05$) the levels of mRNA encoding TNF-α and IL-1β in *S. aureus*-exposed BMECs compared with *S. aureus*-exposed BMEC, but chemokine IL-8 was upregulated ($P < 0.05$). Moreover, the addition of 0.05% TTO enhanced ($P < 0.05$) the expression level of β-casein in *S. aureus*-exposed mammary gland explants compared with *S. aureus*-exposed mammary gland explants. The PMNL incubated with 0.00625, 0.0125, 0.05% TTO had no inhibitory effect on PMNL viability. The expression of IL-1β, IL-6, IL-8, TLR-2, TLR-4, L-SELETIN, IRAKII, TRAF-6, Lysozyme, SOD2 were not altered by incubation of PMNL with 0.05% TTO for 6 h. However, the PMNL incubated with 0.05% TTO for 6 h reduced ($P < 0.01$) the expression level of IL-10, TNF-α, and NFKBIA relative to the control groups. These findings revealed that the use of TTO may be a potent therapeutic tool against bovine mastitis caused by *S. aureus* and thereby mediate protective immunity.

Key Words: tea tree oil, *Staphylococcus aureus*, bovine mammary epithelial cells

388 Mammary gland RNA-seq analysis highlights a protective effect of NutriTek supplementation on udder integrity and health during a *Streptococcus uberis* mastitis challenge in mid-lactating dairy cows. M. Vailati-Riboni*[1], D. Coleman[1], V. Lopreiato[2], A. Alharthi[1], R. Bucktrout[1], E. Trevisi[3], I. Yoon[4], and J. J. Loor[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro, Italy,* [3]*Department of Animal Sciences, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy,* [4]*Diamond V, Cedar Rapids, IA.*

A transcriptomic approach was used to determine the physiological mechanisms associated with the observed protective effect of a *Saccharomyces cerevisiae* fermentation product (NTK, NutriTek, Diamond V, Cedar Rapids, IA) during a mastitis challenge. Eighteen midlactation multiparous Holstein cows (n = 9/group) were fed a control diet (CON) or CON supplemented with 19 g/d NTK for 45 d, and then infected in the right rear quarter with 2500 cfu of *S. uberis*. Mammary gland tissue was collected from the infected quarter at 36 h via repeated percutaneous puncture, before antibiotic treatment was started to stop the infection. RNA was extracted and sequenced on the Illumina HiSeq 4000 system, generating an average of 25 million reads/sample, of which 95% were uniquely mapped, with 77% of gene-assigned read. A linear model with diet as fixed and animal as random effect was fitted. Differentially expressed genes (DEG) were declared at fold change $\geq |2|$ and P-value ≤ 0.05, identifying 150 DEG. The Dynamic Impact Approach was used for pathway analyses to determine effect on biological pathways. NTK supplementation activated genes related to immune cell recruitment (e.g., *GRO1* and *G-CSF*), and increased innate immune cell antibacterial function (e.g., *TNF*, *NOS2*, *CATHL4*). Furthermore, NTK increased expression of genes involved in epithelial tissue protection (e.g., *IL17C*) and anti-inflammatory activity (e.g., *HSP*, *BAG3*, *ATF3*, *p21*). Pathway analysis indicated upregulation of tumor necrosis factor, heat shock protein response, and p21 pathways in the response to a mastitic event in NTK cows. Other pathways for detoxification and cytoprotection functions along with the tight junction pathway were also upregulated in NTK fed cows at 36 h post challenge. Together with the lower quarter somatic cell count and temperature observed during the challenge (presented in a companion abstract), these data shed a light on the possible mechanism of action of NutriTek supplementation in increasing udder health during an intramammary challenge.

Key Words: NutriTek, mastitis, RNA-seq

389 Effects of improving serotonin bioavailability on serotonergic components of the immune system of dairy calves. M. G. Marrero*, A. L. Skibiel, B. Dado-Senn, S. L. Field, and J. Laporta, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dairy calves are born with a naïve immune system and are susceptible to metabolic disorders that can lead to impaired growth and increased mortality rates. Serotonin is a monoamine produced by peripheral tissues and has been proposed to act as an immunoregulatory molecule, but its role in immunity is unclear in dairy calves. Hence, we studied the impact of increased serotonin bioavailability on the immune system of dairy calves by administering a serotonin precursor (5-hydroxy-L-tryptophan, 5-HTP) or a serotonin reuptake inhibitor (fluoxetine, FLX). Bull Holstein calves (21 ± 2 d old) were fed milk replacer (8 L/d) with either saline (CON, 8 mL/d, n = 8), FLX (40 mg/d, n = 8) or 5-HTP (90 mg/d, n = 8) for 10 consecutive d in a complete randomized block design. After 10 d of supplementation, peripheral leukocytes were isolated from whole blood and RNA was extracted to measure gene expression of serotonergic components including serotonin receptors (*5-HTR*), serotonin transporter (*SERT*), tryptophan hydroxylase (*TPH1*), amino acid decarboxylase (*AADC*), monoamine oxidase (*MAO*), interferon gamma (*IFNg*) and interleukins (IL) by RT-qPCR using the Fluidgm Delta-Gene Assay. Data were analyzed using one-way ANOVAs in R. All serotonin receptors were expressed in peripheral leukocytes of dairy calves. Oral supplementation of 5-HTP upregulated gene expression of *5-HTR1A*, *-1B*, *-1D*, *-1F*, *-3B*, *-3C*, and *-4* ($P < 0.04$) and tended to upregulate *5-HT2B* and downregulate *5-HT3A* ($P < 0.06$). Fluoxetine supplementation upregulated gene expression of *5-HT2C* ($P = 0.03$) and downregulated *5-HT2A* and *-3A* ($P < 0.006$). Both treatments upregulated *AADC* and *MAO* gene expression ($P < 0.03$) but no differences were found for *TPH1* and *SERT* expression compared with CON ($P > 0.13$). Expression of *IL-1B*, *-4,* and *-12B* were upregulated by FLX while expression of *IFNg*, *IL-2*, *-4*, *-13*, and *-17A* were upregulated by 5-HTP supplementation ($P < 0.04$). Serotonin activates transcription of serotonergic components and specific immune markers on peripheral leukocytes, suggesting a possible immunomodulatory role in dairy calves.

Key Words: 5-hydroxy-L-tryptophan, fluoxetine, immunity

390 Ex vivo mammalian target of rapamycin (mTOR) pathway activation of bovine immune cell subsets during the transition period.

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Dairy cows experience a nutrient deficit early postpartum when they also exhibit immune dysfunction and inflammation. Previous work raised a possible association with the reduced activation of the nutrient-sensing AKT/mTOR (mTOR) pathway, but responsiveness of the pathway to pro-inflammatory stimulus was not investigated. The objective was to describe differences in activation (expressed as phosphorylation) of AKT kinase and mTOR substrates 4EBP1 and S6RP in immune cell subsets and their responsiveness to LPS during the transition period. Heparinized blood of 14 Holstein cows were taken on d −38 ± 12, −12 ± 4, 7 ± 2, 21 ± 2, and 41 ± 3 relative to calving. Samples were split and pre-treated with PBS or Amino Acid/Glucose solution (AA) (1 h at 37°C) before stimulation with 100 ng/mL LPS or vehicle for 1 h (treatments: PBS, AA, PBS+LPS, AA+LPS). Ratios of phosphorylated to total AKT, 4EBP1, and S6RP were measured by phospho-flow cytometry after fixing and permeabilizing cells. Cell populations were gated based on morphology (neutrophils [PMN], mononuclear cells [MNC]) and cell surface marker staining ($CD14^+$ monocytes). For analysis, PROC MIXED (SAS v. 9.4) with fixed effects of time, treatment, time × treatment, repeated effect of time, and Tukey's test for multiple comparisons was used. Across all time points, PMN had 2.5, 2.9, and 2.2 x higher phospho-ratio of AKT, 4EBP1, and S6RP compared with MNC ($P < 0.01$), and 1.8, 2.6, and 1.3 × higher phospho-ratio for the same proteins compared with $CD14^+$ ($P < 0.01$). Ratios were higher for CD14+ compared with all MNC for AKT and S6RP ($P < 0.01$), but not for 4EBP1 ($P = 0.13$). Expressed as change from baseline (PBS), both time and treatments had an effect on activation, but no interaction was found ($P > 0.08$), indicating activation with LPS was independent of time point. Phosphorylation of mTOR substrates reached the maximum increase from baseline when whole blood was pretreated with AA and then stimulated with LPS. In conclusion, ex vivo activation of the mTOR pathway is highest in PMN, stimulation with LPS reliably induced activation of pathway proteins, responsiveness of target proteins was maintained postpartum, and activation is potentiated by supplying nutrients to immune cells.

Key Words: postpartum inflammation, mammalian target of rapamycin (mTOR), amino acid

391 A comparison of peripheral blood mononuclear cell mitochondrial enzyme activity to genetic markers of lactation performance in high- and low-producing Holstein cows.

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Mitochondria are central to metabolism and are the primary energy producers for all biosynthesis. The objective of this study was to compare mitochondrial enzyme activities of high- and low-producing dairy cows in early lactation to genetic markers of lactation performance. Blood samples were collected from 56 Holstein cows (70 ± 11 DIM) and mitochondria isolated from peripheral blood mononuclear cells (PBMC). Mitochondrial function was assessed by measuring the activity rates of citrate synthase (CS), complex I (CI), complex IV (CIV), and complex V (CV). Milk samples were collected 9 times within a week of blood collection and analyzed for major components using a MilkoScan FT2 by FOSS. Data were analyzed using GLM and the Mixed procedure of SAS (version 9.4, SAS Inst. Inc., Cary, NC) for high or low groups for each production parameter with cow as the experimental unit of interest and dependent variables parity, and DIM as covariates. There were no interactions between milk yield level (high or low) and parity. Complex I and CV activities were lower in low producing cows than high producing cows for both the multiparous and primiparous groups ($P \leq 0.04$) and CV was not influenced by parity for both multiparous and primiparous cows ($P > 0.1$) across production parameters (ECM, milk fat, and total solids) suggesting that it may serve as a marker of a cow's ability to produce regardless of parity. Genetic indices of fluid merit, milk and protein were not correlated with CS, CI, CIV and CV activities ($P > 0.1$). The genetic index for fat was positively associated with CI activity for primiparous cows ($P \leq 0.02$, $R^2 = 0.27$). Comparisons of genetic predictors for milk fat to actual fat yield had positive correlations and were observed only in primiparous cows ($P \leq 0.01$, $R^2 = 0.40$) while multiparous cows showed no associations ($P > 0.1$, $R^2 = 0.05$) suggesting that genetic markers alone may not accurately predict fat production beyond the first lactation and that the use of mitochondrial enzyme activities may provide a better index of cow production potential as it considers both the animal's ability to process nutrients and parity related changes.

Key Words: mitochondria, lactation, enzymes

392 Pharmacological inhibition of the mammalian target of rapamycin (mTOR) pathway alters phenotype and inflammatory response in bovine monocyte derived dendritic cells.

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Immune dysfunction is associated with nutrient deficit in postpartum dairy cows, but the mechanistic link is unclear. The nutrient-sensing mechanistic target of rapamycin (mTOR) signaling pathway is a known regulator of the immune response in other species and our work shows that it is impaired in postpartum dairy cows. Dendritic cells regulate key innate and adaptive immune mechanisms, therefore our objective was to investigate the effect of pharmacological mTOR inhibition in bovine monocyte derived dendritic cells (moDC). Monocytes from 8 cows were differentiated in vitro in the presence or absence of mTOR inhibitors (rapamycin [RAPA], 100 n*M*). On d 7 of culture inhibitors were removed and cells were stimulated with Lipopolysaccharide (LPS, 100 ng/mL) or left as unstimulated controls. Gene expression of *IL-10*, *TNF-α*, *IL-12A* and *IL-12B* was measured after 4h. Expression of surface markers CD14, MHCII, CD40 and CD80 was measured after 24h by flow cytometry. All analysis was done using Friedman's ANOVA, and multiple comparisons corrected with Dunn's procedure. All cows showed an upregulation of surface MHCII, CD40 and CD80 and a downregulation of CD14 in moDC compared with monocyte populations, indicating a mature phenotype. RAPA differentiated cells had a 2.2-fold higher baseline expression of surface MHCII ($P = 0.001$), and a 1.8-fold increased expression of surface CD80 ($P < 0.001$) in response to LPS compared with controls. Stimulation with LPS lead to upregulation of *TNF-α, IL-10, IL-12A* and *IL-12B* gene expression both control and RAPA ($P < 0.03$). Inhibition of mTOR during differentiation induced higher gene expression of *TNF-α, IL-12A* and *IL-12B* ($P < 0.002$) and lower gene expression of *IL-10* ($P = 0.002$) in response to LPS compared with moDC differentiated without inhibitor. Our study shows that inhibition of the mTOR pathway can alter phenotype and cytokine gene expression in bovine moDC in vitro, indicating a more pro-inflammatory profile. An altered inflammatory response of these key regulators could contribute to the immune dysfunction in postpartum dairy cows.

Key Words: immune dysfunction, nutrient signaling, dendritic cells

Production, Management, and the Environment Symposium: Profitability and Sustainability

393 Colostrum management and calf nutrition for profitable and sustainable dairy farms. A. J. Heinrichs*[1], P. S. Erickson[2], H. Chester-Jones[3], and C. M. Jones[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*University of New Hampshire, Durham, NH,* [3]*University of Minnesota Southern Research and Outreach Center, Waseca, MN.*

Early life nutrition and management profoundly impact the health and productivity of dairy calves and by extension affect profitability and sustainability of dairy farms. Research into this topic is active, and members of the USDA Regional Research Project NC2042 have made many contributions specific to management and nutrition, including a colostrum emphasis. Field studies and USDA:NAHMS surveys consistently show that colostrum management has been deficient and contributes to increased calf morbidity and mortality. Heat treatment of colostrum can reduce bacteria loads and increase IgG absorption by the calf despite a small reduction in IgG level. While other colostral proteins are reduced by heat-treating, there have been no reports of long-term impacts on the calf. Investigation into IgG absorption has shown bacteria can directly interfere with IgG absorption, and there may be an upper limit on IgG uptake when feeding superior quality colostrum. Colostrum IgG may be increased by supplementing prepartum diets with nicotinic acid. Additional studies found mixed results on IgG uptake when sodium bicarbonate was added to colostrum replacers and colostrum. In relation to nutrition, a great deal of work has considered the level of feeding and its effect on calf growth and health. Calves can grow faster with increased milk intake; however, at some point, the volume of milk fed affects grain intake and compromises rumen development. This affects weaning age as well as growth and health after weaning. A meta-analysis and a large independent data set evaluated the effect of preweaning average daily gain on subsequent first lactation milk production. Both studies showed a small but significant improvement in milk production as calves grew faster, and both found grain intake was a factor in this improvement. Thus, research indicates that rumen development and the ability to efficiently digest concentrate and limited forage have a large effect on continued growth and calf health. Field studies further support the finding that grain intake at weaning affects growth, age at calving, and first lactation milk production.

Key Words: colostrum, calf nutrition, rumen development

394 Nutrition strategies for improved health, production, and fertility during the transition period. F. Cardoso*[1] and K. Kalscheur[2], [1]*University of Illinois, Urbana, IL,* [2]*Dairy Forage Center ARS-USDA, Madison, WI.*

Dietary formulation and feeding management during the dry period, peripartal period, and early postpartum (fresh) period may facilitate or interrupt many of these steps before pregnancy is established and maintained. There is little evidence that milk yield per se contributes to greater disease occurrence. However, peak disease incidence (shortly after parturition) corresponds with the time of greatest negative energy balance (NEB), the peak in blood concentrations of NEFA, and the greatest acceleration of milk yield. Decreased fertility in the face of increasing milk production may be attributable to greater severity of postpartal NEB resulting from inadequate transition management or increased rates of disease. The depth and duration of NEB is highly related to DMI. Periparturient diseases can be a result from adverse ruminal conditions caused by excessive grain in the precalving or fresh cow diet, perhaps aggravated by overcrowding, heat stress, or other stressors. Others also have implicated inflammatory responses in alterations of metabolism, occurrence of health problems and impaired reproduction. A major area of concern in the fresh cow period is sudden increase in dietary energy density leading to subacute ruminal acidosis (SARA), which can decrease DMI and digestibility of nutrients. Adequate physical form of the diet, derived either from ingredients or mixing strategy, must be present to stimulate ruminal activity and chewing behavior. Dietary starch content and fermentability likely interact with forage characteristics and ration physical form. The comparison of 3 dietary starch contents (primarily from corn starch) in the fresh cow period for cows fed a controlled energy-type ration in the dry period. In conclusion, formulation and delivery of appropriate diets that limit total energy intake to requirements but also provide proper intakes of all other nutrients (including the most limiting amino acids methionine and lysine) before calving can help lessen the extent of NEB after calving. Effects of such diets on indicators of metabolic health are generally positive, suggesting the potential to lessen effects of periparturient disease on fertility.

Key Words: transition period, DMI, negative energy balance

395 Current and future trends of organic dairy in the United States: From feed efficiency to carbon emission. A. F. Brito* and L. H. P. Silva, *University of New Hampshire, Durham, NH.*

Demand for organic milk has grown steadily worldwide, thus creating a niche market opportunity for farmers to transition from conventional to organic certified production. However, surplus of organic milk in the last 2–3 years in the US drove prices down and shrank farmer profitability throughout the country. Currently, transition to organic dairy production has slowed down and the only growing segment of the industry is the "grass-fed" milk market. Farmers shipping "grass-fed" milk are not allowed to feed any source of grain or corn silage with molasses as the sole energy supplement permitted by certifiers and milk processors. While this production system brings challenges including losses in milk yield (MY) and the need for increased forage production, it pays additional premiums that may increase farm profitability. The objective of this paper is to provide data on feed efficiency (FE) using the University of New Hampshire Organic Dairy Research Farm as a case study. Surveys from commercial midwestern and northeastern US organic dairies were used for modeling the impact of "grass-fed" diets on greenhouse gas (GHG) output. Results from 14 experiments (7 published and 7 unpublished) using Jersey cows in confinement (n = 8 studies) or grazing management (n = 6 studies) showed (mean ± SD) DMI of 17.5 ± 2.05 kg/d, MY of 18.3 ± 3.7 kg/d, and ECM of 22.6 ± 4.57 kg/d. Feed efficiency expressed as MY/DMI or ECM/DMI averaged 1.05 ± 0.17 and 1.29 ± 0.20 kg/kg, respectively. Lowered production and FE compared with typical conventional dairies in the US may be related to the use of high forage rations, inclusion of pasture in the diets, increased energy expenditure for grazing, and the lack of digestible forage sources such as corn silage. In fact, our modeling work showed that GHG emissions decreased approximately 2-fold in traditional vs. "grass-fed" organic dairies in part due to the use of corn silage in the diets. In conclusion, MY and FE are lower in organic than conventional dairies. Organic dairy farmers, particularly those shipping "grass-fed" milk should improve forage quality and grazing management and optimize the use of molasses.

Key Words: feed efficiency, organic, pasture

396 The top 5 technologies for the modern cow. A. E. Stone*, *Mississippi State University, Starkville, MS.*

Precision dairy monitoring (PDM) involves the use of technologies to measure physiological, behavioral, and production indicators on individual animals to detect events of interest. Heat, disease, and calving detection are common applications, although heat detection is the most tested and used. Many PDMs are commercially available and are being used in research and on farms. Precision dairy monitoring technologies can be placed on or in the cow, but many technologies are now available in the parlor, in robots, and in an exit or feed alley. Until recently, most PDMs worked with 3-axis accelerometers with different algorithms applied, but advanced imaging is now becoming more commonly used also. A common question from researchers and producers alike is "what is the best technology available?" The top technologies are ones that improve 1) farm efficiency; 2) farm economics; 3) decision making; 4) animal welfare; and 5) producer happiness. Precision dairy monitoring technologies need to be a solution to a problem, not a solution searching for an application, which sometimes occurs particularly when non-agricultural companies start jumping into this market. They also cannot be another problem for a producer to add to their already-full plate. These technologies provide copious amounts of information, which can sometimes be overwhelming. The best PDMs process data and present information in an accurate and easy to understand manner. The producers gaining the most benefit from PDMs are the ones implementing them as part of their everyday standard operating practices and are using 3 of their most important sense to understand the data: common sense, cow sense, and business sense. Implementing PDMs onto a farm is an enormous and potentially expensive decision. As this part of the industry continues to progress, the potential for PDMs are endless. Sound university research and producer feedback are imperative to ensuring that PDMs continue to head in the right direction. This presentation will be aimed at helping weed through the currently available research, discuss current and potential future applications, and explain PDM adoption through the Theory of Diffusion.

Key Words: dairy, precision, technology

397 Decomposing efficiency of milk production and maximizing profit. A. Bach*[1,2], [1]*ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain,* [2]*Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Spain.*

Maximizing milk yield has been the main focus of the dairy industry as it is believed that it maximizes profit mainly through dilution of maintenance costs. Efficiency of milk production has received, until recently, considerably less attention. The most common methods to determine efficiency of milk production are feed efficiency (FE), which is defined as the amount of milk produced relative to the amount of nutrients consumed, and residual feed intake (RFI), which is the difference between observed and predicted DMI, and assumes that animals with a high RFI have a low efficiency. Feed efficiency is affected by 1) ration quality and management (e.g., nutrient balance, mixing order, mixing accuracy, changes in nutrient composition across days, feed availability in the feedbunk), 2) digestive function (e.g., passage rate, rumen fermentation, rumen and hindgut microbiome), 3) feeding behavior (e.g., eating rate, sorting), 4) physical activity of the cow, 5) metabolic function (e.g., homeorhesis, insulin sensitivity, hormonal profile), 6) immune system activity and health, 7) age (e.g., body size, udder vascularization), 8) environmental conditions (e.g., heat stress), and 9) genetics (controlling ultimately most of the previous aspects). Over the years, energy requirements for maintenance have progressively increased, but interestingly, efficiency of nutrient use for milk production has also increased. Empirical evidence from the literature suggests that marginal increases in milk require progressively greater marginal increases in nutrient supply (despite the supposed dilution of maintenance), in other words, the marginal amount of milk produced for every additional Mcal of NEl or every additional gram of metabolizable protein consumed follows the law of diminishing returns. Thus, the dilution of maintenance requirements associated with increases in production is partially overcome by a progressive diminishing biological response to incremental energy and protein supply, and thus FE also follows the law of diminishing returns. Hence, profits associated with improving milk production might, on some occasions, be considerably lower than expected.

Key Words: economics, feed efficiency, residual feed intake

398 Strategies to improve efficiency and profitability of heifer raising. J. L. Anderson*[1], P. S. Erickson[2], K. F. Kalscheur[3], and G. J. Lascano[4], [1]*South Dakota State University, Brookings, SD,* [2]*University of New Hampshire, Durham, NH,* [3]*USDA-ARS Dairy Forage Research Center, Madison, WI,* [4]*Clemson University, Clemson, SC.*

Raising replacement heifers represents a significant investment for producers in time and resources. It is not until the start of lactation that any return on investment is received. Thus, it is critical to the success of the dairy industry that advancements continued to be made to optimize heifer management. The objective of this presentation will be to discuss key research on different aspects of heifer raising strategies that have been conducted by members of the NC-2042 USDA Multistate Project: Management Systems to Improve the Economic and Environmental Sustainability of Dairy Enterprises. A few key areas that will be highlighted specifically in their relationship to improved heifer performance and profitability are: evaluations of different feeding strategies, utilization of alternative forages, and utilization of alternative protein and concentrate feedstuffs. Within our group a variety of feeding strategies such as limit-feeding or precision-feeding versus more moderate limit-fed diets or traditional total mixed rations have been evaluated leading to increased understanding of advantages and disadvantages of each strategy. Research will be reviewed on evaluation of feeding high and low forage diets containing new and developing forage sources such a sorghum silage, sudan grass, and fibrous by-products. Additionally, research has been conducted on effects of feeding co-products such as distillers grains, brewer's grains, oilseed meals, and soy products to heifers in comparison to more traditional concentrate ingredients. In conducting research related to these different feeding strategies and feedstuffs we have gained increased understanding of the relationships of nutrient and energy source utilization and metabolic profile to the development and growth of heifers which can have widespread impacts. In recent years, it has also become apparent the genotype and genetic management will have many implications on heifer performance and influence on nutritional management.

Key Words: dairy heifer, management, efficiency

Joint Reproduction/Animal Health Platform Session: Transition Cow Health and Reproduction

399 Uterine microbiome and uterine disease. K. Galvão*, *University of Florida, Gainesville FL.*

Virtually all cows are infected with bacteria postpartum, and failure to control pathogenic bacterial growth leads to the establishment of disease. Our previous work using 16S rRNA gene sequencing showed that cows have an established uterine microbiota within 20 min of calving. Both cows that remain healthy and cows that develop metritis have sub-communities of bacteria that lead to the development of metritis and commensal sub-communities that are associated with uterine health. However, in cows that develop metritis, there is a dysbiosis in the microbial community in which pathogenic bacteria overtake the uterine commensals. We showed that bacterial relative abundance is similar between healthy and metritic cows up until 2 DPP, after which they deviate in favor of greater abundance of *Bacteroides*, *Porphyromonas* and *Fusobacterium* in cows that develop metritis. We recently confirmed our 16S metagenomic findings using quantitative PCR. At the time of metritis diagnosis (6 ± 2DPP), metritic cows had a greater absolute abundance of total bacteria, and greater abundance of *Bacteroides pyogenes*, *Porphyromonas levii*, and *Fusobacterium necrophorum*, the main species from the genera *Bacteroides*, *Porphyromonas* and *Fusobacterium*, respectively. Previous work using bacterial culture of the postpartum uterus or using PCR to target specific bacteria, pointed to an involvement of *Escherichia coli* and *Trueperella pyogenes*, in the pathogenesis or metritis. However, our work does not show an involvement of *E. coli* or *T. pyogenes* at the time of metritis diagnosis; therefore, our interpretation is that *E. coli* might be a pioneer pathogen that predisposes to uterine colonization by *Bacteroides*, *Porphyromonas*, and *Fusobacterium*, and that *T. pyogenes* would become more important later on, and would be mostly involved in the pathogenesis of endometritis. In summary, our work has increased our understanding of the uterine microbiota in health and disease, and can be used to guide the development of new prophylactic and therapeutic interventions.

Key Words: microbiome, uterine disease, dairy cow

400 Long-term consequences of inflammatory disorders postpartum on fertility. E. S. Ribeiro*, M. R. Carvalho, B. Mion, and J. F. W. Spricigo, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Inflammatory disorders caused by microbial infection, tissue injury, and excessive mobilization of body reserves are prevalent in dairy cows postpartum and cause long-term impairment on fertility. Cows affected by inflammatory disorders postpartum have reduced fertilization of oocytes, reduced survival of zygotes to the morula stage, impaired elongation of preimplantation conceptus, reduced secretion of interferon-tau during the period of maternal recognition of pregnancy, altered transcriptome of conceptus cells, and greater incidence of embryonic and fetal losses. Consequently, the odds of pregnancy per breeding and calving per breeding are reduced by approximately 30 and 42%, respectively. Reduced oocyte competence is a likely reason for the carryover effects of inflammatory disorders on reproduction. Biology of follicular cells and oocytes are affected by inflammation, which might result in reduced developmental competence of oocytes ovulated during the breeding period. Nonetheless, the fact that fertility of lactating cows used as recipients of viable embryos is also affected by postpartum disorders support the idea that altered uterine environment mediates, at least partly, the subfertility of cows that develop inflammatory disorders postpartum. Moreover, negative consequences on fertility seem to go beyond the first breeding postpartum. Cows with clinical diseases in the first 3 wk postpartum had a 19% reduction in pregnancy rate up to 305 DIM, impaired pregnancy per artificial insemination (AI) in breeding performed up to 150 DIM, reduced calving per AI in breeding performed up to 200 DIM, and greater fetal losses in pregnancies established up to 305 DIM. Thus, the negative consequences of postpartum disorders on reproduction might last up to 10 mo after diagnosis and treatment of the problem. Control of inflammation postpartum using drugs or nutraceuticals seem to alleviate the negative effects of postpartum disorders on reproduction but further research is needed to establish firm recommendations. It is increasingly evident that management for optimal fertility of lactating cows starts long before breeding, and requires special attention to management of transition cows.

Key Words: cow, health, fertility

401 Associations between metabolic diseases and reproductive performance of dairy cows using survival analysis and machine learning models. O. Bogado Pascottini[1], M. Probo[2], S. Leblanc[1], G. Opsomer[3], and M. Hostens*[3,4], [1]*Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, ON, Canada,* [2]*Department of Veterinary Medicine, Veterinary Teaching Hospital, University of Milan, Lodi, Italy,* [3]*Department of Reproduction, Obstetrics and Herd Health, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium,* [4]*Department of Farm Animal Health, Faculty of Veterinary Medicine, University of Utrecht | Utrecht, the Netherlands.*

This study aimed to evaluate the associations between individual transition cow conditions and metabolic diseases (MD) and multiple MD (MD+) and the probability of pregnancy in Holstein cows. Kaplan-Meier, Cox proportional hazard, and machine learning models were used to analyze the associations of MD and MD+ with the pregnancy risk from a 1-year cohort with 1,946 calvings. The recorded MD were milk fever, retained placenta, metritis, ketosis, displaced abomasum, and clinical mastitis. Twinning was included as additional event due to its association with multiple MD. The cumulative 60-d milk yield variable (M60) was also included as co-variate for all the models. Survival models were stratified to account for differences by parity. For the machine learning modeling parity was included as a variable of interest. Using Kaplan-Meier models, in primiparous cows, the 120 DIM pregnancy risk was 62% for healthy animals. This was not significantly different for MD (58%) but was reduced for MD+ (45%). Among healthy primiparous cows, 80% were pregnant by 210 DIM, but pregnancy risk at that time was reduced for primiparous cows with MD (72%) and MD+ (62%). In healthy multiparous cows, the 120 DIM pregnancy risk was 53%, which was reduced for MD (36%) and MD+ (30%). The 210 DIM pregnancy risk for healthy multiparous cows was 70%, being significantly higher than the 210 DIM pregnancy risk for multiparous cows with MD (47%) or MD+ (46%). Survival analysis showed that the pregnancy risk was reduced when cows experienced more than one MD, irrespective of parity. Multivariable survival models were not able to accommodate complex MD interactions, which dropped the distinct number of cows per category. Decision tree and random forest models showed that parity was the most influential variable affecting fertility. Decision tree analysis selected ketosis and metritis while random forest also identified mastitis and M60 as important variables interfering with fertility. Machine learning methods helped exploring the complex interactions between the parity and each MD to study their hierarchical effect on the pregnancy risk in dairy cows.

Key Words: transition period, reproduction, decision tree analysis

402 Epidemiology of reproductive disorders in large certified organic dairy farms in northern Colorado.

D. Manriquez*[1], J. Velez[2], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Aurora Organic Dairy, Platteville, CO.*

Management practices in certified organic dairies (ORG) may influence the occurrence of reproductive diseases (RD), contrasted with conventional dairies. The objectives were to report the prevalence of abortion (ABT), retained placenta (RTP), metritis (MET, defined as presence of fetid watery red-brown uterine discharge, fever, and signs of general illness within the first 21 DIM), endometritis (ENDO, defined as the presence of mucopurulent uterine discharge after 21 DIM), and culling due to impaired reproduction (CUR), and to identify risk factors for RD in ORG. A retrospective analysis from farm records on the prevalence of RD and their associations with parity, dry period length, days in closeup groups, pregnancy length, and grazing season was performed in 36,911 lactations from cows calving between January 2016 and December 2018. Five large ORG in Northern Colorado (USA) ranging from 1,900 to 4,100 milking cows were included. Data analyses were performed using PROC FREQ and LOGISTIC in SAS. Prevalence of ABT, RTP, MET, ENDO, and CUR were 3.5, 1.3, 5.4, 8.7, and 7.2%, respectively. Most relevant predictors associated with RD are shown in Table 1. Other variables significantly associated with greater odds of ABT, ENDO, MET and CUR were closeup, calving and dry period occurred during grazing season. Pregnancy length was not associated with any RD. Under organic management, grazing and peripartum managements appear to be potential intervenient variables to improve reproductive efficiency in ORG.

Key Words: organic, reproduction

Table 1 (Abstr. 402). Odds ratios (OR) and 95% CI for ABT, ENDO, MET, RTP, and CUR (only significant and controlling predictors are included)

	ABT		ENDO		MET		RTP		CUR	
	OR	CI	OR	CI	OR	CI	OR	CI	OR	CI
Parity										
1	–				–		–		–	
≥2	1.5	1.2–1.8^{A}			1.8	1.4–2.3^{A}	5.7	2.8–11.7^{A}	2.8	2.4–3.3^{A}
Dry period (d)										
≤30	4	2.8–5.9^{A}							2.6	2.2–3.1^{A}
>30 to ≤60	1.6	1.1–2.3^{B}							1.8	1.6–2.1^{A}
>60	–								–	
Days in closeup										
≤7					2.5	2–3.1^{A}	4.6	3.1–6.8^{A}	0.9	0.8–1.1^{D}
>7 to ≤14					1.2	0.9–1.5^{D}	1.8	1.1–2.7^{C}	1.2	1.1–1.3^{B}
>14					–		–		–	
Calving season										
Fall			2.2	1.9–2.5^{A}	1.7	1.2–2.4^{A}	2.2	1.2–4.3^{C}	1.4	1.2–1.7^{A}
Winter			–		–		–		–	
Spring			1.5	1.2–1.8^{A}	1.5	1–2.1^{B}	3.2	1.6–6.3^{A}	1.6	1.3–2^{A}
Summer			1.5	1.2–1.8	2.2	1.4–3.4^{A}	5.9	2.6–13.3^{A}	1.2	0.9–1.5^{D}

P-value A <0.001; B <0.01; C <0.05; D <0.15.

403 Regardless of bacterial load in the vagina, heat stress increases uterine disease incidence and severity in the dairy cow.

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Uterine disease impacts the dairy industry by reducing milk yield and fertility. Approximately 7d after calving 30% of cows develop metritis due to bacterial infection, that may persist beyond 21d as endometritis. Although heat stress has a negative impact on milk production and immune function, little is known about its effect on uterine health. The focus of this study was to assess uterine disease incidence and quantify bacterial load in vaginal mucus samples of dairy cows during a cool (n = 51, avg max temp: 23°C) and hot season (n = 51, 31°C). We hypothesize that during the hot season uterine disease incidence and severity is increased, as well as the bacterial load in the reproductive tract. Heat abatement was provided to all postpartum cows. Vaginal mucus was scored according to abundance and odor of pus on d7 and 21 postpartum to characterize metritis or endometritis, respectively. Bacterial 16S rRNA was quantified by qPCR in mucus. Categorical variables were compared by χ^2 and GLMM was used for milk data. During the hot season more cows had persistent uterine disease at both d7 and 21 (58.8% vs. 29.4%) and fewer cows recovered from metritis when compared with those in the cool season (21.1% vs. 50.0%). Incidence of endometritis was increased during the hot season (64.7% vs. 43.1%) when compared with cows in the cool season. The concentration of bacterial 16S rRNA in vaginal mucus was higher in cows with endometritis compared with healthy cows at d21 (2.35 vs. 0.04 ng/mg mucus) but did not differ between the hot and cool seasons (2.12 vs. 2.68 ng/mg mucus). Severity of uterine disease was associated with reduced milk production in the

Table 1 (Abstr. 403). Average milk production (kg/d) for 60 DIM

Season	All cows	Mucus score (d21) 0	1	2	3	4
Hot	34.2 ± 1.3	39.8 ± 3.1^{x}	36.3 ± 2.8^{x}	35.7 ± 2.2^{x}	36.2 ± 2.6^{x}	22.8 ± 4.0^{y}
Cool	$39.5 \pm 2.1^{*}$	47.6 ± 3.1^{a}	$44.6 \pm 1.9^{a*}$	36.6 ± 3.0^{b}	30.8 ± 2.6^{b}	37.7 ± 8.9^{ab}

^{a,b} or ^{x,y} $P < 0.05$ within season, $^{*}P < 0.05$ between season.

first 60 DIM which was further compounded in the hot season (Table 1). Our data suggests that heat stress increases incidence of uterine disease despite similar vaginal bacteria load. This work was supported by NICHD R01HD084316.

404 Effects of fully acidified, negative DCAD diets with differing concentrations of dietary calcium fed prepartum on uterine morphology and redox environment of Holstein cows after parturition. K. Ryan[1], A. Guadagnin*[1], K. Glosson[1,2], S. Bascom[2], A. Rowson[2], and F. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Phibro Animal Health Corporation, Teaneck, NJ.*

Diets fed during the transition period can play an important role in the cow's reproductive tract recovery and function following parturition. We aimed to compare the effects of feeding a fully acidified DCAD diet prepartum to Holstein cows (n = 70) at 2 different concentrations of dietary calcium (Ca) inclusion versus a non-acidified, positive DCAD diet prepartum on uterine environment postpartum. Multiparous Holstein cows were enrolled at 50d before expected calving and followed until 75 DIM. Treatments began at 28d before expected calving and were: CON (n = 23), a positive DCAD diet with low dietary Ca (0.4% DM); LOW (n = 22), a fully-acidified, negative DCAD diet (urine pH = 5.7) with low dietary Ca (0.4% DM); HIGH (n = 25), a fully-acidified, negative DCAD diet (urine pH = 5.7) with high dietary Ca (2.0% DM). Endometrial tissue samples were collected at 30 DIM and analyzed for glutathione peroxidase (GPX) and superoxidase dismutase (SOD) activity, and glandular morphology. Data collected were analyzed using PROC MIXED in SAS. Treatment, day and block were considered fixed effects. Cow was the experimental unit and considered as a random effect. Contrasts included CONT1 (CON vs average of LOW and HIGH) and CONT2 (LOW vs HIGH). Cows fed HIGH had greater ($P = 0.02$) epithelial height (22.47 ± 1.08m) than cows fed LOW (18.67 ± 1.08m) and cows fed CON (18.01 ± 1.08 m) tended ($P = 0.06$) to have shorter epithelial height than the average of cows fed LOW and cows fed HIGH. Cows fed HIGH had a greater ($P = 0.05$) number of epithelial cells per gland (25.93 ± 1.07) than cows fed LOW (22.93 ± 1.07). Anti-oxidative enzymes SOD and GPX relieve oxidative stress in cells. Cows fed HIGH had increased ($P = 0.05$) activity of SOD (73.50 ± 2.83%) and decreased ($P < 0.001$) activity of GPX (32.89 ± 5.05%) than cows fed LOW (69.49 ± 2.83% and 68.31 ± 2.83%, respectively). In conclusion, cows fed HIGH had improved glandular epithelial cells, greater SOD activity, and lower GPX activity than cows fed LOW indicating an improved redox environment in the uterine tissue, which may lead to improved postpartum fertility.

Key Words: DCAD, oxidative stress, uterus

405 Aflatoxin compromises development of the preimplantation bovine embryo through mechanisms independent of reactive oxygen production. Y. Jiang*[1], P. J. Hansen[1], I. M. Ogunade[2], X. Yao[1], T. Amaral[1], K. G. Arriola[1], D. Vyas[1], and A. T. Adesogan[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Division of Food and Animal Science, Kentucky State University, Frankfort, KY.*

Aflatoxin is a potent carcinogen often detected in animal feedstuffs. Aflatoxin has been reported to impair fetal development in humans and mice but its actions on development of the preimplantation bovine embryo are not known. The objective was to study the effects of different concentrations of aflatoxin on development of the preimplantation bovine embryo and understand mechanisms underlying these effects. Experiments were conducted with embryos produced in vitro and cultured, beginning after fertilization with various concentrations of aflatoxin. For Experiment 1, embryos were treated with control, AF40 (40 µg/L, aflatoxin B$_1$, AFB$_1$); or AF400 (400 µg/L AFB$_1$, 4) or AF4000 (4000 µg/L AFB$_1$). Proc Glimmix of SAS was used to analyze the data. The statistcal model includes fixed effect of treatment and random effect of replicate. Applying AFB$_1$ at 40, 400, and 4000 µg/L tended to reduce ($P < 0.10$) cleavage rate. As compared with control, AF40 reduced the percent of oocytes becoming blastocysts and the percent of cleaved embryos becoming blastocysts (19.7 vs 8.1% and 30.3 vs 14.3%, respectively, $P < 0.05$). Complete inhibition of blastocyst formation occurred for AF400 and AF4000. Experiments 2 and 3 involved a 2 × 2 factorial design with effects of AFB$_1$ (0 and 40 µg/L) and the antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (0 and 5 µM) and their interaction on production of reactive oxygen species (ROS) in putative zygotes (Experiment 2) and development (Experiment 3). The statistical model included fixed effects of AFB1 and Trolox and their interaction as well as the random effect of replicate. ROS was increased by AF40 ($P < 0.05$) and this effect was reversed by Trolox (AF40 x Trolox; $P < 0.05$). However, Trolox did not prevent the reduction in blastocyst rate caused by AF40 in Experiment 3. Thus, the antidevelopmental effects of AFB$_1$ are not caused solely by increased ROS production. Rather, other underlying mechanisms exist for the adverse effects of aflatoxin on embryonic development.

Key Words: aflatoxin, antioxidant, embryonic development

406 Effect of the treatment with carprofen during early postpartum on the reproductive performance of dairy cows with potential incidence of clinical metritis. A. Rodríguez*[1], M. Solar[2], P. Palma[2], and H. Bustamante[3], [1]*Graduate School, Faculty of Agricultural Sciences, Universidad Austral de Chile, Valdivia, Chile,* [2]*Lecherías del Sur Ltda., Osorno, Chile,* [3]*Veterinary Clinical Sciences Department, Faculty of Veterinary Sciences, Universidad Austral de Chile, Valdivia, Chile.*

After calving, a well described multifactorial inflammatory state can affect the reproductive performance, health and welfare of dairy cows. We hypothesize that an important source of inflammation could come from non-diagnosed and untreated diseases such as clinical metritis (CM). Our objective was to evaluate the effect of the treatment with carprofen during early postpartum on the incidence of CM and subsequent reproductive performance of dairy cows. Fifty-four multiparous Holstein cows were selected upon entry to the close-up group. After calving cows were assigned randomly a one of 2 treatments. CAR (n = 25) received 1.4 mg/kg BW of carprofen (Rimadyl 50 mg/mL) in a single dose between 12 and 48 h postpartum and CON (n = 29) were untreated controls. CM diagnoses were performed weekly starting on d 3–21 postpartum based on the characteristics of the vaginal discharge described by Huzzey et al. (2007). Reproductive data were collected from the farm records and used to calculate conception rate at first service (CRFS), calving to first service interval (CFSI), calving to conception interval (CCI) and services per conception (SC). Data analyses were performed using the GENLINMIXED procedure for SPSS (v.25) considering treatment, CM (presence or absence) and their interactions as fixed effects and cow as a random effect. Significance level was set at $P < 0.05$. CAR treatment decreased the incidence (Table 1) and the risk of developed CM (OR = 0.296, CI 95% OR = 0.09–0.95, $P = 0.04$) compared with the CON treatment. Reproductive performance was not influenced by any treatment (Table 1). Treatment with carprofen decreased the incidence of clinical metritis but did not affect subsequent reproductive performance parameters.

Table 1 (Abstr. 406). Estimated means (with SEM in parentheses) of clinical metritis and reproductive variables in the CAR and CON treatments

	Treatment		
Variable	CAR	CON	*P*-value
Metritis incidence, %	36 (0.09)	65.5 (0.09)	0.04
CRFS, %	36 (0.10)	34.5 (0.09)	0.90
Calving to first service interval, d	55.5 (1.63)	58.3 (1.44)	0.19
Calving to conception interval, d	118.7 (8.15)	120.2 (6.72)	0.88
Services to conception, no.	2.3 (0.29)	2 (0.26)	0.56

Key Words: inflammation, metritis, nonsteroidal anti-inflammatory drug (NSAID)

407 Effects of a new preventive strategy with acetylsalicylic acid on uterine diseases and reproductive performance in dairy cows.

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Uterine diseases negatively affect fertility in dairy cows. Furthermore, it has been suggested that an exacerbated inflammatory response after calving may increase the likelihood of the occurrence of these diseases. The objective of this study was to assess the effects of a new preventive strategy with acetylsalicylic acid (ASA) on the incidence of uterine diseases (i.e., retained placenta, metritis, endometritis), ovarian structures, days in milk to pregnancy and number of services to conception in dairy cows after calving. Dairy cows (n = 246) from one dairy farm located in central Pennsylvania were included in this study. Cows were blocked by parity and randomly assigned to 2 groups: 1) ASP (n = 121): within ~12 h after parturition cows received 1 oral administration of ASA (200 mg/kg of BW; 4 boluses) and a second oral administration 24 h apart; or 2) UNT (n = 125): remained untreated. Metritis was assessed at 7 ± 3 and 14 ± 3 d in milk (DIM) using a Metricheck device. Cows that presented a fetid red-brownish fluid vaginal discharge were classified as having metritis. Endometritis and ovarian structures were assessed through trans-rectal ultrasonography at 50 ± 10 DIM. The incidence of retained placenta was recorded by farm personnel. The data were analyzed using the MIXED and GLIMMIX procedures of SAS as a randomized block design. The treatment variable was used as fixed effect while cow was used as random effect in the statistical models. A lower proportion of cows treated with ASA developed metritis at 7 ± 3 DIM (ASP = 23.74 ± 7; UNT = 47.39 ± 10) and endometritis (ASP = 2.04 ± 2.03; UNT = 14.27 ± 5.05) at 50 ± 10 DIM. Furthermore, cows in the ASP group required less days (ASP = 120 ± 15.2 DIM; UNT = 131 ± 14.9 DIM) and tended to need less services to become pregnant (ASP = 2.66 ± 0.36; UNT = 2.94 ± 0.37) than UNT cows. Results from this study suggest that treatment with ASA after calving may decrease the incidence of uterine diseases during early lactation, and subsequently, enhance reproductive performance of dairy cows.

Key Words: dairy cattle, acetylsalicylic acid, reproductive performance

Ruminant Nutrition 4: Production and Efficiency

408 Improving the efficiency of inefficient lactating cows by increasing dietary forage to concentrate ratio. Y. A. Ben Meir*[1,2], I. Halachmi[3], J. Miron[2], and S. J. Mabjeesh[1], [1]*Hebrew University of Jerusalem, Jerusalem, Israel,* [2]*Agriculture Research Organization, Reashon LeZion, Israel,* [3]*Institute of Agricultural Engineering, Reashon LeZion, Israel.*

The aim of this study was to improve feed efficiency of inefficient (INE) cows through a moderate increase in diet's forages /concentrate ratio. We studied the effects of replacing 8.2% corn grains of the control low fiber diet (LF, 17.5% forage NDF) by 7.5% wheat straw + 0.7% soybean meal in the high fiber diet (HF, 23.4% forage NDF). Previously to the present study, the individual efficiency of 220 cows in ARO herd estimated on a low-roughage TMR. Average residual feed intake (RFI, actual DMI minus predicted DMI according to NRC 2001 equation) of ARO lactating cows was 1.5, and the inefficient cows were characterized by RFI >2.7 (20% least efficient cows). Based on this characterization, 15 pairs of INE multiparous mid-lactating Israeli Holstein dairy cows were selected and paired into 2 groups with similar performance, intake, and efficiency data and were adapted for 2 wk to the 2 dietary treatment. Traits examined during 5 wk of the experiment were: eating behavior, milk production, in vivo digestibility, and estimation of feed efficiency (RFI, ECM/DMI and energy balance). Statistical comparison between treatment analyzed by using Mixed Model with treatment, date and date × treatment as fixed effects and animal as random effect. INE cows fed the HF diet showed slower eating rate (223.1 *vs* 245.9 gDM/min, $P < 0.01$), smaller meal size (5.56 *vs* 5.83 kgDM, $P < 0.01$), longer daily eating time (245.9 *vs* 223.1 min/d, $P < 0.01$), and longer meal duration (53.0 *vs* 46.6 min, $P < 0.01$), compared with INE cows fed the LF diet. Intake of INE cows fed the HF diet was 9.1% lower (29.2 *vs* 32.1 kgDM/d, $P < 0.01$), their dry matter digestibility reduced from 65.7 to 62.2% ($P < 0.01$), and their ECM yield was 7.0% lower (39.8 *vs* 42.8 kg/d, $P < 0.01$), compared with the cows fed the LF diet. Feed efficiency, measured as residual feed intake, ECM/DMI and net energy captured /digestible energy intake, were improved (1.90 *vs* 3.18, $P < 0.01$; 1.36 *vs* 1.33, $P = 0.03$; and 0.495 *vs* 0.447, $P < 0.01$, respectively) in the INE cows fed the HF compared with the cows fed the LF diet. Our results thus show the potential of improving feed efficiency for milk production of INE cows by an increase in forage/concentrate ratio.

Key Words: eating behavior, energy balance, high fiber diet

409 Between-cow variation in the components of feed efficiency. A. Guinguina*[1], T. Yan[2], P. Lund[3], A. Bayat[4], and P. Huhtanen[1], [1]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden,* [2]*Agri-Food and Biosciences Institute, Hillsborough, Co. Down, UK,* [3]*Department of Animal Science, Aarhus University, AU, Foulum, Tjele, Denmark,* [4]*Natural Resources Institute Finland (LUKE), Milk production, Jokioinen, Finland.*

A meta-analysis based on an individual-cow data set was conducted to investigate the between-cow variation in the components of feed efficiency (FE) and to explore the relationships among these components and FE measures. Data were taken from 24 change-over studies consisting of a total of 687 cow/period observations. The experimental diets were based on grass and corn silages, fresh grass, mixture of fresh grass and straw, with cereal grains or by-products as energy supplements, and soybean or canola meal as protein supplements. Average forage: concentrate ratio across all diets on a dry matter basis was 57:43. Variance component and repeatability estimates of FE components were determined using diet, period and cow within experiment as random effects in mixed procedures of SAS. Gross energy intake (GE, MJ/d), and Milk energy (E_l) were more repeatable (≥0.53) experimental measures than [GE digestibility (GED), Fecal energy (FecalE), metabolisable energy (ME), urinary energy (UE), CH_4 energy (CH_4E), heat production (HP) expressed in MJ/kg DM intake], ME requirement for maintenance (ME_m, MJ/kg$^{0.75}$) and the efficiency of ME use for lactation (k_l). Similarly, GE and E_l displayed higher between-cow coefficient of variation (CV = 0.091 and 0.096 respectively) than the rest of the components. A high GED was associated with increased CH_4E (r = 0.35), HP (r = 0.40), ME (r = 0. 93), body reserves deposition (r = 0.29), k_l (r = 0.11), ME_m (r = 0.12) and decreased Fecal-E (r = −0.81). However, no correlation between GED and E_l or UE was observed. Increased GE efficiency (GEeff = E_l/GE) was associated with increases in E_l, CH_4E, HP and mobilization of body reserves, without any effect on Fecal-E, GED, ME, UE, ME_m, and k_l. Low residual feed intake (RFI) was associated with reduced FecalE, UE, CH_4E, HP, ME_m, but increased GED, ME and k_l without any effect on E_l. Clearly, high CH_4 emitters were also high milk producers. Improved GED lead to higher methane and heat production. Because the gastro-intestinal tract tissues have a high metabolic rate, improved GED resulted in increased maintenance requirement. Several mechanisms are involved in the observed differences in FE of dairy cows. In conclusion, ranking of animals for FE can differ depending on the definition used.

Key Words: variation, energy, residual feed intake

410 Effect of adding molasses-based liquid feed to a high-straw dry cow diet on intake and behavior of Holstein dairy cows. C. Havekes*[1], T. F. Duffield[2], A. J. Carpenter[1], and T. J. DeVries[1], [1]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Department of Population Medicine, University of Guelph, Guelph, ON, Canada.*

The objective of this study was to determine the effect of adding molasses-based liquid feed to a high-straw dry cow diet on intake and behavior of dairy cows. Multiparous Holstein cows (n = 40) were enrolled at dry off (~45 d before expected calving) and assigned to 1 of 2 treatments, a high-straw (35% wheat straw on DM basis) dry cow diet with: 1) no molasses (CON; n = 20; 11.6% CP, 1.35 Mcal/kg NE_L) or 2) supplemented with 1 kg/cow/d (DM) of molasses (LF; n = 20; 11.7% CP, 1.38 Mcal/kg NE_L). At calving all cows were fed the same lactating TMR (14.0% CP, 1.64 Mcal/kg NE_L) and followed for 28 d. DMI, feeding behavior, and rumination activity were recorded automatically. TMR and ort samples were collected 2x/wk to determine differences in sorting. A particle separator was used to separate feed samples into 4 fractions: long (>19mm), medium (<19mm, > 8mm), short (<8mm, > 4mm), and fine (<4mm) particles. Feed sorting was calculated as: actual intake of each particle fraction expressed as a % of predicted intake. Data were analyzed in mixed-effect linear models, treating day as a repeated measure. LF cows had greater DMI (15.1 ± 0.26 vs 13.8 ± 0.26 kg/d; $P = 0.001$) and tended to spend less time ruminating (513.9 ± 8.55 vs 534.6 ± 8.54 min/d; $P = 0.1$). LF cows tended to have shorter meals (48.3 ± 4.64 vs. 60.4 ± 4.78 min/meal; $P = 0.08$), a shorter interval between meals (195.2 ± 8.72 vs 217.4 ± 9.0 min; $P = 0.09$) and more frequent meals (6.2 ± 0.79 vs 5.5 ± 0.24; $P = 0.07$), while consuming their feed faster (0.10 ± 0.001 vs 0.08 ± 0.001 kg DM/min; $P = 0.04$)

compared with CON cows. Regardless of treatment, cows sorted against the longest ration particles, with CON cows sorting more against these (81.9 ± 2.5 vs 94.5 ± 2.5%; $P = 0.001$). CON cows did not sort for or against short particles (100.4 ± 0.56%), but LF cows tended to sort against these (98.8 ± 0.56%; $P = 0.06$). Post-calving, LF cows did not sort for long particles (100.3 ± 1.3%), but CON cows continued to sort against these (95.3 ± 1.3%; $P = 0.01$). The results suggest that supplementing a molasses-based liquid feed in high-straw dry cow diets may improve intake and consistency in nutrients consumed during the dry period and in early lactation.

Key Words: dry cow diet, molasses, sorting behavior

411 Effect of *Saccharomyces cerevisiae* fermentation product (XP) supplemented to dairy cows during summer on feed efficiency, oxidative stress, and inflammatory markers.

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Several studies demonstrated improved feed efficiency and beneficial effects on oxidative stress and immune function with supplementation of *Saccharomyces cerevisiae* fermentation product (XP, Diamond V, Cedar Rapids, IA). The objectives were to examine the effects of XP supplemented to high yielding dairy cows during the hot season on feed conversion ratio (FCR) and several markers of oxidative stress and inflammation. Mid-lactation cows (n = 42, 145 DIM and 50.5 kg milk/d) were divided into 2 groups, fed a standard Israeli ration, and supplemented for 10 wks with: Control (CTL), 100 g ground corn; or XP, a blend of 56 g XP + 100 g ground corn per cow per d. Blood samples were taken at d −1, 23, 49 and 56 of the study period. Rumen samples were taken at the 8th wk for pH, ammonia, and VFA measurements. Data were analyzed using the PROC MIXED model of SAS. Milk yields were similar, but milk components of fat (3.76 and 3.57%, respectively; $P < 0.09$) and protein (3.40 and 3.36%, respectively; $P < 0.1$) tended to be higher in the XP cows. The 4% fat-corrected milk (FCM) was 3.2% higher ($P < 0.09$) in the XP cows. Dry matter intake was similar between groups, and FCM FCR (DMI/FCM) tended to be lower ($P < 0.1$) in the XP fed group (690 and 710 g DMI/kg FCM, respectively). Rumen pH was higher in the CTL cows; butyrate concentration was higher in the XP cows, and the total VFA tended to be higher in the XP cows ($P < 0.09$). No differences were observed in tumor necrosis factor α (TNFα) concentrations in blood, but the inflammation marker interleukin-6 (IL6) and oxidative stress marker malondialdehyde (MDA) concentrations tended to be lower ($P < 0.1$) in the XP cows (29.0 and 63.4 pg/mL and 358.0 and 436.4 nM, respectively). In conclusion, supplementation of XP to dairy cows during the hot season tended to increase milk fat and protein content, and FCM yields and FCR. In addition, there were indication of beneficial effects of XP supplementation on oxidative stress and immune function.

Key Words: inflammation, heat stress, *Saccharomyces cerevisiae* fermentation product

412 Effects of supplemental concentrate type on dry matter intake and milk solids production of mid-lactation dairy cattle grazing perennial ryegrass-based pasture.

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The objective of this study was to evaluate factors limiting DMI and milk solid production in dairy cattle grazing perennial ryegrass-based pasture. Eighty dairy cows averaging 98 ± 25 DIM and 518 ± 64 kg of BW were assigned to one of 4 treatments in a randomized block design. Dietary treatments were perennial ryegrass (PRG; DM = 20%, CP = 14%, aNDFom = 44%), PRG plus 4.8 kg DM of citrus pulp and 0.075 kg DM urea (Citrus; DM = 39%, CP = 13.5%, aNDFom = 38%); PRG plus 0.8 kg DM of a heat treated soybean meal (TSBM; DM = 24%, CP = 16%, aNDFom = 42%) and PRG plus 3.1 kg DM of a mix of citrus and heat treated soybean meal (Mix; DM = 32%, CP = 15%, aNDFom = 39%). The trial consisted of a 2-wk covariate period and 10 wk of data collection. Individual cow pasture DMI was obtained on wk 6 utilizing the n-alkane technique. Cattle were milked at 0730 h and 1600 h daily and milk yields recorded. Milk fat, protein and lactose concentrations were determined weekly from one successive PM and AM milking sample from each cow. All data were analyzed using the Proc Mixed procedure in SAS. Total DMI was greater for Citrus than PRG treatment and milk solid production was greater for TSBM and Mix treatments in comparison to PRG (Table 1). The results suggest metabolizable protein was first limiting milk solid production.

Key Words: milk production, metabolizable protein, first limiting

413 Altered rumen fermentation patterns in lactating dairy cows supplemented with phytochemicals improves milk production and efficiency.

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Table 1 (Abstr. 412). Effects of supplemental concentrate type on DMI and milk production of mid-lactation dairy cattle grazing perennial ryegrass-based pasture[1]

Item	PRG	Citrus	TSBM	Mix	SEM
Pasture intake (kg DM d^{-1})	15.6^{a}	12.8^{b}	15.3^{ac}	13.8^{bc}	0.47
Supplement intake (kg DM d^{-1})	0^{a}	4.8^{b}	0.8^{c}	3.1^{d}	0.03
Total intake (kg DM d^{-1})	15.6^{a}	17.6^{b}	16.1^{ab}	16.9^{ab}	0.48
Milk production (kg d^{-1})	18.5^{a}	20.6^{b}	20.3^{b}	21.3^{b}	0.34
Milk solids production (kg d^{-1})	1.41^{a}	1.49^{ab}	1.55^{b}	1.59^{b}	0.03

$^{a–d}$Different superscripts within a row for a given treatment signifies $P < 0.05$.

[1] PRG = perennial ryegrass, Citrus = perennial ryegrass + citrus pulp + urea, TSBM = perennial ryegrass + heat-treated soybean meal, Mix = perennial ryegrass + citrus pulp + heat-treated soybean meal.

Tannins and other phytochemicals can improve RUP in the diet by binding protein and then limiting ruminal degradation, which can improve milk yield and milk protein synthesis. The objective of this study was to evaluate the effects of dietary phytochemicals (tannins and *Capsicum annum*) as rumen modifiers on production parameters and milk efficiency in dairy cows. Twenty-four multiparous Holstein cows (96 ± 16 DIM; mean ± SD) were used in a 3 × 3 Latin Square design with a covariate period. Cows were blocked according to DIM, milk production, and BW and randomly assigned to a treatment sequence (n = 8/group). The experiment consisted of a 14-d covariate period and three 30-d treatment periods. Cows received a basal diet supplemented with soybean meal pellets (SB) as the control diet, phytochemicals (RUM; Rumiviv, CCPA, Janzé, France) pelleted with soybean meal, or expellers soybean meal (ESBM; SoyPlus, Ralston, IA). Milk production and DMI during the last 4 d of each period were used for statistical analysis. Blood and rumen fluid samples were collected on d 27 of each period. Rumen fluid was analyzed for ammonia N and VFA as well as ruminal bacteria via qPCR amplification of 16s rDNA genes. Data were analyzed using the MIXED procedure of SAS. Greater ($P < 0.03$) milk yield (37.9 vs 36 kg/d), ECM (39.7 vs 37.1 kg/d), and protein yield (1.15 vs 1.08 kg/d) were observed in RUM than SB, but RUM and ESBM were similar. Total VFA (118.1 vs 101.5 m*M*), as well as propionate (28.6 vs 24.5 m*M*) and butyrate (10.8 vs 9.4 mM) concentration, were greater ($P \leq 0.04$) in RUM in comparison to SB and ESBM diets. Cows fed RUM had greater ($P \leq 0.05$) BHB (0.49 vs 0.42 mmol/L) than SB and ESBM. *Streptococcus bovis* in rumen fluid was lower ($P \leq 0.01$) in RUM than SB and ESBM diets, and a trend ($P \leq 0.09$) for lower *Selenomonas ruminatium* and *Succinimonas amylolytica* was observed in RUM than SB and ESBM. Overall, data suggest that feeding a mixture of phytochemicals can significantly affect rumen microbiota and fermentation patterns and these were reflected in better milk production and efficiency.

Key Words: phytochemicals, rumen-undegradable protein, microbiota

414 Models to predict feed intake in dairy cows.

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Mathematical models are central to ruminant nutrition. Feed intake (FI) is paramount in the performance of livestock and has been of interest when creating said models. The aim of the present study was to evaluate 4 models in their prediction of FI in dairy cows fed total mixed rations (TMR): BSM-Milk (BioSimetrics Ltd.) a dynamic mechanistic whole cow model, the FI equation part of the CNCPS (Fox et al., 2004), the equation include in NRC (2001) and that in Feed Into Milk (FiM, Thomas 2004). A trial was carried out at SRUC's Dairy Research Centre, Scotland UK. Two contrasting TMR were offered to 2 groups of 40 5 Holstein Friesian cows. The diets were: forage (kg/kg/DM Grass silage 0.40, maize silage 0.23, crimped wheat 0.11, beans 0.25 and min 0.01) and concentrate based (Wholecrop 0.40, Megalac 0.02, whey 0.08, min 0.01 and a concentrate 0.50). Using electronic feeders (HOKO, Insentec, the Netherlands) individual FI were recorded daily. Details of the animals and the TMR were used as inputs. FI predictions obtained were compared with those obtained on-farm. To evaluate the predictions regression analysis, the limits of agreement (LoA) method and the concordance correlation coefficient (CCC) were used. All statistical analyses were carried out using R. The evaluated models predicted FI with different levels of success. Obtained R^2 values were: 0.78 BSM-Milk, 0.48 CNCPS, 0.42 NRC and 0.47 FiM. The CCC were 0.88 BSM-Milk, 0.58 CNCPS, 0.61 NRC and 0.53 FiM. The LoA showed that BSM-Milk predicted FI in average 0.19 higher than observed (limits −3.80 to 4.19), similarly CNCPS predicted FI 0.98 higher than observed (limits −5.06 to 7.03), NRC predictions were −0.41 lower than observed (limits −6.80 to 5.98) and predictions made with FiM were 4.19 higher than observed (limits 0.28 to 8.85). BSM-Milk was the model with the best performance when compared with the rest of the evaluated models. Future research should compare BSM-Milk predictions to those obtained with models that use a more mechanistic approach to FI prediction.

Key Words: feed intake, mathematical model, dairy cow

415 Feed efficiency of slick-hair and wild-type dairy cows under heat stress.

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Dairy cattle in the tropics are exposed to chronic heat stress, which affect DMI, milk yield (MY), and feed efficiency (FE). Our objective was to evaluate FE between dairy cows with 2 genotypes: Slick-hair (SL) and wild-type (WT), under heat stress. SL (n = 8) and WT (n = 8) lactating cows with an average of 140 DIM, were used to evaluate FE for 60 d during April and May of 2018 in Puerto Rico (18. 027435,-67. 094955). Average THI during the experiment was 75.8 ± 0.03. Animals were fed a TMR based on *Brachiaria* spp. forage, soybean meal, steam flaked corn grain, brewers' grains, and minerals. On average the TMR contained 13.9% CP, 1.4 Mcal NEL/kg, 16.5% starch, and 43.1% aNDFom. The DMI was recorded daily with a Calan Feeding System and individual milk yield per cow was recorded daily with the Afimilk system. Milk was chemically analyzed weekly. Energy corrected milk (ECM) was calculated using the formula: ECM (kg/d) = 0.25 × kg of MY + 12.2 × kg of fat yield + 7.7 × kg of protein yield. Weekly average of milk yields and DMI, and weekly pooled milk components data were analyzed using Proc Mixed of SAS using a model that included genotype (SL and WT), week and their interactions as fixed effects. For each parameter week was used as a repeated measurement with cow as the subject. When evaluating MY, there was no interaction between genotype and week ($P = 0.316$). There was no difference in MY between genotypes ($P = 0.598$). Average MY in SL cows was 20.49 ± 0.94 kg with an average of 5.5% ± 0.24 fat and 3.42% ± 0.06 protein, while average MY in WT cows was of 19.49 ± 0.88 kg with an average of 4.86% ± 0.26 fat and 3.16% ± 0.07 protein. There was no difference in protein ($P = 0.233$) or fat ($P = 0.164$) content between the genotypes. Average DMI in SL was 18.72 ± 0.38 kg and WT 19.11 ± 0.38 kg ($P = 0.72$). FE was determined as kg of ECM/ DMI (kg). When evaluating FE, there was no interaction between genotype and week ($P = 0.316$). There was no difference in FE between genotypes ($P = 0.598$). SL cows had an average FE of 1.22 ± 0. 05; while WT cows had an average of 1.11 ± 0.08. In this study, where SL and WT cows were exposed to high THI and a high forage TMR diet, both genotypes presented a similar FE.

Key Words: slick hair, feed efficiency, heat stress

416 Effects of feeding native rumen microorganisms on production efficiency in lactating dairy cows.

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Study objectives were to evaluate effects of an endomicrobial feed supplement (EFS) (Galaxis; ASCUS Biosciences Inc., San Diego, California) comprised of 2 native rumen microbes on production parameters in mid-lactation dairy cows. Forty-seven lactating primiparous and multiparous Holstein cows (629 ± 9 kg BW; parity 1.9 ± 0.1; 119 ± 6 DIM; 45.11 ± 0.92 and 52.73 ± 0.87 kg/d MY for primiparous and multiparous, respectively) were enrolled in a study containing 2 experimental periods (P), during both periods cows were fed the same base TMR ad libitum once daily. During P1 (7 d) baseline data were obtained for covariate analysis. At the beginning of P2 (60 d), cows were randomly assigned to 1 of 2 dietary treatments: 1) a control diet (CON; base TMR; n = 24) or 2) a control diet supplemented with 5 g/d of EFS (EFS; n = 24). Overall, EFS had no effect on milk yield, ECM, DMI, BW, or BCS. However, EFS tended to increase feed efficiency (FE) and total milk solids content when compared with CON cows (2.2 and 1.1%, respectively; $P = 0.08$) and numerically increased protein content compared with CON cows (2.99 vs. 2.96%; $P = 0.13$). Cows from the original data set were retrospectively categorized into 2 milk production groups (MPG) within parity and classified as MPG1 (i.e., lower than median) and MPG2 (i.e., higher than median) based upon median P1 ECM (43.2 and 51.8 kg/d for primiparous and multiparous cows, respectively). EFS supplemented MPG1 cows had increased ECM (4.2%) and FE (2.8%) compared with CON cows ($P = 0.02$ and 0.05, respectively). There was a weak tendency for log somatic cell count to be increased in MPG1 EFS cows as compared with MPG1 CON cows ($P = 0.12$). There were no effects of EFS on production variables for MPG2 cows. In summary, EFS supplementation had little overall effects on production performance when all animals were analyzed. However, EFS supplementation improved milk production efficiency in lower producing primiparous (<43.2 kg/d) and multiparous (<51.8 kg/d) animals. Further investigation is needed to understand production level response differences.

Key Words: endomicrobial feed supplement, Holstein, microbiome

417 Effects of forage particle size on intake, growth performance, and ruminal pH of Holstein dairy calves.

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This study aim was to test the effects of supplementing forage with varying particle sizes on performance and ruminal pH of calves. Holstein calves (n = 40; 48.3 ± 3.2 kg of BW) from 15 to 90d of age were randomly assigned to 1 of 4 treatments (n = 10/treat; 5 males and 5 females): 1) starter without forage supplementation (CON), 2) starter with wheat straw chopped at 1mm geometrical mean particle length (GMPL) (FPS), 3) starter with wheat straw at 4 mm GMPL (MPS), 4) starter with wheat straw at 7 mm GMPL (LPS). The calves had ad libitum access to water, forage, and starter throughout the study. All calves were offered milk 7L/d from 15 to 25 old, 6L/d from 26 to 35d old, 5L/day from 36 to 45d old, and 3L/day from 45d old until weaning at 56d old. Starter and forage intake were recorded daily. Calves were weighed every 10d. ADG and feed efficiency was calculated for 3 periods: preweaning, post-weaning, and overall. Rumen pH was recorded at 35 and 90d of age at 4, 8 and 24h after morning feeding via stomach tube. Data were analyzed using the MIXED procedure of SAS with time as repeated measures for performance variables. Furthermore, a contrast between the control group and all groups fed forage was examined. During the post-weaning and overall periods starter, forage, and total DM intake were not affected by GMPL of wheat straw, but CON calves had lower starter and DM intake ($P = 0.05$) than calves offered forage. Through all 3 periods, ADG did not differ by forage particle size, but CON calves had lower overall ADG than calves fed wheat straw (0.806 kg/d, CON; 0.896 kg/d, MPS; 0.902 kg/d, LPS and 0.930 kg/d, FPS; $P = 0.05$). Feed efficiency did not differ across treatments. Ruminal pH was lower in CON than calves supplemented with forage at 8h after feeding on d 35 (5.50, CON vs 6.08, FPS; 6.16, MPS and 6.01, LPS; $P = 0.05$), 4h after feeding on d 90 (5.32, CON vs. 5.73, FPS; 5.91, MPS and 5.63, LPS; $P = 0.01$), and 8h after feeding on d 90 (5.48, CON vs 5.97, FPS; 6.02, MPS and 5.77, LPS; $P = 0.02$). In conclusion, supplementing wheat straw increased starter intake, improve calf performance, and modified ruminal pH dynamics of dairy calves but no difference was found for different GMPL treatments.

Key Words: chop length, growth, wheat straw

418 Large-scale evaluation of growth performance and health of dairy calves supplemented with condensed whey solubles on a commercial dairy calf ranch.

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Our objective was to determine the effects of supplementation of condensed whey solubles (CWS) on growth performance and health of dairy calves when fed with starter grain mix in a commercial dairy. Calves (2 d old; 350 Crosses, 124 Jersey, 42 Holstein) housed in individual hutches were used in a 12 wk completely randomized design study. Treatments were 1) control (CON) with no supplements, 2) 42 g/d of CWS (CWSL), and 3) 84 g/d of CWS (CWSH). During first 2 wk CWS was fed with milk replacer (MR) and during wk 3–12 CWS was mixed with starter (CP 22%). Calves were fed 1.89 L of MR from wk 1 to 2 and 2.83 L from wk 2 to 8.5, 2 × /d. From wk 8.5 to 11 calves fed 1.89 L MR, 1 × /d then weaned. Starter and water were fed ad libitum. Individual intakes of milk and starter were measured daily. Fecal (0 = firm, 3 = watery) and respiratory scores were recorded daily. Body weights (BW), frame growth and jugular blood samples were taken 1 d at wk 0, 8, and 12 at 3 h after morning feeding of 50 calves per treatment. Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significance was declared at $P < 0.05$ and tendency was $0.05 \leq P < 0.10$. Starter DMI tended to be greater ($P = 0.09$) in CWSL (573, 631 and 543 g/d; SEM = 31.5 for CON, CWSL and CWSH, respectively). Total DMI tended to be greatest ($P = 0.08$) in CON (1242, 1239, and 1143 g/d; SEM = 37.5). Starch intakes (213, 230 and 187 g/d; SEM = 11.3) were greater in CWSL. Sugar intakes (251, 290, and 297 g/d; SEM = 8) were greater in CWSL and CWSH. Calf BW (70, 75, and 69 kg; SEM = 1.4) and ADG (0.99, 1.06 and 0.97 kg/d; SEM = 0.02) were greater in CWSL. Glucose (101, 95, and 97 mg/dL; SEM = 1.54) was greater in CON. B-hydroxybutyrate (BHB; 44, 52, and 46 mg/dL; SEM = 1.93) was greater in CWSL. Less diarrhea incidences (0.17, 0.11, and 0.18%) were observed in CWSL. The gain: feed, withers height, hip width, plasma urea nitrogen and fecal scores were not different among the treatments. Supplementing CWSL improved starter intake, BW, BHB, fecal consistency, health with maintained frame growth.

Key Words: dairy calf, condensed whey solubles, growth performance

419 Effects of corn particle size on feeding behavior, intake, lactation, and metabolic status of early postpartum dairy cows.

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The hepatic oxidation theory states that, especially during the early postpartum (PP), intake is regulated by oxidation of fuels, thus highly fermentable carbohydrates would contribute toward reduced intake. Shifting the site of starch digestion to the small intestine may alleviate the reduced intake in the PP. The aim of the study was to modulate the site of starch digestion using different corn particle sizes, and evaluate its effects on feeding behavior, dry matter intake (DMI), milk yield and composition, and metabolism of dairy cows in the early PP. Jersey cows (n = 117) were used in a randomized block design. Treatment diets, formulated to contain 28% of starch, had either 3 or 6-mm ground corn (fine or coarse), as primary starch source. Diets were fed as total mixed ration (TMR) from calving to 30d PP. Cows were fed a common close-up diet. Intake, milk yield and body weight (BW) were measured daily; milk composition, body condition score (BCS), blood metabolites were obtained weekly. Data were analyzed using PROC GLIMMIX (SAS) with cow as a random factor. Feeding coarsely ground corn (CC) increased DMI (16.08 vs. 17.13 kg/d; $P \leq 0.05$) and milk yield (20.41 vs. 21.70 kg/d; $P \leq 0.05$) during the early PP compared with finely ground corn (CF). Diets did not affect eating (581.13 vs. 583.57 min/d) and rumination time (308.58 vs. 315.35 min/d, CF vs. CC) nor yields of milk protein, ECM, 3.5% FCM or MUN. Milk lactose increased with CC compared with the CF diet (4.61 vs. 4.70%; $P \leq 0.01$); milk fat percentage tended to be higher (5.57 vs. 5.27%; $P = 0.06$) with CF compared with the CC diet, but milk fat yield did not differ. Changes in BW and BCS were greater in cows fed CF (39.92 vs 32.24 kg and 0.23 vs. 0.14 units). Plasma NEFA concentration was lower in the CC fed cows (0.71 vs. 0.56 mmol/L; $P = 0.02$), suggesting that they were in a better metabolic status, thus reducing the mobilization of body reserves. Blood glucose level was not affected by treatment. The lower DMI in cows fed FC compared with cows fed CC supports the hepatic oxidation theory of the control of feed intake.

Key Words: hepatic oxidation theory, transition cow, starch fermentability

Ruminant Nutrition 5: Fat and Lipid Metabolism

420 Abomasal infusion of different exogenous emulsifiers alters fatty acid digestibility and milk fat yield of lactating dairy cows. C. M. Prom* and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of abomasal infusion of emulsifiers on fatty acid (FA) digestibility and production responses of lactating dairy cows. Eight rumen-cannulated multiparous cows (89 ± 13 DIM) were assigned to a treatment sequence in 4 × 4 Latin squares with 18 d periods consisting of 7 d of washout and 11 d of infusion. Treatments were abomasal infusions of water carrier only (CON) or 30 g/d of different emulsifiers enriched in different FA: polysorbate-C16:0 (Tween-40; T40), polysorbate-C18:0 (Tween-60; T60), and polysorbate-C18:1 (Tween-80; T80). Emulsifiers were dissolved in water and delivered at 6 h intervals. Cows were fed the same diet which contained (% DM) 32% NDF, 16% CP, 26% starch, and 3.4% FA (2% DM from a FA supplement containing 34% C16:0 and 47% C18:0). The statistical model included the random effect of cow within square and the fixed effects of treatment, period, square, and their interactions. Pre-planned contrasts included CON vs. average of T40, T60, and T80 (TWEEN); CON vs. T80; and T80 vs. the average of T40 and T60 (T40+T60). Compared with CON, TWEEN did not affect DMI or digestibility ($P > 0.15$), but increased milk fat content ($P = 0.04$) and tended to increase milk fat yield ($P = 0.06$) and 3.5% FCM ($P = 0.09$). Compared with CON, T80 increased total (73.3% vs. 69.3%, $P = 0.02$), 16-carbon (73.1% vs. 69.8%, $P = 0.04$), and 18-carbon (73.0% vs. 68.8%, $P = 0.02$) FA digestibility. T80 tended to decrease DMI ($P = 0.08$) and increase digestibility of DM ($P = 0.09$) and NDF ($P = 0.09$). T80 increased milk fat content (3.70% vs. 3.33%, $P = 0.04$) and yield (1.66 vs. 1.49 kg/d, $P = 0.04$), tended to increase 3.5% FCM ($P = 0.06$), and tended to reduce milk protein yield ($P = 0.06$) compared with CON. Compared with T40+T60, T80 increased total (73.3% vs. 69.4%, $P = 0.01$), 16-carbon (73.1% vs. 70.1%, $P = 0.04$), and 18-carbon (73.0% vs. 68.7%, $P = 0.01$) FA digestibility and tended to increase DM digestibility ($P = 0.06$), but did not affect DMI ($P = 0.19$) or NDF digestibility ($P = 0.29$). In conclusion, T80 infusion increased the digestibility of total, 16-carbon, and 18-carbon FA, compared with control and T40+T60, suggesting that the predominant FA attached to polysorbate affects its ability to improve FA digestibility.

Key Words: emulsifier, fatty acid digestibility, milk fat

421 Effect of olive oil and hydrogenated vegetable oil supplementation on rumen bacterial composition in dairy cows. N. Cancino-Padilla*[1], J. Romero[2], S. A. Huws[3], and E. Vargas-Bello-Pérez[4,1], [1]*Pontificia Universidad Católica de Chile, Santiago, Chile,* [2]*Universidad de Chile, Santiago, Chile,* [3]*Queen's University of Belfast, Belfast, UK,* [4]*University of Copenhagen, Copenhagen, Denmark.*

Diet is the most important factor influencing the rumen microbiome, especially under long-term dietary interventions. Dietary fatty acids can affect the rumen microbiome, and this will depend on their degree of FA saturation. The objective of this research was to study the effect of supplementing the diet of dairy cows with olive oil (OO) and palm oil (HVO) on rumen microbiota. Fifteen Holstein cows averaging (±SD) 189 ± 28 d in milk were assigned to 3 treatment groups. For 63 d animals were fed a control diet (basal diet; 65% forage: 35% concentrate) with no added lipid and 2 supplemented diets (30 g/kg DM). Rumen samples were collected every 21 d for 63 d using an esophageal-ruminal sampling device. Total microbial DNA was extracted from samples for high-throughput sequencing of the 16S rRNA gene through Illumina MiSeq platform. 16S rRNA gene amplicon sequences were quality checked with FASTQC and analyzed by DADA2 and Phyloseq R packages. Taxonomy was assigned using the SILVA Database version 132. A total of 3,762,232 sequences were obtained from 48 samples and clustered into 9829 OTUs. Results revealed the dominance of phyla *Firmicutes* and *Bacteroidetes*, whereby *Firmicutes* was the most prevalent phylum in Control (75.2%), OO (71.1%) and HVO (75.2%). At the genus level, *Succiniclasticum* and *Prevotella* were the most dominant, and these belong to the phyla *Firmicutes* and *Bacteroidetes*, respectively. *Succiniclasticum* increased significantly in relative abundance post OO supplementation ($P < 0.0001$) throughout 63 d, while *Prevotella* increased ($P < 0.0001$) only after 42 d of OO supplementation. Following HVO dietary supplementation, *Succiniclasticum* increased in relative abundance ($P < 0.0001$) in throughout the study period, while *Prevotella* decreased over time ($P < 0.0001$). Olive oil increased the relative abundance of *Succiniclasticum* and *Prevotella*, which are related to methane mitigation and energy supply for the host, respectively. Both roles could be associated with more efficient animals and thus improved milk production. This study was sponsored by a research grant from FONDECYT 1170400, Chile.

Key Words: rumen bacteria, olive oil supplementation

422 Effect of palmitic acid-enriched supplements containing stearic or oleic acid on nutrient digestibility and production responses of low- and high-producing dairy cows. A. M. Burch*, A. Pineda, and A. L. Lock, *Michigan State University, East Lansing, MI.*

We evaluated the effects of fatty acid (FA) supplement blends containing 60% palmitic acid (C16:0) and either 30% stearic (C18:0) or 30% oleic (C18:1) acid on nutrient digestibility and production responses of low- and high-producing dairy cows. Multiparous Holstein cows (118 ± 44 DIM) were randomly assigned to treatment sequences in replicated 3 × 3 Latin squares with 21 d periods. Cows were assigned to one of 2 groups based on milk yield (n = 12/group): a) low group (40.0 ± 2.14 kg/d) and b) high group (54.3 ± 1.87 kg/d). Treatments were: 1) control (CON; diet with no supplemental FA), 2) FA supplement blend containing 60% C16:0 and 30% C18:0 (PA+SA), and 3) FA supplement blend containing 60% C16:0 and 30% C18:1 (PA+OA). Blends were fed at 1.5% DM. The statistical model included the random effect of cow within production group, and the fixed effect of treatment, production group, period, and interactions. Results are presented in the following order: CON, PA+SA, PA+OA. Regardless of production level, FA supplementation reduced DMI (33.2, 32.8, 32.4 kg/d; $P < 0.05$). Compared with CON, PA+SA decreased and PA+OA increased total-tract FA digestibility (71.7, 65.3, 74.1%, $P < 0.001$). Treatment by production group interactions were observed for NDF digestibility ($P = 0.10$), total FA intake ($P = 0.09$), and the yields of 3.5% FCM ($P < 0.01$), ECM ($P < 0.02$), and milk fat ($P < 0.01$). FA treatments increased NDF digestibility in low- (41.8, 43.9, 42.8%, $P = 0.10$) and high-producing cows (41.5, 45.7, 44.0%, $P = 0.10$) and increased total FA intake in low- (753, 1098, 1128 g/d, $P = 0.09$) and high-producing cows (815, 1207, 1237 g/d, $P = 0.09$). In low-producing cows, PA+SA increased 3.5% FCM (41.2, 42.6, 40.9 kg/d, $P = 0.01$), ECM (41.7, 43.0, 41.2 kg/d, $P = 0.02$), and milk fat yield (1.54, 1.60, 1.52 kg/d, $P < 0.01$). In high-producing cows PA+OA increased 3.5% FCM (51.6, 53.1, 54.6 kg/d, $P = 0.01$), ECM (52.1, 53.3, 54.5 kg/d, $P = 0.02$), and milk fat yield (1.80, 1.86, 1.92

kg/d, $P < 0.01$). In conclusion, high-producing cows responded better to a FA supplement containing 60% C16:0 and 30% C18:1, whereas lower-producing cows responded better to a FA supplement containing 60% C16:0 and 30% C18:0.

Key Words: fat supplementation, milk fat, production level

423 Altering the ratio of dietary palmitic and oleic acids impacts nutrient digestibility and energy intake in early lactation dairy cows.

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Fifty-six multiparous cows were used in a randomized complete block design and assigned to 1 of 4 treatments fed from 1 to 24 DIM. The treatments were a control diet (CON; non-fatty acid [FA] supplemented diet) and diets supplemented at 1.5% DM with FA supplement blends differing in the ratio of palmitic (C16:0) and oleic (C18:1) acids. FA treatment diets were: 80:10 (80% C16:0+10% C18:1); 70:20 (70% C16:0+20% C18:1); and 60:30 (60% C16:0+30% C18:1). Digestibility of nutrients was calculated using iNDF as a marker. The statistical model included the random effect of block and cow, and the fixed effects of treatment, time, and their interactions. Results are presented in the following sequence: CON, 80:10, 70:20, and 60:30. Compared with CON, FA-supplemented diets increased the intake of 16-carbon (54, 311, 266, and 256 g/d, $P < 0.01$), 18-carbon (253, 338, 360, and 417 g/d, $P < 0.01$), and total FA (316, 669, 643, and 688 g/d, $P < 0.01$). Increasing C18:1 in FA-supplemented diets increased the intake of total and 18-carbon FA (both linear, $P < 0.01$) and decreased the intake of 16-carbon FA (linear, $P < 0.01$). Compared with CON, FA-supplemented diets increased the digestibility of DM (69.2, 69.9, 70.2, and 71.0%, $P < 0.01$), NDF (37.6, 40.0, 39.9, and 41.3%, $P < 0.01$), 18-carbon (85.7, 88.0, 88.5, and 88.7%, $P < 0.01$), and total FA (82.3, 82.5, 83.8, and 84.9%, $P < 0.01$). Increasing C18:1 in FA-supplemented diets increased digestibility of DM, NDF, total FA, and 18-carbon FA (all linear, $P < 0.01$). Compared with CON, FA-supplemented diets increased the intake of digestible energy (DE; 57.0, 68.1, 65.3, and 69.5 Mcal/d, $P < 0.01$), metabolizable energy (ME; 49.3, 59.2, 56.8, and 60.6 Mcal/d, $P < 0.01$), and net energy of lactation (NE_L; 31.2, 37.1, 36.1, and 38.6 Mcal/d, $P < 0.01$). Increasing C18:1 in FA-supplemented diets increased DE, ME, and NE_L intake (all linear, $P = 0.01$). Our results indicate that feeding FA supplements containing C16:0 and C18:1 increased the digestibility of NDF, FA, and DM and increased energy intake compared with a control diet. Increasing C18:1 in the FA supplement increased DM, NDF, and FA digestibility resulting in increased energy intake.

Key Words: digestibility, fat supplementation, postpartum

424 Dietary fatty acid composition alters digestibility and digestible energy intake in lactating dairy cows.

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We determined the effect of dietary fatty acid (FA) composition on digestibility of dry matter (DM), neutral detergent fiber (NDF), and FA (DMD, NDFD, FAD, respectively) and digestible energy intake (DEI). Our database comprised 423 individual observations from 124 lactating Holstein cows receiving diets that varied in FA composition from 5 studies. Dietary lower and upper quartiles of C16:0, C18:0, C18:1, C18:2, and C18:3 were 0.61 to 1.68, 0.07 to 0.23, 0.63 to 0.79, 1.29 to 1.81, and 0.18 to 0.21% DM, respectively. Starch and forage NDF (fNDF) varied from 26 to 29 and 19 to 21% DM, respectively, and DMI varied from 14 to 40 kg/d. Energy values were determined in feed ingredients, orts, and feces by bomb calorimetry using iNDF as a marker. Meta-regression used the following fixed effects: all the FA variables described above, respective quadratic terms, 2-way interactions between each FA category and starch, fNDF, and DMI. Random effects were study-specific intercepts and slopes on DMI, cow (study), period (study), and block (study). Best fitting prediction equations are presented in Table 1. C16:0 and C18:0 were the dietary FA with the most significant impact on digestibility and DEI. C16:0 increased NDFD and DEI when increased in the diet from 0.33 to 1.27% of DM, while C18:0 decreased DEI when it was increased from 0.35 to 0.88%

Table 1 (Abstr. 424). Best-fit equations for digestibilities and DEI. Coefficients model the effect of deviations from the mean for each variable

Effect	DMD	NDFD	FAD	DEI
Intercept	66.4 ± 0.98	42.5 ± 0.86	76.5 ± 0.60	96.0 ± 1.47
DMI (linear, L)	−0.01 ± 0.06	0.06 ± 0.10	−0.37 ± 0.13	2.83 ± 0.07
DMI (quadratic, Q)	−0.02 ± 0.01	—	−0.03 ± 0.01	—
Starch	1.58 ± 1.03	—	—	3.86 ± 0.94
fNDF	−0.76 ± 1.16	0.96 ± 1.00	−1.49 ± 0.69	0.36 ± 1.02
Palmitic (L)	3.02 ± 0.30	1.71 ± 0.66	−1.71 ± 0.50	0.39 ± 0.85
Palmitic (Q)	−5.66 ± 1.08	—	—	−31.6 ± 3.72
Stearic (L)	−6.48 ± 1.19	16.4 ± 5.40	−11.0 ± 1.10	−16.7 ± 2.01
Stearic (Q)	—	−34.3 ± 10.6	—	—
Oleic (L)	—	5.12 ± 2.11	—	15.2 ± 5.45
Oleic (Q)	—	—	—	−33.9 ± 14.1
Linoleic (L)	—	−4.87 ± 1.24	—	21.4 ± 2.86
Linolenic (L)	—	—	—	325 ± 45.6
Linolenic (Q)	—	—	—	−4527 ± 608
DMI × fNDF	0.20 ± 0.08	0.27 ± 0.13	—	0.25 ± 0.08
Starch × Palmitic	−1.26 ± 0.46	—	—	−3.60 ± 0.80
Starch × Stearic	9.37 ± 2.67	—	—	−18.1 ± 5.95
fNDF × Palmitic	—	1.38 ± 0.65	—	—

of DM. Results could aid the development of models to predict DEI from dietary ingredients including dietary FA.

Key Words: digestibility, fatty acid, energy intake

425 Palmitic fatty acid dosed in continuous culture fermenters increases fiber digestibility estimates.

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Preliminary research dosing palmitic fatty acid (PFA) to continuous culture fermenters contradicted meta-analyses reporting improved total-tract NDF digestibility (NDFd). However, previous experiments dosed fat within pelleted feed and may have inhibited general microbial activity. Our objective was to evaluate whether PFA increases NDFd in continuous culture by dosing PFA supplements as a top dress. In the present study, dual-flow continuous culture fermenters were fed one of 4 treatment diets, either a control (CON) diet, or CON with 85% PFA fat supplement top-dressed at 1, 2, or 3% of the total DM fed (L1, L2, and L3, respectively). The CON diet (37.7 g DM/d) was a 50:50 alfalfa:concentrate pellet mix providing 7.4 g CP, 14.8 g NDF, 11.0 g ADF, and 5.3 g starch fed once daily. Each level of PFA provided 0.4 g of additional fat. Fermenters (n = 8) were run in 4 periods of 2 4 × 4 Latin squares, lasting 11 d each with 4 d sample collection. Buffer dilution and solids passage rate were maintained at 7.0%/h and 5.0%/h, respectively. Data were analyzed using a mixed model with fixed effect of treatment, random effects of fermenter and period, and repeated sampling day. The addition of PFA increased NDFd quadratically ($P = 0.02$; CON: 44.3, L1: 45.9, L2: 45.8, and L3: 44.8%) and addition of PFA tended to increase ADF digestibility quadratically ($P = 0.06$; CON: 49.3, L1: 50.0, L2: 50.1, and L3: 48.3%). Starch digestibility (Avg. = 97.9%) was unaffected ($P > 0.20$) by treatment. Molar proportion of acetate and propionate were cubically affected by dose ($P \leq 0.01$) and isovalerate tended to be cubically affected ($P = 0.08$) but varied no more than 0.1 over all dose levels. No other VFA were affected by dose ($P > 0.20$). Bacterial N flow was not affected ($P > 0.20$) by dose, accordingly, bacterial N yield per NDF or ADF digested (g/kg) tended ($P = 0.06$) to be quadratically affected by PFA dose. There was no effect ($P > 0.20$) of dose on the percent bacterial N from ammonia (Avg. = 69.2%). The present study replicates previous PFA effects on NDFd - indicating potential for this gain to occur within the rumen. Since PFA did not increase bacterial N yield nor efficiency, microbiome analysis may elucidate community shifts.

Key Words: palmitic fatty acid, fermenter, NDF digestibility

426 Effects of fatty acid and one-carbon donor abomasal infusates on hepatic ceramide and phosphatidylcholine levels in lactating dairy cows.

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Dietary fatty acids (FA) and one-carbon donors may influence ceramide and phosphatidylcholine (PC) synthesis in cows with implications for health. In a 5 × 5 Latin square design, 5 rumen-cannulated Holstein cows (214 ± 4.9 DIM; 3.2 ± 1.1 parity) were enrolled in a study with each experimental period lasting 6 d. Abomasal infusates consisted of (1) palmitic acid (PA; 98% 16:0 of total fat), (2) PA + choline chloride (PA+C; 50 g/d choline ion), (3) PA + L-serine (PA+S; 170 g/d L-serine), (4) behenic acid (BA; 92% 22:0 of total fat), and (5) a docosahexaenoic acid algal oil (47.5% DHA of total fat). Emulsions were formulated to provide 301 g/d of total FA and were balanced to provide a minimum of 40 and 19 g/d of 16:0 and glycerol, respectively. Cows were fed a corn silage-based diet. Blood was collected on d 0, 1, 3, 5, and 6. Liver was biopsied on d 6. Plasma choline metabolites, and hepatic ceramides and PC were measured using mass spec. For univariates, ANOVA was performed including fixed effects of treatment and time, and their interaction. Generalized log-transformed lipidomic data were analyzed using multivariate ANOVA. Although treatment did not modify milk yield, milk fat yield and content were lowest for DHA-infused cows ($P < 0.05$). Plasma triglyceride, cholesterol, and insulin levels were lowest with DHA infusion ($P < 0.05$). Plasma choline, betaine, dimethylglycine, and trimethylamine *N*-oxide levels were greatest in cows infused PA+C ($P < 0.01$). Hepatic ceramide-d18:2/22:0 and -d16:1/22:0 levels were elevated in cows infused BA ($P < 0.05$). Infusion of DHA increased hepatic PC containing 4 or more double bonds (52 out of 61 PC; e.g., PC-14:0/22:6 or 22:0/20:4; $P < 0.05$), relative to other treatments. Hepatic PC containing saturated FA were lowest for DHA-infused cows. While PA+C increased hepatic PC with moderate saturation ($P < 0.05$), PA+C decreased highly unsaturated PC when compared with PA ($P < 0.05$). A similar outcome was observed for cows infused PA+S. Abomasal infusion of saturated FA, DHA, and one-carbon donors uniquely modifies hepatic ceramide and PC levels in lactating cows.

Key Words: ceramide, docosahexaenoic acid, phosphatidylcholine

427 Effects of abomasal infusions of fatty acids and one-carbon donors on the plasma and muscle metabolome of lactating cows.

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Dietary fatty acids (FA) influence the composition of sphingolipids and glycerophospholipids, and modulate energy metabolism. In a 5 × 5 Latin square design, 5 rumen-cannulated Holstein cows (214 ± 4.9 DIM; 3.2 ± 1.1 parity) were enrolled in a study with each experimental period lasting 6 d. Abomasal infusates consisted of (1) palmitic acid (PA; 98% 16:0 of total fat), (2) PA + choline chloride (PA+C; 50 g/d choline ion), (3) PA + L-serine (PA+S; 170 g/d L-serine), (4) behenic acid (BA; 92% 22:0 of total fat), and (5) a docosahexaenoic acid algal oil (47.5% DHA of total fat). Emulsions were formulated to provide 301 g/d of total FA and were balanced to provide a minimum of 40 and 19 g/d of 16:0 and glycerol, respectively. Cows were fed a corn silage-based diet. Blood was collected on d 0, 1, 3, 5, and 6. Skeletal muscle was biopsied on d 6. Plasma metabolites and muscle lipids were measured using untargeted metabolomics. Generalized log-transformed data were analyzed using ANOVA (effects of treatment, time, and their interaction). Plasma FA (e.g., DHA and arachidonic acid) increased in response to DHA infusion ($P < 0.05$). Infusing DHA reduced plasma tyrosine, leucine, alanine, and pyruvic, citric, and uric acids, and increased plasma succinic acid ($P < 0.05$). Plasma choline and betaine increased in PA+C, and serine increased in PA+S ($P < 0.05$). Specific DHA-containing plasma triacylglycerols (TAG, n = 44) and PC (n = 8) were only detected in DHA-infused cows. Discriminant analysis revealed a unique lipidomic signature in response to DHA, with most of the treatment differences reflected as changes in plasma PC (111/143; $P < 0.05$). Saturated PC increased in PA (e.g., PC-16:0/20:4; $P < 0.05$), whereas DHA increased very-long chain PC and TAG containing DHA (e.g., PC-40:4 and TAG-16:0/16:0/22:6, respectively; $P < 0.05$). Distinctive treatment effects on muscle lipids were observed in discriminant analysis, characterized by changes in PC, TAG and ceramides (e.g., ceramide-d18:1/22:0 was higher in BA, relative to PA and DHA; $P < 0.05$). We conclude that the abomasal infusion of FA and one-carbon donors modifies the plasma and muscle metabolome.

Key Words: cow, fatty acid, metabolome

428 High oleic soybeans increase milk fat yield at high and low inclusion levels.

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Unsaturated fatty acids (FA) and diet fermentability are key risk factors for milk fat depression. Linoleic acid (C18:2) is the greatest risk. Our hypothesis was that high oleic soybeans would result in higher milk fat yield compared with conventional soybeans. Eighteen non-cannulated multiparous Holstein cows (2.7 ± 0.8 parities; 153 ± 64 DIM; 45.7 ± 7.0 kg milk; 3.47 ± 0.58% milk fat; Mean ± SD at end of pretrial period) were used in a crossover design with 24 d periods. Each period consisted of 2 dietary phases. The first 14 d a diet with 5% roasted soybean in a 31% NDF diet was fed and the last 10 d a diet with 10% roasted soybeans and 28.5% NDF was fed. Treatments were conventional and high oleic roasted soybeans (Plenish, DuPont Pioneer). A common diet was fed for 7 d before the trial and during a 7 d washout between periods. Milk was sampled on d 10, 21, 27, and 31. Data were analyzed using the MIXED procedure of SAS (version 9.3, SAS Institute Inc., Cary, NC). The model included the random effects of period and cow and the fixed effect of soybean type, soybean level, and the interaction of soybean type and level. The preplanned contrasts tested the effect of treatment at each time point. There was no effect of high oleic acid soybeans on DMI or milk yield (averaged > 43 kg/d). Overall, high oleic soybeans increased milk fat percent 0.17 percentage units ($P < 0.05$) and tended to increase milk fat yield 89 g/d ($P = 0.08$) and there was no interaction of soybean type and level. The lower fiber and higher soybean diet increased milk fat concentration 0.2 percentage units and increased milk fat yield 92 g/d (both $P = 0.01$). There was no effect of high oleic soybeans on the concentration of de novo, 16 carbon, or preformed fatty acids in milk fat. The lower fiber and higher soybean diet decreased de novo synthesized FA and mixed source 16 C fatty acids by almost 3 and 9%, respectively, and increased preformed fatty acids over 10%. High oleic acid soybeans decreased *trans*-11 C18:1 and *trans*-10 C18:1 in milk. In conclusion, high oleic soybeans increased milk fat through decreased biohydrogenation induced milk fat depression. Additionally, increasing soybeans increased milk fat through providing preformed fatty acids.

Key Words: milk fat, high oleic, soybeans

429 Effect of oleic acid and lecithin in saturated fatty acid supplements on production and nutrient digestibility in lactating dairy cows.

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Saturated fatty acid (FA) supplements are commonly based on palmitic acid (PA), stearic acid (SA), and oleic acid (OA). Commercial fat supplements reacted with magnesium oxide have also become available. Recent work has indicated that abomasal infusions of OA and chemical emulsifiers increase FA digestibility. Lecithin is a natural emulsifier, but its effect on fat supplements has not been well investigated. Our hypothesis was that the inclusion of OA and lecithin in a FA supplement would increase FA digestibility and milk production without decreasing intake. Treatments were included at 1.75% of the diet and were prills made from a base free FA mixture containing 33.6% PA, 59.4% SA, and 2.9% OA (SF; used as a control), or prills made from the base with 1.9% added OA (SFO), the base FA blended with 10% soybean lecithin (SFOL), the base reacted with 4% magnesium oxide (MS) or the base reacted with magnesium oxide with 2.4% added OA (MSO). Ten multiparous (151 ± 45 DIM) and 5 primiparous (113 ± 23 DIM) Holstein dairy cows were arranged in a 5x5 Latin square design with 14-d periods. Digestibility was calculated using 240 h iNDF with acetone rinse. The statistical model included random effects of cow and period, the fixed effects of treatment and parity, and the interaction of treatment and parity. There were no differences between treatments for DMI (22.3 ± 0.3 kg), milk yield (37.7 ± 0.4 kg), or milk fat and protein concentration or yield ($P > 0.10$). Dry matter and NDF digestibility were also not affected. The MSO, SFOL, and SFO treatments increased total FA digestibility 1.5, 1.3, and 1.0 percentage units compared with MS, respectively. C16 FA digestibility was increased 6.8 and 5.9 percentage units by SFOL and MSO compared with SF, and was also increased 10.4, 9.5, and 7.3 percentage units by SFOL, MSO, and SFO compared with MS, respectively ($P < 0.001$). Digestibility of 18 carbon FA was not affected by treatment ($P = 0.13$). Addition of OA and lecithin increased FA supplement digestibility with no effect on intake.

Key Words: fatty acid digestibility, oleic acid, lecithin

430 Simulating precision feeding of high concentrate diets with high fat inclusion and different unsaturated fat sources in continuous culture fermentors.

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One of the strategies to reduce feed costs and increase efficiency is to control dry matter intake. Diets typically used for limit-feeding are more nutrient dense, allowing an increase in energy and nutrient utilization efficiency while decreasing nutrient loss. The term "Precision-feeding" is a program that provides heifers with the correct amount of nutrients necessary for adequate growth without affecting future performance. The objective of this study was to evaluate the effects on fermentation and nutrient digestion of including different unsaturated fat sources when high concentrate diets with high fat inclusion are used when simulating precision feeding in continuous culture. We hypothesized that incorporating different unsaturated fat sources to the aforementioned program can improve nutrient utilization without affecting rumen fermentation. Four treatments were randomly assigned to 8 continuous culture fermenters for 2 periods of 10 d. Treatments included a high concentrate diet (HC; 65%) with high fat inclusion starting with a basal level of fat as control [3% fat (0% fat inclusion; CON); 9% fat (6% poultry fat inclusion; PF); 9% fat (6% coconut oil inclusion; CO); and 9% fat (6% soybean oil inclusion; SOY)]. Data were analyzed using the MIXED procedure of SAS with repeated measures. The PDIFF option adjusted by Tukey method was included in the LSMEANS statement to account for multiple comparisons. Dry matter, OM, NDF, and ADF apparent digestibility (AD) were higher in CO-and PF-fed fermenters vs SOY and CON (Table 1). Mean culture pH and NH$_3$-N were highest in CO-fed fermenters followed by PF, then SOY, and CON. These results suggest that simulated high concentrate high fat diets can improve nutrient digestibility while affecting rumen fermentation by altering rumen pH and NH$_3$N concentration in continuous culture fermenters.

Table 1 (Abstr. 430). Apparent digestibility of fermenters fed 3 types of fat

	Type of fat				
Digestibility, %	CON 3%	PF 9%	SOY 9%	CO 9%	SE
DM	66.9^c	80.1^a	76.4^b	80.9^a	0.35
OM	75.2^c	85.1^a	81.9^b	85.8^a	0.28
NDF	41.7^c	59.9^a	53.9^b	60.7^a	0.65
ADF	33.9^c	50.6^a	46.6^b	51.2^a	0.81

Key Words: precision feeding, poultry fat, continuous culture

431 Production responses to increasing levels of calcium salts of palm fatty acids in dairy cows grazing tropical pastures.

J. M.

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Fat supplementation is an important strategy to increase energy intake of lactating dairy cows. Previous studies have shown that calcium salts of palm fatty acids (CSPF) increased milk and fat yield, but a dose response of CSPF is not well established in dairy cows grazing tropical pastures. Therefore, we evaluated the effects of CSPF on production responses of lactating dairy cows grazing tropical pastures. Forty Holstein × Jersey crossbred cows averaging (mean ± SEM) 20.4 ± 0.35 kg of milk and 20 ± 5 DIM were distributed in a randomized block design to receive one of 4 CSPF levels: Control (no CSPF added); 200 g/d; 400 g/d; and 600 g/d. Dietary treatments were fed for 90 d. Statistical analysis was performed using the MIXED procedure of SAS, with repeated measures, including fixed effects of CSPF and time, and random effects of cow, block, and cow nested in block. Significant differences were declared at $P \leq 0.05$, and tendencies at $P \leq 0.10$. CSPF linearly increased milk yield (from 22.8 to 26.3 kg/d; $P = 0.01$), tended to increase fat yield (from 0.79 to 0.88 kg/d; $P = 0.08$), and did not affect the yields of protein (0.73 kg/d; $P = 0.8$), casein (0.55 kg/d; $P = 0.9$), lactose (1.12 kg/d; $P = 0.1$) and total solids (2.88 kg/d; $P = 0.2$). For milk component concentrations, CSPF did not affect milk fat (3.38 g/100g; $P = 0.3$), had a negative linear effect on protein (from 3.30 to 2.82 g/100 g; $P < 0.01$) and casein (from 2.45 to 2.11; $P < 0.01$), and had no effect on total solids (11.82%; $P = 0.7$) and MUN (13.45 mg/dl; $P = 0.2$). In conclusion, increasing CSPF increased milk and milk fat yield, maintained fat concentration, did not alter milk protein yield and reduced protein concentration.

Key Words: calcium salts, fat supplementation, grazing cow

Animal Behavior and Well-Being 2

W1 Tryptophan supplementation in calf milk replacers at weaning as an attempt to facilitate weaning. M. Terré[1], A. Bassols[2], M. Vidal[1], and A. Bach*[3,1], [1]*Institut de Recerca i Tecnologia Agroalimentàries, Caldes de Montbui, Barcelona, Spain,* [2]*Universitat Autònoma de Barcelona, Bellaterra, Barcelona, Spain,* [3]*Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

Tryptophan is a precursor of serotonin, a neurotransmitter that participates in the control of the affective state of the animal. We hypothesized that Trp supplementation could help dairy calves to cope with weaning stress. Twenty-seven Holstein male calves (48.2 ± 0.79 d old; 82.1 ± 2.57 kg BW) were used to evaluate the effects of Trp supplementation at the rate of 4.5 g/d via milk replacer (MR) on performance and behavioral parameters the weeks around weaning. All calves received the same feeding program (6 L/d at 15% DM from d 1 to 7, 4 L/d at 15% DM from d 8 to 14, and 2 L/d at 15% DM in one feeding until d 21 of study) and were weaned 21 d after the beginning of the study (around 63 d of life). Calves were fed starter feed (18% CP and 16.1% NDF) and chopped straw ad libitum. Animals were weighed weekly, DM intakes recorded daily, lying behavior was recorded using accelerometers throughout the study, and a scan sampling was performed twice a week, 1 h after the morning feeding to record behavioral activity (non-nutritive oral behaviors, suckling a neighbor calf, standing, resting, rumination, vocalizations, eating, and drinking). Data were analyzed with a mixed-effects model with repeated measures. Tryptophan supplementation did not affect calf performance nor concentrate and MR intake, but straw intake tended ($P = 0.07$) to be greater in non-supplemented compared with Trp-supplemented calves (153 vs 129 ± 9.0 g/d, respectively). Lying time decreased, lying bouts increased, and lying duration decreased when changes in the MR feeding program occurred, independent of treatment. Similarly, differences in behavioral observations occurred along days of study, with no impact of Trp-supplementation. The main changes observed in calf behaviors were an increase in vocalizations and standing time 1 h after the morning feeding at weaning, but again these changes were independent of treatment. Supplementing 4.5 g/d of Trp via MR between 48 and 62 d of life had no effect on performance and behavior in calves around weaning.

Key Words: calf, tryptophan, weaning

W2 Are fly avoidance behaviors of dairy cows housed on pasture influenced by the use of mesh fly leggings? R. Perttu*, B. Heins, H. Phillips, and M. Endres, *Department of Animal Science, University of Minnesota, St. Paul, MN.*

The objective of this study was to evaluate the effects of mesh fly leggings (Shoofly Leggins; Stone Manufacturing & Supply, Kansas City, MO) on number of flies and fly avoidance behaviors of pastured dairy cows. The study was conducted at the University of Minnesota West Central Research and Outreach Center (Morris, MN, USA) from June to July 2017. In this replicated crossover design study, dairy cows housed on pasture (n = 80) were randomly assigned to 1 of 2 treatment groups: leggings (on all legs) and control (no leggings). Cows were exposed to their treatment for a 2-week period, then switched treatments every period for a total of 4 periods (2 replicates per treatment). Counts for face, horn, and stable flies were recorded on all cows twice daily (0930 to 1230 and 1330 to 1630), 3 times per week. A random subset of 40 focal cows was observed in 5-min intervals for frequency of foot stomps, head tosses, skin twitches, and tail swishes. Period means were used for the analysis using PROC GLIMMIX of SAS. Poisson models were built for fly count data with fixed effects of treatment, time of day, treatment and time of day interaction, period within replicate, replicate, and order of treatment, and a random effect of cow. Head toss, skin twitch, and tail swish behaviors were similar between treatment groups and time of day. Leg stomps were greater ($P < 0.001$) for the control group than the leggings group (mean ± SE; 2.8 and 2.1 ± 0.3 per observation, respectively), and leg stomps were greater ($P < 0.001$) in the afternoon than in the morning (2.8 and 2.1 ± 0.3 per observation, respectively). The number of stable flies was a predictor ($P < 0.0001$) of all observed behaviors and the number of horn flies was a predictor ($P < 0.05$) of head toss, skin twitch, and tail swish behaviors. The number of stable flies on cows was greater ($P < 0.001$) in the afternoon compared with the morning (20.6 ± 0.8 and 15.0 ± 0.6 per cow, respectively). The results of this study indicate that flies cause fly avoidance behaviors in cows regardless of the use of leggings. However, leggings effectively reduce leg stomps and may offer some relief to dairy cows on pasture.

Key Words: fly avoidance, behavior

W3 Public acceptance of dairy calf housing options. R. Perttu*, B. Ventura, and M. Endres, *Department of Animal Science, University of Minnesota, St. Paul, MN.*

The objective of this study was to understand public acceptance of various forms of dairy calf housing. Adults (age 18 and up, n = 1310) were invited to complete a 28-item survey at the Minnesota State Fair (St. Paul, MN) in summer 2018. The survey was administered via Qualtrics using iPads and, in addition to collecting demographics, presented 3 images of calf housing options [individual (“IH”), pair (“PH”), or group (“GH”)] and asked participants to likert rate their acceptance for each option. Most participants (median age = 45 – 54 yr) were female (65%), urban residents (82%) who had completed a bachelor’s degree (42%) and owned a pet (94%). Most participants did not have a loved one working in dairy industry (78%), nor did most have any experience handling livestock (81%), though they had visited a livestock farm in the past (63%). Data were analyzed using PROC GLIMMIX of SAS, and multinomial models were built with rank of housing acceptance as the dependent variable. Overall, highest acceptance was found for GH (75.8% that agreed or strongly agreed about acceptability), followed by PH (66.0%) and IH (31.5%). Acceptance of IH was associated with sex, urban v. rural background, previous livestock experience, and knowing an individual in the dairy industry. More females disagreed that IH was acceptable compared with males (52.1 ± 0.01% vs. 37.6 ± 0.02%, respectively, $P < 0.0001$). Acceptance of IH was also less frequent among urban residents (28.8 ± 0.01% vs. 43.0 ± 0.03% of rural residents, $P < 0.0001$). Those reporting loved ones in the dairy industry were likewise less accepting of IH (50.3 ± 0.02% vs. 35.1 ± 0.03% of those lacking loved ones working in dairy, $P < 0.0001$), as were participants reporting livestock experience (49.2 ± 0.03% vs. 27.3 ± 0.01% of those without livestock experience, $P < 0.0001$). These findings suggest that group housing may be the most socially accepted form of dairy calf housing, while individual housing of calves appears less acceptable. Demographic factors, including sex, rural v. urban background, and livestock association appear to play a role in predicting acceptance of calf housing systems. Further qualitative analysis will be used to identify reasons underlying adult acceptance of calf housing options.

Key Words: public perceptions

W4 Udder wetness and behavioral responses to showers in the milking parlor.

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Sprinklers for cooling dairy cattle are conventionally found in the holding pen or home pens, but these potentially waste water when cows are outside the spray radius. A dairy farm developed a novel system with showers above each milking parlor stall to target spray on the cows' backs. Our objective was to evaluate how well the spray was targeted with minimal water landing on the udder or head. Eight pens (n = 8) of lactating Holsteins were evaluated during 1 milking/d when cows were sprayed vs. unsprayed (control) on 3 d each. Four focal stalls/parlor side (balanced among the 12 stalls/side across days) were scored for presence of water on the udder with both live observations and digital photographs (standard and infrared) using a standardized scoring system. Thirty focal cows were video recorded during treatment (57–67 s) application. The frequency (bouts/min) of ear flicks, head shakes, and head lowering were measured along with proportion of time for the latter. Linear mixed models were used to evaluate treatment differences within each time period. Before treatment, the percentage of wet udders was the same in the spray and control treatments (0.4 ± 0.3 vs. 0.5 ± 0.3%, mean ± SE; $P = 0.08$). After treatment, more udders were wet in the spray vs. control treatments (20.6 ± 2.3 vs. 0.5 ± 2.4%; $P < 0.001$), of which 9.5% (2% of cows) had streams of water reaching the teats or teat cups. Overall, we rarely observed wetness on the udder that could potentially wet the teats. During the spray, cows spent less time with their heads lowered but shook them more frequently in the spray vs. control treatment (1.8 ± 2.5 vs. 11.2 ± 2.6% of time, 0.15 ± 0.04 vs 0.03 ± 0.04 bouts/min, respectively; $P < 0.048$). However, there was no difference in ear flicks or head lowering between the treatments (ear flicks: 0.11 ± 0.05 vs. 0.07 ± 0.05, head lowering: 0.26 ± 0.07 vs. 0.27 ± 0.07 bouts/min; $P > 0.57$). Although cows occasionally shook their heads more during spray application, they did not show other behaviors consistent with avoidance of water on the head. On the whole, our findings support that the showers, aimed toward the cows' backs, rarely sprayed the heads or udders.

Key Words: heat stress, soaker, hygiene

W5 Effect of using fresh ryegrass/berseem clover as forage basis for TMR on dairy cow behavior.

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Cows have a natural selection activity regarding their feed as they can make dietary choices or be reluctant facing a new diet. Thus, a change in the diet composition can change their feeding behavior, affecting feed intake. The objective of the study was to evaluate the effect of using fresh ryegrass/berseem clover (MIX) and alfalfa hay/maize silage (CON) as forage basis for TMR on dairy cow behavior. Lactating dairy cows (n = 24) were blocked according to productive traits and allocated to each TMR diet for 10 weeks. Cows were kept in individual pens (6 × 3.5 m) with free access to the TMR and water. Cow behavior was assessed through video recordings between milking times (10 to 14 h and 16 to 20 h) by scan sampling every 10 min during 4 d in wk 1, 6 and 8. Behaviors: eating, ruminating or idling, walking and other (e.g., grooming, drinking) were registered. The occurrence of social interactions with neighboring cows were registered during 60 s every 10 min. Individual (n = 16) lying time was recorded over 4 d/week using HOBO dataloggers fitted to the cow's hind leg. Individual feed intake and milk production was daily recorded. Data were analyzed using generalized linear mixed models, including the week, diet type and its interactions as fixed effects and block as random effect. The day within week was used as a repeated measure. Ingestive behaviors, eating (33.9 ± 4.1%), ruminating (17.2 ± 2.4%) and idling (40.0 ± 6.6%) were not affected by TMR diet ($P < 0.05$). Feed intake tended to be higher with CON than with MIX diet (30.4 vs 25.1 ± 1.8 kg DM/d $P = 0.05$). There was no effect of the TMR diet on lying time (11.7 ± 0.38 h/d), number of lying bouts (6.9 ± 0.33/d), or bout duration (1.7 ± 0.06 h/bout). Social interactions (1.74 ± 0.65) and walking behavior (0.35 ± 0.01%) were not influenced by TMR diet ($P < 0.05$), but cows showed a higher frequency of "other" behaviors when fed with CON than when fed with MIX diet (9.2 vs 7.6 ± 0.07%; $P < 0.05$). We conclude that the TMR diets used in this study had minimal effects on dairy cow behavior. The study was supported by FONDECYT 11160697

Key Words: fresh forage, dairy cow, behavior

W6 Characterization of use of a molasses-based feeding enrichment and effects on feeding behavior in group-housed dairy calves.

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We examined the behavioral effects of providing group-housed calves access to a molasses-based feeding enrichment. Holstein heifer and bull calves (n = 32) were group housed (4 calves/pen) at 16 ± 2 (mean ± SD) d of age and provided milk and starter *ad libitum* via automated feeders. Calves were incrementally weaned over 9 d beginning at 6 wk of age. Pens (n = 4/treatment) were randomly assigned to receive a plastic 20 L tub filled with a hardened molasses-based product (1 tub/pen, hanging on the pen fence) or no enrichment. The tub was weighed weekly to calculate average daily intake. Cameras were mounted above each pen and behavior of the calves that received the tub was continuously recorded for one 24 h period during wk 5 of life. Recorded behaviors included: nutritive visits (calf's mouth in contact with the tub contents), tub-directed non-nutritive oral behaviors (calf's mouth in contact with plastic part of the tub), and head-initiated tub contact (any part of the calf's head, other than the mouth, in contact with the tub). Milk and starter intake and meal characteristics were collected from the automated feeders for the pre-weaning and weaning periods. These data were summarized by pen and week, and analyzed separately by stage (preweaning and weaning) in a repeated measures general linear mixed model. Calves showed interest in the nutritional enrichment, having 25.3 ± 14.2 (mean ± SD; min 7, max 45) nutritive visits/d, and spending 11.3 ± 9.1 min/d during nutritive visits (min 1.2 and max 26.7 min). Calves also directed non-nutritive oral behaviors toward the tub (68.7 ± 48.9 s/d) and pushed on it with their head (106.3 ± 78.2 s/d). Intake from the tub increased over time (15.6 g/calf/d during the first week in the pen vs. 115.1 g/calf/d during weaning; SE = 18.3; $P = 0.002$). Milk intake (8.0 L/d; SE = 0.66; $P = 0.88$) and feeder visit frequency (11.5 visits/d; SE = 1.6; $P = 0.26$) were similar between treatments. Starter intakes were also similar during both preweaning and weaning ($P > 0.22$). These results suggest that group-housed calves make use of nutritional enrichment, and further work is needed to evaluate effects on a broader range of behaviors.

Key Words: dairy calf, nutritional enrichment, feeding behavior

W7 Epidemiology of lameness during the dry period.

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Lameness during the dry period of dairy cows has received little attention in dairy research. The aim of this study was to describe: 1) lameness incidence and cure rates during the dry period, and 2) the risk factors for the onset and cure of lameness during this period. We followed 455 dairy cows on 6 freestall dairy farms in British Columbia, Canada. Cows were gait scored (1 to 5) weekly for 8 wk before calving. Body condition score (<3.0 = thin, 3.0 to 3.5 = good, > 3.5 = fat) was assessed 1 wk after enrolment. Parity was retrieved from farm records. Onset of lameness and lameness cure rate were assessed based on changes in gait scores over the study period. Cows were considered lame when gait score was ≥4, or when scored 3 on 2 consecutive assessments. Likewise, cows were considered sound when gait was ≤2 for 2 consecutive scorings. Approximately 45% of cows were hoof-trimmed before enrolment (mean ± SD = 20 ± 12 d before). Two multilevel logistic regression models using farm as random effect were fitted to assess risk factors for: 1) lameness onset (becoming lame vs remaining sound) and, 2) lameness cure (curing lameness vs remaining lame). Lameness Incidence rate was 8.2 cases/wk and cure rate was 7.1 cases/wk; cumulatively these cases resulted in 50% of cows becoming lame and 36% of cows curing from lameness during the dry period. We noted an interaction between parity and hoof-trimming; multiparous cows had higher odds (OR: 4.7; 95% CI: 1.4 – 17.2; $P = 0.02$) of becoming lame if they were trimmed, while primiparous cows showed the opposite pattern (OR: 0.2; 95% CI: 0.1 – 0.7; $P = 0.02$). Multiparous cows had lower odds (OR: 0.3; 95% CI: 0.1 – 0.9; $P = 0.04$) of being cured compared with primiparous cows, and thin cows had lower odds (OR: 0.2; 95% CI: 0.1 – 0.9; $P = 0.04$) of being cured compared with cows in good body condition. In conclusion, the lameness incidence was high during the dry period. Hoof-trimming in the weeks before dry-off was associated with reduced odds of becoming lame during the dry period for primiparous cows but not for multiparous cows.

Key Words: hoof-trimming, cure rate, incidence rate

W8 Effects of providing social and nutritional enrichment to dairy calves on learning behavior. K. L. Kutina* and J. M. Huzzey, *California Polytechnic State University, San Luis Obispo, CA.*

Dairy calves are raised in conditions that limit natural behavior; this may have consequences on later behavior and performance. Research shows that social housing improves learning in calves; however, little research has explored the interactions between different types of enrichment on cognition. Calves likely have an innate desire to suckle that extends beyond what could be provided from milk feeding alone; providing water from a nipple may be another important enrichment. The objective of this study was to measure the effects of both a water and social enrichment on calf learning behavior. The enrichments included 1) water provided from a nipple vs. a bucket, and 2) visual and tactile access to a partner vs. being isolated in a single hutch. A total of 24 Holstein and Jersey dairy calves were evenly distributed into 4 treatments at birth [Individual/Bucket (IB), Paired/Bucket (PB), Individual/Nipple (IN), Paired/Nipple (PN)]. At wk 8 calves began a visual discrimination task where they had to distinguish between an "X" and "O" cue to receive a milk reward. Testing occurred daily for 14 d and each session consisted of 12 trials. Learning was achieved when calves reached an 80% learning criterion (10/12 correct choices) for 3 consecutive d. Time to reach the learning criterion was analyzed using the Wilcoxin Signed Rank test and learning curves were compared using a mixed model in SAS. Calves in the IN treatment reached the learning criterion faster than IB or PB calves (11.5 ± 1.4 d vs. 16.7 ± 0.8 d and 17 ± 0 d, respectively; $P < 0.05$), while PN calves reached the learning criterion faster than PB calves (14.8 ± 1.3 d vs. 17 ± 0 d respectively, $P < 0.05$). Across the 14-d testing period calves in the IN treatment tended to perform better than calves in the other 3 treatments ($P = 0.07$). When reared individually there was no advantage to learning when calves had a nipple vs. a bucket ($P = 0.3$); however, among the paired calves, those that had the nipple made more correct choices over time than the PB calves ($P = 0.05$). These results indicate that social and nutritional enrichments are important for calf cognitive performance.

Key Words: dairy calf, enrichment, cognition

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W9 Abundance of microbial virulence genes in rectal swabs from US dairy cows varies by stage of lactation.

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Gastrointestinal disease can arise at any time during a dairy cow's lactation cycle, yet periods of high feed intake and production or greater metabolic demand can increase susceptibility to opportunistic infection. *Clostridium*, *E. coli*, *Salmonella*, and mycotoxin-producing fungi are commonly found in the GI tract, however fluctuation in abundance of these microorganisms or their virulence genes at different stages of lactation remains unclear. The objective of this study was to quantify common microbial marker and virulence genes in rectal swabs from dairy cows during 3 stages of lactation. Rectal swabs were obtained from 370 dairy cows at 26 commercial farms across major US dairy-producing regions, and classified into 3 groups by days post-parturition at time of sampling: fresh (d1–21, n = 95), early lactation (d22–100, n = 146) and late lactation (>101d, n = 129). Genomic DNA was extracted from rectal swabs and genus- or species-specific marker or virulence genes from *E. coli, Salmonella, Clostridium*, and *Aspergillus* were quantified using a panel of qPCR assays. Data were log-transformed and the Kruskal-Wallis test, followed by Mann-Whitney pairwise comparisons were performed to detect differences in gene quantity between lactation stage groups. Cows in fresh and early lactation periods harbored higher quantities ($H = 7.51$, $P = 0.02$) of *EAST1*, a heat-stable enterotoxin gene in enteroaggregative *E. coli,* compared with cows in late lactation (2.06 and 2.13 vs. 1.82 $\log_{10}$ gene copies respectively). Similarly, quantities of *Aspergillus* 18S rRNA gene ($H = 12.93$, $P < 0.01$) and *C. difficile* toxin A gene *tcdA* ($H = 7.52$, $P = 0.02$) were elevated in fresh and early lactation groups. Fresh cows swabs also contained highest ($H = 6.91$, $P = 0.03$) quantities of the *C. perfringens* α toxin gene *cpa* compared with cows in early and late periods (0.76 vs. 0.35 and 0.41 $\log_{10}$ copies respectively). These results indicate that harborage of several opportunistic microorganisms and their virulence genes may be greater during the fresh and early lactation periods, providing insight into the increased vulnerability of cows during these stages.

Key Words: virulence gene, *Escherichia coli*, *Clostridium*

W10 Microbial virulence gene abundance in rectal swabs from US dairy cows with or without gastrointestinal disease symptoms.

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Clostridium, *Salmonella*, *E. coli* and mycotoxin-producing fungi are often involved in opportunistic infection of the GI tract in many livestock species including dairy cattle. However, their abundance in lactating dairy cows with gastrointestinal disease symptoms is poorly understood. The objective of this survey was to quantify common microbial marker and virulence genes in rectal swabs from healthy dairy cows (n = 206) and cows with GI symptoms (n = 159) from commercial farms across 10 US states. The GI symptom group included cows with diarrhea, indigestion, ketosis, displaced abomasum and other digestive issues reported by producers. Genomic DNA was extracted from rectal swabs and tested with a panel of qPCR assays for genus- or species-specific marker or virulence genes from *E. coli, Salmonella, Clostridium* spp., and *Aspergillus*. The proportion of positive samples and the quantitative results for each gene in the healthy and GI groups were compared using Fisher's Exact test and Mann-Whitney tests respectively. Prevalence of *stx2*, typically associated with Shiga toxin-producing *E. coli*, the *C. perfringens* α toxin gene *cpa*, and the *C. difficile* toxin B gene *tcdB* were higher in swabs from sick cows ($P < 0.05$). Sick cows also harbored higher quantities ($P < 0.01$) of the *E. coli* 16S rRNA gene (2.81 vs. 2.27 $\log_{10}$ copies), *EAST1*, a heat-stable enterotoxin gene in enteroaggregative *E. coli,* (2.24 vs. 1.82 $\log_{10}$ copies), and *cpa* (0.74 vs. 0.30 $\log_{10}$ copies). Isolates of *E. coli* and *Clostridium* spp. were cultured from swabs and tested in agar cross-streak assays for growth inhibition by several novel *Bacillus* spp. strains. 813 of 853 *E. coli* and 390 of 394 *C. perfringens* isolates were inhibited by at least one *Bacillus* strain in cross-streak assays. This survey indicates that cows with gastrointestinal symptoms harbor elevated virulence gene quantities from multiple microorganisms, providing new targets for risk assessment, while in vitro inhibition testing shows promise of potential biological solutions for control of opportunistic microbial growth.

Key Words: *Escherichia coli*, *Clostridium perfringens*, gut health

W11 Prevalence of five enteric pathogens on Ohio dairy farms.

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Calf diarrhea remains one of the main reasons for productivity and economic losses on US dairy operations. The majority of pre-weaned calf mortality (56.4%) is attributed to diarrhea or other digestive problems (USDA, 2014). Five enteric pathogens are commonly associated with diarrhea in dairy calves, including bovine rotavirus, bovine coronavirus, *Escherichia coli*, *Salmonella* spp., and *Cryptosporidium parvum*. However, pathogen-associated differences in health outcomes and case fatality rates have not been well characterized. The objective of this study was to estimate the prevalence of diarrheal pathogens on Ohio dairy farms, and longitudinally measure the health outcomes for diarrheal illnesses. For this study, fecal samples were collected from 277 clinically ill calves across 5 different farms on the first day of diarrheal diagnosis. Genomic techniques, including RT-PCR and ddPCR were used to test for the presence of the 5 enteric pathogens. A Poisson regression model was used to analyze the relative risk of mortality by pathogen and a survival analysis with a Cox regression model was used to analyze time to return to a healthy clinical status by pathogen. Rotavirus was the most prevalent at 75.5% (209/277), followed by K99$^+$ *E. coli* at 42.8% (115/269), *C. parvum* at 28.0% (65/232), coronavirus at 10.1% (28/277), and *Salmonella* had the smallest prevalence at 3.7% (10/269). Risk of mortality was significantly higher for calves infected with *E. coli* and *Salmonella* with relative risks of 4.32 (95%CI: 1.08, 17.27) and 10.98 (95%CI: 2.39, 50.53) respectively ($P = 0.038$ $P = 0.002$). The pathogens did not, however, have any statistically significant effect on time to return to a healthy clinical status. Only farm was a significant predictor of time to return to health ($P = 0.0139$). The results suggest that rotaviral infections are prevalent and widely distributed across farms; however, mortality is more commonly associated with *Salmonella* and K99$^+$ *E. coli* infections. Specific prevention and rapid

differentiation from other causes of calf diarrhea are important to reduce risk of mortality in pre-weaned calves with diarrhea.

Key Words: calf diarrhea, prevalence, enteric pathogen

W12 Advanced molecular spectroscopic techniques for screening mycotoxin concentrations in feed grains for dairy cows in western Canada. H. Shi[1,2] and P. Yu*[1], [1]*Ministry of Agriculture Strategic Feeds Research Chair Program, Department of Animal and Poultry Science, College of Agriculture and Bioresources, University of Saskatchewan, Saskatoon, SK, Canada* [2]*College of Life Science and Engineering, Foshan University, Foshan, Guangdong.*

The application of traditional methods to detect feed mycotoxin is time consuming and requires a high level of experience and expertise. The objective of this study was to test possibility of using advanced molecular spectroscopic techniques to screen mycotoxin concentrations in feed grains. Barley and wheat grains are ranked as the most important feed crops for dairy cows in western Canada. However, they have been suffering from mycotoxins contamination for a long time. In this study, a total of 80 wheat and 42 barley samples were collected and detected for 6 major ergot alkaloids and 12 common mycotoxins concentration by liquid chromatography- tandem mass spectrometry. The near-IR (NIR; 680–2500 nm) and mid-IR spectra (MIR; 4000–700 cm^{-1}) of all samples were all collected with the grading NIR and FTIR. All spectra were averaged from 3 repeat NIR or FTIR measurements, each recorded from a new sub-sample. The final spectra data were imported into the UnscramblerX v10.3. Preliminary descriptive analyses were performed by both graphic tools and numerical results. To remove the spectral baseline shift, noise, and light scatter effects, 9 preprocessing methods were applied, including baseline offset, standard normal variate (SNV), detrending, SNV+ detrending, multiplicative scatter correction, first derivative, second derivative, first derivative + SNV, and second derivative + SNV. The NIR and MIR spectra were calibrated with EAs reference values using PLS technique based on different spectral preprocessing methods and selected wavelength ranges. The possibility whether we could develop fast screening methods for wheat and barley major 6 ergot alkaloids and 12 common mycotoxins detecting by NIR and MIR were revealed in this study. In total ergot alkaloids, R^2_C for calibration was less than 0.55 and 0.96, R^2_{Cv} for cross validation was less than 0.14 and 0.96, R^2_p for external prediction was NA, for barley and wheat, respectively. In general, the PLS models developed showed relatively weak cross-validation performance. More efforts are required to explore the direct detection limit of the NIR and ATR- FT/MIR techniques for the quantification in different sample matrix.

Key Words: feed, ergot alkaloids and mycotoxins, molecular spectroscopy

W13 Toxicity of deoxynivalenol and fumonisin B_1 in primary bovine rumen epithelial cells and a calf intestinal epithelial cell line. N. Reisinger*[1], D. Baranski[1], S. Schürer-Waldheim[1], D. Wendner[1], G. Antonissen[2], E. Mayer[1], and V. Nagl[1], [1]*BIOMIN Research Center, Tulln, Austria,* [2]*Department of Pharmacology, Toxicology and Biochemistry, Department of Pathology, Bacteriology and Avian Diseases, Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium.*

The bovine rumen is capable to detoxify certain mycotoxins to less toxic metabolites. However, during rumen disturbance e.g., sub-acute ruminal acidosis, the ability of detoxification might decrease. There is only a limited number of studies available evaluating the effect of mycotoxins on rumen and intestinal epithelium of cattle. The aim of the study was therefore to access the toxicity of 2 common mycotoxins: deoxynivalenol (DON) or fumonisin B_1 (FB_1) in primary bovine rumen epithelial cells (REC) and in a calf intestinal epithelial cell line (CIEB). REC were isolated from rumen tissue of dairy cows by enzymatic dissociation with trypsin. CIEB is a spontaneously immortalized cell line derived from the small intestine of a calf. Both cells types were characterized via immunostaining for cytokeratin (epithelial cell marker). For toxicity studies, cells were seeded in 96 well plates (2×10^4 cells/well) for 24 h. Thereafter, cells were incubated with 0 to 25 µM DON and FB_1 (n = 6). After 48 h of incubation, the water-soluble tetrazolium salt (WST-1; Mitochondrial metabolism) assay, the neutral red (NR; Lysosomal Activity) assay as well as the sulforhodamine B (SRB; Total protein synthesis rate) assay were performed. Statistical evaluation of data was performed with GraphPad Prism software (Version 7). Analysis of variance or Kruskal-Wallis test was used for data evaluation, depending if data were normally distributed or not. Data were considered as significant if $P < 0.05$. REC as well as CIEB were positively stained for cytokeratin, and therefore confirmed as epithelial cells. DON had the greatest effect on mitochondrial metabolism in REC starting at a concentration of 1 µM ($P < 0.05$) and in CIEB at a concentration of 0.39 µM ($P < 0.05$). FB_1 had the greatest effect on lysosomal activity in REC starting at a concentration of 3.13 µM ($P < 0.05$) and in CIEB at a concentration of 6.25 µM ($P < 0.05$). Taken together, DON and FB_1 had a toxic effect on bovine rumen as well as calf intestinal epithelial cells.

Key Words: in vitro, mycotoxin, digestive epithelium

W14 In vitro evaluation of anti-inflammatory activity of glycerol monolaurate, lauric acid, and methyl laurate. L. K. Mamedova*[1], G. Davis[1], C. C. Elrod[2], and B. J. Bradford[1], [1]*Kansas State University, Manhattan, KS,* [2]*Natural Biologics, Inc, Newfield, NY.*

Glycerol monolaurate (GML) is a natural surfactant comprised of a monoester of glycerol and the medium-chain fatty acid lauric acid (LA). Both GML and LA have bacteriostatic properties, but less is known about the effects of these nutrients on immune or intestinal epithelial cells. The first objective of this study was to assess impacts of GML, LA, and methyl laurate (ML) on inflammatory signaling in RAW 264.7 murine macrophages transfected with a vector that drives expression of alkaline phosphatase (AP) upon activation of NFκB. RAW cells were challenged with 0.1 µg/mL LPS or not for 6 h. Medium was then replaced to provide varying doses of GML, LA and ML (0, 0.5, 2.5, 12.5, 62.5, and 312.5 µM) in 0.06% dimethyl sulfoxide (DMSO) for an additional 4 h (2 × 3 × 6 factorial, n = 6). In addition to AP activity, resazurin metabolism was used to assess cell viability. As expected, LPS treatment significantly increased AP activity and decreased cell viability, whereas DMSO mitigated the loss of viability from LPS. Therefore, the DMSO control served as the reference point for evaluating treatment effects. Increasing doses of GML, LA, and ML all decreased AP activity in a log-linear manner in the presence of LPS ($P < 0.001$), with significant effects detected at 62.5 and 312.5 µM for all 3 compounds ($P < 0.05$, Tukey test). No treatment effects were detected in the absence of LPS, and cell viability measures did not indicate toxic effects of these compounds. Second, we determined whether these compounds influence tight junction integrity in LPS-challenged intestinal cells. Caco-2 human colon cancer cells were cultured in transwell inserts for 10 d. Cells were treated with 312.5 µM GML, LA, or ML for 48 h, with or without addition of 1 µg/mL LPS for the last 24 h (n = 3). Permeability was measured by the transepithelial flux of fluorescein-sulfonic acid. LPS increased monolayer permeability, whereas all 3 compounds significantly attenuated this response ($P < 0.05$), with LA and GML having a greater effect than ML. The results demonstrate that LA and 2 of its esters have anti-inflammatory impacts on macrophages and reduce permeability of colonocytes challenged with LPS.

Key Words: intestinal permeability, macrophage, bioactive

W15 Effect of a *Bacillus*-based direct-fed microbial on production, health, and reproduction in lactating dairy cattle: A meta-analysis.

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This study investigated the effects of a *Bacillus*-based direct-fed microbial (DFM; Strateris ECL, United Animal Health Inc., Sheridan, IN) supplementation on production, health, and reproduction of lactating dairy cows. The DFM was fed at 15 g/hd/d (7.35×10^9 cfu/cow/d) to a total of 18,724 dairy cows in 5 different farms located across major dairy-producing regions of the US (WI, OH, MN, MI and ID). A longitudinal design was used with monthly milk production, components, health, and reproductive metrics monitored via Dairy Comp 305 or PCDART. Reproductive metrics, when available, were evaluated during the same monthly time period over consecutive years. A meta-analysis was then performed on the entire data set comparing 3 mo pre- and post-DFM supplementation. Production, components and reproduction data were analyzed by the PROC MIXED of SAS, with fixed effects of treatment*farm and treatment. Health metrics were evaluated by the PROC GLIMMIX of SAS with the interaction of treatment*farm and treatment as fixed effects. DFM supplementation reduced ($P < 0.05$) the incidence of ketosis (18 ± 1.3% vs. 12.5 ± 1.4%), retained placentas (13.5 ± 1% vs. 9.7 ± 0.9%) and decreased ($P = 0.0342$) somatic cell counts (SCC, 438 ± 10.1 vs. 404 ± 10.7*1000 cells/mL). DFM increased ($P < 0.05$) both milk fat (4.05 ± 0.03 vs. 4.30 ± 0.03) and protein (3.2 ± 0.01 vs. 3.3 ± 0.01) percentages, resulting in 1.1 kg higher ($P = 0.0387$) energy corrected milk (ECM, 35.3 ± 0.4 vs. 36.4 ± 0.4 kg/hd/d). DFM supplementation resulted in increased ($P < 0.05$) overall heat detection (52.6% vs. 57.3%), conception rate (36% vs. 43.8%) and 21-d pregnancy rate (17.9% vs. 23.9%) and reduced ($P < 0.05$) services per conception (2.8 vs. 2.3) and abortions (4.1 ± 0.3% vs. 3.1 ± 0.2%). Feeding a *Bacillus*-based DFM to dairy cattle for 3 mo improved transition cow health, reduced SCC, increased milk fat and protein, ECM, and enhanced reproductive performance. The impact on health and production are probably the secondary and tertiary effects of improved gastrointestinal health.

Key Words: direct-fed microbial, health, performance

W16 Feeding whey-based colostrum replacer for the first 14 days of life improves dairy calf performance.

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While data surrounding the use of colostral immunoglobulins (IgG) to calves to achieve passive transfer of immunity is abundant, data supplementing calves with colostral immunoglobulins after 24 h of age is a seldom researched disease prevention strategy to reduce antibiotic use. The study objectives were to evaluate the effect of supplementing calves with a concentrated, whey-based IgG, from birth to d 14 on intake and performance. The IgG was from a commercially available colostrum replacer product (Premolac, Zinpro Corp., Eden Prairie, MN; PZ). Upon arrival at the facility, 1,037 newborn, Holstein calves were randomly assigned to 1 of 3 treatments of IgG: 1) 0 g (CON; negative control), 2) 10 g (CR10; 23 g PZ), or 3) 20 g (CR20; 46 g PZ)/d. Treatments were added to milk replacer and fed 2×/d to individually housed calves. Calf BW was recorded on d 0, 15, pre-weaning (d 53) and hutch exit (d 69). Grain intake was measured 1x/wk. All milk replacer refusals were recorded. Continuous outcomes were analyzed using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Percentage of calves that refused milk and feeding refusal number were analyzed using the GLIMMIX procedure with a binary distribution or a Poisson distribution and log link, respectively. Supplementing IgG from PZ reduced the percentage of calves that had a milk refusal event in at least one meal during the first 3 weeks of age (38.8, 23.3 and 20.0% for CON, CR10, and CR20 respectively; $P < 0.01$). Among refusals, CR20 calves refused less feedings (1.5 vs. 2.1; $P = 0.03$) and less total milk (1.51 vs. 1.99 L; $P = 0.04$) compared with CON calves. Grain intake was similar among treatments ($P = 0.29$). Calves fed CR20 were heaviest at d 15 (43.5, 42.9 and 42.2 for CR20, CR10, and CON respectively; $P < 0.01$). Total gain and ADG from d 0 to 14 were greatest for CR20, intermediate for CR10, and lowest for CON calves (3.6, 3.0 and 2.2 kg and 0.24, 0.20 and 0.15 kg/d respectively; $P < 0.01$). Supplementing PZ, especially 20 g of IgG from PZ, d 0 to 14 improved growth and reduced milk refusals in Holstein calves.

Key Words: colostrum replacer, calf, immunoglobulin

W17 Identification of biomarkers associated with mortality in grain-fed veal calves.

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The objective of this prospective cohort study was to identify biomarkers associated with mortality in grain-fed veal calves. Upon arrival at a grain-fed veal facility in Ontario, blood was collected from the jugular vein of each calf into a 10-mL sterile blood collection tube without an anticoagulant. Blood was allowed to clot and then centrifuged at 1,500 × *g* for 15 min. Serum was separated and stored at −20°C until analysis at a commercial reference laboratory (Animal Health Laboratory, Guelph, Ontario, Canada). Several metabolites were measured including creatine kinase, cholesterol, haptoglobin, manganese, serum total protein, iron, cobalt, zinc, selenium, and molybdenum. Univariable mixed logistic regression models were created to evaluate metabolic biomarkers associated with mortality. A lowess smoother curve was generated to assess the linearity of each predictor variable to the outcome on a log odds scale. If a variable failed to meet the linearity assumption, the variable was categorized into quartiles. A total of 909 calves of unknown age had blood collected at arrival from January to December 2017. Of the calves examined, 67 calves (7.5%) died over the 11-week period under observation. The level of cholesterol, haptoglobin, and iron were associated with mortality. For every 1 mmol/L increase in cholesterol, the odds of mortality are reduced (Odds ratio (OR): 0.57; 95% CI: 0.37–0.91; $P = 0.02$). Compared with the referent category (less than 0.15 g/L), if the calf had a haptoglobin concentration between 0.15 and 0.16 g/L (OR: 2.24; 95% CI: 1.02–4.89; $P = 0.04$) or 0.19–3.3 g/L (OR: 2.38; 95% CI: 1.01–5.58; $P = 0.047$), calves had an increased odds of dying. Compared with the referent category (less than 2.1 mg/mL), calves with iron concentrations of 2.8–3.6 mg/mL (OR: 2.12; 95% CI: 1.03–4.37; $P = 0.04$) had a greater risk of mortality. These results demonstrate that cholesterol, haptoglobin, and iron could serve as biomarkers to identify calves at high-risk of mortality when measured at arrival to a veal facility.

Key Words: male dairy calf, mortality, biomarker

W18 Transcriptional comparison between total RNA and mRNA isolated from same fecal samples of neonatal dairy calves.

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Inflammatory-related genes are commonly expressed at a low abundance. However, these can still be detected in fecal RNA isolated from dairy calves, which contains a negligible amount of RNA from immune cells under non-diarrheic conditions. Additionally, genes related to common functions of the gastrointestinal (GI) tract were observed in total RNA from fecal samples. However, fecal RNA isolation remains a challenge, because of the potential enrichment of bacterial RNA, which can dilute the targeted eukaryotic RNA and consequently dampen the sensitivity of the fecal RNA method. Therefore, our objective in this study was to determine the differential eukaryotic RNA enrichment in total RNA vs mRNA from same fecal samples of healthy neonatal dairy calves. To test this, 200 mg of feces were used from 6 neonatal Holstein calves for total RNA isolation, using a Trizol based method along with the RNeasy Plus Mini Kit (Qiagen). Then, 45 µg of fecal total RNA was used to isolate mRNA through a magnetic selection using Dynabeads Oligo (dT)25 (Invitrogen). The cDNA synthesis was performed using the SuperScript IV reverse transcriptase (Invitrogen). The standard curve was composite from all samples including cDNA from total RNA and mRNA. The internal control genes used in this experiment were *B2M*, *ACTB*, *GAPDH*, *RPS9*, and *PPIA*. Normalized gene expression data were log-transformed before statistical analysis using the Proc Mixed of SAS (SAS 9.4). Expression of genes specific for epithelial cells including cytokeratin 8 (*KRT8)* and aquaporin (*AQP3*) as well as inflammatory-related genes (*TLR4* and *IL1B*) were evaluated. The expression of *KRT8* was greater ($P = 0.03$) in fecal mRNA than in fecal total RNA. A trend ($P = 0.09$) was observed for greater expression of *TLR4* in fecal total RNA than in fecal mRNA. The expression of *AQP3* and *IL1B* was not different. Greater expression of *KRT8* in mRNA than in total RNA suggests that this additional selection of gene transcripts within fecal RNA might improve the sensitivity of this method and consequently the accuracy and robustness. These results further confirms that the fecal RNA method has a potential to be used as a tool to evaluate GI tract molecular adaptations in dairy calves.

Key Words: calf, fecal RNA, gastrointestinal tract

W19 Comparative transcriptomic analysis of epithelial cell markers across gastrointestinal tissues and fecal RNA isolated from dairy calves.

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Fecal RNA method can be used to evaluate biological adaptations of the gastrointestinal (GI) tract of dairy calves through gene expression analysis. A limitation with this method is the current lack of data indicating how the transcriptomic profile observed in fecal RNA mirrors that in specific sections of the GI tract. Therefore, our objective in this study was to compare the transcription of gene markers for GI epithelial cells, fatty acid binding protein 2 (*FABP2*) and cytokeratin 8 (*KRT8*) in fecal RNA against several GI tract sections in dairy calves. To test this, postmortem samples were collected from ruminal epithelium, cecum, large intestine, duodenum, jejunum, ileum, and feces from 6 healthy male Jersey calves (5 wk of age) for total RNA isolation. The standard curve was composite from all samples including cDNA from tissues and fecal. The internal control genes used in this experiment were *B2M*, *ACTB*, *GAPDH*, *RPS9*, *RPS15A*, and *PPIA*. Normalized gene expression data were log-transformed before statistical analysis using Proc Mixed of SAS. The expression of *FABP2* was greater ($P < 0.01$) in the duodenum tissue than in GI section associated with fermentation (i.e., rumen, large intestine, and cecum). Within the small intestine the mRNA expression of *FABP2* was greater ($P = 0.01$) in duodenum than in jejunum, but not different than ileum. In fecal RNA, the *FABP2* expression was greater ($P \leq 0.03$) than in GI section related to fermentation. However, *FABP2* was similar ($P = 0.3$) between fecal RNA and ileum. The expression of *KRT8* was greater ($P \leq 0.02$) in cecum and large intestine than in rumen and jejunum. Among the small intestine sections *KRT8* was greater ($P = 0.03$) expressed in duodenum than in jejunum. The fecal RNA had greater ($P \leq 0.02$) expression of *KRT8* than jejunum and ileum. In contrast, the *KRT8* expression in fecal was not different than the transcripts observed in cecum and large intestine. Since the transcription of the genes specific for GI epithelial cells were significant observed in the RNA isolated from feces, these preliminary data further confirms that fecal RNA has a potential to be used as a tool to evaluate molecular adaptations in the GI tract of dairy calves.

Key Words: calf, fecal RNA, gastrointestinal tract

W20 Milk metabolites as noninvasive indicators of nutritional status of mid-lactation Holstein and Montbéliarde cows.

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The objective was to investigate the effects of feed restriction on concentrations of selected milk metabolites in mid-lactation Holstein and Montbéliarde cows, and explore their correlations with energy balance. Nine Holstein and 10 Montbéliarde cows (165 ± 21 DIM) underwent 6 d of feed restriction during which feed allowance was reduced to meet 50% of NE_L requirements calculated before initiation of the challenge. The experiment was divided in 4 periods: Control (CONT; d −3 to −1), restriction (REST; d 1 to 6), WK1 (d 7 to 13) and WK2 (d 18). Milk concentrations of β-hydroxybutyrate (BHB), glucose, glucose-6-phosphate (Glu6P), isocitrate and glutamate were measured and statistical analyses performed using mixed models of SAS with fixed effects of period and breed, and the random effect of cow. Relationships among variables were explored by Spearman correlations. Feed restriction induced a negative EB, increased Glu6P and isocitrate (+38% and +39%, respectively) and decreased BHB, glucose and glutamate concentrations in milk (−20%, −57% and −65%, respectively) compared with pre-challenge values (Table 1). All milk metabolites were significantly correlated with EB (0.46, 0.62, −0.25, −0.41, 0.59 for BHB, glucose, Glu6P, isocitrate and glutamate, respectively). Results suggest that milk metabolites may be used as noninvasive indicators of nutritional status of mid-lactation cows.

Key Words: milk metabolite, dairy cow, energy balance

W21 Use of circulating metabolites and milk production variables to generate linear regression models for prediction of postpartum liver triglycerides.

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Given the potential impacts of liver triglyceride (lvTG) accumulation on hepatic metabolism, the ability to diagnose fatty liver without a liver biopsy could be advantageous in both the research and applied settings as accumulation of lvTG can only be diagnosed by liver biopsy. Since fatty liver is related to the overall metabolic status of the cow, the

Table 1 (Abstr. W20). Effects of feed restriction on energy balance and milk metabolite concentrations in mid-lactation cows

		Period[1]					*P*-value		
		CONT	REST	WK1	WK2	SEM	Breed	Period	Breed × Period
	HOLS	$46^{a,y}$	-40^{b}	$41^{a,y}$	41^{a}	4.0			
EB (MJ/d)	MONT	31^{x}	−40	29^{x}	42	3.6	0.06	0.001	0.04
	HOLS	0.05^{b}	0.04^{c}	0.06^{a}	0.05^{b}	0.01			
BHB (mM)	MONT	0.05	0.04	0.06	0.05	0.01	0.93	0.001	0.55
	HOLS	0.54^{a}	0.22^{c}	0.47^{b}	0.51^{ab}	0.03			
Glucose (m*M*)	MONT	0.47	0.21	0.45	0.45	0.02	0.15	0.001	0.17
	HOLS	0.02^{b}	0.03^{a}	0.01^{c}	0.03^{a}	0.01			
Glu6P (m*M*)	MONT	0.04	0.05	0.02	0.05	0.01	0.24	0.001	0.12
	HOLS	0.12^{c}	0.17^{a}	0.13^{c}	0.13^{b}	0.01			
Isocitrate (m*M*)	MONT	0.14	0.19	0.13	0.16	0.01	0.05	0.001	0.29
	HOLS	0.39^{a}	0.14^{c}	0.39^{a}	0.33^{b}	0.06			
Glutamate (m*M*)	MONT	0.38	0.13	0.38	0.31	0.06	0.77	0.001	0.99

[a–d]Period LSMEANS not sharing a common superscript differ ($P \leq 0.05$).

[y,x]Breed LSMEANS not sharing a common superscript differ within the period ($P \leq 0.05$).

objective of this study was to determine if the concentration of lvTG could be predicted from milk production variables and circulating blood metabolites related to energy balance and liver health. Blood and liver samples were taken at −14, +1, and +14 d relative to calving (DRTC) from multiparous Holstein cows (n = 37) enrolled in 2 previously reported studies. Daily milk production and weekly milk composition were collected. Liver TG (% dry matter) was quantified and serum was analyzed for aspartate amino transferase (AST), alanine amino transferase (ALT), albumin (alb), BHB, BUN, and triglyceride (TG). Plasma was analyzed for glucose (glc) and nonesterified fatty acids (NEFA). Through the PROC REG procedures of SAS (9.4), forward stepwise linear regression models utilizing a $P < 0.1$ and minimum AIC inclusion criterion were fit to predict either +1 or +14 DRTC, or maximum lvTG% from the analyzed blood metabolites and milk variables. Two types of models were explored; 1) a predictive model that used prepartum metabolites to predict maximum lvTG% and 2) a diagnostic model that utilized +1 and +14 DRTC metabolites and milk variables to predict respective lvTG%. Maximum lvTG% was 18.9 ± 1.4% and occurred at +14 ± 1 DRTC. Diagnostic models at +1 DRTC included ALT and AST ($R^2 = 0.23$) and +14 DRTC included blood TG, NEFA, glc, and cumulative milk yield to date ($R^2 = 0.66$). The predictive regression model for maximum lvTG% based on −14 DRTC metabolites included ALT, alb, BUN, and TG ($R^2 = 0.41$). Overall, postpartum diagnostic models were stronger than predictive models; however, additional metabolites should be explored to improve the ability to diagnose or predict lvTG%.

Key Words: transition cow, biomarker, fatty liver

W22 Antimicrobial usage for the treatment on respiratory diseases in calves: A systematic review.

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Our objective was to conduct a systematic review of the quality of previous publications that evaluated the efficacy of antimicrobials for the treatment of bovine respiratory disease (BRD) in calves. The literature search strategy, based on population, intervention, and outcome of studies written in English from CabDirect, PubMed, Web of Science and Scopus, was conducted on December 2018; a total of 2,058 publications were retrieved. Publications of interest were clinical trials and experimental challenges that used antimicrobials to treat BRD in calves <6 mo. Thirty-four manuscripts containing 37 trials were retained after screening the titles (n = 901), the abstracts (n = 308) and the full papers. The selected trials included clinical trials (n = 22) and challenge trials (n = 15) that dated back from 1979. The median number of animals enrolled was 49 and ranged from 11 to 696 calves. Seventeen manuscripts were either funded or had authors affiliated with pharmaceutical companies. A total of 29 trials were randomized but only 14 of those were blinded. Fifteen trials included a negative control treatment; but only 6 were randomized and blinded. Trials with negative control evaluated the efficacy of: one (n = 3) or more (n = 2) antimicrobials, anti-inflammatories combined with antimicrobials (n = 2), various dosages or timing of treatments (n = 7), or combination of antimicrobial treatments (n = 1). Macrolides were the most common antimicrobial class evaluated (n = 14). The length of the observational period for health outcomes ranged from 3 d to 8 wks. Fever was the most frequent clinical sign of BRD evaluated (n = 26). Only 8 trials evaluated clinical signs of respiratory disease using a scoring tool. In addition to clinical signs, 13 trials performed pathological examination of euthanized calves. Although considerable numbers of studies have been conducted on antimicrobial use for BRD in calves, very few studies were controlled and randomized. Future research on BRD should follow standardized methods for the evaluation of clinical outcomes. Funding provided by CDFA–AUS project.

Key Words: antimicrobial, calf, respiratory disease

W23 Effect of acupuncture therapy in dairy cows affected by pyometra: A randomized controlled clinical trial.

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Pyometra (PYO) is a uterine disease characterized by the accumulation of purulent or mucopurulent material within the uterine lumen in the presence of an active corpus luteum (CL). Due to prohibited use of artificial hormones in US certified organic dairies, conventional therapies for treatment of PYI are not applicable. The objective of this study was to evaluate the efficacy of 2 acupuncture procedures on the treatment of persistent CL in cows with PYO. We hypothesized that acupuncture would reduce CL diameter and serum progesterone (P4) concentrations resolving the PYO. Holstein cows with PYO, at a USDA certified organic dairy farm in Northern Colorado were enrolled in a randomized controlled clinical trial and assigned into 1 of 3 treatments: (1) control pyometra (CP; no treatment; n = 17); (2) electroacupuncture (EAP; n = 15); and (3) laser acupuncture (LAP; n = 15). Each cow received 3 20-min acupuncture sessions on alternate days. Cows had blood samples collected for determination of serum progesterone concentration at enrolment (−3 d) and at d 0, d 2, d 4, d 11, d 18, and d2 5 after first treatment. CL diameter was determined by transrectal ultrasonography at −3 d, d 0, d 2, d 4, d 11, and d 18. Logistic regression was used for the analysis of binary outcome variables, whereas continuous variables were evaluated by ANOVA and by repeated measures analyses, accounting for baseline data (CL diameter and serum P4 concentration). Average ± SE for CL diameter change from d0 to d18 were 1.0 ± 1.0 mm, 0 ± 1.0 mm, and −0.33 ± 1.0 mm for CP, EAP, and LAP, respectively. None of the cows had serum P4 values <1 ng/mL by the end of the monitoring period and average ± SE P4 concentration change from d 0 to d 25 were −4.2 ± ng/mL, −0.7 ± ng/mL, and 4.5 ± ng/mL for CP, EAP, and LAP, respectively. The repeated measures analysis indicated no differences in time for CL diameter or serum P4 concentrations among groups. Two cows in EAP and 1 cow in LAP conceived 38 d, 68 d, and 38 d, after treatment completion. In conclusion, acupuncture was not an effective treatment for persistent CL in cows with PYO during the monitoring period.

Key Words: acupuncture, pyometra, corpus luteum

W24 Colostrum supplementation with omega-3 fatty acids and α-tocopherol decreases indicators of oxidative stress and alters plasma fatty acid profile in newborn calves during the first week of life.

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Oxidative stress (OS) occurs when antioxidants fail to neutralize an overabundant concentration of reactive oxygen species, resulting in damage to cellular components. This phenomenon is prevalent in neonatal calves, potentially causing disease vulnerability and immune dysfunction. Past studies have shown the benefits of fish and flax oil on calf health and growth due to their omega-3 fatty acids (n-3 FA); these metabolites may mediate inflammation and OS through anti-inflammatory and antioxidant properties. We hypothesized a 60 mL fish and flax oil colostrum supplement would improve indicators of calf health and plasma concentrations of n-3 FA during the first week of life. Sixteen Holstein calves were blocked by sex and birth date and randomly assigned to control (no supplement; Con), or fish and flax oil (FFtrt) supplemented in first colostrum (3 L within 6 h, > 22% on Brix). FFtrt was a 60 mL 1:1 blend of oils with 200 mg α-tocopherol. Blood was sampled on d 1, 2, 4, 7, 14, and 21 after birth for assessment of passive transfer, oxidant status, and FA profile. Health was scored daily. Hip height and body weight were recorded weekly. Data were analyzed with a mixed procedure in SAS 9.4 including treatment, sex, and day as fixed effects and calf and block as random effects. FFTrt did not alter concentration of total protein in blood serum, prevalence of diarrhea, or rate of growth ($P > 0.10$), but tended to improve nasal scores ($P = 0.07$). FFtrt increased plasma concentrations of n-3 FA as much as 90% by 1 d of age ($P < 0.01$). FFtrt decreased oxidant status index (OSi) by 55% by 2 d of age (Con: 73, FFtrt: 32 OSi; $P < 0.01$) and remained decreased overall in the first week of life (Con: 74, FFtrt: 50 OSi; $P < 0.01$). OSi and FA concentrations returned to control values by d 14. In conclusion, a colostrum supplement of n-3 FA and α-tocopherol decreased oxidant status and increased plasma n-3 FA concentrations in the first week of life and has the potential to improve health of neonatal calves.

Key Words: omega-3, colostrum, oxidative stress

Forages and Pastures 2

W25 In vitro degradability of Guinea grass (*Megathyrsus maximus*) silage with different inclusion levels of hulls from pineapple, passion fruit, and banana. I. Espinoza*, A. Sánchez, M. Medina, L. Montenegro, M. Romero, A. Barrera, L. Godoy, and E. Torres, *Universidad Técnica Estatal de Quevedo, Quevedo, Los Ríos, Ecuador.*

Ecuador has a diversity of climatic conditions allowing to potentiate the production of tropical fruits throughout the year, some of which are part of industrial processes of concentrates or fruit juice and generate several byproducts or agroindustry residues ranging from 30 to 50% in pineapple ; 65 to 70% in passion fruit and 40.5% in banana. Guinea grass (GG) with different inclusion levels of hulls from pineapple (PH), passion fruit (PFH) and banana (BH) were evaluated using silages, with the following treatments T0: GG 100%; T1: GG 85% + PH 15%; T2: GG 85% + PFH 15% and T3: GG 85% + BH 15%, with 6 replications per treatment. The silages were made in mini silos with 3 kg of capacity, fermented for 60 d and the degradability with the in vitro incubation system with incubation periods of 0; 3; 6; 12, 24; 48 and 72 h. A Completely Randomized Design and Tukey test ($P < 0.05$) for the means comparison were performed. All the treatments had similar DM degradabilities at 72 h of incubation with a mean of 61.58 ± 0.85%. Degradability of NDF was also similar across treatment with a mean of 51.57 ± 0.47%. T2 reach the highest In vitro degradability of the ADF with contents 0f 49.07 ± 0.72%. Guinea grass is a very usable forage, however, converted into silage with inclusion of pineapple and passion fruit residues, it presented the best degradability coefficients.

Key Words: byproduct, agroindustry residues, Guinea grass

W26 Evaluation of the bale-cutting mechanism for alfalfa-orchardgrass baled silages. W. Coblentz*[1] and M. Akins[2], [1]*US Dairy Forage Research Center, Marshfield, WI,* [2]*University of Wisconsin, Madison, WI.*

The production of baled silages is increasingly popular; however, some management considerations and fermentation characteristics are notably different from traditional precision-chopped silages. Our objectives were to compare the silage fermentation characteristics and nutritive value of cut and uncut alfalfa (*Medicago sativa* L.)-orchardgrass (*Dactylis glomerata* L.) baled silages packaged at moisture concentrations ranging from about 40 to 70%. Thirty-one 1.2 × 1.2-m round bales made from a mixed alfalfa-orchardgrass stand (56.5% alfalfa; 42.7% orchardgrass) were baled with or without engagement of the bale-cutting system over 4 bale-moisture groupings (67.5, 61.5, 51.1, and 45.8%). Bale-cutting improved initial wet bale weights (668 vs. 641 kg; $P = 0.003$), but only numerical differences ($P \geq 0.144$) were observed for initial dry bale weights (287 vs. 278 kg) and initial DM density (192 vs. 188 kg DM/m^3). Overall, the effects of initial bale moisture and cutting had little meaningful effect on silage nutritive value, except that silage fermentation reduced concentrations of neutral-detergent insoluble CP (% of CP) compared with pre-ensiled forage by 48 to 68%. Following fermentation, modest improvements were noted within cut bales for lactic acid, percentages of total VFA comprised of lactic acid, and a more acidic final silage pH. Final silage pH was best related to initial bale moisture with simple linear models for both cut ($Y = -0.031x + 7.50$; $R^2 = 0.847$) and uncut ($Y = -0.033x + 7.74$; $R^2 = 0.869$) silages. Tests of homogeneity indicated that slopes did not differ ($P = 0.653$) based on bale-cutting engagement, but intercepts were different ($P = 0.024$). For the silages in this study, the cumulative effects of improved fermentation within cut forages represented pH declines of about 0.10 to 0.16 pH units over initial bale moistures ranging from about 40 to 70%. Although there may be practical or logistical reasons for bale cutting, such as easing incorporation into TMRs, improvements in silage fermentation observed in this study were very modest, and do not really justify engagement of cutting systems solely for that specific purpose.

Key Words: baled silage, fermentation, pH

W27 Effects of the application of a silage inoculant (*Lactobacillus hilgardii* and *Lactobacillus buchneri*) on corn silage aerobic stability and performance of dairy cows. A. Bach*[1,2], E. Cheveaux[3], G. Elcoso[4], and J. Malló[4], [1]*ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona,Spain,* [2]*IRTA, Department of Ruminant Production, Caldes de Montbui, Spain,* [3]*Lallemand Animal Nutrition, Toulouse, France,* [4]*Blanca from the Pyrenees, Hostalets de Tost, Spain.*

Two experiments were conducted to assess the impact of adding an inoculant on corn silage (CS) aerobic stability and performance of dairy cows. A total of 166,880 kg of whole corn plant (36.5% DM) were harvested from the same field on the same day and ensiled in 2 adjacent bunker silos. One silage (81,240 kg) remained untreated (CTR), and the other (85,560 kg, INL) was treated with 300,000 cfu/g of fresh matter, targeting, for every gram of CS, 150,000 cfu of *Lactobacillus hilgardii* CNCM I-4785 and 150,000 cfu of *Lactobacillus buchneri* NCIMB 40788. Inoculation rate was 100 mL of water/ton. In Experiment 1, at silo opening (300 d after ensiling) and fortnightly for 60 d, 2 samples of 500 g were taken to determine aerobic stability in quadruplicates by placing 3.2 ± 0.21 kg of CS in insulated plastic boxes kept at room temperature for 96 h and electronically monitoring temperature continuously. Counts of yeast, molds, and lactic acid bacteria (LAB) were also performed at these time points. In Experiment 2, 60 cows (597 ± 67 kg of BW; 127 ± 41 DIM, producing 35.3 ± 8.01 kg of milk/d) were randomly allocated to a control TMR (untreated silage) or to an identical TMR (15.5% CP, 35.0% NDF, 38.2% CS, in a DM basis) containing CS inoculated as described above for 8 wk. Individual intake, milk production and composition were monitored daily. Data were analyzed using a mixed-effects model including the effect of box (Exp. 1) or cow (Exp. 2), treatment, and their 2-way interaction, with time as a repeated measure. In Exp. 1, counts of LAB, molds, and yeast were lower ($P < 0.01$) in INL (69 cfu/g, 68×10^3 cfu/g, 131×10^3 cfu/g, respectively) than in CTR (307 cfu/g, 212×10^3 cfu/g, 996×10^3 cfu/g, respectively) at 96 h of aerobic exposure, and time to reach instability (i.e., 3°C above ambient temperature) was shorter ($P < 0.01$) in CTR (29.8 ± 3.4 h) than in INL (44.8 ± 3.4 h). In Exp. 2, there were no differences in DMI, yield, or milk composition, but feed efficiency was greater in INL than in CTR cows during wk 7 and 8 of study. It is concluded that inoculating CS with *L. hilgardii* and *L. buchneri* improves aerobic stability of CS and feed efficiency of dairy cows over time.

Key Words: conservation, feed efficiency, heterofermentative inoculant

W28 Effect of ensiling time and temperature on the fermentation and nutritive value of a corn hybrid containing an α-amylase enzyme trait (Enogen Feed Corn). E. Hellings[1], K. Moyer*[1], É. da Silva[1], C. Novinski[3,1], Y. Li[2,1], W. Sá[4,1], R. Mester[1], and L. Kung Jr.[1], [1]*University of Delaware, Newark, DE,* [2]*Heilongjiang Bayi*

Agricultural University, Daqing, Heilongjiang, China, [3]Federal University of Paraná, Curitiba, Paraná, Brazil, [4]Federal University of Paraíba, João Pessoa, Paraíba, Brazil.

We evaluated the effect of time and storage temperature on the nutritive value of a corn hybrid containing an α-amylase enzyme trait (E; Syngenta Seeds, LLC, Minnetonka, MN) compared with its isogenic line (C). Whole-plant corn (38–39% DM) was packed in 7.5 L laboratory silos (208 kg of DM/m^3) and ensiled at 22°C (low temperature) or at 40°C for 0 to 28 d, then at 32°C (high temperature) before opening after 28, 123 and 242 d of ensiling. Data were analyzed using the Fit Model in JMP (SAS Institute Inc., Cary, NC) with the main effects of hybrid, day, temperature, and their interactions. Concentrations of starch were not affected by length of storage, temperature, or hybrid. Water-soluble carbohydrates (WSC) were higher in E than C stored at the higher vs. lower temperature ($P < 0.005$), but were unaffected by storage length. Lactic acid was lower in E than C ($P < 0.05$), but pH remained the same. Acetic acid was not affected by temperature or hybrid but increased at 123 d ($P < 0.005$). Washout of starch from in situ bags at 0 h from fresh plants was 25.55% for E compared with 22.55% for C. There was no ensiling day (28 to 242 d) effect on 0 h loss of starch, but E stored at a higher temperature had more ($P < 0.05$) loss (55.67%) than C (32.24%). Seven h in situ starch digestion (STRD) of fresh plants was 54.39% for C compared with 73.15% for E. STRD was affected by all main effects. For hybrids, E had higher STRD than C (85.92 vs. 83.96%, $P < 0.05$). STRD increased with ensiling time (81.54% at 28 d, 85.39% at 123 d, and 87.88% at 242 d, $P < 0.0001$). Corn stored at a higher temperature had greater STRD than corn stored at a lower temperature (88.13 vs. 81.75%, $P < 0.0001$). Type of hybrid did not affect the aerobic stability (h before a 2°C rise after exposure to air at 21°C) of silages. Greater WSC and STRD in E stored at elevated temperatures suggests expression of α-amylase activity during storage. The corn hybrid containing an α-amylase enzyme trait shows promise in providing increased STRD while maintaining a favorable fermentation during ensiling.

Key Words: corn silage, α-amylase, starch digestibility

W29 Survey of physical and chemical characteristics of sorghum silage in California.

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The aim of this study was to describe sorghum silage quality and density of silage in storage structures in California's San Joaquin Valley. Silage in 15 structures was sampled (7 piles, 8 bags). Open faces were cored once during feed out. Each coring event consisted of sampling 3 premarked locations which were 0.25, 0.50, and 0.75 of the distance across the face at approximately 1.5 m above grade. Bags were cored twice at the 0.50 location, at approximately 0.30 m above the original core location, to ensure enough material for chemical analysis. Immediately upon removal of each 25.4 cm core sample, the sample was weighed and a small portion of silage from each core was used for pH determination. Within each structure, samples were pooled across coring locations for nutrient analysis (Table 1) and berry processing score (BPS). The overall density for each silage structure was calculated as an average of the coring locations. Descriptive statistics were calculated in Excel. Average density of sorghum piles was 609 kg of WW/m^3, with a range of 432 to 705 kg of WW/m^3. Bagged sorghum averaged 513 kg of WW/m^3, with a range of 433 to 561 kg of WW/m^3. Average sorghum silage pH was 3.9, with a range of 3.5 to 4.7. Berry processing score of sorghum, the percent of starch passing a 1.18mm screen, averaged 28%, with a range of 12 to 41%. Four of the 7 sorghum piles met the minimum density required (640 kg of WW/m^3) by current California air district regulations (bagged silage is exempt). Variable BPS results may justify current recommendations to adjust starch availability to zero when incorporating sorghum silage into rations. There is a need to describe sorghum silage quality in California, as sorghum is a minor agronomic crop gaining interest due to water shortages and future water regulation.

Table 1 (Abstr. W29). Sorghum nutrient composition (n = 15) in California's San Joaquin Valley

		% of DM						NDFD 30, % of NDF
	DM %	CP	ADF	NDF	Starch	NFC	Ash	
Mean	29.3	8.5	33.9	49.1	13.1	28.9	11.7	48.9
Median	29.7	8.9	34.2	47.7	10.1	29.3	10.9	48.6
Minimum	22.8	5.6	28.4	42.2	1.4	15.3	8.9	32.2
Maximum	35.2	11.8	41.3	59.4	29.3	41.2	14.5	57.5
SD	3.4	1.8	4.3	5.2	8.2	7.9	1.8	6.6

Key Words: sorghum, silage, California

W30 Probiotic potential of *Lactobacillus plantarum* and its effectiveness on low-moisture silage development.

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There is an increasing demand for high-quality silage production with improved nutrients as a feed resource for livestock. The addition of lactic acid bacteria (LAB) is one way to enhance silage quality via increasing lactic acid content. The objective of the present study was to isolate and identify potent LAB and investigate their effects on in vitro fermentation in low moisture Italian ryegrass (IRG) silage. LAB strains were isolated from alfalfa (*Medicago sativa*) using a selective media and then the antifungal activity were assessed by pour plate method; selected LAB were characterized and identified by biochemical and 16S rRNA sequencing. For silage production, low moisture (<50%) IRG was chopped and packed in sterile air-tight polypropylene bags with selected LAB colonies and sealed in an anaerobic condition using a vacuum-sealing machine. After a month, the number of LAB colonies and fermentative metabolites was analyzed in the samples. Statistical analysis was performed on all variables using SPSS/PC. One-way ANOVA and Duncan multiple range tests were used with 5% probability level. Thirty LAB strains were isolated and screened for their antifungal activity against *Aspergillus* species; among these strains, 2 strains exhibited strong antifungal activity and named as KCC-37 and KCC-38. 16srRNA sequence revealed that KCC-37 and KCC-38 were *Lactobacillus plantarum*. Both strains were able to survive under harsh conditions of GIT tract. Low moisture IRG with either KCC-37 or KCC-38 reduced pH of the silage and improved the organic acids productions than the non-inoculated samples. Overall, these isolated strains could be used as an additive for the improvement of the fermentation quality of the low moisture silage.

Key Words: *Lactobacillus plantarum*, probiotics, silage fermentation

W31 Influence of microbial inoculation and length of storage on fermentation profile, N fractions, and ruminal in situ starch disappearance of whole-plant corn silage.

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The objective of this study was to determine the effects of storage length and microbial inoculation on the fermentation profile, N fractions, and ruminal in situ starch disappearance of whole-plant corn silage. Whole-plant corn (33.3 ± 1.0% DM) was ensiled for 0, 30, 60, 90 or 120 d in quintuplicate vacuum-sealed pouches untreated (CON) or inoculated with: *Lactobacillus plantarum* CH6072 at 1×10^5 and *Enterococcus faecium* CH212 at 5×10^4 cfu/g of fresh forage (LPEF); *L. buchneri* LB1819 and *Lactococcus lactis* O224 at 1.5×10^5 cfu/g (LBLL); and *E. faecium* CH212 at 1.5×10^5 cfu/g (EF). Data were analyzed as a completely randomized design with a 4 × 5 factorial arrangement of treatments using PROC GLIMMIX. The model included microbial inoculation, storage length, and their interactions as fixed effects. Silage pH was greater for LBLL compared with the other treatments ($P < 0.01$). Total acids (as % of DM) were greatest in LPEF (7.27 ± 0.12), intermediate in CON and LBLL (7.15 and 6.83 ± 0.12, respectively), and lowest in EF (6.71 ± 0.12; $P < 0.001$). Ammonia-N (as % of N) was greatest in CON (5.27 ± 0.08), intermediate in LPEF and LBLL (4.87 ± 0.08 for both), and lowest in EF (4.62 ± 0.08; $P < 0.01$). Silage pH decreased quadratically as storage length increased from 30 to 120 d ($P < 0.01$). Ammonia-N increased quadratically with storage length ($P < 0.01$). Interactions between microbial inoculation and storage length were observed for lactic acid ($P = 0.05$), acetic acid ($P = 0.09$), and ruminal in situ starch disappearance ($P = 0.01$). Lactic acid concentrations were reduced with LBLL compared with the other treatments at 30, 90, and 120 d. Acetic acid concentrations were similar between all treatments at 30 d, but were greater for LBLL after 60 d. Starch disappearance was reduced for CON compared with the other treatments at 60 and 90 d but was similar across all treatments at 120 d. Although fermentation profile was improved with the use of microbial inoculants, minimal benefits of inoculants on starch disappearance were observed.

Key Words: microbial inoculation, corn silage, fermentation profile

W32 Evaluation of a novel pocket-sized micro-spectrometer as a rapid method to determine dry matter in silages. S. Ostrom, M. Daynuah, M. Messman*, T. Da Silva, and G. Schroeder, *Cargill Animal Nutrition, Elk River, MN.*

Frequent determination of dry matter (DM) content of ensiled forages is imperative for precise mixing of TMR diets. The methods for on-farm DM determination should be fast, easy to use, and comparable with the standard oven-drying measurements. The objective of this study was to compare a novel method using a pocket-sized micro-spectrometer combined with cloud-based calibrations (Reveal, Cargill Inc.; 1,417 samples included in the calibration) with other rapid methods: 1) Koster tester run for 30 min (KT 30 min); 2) Koster tester run until constant weight or maximum time of 70 min (KT final); 3) laboratory near infrared spectroscopy (DS2500 model, Foss) calibrated using 11,728 samples oven-dried at 60°C for 16 h (Lab NIR); and 4) 60°C oven for 16 h (Oven DM). Samples of corn, alfalfa, and grass silages (10 samples per forage class) were sourced from dairy farms and selected to represent a large range of DM (ranged from 19 to 63%). Concordance correlation coefficients (CCC) between methods was calculated using epiR package of the R software. The CCC indicated that all methods were highly correlated with Oven DM (considered standard method in this study) and among each other when compared within forage class (data not shown) and when combining all samples in one set (all CCC >0.90). Results of DM content obtained with each method were compared with Oven DM using a *t*-test for paired means (Table). KT 30 min overpredicted DM by 8% ($P < 0.05$), indicating that 30 min was not sufficient time to accurately measure DM content. Compared with Oven DM, KT Final resulted in lower DM values ($P < 0.05$), likely associated with the fact that Oven DM was determined at 60°C for 16h and not at higher temperatures. Dry matters obtained by Lab NIR and Reveal were not different from Oven DM, indicating that Reveal is a valid alternative for fast determination of DM content in forages on farms.

Table 1 (Abstr. W32).

	Mean DM, %	SD	Mean difference with oven DM,[1] %	SD	*P*-value	95% CI for differences
Oven DM	37.7	13.2	—	—	—	—
KT 30 min	40.7	11.1	2.91	3.91	0.01	1.43 to 4.40
KT Final	36.6	12.3	−1.19	2.77	0.03	−2.25 to −0.14
Reveal	37.4	11.6	−0.36	3.23	0.55	−1.59 to 0.86
Lab NIR	38.3	13.3	0.49	3.04	0.39	−0.66 to 1.65

[1] t-test for paired means (oven DM vs. the rest).

Key Words: DM determination, near infrared, silage

W33 The effects of recombinant bacterial expansin-like protein and a fibrolytic enzyme on in vitro nutrient digestibility and preingestive hydrolysis of alfalfa silage. J. E. Blajman[1], A. A. Pech-Cervantes*[2], M. Irfan[3], C. F. Gonzalez[3], D. Vyas[2], K. G. Arriola[2], N. Dilorenzo[4,2], Y. Jiang[2], F. X. Amaro[2], and A. T. Adesogan[2], [1]*National Institute of Agricultural Technology EEA Rafaela, National Council of Scientific and Technical Research, Rafaela, Argentina,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Department of Microbiology and Cell Science, Gainesville, FL,* [4]*Department of Animal Sciences, North Florida Education Center University of Florida, Marianna, FL.*

The aim of this study was to examine if effects of an exogenous fibrolytic enzyme (EFE) on in vitro digestibility and preingestive hydrolysis of alfafa silage can be synergistically increased by different doses of a recombinant bacterial expansin-like protein (BsEXLX1). Treatments were arranged in a 2 × 4 factorial arrangement with 2 levels of a cellulase-xylanase EFE (0, 2.3 mg/g DM) and 4 levels of BsEXLX1 (0, 0.106, 0.212, and 0.424 mg/g DM). In Experiment 1 (E-1), dried, ground (1 mm) alfalfa silage samples were preincubated in sodium citrate buffer for 1 h at room temperature in quadruplicate with or without the treatments followed by incubation in rumen fluid for 24 h at 39°C. Gas production (GP), in vitro true digestibility, CH_4 emissions, and VFA profile were measured after 24 h. In Experiment 2 (E-2), alfalfa silage was incubated with or without the treatments in deionized water containing 0.02% sodium azide for 24 h at 25°C and preingestive hydrolysis was measured. Data were analyzed using NLME package of R studio with EFE, BsEXLX1 and interaction as fixed effects and run as a random factor in the model. In E-1, EFE increased GP ($P = 0.02$) and reduced pH ($P < 0.01$) compared with the control; however, no effects were observed on in vitro DM, NDF, and ADF digestibility. Similarly, the combination of EFE and BsEXLX1 had no effects on digestibility of DM, NDF, and ADF or VFA and NH_3-N concentrations. Methane emissions tended to increase with EFE ($P = 0.08$) treatment while BsEXLX1 supplemented at 0.212 and 0.424 mg/g DM significantly increased CH_4 emissions ($P < 0.01$), compared with the control. During preingestive hydrolysis (E-2), EFE increased DM, NDF, ADF and HEM hydrolysis ($P < 0.01$); however, no synergistic improvements were observed when BsEXLX1 was added. Adding EFE increased release of reducing sugars compared with control ($P < 0.01$) but no effects were observed by adding BsEXLX1 (*P*

= 0.80). In conclusion, EFE increased preingestive hydrolysis of alfalfa fiber but adding BsEXLX1 did not synergistically increase the response.

Key Words: alfalfa silage, enzyme, expansin

W34 Aerobic stability of sorghum silages inoculated with wild strains of *Lactobacillus buchneri*.

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The objective of this study was to evaluate the effect of microbial inoculant containing new strains of *Lactobacillus buchneri* (LB) isolated in tropical conditions on the aerobic stability (AS) of sorghum silages in different fermentation periods. A 3 × 5 factorial arrangement was used, with 3 fermentation periods (P) (20, 40 and 60 d), and 5 inoculants (I) (control, commercial inoculant, LB strains 50.4, 90.14, and 45.22), in a completely randomized design, with 4 replicates. The sorghum was mixed either with the inoculants or with distilled water (control) in approximately 7 kg and packed in plastic buckets (mini-silos). The commercial inoculant used was Lalsil AS (LAS) (Lallemand, Brazil). The strains were isolated from sorghum silages. For all treatments, the theoretical application rate was 1.0×10^6 cfu/g fresh forage. After 20, 40 and 60 d of fermentation, 2 kg of silage from each silo were collected and placed in plastic buckets without a lid for 7 d at 22°C, to determine the AS. Temperatures were measured every 10 min using data loggers inserted into the silage mass at geometric center. Silage samples after 7 d of air exposure were analyzed for pH, and yeast and molds counts. The estimated means were compared by Tukey's test considering a significance level of 0.05. The AS, pH, yeast population, and maximum temperature reached (MT) were affected ($P < 0.05$) by I × P interaction. Mold population was affect ($P < 0.05$) by both P and I effects. At d 20 of fermentation, the silages treated with LB 90.14 and 45.22 remained stable (>160 h) for 7 d after air exposure, consequently had the lowest pH, MT and yeast counts (3.59, 23.7 h and 5.22 log cfu/fresh weight, respectively). After 40 and 60 d of fermentation inoculated silages remained stable after air exposure and had the lowest pH and yeast counts. Molds population reduced throughout the fermentation period and the highest counts was observed in non-inoculated silage. The addition of LB 90.14 and 45.22 strains improved the aerobic stability of sorghum silages stored for a short period. Supported by CNPq, CAPES, and INCT-CA

Key Words: mold, pH, yeast

W35 Effect of wild strains of lactic acid bacteria on the fermentation profile of alfalfa silage.

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Ensiling is a helpful process to ensure food availability and support animal production. However, a good fermentation is required to avoid nutritional and economic losses. Lactic acid bacteria (LAB) are used to favor homolactic fermentation and inhibit the growth of undesirable microorganisms. The objective of this study was to evaluate the changes on the fermentation profile, chemical composition and microbial populations of alfalfa silages harvested in January (cut 1, C1) and March (cut 2, C2), treated with LAB strains. The alfalfa was harvested at the early flowering stage, using a backpack mower, in 2016. The LAB strains used were isolated from alfalfa silage in a prior study and the commercial inoculant (CI) was the Sil All (Alltech, Brazil) which contains *Lactobacillus plantarum*, *Pediococcus acidilactici*, *Enterococcus faecium*, and *L. salivarus* ssp. *salivarus*. The inoculants (I) evaluated were: 1. No inoculant (Control); 2. *L. plantarum* (AV 14.17); 3. Mixture of *L. plantarum*, *L. brevis* and *P. acidilactici* (Combo); and 4. CI. The experiment was analyzed as a completely randomized design in a 4 × 2 factorial arrangement, with 4 replicates. All inoculants were applied at the rate of 10^6 cfu/g fresh matter. The silages were stored for 90 d. The means were separated by Tukey's test ($P \leq 0.05$). There were I × C interactions for WSC, lactic to acetic acid ratio (L:A), NDF and ADF. Silage from C2 treated with AV 14.17 had higher WSC, while both silages treated with AV 14.17 and Combo had higher L:A. Additionally, lower NDF and ADF contents were found in silages inoculated with CI and Combo, respectively. The silage treated with the AV 14.17 had the lowest pH, with average of 4.41. NH_3-N was lower in silages treated with Combo and CI, as well as, in silages of C2 compared with C1. The DM was higher for silages treated with inoculants (376.6 vs. 370.9 g/kg). It was observed the highest CP value for C2 silages (150.3 g/kg of DM). The C2 silages had higher lactic and lower acetic acids. The LAB and yeast counts were higher and mold count was lower in C1 silages. The wild LAB strains improved the fermentation profile on alfalfa silage in tropical conditions. Supported by CNPq, CAPES, and INCT-CA

Key Words: organic acid, chemical composition, microbial population

W36 Reduced-lignin and normal alfalfa in monoculture and in binary mixtures with perennial grass.

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Nutritive value improvements in both alfalfa and perennial grasses have the potential to significantly increase economic returns when fed to dairy cattle. Our goal was to assess yield and nutritive value of genetically modified reduced-lignin alfalfa in monoculture and in binary mixtures with perennial grass. A HarvXtra-type alfalfa (Hx14376) and a conventional alfalfa (WL355RR) were sown in small plots as pure stands and in mixture with festulolium (Fojtan), meadow fescue (Driftless), or orchardgrass (Dividend VL). Studies were sown in the spring of 2016 in Ithaca, NY, Lexington, KY, and Rosemount, MN, and harvested in 2016 (NY and KY only), 2017, and the spring of 2018. Two harvests were taken in the seeding year, and 3 harvests (MN), 4 harvests (NY), and 4 (flower stage) or 5 (bud stage) harvests (KY) were taken in 2017. Plots were harvested at alfalfa first bud and first flower stages in NY and KY, and harvested at first flower stage in MN. Alfalfa-grass mixtures outyielded pure alfalfa ($P < 0.05$) for 3 of 8 site-year combinations, but never yielded less than pure alfalfa stands ($P < 0.05$). Delayed harvest to flower stage for alfalfa resulted in increased yield if an equal number of cuttings were taken for both bud and flower stages, but reduced yield if more harvests could be taken from the bud stage than flower stage over the season ($P < 0.5$) (e.g., KY in 2017). Orchardgrass proportion of mixtures greatly increased between 2016 and 2018, from 10% to over 80% grass in NY, and from 19% to 62% grass in KY. Minnesota had no 2016 data, but orchardgrass mixtures had only 15% grass in 2018. Reduced-lignin alfalfa averaged (annually) between 11.0 and 18.5% less lignin over the 3 years of the study, and averaged between 5.8 and 10.6% greater fiber digestibility, compared with WL355RR. Alfalfa nutritive value response was relatively consistent across regions, but grass response was variable across regions for both grass proportion in mixtures and nutritive value. Perennial grasses grown with alfalfa must be evaluated for both yield and nutritive value on a regional basis to produce meaningful results.

Key Words: reduced lignin, nutritive value, fiber digestibility

W37 An evaluation of *Lactobacillus hilgardii* 4785 to improve the aerobic stability of corn silage.

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We evaluated the effect of various microbial inoculants (Lallemand Animal Nutrition, Milwaukee, WI) on the fermentation and aerobic stability of corn silage. Corn silage (33% DM) was untreated (UN), treated with *Lactobacillus buchneri* 40788 (LB; 150,000 cfu/g of fresh forage wt.), treated with *L. hilgardii* 4785 (LH; 150,000 cfu/g), treated with LB + LH (LBLH; 150,000 cfu of each/g), or treated with LB500 (LBC; *L. buchneri* 400,000 and *Pediococcus pentosaceus* 100,000 cfu/g of fresh material). Five individually replicated lab silos (7.5 L) for each treatment were packed (208 kg DM/m^3) and ensiled for 34 and 99 d between 21 and 23°C before testing for aerobic stability (ASb, h before a 2°C increase above baseline after exposure to air at 21°C.). An additional set of silos containing UN, LBLH, and LBC silages were prepared and subjected to ASb at a higher temperature of 29°C at 99 d. Data were analyzed using the Fit Model in JMP (SAS Institute Inc., Cary, NC) as a 5 × 2 factorial arrangement of treatments with the main effects of treatment, day, and their interaction. Lactic acid was higher ($P < 0.01$) in LH silages (6.11%) compared with LBLH and LBC silages, but similar to UN and LB silages at 99 d. Concentrations of acetic acid were numerically highest for LBLH (1.71%) of all treatments and lowest for UN (1.25%) and LB (1.22%). After 99 d, concentrations of 1,2-propanediol were higher ($P < 0.01$) in inoculated silages (0.77 to 1.12%) compared with UN (0.04%). At 34 d, silage treated with LBLH had the highest ASb (127 h, $P < 0.01$) compared with all other treatments. Treatment with LBC (87 h) also was more stable than UN (36 h), LB (44 h) and LH (49 h). At 99 d, ASb ($P < 0.01$) was higher in all inoculated silages (mean >360 h) compared with UN (79 h). When silages were exposed to the higher temperature during aerobic spoilage, treatment with LBLH and LBC had lower ASb than when spoiled at the cooler temperature but they were still markedly more stable than UN ($P < 0.001$). These data suggest that combining LB and LH results in synergistic effects on stimulating ASb after a relatively short time of ensiling.

Key Words: *Lactobacillus hilgardii*, silage, aerobic stability

W38 Evaluation of additives containing *Lactobacillus buchneri* on the fermentation of corn silage.

E. M. Santos[2,3], K. Z. Moyer[3], E. A. Hellings[3], D. M. Costa[1,3], N. A. Moyer[3], M. A. Davidson[3], and L. Kung Jr.*[3], [1]*Universidade Federal da Lavras, Lavras, MG, Brazil,* [2]*Universidade Federal da Paraíba, João Pessoa, Paraíba, Brazil,* [3]*University of Delaware, Newark, DE.*

We evaluated the effect of 2 additives (American Farm Products, Saline, MI) on the fermentation and aerobic stability (ASb) of corn silage. Whole-plant corn (31% DM) was untreated (CN) or treated with Silage-Pro B, (SPB; 400,000 cfu of *L. buchneri*/g of fresh forage wt., 50,000 cfu of *Pediococcus pentosaceus*, 50,000 cfu of *P. acidilactici,* and isolated enzymes from *Aspergillus oryzae* and *Bacillus subtilis* including amylase, cellulase, and hemicellulose), or treated with SPB (but with a lower application rate [200,000 cfu/g] of *L. buchneri* (SPBL). Five replicated silos for each treatment were packed into 7.5 L silos (208 kg of DM/m^3) that remained sealed until opening at 90 d of ensiling. An additional set of treatments was packed in silos with 3, 1.60-cm diameter holes plugged with stoppers and silicone glue, 2 located on the bottom of the silos at opposing ends, and one on the lid that were opened during air stress (AS) on d 14, 28, and 84. Silages were analyzed for fermentation end products and ASb (h before a 2°C increase above baseline after exposure to air at 21°C). Data were analyzed using the Fit Model in JMP (SAS Institute Inc., Cary, NC) as a 3 × 2 factorial arrangement of treatments with the main effects of treatment, air stress, and their interaction. The ASb ($P < 0.001$) of non-AS silages were 112, 285 and 276 h for CN, SPB and SPBL, respectively. With AS, ASb ($P < 0.001$) was 21, 171 and 254 h for CN, SPB and SPBL, respectively. In non-AS silages, inoculated silages had higher ($P < 0.001$) amounts of acetic acid and 1,2 propanediol (1,2PD) than CN. However, AS increased ($P < 0.001$) acetic acid in CN so it was not different from SPB but it was higher ($P < 0.001$) than SPBL. 1,2PD was not detected in CN that was AS and inoculated silages had high ($P < 0.001$) amounts of this compound. In non-AS silages, only treatment with SPBL was lower in ethanol than CN, but inoculation consistently decreased the concentration of ethanol in AS silages compared with CN. Inoculants markedly improved aerobic stability of corn silage even if air stressed during storage.

Key Words: air stress, corn silage, *Lactobacillus buchneri*

W39 Particle size distribution of whole-plant corn silage harvested with pull-type or self-propelled forage harvesters.

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The particle size distribution (PSD) of whole-plant corn silage (WPCS) can be a major factor on the supply of physically effective NDF (peNDF) for dairy cattle. The majority of Brazilian dairy farms have pull-type forage harvesters (PTH) as the main equipment. The PTH usually do not have crop-processing rolls and kernel processing is achieved by shortening the theoretical length of cut. Obtaining WPCS with high proportions of long NDF and processed kernels is a challenge in PTH compared with self-propelled harvesters with crop-processing rolls (SPH). The objective of this survey was to evaluate the PSD of WPCS harvested with PTH or SPH. Data were obtained from nutrition consultants and farms located in 6 Brazilian states (MG, SP, ES, SC, PR, GO) from May 2014 to August 2018. The PSD was evaluated with the 19 mm (Top) and 8 mm (Middle) diameter mesh screens and Pan of the Penn State Particle Separator. The number of samples was 195 for PTH and 714 for SPH. The PSD (% of as fed) had normal distribution for PTH and SPH and were, respectively [Mean ± SD (Min–Max)]: Top: 9.7 ± 5.9% (0.2–27.9) and 11.4 ± 4.9% (2.4–28.8). Middle: 55.3 ± 8.5% (27.5–73.6) and 68.4 ± 5.8% (49.2–82.6). Pan: 35.0 ± 8.3% (21.5–63.4) and 20.2 ± 4.0% (10.1–34.6). The linear correlation coefficients between particle sizes were ($P < 0.01$): Top vs Pan: −0.32 for PTH and −0.16 for SPH. Top vs Middle: −0.37 for PTH and −0.74 for SPH. Middle vs Pan: −0.75 for PTH and −0.54 for SPH. The poorest correlations were between Top and Pan for both harvesters. The highest correlations were between Middle and Pan for PTH and between Top and Middle for SPH, suggesting that how PSD would change in response to variation in equipment processing is dependent on the type of harvester. Assuming that obtaining the largest proportion of WPCS particles >8 mm would be desirable to achieve high forage peNDF, reasonable long-particle goals (Mean – 1 SD) would be less than 16% of particles in Pan for SPH and less than 27% in Pan for PTH. Silages harvested with PTH had shorter particle size than silages harvested with SPH.

Key Words: corn silage, particle size, effective fiber

W40 Impact of roll gap and theoretical length of cut settings, and storage length on fermentation profile and berry processing score of whole plant sorghum silage. C. L. McCary*, C. Heinzen Jr., B. A. Saylor, and L. F. Ferraretto, *University of Florida, Gainesville, FL.*

This study aimed to quantify the effects of roll gap (RP) and theoretical length of cut (TLOC) settings, and storage length on fermentation profile, and berry processing score (BPS) of whole plant sorghum silage. Sorghum plants from 4 replicated plots were harvested and processed to achieve 1 of 4 treatments which were a combination of 2 RP settings (1 or 3 mm) and 2 TLOC (15 or 22 mm). Plants were handfed into the chopping cylinder of a self-propelled forage harvester and 800 g samples were sealed in vacuum bags in quadruplicate. Mini silos were allowed to ferment for 0, 30, or 90 d. Measurements of BPS were performed to determine the percentage of starch passing through a 1.7-mm sieve. Data were analyzed as a complete randomized design with a 2 × 2 × 3 factorial arrangement of treatments using PROC GLIMMIX. The model included TLOC, RP, storage length and their interactions as fixed effects. A 3-way interaction occurred for lactic ($P < 0.01$) and acetic ($P < 0.01$) acids, and mold count (cfu/g; $P < 0.04$). Lactic acid concentrations increased with storage length and at 90 d the concentrations were greatest with the combination of a 1-mm RP and a 22-mm TLOC. Likewise, acetic acid increased until 30 d with the greatest concentrations observed for 3 mm RP coupled with a 22-mm TLOC. Mold counts were reduced within all treatments as storage length progressed. A RP by storage length interaction was observed for pH ($P < 0.01$). The pH with a 1-mm RP was greater at 0 d but lower at 30 d compared with the pH with a 3-mm RP. An interaction between TLOC and RP was observed for BPS ($P < 0.02$). The combination of a 1-mm RP with 15-mm TLOC provided the greatest BPS (25.69% vs. 21.50%). These results demonstrate that reduced RP and TLOC settings, in conjunction with extended storage length, improves the fermentation profile of whole plant sorghum silage. Additionally, the utilization of reduced RP and TLOC settings increase BPS in whole plant sorghum silage.

Key Words: berry processing score, roll gap setting, theoretical length of cut

W41 Dairy heifer growth while grazing meadow fescue or orchardgrass. C. Hribar*[1], G. Brink[2], J. Bleier[2], M. Casler[2], W. K. Coblentz[3], R. K. Ogden[3], J. C. Cavadini[4], and M. S. Akins[1], [1]*University of Wisconsin- Madison, Madison, WI,* [2]*USDA Dairy Forage Research Center, Madison, WI,* [3]*USDA Dairy Forage Research Center, Marshfield, WI,* [4]*University of Wisconsin Marshfield Agricultural Research Station, Stratford, WI.*

Grazing operations are interested in grasses that will promote livestock performance and thrive under a management intensive grazing system. Our objective was to evaluate pasture productivity and dairy heifer growth when grazing either meadow fescue (MF; variety Hidden Valley) or orchardgrass (OG; variety Haymaster) at the University of Wisconsin Marshfield Agricultural Research Station. Six pastures (1 ha each) were used with 3 blocks (2 pastures/block) and grass treatment randomized within each block. Pre-pubescent heifers (n = 24) were blocked by weight (8 heifers per weight block; low, medium, high) and randomly assigned to graze MF or OG in each block with 4 heifers per pasture. Three years of study were completed with different heifers used each year. No supplemental concentrates were provided. Mineral was provided daily to meet mineral and vitamin needs. Body measurements were taken at the start and end of the grazing season. Grazing commenced in late May. Forage height measures were taken with a rising plate meter. Calibration of height measures to estimate forage availability was done by clipping 3 locations in each pasture. Data were analyzed using Proc Mixed (SAS v9.4) as a randomized complete block design with year as a repeated measure. Meadow fescue had lower forage availability (849, 865, and 1423 kg/ha in 2016, 2017, and 2018, respectively) than OG (1022, 891, 1471 kg/ha for 2016, 2017, and 2018, respectively). Average daily gains were similar ($P = 0.14$) for heifers grazing MF (0.78 kg/d) and OG (0.74 kg/d). Hip height gains and body condition change were also similar between MF and OG ($P > 0.28$).Both Based on these results, both grass species are suitable forage sources for grazing young heifers.

Key Words: grazing, meadow fescue, orchardgrass

W42 Use of machine learning to predict feed components via near-infrared spectroscopy. J. R. R. Dorea*[1], J. Karlen[2], and G. J. M. Rosa[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*Rock River Laboratory Inc., Watertown, WI.*

Near-infrared spectroscopy (NIRS) has been widely used for feed analyses. Traditionally, partial least squares (PLS) has been used as the statistical method for prediction using spectral data. However, only linear relationships between spectral wavelengths and response variables are explored in PLS models. The objective of this study was to evaluate the ability of artificial neural networks (ANN) to generate accurate predictions of feed components using NIRS data. Feed samples were scanned in a NIRS instrument and analyzed with wet chemistry for: acid detergent fiber (ADF, n = 16,032), neutral detergent fiber (NDF, n = 19,942), ash (n = 11,165), calcium (Ca, n = 50,372), potassium (K, n = 50,438), water soluble carbohydrate (WSC, n = 1,982), mannitol (MAN, n = 2,836), starch (STA, n = 18,694) and butyric acid (BA, n = 12,763). A Bayesian Network approach was implemented to investigate statistical dependences among wavelengths for selection of more informative predictors using the concept of Markov Blanket (MB). The use of MB for variable selection was combined with 2 predictive approaches: PLS and ANN. Prediction quality was assessed by randomly splitting the data set into training and test sets (70% and 30% of the data set, respectively). The ANN and PLS results using all wavelengths (ANN_{all} and PLS_{all}) were similar across feed components. The use of MB coupled with ANN (ANN_{mb}) yielded more accurate and precise predictions compared with MB with PLS (PLS_{mb}). ANN_{mb} performed better than the traditional approach (PLS_{all}) in terms of R^2 and root mean square error of prediction (RMSEP) for: mannitol (ANN_{mb}: $R^2 = 0.71$, RMSEP = 0.71%, PLS_{all}: $R^2 = 0.25$, RMSEP = 1.7%), WSC (ANN_{mb}: $R^2 = 0.73$, RMSEP = 5.1%, PLS_{all}: $R^2 = 0.57$, RMSEP = 7.3%), Ca (ANN_{mb}: $R^2 = 0.71$, RMSEP = 0.82%, PLS_{all}: $R^2 = 0.57$, RMSEP = 0.92%), BA (ANN_{mb}: $R^2 = 0.71$, RMSEP = 0.42%, PLS_{all}: $R^2 = 0.64$, RMSEP = 0.86%), and similar for: STA (ANN_{mb}: $R^2 = 0.97$, RMSEP = 3.7%, PLS_{all}: $R^2 = 0.94$, RMSEP = 5.0%), ADF (ANN_{mb}: $R^2 = 0.92$, RMSEP = 4.0%, PLS_{all}: $R^2 = 0.88$, RMSEP = 5.0%), and NDF (ANN_{mb}: $R^2 = 0.90$, RMSEP = 4.8%, PLS_{all}: $R^2 = 0.87$, RMSEP = 5.4%). In summary, combining MB with ANN is an effective way to reduce data dimension and increase quality of NIRS-based predictions.

Key Words: artificial neural networks (ANN), near-infrared spectroscopy (NIRS), machine learning

W43 Effect of ammonia fiber expansion (AFEX) treatment on the digestibility and feeding value of rice straw for young dairy steers. B. Van Soest*[1], F. Teymouri[2], D. Haddad[2], V. Bringi[2], and M. VandeHaar[1], [1]*Michigan State University, East Lansing, MI,* [2]*Michigan Biotechnology Institute, Lansing MI.*

Ammonia fiber expansion (AFEX) is a promising method to enhance fiber digestibility of crop residues. Our objective was to determine if the AFEX process would enhance the feed value of rice straw fed to young Holstein steers. Holstein steers (n = 16; BW = 150 kg with SD of 20) were blocked by BW and randomly assigned within block to control or AFEX diets. The control contained 49% chopped untreated rice straw, 23% corn silage, 23% modified distillers grains, 2% soybean meal, 1.0% urea, and 1.5% minerals and vitamins and was calculated to contain 54% NDF, 17% CP, and 8% starch. The AFEX process increased CP of straw from 5 to 16% and in vitro NDF digestibility at 240 h from 62 to 88%. Diets were fed at same straw content but without added urea in the AFEX diet. Calves were fed once daily for ad libitum intake and slaughtered after 7 wk. Calves were weighed 3 consecutive days at the start, at 1 wk, and at end of the study, and once weekly during the study. Digestibility was assessed by samples every 15 h for 5 d in wk 5, and undigested NDF (240h) was used as a marker for calculations. Apparent total-tract digestibilities for DM and aNDF were greater in the AFEX than control diet (58% vs. 48% for DM at $P = 0.003$; 55% vs. 45% for aNDF at $P = 0.002$). Compared with control, AFEX tended to increase DMI (9.8 vs. 9.0 kg/d, $P = 0.11$) and average daily gain (0.81 vs 0.66 kg/d; $P = 0.10$). Carcass weight was 48% of BW for both groups, and mirrored changes in gain, but was not significantly different for the 2 treatments. Compared with control, AFEX resulted in 40% larger liver ($P < 0.01$), 17% larger kidney ($P = 0.13$), and 9% larger heart ($P = 0.02$). We conclude that the AFEX process improves the digestibility of rice straw and likely converts it into a more useful feed for ruminants when fed at half the total diet. This study supports the idea that the AFEX technology could enhance the production of ruminants in areas with limited feed resources and plentiful supplies of poor-quality crop residues.

Key Words: forage, ammonia fiber expansion, rice straw

Growth and Development: Milk and Milk Replacer

W44 Effects of breed and health incidences on total milk consumption and predicted body weight of Holstein and Angus × Holstein F_1 calves during the preweaning period. T. S. Steckler* and J. P. Boerman, *Department of Animal Sciences, Purdue University, West Lafayette, IN.*

The objective of this study was to compare the impact of milk consumption, health incidences, and growth between Holstein and Angus × Holstein F_1 calves fed through automated calf feeders. Daily milk consumption, serum total protein (STP), pneumonia and scours incidences, birth weights and weaning weights were recorded on a commercial dairy farm from October 1, 2017 to January 30, 2019. Calves (n = 4,185; Holstein n = 2,912, Angus × Holstein F1 n = 1,273) were fed pasteurized waste milk with a 30% protein, 5% fat milk enhancer added at 20 g/L of milk through a Förster-Technik automated calf feeding system (pens = 16; feeders per pen = 2) for an average of 68 d. STP was taken between 1 and 7 d after birth (6.8 ± 0.69 g/dL, mean ± SD). Daily BW was estimated for individual animals using Legendre polynomials to best model growth data. The effects of feeder, days on feeder, incidences of pneumonia and scours, STP, breed and sex were evaluated using a multiple regression model to predict body weight and total milk consumption. When predicting total milk consumption, 4 variables were highly significant: feeder, pneumonia incidences, STP, and days on feeder (all $P < 0.0001$; $R^2 = 0.56$). For each additional day on feeder, total milk consumption increased by 5.7 L and calves diagnosed with pneumonia had reduced total milk consumption (0 cases = 484, 1 case = 468, 2 cases = 458, 3 cases = 440 and ≥ 4 cases = 420 L). Six variables contributed to predicted body weight at 60 d: feeder, sex, total consumption of milk, and pneumonia incidences (all $P < 0.0001$), breed ($P < 0.04$), and STP ($P = 0.10$; entire model $R^2 = 0.38$). Calves were 7.0 kg less at 60 d if they were treated for pneumonia 3 times compared with calves with no treatment for pneumonia during the pre-weaning period. Holstein calves were 1.4 kg larger at 60 d than Angus × Holstein F_1 calves and males were 4.5 kg larger than females. Pneumonia incidences negatively affected both milk consumption and predicted BW at 60 d. Overall, there were no breed differences in milk consumption between Holstein and Angus × Holstein F_1 calves and Holsteins had a slight advantage in predicted BW at 60 d.

Key Words: autofeeder, growth, Angus × Holstein F_1

W45 Effects of porcine plasma or combined sodium butyrate and *Bacillus subtilis* on growth and health of dairy calves. D. Wood*[1], R. Blome[1], A. Keunen[2], D. Renaud[3], J. Campbell[4], and J. Crenshaw[4], [1]*Animix LLC, Juneau, WI,* [2]*Mapleview Agri Ltd., Palmerston, ON, Canada,* [3]*Population Medicine, University of Guelph, Guelph, ON, Canada,* [4]*APC Inc., Ankeny, IA.*

The objective of this study was to evaluate the health and performance effects of incorporating 2 common feed additives, *Bacillus subtilis* (1.3 million cfu/g) and 70% sodium butyrate at 2kg/MT (FA), or spray dried porcine plasma (SDPP) at 5% of the milk replacer formula (MR). Holstein male calves (n = 158) were randomly assigned to receive one of 3 formulas containing SDPP, FA, or a control group with no feed additives or non-milk proteins. Calves were housed individually until weaning at d 49, then commingled into consecutive groups of 5 until d 78. MR was formulated with 26% crude protein (CP) and 17% fat and fed at 0.7 kg powder / d in 2 feedings for 5 weeks followed by 0.45 kg of MR powder / d over a 2-week weaning period. Calf starter (18% CP) was fed for 21 d followed by a mixed corn and supplement (18.1% CP) ration for the remainder of the study. For the first 28 d following arrival, calves were scored for fecal consistency on a scale of 0 to 3 with 0 being normal manure and 3 meaning watery feces. Calves were observed daily for symptoms of respiratory disease. Body weights were recorded at arrival, 49, and 78. A Cox proportion hazard model was built to evaluate the impact of treatment groups on the risk of morbidity, and mortality over the growing period, whereas a mixed linear regression model was built to evaluate the effect on average daily gain. To evaluate the effect of treatment group on fecal score, a generalized linear model with a logit link and binomial family was used. There was no difference between the groups with respect to body weight ($P = 0.56$), or level of serum total protein ($P = 0.48$) measured at arrival. Calves in the FA group had an increased risk of mortality ($P = 0.02$) when compared with the control group. SDPP fed calves had a reduction in the number of days with a fecal score of 3 ($P = 0.03$). There were no significant differences between groups with respect to treatment for diarrhea, respiratory disease, or average daily gain. This study suggests that feeding plasma in milk replacer may help reduce the severity of diarrhea, whereas, adding the FA could result in a higher risk of mortality.

Key Words: calf, health, growth

W46 Effects of milk replacer feeding rate and functional fatty acids on digestion in Jersey calves. F. X. Suarez-Mena*, T. S. Dennis, K. M. Aragona, T. M. Hill, W. Hu, J. D. Quigley, and R. L. Schlotterbeck, *Nurture Research Center, Provimi, Cargill Animal Nutrition, Brookville, OH.*

This study evaluated the effects of 2 milk replacer (MR) feeding rates and addition of functional fatty acids (FA) to calf feeds on total track digestion (TTD). Male Jersey calves (n = 48; initially 30 ± 2.4 kg BW; 3 to 7 d of age) were randomly assigned to 4 treatments in a 2 × 2 factorial arrangement of MR feeding rate (454 g for 42 d, then 227 g for 7 d [Low]; or 454 g for 3 d, 568 g for 4 d, 681 for 35 d and 341 for 7 d [High]) and supplementation with (FA+) or without (FA-) a FA blend (NeoTec5g, Provimi) added to MR and CS. Milk replacer (24% CP, 21% fat DM) was reconstituted to 14% solids and fed in 2 equal feedings for 42 d and a.m. only for 7 d. Textured calf starter (CS; 20% CP, 39% starch DM) and water were offered for ad libitum consumption. From d 57–112, CS was mixed with 5% chopped grass hay. Up to d 56, calves were housed individually and from d 57–112 in groups (n = 4/pen). Total-tract digestion was measured from 5 calves/treatment at wk 3 and 7, and from all pens at 9, 11, and 15 wk using acid insoluble ash as a digestion marker. Data were analyzed as a completely randomized design with repeated measures when appropriate, with calf as the experimental unit d 1–56 and pen thereafter. No differences were detected in CS intake or performance. No TTD differences were detected at wk 3. Shortly after weaning (7 wk) TTD of DM, OM, starch, NDF and ADF were greater ($P < 0.05$) for calves fed Low; and TTD of DM, OM, NDF, ADF and fat were greater ($P < 0.05$) for calves fed FA+. Average TTD for DM, OM, starch, NDF, ADF and fat at 7 wk were 79, 80, 97, 40, 28 and 75%, respectively. Combined group pen TTD (wk 9, 11 and 15) of DM, OM, NDF and ADF were greater ($P < 0.05$) for calves fed Low; and DM, OM, sugar, NDF, ADF, CP and fat were greater ($P < 0.05$) for calves fed FA+. Average TTD for wk 9, 11 and 15 of DM, OM, sugar, NDF, ADF, CP and fat were 78, 80, 95, 47, 39, 78, 67%, respectively. In this study, feeding Jersey calves more MR had a negative impact on post-weaning digestion of feeds, whereas supplementing the diets with functional FA improved digestion.

Key Words: feeding rate, digestibility, Jersey calf

W47 Different milk replacer induces changes in growth performance and rumen bacterial diversity of dairy bull calves.

Y. Zhang[1], D. Jin[1], J. Cheng[2], N. Zhang[1], Y. Zhang[2], and J. Wang*[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Institute of Animal Husbandry and Veterinary Science, Shanxi Academy of Agricultural Science, Taiyuan, China.*

The diets of calves are predominantly milk, and the beginning of solid feed intake triggers a critical process of the activity of the rumen microbiota and ruminal fermentation. However, little in know about the rumen bacterial diversity changes in dairy bull calves induced by milk replacer and other starters such as solid pellets or hay. The effects of replacing part milk replacer with pellet diet and Chinese wildrye in the diet on growth performance and rumen bacterial composition of dairy bull calves were analyzed. Thirty-two newborn Holstein bull calves with initial weight of (42 ± 5) kg were randomly allocated to 4 dietary treatments: Group MFC were fed milk replacer, pellet diet and Chinese wildrye, group MFR were fed milk replacer and Chinese wildrye, group MCO were fed milk replacer and pellet diet, group MIL were fed milk replacer only. One week for pretrial and 7 weeks for experiment. At the end of the experiment, 3 calves from each treatment were slaughtered to determine the growth performance and slaughter traits, and rumen bacteria composition were analyzed by using 16S rRNA gene sequencing. Results showed that body weight, withers height, body length and heart girth were higher ($P < 0.05$) in MCO and MFC group compared with those in MIL and MFR group. Besides, fresh weight of liver, spleen, lung, kidney, rumen, reticulum and abomasum were also higher ($P < 0.05$) in MCO and MFC group. Sequencing results showed that both at phylum and genus level, group MIL and MFR cluster together, while group MCO and MFC cluster together. *Bacteroidetes* and *Proteobacteria* showed higher abundance in MIL and MFR groups, and *Firmicutes* showed higher abundance in MFC and MCO groups. The abundance of genera such as *Dialister*, *Acidaminococcus* and *Mitsuokella* were higher ($P < 0.05$) in the MCO and MFC group. Our results showed that the growth performance of calves could be increased by feeding milk replacer with pellet diet and Chinese wildrye, and also indicated that this may associate with changes of the rumen bacterial diversity or abundance, especially bacteria from the phylum *Firmicutes*.

Key Words: diet composition, rumen, bacteria diversity

W48 Effects of feeding Holstein calves 3% wheat protein in milk replacer.

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Following birth and colostrum feeding, many calves are fed a commercial milk replacer (MR). MR including non-milk proteins such as wheat, can be perceived as a cost-effective way of raising calves. The objective of this 78d study was to evaluate the health and performance of Holstein male calves fed one of 2 MR treatments differing in CP source. Milk proteins supplied 100% of the CP in MLK. Hydrolyzed wheat supplied 3% of the CP in WHT, with the remainder from milk proteins. Both MR contained 25% CP (as-fed) and 19% animal fat (as-fed). Calves (n = 240), were sourced from dairy farms or auction at approximately 7 d of age. Calves were randomized upon arrival based on BW, source, and serum total protein and fed individually until weaning at d 49, then co-mingled into consecutive groups of 5 until d 78. Both groups were offered: 0.52kg MR for wk 1 and 2; 0.65kg MR for wk 3; 0.90kg MR for wk 4 and 5, followed by a 2 wk weaning period of 0.45kg of MR powder at 13% solids, daily. Calf starter (21% CP, as-fed) was fed until d 28, followed by a corn and supplement ration (18.1% CP, as-fed) until d 78. Milk intake was not different ($P = 0.76$), however, grain consumption was higher in the preweaning period in the MLK ($P = 0.02$) but not in the post weaning period ($P = 0.67$). Fecal scores were recorded for the first 28 d and calves were observed for respiratory disease daily for 78 d. Body weight was recorded at arrival, on d 49, and d 78. Cox proportional hazard models were built to evaluate impact of treatment on mortality and morbidity, whereas, a mixed linear regression model evaluated the effect of treatment on ADG. No differences were observed between treatment groups with respect to diarrhea, respiratory disease, or mortality. No difference was found in gain from arrival to d 49, however, from d 49 to d 78, calves in the WHT gained 0.08 kg/d less compared with the MLK ($P = 0.004$) when using a random effect controlling for the group the calf was contained within. The outcome suggests that feeding calves milk replacer with 3% wheat protein results in reduced ADG in the post-weaning period, with no effects on diarrhea, respiratory disease, or mortality.

Key Words: milk replacer, dairy calf, protein

W49 Effect of tributyrin supplementation in milk replacer on growth performance, and plasma glucagon-like peptide-2 concentration in preweaning dairy calves.

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The objective of this study was to evaluate the effect of tributyrin (TB) supplementation in milk replacer (MR) on growth performance and plasma glucagon-like peptide-2 (GLP-2) concentration in preweaning dairy calves. Twenty Holstein heifer calves were supplemented with 0.3% palm oil (Control; n = 10) or TB (TB; n = 10) on dry matter basis. The MR supplemented with palm oil or TB, and containing 28.0% CP and 15.0% fat was offered at 578 g/d (dry matter basis) from 7 to 13 d of age, 770 g/d from 14 to 20 d of age, 1,156 g/d from 21 to 41 d of age, 770 g/d from 42 to 48 d of age, and 578 g/d from 49 to 55 d of age, then weaned on 56 d of age. All calves were fed calf starter ad libitum and chopped hay limited 200 g/d (as fed basis). Body weight were measured weekly from 7 to 56 d of age. Blood samples were collected weekly from 7 to 56 d of age and assayed for plasma GLP-2 and metabolite concentrations. A mixed model was used to determine the effects of treatment and day as repeated measures, and their interaction. Average daily gain did not differ between control (0.78 ± 0.03 kg/d; LSM ± SE) and TB (0.72 ± 0.03 kg/d). Dry matter intake (DMI) of MR and hay did not differ between the 2 treatments. However, interaction effects between treatment and day about DMI of calf starter, total DMI and intake of metabolizable energy (ME) were observed ($P < 0.05$), and these were lower for TB calves at 46, 47, from 50 to 55 d of age compared with control calves. Plasma GLP-2 concentration was higher for TB calves (0.59 ± 0.05 ng/mL) compared with control (0.41 ± 0.05 ng/mL) calves ($P < 0.05$). Blood glucose and serum β-hydroxybutyric acid concentrations did not differ between the 2 treatments. In conclusion, MR supplemented with TB increased plasma GLP-2 concentration. Despite the decreased ME intake in preweaning dairy calves fed TB, no difference in growth performance was observed which may be related to GLP-2 action on gut development and nutrient absorption.

Key Words: calf, tributyrin, glucagon-like peptide-2

W50 Use of body measurements to estimate live weight of Holstein dairy calves in the pre-weaning period. M. Hasnaoui*[1], D. Santschi[2], S. Plante[1], E. Vasseur[3], A. Bregard[1], S. Binggeli[1], and É. Charbonneau[1], [1]*Université Laval, Québec, QC, Canada,* [2]*Valacta, Ste-Anne-de-Bellevue, QC, Canada,* [3]*McGill University, Ste-Anne-de-Bellevue, QC, Canada.*

The evaluation of body weight (BW) is one of the most effective ways to assess proper growth of calves and ultimately management. Although a scale is the best way to achieve this measure, most small dairy herds still rely on heart girth (HG) measurements with a tape to estimate calves BW. The equations linking HG measurements with BW were developed using animals of several weights, but rarely pre-weaning calves. The aim of this study was to validate the use of HG measurement for dairy calves in the pre-weaning period to estimate their BW and verify if other body measurements would also be effective to predict pre-weaning BW of calves. A database was built by weighting on a scale and measuring different parameter related to body size (HG circumference, withers height, hip height, and at the hip width) of 329 Holstein dairy heifers of 2 dairy farms in Quebec, Canada. The measures were taken 3 times per week during the first 3 weeks of life and every 2 weeks until the week following weaning at 76 d of age. Preliminary analyses with Pearson correlations were performed to assess the relationship between body weight and independent variables. Simple regressions were then performed using the MIXED procedure of SAS to predict body weight with calf as a random effect. Five-fold cross-validation was used for each independent variable to evaluate the equations. The prediction equation for BW resulting in the highest r (0.99) and the lowest RMSPE (6.87) was using HG. The prediction equation with HG was BW (kg) = $119.04 - 3.3089 \times \text{HG (cm)} + 0.02959 \times \text{HG}^2$. Further analyses showed no mean bias (0.11 kg; $P = 0.51$) or linear bias (−0.002 kg, $P = 0.47$) for the proposed equation. In contrast, the most commonly used equation (Heinrichs et al., 1992; J. Dairy Sci. 75:3576–3581) exhibited a mean bias of 0.63 kg ($P < 0.001$) and a linear bias of −0.045 ($P < 0.001$). Predictions were also possible with the other parameters measured (hip width: r = 0.97, RMSPE = 9.92; withers height: r = 0.97, RMSPE = 10.82; hip height: r = 0.96, RMSPE = 11.38). The results of this study confirm the possibility to use HG or other body parameters to predict pre-weaning BW of calves.

Key Words: body weight, dairy calf, heart girth

Lactation Biology 1

W51 Differential effects of the lactogenic hormones on mechanistic target of rapamycin complex 1 (mTORC1) signaling in primary bovine mammary epithelial cells. V. Pszczolkowsky[1], L. Hernandez[1,2], and S. Arriola Apelo*[1,2], [1]*Endocrinology and Reproductive Physiology Graduate Training Program, University of Wisconsin-Madison, Madison, WI,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Lactogenic differentiation of primary bovine mammary epithelial cells (pBME) with the hormones prolactin (PRL), cortisol (COR), and insulin (INS) is an important in vitro model of lactation. Observations from undifferentiated mammary cell models, such as MAC-T, are frequently extrapolated to lactogenic conditions. The mechanistic target of rapamycin complex 1 (mTORC1) integrates hormonal and nutritional signals, including INS and essential AA (EAA), to regulate a myriad of metabolic pathways, such as translation of milk proteins. The precise role of mTORC1 in governing milk production is under investigation, but whether lactogenesis affects mTORC1 signaling in pBME has not yet been established. The objective of this study was to determine if lactogenic hormone treatment alters mTORC1 activity as determined by phosphorylation of its canonical substrate S6 kinase 1 (S6K1). Monolayer pBME were grown to 70% confluency in DMEM/F12 containing 10% fetal bovine serum (FBS). Confluent cells were FBS and EAA starved for 16 h, and treatments were applied for 4 h. Proteins were isolated from the cell lysate, and S6K1(T389) phosphorylation was determined by Western blotting. First, undifferentiated pBME were treated with PRL (100 ng/mL), COR 100 (μg/mL), INS (100 nM) or the triple hormone cocktail. Treatment effects were analyzed by ANOVA and means were separated by Tukey-HSD. Only INS, alone and in combination with PRL and COR, increased ($P < 0.05$) S6K1 phosphorylation compared with control, PRL, or COR, the latter 2 of which had no effect ($P > 0.05$). Second, we asked if INS interfere with EAA (3 mM) stimulation of mTORC1, using 2-way ANOVA. Surprisingly, INS potentiated the effect of EAA on S6K1 phosphorylation (EAA × INS, $P < 0.01$). Finally, using the triple hormone cocktail, we tested if extended lactogenic induction (16 h vs 4 h) further affected mTORC1 stimulation by EAA (3 mM) with 2-way ANOVA. Length of lactogenic treatment did not affect S6K1 phosphorylation ($P > 0.05$) or the significant stimulation by EAA (Time × EAA $P > 0.05$, EAA $P < 0.01$). Our results support the extrapolation from nonlactogenic models. More importantly, in line with our previous results in MAC-T cells, we provide evidence of synergistic effects of INS on EAA stimulation of mTORC1 signaling.

Key Words: primary bovine mammary epithelial cells (pBME), lactogenic hormone, mechanistic target of rapamycin complex 1 (mTORC1)

W52 Relationships between blood metabolites and milk fat-to-protein ratio in dairy cows during early lactation. E. H. Cabezas-Garcia*[1], A. W. Gordon[2], F. J. Mulligan[3], and C. P. Ferris[1], [1]*Agri-Food and Biosciences Institute, Hillsborough, Co. Down, UK,* [2]*Agri-Food and Biosciences Institute, Belfast, Co. Antrim, UK,* [3]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland.*

Both milk fat-to-protein ratio (FPR) and blood metabolites such as β-hydroxy butyrate (BHB), glucose, and nonesterified fatty acids (NEFA) are frequently suggested as indicators of energy status in lactating cows. This study used a meta-analysis to identify if relationships exist between blood metabolites and FPR. The data set was collected from 20 experiments (67 treatments, 817 cows, mostly Holstein Friesian). Perennial ryegrass silage was the predominant forage source in all experiments (mean forage-to-concentrate DM ratio of 47:53). Samples for milk composition analysis were collected at least once every 2 weeks. Blood samples were normally taken between 1 and 2 h before feeding (sampling occasions = 894), while the frequency of blood sampling varied between studies (normally one sample every 14 to 28 d). Data were split into 3 periods (1–4, 5–8 and 9–12 weeks post calving). Relationships between individual blood parameters and FPR were examined using a Linear Mixed Model (REML estimation method). Study and cow within study were fitted as random effects in the model. The mean FPR (±SD) was 1.28 ± 0.215 for the entire 12 weeks period. For all blood parameters evaluated, relationships with FPR were most robust within each of the 3 time periods ($R^2 \geq 0.69$) compared with the entire period (wk 1–12; $R^2 \geq 0.53$). Beta-hydroxy butyrate was positively related to FPR whereas there was a negative relationship with glucose. Conversely, NEFA models were only significant ($P = 0.05$) for the wk 1–4 post calving, showing a positive relationship with FPR. Regression slopes of the linear equations were statistically different between the periods for both BHB and glucose ($P < 0.05$). Fat-to-protein ratio responses per unit of glucose (mmol/L) were more negative at wk 1–4 weeks (−0.045 FPR) compared with the observed responses after 5 wk post calving (−0.030 FPR). The results of this study indicate that empirical models could be established between BHB and glucose, and FPR, and it may be possible to integrate these to improve our understanding of energy balance during early lactation.

Key Words: blood metabolite, fat-to-protein ratio

W53 Regulation of mammary gene expression during prolonged exposure to heat stress. R. O. Rodrigues*, E. M. Shangraw, L. K. Hirtz, P. R. F. Adkins, and T. B. McFadden, *University of Missouri, Columbia, MO.*

We investigated the effects of prolonged exposure to heat stress on mammary gene expression in lactating dairy cows. Eighteen mid-lactation Holstein cows were housed in tie-stalls in environmental chambers and subjected to one of the 3 treatments: 1) hyperthermia and ad libitum feed intake (HS), 2) normothermia and pair-feeding relative to HS (PF), or 3) normothermia and ad libitum feed intake (CT). Hyperthermia was modulated by exposing cows to 14 d of programmed constant heat stress (THI~80) to achieve and maintain elevated rectal temperature (~40°C). For normothermia, cows were exposed to constant thermoneutrality (THI~66) to maintain normal rectal temperature (~38.5°C). After the experimental period, all cows were placed on CT for 8 d to evaluate recovery. Mammary biopsies were obtained from randomly selected, alternating rear mammary glands on d 14 and 22 (last day of treatments and 8-d recovery post-treatment, respectively), then mRNA was isolated and sequenced. Genes were considered differentially expressed (DE) when $P < 0.01$ and false discovery rate ≤ 0.10. On d 14, over 75 genes were DE between HS and PF or CT, of which 23 were solely induced by HS. These genes represent upregulation of functional clusters such as superoxide metabolic process and response to reactive oxygen species, and downregulation of a cluster including extracellular space. During recovery of mammary function in HS cows, 7 DE genes were downregulated and 82 upregulated from d 14 to 22. Annotated functions of downregulated genes included superoxide formation, cell death, and others, whereas upregulated functions included clusters such as cell adhesion, collagen fibril organization, extracellular matrix organization and response to amino acid stimulus, and pathways such as protein digestion and absorption, ECM-receptor interaction, focal adhesion, PI3K-Akt signaling pathway, proteoglycans, and more. Only 16 genes were DE between PF and CT on d 14, and none on d 22. We conclude that effects of prolonged exposure to heat stress on mammary gene expression are distinct from the effects of feed restriction, in lactating dairy cows.

Key Words: feed restriction, hyperthermia, RNA-sequencing

W54 Regulation of mammary function during early exposure to heat stress in dairy cows. R. O. Rodrigues*[1], J. R. Scaliante Jr.[2], E. M. Shangraw[1], L. K. Hirtz[1], and T. B. McFadden[1], [1]*University of Missouri, Columbia, MO,* [2]*Sao Paulo State University, Araçatuba, SP, Brazil.*

We investigated the effects of early exposure to heat stress on total mammary blood flow, trans-mammary disappearance of metabolites and mammary gene expression profiles in lactating dairy cows. Six mid-lactation Holstein cows were housed in tie-stalls in environmental chambers at constant thermoneutrality (TN; THI~65) for 5 d to maintain normal rectal temperature, then exposed to 5 d of programmed constant heat stress (HS; THI~75) to achieve and maintain elevated rectal temperature. Total mammary blood flow was measured throughout the trial by transrectal scanning of both right and left external pudic arteries using color Doppler ultrasound equipped with a convex probe. Mammary arteriovenous blood samples were collected, analyzed for concentration of metabolites, and trans-mammary metabolite disappearance was calculated. Mammary biopsies were obtained from randomly selected, alternating rear mammary glands on d −1 and 5 relative to HS, then mRNA was isolated and sequenced. Genes were considered differentially expressed (DE) when $P \leq 0.002$ and FDR ≤ 0.10. Rectal temperature increased whereas feed intake and milk yield decreased ($P < 0.001$) in HS compared with TN (39.7 vs 38.5 ± 0.1°C, 15.9 vs 22.4 ± 0.4 kg/d, and 29.2 vs 36.7 ± 0.8 kg/d, respectively). HS reduced ($P = 0.04$) total mammary blood flow (543 vs 466 ± 22 L/h for TN and HS, respectively). Relative trans-mammary disappearance of glucose and triglycerides did not differ between HS and TN, averaging 20.0 ± 1.8% and 44.6 ± 3.5%, respectively. Compared with TN, 402 DE genes were downregulated in HS, which represent functions such as metabolic pathways, carbon metabolism, glycolysis and gluconeogenesis, biosynthesis of amino acids, protein processing in the endoplasmic reticulum, and phagosome, among others. In addition, 452 DE genes were upregulated in HS; annotated functions include focal adhesion, hippo signaling pathway, ECM-receptor interaction, proteoglycans, ribosome, regulation of actin cytoskeleton, and more. Early exposure to heat stress reduced total blood flow and altered gene expression in the mammary gland but did not affect relative nutrient uptake.

Key Words: feed restriction, mammary uptake, mammary blood flow

W55 Effects of extracellular branched-chain amino acid availability on the abundance of glucose transporter 1 (GLUT1) in bovine mammary epithelial cells. J. V. V. Silva*[1], S. Ganesan[1], C. A. Kaya[2], H. K. J. P. Wickramasinghe[1], and J. A. D. R. N. Appuhamy[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Dicle University, Diyarbakir, Turkey.*

Glucose transport across the plasma membrane is suggested to be a rate-limiting step in milk synthesis. Glucose transporter 1 (GLUT1) is a predominant glucose transporter in the lactating bovine mammary glands. Branched-chain amino acids (BCAA) increased GLUT1 expression and the translocation to plasma membrane in muscle and intestinal tissues in rats and pigs. An abomasal infusion of casein however decreased the clearance of glucose by the mammary glands in cows infused with starch. The objective of this study was to examine the impact of BCAA on the abundance of GLUT1 in bovine mammary epithelial cells (BMEC). Primary BMEC from 4 passages ($n = 4$) were treated with media rich in all essential amino acids (+EAA), deficient in only BCAA (−BCAA), or deficient in all essential amino acids (−EAA) for 24 h. The amino acid concentrations in –BCAA and –EAA were 10% of +EAA. All media contained bovine insulin (100 μg/L) and D-glucose (17.5 mmol/L). Cell membrane and cytosolic protein fractions were extracted and 15 μg of protein from each fraction was subjected to Western immunoblotting analyses. The abundance of total GLUT1 (GLUT1-t) was calculated by adding together the abundance of GLUT1 in cytosolic (GLUT1-c) and cell membrane (GLUT1-m) protein fractions. The GLUT1-m was expressed as a fraction of GLUT1-t to describe the intensity of translocation of GLUT1 from cytoplasm to the cell membrane. Similarly, the abundance of β-actin in both protein fractions were determined. The ratio of β-actin in the membrane fraction to total β-actin was calculated and used to explain potential cytosolic protein contaminations in the membrane protein fraction. Treatment effects were analyzed using the MIXED procedure in SAS including treatment, corresponding β-actin value as a covariate and cell passage (random effect). –BCAA or –EAA did not affect GLUT-m ($P = 0.114$), GLUT-c ($P = 0.248$), and GLUT-t ($P = 0.845$). Each of –BCAA and –EAA equally increased the fraction of GLUT1 found in the cell membrane by 2-fold ($P < 0.001$). The results highlighted that extracellular BCAA deficiency did not likely affect the expression of GLUT1 but potentially enhanced the translocation of GLUT1 from cytoplasm to the cell membrane in BMEC.

Key Words: glucose, bovine, transporter

W56 Milk fatty acid profile of 32 inbred mice strains and in silico genome-wide association analysis to locate significant SNP associated with fatty acid variability. C. I. Matamoros*[1], K. E. Robinson[1], D. L. Hadsell[2,3], and K. J. Harvatine[1], [1]*Department of Animal Science, The Pennsylvania State University, University Park, PA,* [2]*USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX,* [3]*Department of Molecular and Cellular Biology, Baylor College of Medicine, Houston, TX.*

Considerable variation in maternal ability and milk fat concentration has been reported between mouse strains (*Mus musculus*). Differences in milk fatty acid (FA) profile, including the FA synthesized de novo in the mammary gland, has not been well investigated. Our objective was to characterize the FA profile of different inbred strains and utilize genomic data to determine single nucleotide polymorphisms (SNP) that influence FA profile. For this objective, 32 mice strains from the mouse diversity panel were utilized and crossbred with CD-1 males. At 1 d postpartum litters were replaced with 10 one-day-old CD1 pups to standardize size and genetic background of litter and milk samples were collected at d 10. Milk FA were extracted with hexane:isopropanol, transmethylated with sodium methoxide, and quantified by GC. Distribution analysis was conducted in JMP Pro 13 and genome-wide association analysis was done in R (ver. 3.5.1) with EMMA package. Association analysis was conducted with 325,015 SNP that were filtered from the Broad2 and CGD-MDA1 data sets (minor allele frequency >0.05, no call rate <0.2, and removal of the Y chromosome and mitochondrial variants). Population structure effect was corrected by utilizing a kinship matrix of the strains in the association analysis. A genome-wide threshold of 1×10^{-6} was used to identify significant SNP. There was a wide distribution in the concentration of de novo [<16 C; mean = 29.92, 95% CI (30.45, 29.39), Range (15.88, 45.80)], mixed [16 C; mean = 28.51, 95% CI (28.92, 28.11), Range (19.58, 43.15)], and preformed FA [>16 C; mean = 38.53, 95% CI (38.97, 38.10), Range (54.22, 28.92)]. From 1,648 significant SNP, a strong signal ($P = 5 \times 10^{-9}$) in chromosome 2 associated with many de novo FA was identified in position 3–4 mbp. In-depth analysis shows that thioesterase II (*Olah*), an enzyme in mammary de novo lipogenesis, resides in that region. There is a wide variation in milk fat concentration between strains and differences in de novo FA are associated with specific genetic differences.

Key Words: milk fat, genome-wide association study (GWAS), *Olah*

W57 Intramammary lipopolysaccharide infusion elicits local or systemic effects depending on milk component. E. Shangraw*[1], R. Rodrigues[1], M. Witzke[1], H. Linder[1], R. Choudhary[2], A. Spitzer[2], F.-Q. Zhao[2], and T. McFadden[1], [1]*University of Missouri, Columbia, MO,* [2]*University of Vermont, Burlington, VT.*

Lipopolysaccharides (LPS) induce acute mastitis in dairy cows. A single quarter exposed to LPS will exhibit a local response, but neighboring glands may also show a systemic response. Our objective was to characterize the early responses of milk components to intramammary LPS challenge, aiming to distinguish between local and systemic effects. Ten multiparous cows, >70 DIM, were blocked based on milk yield, parity, and DIM. One cow of each block (T) received an infusion of 50 µg LPS in 10 mL of saline in both front and rear quarters of a randomly selected half-udder (TL); the contralateral quarters received 10mL saline (TS). In parallel, the other cow within block (C) received either 10 mL of saline (CS) or no infusion (CN) into respective half-udders. Infusions were given immediately after morning milking (0h). After sanitizing and stripping teats, samples of foremilk (~30 mL) were taken before twice-daily quarter-milking (−24, 0, 12, 24 h relative to infusion) from front quarters only. Additional foremilk samples were collected at 3 and 6 h. Milk composition was similar between all quarters before infusions. SCC and protein content indicated a local response to LPS: SCC was higher in TL milk compared with TS by 6 h (6.48 vs 5.25 log10 cells/mL, $P < 0.001$) and remained elevated at 24 h ($P < 0.001$); protein was transiently higher in TL compared with TS (3h; 3.1 vs 2.9%, $P < 0.05$). Systemic effects were observed for fat content and milk urea nitrogen (MUN): in both TL and TS, fat was lower than in CS and CN (3 h: 3.3, 3.7 vs 6.7, 6.5%, $P < 0.01$; 6 h: 2.9, 3.2 vs 4.7, 4.8%, $P < 0.05$) and MUN was lower (12 h: 9.3, 8.8 vs 12.9, 12.3 mg/dL, $P < 0.05$), respectively. Both local and systemic effects were observed for lactose content: at 6 and 24 h, lactose in TL was lower than in TS (6 h: 3.8 vs 4.2%, $P < 0.05$; 24 h: 2.9 vs 3.5%, $P < 0.01$), with both lower than C quarters by 12 h ($P < 0.01$). In summary, intramammary LPS rapidly altered milk composition with significant changes in T glands by 3 h. Analyzing composition of foremilk revealed distinct local or systemic effects of LPS.

Key Words: mastitis, early response, milk composition

W58 Correlations of feed efficiency measures to parameters of the Dijkstra lactation model in dairy cattle. D. J. Seymour*[1,2], A. Cánovas[2], T. C. S. Chud[2], J. P. Cant[1], V. R. Osborne[1], F. S. Schenkel[2], D. Hailemariam[3], E. E. Connor[4], J. E. Pryce[5,6], E. Wall[7], S. Wegmann[8], J. Lassen[9], and F. Miglior[2], [1]*Centre for Nutrition Modelling, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [2]*Centre for Genetic Improvement of Livestock, Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada,* [4]*Animal Genomics & Improvement Laboratory, USDA ARS, Beltsville, MD,* [5]*Agriculture Victoria, Agribio, Centre for AgriBioscience, Bundoora, VIC, Australia,* [6]*School of Applied Systems Biology, La Trobe University, Bundoora, VIC, Australia,* [7]*Animal & Veterinary Sciences, Scotland's Rural College, Edinburgh, UK,* [8]*Qualities AG, Zug, Switzerland,* [9]*Viking Genetics, Randers, Denmark.*

There is currently a renewed interest in assessing feed efficiency in dairy cattle, predominantly in the field of animal breeding. As feed efficiency is directly related to milk yield, it is possible that different aspects of an animal's lactation curve could be indicative of whether an animal is more or less feed efficient. Feed efficiency estimates can also be inflated before peak milk yield due to negative energy balance. The objective of this study was to evaluate the relationship between measures of post-peak lactation feed efficiency and parameter estimates of the Dijkstra lactation curve model. Data pertaining to calving, dry matter intake, milk yield and milk composition were obtained from a total of 1,933 cows from the Efficient Dairy Genome Project database, spanning 6 different research herds and 15 years. The Dijkstra model was fit to individual multiparous lactations up to 305 d in milk, estimating parameters for initial milk yield ($M0$), days to peak yield (Tp), peak milk yield (Mp), cell proliferation at parturition (uT), exponential decay (k), and rate of mammary cell death per day (L; lactation persistency). Feed efficiency (gross feed efficiency [GFE], average return over feed costs [ROFC], and residual feed intake [RFI]) were calculated for the period after peak milk yield for each lactation where model parameters were successfully estimated (n = 1,004). GFE and RFI were associated with $M0$ (r = −0.084; $P < 0.01$) and Tp (r = 0.068; $P = 0.04$), respectively. ROFC was correlated ($P < 0.001$) with $M0$ (r = 0.217), Mp (r = 0.615) and L (r = −0.174). All measures were associated ($P = 0.05$) with k (GFE: r = 0.061; ROFC: r = 0.113; RFI: r = −0.097). These results suggest that while improved return over feed cost is associated with improved persistency of lactation and increases in both initial milk yield and peak milk yield, other aspects of the lactation curve are generally unrelated to feed efficiency after peak production in multiparous cattle. Further investigation with a more robust measure of feed efficiency is warranted.

Key Words: dairy cattle, feed efficiency, lactation curve

W59 Bovine milk proteome: Pooling samples within day does not account for diurnal mammary fluctuations. M. Honan*, M. Fahey, and S. Greenwood, *The University of Vermont, Burlington, VT.*

Proteomic studies of bovine milk frequently analyze daily milkings as one pooled sample thus not accounting for possible diurnal fluctuations that may be occurring in the mammary gland. Daily variation in the high abundance proteins (caseins, lactalbumin and lactoglobulin) has been reported, yet little investigation of the impact of diurnal rhythm on the rest of the milk proteins (the low abundance proteins; LAP) has been done. This study investigates diurnal variation of the skim milk LAP profile. The 5-d trial included 3 d used for collection. Milk samples were collected at both AM and PM milkings (0400 and 1500 h) from mid to late lactation multiparous Holsteins (n = 3; DIM = 219.3 ± 77.7). Cows were all housed the same facility, managed under the same protocol, and ad libitum fed the same TMR. Milk samples were flash frozen and stored at −80°C until analysis. Samples were pooled according to time of collection across the week within cow before the addition of a protease inhibitor cocktail and skimmed by repeated centrifugation (4,000 × *g*). The LAP were isolated through $CaCl_2$ precipitation of the high abundance proteins followed by ultracentrifugation (189,000 × *g* for 60 min). The supernatant was lyophilized and reconstituted in PBS and samples were labeled using Tandem Mass Tag (TMT). Protein abundances were determined using LC-MS/MS and PROC MIXED of SAS was used to compare protein abundances in AM versus PM milk samples. A total of 262 proteins were identified using SEQUEST and Mascot within Proteome Discoverer 2.2. Of those proteins, 3 differed in abundance between AM and PM milk samples. Alpha-1-antiproteinase, isocitrate dehydrogenase [NADP], and keratin: type II cytoskeletal 59 kDa, component IV all decreased in abundance from AM to PM milking. The affected proteins are diverse and not comprised in a single gene ontological classification. Results from this trial offers insight into mammary gland changes that may not be accounted for having a single pooled sample to represent a 24-h period that possibly should be considered for future biomarker research. However, these results also suggest that the low abundance skim milk proteome is not significantly affected by time of day.

Key Words: liquid chromatography-tandem MS (LC-MS/MS)

W60 Stearic acid supplementation (C18:0) does not overcome the CLA *trans*-10,*cis*-12-induced milk fat depression in early lactating dairy ewes. G. C. Aguiar, R. Horstmann, C. G. Padilha, D. T. C. Bessani, and D. E. Oliveira*, *Santa Catarina State University, Lages, Santa Catarina, Brazil.*

Conjugated linoleic acid (CLA) *trans*-10,*cis*-12 is able to cause milk fat depression (MFD) in lactating cows, goats and ewes. The objective of this study was evaluate if stearic acid (C18:0) is able to overcome the MFD caused by CLA *trans*-10,*cis*-12. Twenty-eight Lacaune sheep (36 ± 3 DIM; 73 ± 8.1 kg BW) were used in a completely randomized design (n = 7/treatment) for 21 d (7 d of adaptation and 14 d of data collection). The animals were submitted to 4 treatments: a) Control (no CLA or C18:0); b) CLA (27 g of CLA, 29.9% of *trans*-10,*cis*-12 orally fed); c) C18:0 (32 g of C18:0, 87%, mixed to the concentrate) and; d) CLAC18:0. Milk yield was measured and milk samples, individually, were obtained each 2 d. The data were analyzed using the MIXED procedure of the SAS, using treatments as fixed effect and the animal as random. Compared with Control, CLA and CLAC18:0 reduced milk fat content by 12 ($P = 0.0003$) and 14% ($P = 0.0001$), respectively, with no difference between CLA and CLAC18:0 ($P = 0.50$). The lactose content was increased in CLA compared with C18:0 and CLAC18:0, respectively, in 2 ($P = 0.05$) and 7% ($P = 0.0001$), and reduced in 6% for CLAC18:0 compared with Control ($P = 0.0001$). The CLA and CLAC18:0, in comparison to Control decreased the content of total solids in of 4 ($P = 0.011$) and 7% ($P = 0.0001$). There was no difference on milk and protein yield. Overall, C18:0 combined with CLA *trans*-10,*cis*-12 was not able to inhibit CLA-induced MFD.

Key Words: fatty acid, lipid supplementation, milk composition

W61 Stearic acid (C18:0) does not overcome the downregulating gene expression effect of conjugated linoleic acid (CLA) *trans*-10,*cis*-12 on lipogenic genes in early lactating dairy ewes. G. C. Aguiar, R. Horstmann, C. G. Padilha, and D. E. Oliveira*, *Santa Catarina State University, Lages, Santa Catarina, Brazil.*

Stearic acid (C18:0) has shown be able to increase milk fat in ruminants but the results are variable. This study evaluated the effect of feeding stearic acid (C18:0) and its interaction with CLA *trans*-10,*cis*-12 on the transcription of genes involved in milk fat synthesis in ewes. Twenty-eight Lacaune ewes (36 ± 2 DIM; 73 ± 9 kg BW) were randomly assigned to one of the following treatments (7/treatment) for 21 d (7 d for adaptation and 14 d of data collection): 1) Control (no CLA or C18:0); 2) C18:0 (32 g/d, 87%); 3) CLA (27 g/d orally dosed containing 29.9% *trans*-10,*cis*-12 CLA,); and 4) CLAC18:0. Mammary biopsies were taken at d 14 of data collection, RNA was extracted, cDNA synthesized and RT-qPCR analysis conducted for ACACAα PII, FASN, SCD, LPL, CD36, FABP (3 and 4). The data were analyzed using the MIXED procedure of SAS using treatment as fixed effect and animal as random. The geometric mean of the housekeeping genes (S18 and actin-β) were used to normalize the gene expression. Compared with Control, C18:0, CLA and CLAC18:0 reduced the expression of ACACAα PII in 1.8-, 2.3- and 1.4-fold ($P = 0.01$ for all), respectively, and CLA reduced expression by 1.6-fold ($P = 0.01$) compared with CLAC18:0. Compared with Control, CLA reduced FASN and LPL by 1.7-fold ($P = 0.02$) and CLAC18:0 reduced LPL expression by 1.3-fold ($P = 0.05$). Expression of CD36 in C18:0 was increased, compared with Control and CLAC18 in 1.8-fold ($P = 0.05$) and 2.4-fold ($P = 0.01$), respectively. SCD expression was, respectively, decreased in 1.6-, 1.6- and 1.6-fold ($P = 0.01$ for all) in C18:0, CLA and CLAC18:0 when compared with Control. The FABP4 was 2.1-fold ($P = 0.01$) higher in C18:0 compared with CLAC18:0. Overall, C18:0 was not able to overcome the downregulating effect of CLA *trans*-10,*cis*-12 on mammary lipogenic genes when combined with C18:0

Key Words: lipid supplementation, mammary gland, nutrigenomics

W62 The effects of incomplete milking and increased milking frequency on milk production rate and milk composition. J. Kuehnl*[1], M. Connelly[1], A. Dzidic[2], M. Lauber[1], M. Klister[1], E. Olstad[1], M. Balbach[1], E. Timlin[1], V. Pszczolkowski[1], P. Crump[1], D. Reinemann[1], and L. Hernandez[1], [1]*University of Wisconsin-Madison, Madison, WI,* [2]*University of Zagreb, Croatia, Zagreb, Croatia.*

Incomplete milking (IM) and increased milking frequency (IMF) exert different effects on milk yield and mammary physiology. The objectives of this experiment were to determine the effects of IM and IMF on milk production rate (MPR) and milk composition and whether milking 3 times daily (3×) could rescue the negative production effects of IM. Twenty-two multiparous cows (mean ± SEM; 2.73 ± 0.18 parity) were enrolled from 5 to 47 DIM utilizing a 2 × 2 factorial design. Eleven cows were randomly assigned to be milked 2 times (2×) daily and 11 cows were randomly assigned to be milked 3×. Within each cow, a half-udder was randomly assigned to IM (30% milk remaining in the gland, MRG), while the other half-udder was completely milked (CM). Half-udder IM was achieved via weekly calibrations of target milk yields for each quarter. Control quarters were CM until milk flow had ceased visually. Quarter-level milk yields were recorded at each milking. Quarter-level milk samples were collected twice weekly at the beginning of the morning milking to determine milk fat, protein, lactose, and SCC. Data were analyzed using the MIXED procedure of SAS. Cows milked 2× and 3× produced 1.81 ± 0.06 and 1.97 ± 0.06 kg milk/h, respectively ($P = 0.06$). Half-udders that were CM and IM produced 1.09 ± 0.03 and 0.80 ± 0.03 kg milk/h, respectively. An interaction was detected between IM and week of lactation for MPR ($P = 0.04$). Half-udders that were IM had increased milk fat percent (2.15 ± 0.07% vs. 1.43 ± 0.07%; $P < 0.0001$), decreased lactose percent (4.75 ± 0.03% vs. 4.99 ± 0.03%; $P < 0.0001$), increased milk log10SCC (4.22 ± 0.05 vs. 4.41 ± 0.05; $P = 0.0004$), and no difference in milk protein percent compared with CM half-udders. Cows milked 3X had increased milk fat percent (1.93 ± 0.09% vs. 1.65 ± 0.09%; $P = 0.047$), decreased milk lactose percent (4.80 ± 0.04% vs. 4.93 ± 0.04%; $P = 0.04$), and no difference in milk protein percent or milk SCC compared with cows milked 2×. No interaction was detected between IM and IMF for MPR or milk components. Our results indicate that 30% MRG had an irreversible impact on milk yield as IMF was not able to reverse the milk yield loss that occurs with the IM treatment.

Key Words: incomplete, frequency, production

W63 Associations between body condition score at parturition and microRNA profile in colostrum of dairy cows.

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MicroRNA (miRNA) are abundant in milk, and various miRNA species regulate functions including lactation and immunity. The study objective was to determine the miRNA profile in colostrum of over-conditioned cows compared with cows of more moderate BCS at calving. Multiparous cows with either high (≥4.0; n = 7) or moderate BCS (2.75–3.50; n = 9) in the week before parturition were selected from a commercial dairy herd. Blood was sampled within 24 h after calving and analyzed for serum free fatty acids (FFA). Colostrum was collected within 20 h of calving; miRNA was isolated from colostrum samples after removing milk fat and cells. MicroRNA were sequenced and reads were mapped to the bovine genome and to the existing database of miRNA at miRBase.org. Two programs, Oasis 2.0 and miRDeep2, were employed in parallel for mapping reads to the bovine genome. Analysis of miRNA count data was performed using DEseq2. After removing miRNA with less than 10 counts across all samples, 343 and 415 known miRNA mapping to miRBase were detected from Oasis and miRDeep2, respectively. Identification of differentially expressed miRNA from DEseq2 was not affected by the differences in number of miRNA detected by the 2 mapping programs. Most abundant miRNA included miR-30a, miR-148a, miR-181a, let-7f, miR-26a, miR-21, miR-22, and miR-92a. Large-scale shifts in miRNA profile were not observed; however, colostrum of cows with high BCS contained fewer miR-486 (fold change −2.15; false discovery rate $P = 0.06$), which has been linked with altered glucose metabolism. Colostrum from cows with elevated FFA (greater than 700 μ*M*) contained fewer miR-10b (fold change −2.48; false discovery rate $P < 0.01$), which may have regulatory effects on inflammatory signaling and lipid metabolism. These miRNA may provide a link between periparturient cow adiposity and mammary nutrient metabolism. Potential functions of abundant miRNA suggest involvement in development and maintenance of cellular function in the mammary gland, with the additional possibility of influencing neonatal tissue and immune system development.

Key Words: RNA silencing, transition cow, adipose

W64 Effects of extracellular branched-chain amino acid availability on the abundance of proteins regulating fat synthesis in bovine mammary cells: A proteomics analysis.

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Regulation of milk fat synthesis relies on the abundance of enzymes involved in de novo synthesis of fatty acids, lipid synthesis, and fat droplet formation. Branched-chain amino acids (BCAA) are essential amino acids (EAA), shown to modulate lipogenesis in liver and adipose tissues. A little is known about a link between BCAA and milk fat synthesis. Objective of this study is to examine the impact of extracellular BCAA availability on the abundance of proteins involved in milk fat synthesis in bovine mammary epithelial cells (BMEC). Primary BMEC from 4 passages were cultured in medium rich in all EAA (+EAA), medium deficient in only BCAA (−BCAA), and medium deficient in all EAA (−EAA) for 24 h on 4 separate days (n = 4). Protein (50μg) from each replicate was subjected to a LC-MS-based proteomics analysis. A comparison of primary peptide sequences with the Mascot software identified 3500 proteins. Those proteins were ranked based on an index developed by combining 3 parameters (coverage, protein score, and PSM score). The top 1000 proteins were chosen for further analyses. The abundance of each protein was normalized against the abundance of cytosolic actin, which was the highest abundant protein in the data. The treatment effects (+EAA vs. –BCAA and +EAA vs. −EAA) on the abundance of normalized proteins were analyzed using MetaboAnalyst 3.0 software. −BCAA altered the abundance of 21 proteins, 19 of which were downregulated ($P < 0.05$). Those downregulated proteins included 9 enzymes involved in pathways related to fatty acid synthesis, lipogenesis, and milk fat droplet formation (Acetyl-CoA acetyltransferase 2, Acyl-CoA synthetase-short chain family member 2, Fatty acid synthase, Fatty acid-binding protein, ATP-citrate synthase, Diphosphomevalonate decarboxylase, Isopentenyl-diphosphate Delta-isomerase 1, Farnesyl pyrophosphate synthase, and HMG-CoA synthase). The abundance of those proteins were not significantly different from the abundance in –EAA. There was a strong link between extracellular BCAA availability and the abundance of proteins involved in formation of milk fat, independent of the availability of other EAA.

Key Words: milk fat synthesis, regulation, enzyme

W65 Conjugated linoleic acid (CLA) alters the milk fat composition in lactating sows.

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Dietary conjugated linoleic acid (CLA) is known to change lipid metabolism in the mammary gland of different animal species and, there has recently been an interest regarding its effect on milk fat synthesis modulation in sows. The objective of this study was to evaluate the effect of CLA on the fatty acid profile of sow milk. Twenty multiparous sows from a commercial line, with a mean body weight (BW) of 200 ± 10 kg were randomly assigned to 1 of the 2 treatments (n = 10/treatment) for 18 d: 1) Control, without CLA; and 2) 1% of CLA (29.9% of *trans*-10,*cis*-12 and 29.8% of *cis*-9,*trans*-11) mixed in the ration. The diets were formulated to meet the nutritional requirements. The animals were kept in a controlled environment (temperature, humidity, and ventilation) and the CLA treatment was fed from d 7 through d 25 of lactation. Milk samples were collected from all sows at d 25 to evaluate milk fatty acid profile by gas chromatography. Data were analyzed as a complete randomized design using the Mixed Procedure of SAS. Dietary CLA increased the C14:0 ($P = 0.003$), C18:0 ($P < 0.0001$), C18:1 *trans* ($P = 0.0004$), C20:0 ($P < 0.0001$), C20:4n-6 ($P = 0.03$), *cis*-9,*trans*-11 CLA ($P = 0.0004$), and saturated fatty acids (SFA) proportions ($P < 0.0001$) and reduced the C14:1 ($P = 0.0002$), C16:1 ($P = 0.0001$), C18:1 n-9 ($P = 0.03$), C18:2n-6 ($P = 0.03$), C18:3n-3 ($P = 0.03$), and monounsaturated fatty acids (MUFA) ($P < 0.0001$) proportions. The *trans*-10,*cis*-12 isomer was not detectable in the Control treatment, but in the CLA treatment the mean concentration was 0.58%. No alteration in total polyunsaturated fatty acids concentration was observed with the CLA treatment. The distinct shift toward higher deposition of SFA and lower deposition of MUFA indicated a potential downregulation of desaturation by dietary CLA.

Key Words: fatty acids profile, mammary gland, milk fat synthesis

W66 ***Trans*-10,*cis*-12 conjugated linoleic acid (CLA) reduces gene expression of *ACACA*, *FASN*, *SCD*, *LPL*, *AGPAT6*, *DGAT1*, *CSN2*, and *LALBA* in the mammary gland of sows.** E. C. Sandri[1], K. J. Harvatine[2], and D. E. Oliveira*[1], [1]*Santa Catarina State University, Lages, Santa Catarina, Brazil,* [2]*Penn State University, State College, PA.*

The conjugated linoleic acid (CLA) *trans*-10,*cis*-12 inhibits fat synthesis in the mammary gland of bovines, ewes, and goats, at least in part, by regulating the expression of genes involved in lipid metabolism. However, in sows, studies about CLA regulation of lipogenic gene expression are scarce. The objective of this study was to evaluate the effect of *trans*-10,*cis*-12 CLA on mammary and adipose lipogenic gene expression of sows. Twenty multiparous commercial line sows with a mean body weight (BW) of 200 ± 10 kg were randomly assigned to one of the 2 treatments (n = 10/treatment) for 18 d: 1) Control (no CLA added) and 2) CLA at 1% of diet DM (29.9% of *trans*-10,*cis*-12 and 29.8% of *cis*-9,*trans*-11) mixed in the ration. The diet was formulated to meet the nutritional requirements, the sows were kept in a controlled environment (temperature, humidity and ventilation) and the CLA treatment was administered from d 7 through d 25 of lactation. Mammary and adipose tissue biopsies were taken on d 25 of each experimental period for subsequent RNA extraction and analysis of gene expression by SYBR Green Real-Time RT-PCR. Data were analyzed as a complete randomized design using the Mixed Procedure of SAS. CLA reduced mammary expression of all lipid enzymes evaluated (ACACAα: −37%, $P = 0.003$; FASN: −64%, $P = 0.002$; SCD1: −52%, $P = 0.003$; LPL: −26%, $P = 0.03$; AGPAT6: −15%, $P = 0.02$; and DGAT1: −27%, $P = 0.02$), except FABP3 ($P = 0.09$). Mammary expression of milk proteins, β-casein and α-lactalbumin, were reduced by the CLA treatment ($P = 0.0004$ and $P = 0.005$, respectively), compared with Control. CLA treatment had no effect on expression of the same lipogenic genes in adipose tissue. As in ruminants, in sows mammary expression of genes involved in the evaluated lipogenic pathways were reduced by CLA, and a greater magnitude was observed in genes from the de novo pathway synthesis

Key Words: lipogenesis, mammary metabolism, milk fat depression

W67 **Leucine stimulates mammalian target of rapamycin (mTOR) signaling pathway to regulate κ-casein synthesis via activating LAT1 and LARS in bovine mammary epithelial cells.** H. R. Wang*, H. Zhang, F. F. Zhao, Y. Q. Jin, H. Sun, X. Z. Zhang, and Y. T. Fan, *Yangzhou University, Yangzhou, China.*

Leucine, as a signal regulator, could regulate milk casein synthesis by mammalian target of rapamycin (mTOR) signaling pathway. It is transported into cell via L system amino acid transporter 1 (LAT1) accompanying the output of glutamine. However, the upstream signals in the mTOR activation by leucine are still unclear in bovine mammary epithelial cells (BMECs). This study investigated whether LAT1 and leucyl-tRNA synthetase (LARS)-induced mTOR signaling could regulate κ-casein synthesis in BMECs. The BMECs were established by enzymatic digestion of mammary tissue from mid-lactation cows. According to the cell viability, the mRNA expression of LAT1 and LARS in medium lacking leucine and glutamine, 12 h post-starvation was considered the optimal starvation time to determine the cellular signal of leucine influence on κ-casein synthesis with a deficiency of leucine or glutamine. Passage 2 mammary epithelial cells were divided into 4 groups, including the entire AA profile (CTL), leucine deficiency (CTL−Leu, −Leu), glutamine deficiency (CTL−Gln, −Gln), and deficiency of both leucine and glutamine (CTL−Leu−Gln, −Leu−Gln) treatments. The LAT1, ASC system amino acid transporter 2 (ASCT2), LARS, mTOR, ribosomal protein S6 kinase 1 (S6K1) proteins, and the κ-casein were determined. All data were analyzed using one-way ANOVA by SPSS 16.0 (SPSS Inc., Chicago, IL). The results showed that compared with the CTL treatment, LAT1 and LARS proteins were significantly decreased in −Leu and –Leu−Gln treatments ($P < 0.05$). The phosphorylation mTOR was significantly decreased by the −Gln and –Leu−Gln treatments ($P < 0.05$), and there was a similar trend ($P = 0.057$) in the −Leu treatment. The phosphorylation of S6K1 was significantly decreased in −Leu, −Gln and –Leu−Gln compared with CTL and later reduced the κ-casein protein expression ($P < 0.05$). These results suggested that κ-casein synthesis was affected by leucine stimulated mTOR signaling pathway via activating LAT1 and LARS in BMECs.

Key Words: bovine mammary epithelial cell, leucine, κ-casein

Physiology and Endocrinology 2

W68 Nerve growth factor-β effects on steroidogenesis and angiogenic markers in the bovine pre-ovulatory follicle. J. L. Stewart[1,2], L. Gao[3], J. A. Flaws[3], I. F. Canisso[1,3], and F. S. Lima*[1,3], [1]*Department of Veterinary Medicine, University of Illinois, Urbana, IL,* [2]*Department of Large Animal Clinical Science, Virginia Polytechnic Institute and State University, Blacksburg, VA,* [3]*Department of Comparative Biosciences, University of Illinois, Urbana, IL.*

Nerve growth factor-β (NGF) is a seminal plasma protein that induces ovulation and has a luteotrophic effect in camelids, but little is known regarding the interactions of seminal plasma-derived NGF on the pre-ovulatory follicle in the bovine species. Our objectives were to assess the effects of purified bovine NGF on steroidogenesis and angiogenic markers in the bovine pre-ovulatory follicle. Two Holstein heifers were synchronized, and ovariectomy was performed via colpotomy when a pre-ovulatory follicle >12 mm was present. The pre-ovulatory follicle was excised from the ovary, fluid was aspirated, and the remaining tissue was dissected. The theca interna with adherent granulosa cells was peeled from the theca externa and surrounding stromal tissue and cut into small pieces (average weight: 5.3 ± 0.7 mg). The follicle tissue pieces ($n = 24$) were incubated in 0.5 mL of Eagle's MEM culture medium supplemented with 1% L-glutamine, 1% nonessential amino acids, 1% penicillin-streptomycin, 1% insulin-transferrin-selenium, 10% fetal bovine serum, 40 ng/mL cortisol, 4 ng/mL LH, and 4 ng/mL FSH. Culture wells were either supplemented with 100 ng/mL NGF ($n = 12$) or left as an untreated control ($n = 12$). Medium was withdrawn and replaced with fresh medium at 3, 6, 12, 24, 48, and 72 h of culture and frozen at −80°C for hormone analysis and follicle tissue pieces were frozen to measure steroidogenic and angiogenic gene expression using qPCR. A general linear mixed model with repeated measures for hormone data and Kruskal-Wallis rank sum test for non-parametric data were performed. Treatment with NGF increased testosterone and ($P < 0.01$) and steroidogenic enzyme 17β-hydroxysteroid dehydrogenase ($P = 0.04$) in the follicle wall extracts. There was no effect of NGF on progesterone and estradiol ($P \geq 0.14$) or other steroidogenic enzymes ($P \geq 0.31$). Treatment with NGF reduced ($P = 0.02$) fibroblast growth factor 2 (*Fgf-2*) but did not alter other angiogenic factors ($P \geq 0.44$). In conclusion, NGF affected steroidogenesis increasing testosterone production and reduced *Fgf-2*, a marker of cell remodeling.

Key Words: nerve growth factor-β, follicle, bovine

W69 Nerve growth factor-β increases small luteal cell number and steroidogenic activity in the bovine corpus luteum. J. L. Stewart[1,2], V. R. G. Mercadante[3], N. W. Dias[3], S. Stella[1], L. Cunha*[1], I. F. Canisso[1], and F. S. Lima[1], [1]*Department of Veterinary Medicine, University of Illinois, Urbana, IL,* [2]*Department of Large Animal Clinical Science, Virginia Polytechnic Institute and State University, Blacksburg, VA,* [3]*Department of Animal Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Nerve growth factor-β (NGF) is a seminal plasma protein that stimulates bovine theca cell proliferation and steroidogenesis in vitro and is luteotrophic in vivo, yet there is little is known on how it alters ovulation and corpus luteum formation in cattle. Our objectives were to assess the effects of systemic purified bovine NGF on vascularity and steroidogenesis of the pre-ovulatory follicle and subsequent corpus luteum (CL) in cattle. Holstein heifers ($n = 12$) were synchronized using a 5-d CIDR-Synch and randomly allocated to 1 of 2 treatment groups: (1) CONT, 12 mL of PBS; or (2) NGF, 250 µg purified NGF in 12 mL of PBS administered intramuscularly at time 0 (presence of pre-ovulatory follicle). A second replicate was performed using a crossover ~1 mo after the first replicate. Transrectal ultrasonography with Doppler and blood sampling were performed every 4 h from 0 to 32 h to evaluate follicle size and vascularity, ovulation time, and estradiol concentrations. Ultrasonography was then performed daily to assess CL size and vascularity, and blood was obtained every 2 d to measure progesterone concentrations. On d 9 and 14, a CL biopsy was performed to assess mRNA expression of steroidogenic enzymes and LH receptor and the histological ratio of small to large luteal cells. Statistical analyses were performed using a general linear mixed model with repeated measures using R Version 3.4.3. Treatment with increased follicle diameter ($P = 0.02$) but did not alter estradiol ($P = 0.95$) or ovulation time ($P = 0.42$). Treatment with NGF tended to increase CL diameter ($P = 0.10$) and increased progesterone concentrations ($P = 0.04$). There was a higher percentage of small luteal cells ($P < 0.01$) and a tendency for increased LH receptor gene expression ($P = 0.09$) in NGF-treated heifers. Steroidogenic acute regulatory protein and 3β-hydroxysteroid dehydrogenase were increased ($P \leq 0.05$) in NGF-treated heifers. Treatment with NGF did not alter the vascularity of the follicle or CL ($P \geq 0.16$). Purified NGF interacted with the pre-ovulatory follicle and altered downstream CL formation and function.

Key Words: bovine, luteotrophic, nerve growth factor-β

W70 Pegbovigrastim treatment alters gene expression profiles of leukocytes in Simmental and Holstein cows after calving. V. Lopreiato[1], A. Minuti[2,3], D. Britti[1], F. Trimboli[1], F. Piccioli Cappelli[2], J. J. Loor[4], and E. Trevisi*[2,3], [1]*Interdepartmental Services Centre of Veterinary for Human and Animal Health, Department of Health Science, Magna Græcia University, Catanzaro, Italy,* [2]*Department of Animal Sciences, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy,* [3]*Proteomics and Nutrigenomics Research Center (PRONUTRIGEN), Università Cattolica del Sacro Cuore, Piacenza, Italy,* [4]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL.*

Pegbovigrastim is a commercial long-acting analog of bovine granulocyte colony-stimulating factor. We hypothesized that pegbovigrastim (Imrestor; Elanco) administered at approximately 7 d before expected calving and within 24 h after calving affects expression profiles of genes involved in leukocyte function. Cows were randomly allocated into 1 of 2 groups: control [CTR; 13 Holstein (7 primiparous and 6 multiparous) and 13 Simmental (6 primiparous and 7 multiparous)] and pegbovigrastim [PEG; 13 Holstein (6 primiparous and 7 multiparous) and 13 Simmental (6 primiparous and 7 multiparous)]. Blood was collected on d 3 after calving in PAXgene tubes (Preanalytix) to measure mRNA expression of 36 genes. The final data were normalized using the geometric mean of 3 internal control genes: *ACTB*, *SDHA*, and *YWHAZ*. Data were subjected to ANOVA and analyzed with PROC MIXED of SAS. Treatment, breed, parity, and their interaction were the fixed effects. Compared with CTR, PEG cows had greater expression ($P < 0.01$) of genes involved in recognition and immune modulation (*CD14*, *CD16*, *MYD88*, *TLR2*, and *TLR4*), cell adhesion (*ITGB2*, *ITGAL*, *TLN1*, *SELL*, *SELPLG*, and *CD44*), antimicrobial activity (*MMP9*, *LTF*, *LYZ*, and *LCN2*), and inflammation (*CASP1*, *TNFRSF1A*, *IL1B*, *IL1R*, *IL18*, *IRAK1*, *NRLP3*, and *S100A8*). Pegbovigrastim also led to lower expression of *RPL13A*, *ALOX15*, *IL8*, *TLN2*, and *TNF* ($P < 0.05$). Expression of *IDO1*, *RPL13A*, *ALOX5*, *CD44*, *CX3CR1*, *ITGB2*, and *TNF* was greater in Simmental compared with Holstein ($P \le 0.05$), whereas expression of *TLR2*was lower ($P < 0.05$). Compared with multiparous cows, primiparous had greater expression of *IL1B*, *IL18*, *MYD88*, *SELL*, and *TLR2* ($P < 0.05$), and lower expression of *MMP9* ($P < 0.05$). Overall, pegbovigrastim have elicited an improvement of migration, adhesion, and antimicrobial ability, and an enhanced inflammatory response at 3 d after calving, that in turn might be beneficial for mounting a better response after the calving event and the homeorhetic adaptation to lactation. These preliminary results also unravel a different molecular response at innate and adaptive immunity level from different cattle breeds under the dairy management.

Key Words: gene expression, pegbovigrastim, leukocyte

W71 A lateral flow-based portable platform for quantification of circulating concentrations of progesterone. M. Masello*[1], E. M. Schillkowsky[1], Z. Lu[2], D. Erickson[2], J. Gavalchin[1], and J. O. Giordano[1], [1]*Department of Animal Science, Cornell University, Ithaca NY,* [2]*Sibley School of Mechanical and Aerospace Engineering, Cornell University, Ithaca NY.*

Our objective was to develop and validate a disposable fluorescence-based test strip coupled with a portable imaging device for quantification of plasma progesterone (P4). First, we developed and optimized a competitive lateral flow immunoassay (LFIA) test strip to measure P4 in bovine plasma. The LFIA design included a sample pad, a conjugate pad that dry-stores Rphycoerythriin-anti-P4 conjugates, a glass-fiber spacer pad, a nitrocellulose membrane with printed test and control lines, and a cellulose-fiber absorbent pad. To perform a test, 20 µL of plasma and 50 µL of running buffer (RB) were added on the sample pad, and the test strip was left in a light-free environment. After 3 min, 45 µL of RB were added to initiate sample flow. After allowing 15 min to stabilize the colorimetric signal, strips were inserted into an LFIA reader to determine P4 concentration based on test-to-control-line signal (T/C ratio). The reader was linked to a laptop to interpret and display results. In a series of experiments (n = 6), the ability of the LFIA to predict the presence of a functional (P4 ≥ 1 ng/mL) corpus luteum (CL) in bovine plasma samples was evaluated. For each experiment, a calibration curve was constructed using plasma with known concentrations of P4 (0.1–3.7 ng/mL; n = 5). There was a linear relationship between average T/C ratio and P4 levels (average $R^2 = 0.82$; range 0.44–0.99). The T/C ratio decreased as P4 concentrations increased. Next, plasma samples from lactating dairy cattle (n = 58) were tested in triplicate to predict the presence or absence of a functional CL using a radioimmunoassay for P4 as reference test. Overall, the LFIA assay correctly classified 90% ($P < 0.01$; 95%CI 79–96) of the samples, with 93% sensitivity ($P < 0.01$; 95%CI 77–99), 86% specificity ($P < 0.01$; 95%CI 68–96), 87% positive predictive value ($P < 0.01$; 95%CI 70–96) and 93% negative predictive value ($P < 0.01$; 95%CI 76 to 99). The agreement between the LFIA and the reference test was substantial (kappa = 0.79; 95%CI 0.64–0.95; $P < 0.01$).The intra-assay CV averaged 16.0% (range 0.5–42.8%). These data suggest that the current LFIA system can accurately predict the presence of a CL based on circulating concentrations of P4. Supported by USDA-NIFA Project 2016–08814.

Key Words: lateral flow immunoassay, progesterone, corpus luteum

W72 Increasing supply of Met and Arg relative to Lys while maintaining Thr:Phe, Lys:Thr, Lys:His, and Lys:Val ratios alters casein protein abundance via amino acid-induced upstream mechanistic target of rapamycin signaling pathway and circadian clock in bovine mammary epithelial cells. L. Y. Hu[1,2], Y. F. Chen[1], I. M. Cortes[2,3], D. N. Coleman*[2], H. Y. Dai[2,4], C. Parys[5], M. Z. Wang[1], and J. J. Loor[2], [1]*College of Animal Science and Technology, Yangzhou University, Yangzhou, Jiangsu, China,* [2]*Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL,* [3]*Agricultural and Animal Production Department, UAM-Xochimilco, Mexico City, Mexico,* [4]*College of Veterinary Medicine, Nanjing Agricultural University, Nanjing, Jiangsu, China,* [5]*Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.*

Methionine (Met) and arginine (Arg) regulate casein synthesis partly via alterations in activity of the mechanistic target of rapamycin (mTOR) pathway. Whether upstream regulators and cellular energy status contribute to those responses is unknown. Recent data highlighted a putative role of the circadian clock in milk protein synthesis partly via mTOR. The objective was to determine if greater supply of Met and Arg alone or in combination altered mRNA, protein abundance and phosphorylation status of mTOR-related targets. Primary bovine mammary epithelial cells (BMEC) were incubated (n = 5 replicates/treatment) for 12 h with control medium (IPAA; Lys:Met 2.9:1, Lys:Arg 2:1) or medium supplemented with Met (LM2.5; Lys:Met 2.5:1, Lys:Arg 2:1), Arg (LA1; Lys:Met 2.9:1, Lys:Arg 1:1), or LM2.5+LA1 (Lys:Met 2.5:1, Lys:Arg 1:1). Ratios of Thr:Phe (1.05:1), Lys:Thr (1.8:1), Lys:His (2.38:1), and Lys:Val (1.23:1) were the same across treatments. Target genes were measured by RT-PCR and proteins by Western blotting. Data were analyzed as a 2 × 2 factorial arrangement of treatments with the MIXED procedure ofSAS 9.4.Phosphorylation ratio of p-TSC2:total (t) TSC2 (negative regulator of mTOR) was greater with LA1 ($P = 0.001$), while p-AKT:tAKT and p-AMPK:tAMPK was lower ($P = 0.05$, $P < 0.01$). In spite of this, ratio of p-mTOR:tmTOR nearly doubled with LA1 and LM2.5 (interaction $P < 0.05$), but such response did not prevent a decrease in casein protein abundance (CSN1S1, interaction $P = 0.03$). Abundance of PER1 protein nearly doubled with Met or Arg alone or in combination (interaction $P < 0.01$), but only LM2.5+LA1 was able to double abundance of CLOCK protein ($P < 0.01$). Overall, data suggest that greater supply of Met or Arg relative to Lys while holding the ratios of other essential amino acids to Lys constant could influence casein synthesis. The underlying mechanisms seem to encompass mTOR, CLOCK, and intracellular energy metabolism.

Key Words: Met and Arg availability, mechanistic target of rapamycin (mTOR) signaling pathway, circadian clock

W73 Novel phospho-proteomic analysis of abdominal and subcutaneous adipose tissues from dairy cows supplemented with conjugated linoleic acid during the transition period. M. Zachut*[1], G. Kra[1], Y. Levin[2], A. Tröscher[3], L. Vogel[4], M. Gnott[4], and H. Hammon[4], [1]*Agriculture Research Organization, Volcani Center, Rishon LeZion, Israel,* [2]*The Nancy and Stephen Grand Israel National Center for Personalized Medicine, Weizmann Institute of Science, Rehovot, Israel,* [3]*BASF SE, Lampertheim, Germany,* [4]*FBN, Dummerstorf, Germany.*

Phospho-proteomics is a new frontier in omics-research that reveals the full repertoire of phosphorylation sites in proteins. Conjugated linoleic acid (CLA) supplementation reduces energy output in milk, which could increase fat storage in transition cows, but it is also known to promote lipolysis in adipose tissue (AT). Our objective was to elucidate the effect of CLA on protein activation in AT of transition cows by phospho-proteomics. Ten rumen cannulated Holstein cows were fed a corn silage-based TMR with low fat content and daily abomasally supplemented from wk 9 prepartum until slaughtering at 63 d postpartum with coconut oil (CTL, n = 5; 45.5% C12:0; 16.9% C14:0; 76g/d) or Lutalin (CLA, n = 5; *c*9, *t*11 and *t*10, *c*12, 10g/d). Subcutaneous (S) and abdominal (A) AT were collected and frozen (−80°C). Proteins were extracted for global quantification of the phospho-proteome by enrichment of phospho-peptides (PP) by Fe immobilized metal ion affinity chromatography (Fe-IMAC) followed by discovery analysis. Data were analyzed for the effect of fat depot (S vs. A), treatment (CLA vs. CTL), and their interaction by 2-way ANOVA, and bioinformatics analysis was conducted by Ingenuity (Qiagen). Overall, CLA cows had lower fat and energy output in milk compared with CTL based on weekly milk components, and had more total and abdominal AT than CTL ($P < 0.005$). A total of 5,919 PP were identified in AT. The abundance of 854 PP (14.4%) was statistically different between CLA and CTL ($P < 0.05$, FC ± 1.5). Increased protein phosphorylation, i.e., higher abundance of several PP, was found in lipid-metabolism proteins in CLA vs. CTL: 7 PP were more abundant in acetyl-CoA carboxylase 1 (ACACA); 9 PP in fatty acid synthase (FASN); 4 PP in hormone sensitive lipase (HSL); and 3 PP in perilipin (PLIN). Increased total abundance of FASN and HSL was found by immunoblots in CLA vs. CTL AT ($P < 0.02$). Top canonical pathways enriched in CLA AT were protein kinase A signaling and insulin receptor signaling. These novel findings provide insight to the molecular mechanism by which CLA might stimulate both lipogenesis and lipolysis in AT.

Key Words: proteomics, adipose, CLA

W74 Effect of 2-hydroxy-4-(methylthio)butanoate supplementation on rumen bacterial populations in dairy cows when exposed to diets with risk for milk fat depression.

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Diet-induced milk fat depression (MFD) is a condition marked by a reduction in milk fat yield achieved by altering dietary conditions including increasing unsaturated fatty acids and fermentability of carbohydrates. 2-Hydroxy-4-(methylthio) butanoate (HMTBa) is a methionine analog that has been observed to reduce diet-induced MFD in dairy cows. Our hypothesis was that the reduction in diet-induced MFD by HMTBa was due to changes in the rumen microbiota. To test this, 22 high-producing cannulated Holstein dairy cows were arranged in a randomized block design and assigned to either control of HMTBa supplementation (0.1% of diet DM). Cows were then exposed to 3 diets with either a low risk (32% NDF, no added oil; fed d 1 to 14), a moderate risk (29% NDF and 0.75% soybean oil; fed d 15 to 21), or a high-risk (29% NDF and 1.5% soybean oil; fed d 21 to 28) for diet induced MFD. Rumen samples were collected on d 0, 14, 21 and 28, DNA extracted, V1-V2 region of the 16S rRNA gene PCR amplified, sequenced on Illumina MiSeq platform, and subjected to bacterial diversity analysis using the QIIME pipeline. The α diversity estimates (species richness) and shannon diversity were decreased in control compared with HMTBa ($P < 0.05$). Bacterial community composition also differed between control and HMTBa based on both unweighted uniFrac (presence/absence) and weighted uniFrac (relative abundance of commonly detected bacteria) distances ($P < 0.01$). Within the HMTBa group, there were no differences between d 0 and d 14, 21 and 28; however, in the control group, d 0 samples were different ($P < 0.05$) from d 14, 21 and 28. Bacterial genera including *Dialister*, *Meghasphaera*, *Lachnospira*, and *Sharpea* were increased in control compared with HMTBa ($P < 0.05$). Interestingly, these genera were positively correlated with milk fat *trans*-10, *cis*-12 CLA and *trans*-10 C18:1, isomers associated with MFD. It can be concluded that diet-induced MFD is accompanied by significant alterations in the bacterial community and HMTBa supplementation reduced rumen microbial perturbations when increasing dietary risk factors.

Key Words: biohydrogenation, milk fat depression, rumen bacteria

W75 Effect of postpartum meloxicam administration to ewes on inflammatory status, plasma fatty acid concentrations, and oxylipid biosynthesis.

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The effect of postpartum meloxicam administration on ewe inflammatory status and plasma fatty acid (FA) and oxylipid concentrations were evaluated in 34 Hampshire and Hampshire × Suffolk ewes rearing singles or twins. After lambing, ewes (94 ± 17 kg BW) were sequentially assigned within type of birth to control (n = 16) or meloxicam orally administered on d 1 and 4 of lactation (MEL; 90 mg, n = 18). Blood was sampled on d 1 (before treatment) and d 4, and plasma was analyzed for haptoglobin (Hp) by colorimetric assay, FA profile by LC-MS, and oxylipids by LC-MS/MS. Results were analyzed in a mixed model with d 1 values as covariates. Plasma Hp concentrations tended to be less for MEL ewes ($P = 0.06$), and a covariate x treatment effect ($P = 0.04$) showed that CON ewes with greater d 1 Hp concentrations were also elevated on d 4, which was not the case for MEL. Among FA, MEL increased arachidonic acid (AA) concentration by more than 4-fold in ewes rearing singles ($P < 0.01$ main effect and interaction). MEL decreased concentrations of 9,10-DiHOME, $PGF_{2\alpha}$, 8-iso-PGE_2, and 8,9-DHET ($P < 0.05$). Nine oxylipids had interactions of treatment with d 1 Hp ($P < 0.05$), all of which revealed positive associations between d 1 Hp and d 4 oxylipid concentrations for CON, but neutral or negative relationships for MEL. MEL decreased 13-HODE/13-oxoODE ($P = 0.04$), tended to increase 9-HODE/9-oxoODE ratio ($P = 0.06$; both dependent on d 1 values), and tended to decrease 13-HODE/9-HODE ratio (depending on d 1 Hp, $P = 0.08$), indicating progressive metabolism of linoleic acid-derived oxylipids occurred by enzymatic oxidation after MEL treatment. The diversity of affected oxylipids suggested that MEL not only inhibited cyclooxygenase activity, but may also have reduced cytochrome P450 and nonenzymatic oxidative reactions, potentially due to an improved redox state. In conclusion, postpartum MEL treatment of ewes decreased plasma concentrations of Hp and several oxylipids, with the greatest effect in ewes with biomarkers reflecting a greater inflammatory state before treatment.

Key Words: nonsteroidal anti-inflammatory drug, inflammation, lactation

W76 Adipose tissue and plasma fatty acid profile during the peripartum period differ by parity but not by late-lactation dietary fatty acid profile.

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Some dietary fatty acids (FA) may influence adipose tissue FA composition. The objective of this study was to compare the long-term effects of late lactation fat feeding on subcutaneous adipose and plasma FA profiles during the peripartum period in multiparous (MP) cows. To determine if there is an influence of mobilization cycle, a secondary objective was to compare MP and primiparous (PP) adipose tissue FA profile. Multiparous cows were assigned to either a C16 (pure palmitic acid + concentrate pellet) or C18 (concentrate pellet with 50% FA [78% C18, 22% C16]) supplemented diet (2.3% DM added FA) for the last

50 ± 8 d of late lactation. Cows were pen fed (2/treatment) until dry off and then combined during the dry period. At −31 (±1) days relative to calving (DRTC), MP cows were cohoused with PP cows. Subcutaneous adipose biopsies were taken from MP cows before enrollment, and from all cows at −31 DRTC. Blood samples were taken at −31 and 5 DRTC. Fatty acid profiles were determined via GC. Data were analyzed in SAS 9.4 using PROC MIXED with a fixed effect of treatment (combination of parity and dietary fat; experimental units n = 6), with preplanned contrasts of: MP vs. PP, and MP C16 vs. MP C18. There was no effect of diet on MP cow adipose FA profile at enrollment or −31 DRTC ($P > 0.10$), but MP cows had greater C16:0 (27.4 vs. 25.3 ± 0.4 g/100g FA; $P = 0.04$) and less C18:0 (10.3 vs. 12.5 ± 0.6 g/100g FA; $P = 0.08$) and C18:2 (1.6 vs. 2.6 ± 0.1 g/100g FA; $P < 0.01$) than PP cows at −31 d. At 5 DRTC, MP cows had increased plasma C16:0 (18.2 vs. 16.2 ± 0.3 g/100g FA; $P = 0.04$) and C18:1 (20.7 vs. 15.4 ± 0.7 g/100g FA; $P = 0.04$) but decreased C18:0 (16.2 vs. 18.6 ± 0.3 g/100g FA; $P = 0.03$) in agreement with increased ($P < 0.05$) NEFA. The difference in dietary FA profile during late lactation was either not great enough to alter subcutaneous adipose tissue profile, or adipose tissue metabolism regulated the profile stored. The difference between MP and PP animals suggests that over lactations, there are influences of diet and mobilization cycles on adipose tissue FA profile in MP animals.

Key Words: palmitic acid, transition cow

W77 Calcium propionate supplementation leads to broad downregulation of hypothalamic pathways in lambs.

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Our objective was to investigate the effect of propionate on the hypothalamic regulation of food intake in the context of the hepatic oxidation theory in ruminants. Nine Rambouillet lambs (27.93 ± 4.6 kg) were randomly assigned to one of 3 experimental diets (n = 3/diet): T1, a control diet containing 93% alfalfa hay and 7% molasses, without supplementation of Calcium-Propionate (CaPr); T2, T1 + 30 g/kg DM of CaPr; and T3, T1 + 35 g/kg DM of CaPr. Diets were offered ad libitum as a total mixed ration. The lambs were housed in individual cages equipped with feed and water bowls. Lambs were first adapted to their diets (without CaPr) for 10 d, and the experimental period lasted 42 d (lambs will receive treatments during this period). On d 42, lambs were euthanized, the hypothalamus collected, and RNA was extracted and sequenced on the Illumina HiSeq 4000 system, generating an average of 50 million reads/sample, of which 75% where uniquely mapped, with 56% of gene-assigned read, with a total of 15608 uniquely identified genes. A linear model with CaPr as fixed and animal as random effect was fitted. Differentially expressed genes (DEG) were declared at fold change ≥ |2| and P-value ≤0.05. The Dynamic Impact Approach was used for pathway analyses using the KEGG database. Two contrast yielded the highest number of DEG: T2+T3vsT1 (overall effect of CaPr infusion vs control), and T3vsT1 (3.5% CaPr vs control), with 1200 and 919 DEG, respectively. Independently of the contrast, pathway analysis revealed a broad downregulation of all pathways in animals that received CaPr. The upregulated pathways in CaPr lambs compared with control (T3vsT1) were galactose, glyoxylate and dicarboxylate, β-alanine, and porphyrin metabolism, terpenoid backbone biosynthesis, peroxisome, ferroptosis, and one carbon pool by folate. In addition, ether lipids and sphingolipid metabolism, mucin type O-glycan and heparan sulfate glycosaminoglycan biosynthesis were upregulated in both contrasts. The transcriptome analysis highlighted an extended downregulation of all hypothalamic activities in ewes that received CaPr infusions, independently of the dose. Further work is needed to assess the hypothalamic mechanisms involved in feed intake regulation.

Key Words: propionate, hypothalamus, RNA-seq

W78 Comparison of telomere lengths in blood leukocytes and in nasal and vaginal epithelial cells from water buffalos (*Bubalus bubalis*) of different ages.

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Telomeres are short and repetitive sequences at the ends of linear chromosomes which shorten with every cell division in vitro. Telomere length (TL) is reported to decrease with age and stress. The domesticated water buffalo (*Bubalus bubalis*) is the second important milk-producing animal worldwide. The productive lifespan of water buffalo cows is reported to be longer than that of dairy cows (*Bos taurus*). With this background, we aimed to compare in TL in blood leukocytes obtained from water buffalos across different ages. In addition, we tested the suitability of assessing TL in DNA derived from nasal and vaginal swabs as potential non-invasive alternatives for blood telomeres. The respective samples were collected from 20 calves (3 mo of age), 20 heifers (2 years old), 20 cows (first lactation, 3 years old age), and 13 cows (third lactation, about 5 years old) and TL was assessed by qPCR with β-globin as reference. One-way ANOVA with Bonferroni post-hoc tests was used to test for differences between the age groups. Pearson correlations were calculated to assess associations of TL obtained in different sample matrices (blood versus smears). The TL in blood leukocytes from water buffalo calves, heifers, and from cows in their first lactation was not different, but shorter telomeres were observed in cows in their third lactation. The results thus support an age-dependent decrease of TL in water buffalos. The TL recorded in leukocytes were weakly correlated with TL measured in DNA from nasal swabs ($r = 0.327$; $P = 0.025$), but not with TL from vaginal swabs. The weak and absent correlation of TL in nasal vaginal and nasal epithelial cells, respectively, and the unease of collecting nasal swabs from buffaloes, incapacitated these swabs as suitable alternatives for blood cells when assessing TL.

Key Words: telomere length, water buffaloes, age

W79 Post-ruminal choline supply during a feed restriction-induced negative nutrient balance alters components of hepatic mechanistic target of rapamycin (mTOR) signaling and plasma amino acids in Holstein cows.

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The objective was to investigate the effects of post-ruminal choline (CHL) supply during a feed restriction-induced negative nutrient balance (NNB) on hepatic abundance and phosphorylation of mTOR (mechanistic target of rapamycin)-related signaling proteins and plasma AA concentrations. Ten primiparous rumen-cannulated Holstein cows (158 ± 24 DIM) were used in a replicated 5 × 5 Latin square design with 4 d of treatment and 10 d of recovery (14 d/period). Treatments were unrestricted intake with abomasal infusion of water, restricted intake (R; 60% of net energy for lactation requirements to induce NNB) with abomasal infusion of water (R0) or R plus abomasal infusion of 6.25, 12.5, or 25 g/d CHL ion. Liver tissue was collected on d 5 after infusions ended and blood on d 1–5. Statistical contrasts were A0 vs. R0 (CONT1), R vs. the average of CHL dose (CONT2) and tests of linear and quadratic effects of CHL dose. Although R tended to decrease the ratio of p-mTOR:total (t) mTOR (CONT1; $P = 0.08$), ratios of p-RPS6KB1:tRPS6KB1, p-EEF2:tEEF2, and p-EIF2:tEIF2 were greater ($P < 0.05$). Among those, supply of CHL led to decreases in p-EEF2:tEEF2 (CONT2; $P = 0.04$), p-EIF2:tEIF2 ($P < 0.001$) and tended to decrease p-EIF4BP1:tEIF4BP1 ($P = 0.07$). However, the effect was quadratic only for p-EEF2:tEEF2 ($P = 0.02$) and p-EIF2:tEIF2 ($P < 0.001$), reaching a nadir at 6.25 to 12.5 g/d CHL ion. The ratio of p-RPS6KB1:tRPS6KB1 was not affected by supply of CHL and was close to 2-fold higher at 25 g/d CHL vs. A0. Plasma Met concentration decreased with R (CONT1; $P < 0.001$) but increased linearly with CHL ($P = 0.03$). Restriction also increased plasma 3-methyl-histidine (CONT1; $P < 0.001$). Data suggest that dephosphorylation of EEF2 due to enhanced CHL supply along with greater p-RPS6KB1 potentially helped maintain or increase protein elongation during NNB. This idea is partly supported by the increased circulating Met. However, changes in initiation factors and initiation binding proteins indicated that CHL did not enhance initiation of protein synthesis.

Key Words: amino acid, protein synthesis

W80 Lipogenic effects of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 conjugated linoleic acids on 3D cultured omental and subcutaneous adipocytes derived from lactating dairy cows.

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The anti-lipogenic effects of *trans*-10,*cis*-12 conjugated linoleic acid (T10C12) are well characterized in monogastric species. Similarly, the impact of abomasal infusion of T10C12 on milk fat synthesis in lactating dairy cows is well established. However, the effect of T10C12 on the lipogenic capacity of bovine adipocytes from omental (OM) and subcutaneous adipose tissues (AT) of lactating dairy cows is unknown. Our objective was to evaluate the effects of T10C12 on the lipogenic activity of 3D cultured bovine adipocytes. OM and tailhead (TH) AT samples were collected from 5 lactating non-gestating mature Holstein dairy cows. AT samples were digested with collagenase type II to harvest stromal vascular cells. Preadipocytes were selected by outgrowth of plastic adherent cells and then seeded 3 dimensionally using collagen type I gels. Cells were induced to differentiate using standard adipogenic medium for 14 d. During induction, adipocytes were supplemented with 50 µM T10C12 or 50 µM *cis*-9,*trans*-11 (C9T11) using fatty acid free bovine serum albumin (BSA) as carrier. Triglyceride (TAG) accumulation was evaluated quantitatively (unit = µM/µg DNA) using a fluorometric adipogenic kit. Statistical analysis was performed using linear mixed models. Independent of treatment, adipocytes from TH accumulated more TAG than those from OM (28.3 ± 2.92 vs. 20.2 ± 2.92; $P < 0.01$). Across sites, adipocytes treated with T10C12 had less TAG (18.1 ± 3.21) than those exposed to BSA (25.8 ± 3.21) or C9T11 (28.8 ± 3.21; $P < 0.05$). TH adipocytes treated with C9T11 accumulated more ($P < 0.05$) TAG (35.17 ± 4.12) than those exposed to BSA (27.9 ± 4.12). In turn, T10C12 treated TH adipocytes had lower TAG content (21.9 ± 4.12) than cells that were BSA treated. OM adipocytes exposed to T10C12 (14.3 ± 3.71) had lower TAG compared with those treated with C9T11 (22.3 ± 3.71) and BSA (23.9 ± 3.71). Results suggests that T10C12 has a marked anti-lipogenic effect in bovine adipocytes from OM and TH, similar to that observed in monogastric species, and that C9T11 may enhance lipogenesis in the subcutaneous TH AT depot of lactating dairy cows.

Key Words: conjugated linoleic acid, adipocyte, lipogenesis

W81 Increasing supply of methionine and arginine at constant Thr:Phe, Lys:Thr, Lys:His, and Lys:Val ratios alters inflammatory and oxidative stress responses during a lipopolysaccharide challenge in bovine mammary epithelial cells.

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We evaluated whether increased supply of Met and Arg alone or in combination altered inflammation and oxidative stress responses in primary bovine mammary cells (BMEC) challenged with lipopolysaccharide (LPS). The BMEC (n = 4 replicates/treatment) were incubated for 12 h with control medium (IPAA, Lys:Met 2.9:1, Lys:Arg 2:1) or medium supplemented with Met (LM2.5; Lys:Met 2.5:1, Lys:Arg 2:1), Arg (LA; Lys:Met 2.9:1, Lys:Arg 1:1), or LM2.5+LA (Lys:Met 2.5:1, Lys:Arg 1:1). Ratios of Thr:Phe (1.05:1), Lys:Thr (1.8:1), Lys:His (2.38:1), and Lys:Val (1.23:1) were constant across treatments. Cells were then incubated with or without LPS (1,000 ng/mL) for another 6 h. Data were analyzed as a 2 × 2 × 2 factorial arrangement of treatments with the MIXED procedure of SAS 9.4. Target genes were measured by RT-PCR and proteins by Western blotting. LPS downregulated mRNA abundance of the amino acid transporters *SLC7A5*, *SLC7A1* and *SLC36A1*. However, Arg supply alone attenuated the downregulation of *SLC36A1* mRNA and SLC7A1 mRNA. LPS upregulated mRNA abundance of *KEAP1* and protein abundance of CUL3, and both of them inhibit the antioxidant transcription factor NFE2L2 which was associated with lower protein abundance of NFE2L2 and the antioxidant proteins NQO1 and GPX1. Protein abundance of phosphorylated p65 (RELA; NFKB1 activator) was greater after LPS stimulation, but the response was attenuated by supply of Met alone. Similar responses to LPS and increased supply of Met plus Arg were observed for the ratio of p-RELA to total RELA. mRNA abundance of proinflammatory cytokines and chemokines (*IL1B* and *CXCL2*) was greater after LPS stimulation, but the response was attenuated with greater supply of Met and Arg alone. Although greater supply of Met and Arg could not rescue the inhibition of antioxidant mechanisms controlled by NFE2L2, overall, the data suggest that these amino acids dampened the proinflammatory responses triggered by LPS. One of the underlying mechanisms was through the control of NFKB1 activity and abundance of proinflammatory cytokines and chemokines.

Key Words: amino acid, lactation, mastitis

W82 Identification of novel real-time quantitative PCR reference genes for bovine corpus luteum via whole-transcriptome RNA sequencing.

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Real-time quantitative PCR (RTqPCR) is a valuable tool to study gene expression in tissues. However, tissues from dynamic physiological states pose challenges to normalization, and thus analysis, as traditional reference genes may be unstable across physiologic states. The corpus luteum (CL) is dynamic throughout luteolysis and maintenance in pregnancy. Thus, there is a need to identify stable genes throughout physiologic conditions for use as references genes in RTqPCR analysis. Stable genes were discovered with whole transcriptome RNA sequencing (RNaseq) and validated with RT-qPCR. CL biopsies collected from 5 states were subjected to RNA-seq analysis: CL from pregnant animals during (n = 5, d 20 ± 0) and after (n = 4, d 55.3 ± 3.4) secretion of interferon-tau, and CL before (n = 10), during (n = 8), and after (n = 5) functional luteolysis (luteolytic progression based on circulating progesterone and prostaglandin F2 α metabolite). Potential reference genes were identified by ANOVA analysis of normalized read counts calculated by Cufflinks. Seventy-seven genes had a *P*-value greater than 0.1 and standard deviation less than 20% of mean read count. Genes were further analyzed with the R/Bioconductor package DEseq2 by randomly assigning samples to 2 groups. The 6 genes with the highest *P*-values were further analyzed (RPL4, UQCRFS1, COX4I1, RPS4X, SSR3, and CST3) with RT-qPCR analysis of an independent set of CL samples from first (n = 4) and second month (n = 4) pregnant cows, and CL before (n = 5), during (n = 5), and after (n = 5) functional luteolysis. Gene stability from PCR was calculated with the algorithms geNorm and NormFinder. Four genes were consistently more stable than ACTB and GAPDH, the most common reference genes in bovine CL literature, regardless of physiologic state based on RT-qPCR analysis: RPL4, COX4I1, SSR3, and RPS4X. In CL tissue from pregnancy, CST3 had highest stability. The identification of these novel reference genes will aid accurate normalization of RT-qPCR results. Furthermore, analyses shed light into the effects of luteolysis and pregnancy on stability of gene expression in the CL.

Key Words: corpus luteum, PCR

W83 Whole-transcriptome RNA-sequencing analysis of the corpus luteum throughout physiologic luteolysis in dairy cows.

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Corpus luteum (CL) regression is required for ovulation, with incomplete luteolysis associated with decreased fertility. Changes in gene expression have been correlated with CL from known days of the cycle and circulating progesterone (P4), and from exogenous prostaglandin $F_{2\alpha}$ (PGF) induced regression. However, characterization of gene expression in physiologic regression with knowledge of PGF exposure is lacking. To address this, daily CL biopsies were collected with bihourly blood samples for 74 h from d 18–21, and circulating P4 and PGFM (PGF metabolite) analyzed. Hormone profiles were used to classify biopsies in 3 groups: control: CL unexposed to PGF in the previous 24 h (C; n = 6); EL: CL with exposure to ≥1 PGFM pulse (n = 8); and LL: when P4 < 1 ng/mL (n = 5). Whole transcriptome RNA-seq raw data were aligned to bovine reference genome (NCBI, UMD3.1) with Tophat, followed by differential gene expression analysis using Cufflinks. Compared with control, 173 genes were differentially expressed (DE: Q < 0.05) in EL (103 upregulated, 70 downregulated), while 4615 were DE in LL (2455 upregulated, 2160 downregulated), with 161 genes DE in both EL and LL. Of these, 145 (84% DE genes in EL) displayed an increased fold change from control in LL compared with EL, showing most DE genes in early regression are further up or downregulated as regression progresses. For upregulated genes, enriched pathways in LL included hemostasis ($P = 4.0E-8$), extracellular matrix organization ($P = 4.9E-8$), and innate immune system ($P = 2.1E-6$), while pathways in downregulated genes were enriched in cholesterol biosynthesis ($P = 7.1E-12$), metabolism of lipids and lipoproteins ($P = 1.0E-17$), and steroid biosynthesis ($P = 3.2E-6$). While these results are largely confirmatory of pathways associated with luteolysis, this is the first study to characterize physiologic regression with serial biopsies and known PGF exposure. Furthermore, the small number of genes altered after small PGF pulses but before the drop in P4 associated with luteolysis suggests further inquiry into the role of small PGF pulses in physiologic luteolysis is warranted.

Key Words: corpus luteum, luteolysis

W84 Feeding NutriTek improves udder health and systemic response during a *Streptococcus uberis* mastitis challenge in mid-lactating dairy cows.

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Eighteen mid-lactation multiparous Holstein cows (n = 9/group) were used to determine the effects of a *Saccharomyces cerevisiae* fermentation product (NTK, NutriTek, Diamond V, Cedar Rapids, IA) on the response to a mastitis challenge. Cows were fed the control diet (CON) or CON supplemented with 19 g/d NTK for 45 d (phase 1, P1), and then infected in the right rear quarter with 2500 cfu of *S. uberis* (phase 2, P2). Antibiotic treatment was started 36 h post-challenge until the end of P2 (9 d post challenge). Milk yield (MY) and DMI were recorded daily. Milk samples for somatic cell count were collected 3 times daily, and rectal and udder temperature, heart and respiration rate were recorded every 6 h during the challenge period. Blood samples for metabolites and immune function analysis were collected at 0, 15, and 36 h post-challenge. Data were analyzed by phase using the PROC MIXED procedure in SAS. Cow was used as random effect, while diet, time, and their interaction were used as fixed effects. DMI and MY were not affected by diet ($P > 0.05$) in P1, but an interaction of diet ´ time was recorded in P2 (MY, $P = 0.01$; DMI, $P = 0.11$) indicating a better recovery from the challenge in NTK compared with CON. NTK significantly reduced (P2, $P < 0.05$) the somatic cell score and temperature of the infected quarter during the challenge, while rectal temperature was significantly reduced (P2, $P < 0.05$) at the 24 h mark. No effects (P2, $P > 0.05$) were recorded for circulating neutrophil and monocyte oxidative burst activity; however, NTK reduced (P2, $P < 0.05$) their activation as indicated by lower phagocytosis response compared with CON at 36 h post challenge. Furthermore, NTK cows had greater (P2, $P < 0.05$) plasma concentrations of Ca (P2, $P < 0.05$), suggesting a better systemic inflammatory status, supporting the hypothesis of a better control of the infection at the level of the mammary gland. Overall, results indicate a protective effect of NutriTek supplementation on udder and systemic health during mastitic events.

Key Words: NutriTek, mastitis, udder health

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W85 Rumen-protected methionine product in lactating dairy cows. V. Sáinz de la Maza*[1], B. Rossi[2], R. Paratte[2], A. Piva[2,3], and E. Grilli[3,4], [1]*Department of Animal Production, University of Lleida, Lleida, Spain,* [2]*Vetagro S.p.A, Reggio Emilia, Italy,* [3]*DIMEVET, University of Bologna, Ozzano dell'Emilia, Bologna, Italy,* [4]*Vetagro Inc., Chicago, IL.*

Methionine is the first limiting amino acid for milk and protein production in lactating dairy cows fed corn-based diets. A field trial was conducted in a commercial dairy farm in Northeast Spain with the aim to evaluate the effect of supplementing a rumen-protected methionine product (Timet; Vetagro S.p.A.; Reggio Emilia, Italy) on lactation performance in high-yielding dairy cows. Ninety-nine multiparous Holstein cows were used in a 3-period switchback design: Control 1 (CTR1, 21 d), Timet (TMT, 21 d) and CTR2 (12 d). All the cows that entered the study were between 0 and 120 DIM. Cows were fed a TMR formulated to provide 16.7% CP, 29% starch, and 32% NDF with 2793 g/d of MP and Lys:Met 2.93. During TMT treatment, Timet (55% of DL-Methionine) was supplemented 25 g/d per head. Milk yield was recorded daily and milk was sampled for protein, fat, and urea every 2 d. Data were analyzed using MIXED model ANOVA including cow as random effect (JMR pro 13). Although there was not significant difference in milk yield, milk protein was significantly increased by TMT (3.43%) compared with CTR1 (3.35%) and CTR2 (3.38%) ($P < 0.05$), and milk fat was also significantly increased by TMT (3.75%) compared with CTR1 (3.63%) and CTR2 (3.71%) ($P < 0.05$). During TMT treatment it was possible to observe a trend in lowering milk urea (180.8 mg/L) compared with CTR1 (193.0 mg/L) and CRT2 (189.9 mg/L) ($P = 0.43$). In conclusion, addition of Timet to a corn-based diet increased milk protein and fat concentration.

Key Words: rumen-protected methionine, milk protein, milk fat

W86 Relationship between climate variations and milk composition of Ayrshire cows in tropical conditions. C. Bespalhok Jacometo*, J. Corrales Álvarez, J. Ramírez Días, and A. Navas Panadero, *Programa de Zootecnia, Facultad de Ciencias Agropecuarias, Universidad de La Salle, Bogotá DC, Colombia.*

Milk production in Colombia is mainly located at high-altitude areas, where environmental temperatures are generally low. However, in the last years, it is possible to detect high temperature combined to high relative humidity at these regions, leading animals to experience some level of heat stress. Therefore, the aim of this study was to identify the alterations in milk yield and composition of Ayrshire cows under heat stress. Data were provided by the Colombian National Union of Cattle Association and Colombian Ayrshire Producers Association. A total of 10,846 milking control records, representing 2,090 cows, from 3 different Colombian Departments (Antioquia, Boyacá and Cundinamarca), in a 2-year period, were analyzed. Milk composition was determined monthly by an automated method from a certified laboratory. The temperature-humidity index (THI) was calculated based on climatic parameters including environmental temperature and humidity, obtained from weather stations belonging to the Institute of Hydrology, Meteorology and Environmental Studies, located close to the evaluated herds. Data were analyzed using a GLM model, including month within year of evaluation, nutrition (pasture or pasture + supplementation), number of calving, days in milk, and THI as fixed effect and cow as random. For this study, THI threshold of 72 was stablished, indicating that cows were under a mild heat stress when THI > 72. During the evaluated period, environmental temperature ranged from −0.12°C to 32.69°C, the relative humidity from 0% to 73%, and THI from 58 to 83. When cows were exposed to a THI > 72 it was observed a reduction in milk yield of 0.9 kg/d (15.6 to 14.7 kg/d; $P < 0.001$), in milk protein yield of 21.1 g/d (495.8 to 474.7 g/d;$P = 0.02$) and in total solids of 71.4 g/d (1,904.3 to 1,832.9 g/d; $P = 0.04$) were observed. Also, there was a trend ($P = 0.07$) for a reduction in fat yield (561.5 to 539.2 g/d). Overall, data suggest that milk production and composition of Ayrshire cows, located in high-altitude areas of Colombia are negatively affected by a mild heat stress, which deserves further investigations to mitigate these effects.

Key Words: heat stress, milk protein, milk yield.

W87 Effects of milk replacer feeding rate on blood metabolites, insulin, and cortisol during an ACTH challenge of preweaning dairy calves during summer. R. M. Orellana Rivas*, G. H. Komori, V. V. Beihling, T. N. Marins, J. K. Bernard, and S. Tao, *University of Georgia, Tifton, GA.*

To evaluate the effect of milk replacer (MR) feeding rate on cortisol and metabolic responses to an ACTH challenge, 39 Holstein calves (BW = 40.8 ± 5.2 kg) were randomly assigned to 3 dietary treatments: control (CON, 0.57 kg/d of a 20% CP and 20% fat MR), intermediate (IL, 0.68 kg/d of a 26% CP and 17% fat MR [26:17]), or high (HL, 0.79 kg/d of 26:17) at 2 d of age. Calves were housed in polyethylene hutches and managed similarly throughout the trial. Milk replacer was offered twice daily (12% solids) until d 42 when MR was fed once daily to decrease intake by 50%. Calves were weaned at d 49 and remained on trial until d 56. Plasma was collected at d 2, 5, 10, 14, 28, 42, 43, 45, 47, 49, 51, and 56 for analysis of cortisol. A subset of calves (n = 8/treatment) was subjected to an ACTH challenge at d 40. Plasma was collected at −30, −15, −5, 5, 10 15, 30, 45, 60, 90 and 120 min relative to ACTH infusion (0.125 IU/kg of BW) for analyses of cortisol, insulin and metabolites. Data were analyzed using the mixed procedure of SAS. During the experiment, the average temperature-humidity index was 75 inside and outside hutches and the average calf rectal temperature was 39.6 ± 0.4°C indicating consistent exposure to heat stress. Feeding rate of MR did not influence ($P > 0.13$) plasma cortisol concentrations during the experiment or the ACTH challenge. The ACTH infusion decreased insulin concentration and increased nonesterified fatty acid concentration ($P < 0.01$). After ACTH infusion, the plasma glucose concentration of HL calves was greater compared with CON and tended to be greater than IL ($P = 0.08$). Insulin concentrations were greater for HL compared with CON and IL ($P = 0.03$). Plasma nonesterified fatty acid concentrations of CON calves were greater than HL and tended to be greater than IL at 15 min and were greater than IL at 45 min following infusion (treatment × time, $P = 0.02$). Feeding increasing amounts of MR had no effect on plasma cortisol concentrations during the preweaning period and during an ACTH challenge; however, higher MR allowance may alter metabolism during acute heat stress.

Key Words: summer, milk replacer, stress

W88 Impact of cold stress on rumen fermentation parameters and enteric methane production in Holstein and Jersey steers. M. Islam*[1], S. H. Kim[1], L. L. Mamuad[1], S. C. Ramos[1], Y. I. Cho[1], E. T. Kim[2], and S. S. Lee[1], [1]*Department of Animal Science and Technology, Sunchon National University, Suncheon, Republic of Korea,* [2]*Dairy*

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This study was compared the rumen fermentation parameters and enteric methane production between Holstein and Jersey steers under cold stress feeding same total mixed ration (TMR). In this study, 3 Holstein (508.92 ± 7.95) and 3 Jersey (392.75 ± 30.85) steers were used under a completely randomized design. The animals were subjected for 2 periods of 7 d adaptation period and 5 d experimental period in winter. For each experimental period, the methane (CH_4) was measured at the first 3 d using an automated head chamber (GreenFeed) system and the collection of rumen fluids at 0, 3, and 6 h at the last 2 d. The rumen fluids were analyzed for pH, ammonia-nitrogen (NH_3-N) and volatile fatty acids (VFAs) concentrations. The average ambient temperature, relative humidity of and temperature humidity index (THI) were recorded accordingly, 3.98°C, 59.99%, 45.98. All the animals were fed with TMR diet containing 66.97% dry matter (DM), 16.22% crude protein, 3.11% crude fat, and 14.89% crude fiber. The dry matter intake (DMI) and daily weight gain were also recorded. The results showed that DMI (kg/d) was significantly higher ($P < 0.05$) in Holstein (13.42) than that of Jersey (9.66). The numerical value of daily weight gain (kg/d) was double in Holstein (1.22) compare with Jersey (0.60). The CH_4 production in Holstein had 154.17 g/d while Jersey had 136.83 g/d but was not significantly different to each other. Conversely, CH_4 yield in Jersey (15.01 g/kg DMI) was significantly higher ($P < 0.05$) in comparison to Holstein (11.63 g/kg DMI). The pH values, NH_3-N, acetate, propionate, and total VFA concentrations did not differ significantly. However, Jersey produced more butyric acid with 57.33, 42.24, and 43.70 mmol/L ($P < 0.05$) than Holstein with 39.41, 31.09, and 35.57 mmol/L at 0, 3, and 6 h collection times, respectively. Overall, results imply that cold stress has a potential effect on rumen fermentation and methane emission in Holstein and Jersey steers.

Key Words: cold stress, enteric methane production, volatile fatty acids

W89 An investigation of dairy calf management practices, colostrum quality, and occurrence of failure of passive transfer and enteropathogens among Australian dairy farms.

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Calf pre-weaning morbidity and mortality rates have been reported to be high in several countries. However, limited data have been reported on calf morbidity and mortality rates in Australian dairy farms. The aims of this study were to (i) investigate current calf management practices on dairy farms in Australia and their association with herd-level morbidity and mortality using a questionnaire-based cross-sectional study; and (ii) to estimate the prevalence of enteropathogens causing diarrhea, failure of passive transfer of immunity, and poor colostrum quality in a sample of Australian dairy farms. One-hundred and 6 completed questionnaires and samples from 23 farms were analyzed (202 fecal, 253 calf serum, and 221 colostrum samples). Morbidity and mortality rates reported by farmers in pre-weaned heifers were 23.9% and 5.8%, respectively. These rates were above the Australian Dairy industry targets in 75.5% and 66.7% of respondents. The zoonotic pathogens *Cryptosporidium* spp. and *Salmonella* spp. were the most prevalent enteropathogens, with true prevalence of 40.9% and 25.2%, respectively. Salmonella O-group D was the most prevalent at 67.9% of Salmonella-positive samples, followed by O-groups B (17.9%) and C (10.7%). Failure of passive transfer of immunity was observed in 41.9% of samples (mean herd prevalence of 36.2%) and only 19.5% of colostrum samples met the standards of immunoglobulin content and microbiological quality. Collectively, these data indicate that there is still considerable room for improvement in calf rearing practices on Australian dairy farms, particularly with regards to colostrum management and feeding hygiene.

Key Words: calf health, diarrhea, neonatal disease

W90 Management factors affecting dairy calf growth in northeastern Italy.

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Calf management, especially from birth to weaning, is extremely important to guarantee optimal health, growth and future performance of replacement heifers. The aim of this study was to evaluate the management procedures in the very first stages of the calf's life until weaning and identify those that affect calf growth the most. The trial was conducted on 31 dairy farms, milking from 35 to 600 dairy cows and producing on average from 17 to 40 kg/head/day. From July to December 2018, each farmer was interviewed about all procedures related to calf management from birth to weaning. Some of the procedures evaluated were navel disinfection, separation from the dam, colostrum management, disease prevention, bedding removal, cleaning of housing facilities and calf feeding. The facilities were thoroughly described, measured and assessed for cleanliness. Female calves between 1 and 70 d of age were measured for weight and height at withers. A stepwise regression was performed to identify the principal factors affecting average daily gain (ADG) the most. Farms were grouped by size and milk yield and were tested for differences in ADG (ANOVA) or management procedures (Kruskal-Wallis test). Altogether 271 calves were measured. On average, body weight at weaning was 89.7 ± 15.1kg, and average daily gain in the first 2 mo of life was 0.77 ± 0.19 kg/d. Of all factors considered to explain the ADG until weaning, the stepwise regression selected the amount of colostrum given within 6 h from birth, dam vaccination against enteric diseases, frequency at which colostrum quality is measured and type of colostrum given ($R^2 = 0.98$). None of the factors related to the facilities' cleanliness, animal density or concentrate feeding were selected. Regarding the selected factors, the 26% of the farms fed more than 3L of colostrum within 6 h from the birth, 55% vaccinated the dams against enteric diseases, 16% constantly measured the colostrum quality and 48% fed only maternal colostrum. Farm size and milk yield did not affect ADG nor selected factors, except for frequency at which colostrum quality was measured, that was higher ($P = 0.027$) in high yield farms.

Key Words: calf, management, growth

W91 Effects of metritis treatment strategies on health, reproductive, and productive parameters of Holstein cows. V. R. Merenda*[1], D. Lezier[1], A. Odetti[1], C. C. Figueiredo[1], C. A. Risco[1], and R. C. Chebel[1,2], [1]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Objectives of the study were to compare the effects of 2 metritis treatments on health, reproductive, and productive parameters of Holstein cows. Cows from 2 herds were examined at 4, 6, 8, 10 and 12 DIM for metritis (fetid, watery, red/brown uterine discharge). On the day of enrollment (d 0), cows were paired by parity and severity of metritis {metritis vs. puerperal metritis [rectal temperature (RT) ≥ 39.5°C]} and assigned randomly to Ampicillin (AMP) or Ceftiofur Crystalline Free Acid (CCFA) treatments. Cows enrolled in the AMP (n = 307) treatment were moved to a hospital pen where they were treated once daily for 5 d and were moved back to their original pen 72 h after the last treatment (d 7). Cows enrolled in the CCFA (n = 306) treatment remained in their original pen and received 2 doses of CCFA, 72 h apart. Contemporary healthy cows (POS = 636) were paired by parity with metritic cows and were enrolled in the study. On d 4, 6 and 11 after enrollment, cure was defined based on uterine discharge alone (no fetid, watery, red/brown uterine discharge) and uterine discharge and RT (<39.5°C). Cows were examined at 28 ± 3 DIM for purulent vaginal discharge (PVD) and at 35 ± 3 DIM for endometritis. All cows were enrolled in the Presynch-Ovsynch at 37 ± 5 DIM and pregnancy was diagnosed at 40 ± 3 and 60 ± 7 d after AI. Individual milk yield was measured monthly and weekly milk yield from calving to 14 wk postpartum are reported. Treatment did not affect cure rates on d 4 ($P \geq 0.67$), 6 ($P \geq 0.37$), and 11 ($P \geq 0.43$), but CCFA cows had ($P < 0.01$) lower RT than AMP cows (102.1 ± 0.1 vs. 102.4 ± 0.1°C). Cows in the AMP treatment had higher ($P = 0.05$) incidence of PVD (79.3 vs. 70.7%) and tended ($P = 0.06$) to have higher incidence of endometritis (72 vs. 56.9%). Likelihood of pregnancy at 40 d after first AI was not ($P = 0.14$) affected by treatment but, at 60 d after AI, cows treated with CCFA were ($P = 0.05$) less likely to be pregnant (22.3 vs. 28.4%). Finally, treatment had no effect on milk ($P = 0.40$). In conclusion, despite slight improvements in uterine health, CCFA treatment resulted in reduced pregnancy to first AI compared with AMP treatment.

Key Words: Holstein, metritis therapy, performance.

W92 Hair coat color comparisons between slick and wild type-haired Puerto Rican Holstein cows. G. Soriano-Varela*, I. Colón-Rodríguez, M. Rivera-Barreto, V. Blas-Rosado, C. Luciano-Ramos, J. Nieves-Rivera, Y. Sein-Rivera, K. Domenech-Pérez, and H. Sánchez-Rodríguez, *University of Puerto Rico, Mayaguez Campus, Mayaguez, PR.*

A previous study suggested that under tropical conditions, Holstein cows with a high percentage of black color in their hair coats have larger productive lives than similar animals with a predominantly white coat color. Because the slick-haired (SLICK) Puerto Rican Holstein cows are better adapted to such environmental conditions than their wild type-haired (WT) counterparts, questions about other possible adaptations besides a shorter hair coat remain. In this regard, visual observations from our group suggested that SLICK cows present highly black-colored coats. Thus, the aim of this study was to quantify and compare the proportion of black and white colors in the coats between 18 SLICK and 53 WT lactating Puerto Rican Holstein cows. Cows were obtained from Lajas Agricultural Experiment Station of the University of Puerto Rico. Photos were taken from the left and right sides of each cow, close to a board of 43.18 cm in length for scaling purposes. Images were analyzed with ImageJ software (v 1.31) to determine the total area, as well as the black and white-colored spots areas on each side. These dimensions were used to calculate the respective black and white percentages. Data were analyzed using the GLIMMIX procedure in SAS. When taking into consideration the 71 evaluated cows, no differences were observed between the left and right sides for black ($P = 0.9570$) or white ($P = 0.3324$) colors; thus, sides were averaged for further analysis. Similarly, no differences were detected in the proportion of colors between SLICK and WT cows in both, white (27.68 ± 21.81 vs. 28.59 ± 22.51%, respectively; $P = 0.8760$) and black percentages (71.18 ± 21.51 vs. 71.09 ± 22.59%, respectively; $P = 0.9877$). In fact, both SLICK and WT cows showed a greater percentage of black relative to white (71.11 ± 22.27 vs. 28.35 ± 22.29%, respectively; $P < 0.0001$). Probably, after living in Puerto Rico for multiple generations, this characteristic has been achieved by both hair coat groups as a possible adaptation to withstand tropical weather conditions. Greater pigmentation may protect against chronic solar exposure.

Key Words: slick-haired cow, coat color, heat stress

W93 An observational study of cow contact resistance conditions on commercial dairy farms in Idaho. R. Norell*[1], J. Wilson[2], M. de Haro Marti[3], M. Chahine[2], and A. Ahmadzadeh[4], [1]*University of Idaho, Idaho Falls ID,* [2]*University of Idaho, Twin Falls, ID,* [3]*University of Idaho, Gooding, ID,* [4]*University of Idaho, Moscow, ID.*

The electrical resistance of dairy cows is decreased with wet haircoats, standing on wet flooring, and touching wet metal. We assessed electrical contact conditions on 27 commercial dairies by observing wetness of cow contact areas within cattle housing and the milking parlor plus measured resistance of water from water troughs. Cattle housing on survey farms was 100% open lot (n = 10), 100% freestall (n = 10), or both (n = 7). Herd size ranged from 300 to 4000 cows and parlors were parallel (n = 24) or herringbone (n = 5). Composite water samples were collected from 18 dairies. The electrical resistance of each water sample was measured by inserting a 10 × 10 cm aluminum probe at 2 angles on the water surface (45 and 90 degrees) and at 2 water depths (8.9 and 17.8 cm). Data were analyzed as a 2 × 2 factorial in SAS, blocking on dairy. During our freestall observations, all of the holding corrals, transfer lanes, cow alleyways and crossovers with waterers were wet from urine and feces. Open lots and feed lanes were wet from recent rain or melting snow. Median and range in percentage of wet parlor splash plates, cows touching the splash plate, and wet rear udders during milking were: 48%, 48 to 92%; 54%, 0 to 91%; and 44%, 17 to 93%, respectively. The median percentage of cows touching the splash plate was lower in herringbone (7%) versus parallel parlors (61%) and lower for first-lactation cows (15%) than mature cows (64%). Wet splash plates and udders were more common from cows housed in open lot pens (median = 72%) during wet conditions versus covered freestalls (median 25%). Measured resistance of water samples ranged from 33 to 110 ohms and were significantly lower ($P < 0.001$) at 45° versus 90° and significantly higher ($P < 0.001$) at a depth of 17.8 cm than at 8.9 cm. In conclusion, the electrical resistance of Idaho dairy cows is decreased during the winter months due to wet conditions. The potential for wet contact of the udder with parlor splash plate varies with cow size, parlor type, and environmental conditions. Water resistance varies widely between locations and should be considered during farm evaluations.

Key Words: electrical resistance

W94 A postbiotic additive from *Aspergillus oryzae* decreased body temperature and increased milk production of lactating

dairy cows exposed to heat stress. J. Kaufman*[1], H. Bailey[1], P. De Toledo Shimoda[1], F. Bargo[2,3], I. Ipharraguerre[4], G. Pighetti[1], and A. Ríus[1], [1]*University of Tennessee, Knoxville, TN,* [2]*Biozyme Inc., St. Joseph, MO,* [3]*Universidad de Buenos Aires, Argentina,* [4]*University of Kiel, Germany.*

The objective of this study was to assess the effect of a postbiotic additive from *Aspergillus oryzae* (AO) on milk production and body temperature in dairy cows exposed to heat stress. Forty-eight Holstein cows (105 ± 27 SD days in milk, 704 ± 23 kg body weight) were used in a completely randomized design for 36 d. Cows were randomly assigned to 1 of 4 treatments: 0 g/d (control; CTL), 3 g/d (low), 6 g/d (medium), and 18 g/d (high) of AO (Biozyme Inc., St. Joseph, MO). A 41% forage and 59% concentrate total mixed ration (18.1% CP, 33.0% NDF, 1.61 Mcal/kg NEL) was fed twice daily, and AO was top-dressed after each feeding. Cows experienced warm climate during June and July 2018 with heat abatement (i.e., fans and misters) from d 1 to 10 (period 1). On d 11 to 36, heat abatement was removed to increase heat stress (period 2). Feed ingredients and milk samples were collected and analyzed. Body temperature and respiration rate were monitored. Energy-corrected milk (ECM) was estimated from milk measurements. Treatment means were analyzed for each period using a mixed-effect model with polynomial contrasts for the increasing doses of the AO postbiotic using SAS. Assessment of temperature-humidity index showed that heat stress was mild in period 1 (74.6 ± 2.4 SD) whereas its intensity increased in period 2 (77.3 ± 4.2 SD). In period 1, AO decreased ($P < 0.01$) afternoon vaginal temperature following a cubic response. Additionally, AO tended to quadratically increase ($P = 0.06$) yields of milk and ECM by 3.2 and 3.6 kg/d (CTL = 40.9 and 37.7 kg/d). The AO postbiotic did not affect DMI, milk protein and fat yield, milk protein, fat content, and SCC. In period 2, AO quadratically increased ($P < 0.01$) yields of ECM, milk protein, and fat by 3.8, 0.08, and 0.16 kg/d (CTL = 36.4, 0.98, and 1.37 kg/d). Furthermore, AO linearly decreased SCC ($P = 0.02$) and morning vaginal temperature ($P < 0.01$) but did not affect DMI. In summary, the AO postbiotic increased milk yield parameters and reduced vaginal temperature in heat-stressed cows.

Key Words: heat stress, milk production, prebiotic

W95 Supplements of biotin, folic acid and vitamin B_{12}: Their effects on cow metabolism during the transition period. M. Duplessis*, H. Lapierre, and C. L. Girard, *Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Given their related metabolic roles, the aim of this study was to determine if a low biotin (B8) supply reduced the effects of a combined supplement of folic acid (B9) and vitamin B_{12} (B12) during the transition period on cow productivity and metabolism. Cows (n = 32) were assigned to either one of the following treatments: 1) No supplement (CON); 2) 20 mg/d of top-dressed B8 (B8); 3) 2.6 g/d of top-dressed B9 and 10 mg/wk of intramuscular injection of B12 (B9B12); 4) B8 with B9B12 (B8B9B12) from 3 wk before the expected calving date until 3 wk postpartum. According to a 2 × 2 factorial arrangement, Proc MIXED of SAS with repeated measures was used to analyze data (log-transformed when needed). Supplementary B8, B9 and B12 increased their respective plasma concentrations throughout the trial ($P \leq 0.006$). No supplementation effect was noted on prepartum plasma concentrations of glucose, insulin, free fatty acids (FFA) and β-hydroxybutyrate (BHB; $P \geq 0.11$). With similar dry matter intake and plasma concentrations of glucose and BHB from calving to 3 wk postpartum ($P \geq 0.29$), supplementary B9B12 increased body weight (BW) loss, milk and total solid (TS) yields and plasma concentration of FFA and decreased plasma concentration of insulin for cows not receiving B8 ($P \leq 0.09$; Table 1) whereas it had no effect when cows received B8 ($P \geq 0.1$; B8xB9B12 interaction, $P \leq 0.07$). These results suggested that B9B12 supplements increased productivity and altered energy metabolism in early lactation but, under the current conditions, when combined with B8, these effects were not observed.

Key Words: cyanocobalamin, cattle, peripartum

W96 Effect of rearing method of calves during liquid milk period on their growth. J. Broucek*[1], M. Uhrincat[1], A. Hanus[1], P. Kisac[1], and M. Soch[2], [1]*National Agricultural and Food Centre, Research Institute of Animal Production Nitra, Luzianky, Slovakia,* [2]*South Bohemia University, Ceske Budejovice, Czech Republic.*

The objective was to determine the impact of the length of calf receiving milk from its own mother or nursing cow and the sire lineage on the live body growth. 105 Holstein calves (53 M, 52 F, descended from 4 sires) were assigned to one of 3 rearing treatments: M, n = 36, M,F 18, pen with mother (milked from 2nd d) to 21st day, suck a mother's udder 3 times per d, then group pen (6 kg milk per d, bucket with nipple); N, n = 34, M,F 17, after 3 d with own mother in pen with nursing cow, calves could drink at any time, usually 3–5 times per d, number of calves per nursing cow determined according to milk yield (6 kg milk per d and calf); H, n = 35, M 18, F 17, after having nursed their dams in individual pen for 24 h in hutches from 2nd to 56th d (bucket with nipple, milk

Table 1 (Abstr. W95). Weekly average of milk and TS yields and postpartum plasma concentrations of metabolites and BW loss following B8, B9B12 supplements, or both (mean and 95% CI)

	Treatment				*P*-value		
Item	CON	B8	B9B12	B8B9B12	B8	B9B12	B8 × B9B12
Milk, kg/d	40.1 (36.2–44.0)	43.3 (39.0–47.5)	45.5 (41.8–49.2)	41.0 (37.1–44.9)	NS	NS	0.06
TS, kg/d	5.1 (4.6–5.6)	5.2 (4.7–5.7)	5.8 (5.4–6.2)	5.0 (4.6–5.5)	NS	NS	0.07
Insulin, µU/mL	5.3 (4.0–7.1)	4.1 (3.0–5.7)	3.4 (2.6–4.5)	5.6 (4.2–7.4)	NS	NS	0.02
FFA, µ*M*	218 (178–266)	314 (252–391)	276 (228–334)	251 (205–307)	NS	NS	0.03
BW loss, kg	15 (1–28)	26 (11–40)	38 (26–51)	23 (9–36)	NS	NS	0.05

replacer, 6 kg per day), then loose housing pen to weaning (bucket with nipple, milk replacer, 6 kg per d). Group M allowed 21 d suckle, 63 d bucket fed; N allowed 84 d suckle; H allowed 1 d suckle, 83 d bucket fed. All calves were weaned at 84th day. From the 2nd day until weaning the calves were offered concentrate mixture and alfalfa hay *ad libitum*. After weaning from milk feeding, all calves were kept sex separately in age-balanced groups in bedded pens with the same ration. The data were analyzed using a General Linear Model ANOVA. The starting live body weight was not statistically different. At the weaning, the highest live body weight was recorded in group N and the lowest in group H (N 109.85 ± 13.94 kg, M 94.97 ± 16.69 kg, H 80.80 ± 10.87 kg, $P < 0.001$). The average daily gains from the birth to weaning were 0.81 ± 0.22 kg (N), 0.67 ± 0.31 kg (M), 0.48 ± 0.12 kg (H), ($P < 0.001$). A significant difference ($P < 0.001$) was found also for the period from birth to 180th day of life (N 0.82 ± 0.33 kg, M 0.75 ± 0.39 kg, H 0.67 ± 0.36 kg). Trend of higher growth of N and M groups was maintained also in the later period. We did not obtain any sufficient evidence of effects exerted by the sire lineage. The results indicate that the feeding method used to rear calves may have a significant impact on their later performance. This study was possible through projects APVV 15–0060 and QK1910438.

Key Words: weaning, milk nutrition, housing.

W97 Hair diameter comparisons between slick and wild type-haired lactating Puerto Rican Holstein cows.

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In Puerto Rico, a shorter hair coat has been associated with a superior thermoregulatory capacity in slick (SLICK) Holstein cows relative to their wild type-haired (WT) counterparts. However, other hair properties may be associated with this tropical adaptation. For example, a thicker hair diameter has been reported as an adaptation of tropical Brazilian Holstein cattle. Thus, the aim of this study was to compare the hair diameter values between SLICK and WT Holstein cows in Puerto Rico. For this, 15 SLICK (2.77 ± 0.44 lactations; 595.05 ± 25.86 kg of body weight) and 19 WT (3.00 ± 0.44 lactations; 620.91 ± 21.44 kg of body weight) lactating Holstein cows from Lajas Agricultural Experiment Station (University of Puerto Rico) were compared. After local anesthesia infiltration, a skin biopsy (6 mm in diameter) was collected from the right shoulder of each cow. Skin samples were preserved in 10% formalin in histological cassettes for further analysis. Multiple photographs of each sample were taken perpendicular to the skin surface and compressed using a Canon EOS 7D Mark II camera and the Camlift V2.7.0 Zerene Stacker (64-bit) software. The measurement scale was set at 0.05 mm by Photoshop 6.0 (Adobe, San Jose, CA). The diameter at the base of 5 randomly selected hairs per biopsy was measured using the ImageJ software (v. 1.31). Measurements were replicated 5 times and averaged to obtain one value per skin sample (PROC MEANS). Data were analyzed by the GLIMMIX procedure in SAS. No differences in hair diameter were observed between SLICK and WT cows (0.0858 ± 0.0014 vs. 0.0838 ± 0.0014 mm, respectively; $P = 0.6291$). The observed diameter values in both hair coat groups coincide with those previously published by others in tropically adapted Brazilian Holstein cattle. Thus, it the evaluated WT cows might also have achieved considerable adaptations to tropical weather, avoiding any possible differences between phenotypes.

Key Words: slick-haired cow, hair diameter, heat stress

W98 Effect of on-farm, component-based milk replacer compared with commercial agglomerated milk replacer on growth performance and feed efficiency of dairy calves.

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The objective of this study was to evaluate the growth performance and feed efficiency of dairy calves fed a commercial agglomerated milk replacer (CMR) compared with a component-based milk replacer formula, mixed on farm, using high quality, individually-sourced ingredients (PDS). Both milk replacer (MR) formulas contained 24% crude protein and 24% fat on a DM basis and were devoid of feed additives or medications. A total of 64 neonate Holstein bull calves (2–3 d of age, initial body weight (BW) 38.9 ± 0.24 kg) were blocked by weight and allotted to one of 2 MR treatments. Calves on each treatment received similar milk solids and volume per day throughout the trial, were weaned on d 42 in a step-down manner by reducing milk solids and volume from d 35 to 42, and exited the facility on d 49. Calves were offered ad libitum access to a commercial starter feed (18% CP) beginning on d 5. Calf growth and starter feed intakes were monitored weekly. Growth performance, intakes, and feed efficiency data were analyzed by the PROC MIXED of SAS. Intake of daily milk solids did not differ between treatments, but PDS increased ($P < 0.05$) average daily starter intakes at d 42 (0.30 vs. 0.20 ± 0.03 kg) and d 49 (0.51 vs. 0.40 ± 0.03 kg); and total intakes (milk solids + starter) at d 42 (1.01 vs. 0.92 ± 0.03 kg) and d 49 (1.12 vs. 1.01 ± 0.03 kg) versus those fed CMR. Compared with CMR, calves fed PDS had improved ($P < 0.05$) ADG on d 28 (0.53 vs. 0.45 ± 0.02 kg), d 42 (0.62 vs. 0.52 ± 0.02 kg), and d 49 (0.65 vs. 0.57 ± 0.02 kg). The PDS-fed calves also had improved gain:feed ($P < 0.05$) on d 28 (0.639 vs. 0.554 ± 0.015) and d 42 (0.620 vs. 0.560 ± 0.013) compared with CMR. These improvements resulted in increased ($P < 0.05$) BW on d 28 (53.7 vs. 51.6 ± 0.5 kg), d 42 (65.1 vs. 60.9 ± 0.9 kg), and d 49 (70.9 vs. 67.0 ± 1.3 kg) for calves fed the PDS formula. These data indicate an on-farm, component-based MR based on individual, high quality ingredients improved performance and feed efficiency of calves compared with a commercial agglomerated MR of similar nutrient content.

Key Words: calf, milk replacer, component-based

W99 A survey of diet characteristics related to feed particle size on buffalo farms in southern Italy.

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Over 10% of the milk used by humans worldwide comes from buffalo. In Italy, a major part of that buffalo milk is used to manufacture mozzarella cheese. The objective of this study was to evaluate feeding program characteristics of buffalo farms in the Italian provinces of Caserta and Salerno, particularly forage particle size as it may affect milk production and quality. Thirty-nine farms were studied and each visited twice to collect feeding information, production data, and feed samples to evaluate particle size, diet components, and diet composition. Herds averaged 136 lactating and dry cows (range 66 to 570), with mean daily milk/cow of 7.70 kg (6 to 12 kg/d) containing 8.46% fat (7.4 to 9.3%), 4.72% protein (4.4 to 5.0%), and 4.85% lactose (4.6 to 6.3%). Average milk production/lactation was 2,274 kg (1,800 to 3,100 kg), with average lactation length of 293 d and 409 d average lactation interval. All farms used a total mixed ration; 6 used only dry hay and concentrate and the remaining 33 used a combination of dry hay, silage, and concentrates. Samples of total mixed rations were analyzed for nutrient composition and particle size. Samples averaged 51% dry matter, 13.8% crude protein, 57% neutral detergent fiber, and 26% acid detergent fiber with wide ranges of values for all nutrients measured. Particle size analysis showed on average 45.6% of the sample was retained on the 19-mm sieve (range 12.3 to 78.7%), 20.4% on the 8-mm sieve (2.4 to 50.5%), 10.4% on the 4-mm sieve (3.2 to 20.7%), and 23.0% on the bottom pan (5.4 to 22.7%). Overall, no significant correlations between diet composition or particle size and production were detected. This study showed that feeding systems used for buffalo in the mozzarella cheese making areas of Italy are quite varied.

Key Words: feed particle size, buffalo

W100 Effects of a *Saccharomyces cerevisiae* fermentation product in heat-stressed dairy cows. M. Al-Qaisi*[1], E. A. Horst[1], E. J. Mayorga[1], B. M. Goetz[1], M. A. Abeyta[1], C. S. McCarthy[1], M. R. O' Neil[1], I. Yoon[2], H. A. Ramirez-Ramirez[1], L. L. Timms[1], and L. H. Baumgard[1], [1]*Department of Animal Science, Iowa State University, Ames, IA,* [2]*Diamond V, Cedar Rapids, IA.*

Study objectives were to evaluate the effects of supplementing a *Saccharomyces cerevisiae* fermentation product (SCFP) on metabolism, the acute phase protein response (APPR), and production parameters during heat stress (HS). Twenty multiparous, lactating Holstein cows were randomly assigned to 1 of 2 dietary treatments: 1) a control diet (CON; n = 10) or 2) a control diet supplemented with 19 g/d of SCFP (n = 10; NutriTek; Diamond V, Cedar Rapids, IA). Cows were fed their respective diets for 21 d before study initiation. The trial consisted of 2 experimental periods (P). During P1 (4 d), cows were fed ad libitum and housed in thermoneutral conditions (TN). During P2 (7 d), HS was induced by using an electric heat blanket (EHB; Thermotex Therapy Systems Ltd. Calgary, AB, Canada). Cows were fitted with the EHB for the entirety of P2. Rectal temperature (Tr), respiration rate (RR), and skin temperature (Ts) were obtained twice daily (0600 and 1800 h) during both TN and HS. Overall, the HS increased Tr, RR, and Ts (1.4°C, 54 breaths/min and 4.8°C, respectively; $P < 0.01$) relative to TN but there were no dietary treatment differences detected. Compared with TN, HS decreased DMI and milk yield (36 and 26%, respectively; $P < 0.01$), and the decrease was similar between dietary treatments. During HS, milk fat and MUN increased (17 and 30%; respectively; $P < 0.01$) and milk protein and lactose decreased (7 and 1.7%; respectively; $P < 0.01$). Milk SCC was not affected by HS but was decreased (19%; $P = 0.07$) in SCFP-fed cows. During HS, circulating cortisol increased (48%; $P = 0.04$) in CON-fed cows, but did not change in SCFP-fed cows. During HS, circulating lipopolysaccharide binding protein and serum amyloid A (SAA) increased (2-and 4-fold, respectively $P < 0.01$) and SAA decreased on d 5 of HS in SCFP-fed cows (33%; $P = 0.07$) relative to CON. During HS, circulating white blood cells and neutrophils increased (9 and 26%; respectively; $P \leq 0.05$) in SCFP-fed cows compared with CON cows. In conclusion, HS caused an APPR and feeding SCFP blunted the cortisol and SAA response and mediated leukocyte dynamics during HS.

Key Words: electric heat blanket, *Saccharomyces cerevisiae* fermentation product, dairy cow

W101 Effects of a new preventive strategy with acetylsalicylic acid on daily milk yield, milk conductivity and rumination in dairy cows after calving. A. Ludwikowski*[1], A. A. Barragan[1], E. Hovingh[1], S. Bas[2], S. Takitch[3], J. Zug[4], and S. Hann[4], [1]*Department of Veterinary and Biomedical Sciences, Penn State University, University Park, PA,* [2]*Phytobiotics Futterzusatzstoffe GmbH Bvd, Córdoba, Argentina,* [3]*Department of Animal Science, Penn State University, University Park, PA* [4]*Zugstead Farm, Mifflintown, PA.*

The transition period is one of the most challenging times for dairy cattle, and if excellent management is not in place, cows may not be able to cope with these challenges. It has been reported that preventive treatment of postpartum cows with anti-inflammatory drugs may decrease pain and inflammation, enhancing cow welfare and performance during the early lactation period. However, these strategies involve many interventions, requiring extra labor, and are time-consuming. The objective of this study was to assess the effects of new preventive strategy with acetylsalicylic acid (ASA) on daily milk yield, milk conductivity and rumination in dairy cows after calving. Dairy cows (n = 246) from one dairy farm located in central Pennsylvania were included in this study. Cows were blocked by parity and randomly assigned to 2 groups: 1) ASP (n = 121): within ~12 h after parturition cows received 2 treatments with ASA (200 mg/kg; 4 boluses) 24 h apart; or 2) UNT (n = 125): remained untreated. Daily milk yield, milk conductivity and rumination were collected using electronic monitors (Afimilk, Kibbutz Afikim, Israel) from calving to 60 d in milk (DIM). The data were analyzed using the MIXED procedure of SAS as a randomized block design with repeated measures. Cows treated with ASA tended to produce more milk (ASP = 32.16 ± 0.55 kg/d; UNT = 31.09 ± 0.59 kg/d) during the first 30 DIM, with no significant differences being observed between 30 and 60 DIM. There was no difference in daily milk conductivity and rumination in cows treated with ASA compared with UNT cows. The results of this study support previous findings showing that the use of anti-inflammatory drugs after calving may increase milk production. However, further research is needed to elucidate the mechanisms utilized by the cow to produce the observed increase in milk yield.

Key Words: dairy cattle, acetylsalicylic acid, milk yield

Reproduction 1

W102 The association between serum anti-Müllerian hormone (AMH) concentration and fertility, and genomic heritability and genome-wide associations for serum AMH in Irish dairy cows. M. Gobikrushanth[1,2], D. Purfiled[2], E. Canadas[2], M. Herlihy[2], J. Kenneally[2], M. Murray[3], F. Kearney[4], M. Colazo[5], D. Ambrose[5,1], and S. Butler*[2], [1]*University of Alberta, Edmonton, AB, Canada,* [2]*Teagasc, Moorepark, Cork, Ireland,* [3]*Teagasc, Dunsany, Meath, Ireland,* [4]*Irish Cattle Breeding Association, Bandon, Cork, Ireland,* [5]*Alberta Agriculture and Forestry, Edmonton, AB, Canada.*

Objectives were to (1) characterize the distribution and variability of serum anti-Müllerian hormone (AMH) concentration, (2) examine the association between serum AMH and reproductive outcomes, (3) estimate genomic heritability for serum AMH, and (4) identify putative single nucleotide polymorphisms associated with phenotypic variation in serum AMH in Irish dairy cows. Serum AMH (pg/mL) was determined from a single blood sample collected between 7 and 13 d after first insemination at detected estrus (IDE) in 2,628 dairy cows managed under pasture-based seasonal-calving system. Overall, serum AMH had a positively skewed distribution with the mean (±SEM), median, minimum and maximum concentrations of 326 ± 4, 268, 15 and 2,863 pg/mL, respectively. Cows were categorized into low (<150 pg/mL; n = 526, lowest 20%), intermediate (≥150 to ≤ 461 pg/mL; n = 1,576, intermediate 60%) and high serum AMH (>461 pg/mL; n = 526, highest 20%) groups and associations with reproductive outcomes were tested. Cows with high and intermediate serum AMH had 1.5 times greater odds of pregnancy within 84-d after mating start date compared with cows with low serum AMH (91 and 90 vs. 86%, respectively); however, pregnancy to first IDE and pregnancy rate within 21 and 42-d after mating start date did not differ between AMH categories. Serum AMH was moderately heritable (genomic heritability estimate of 0.40 ± 0.05), and 68 single nucleotide polymorphisms across *Bos taurus* autosomes 7 and 11 were associated with phenotypic variation in serum AMH. Serum AMH concentration had high variability and moderate heritability; however, a reduced fertility in the low serum AMH group was only evident at the end of the breeding season.

Key Words: ovarian reserve, reproductive phenotype, antral follicle count

W103 Association between milk yield and fertility by health status during early lactation. P. Pinedo*[1], J. Santos[2], G. Schuenemann[3], R. Bicalho[4], R. Chebel[2], K. Galvao[2], R. Gilbert[8], S. Rodriguez-Zas[5], G. Rosa[6], C. Seabury[7], and W. Thatcher[2], [1]*Colorado State University, Fort Collins CO,* [2]*University of Florida, Gainesville, FL,* [3]*The Ohio State University, Columbus, OH,* [4]*Cornell University, Ithaca, NY,* [5]*University of Illinois, Urbana-Champaign, IL,* [6]*University of Wisconsin, Madison, WI,* [7]*Texas A&M University, College Station, TX,* [8]*Ross University, St. Kitts, West Indies.*

Opposite trends for milk yield and fertility traits have been reported. However, the confounding effect of health status adds complexity to these associations. Our objective was to test the association between milk yield during early lactation and fertility variables, considering the effect of health during the first 50 DIM. Holstein cows (n = 11,733) calving in 16 farms in 4 regions (NE, MW, SE, and SW) were enrolled at parturition and monitored weekly for reproductive and health events. Pregnancy was diagnosed by ultrasonography on d32 after AI and monthly DHI milk yields were available. Cows with dystocia, twins, retained fetal membranes, metritis, clinical endometritis, subclinical ketosis, mastitis, displaced abomasum, or pneumonia within 50 DIM were considered as unhealthy (UH50; n = 6,437) and the remaining cows were identified as healthy (HT50; n = 4,052) to control for the effect of disease. Daily average milk until 90 DIM (MLK90) was categorized by quartiles into low, mid, and high. Fertility variables were pregnancy to first AI (PAI1) and pregnant at 305 DIM (P305). Logistic regression and ANOVA were used for the analyses, with parity number and calving season included as fixed effects and farm as a random effect in the models. Logistic regression and ANOVA results for UH50 and HT50 cows are presented in Table 1. Odds of PAI1 did not differ for UH or HT populations between MLK90 levels; in contrast, odds of P305 increased sequentially from low to high MLK90 categories. Similarly, MLK90 was greater in cows pregnant at 305 DIM in both populations.

Key Words: milk, fertility

W104 Use of Zn^{2+} chelators to improve bovine artificial oocyte activation. V. Negron-Perez*, K. Uh, and K. Lee, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Table 1 (Abstr. W103). Odds (95%CI) of pregnancy at first AI and at 305 DIM and average MLK90 for different levels of milk yield (90 DIM) and pregnancy status in UH50 and HT50 subpopulations

	UH50			HT50	
MLK90 (kg)	PAI1	P305	MLK90 (kg)	PAI1	P305
Low (<32.7)	0.91 (0.78–1.06)	0.39 (0.33–0.46)	Low (<33.9)	1.06 (0.83–1.36)	0.50 (0.36–0.70)
Mid (32.7–45.8)	0.97 (0.86–1.08)	0.76 (0.66–0.86)	Mid (33.8–47)	0.97 (0.82–1.16)	0.84 (0.67–1.06)
High (>45.8)	Referent	Referent	High (>47)	Referent	Referent
	MLK90 (kg)-UH50			MLK90 (kg)-HT50	
Pregnant: no	38.5 ± 0.16	37.8 ± 0.27		39.6 ± 0.22	36.4 ± 0.23
Pregnant: yes	38.2 ± 0.20	38.5 ± 0.14		39.3 ± 0.28	38.5 ± 0.12
P-value	0.21	0.02		0.36	<0.0001

Artificial oocyte activation is necessary during somatic cell nuclear transfer (SCNT) and can improve development of intracytoplasmic sperm injection embryos. Recent studies indicate that zinc chelators effectively activate porcine embryos by possibly decreasing the mitotic inhibitors and maturation promoting factor activity. The study objective was to optimize the use of Zn^{2+} chelators to activate bovine oocytes; TPEN [*N*,*N*,*N*,″*N*′-tetrakis(2-pyridylmethyl)-1,2-ethanediamine], TPA [tris(2-pyridylmethyl)amine] and PHEN (1, 10-phenanthroline) were tested. All experiments were completed in 7 replicates including: PHEN 20, 400 or 650 µM; TPA 5, 50 or 100 µM; or TPEN 5 or 100 µM; in vitro fertilized (IVF) as a positive control; ionomycin (ion) followed by DMAP (6-dimethylaminopurine) as a positive control; and dimethyl sulfoxide as negative control. Changes in Zn^{2+} intracellular levels post-treatment and blastocyst formation at d 9 post-activation were evaluated. Generalized linear mixed models on SAS were used to identify statistical differences (treatment was fixed). When treated with any of the chelators, the level of intracellular Zn^{2+} in oocytes was significantly reduced ($P < 0.001$). All chelators were able to induce artificial activation as indicated by cellular divisions; however, frequency of blastocyst formation was lower compared with the positive controls. Blastocyst production rate was < 10% overall on activated oocytes whereas 22% of the embryos were blastocysts in both positive control groups. When oocytes were exposed to trio-conditions (*i.e.* ion followed by a chelator+DMAP mix), blastocyst rate (>25%) was comparable to the positive controls. Interestingly, total cell number in blastocysts showed that embryos treated with TPA100µM were closer to IVF embryos than ion + DMAP (88 ± 11 *vs* 93 ± 15 *vs* 72 ± 14, respectively; $P < 0.06$). This study demonstrates that Zn^{2+} chelators can effectively reduce the level of intracellular Zn^{2+} in bovine oocytes and be used to enhance development of parthenogenetic embryos. Further studies will focus on evaluating embryo quality to assess the potential use of Zn^{2+} chelators to improve SCNT derived bovine embryos.

Key Words: oocyte activation, Zn^{2+} chelator, bovine

W105 Effects of nerve growth factor-β added to extenders for cryopreservation of electro-ejaculated and epididymal harvested bull semen.

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Nerve growth factor-β (NGF) is a seminal plasma protein associated with improved sperm membrane integrity and motility in mammalian species. Greater NGF concentration has been identified in the seminal plasma from bulls with positive sire conception rate. Seminal plasma NGF mRNA expression was also found to be positively associated with the maintenance of post-thaw functional membrane integrity in bull spermatozoa, suggesting that it could impact cryotolerance. Semen collected by electroejaculation (EEJ) and epididymal-harvested sperm (EPI) are not the most common methods used in the AI industry, but both methods can be models to test the NGF impact on cryotolerance. Our objectives were to compare post-thaw semen quality from EEJ and EPI from bulls with purified NGF before cryopreservation. Semen was obtained from Angus x Simmental crossbred bulls (n = 10) by EEJ and EPI 3 d later. Semen samples were incubated with a commercial extender having 0 ng/mL (CONT), 0.5 ng/mL (LOW), 5 ng/mL (MED), or 50 ng/mL (HIGH) of purified NGF before cryopreservation. Sperm motility was assessed in each sample before treatment and cryopreservation and at post-thaw. Flow cytometry was used for post-thaw assessment of sperm viability, acrosome integrity, and chromatin stability. Kruskal-Wallis rank sum test and ANOVA were used for the statistical analysis. Post-thaw sperm motility and velocity parameters (VCL) were decreased, while linearity (LIN) was increased in HIGH versus CONT EEJ samples ($P < 0.01$), but no differences were observed in EPI samples ($P = 0.22$). HIGH EEJ samples had a lower amplitude of lateral head displacement (ALH) at 2.5 and 3 h post-thaw ($P < 0.01$). Post-thaw sperm viability, acrosome integrity, and DNA fragmentation index were not affected by NGF treatment in either EEJ or EPI ($P \geq 0.15$). Treatment with NGF did not improve cryotolerance of sperm collected by electroejaculation or epididymal harvest in bulls. Supplementation with high concentrations of NGF decreased post-thaw VCL and ALH and increased LIN, which may suggest a role in preventing premature sperm hyperactivation and capacitation.

Key Words: motility, nerve growth factor-β, spermatozoa

W106 Phenotyping the expression of estrus behavior in dairy cows using novel heat detection technology.

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The objectives were to quantify mounting behavior associated with estrus and to estimate the maximum heritability of this trait. Holstein-Friesian cows (n = 1,133) across 5 grazing herds located in the United States, New Zealand, and Ireland, were fitted with a FlashMate (Farmshed Labs Limited, Hamilton, New Zealand), a touch activated mounting detection device, to record the number of contacts and duration of the contacts received at estrus. FlashMates were removed after approximately 60d of breeding. Data were extracted from each device for individual cows. A peak detection program in SAS 9.4 (SAS Institute Inc., Cary, NC) was used to identify individual peaks and to calculate the sum of the contact time, in seconds, and the number of times contacted during estrus. Across all farms, there were 91 cows (8%) with no peak detected and presumed anestrus. The data from the remaining cows (n = 1042) with at least one peak (total of 1,758 peaks) were analyzed for the effects of farm and peak number by using PROC MIXED of SAS. There was an effect of farm on the duration of contacts (range of 37.8 ± 8.3 to 118.3 ± 2.2 s; $P < 0.0001$) and the number of contacts received (range of 16.9 ± 2.4 to 37.8 ± 0.6; $P < 0.0001$). The peak number (first and second) was not significant. The number of days between the first and the second estrus detected was affected by farm (range of 18.2 ± 2.1 to 25.1 ± 0.5 d; $P < 0.001$). The intraclass correlation coefficient (maximum heritability) was 21.1% for the number of contacts received and 18.7% for the duration of contacts. In conclusion, the number of contacts received, the duration of each contact, and the time between peaks differed between farms. The maximum heritability of the estrus traits measured was approximately 20%. Novel technology for estrus detection allows collection of estrus phenotypes in a large number of cows suitable for in-depth studies.

Key Words: estrus detection, heritability, FlashMate

W107 Characterizing estrus behavior in Holstein heifers based on an ear-attached movement sensor.

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Optimizing the use of electronic aids for estrus detection in dairy heifers will have direct implications for increased reproductive efficiency as well as more sustainable reproductive management, as hormonal interventions can be minimized. The objective was to describe estrus-related changes in rumination, eating, and high-active time in Holstein heifers using an ear-attached movement sensor whose application has been previously described in lactating cows. Starting at 6 mo of age, outdoor group-housed Holstein heifers (n = 22) received an ear-attached movement sensor (CowManager Sensor, Geryerscop, UT, the Netherlands), and were subjected to weekly ovarian ultrasonography until 2 consecutive ovulations were confirmed. Starting 1 wk after the second ovulation, ovaries were scanned every 2 d until 2 subsequent ovulations were confirmed, monitoring ovarian dynamics throughout 1 complete estrous cycle. Data were evaluated keeping days of estrus (defined as day of highest activity preceding ovulation) and ovulation as reference points, and analyzed using the GLIMMIX procedure of SAS. Mean (±SE) daily rumination time was decreased (295.2 ± 9.6 vs. 427.2 ± 12.0 min/d; $P < 0.01$) while eating time was unexpectedly increased (386.4 ± 14.4 vs. 338.4 ± 14.4 min/d; $P = 0.02$) on day of estrus compared with the mean activity over the 5 d preceding. As expected, high-active time was increased (487.2 ± 16.8 vs. 247.2 ± 16.8 min/d; $P < 0.01$) on day of estrus compared with the 5 d prior. Furthermore, high-active time was markedly increased (376.8 ± 33.6 vs. 216.0 ± 9.6 min/d; $P < 0.01$) during the 2 d preceding day of ovulation. In summary, rumination time was decreased on day of estrus relative to the 5 d preceding, while high-active time was increased on day of estrus relative to the 5 d preceding estrus as well as during the 2 d preceding ovulation. Significant changes in rumination, eating and activity were documented in Holstein heifers by an ear-attached movement sensor, which can be a useful tool for reproductive management.

Key Words: activity monitoring, behavior, estrus detection

W108 Ano-genital distance as a possible indicator of embryo yield and viability in superovulated Holstein cows—A preliminary report.

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Ano-genital distance (AGD) is the distance from the center of the anus to the base of the clitoris in dairy cows, and considered an indicator of prenatal androgen exposure. In our previous work (J Dairy Sci 2017; 100:9815–23) first- and second-parity Canadian Holstein cows with long AGD had lower pregnancy outcomes to first and subsequent artificial inseminations compared with those with short AGD. Consequently, we hypothesized that cows with long AGD have reduced embryo yield and viability compared with cows with short AGD. To determine if embryo characteristics (yield and viability) differed among cows of long and short AGD categories, AGD was measured using digital calipers in 10 Holstein cows that had been previously subjected to superovulation and embryo collection. Cows were classified into either short (≤127 mm; n = 5) or long (>127 mm; n = 5) AGD categories based on the optimum (127 mm) AGD threshold that predicted pregnancy outcomes in our previous report. Data were analyzed using FREQ and GENMOD procedure of SAS. The overall number of structures, fertilized ova, and viable embryos recovered were 73, 54, and 49, respectively. The proportion of total structures tended ($P = 0.09$) to be greater for cows with short AGD than those with long AGD (58 [42/73] vs. 42 [31/73] %). The proportion of fertilized ova (61 [33/54] vs. 39 [21/54] %) and viable embryos (63 [31/49] vs. 37 [18/49] %) were greater ($P \leq 0.05$) for cows with short AGD than those with long AGD. However, within short and long AGD categories, the proportion of fertilized ova out of total structures collected did not differ (79 [33/42] *vs*. 68 [21/31] %). Likewise, the proportion of viable embryos out of total structures (74 [31/42] vs. 58 [18/31] %) and the proportion of viable embryos out of fertilized ova (94 [31/33] vs. 86 [18/21] %) from each of short and long AGD categories did not differ. In summary, preliminary findings indicate that superovulated Holstein cows with short AGD have greater embryo yield and viability than cows with long AGD. These results need to be confirmed with a larger sample size.

Key Words: embryo characteristics, fertilization, fertility.

W109 Effects of fully-acidified, negative DCAD diets with differing concentrations of dietary calcium fed prepartum on the dominant follicle of the first follicular wave after parturition and pregnancy in Holstein cows.

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Diets fed during the transition period can play an important role in the cow's follicular dynamics following parturition. We aimed to compare the effects of feeding a fully-acidified, negative DCAD diet prepartum to multiparous Holstein cows (n = 70) at 2 concentrations of dietary calcium (Ca) inclusion versus a non-acidified, positive DCAD diet prepartum on follicular dynamics and pregnancy postpartum. Treatments began at 28d before expected calving and were: CON(n = 23), a positive DCAD diet with low dietary Ca (0.4% DM); LOW(n = 22), a fully-acidified, negative DCAD diet (urine pH = 5.7) with low dietary Ca (0.4% DM); and HIGH(n = 25), a fully-acidified, negative DCAD diet (urine pH = 5.7) with high dietary Ca (2.0% DM). Follicular development was monitored via ultrasound every 2 d starting at 7 DIM until ovulation of the first dominant follicle (DF). Data were analyzed using the MIXED, LIFETEST, and PHREG procedures in SAS. Association between treatments and days to first ovulation and pregnancy per first timed AI (P/AI) were assessed using Kaplan Meier curves and Cox's proportional hazard regression. Contrasts included CONT1 (CON vs the average of LOW and HIGH) and CONT2 (LOW vs HIGH).Cows fed CON (18.95 ± 0.9 d) had increased ($P = 0.01$) days to first ovulation than cows fed LOW (17.13 ± 0.6 d) and HIGH (16.12 ± 0.4 d).There was no treatment effect on maximum DF diameter (CON = 17.87mm, LOW = 18.33mm, and HIGH = 17.56mm; SEM 0.44; $P = 0.44$, CONT1 and $P = 0.16$, CONT2).There was a tendency for a treatment × days relative to ovulation interaction ($P = 0.11$) indicating that cows fed CON had a slower rate of growth in the 4 d before ovulation of the first DF than cows fed LOW or HIGH. Cows fed CON (4/19 P/AI) tented to had lower P/AI ($P = 0.11$; 95CI = 1.02 – 16.6) than cows fed HIGH (11/21 P/AI) but not LOW (8/20 P/AI). In conclusion, cows fed HIGH and LOW had improved days to first ovulation than cows fed CON. Cows fed HIGH tended to be more likely to become pregnant than cows fed CON. Overall, cows fed a fully-acidified, negative DCAD diet prepartum had improved reproductive performance postpartum.

Key Words: dietary cation-anion difference (DCAD), follicle, pregnancy

W110 Effects of parity, season and region on fertility of lactating dairy cows submitted to a Double-Ovsynch protocol for first timed-AI.

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Our objective was to determine the effects of parity, US region and season on pregnancy per AI (P/AI) in lactating dairy cows submitted to Double-Ovsynch (DO) for first timed AI (TAI). Records for first TAI events (60 to 97 DIM; n = 19,298) of cows submitted to DO TAI were collected from on-farm computer records from farms in CA (n = 3), NY (n = 2), and WI (n = 5) between May 2016 and April 2017. Average herd size was 2,355 lactating cows (range: 892 to 3,632 cows). The average submission risk to DO for first AI in the studied farms was 90.9% (n = 21,210). Overall, P/AI was 44.5% and ranged from 37.5% to 54.2% among farms. Logistic regression was used to test the effect of parity (1, 2, and ≥3), region, season of TAI, and region × season of TAI interaction within parity. Primiparous cows (48.4%) had more P/AI ($P < 0.01$) than cows in parity 2 (42.5%) and parity ≥3 (40.8%). Cows in CA (40.8%) had a fewer P/AI ($P < 0.01$) than cows in NY (45.6%), and WI (46.8%). Primiparous cows from CA farms had fewer P/AI when TAI occurred during the summer than other seasons; however, season did not affect P/AI in primiparous cows from NY and WI dairies (Table 1). Regardless of region, parity-2 cows receiving TAI during summer had fewer ($P < 0.01$) P/AI than cows receiving TAI during winter and spring. Cows of parity ≥3 in CA had fewer P/AI during all TAI seasons than cows of parity ≥3 in NY and WI except during the summer when cows of parity ≥3 in NY and WI had fewer P/AI than in other TAI seasons. In conclusion, cows submitted to first TAI after DO had overall high P/AI across a wide variety of parities, regions and TAI seasons. Moreover, TAI season did not affect P/AI of primiparous cows submitted to DO for first TAI in the Midwest and Northeast US.

Table 1 (Abstr. W110). Effects of region and season within parity on P/AI

		P/AI, %			
Parity	State	Winter	Spring	Summer	Autumn
1	CA	47.1^{a}	40.5^{b}	33.1^{c}	45.6^{b}
	NY	48.8^{a}	49.0^{a}	44.7^{ab}	47.6^{ab}
	WI	53.8^{a}	51.6^{a}	52.2^{a}	52.2^{a}
2	CA	43.7^{ab}	41.1^{b}	34.5^{c}	39.3^{b}
	NY	46.7^{a}	41.6^{b}	38.8^{b}	46.6^{a}
	WI	46.1^{a}	48.9^{a}	37.2^{bc}	45.9^{a}
≥3	CA	39.8^{b}	33.7^{c}	38.6^{b}	36.0^{b}
	NY	42.5^{b}	44.0^{ab}	38.8^{b}	51.6^{a}
	WI	48.0^{a}	45.9^{a}	35.6^{b}	41.4^{ab}

$^{a–c}$Means within parity with different superscripts differ ($P < 0.05$).

Key Words: double-Ovsynch, fertility, timed AI

W111 Association between reproductive efficiency of heifers and their reproductive performance as lactating cows. B. Mion*, M. R. Carvalho, and E. S. Ribeiro, *Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada.*

Our objective was to investigate whether reproductive efficiency of nulliparous heifers is predictive of their reproductive performance during the first lactation. Pubertal heifers (n = 2,796) were categorized based on their pregnancy success as: 1) highly fertile - pregnant at the first breeding (HF; n = 1,327); 2) moderately fertile - pregnant at the second or third breeding (MF; n = 899); or 3) subfertile - pregnant at fourth or later breeding (SF; n = 529). Pregnancy diagnosis was performed 45d after breeding. Heifers that failed to become pregnant were excluded (n = 41). Heifers diagnosed pregnant were followed up to 305DIM in their first lactation, and all reproductive events were recorded. Data were analyzed using the PROC GLIMMIX of SAS fitting the adequate data distribution. Statistical models included the effects of heifer category, season of birth, and their interaction. Age at first breeding was similar between groups and averaged 397 ± 0.7d. However, as a result of our experimental design, HF became pregnant earlier than MF, which became pregnant earlier than SF (397 vs 435 vs 508d, respectively; $P < 0.01$). During first lactation, however, heifer category was not associated with the proportion of cows inseminated (HF = 93 vs MF = 92 vs SF = 90%; $P = 0.15$), time to first insemination (HF = 79 vs MF = 78 vs SF = 78d; $P = 0.12$), time to pregnancy (HF = 120 vs MF = 118 vs SF = 115d; $P < 0.01$), pregnancy per breeding (HF = 37 vs MF = 36 vs SF = 39%; $P = 0.57$), pregnancy loss (HF = 13 vs MF = 19 vs SF = 15%; $P = 0.22$) and calving per breeding for the first breeding postpartum (HF = 32 vs MF = 29 vs SF = 33%; $P = 0.30$), and hazard ratio for pregnancy up to 305DIM (HF = 1.0 vs MF = 0.99 [0.90–1.09] vs SF = 0.92 [0.82–1.04]; $P = 0.39$). The only difference observed was the proportion of cows diagnosed pregnant by 305DIM, which was greater for HF compared with MF and SF (HF = 91 vs. MF = 92 vs. SF = 87%; $P = 0.04$). In conclusion, the association between reproductive efficiency of dairy heifers and their reproductive efficiency as first lactation cows was weak and not predictive, which supports the idea that factors affecting fertility in dairy cattle are mostly distinct between heifers and cows.

Key Words: fertility, heifer, cow

W112 Length of follicular and luteal phase is associated with estrous expression. A. M. L. Madureira*, T. A. Burnett, J. W. Bauer, W. A. Gomes, and R. L. A. Cerri, *The University of British Columbia, Vancouver, BC, Canada.*

This study evaluated the association between estrous expression and the duration of the current and previous follicular and luteal phases. A total of 169 events from Holstein cows were enrolled. Cows were monitored continuously by an accelerometer. Ovaries were scanned from the day of first alert until pregnancy as follows: day of alert (0 d), 24 h, 48 h, 10, 14, 17, 21, 24, 28, 31, 35, 38 and 42 d post-alert. Blood samples for progesterone (P4) analysis were collected at the same times as described above. Area under the curve (AUC) was calculated for P4 concentration over the total cycle. Duration of the follicular phase (FPh) was considered as the day of luteolysis until next ovulation and luteal phase (LPh) as the time of ovulation until next luteolysis. Luteolysis was considered as 2 consecutive small CLs (<25mm). Estrous expression was classified as high (≥80 index) and low (<80 index) using the median. The LPh was categorized as short (<18 d) or long (≥18 d) also using the median. Data were analyzed by the GLIMMIX procedure of SAS with estrus event as experimental unit and cow as a random effect. An average of 4.4 ± 2.1 estrous cycles per cow were recorded. Each unit increase of AUC of P4 was associated with a 0.15-d increase in the LPh ($P < 0.01$). The duration of the preceding LPh had an impact on subsequent FPh, as an increase of 1 d of the LPh was associated with a 0.30 d increase in the FPh. Cows with high estrous expression had shorter LPh, FPh and overall length of the estrous cycle when compared with cows that had low estrous expression (21.3 ± 1.1 vs. 25.6 ± 1.9d, $P = 0.06$; 6.5 ± 0.5 vs. 8.4 ± 0.9 d, $P = 0.10$; and 27.7 ± 1.7 vs. 37.5 ± 3.1 d, $P = 0.02$, respectively). Higher concentrations of P4 at the time of estrus were associated with a shorter LPh (slope = −0.10; $P < 0.01$) but not FPh. Shorter LPh before AI had higher pregnancy per AI (P/AI) when compared with cows with a longer LPh (50.5 ± 7.0 vs. 27.9 ± 8.0%, respectively; $P = 0.03$). Additionally, for every day increase in FPh duration there was a tendency for 2.5% increase in P/AI ($P = 0.10$). In conclusion, the duration of the luteal phase is affected by P4 during the preceding cycle and at the time of estrus, as well as by the intensity of estrous expression detected by an automated activity monitor.

Key Words: estrous expression, fertility, estrous cycle

W113 Transcriptome of corpus lutea in pregnant and nonpregnant cows at late diestrus.

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Our objectives were to quantify and characterize potential differences in transcriptome of the corpus luteum (CL) in pregnant (P) and nonpregnant (NP) cows at late diestrus. The estrous cycle of primiparous cows (n = 35) were synchronized using the Presynch-CIDRSynch protocol. On the day of the last GnRH (d 0), 22 cows were selected randomly to be inseminated (AI), while the other cows remained as a nonbred NP group (n = 13). On d 17, all cows were slaughtered and those with an elongating conceptus were classified as P (n = 14). The CL was dissected, weighed, and stored at −80°C. A subsample of CL from P (PCL = 8) and NP (NPCL = 4) cows were subjected to transcriptome analysis using Affymetrix Gene Chip Array. Data were analyzed using Bioconductor software in R. The GCRMA function was used to preprocess the data, and the Limma package was used to fit a linear model and adjust variances by empirical Bayes adjustment. Moderate *t*-test was performed and *P* values were adjusted for multiple testing using the BH false discovery rate. Adjusted $P < 0.05$ and fold change > 1.5 characterized significant differences. A total of 106 transcripts were differently expressed, 67 upregulated and 39 downregulated in PCL compared with NPCL. Differently expressed genes (DEG) were associated with lipid metabolism, small molecule biochemistry, cell-to-cell signaling and interaction, and cell morphology. Among DEG with increased expression in PCL were *APLNR* (2-fold), *HPGD* (2-fold), and *AKR1C4* (1.8-fold). The first is relevant for angiogenesis, and the latter 2 are related to metabolism and degradation of prostaglandins. Among DEG with reduced expression in PCL were *BOLA* (4.3-fold), *SERPINA14* (1.8-fold), and *FAS* (1.6-fold). The first is important for antigen presentation and might indicate the abundance of immune cells in the CL, and the latter 2 are important cell survival and tissue remodeling. In conclusion, transcriptome of CL in P and NP cows at late diestrus have important differences that seem to be unrelated to luteolytic signals in NPCL and suggest the presence of endocrine signals derived from the pregnant uterus in the PCL during maternal recognition of pregnancy.

Key Words: corpus luteum, pregnancy, transcriptome

W114 Associations between maternal characteristics and health, survival, and performance of heifers.

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Our objective was to investigate whether health, survival and performance of heifers from birth up to first lactation are associated with parity and health status of the dam. Holsteins heifers (n = 2,669) were categorized as 1) daughters of primiparous cows that, consequently, were not lactating during gestation (Prim-NoL; n = 1,322); 2) daughters of multiparous cows that did not have any clinical diseases in the previous lactation (Mult-NoCD; n = 841); and 3) daughters of multiparous cows that had at least one clinical disease in the previous lactation (Mult-CD; n = 506). Clinical diseases included retained placenta, metritis, mastitis, lameness, digestive and respiratory problems. Data from daughters included genotypic and phenotypic characteristics at birth, morbidity, reproductive performance, and culling from birth up to 305 DIM of first lactation, and milk production in the first lactation. Orthogonal contrasts evaluated the effects of the parity of the dam (Prim-NoL vs. Mult-NoCD+Mult-CD) and clinical disease in the previous lactation (Mult-NoCD vs. Mult-CD). Compared with daughters of multiparous cows, Prim-NoL were lighter at birth (36 vs. 41 kg; $P < 0.01$), had better genetics for production traits (EBV for milk: 942 vs. 806 kg; $P < 0.01$), were less likely to leave the herd as a heifer (18 vs. 27%; $P < 0.01$) and as a first-lactation cow (11 vs. 15%; $P = 0.02$), less likely to have pregnancy losses as a heifer (9 vs. 14%; $P < 0.01$) and clinical diseases as a first-lactation cow (31 vs. 37%; $P = 0.02$), and had reduced performance in the first lactation when considering their genetic potential (adjusted 305-d yield: 11214 vs. 11400 kg; $P < 0.01$). Compared with Mult-NoCD, Mult-CD were less likely to have diarrhea as a heifer (20 vs. 26%; $P = 0.02$) and a clinical disease as a first lactation cow (32 vs. 40%; $P = 0.02$), but were also more likely to leave the herd as a heifer (32 vs. 24%; $P < 0.01$) even though genetic merit for production traits were similar. In conclusion, parity and health status of the dam in the previous lactation were associated with morbidity and performance of heifers, and might represent factors affecting developmental programming in utero.

Key Words: heifer, developmental programming

W115 The reproductive and economic impact among 6 reproductive programs for lactating dairy cows including a sensitivity analysis of the cost of hormonal treatments.

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Hormonal synchronization protocols can dramatically increase the reproductive efficiency of high-producing dairy cows, yet some farmers continue to question the economics of these programs based on the cost of hormonal treatments. An economic simulation model considering hormonal treatments and labor was used to compare 6 protocols for timed AI (TAI) in a year-around calving system: PreSynch-OvSynch with estrous detection (ED) before and after first TAI [Conception Rate (CR) 35%; for ED, Service rate (SR) 60% and CR 30%]; Presynch-Ovsynch with different CR (35%, 40%, or 45%); and Double-OvSynch+PGF, (CR = 50%) with the following aims: 1) assess the economic impact of using more intensive synchronization programs and 2) quantify the effect of increasing the cost of GnRH and $PGF_{2\alpha}$ on the profitability of more intensive reproductive programs for high-producing dairy cows housed in confinement systems and managed for year-around calving in the US market ($2.60/GnRH dose and $2.30/ $PGF_{2\alpha}$ dose) and in the EU market ($6.70/GnRH dose and $5.10/ $PGF_{2\alpha}$). We confirmed that PreSynch-OvSynch protocols require fewer hormonal treatments than the Double-OvSynch+PGF protocol (1.4 to 3.0 per cow per yr); however, the Double-OvSynch+PGF protocol yielded more profit/cow per yr than PreSynch-OvSynch protocols with ED or 100% TAI after Presynch-Ovsynch protocols ($21.2 to $46.2 in the US market and $5.4 to $36.1 in the EU market). Including ED after first TAI was more profitable than including ED before first TAI or not including ED at all. The sensitivity analyses indicated that hormone costs would need to be 5 to 14 times higher in the US market and 2 to 6 times higher in the EU market for the Presynch-Ovsynch protocols to exceed the profitability of a Double-OvSynch+PGF protocol. Switching from Presynch-Ovsynch to Double-OvSynch+PGF requires a similar number of $PGF_{2\alpha}$ treatments but more GnRH treatments. Our economic analysis consistently found that reproductive programs using more hormonal treatments but yielding greater reproductive performance are more profitable for high-producing dairy cows housed in confinement systems and managed for year-around calving

Key Words: reproduction, synchronization, economics

Ruminant Nutrition: Fat and Lipids

W116 Relationship between body condition score and ultrasound measurement of backfat thickness of Holstein dairy cows in a grazing-based system. G. V. Kozloski*[1], L. Wlodarski[1], D. S. Zeni[2], J. A. R. Rosback[1], and W. M. Gräf[1], [1]*Universidade Federal de Santa Maria, Santa Maria, RS, Brazil,* [2]*Instituto Federal Farroupilha, Santa Maria, RS, Brazil.*

The body condition score (BCS) is a practice and common approach for evaluating the nutritional status of dairy cows which, however, has been questioned because of its subjectivity. Alternatively, backfat thickness (BFT) is a reliable parameter to assess the change of the energy status of dairy cows and can be objectively measured by ultrasonography. A high correlation between BCS index and BFT has been obtained in Holstein dairy herds where cows were fed with total mixed ration. The objective of the present study was to determine the relationship between these variables as well as how much they vary throughout the lactation of cows in a grazing-based system. Paired measurements of BCS and BFT were performed monthly from October 2017 to September 2018 in 128 cows of a commercial herd in Southern Brazil. The diet of cows was based on grazing tropical grasses from October to April and temperate grasses from May to September, supplemented at variable rates with concentrate, maize silage and/or grass hay. BCS was estimated using a 5-point scale and BFT (mm) was obtained using a 5-MHz linear transducer positioned vertical to a line between the tuber coxae and tuber ischia at the sacral site. Both variables were measured 4 to 7 repeated times in each cow throughout the experimental period. Pearson correlation and linear regression analysis were performed on data set. The minimal and maximal values of the variables were: DIM: 7 to 303; milk production (L/d): 6.3 to 36.0; BCS: 1.25 to 4.00 and BFT (mm): 2.8 to 30.4. There was a significant ($P < 0.05$) low correlation (r) between DIM and either BCS (r = 0.23) or BFT (r = 0.27) as well as between BCS and BFT (r = 0.33). The linear regression between BFT and BCS, which model included the cows as a random class variable, was: BFT (mm) = 2.3 + 5.2BCS ($P < 0.05$, RMSE = 2.79). In conclusion, in a grazing-based system where herbage allowance and quality are broadly variable throughout the year, the change in either BCS or BFT of cows was only weakly related to their lactation stage. Moreover, the change in BFT of cows was not accurately detected as a change in their BCS.

Key Words: backfat thickness, body condition score, dairy cow

W117 Effects of increasing levels of calcium soap of fatty acid supplementation on lactation performance in dairy buffaloes. Hifzulrahman*[1], M. Abdullah[1], J. Bhatti[1], T. Pasha[2], M. Akhtar[2], Z. Ali[3], M. Saadullah[1], and M. Haque[2], [1]*Department of Livestock Production, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan,* [2]*Department of Animal Nutrition, University of Veterinary and Animal Sciences, Lahore, Punjab, Pakistan,* [3]*Applied Chemistry Research Center, Pakistan Council of Scientific and Industrial Research Laboratories Complex, Lahore, Punjab, Pakistan.*

The effects of feeding varying levels of Ca salts of fatty acids on production responses were less thoroughly reported in lactating dairy buffalo compared with the dairy cow. The objective of this study was to investigate the effect of increasing level of Ca salts of palm fatty acid (Ca-FA) on dry matter intake, milk yield, milk fat, and milk fatty acid (FA) profile in lactating Nili Ravi buffalo. Twelve multiparous early-lactating Nili Ravi buffaloes received 4 treatments in a 4 × 4 Latin square design with a period length of 21-d. The 4 diets were designed to provide 0, 200, 400, and 600 g of Ca-FA per day/buffalo. Milk yield and 3.5% fat-corrected milk yield were increased by 2.00 (quadratically, $P = 0.04$) and 6.20% (quadratically, $P < 0.01$), respectively, with the increasing Ca-FA intake. However, the response was maximized with the 400 g/d of Ca-FA intake by 16.1% on 3.5% fat-corrected milk yield. Milk fat content and yield were increased by 3.20 (quadratically, $P = 0.04$) and 8.20% (quadratically, $P = 0.01$), respectively, with the Ca-FA addition. Similarly, the increase in milk fat content and yield were maximum by 7.90 and 18.9%, respectively, with the 400 g/d of Ca-FA intake. The Ca-FA supplemental levels decreased the content and yield of de novo milk FA by 21.7% (linearly, $P < 0.01$) and increased the content and yield of preformed milk FA by 10.0% (linearly, $P < 0.05$) and the increase in milk C16:0 content was 9.32% (linearly, $P < 0.01$). Milk-feed-ratio were decreased for de novo milk FA (C12:0 and C14:0), C16:0, and preformed milk FA (C18:0 and C18:1) (linearly, $P < 0.01$) with increasing Ca-FA intake. In conclusion, increasing Ca-FA intake increased milk and milk fat yield and responses were maximized with the 400 g/d of Ca-FA supplemental level. Data were analyzed using the GLIMMIX procedure of SAS University Edition (SAS Institute Inc., Cary, NC), with main effects of period and treatments, whereas buffaloes were designated as random effect in the model. Treatments were compared with linear and quadratic polynomial contrasts to examine the response surface for the level of Ca-FA. When a significant effect ($P \leq 0.05$) of dietary treatment was observed, means were compared using the Tukey's test.

Key Words: calcium soap, milk yield, buffalo

W118 Responses in performance and feed intake of early-lactation dairy cows supplemented with linseed oil coated with vegetable fat or extruded linseed. J. M. Ruiz-Rodriguez[1], M. Puyalto[2], J. J. Mallo[2], G. Elcoso[3], and A. Bach*[4,5], [1]*Department of Agrarian Production. Polytechnic University of Madrid, Madrid, Spain,* [2]*Norel S.A., Madrid, Spain,* [3]*Blanca from the Pyrenees, Hostalets de Tost, Spain,* [4]*Department of Ruminant Production, IRTA, Barcelona, Spain,* [5]*Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

The aim of the study was to evaluate the effects of supplementing extruded linseed or linseed oil coated with hydrogenated palm fatty acid distillates (HPFAD) on DMI and milk yield of early-lactation dairy cows. Sixty-three Holstein cows (591 ± 84 kg BW, 42 ± 24.8 DIM, 37.2 ± 10.6 kg of milk/d) were randomly distributed in 3 groups (n = 21) and exposed for 84 d to 3 treatments following a complete randomized design. Treatments consisted of supplementation with 500 g/d of HPFAD (CTR), 350 g/d of extruded linseed and 390 g/d of HPFAD (EXT), or 500 g/d of HPFAD coated linseed oil (HFL). Both, HFL and EXT diets provided the same amount of linolenic acid. Cows were fed a TMR (15.5% CP, 33.7% NDF, 1.65Mcal of NEl/kg; DM basis) twice daily. On a daily basis, BW, DMI, milk yield, and milk fat and protein contents were determined individually. Animal was the experimental unit and data were analyzed using a mixed-effects model for repeated measures. Feed intake was lower ($P < 0.01$) in HFL (22.8 ± 0.54 kg/d) than in CTR (24.9 ± 0.54 kg/d) or EXT (25.7 ± 0.54 kg/d) cows, and it was affected by an interaction ($P < 0.01$) between treatment and time due to a lower increase in DMI in HFL as the study progressed. Milk yield and milk protein content did not differ among treatments. Milk fat content ($P = 0.02$) was lower in EXT (3.33 ± 0.06%) compared with CTR (3.55 ± 0.06%) cows, with HFL cows (3.36 ± 0.06%) not differing with either treatment. Milk fat yield ($P < 0.01$) and ECM ($P = 0.02$) were affected by an interaction between treatment, week and parity with multiparous cows on CTR producing more fat and ECM at the beginning of the study and multiparous cows on HFL producing more fat and ECM toward the end. As a result, feed efficiency (ECM/DMI) was greatest ($P < 0.01$) in HFL (1.83 ± 0.04), with no differences between CTR (1.63 ± 0.04) and EXT (1.66 ± 0.04) cows. Although HFL cows had a lower DMI, yield performance and BW were not affected along the study. We conclude that HFL has the potential to improve FE through a decrease in DMI while sustaining milking performance.

Key Words: linseed, linolenic acid, palm fatty acid distillates

W119 Impact of feed intake and fiber digestibility on milk fatty acid profile and yield. J. de Souza*[1,2] and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined the impact of feed intake (DMI) and total-tract fiber digestibility (NDFd) on milk fatty acid (FA) profile and yield responses in dairy cows. Our analysis used individual observations (n = 808) from 169 Holstein cows from 11 studies. Diets (% DM) contained (mean ± SD) 30.1 ± 1.94 NDF, 26.9 ± 2.09 starch, and 4.12 ± 0.78 total FA. DMI averaged 27.2 kg/d (range 14 to 39 kg/d) and NDFd averaged 40.4% (range 28 to 58%). Milk FA were classified as: <16C (summation of FA lower than 16-carbon, de novo FA), 16C (summation of 16-carbon FA, mixed FA), >16C (summation of FA greater than 16-carbon, preformed FA), and are reported in g/100 g FA, g/100 g milk, and g/d. Mixed model regressions were developed taking into account experiment, period within experiment, and cow within experiment as random factors. Increasing DMI linearly increased <16C milk FA as g/100 g FA [$17.6 \pm 0.95 + 0.24 \pm 0.02 \times \text{DMI}$, $P < 0.001$], g/100 g milk [$0.25 \pm 0.03 + 0.02 \pm 0.002 \times \text{DMI}$, $P < 0.001$], and g/d [$157 \pm 5.89 + 7.57 \pm 0.95 \times \text{DMI}$, $P < 0.001$]. Increasing DMI linearly decreased >16C milk FA as g/100 g FA [$45.4 \pm 1.43 - 0.21 \pm 0.03 \times \text{DMI}$, $P < 0.001$] and g/100 g milk [$31.3 \pm 0.83 - 2.44 \pm 0.37 \times \text{DMI}$, $P < 0.001$]; however, increasing DMI linearly increased >16C milk FA as g/d [$451 \pm 50.5 + 5.39 \pm 1.20 \times \text{DMI}$, $P < 0.001$]. Increasing DMI was not associated with changes in 16C milk FA ($P > 0.50$). Increasing NDFd linearly increased <16C milk FA as g/100 g milk [$0.70 \pm 0.05 + 0.002 \pm 0.0009 \times \text{NDFd}$, $P = 0.05$]; however, NDFd was not associated with <16C milk FA as g/100 g FA ($P = 0.73$) or g/d ($P = 0.37$). Increasing NDFd linearly increased 16C milk FA as g/100 g FA [$32.5 \pm 1.61 + 0.08 \pm 0.02 \times \text{NDFd}$, $P < 0.001$], g/100 g milk [$0.98 \pm 0.06 + 0.005 \pm 0.001 \times \text{NDFd}$, $P < 0.001$], and g/d [$489 \pm 39.0 + 1.39 \pm 0.72 \times \text{NDFd}$, $P = 0.05$]. Increasing NDFd linearly decreased >16C milk FA as g/100 g FA [$44.4 \pm 1.73 - 0.09 \pm 0.02 \times \text{NDFd}$, $P < 0.001$]; however, NDFd was not associated with > 16C as g/100 g milk ($P = 0.97$) or g/d ($P = 0.41$). Results demonstrate that DMI is important for predicting the concentration and yield of de novo and preformed milk FA, while NDFd effects on milk FA depend upon the unit that these FA groups are reported in. The interpretation of relationships between DMI and NDFd with milk FA must consider the unit that milk FA are reported in.

Key Words: fatty acids, fiber digestibility, milk fat

W120 Degree of esterification and fatty acid profile of C16:0-enriched supplements impact fatty acid digestibility in lactating dairy cows: A meta-analysis. J. de Souza*[1,2], N. R. St-Pierre[2], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined apparent total-tract fatty acid (FA) digestibility of lactating dairy cows fed palmitic acid (C16:0)-enriched supplements differing in their degree of esterification and FA profile. Our analysis utilized individual observations (n = 385) of mid-lactation Holstein dairy cows from 7 Latin square design studies. Diets (% DM) contained (mean ± SD) 30.6 ± 3.66 NDF, 27.1 ± 2.16 starch, and 4.0 ± 0.97 total FA. C16:0 supplements were classified based on the degree of esterification and FA profile as follows: a) C16:0_FFA (prilled free FA supplements containing ~85% C16:0 and ~6.0% C18:1); b) C16:0_TAG (prilled triglyceride supplements containing ~80% C16:0 and ~13% C18:1); and c) C16:0_blend (blend of free FA and Ca-salt supplements containing ~80% C16:0 and ~10% C18:1). Data were analyzed using a mixed model including treatment as a fixed effect and study, cow (study), and period (study) as random effects. To estimate supplement FA digestibility, we used a Lucas test with a regression of the intake of supplemental FA (g/d) on supplemental absorbed FA (g/d); slopes indicate true digestibility and intercepts endogenous synthesis. Apparent total-tract FA digestibility (FAd; %) for control diets (no supplemental fat) was 76.1 ± 0.63 (mean ± SEM) and was not different across studies ($P = 0.85$). We did not observe differences in FAd between control and C16:0_blend supplemented diets (76.1 ± 0.63 vs. 76.3 ± 0.89, $P = 0.92$). In contrast, compared with control, C16:0_FFA (76.1 ± 0.63 vs. 71.9 ± 0.66; $P < 0.01$) and C16:0_TAG (76.1 ± 0.63 vs. 69.8 ± 0.77, $P < 0.01$) supplemented diets decreased FAd. C16:0_blend supplemented diets increased FAd compared with C16:0_FFA ($P < 0.01$) and C16:0_TAG ($P < 0.01$) supplemented diets. Additionally, C16:0_FFA supplemented diets increased FAd compared with C16:0_TAG ($P < 0.05$) supplemented diets. Digestibility for supplemental fats estimated by Lucas test were 69.2 ± 1.26, 60.6 ± 1.47, and 76.4 ± 1.15 for C16:0_FFA, C16:0_TAG, and C16:0_blend, respectively. Our results demonstrate that the degree of esterification and FA profile of C16:0-enriched supplements impact total FA digestibility.

Key Words: digestibility, meta-analysis, palmitic acid

W121 The yields of individual de novo and preformed milk fatty acids are differentially regulated in response to changes in the intake of palmitic acid in dairy cows. J. de Souza*[1,2] and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*Perdue AgriBusiness, Salisbury, MD.*

We determined how the intake of palmitic acid (C16:0) alters the yield of individual milk fatty acids (FA) in dairy cows. Our analysis used individual observations (n = 1202) from 212 Holstein cows from 14 studies. Diets (% DM) contained (mean ± SD) 30.6 ± 3.66 NDF, 27.1 ± 2.16 starch, and 4.0 ± 0.97 total FA. Intake of C16:0 averaged 371 g/d and ranged from 60 to 923 g/d. Milk FA were determined by gas-liquid chromatography. Mixed model regressions were developed taking into account study, period within study, and cow within study as random factors. All equations report the relationship between C16:0 intake (g/d) and yield of milk FA (g/d). Overall, no relationship was observed between the intake of C16:0 and the yield of de novo milk FA (<16-carbon FA; $P = 0.60$) or the yield of preformed milk FA (>16-carbon FA; $P = 0.54$); however, increasing 16:0 intake linearly increased the yield of 16-carbon milk FA [$468 \pm 3.23 + 0.23 \pm 0.008 \times 16{:}0$ intake, $P < 0.001$, $R^2 = 0.43$]. Among individual de novo synthesized milk FA, increasing C16:0 intake linearly increased the yield of milk C4:0 [$43.6 \pm 0.12 + 0.092 \pm 0.0003 \times 16{:}0$ intake, $P < 0.001$, $R^2 = 0.45$] and C6:0 [$26.8 \pm 0.07 + 0.008 \pm 0.00003 \times 16{:}0$ intake, $P < 0.001$, $R^2 = 0.32$]. No relationship was observed between the intake of C16:0 and yield of milk C8:0 ($P = 0.95$) or C10:0 ($P = 0.32$). Increasing C16:0 intake linearly decreased the yield of milk C12:0 [52.1 ± 0.16 − 0.008 ± 0.0004 × 16:0 intake, $P < 0.001$, $R^2 = 0.24$], and C14:0 [166 ± 0.40 - 0.008 ± 0.00009 × 16:0 intake, $P < 0.001$, $R^2 = 0.25$]. Among individual preformed milk FA, increasing C16:0 intake linearly increased the yield of milk *cis*-9 C18:1 [272 ± 1.38 + 0.04 ± 0.003 × 16:0 intake, $P < 0.001$, $R^2 = 0.18$] and tended to decrease the yield of milk C18:0 [145 ± 10.8 - 0.009 ± 0.0005 × 16:0 intake, $P = 0.06$, $R^2 = 0.10$]. Our results demonstrate that increasing dietary intake of C16:0 improves milk fat yield due to an increase in the yield of 16-carbon milk FA. Although C16:0 intake does not affect the yield of total de novo FA or preformed FA, specific changes in the yield of some individual de novo and preformed milk FA occurs, which is likely associated with mammary gland plasticity to maintain milk fluidity.

Key Words: fatty acids, meta-analysis, milk fat

W122 Milk fatty acids profile, blood serum, and oocyte quality of early-lactation dairy cows supplemented with linseed oil coated with vegetable fat or extruded linseed. J. Ruiz Rodríguez[1], M. Puyalto[2], J. Mallo[2], G. Elcoso[3], and A. Bach*[4,5], [1]*Department of Agrarian Production, Polytechnic University of Madrid, Madrid, Spain,* [2]*Norel S.A., Madrid, Spain,* [3]*Blaca from the Pyrenees, Hostalets de Tost, Spain,* [4]*Department of Ruminant Production, IRTA, Caldes de Montbui, Spain,* [5]*ICREA, Institució Catalana de Recerca i Estudis Avançats, Barcelona, Spain.*

The aim of the study was to evaluate the effects of supplementing extruded linseed or linseed oil coated with hydrogenated palm fatty acid distillates (HPFAD) on milk fatty acid (FA) profile, oocyte quality and glucose, immunoglobulin IgG1 and NEFA blood serum of early-lactation dairy cows. Sixty-three Holstein cows (591 ± 84 kg BW, 42 ± 24.8 DIM, 37.2 ± 10.6 kg of milk/d) were randomly distributed in 3 groups (n = 21) and exposed for 84 d to 3 treatments following a complete randomized design. Treatments consisted of supplementation of 500 g/d of HPFAD (CTR), 350 g/d of extruded linseed and 390 g/d of HPFAD (EXT), or 500 g/d of HPFAD coated flaxseed oil (HFL). Both, HFL and EXT provided the same amount of C18:3. Cows were fed a TMR (15.5% CP, 33.7% NDF, 1.65Mcal of NEl/kg; DM basis) twice daily. Milk FA profile was determined at d 0, 5, 10, 28, 56 and 84. Blood glucose, NEFA, and IgG1 concentrations were determined at d 0, 28, 56 and 84. Oocyte pickup was performed at d 50 and oocyte quality was evaluated morphologically. Animal was the experimental unit and milk FA profile and serum results were analyzed using a mixed-effects model for repeated measures. Oocyte quality was analyzed using ordinal logistic regression. Results for FA are shown in Table 1. Serum glucose concentration was steady throughout the study in HFL cows, but it progressively decreased in CTR and EXT cows. Serum NEFA concentrations progressively decreased as lactation progressed. Serum IgG1 concentration in EXT cows was low except for an increase at d 84, whereas it remained steady in CTR and HFL cows. The odds of having second quality category oocytes was greater (1.62 more chances) in HFL and EXT compared with CTR. We conclude that HFL and EXT improve milk FA profile and show potential to improve oocyte quality in early-lactation cows.

Table 1 (Abstr. W122). Milk FA composition per treatment

Fatty acid, %	CTR	EXT	HFL	SE	*P*-value
C18:3	0.48^a	0.64^b	0.67^b	0.05	<0.0001
C20:5	0.059^b	0.070^a	0.069^a	0.001	0.01
Total unsaturated	3.96^b	4.35^a	4.25^a	0.07	<0.01
Total n-3	0.60^b	0.80^a	0.83^a	0.01	<0.0001

Key words: linseed, linolenic acid, palm fatty acid distillates (PFAD)

W123 Lipid-coat protection of sodium selenite and copper sulfate from microbial fermentation impacts VFA synthesis and nitrogen metabolism in a dual-flow continuous culture system. J. A. Arce-Cordero*[1], H. F. Monteiro[1], A. L. Lelis[1], R. Restelatto[2], L. R. Lima[3], V. L. N. Brandao[1], L. G. Silva[1], H. Leclerc[4], and A. P. Faciola[1], [1]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [2]*Department of Animal Sciences, Federal University of Parana, Curitiba, PR, Brazil,* [3]*Department of Animal Sciences, Federal University of Mato Grosso, Cuiaba, MT, Brazil,* [4]*Jefo, St. Hyacinthe, QC, Canada.*

Antimicrobial effects of $CuSO_4$ and Na_2SeO_3 have been reported in the literature. As common sources of Cu and Se for ruminants, we aimed to evaluate the effects of lipid-coat protection of $CuSO_4$ and Na_2SeO_3 on microbial fermentation in a dual-flow continuous culture system. We used 8 fermenters in a 4 × 4 duplicated Latin-square design with a 2 × 2 factorial arrangement of treatments (2 mineral sources × 2 protection levels). Treatments were: 1) unprotected Cu and Se, 2) protected Cu + unprotected Se, 3) protected Se + unprotected Cu, 4) protected Cu and Se. Main effects of protection of Cu, Se, and their interaction were tested. Fermenters were fed 106 g DM/d and all diets had the same nutrient composition (16% CP, 31% NDF, 29.5% starch, 1.6 MCal ENl/kg, 18 ppm Cu, and 0.4 ppm Se). Experimental period length was 10 d (7 d of adaptation and 3 d for sample collections). Daily pooled samples of effluents were analyzed for VFA, NH_3-N, soluble Cu and Se, ruminal true digestibility of nutrients, and flows (g/d) of total N, NH_3-N, non-ammonia N (NAN), bacterial N, dietary N, RDP-N supply and bacterial efficiency. Kinetics of pH, VFA, and NH_3-N was evaluated as repeated measures in samples collected daily at 0, 1, 2, 4, 6, and 8 h post morning feeding. Protection of Cu, Se, or their interaction did not affect pH and NH_3-N kinetics. Protection of Se tended to reduce: NH_3-N effluent concentration ($P = 0.09$), NH_3-N flow ($P = 0.07$), and RDP-supply ($P = 0.06$); and tended to increase flows of: NAN ($P = 0.06$) and dietary N ($P = 0.06$). For VFA kinetics, protection of Cu reduced acetate % ($P = 0.02$), increased butyrate % ($P = 0.04$), and tended to decrease acetate:propionate ($P = 0.06$). Protecting both Cu and Se reduced isovalerate % ($P = 0.05$) and tended to reduce BCVFA % ($P = 0.07$). Our results suggest that lipid-coat protection of $CuSO_4$ and Na_2SeO_3 might benefit ruminal fermentation through increased efficiency of VFA synthesis and N utilization, respectively.

Key Words: minerals, ruminal fermentation, selenium

W124 Bioequivalence test of neutral detergent fiber analysis with or without an acetone wash of feed ingredients, orts, and feces from cows fed fat-supplemented diets.

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We evaluated whether neutral detergent fiber (NDF) and indigestible NDF (iNDF) determination without an acetone wash step of feed, orts, and feces from cows fed fat supplemented diets is equivalent to the original method using acetone. Thirty-two samples of feeds, orts, and feces were obtained from a 4 × 4 Latin square design study with 8 cows that determined effects of fatty acid (FA) supplements with different ratios of stearic (SA) and oleic (OA) acids on nutrient digestibility. Treatments were a non-FA supplemented control diet (CON) and 3 diets incorporating FA supplements (1.5% DM) containing 50% SA + 10% OA, 40% SA + 20% OA, and 30% SA + 30% OA. Analysis of NDF proceeded with heat-stable α-amylase, sodium sulfite, filtering process on glass crucible, and washing step (WS) with water and acetone (AC) or with water only (WA). Ash content was excluded from the NDF. A 240-h in vitro fermentation was used to determine iNDF. Bioequivalence testing with a 90% confidence interval (CI) was used to verify if NDF determination using WA is equivalent to AC. For this, it was determined if the lower (L) and upper (U) limit of the mean difference (MD) falls into an equivalence interval $< -2 < +2$. Statistical analysis was performed using PROC MIXED of SAS, including fixed effects of diet, WS, their interactions, and random effects of cow and period. Overall, dietary NDF content determined from AC and WA analysis of feed ingredients was 32.4% and 32.3% (SEM ± 0.03), respectively. NDF content in control (33.3%; SEM ± 0.04) or FA treatments (32.1%; SEM ± 0.03) was not affected by WS. NDF intake did not differ between AC and WA (10.0 vs. 9.98 kg/d, $P = 0.84$). Interactions between WS and FA treatments were tested for NDF and iNDF in orts and feces; all interactions were bioequivalent. Independent of treatment, WA was equivalent to AC for NDF content of orts (MD = 0.46; L = −0.26; U = 1.18) and feces (MD = 0.97; L = 0.46; U = 1.48), and for iNDF content of orts (MD = 0.36; L = −0.14; U = 0.87) and feces (MD = 0.60; L = −0.43; U = 1.64). In conclusion, NDF analysis with or without acetone wash was equivalent for determination of NDF intake and the content of NDF and iNDF of orts and feces of dairy cows fed FA supplemented diets.

Key Words: acetone, bioequivalence, NDF

W125 Oleic acid supplementation alters adipose tissue lipolytic responses and insulin sensitivity in early-lactation dairy cows.

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We previously reported that postpartum supplementation of oleic acid (*cis*-9 C18:1; OA) increased milk yield and reduced plasma NEFA and body weight loss; however, the mechanisms driving these responses are unknown. In rodent adipose tissue (AT), OA enhances lipogenesis and potentiates the effects of insulin; however, responses to OA supplementation in bovine AT have not been characterized. Our objective was to determine the effects of postpartum OA supplementation on AT responses to isoproterenol and insulin. Multiparous cows were infused abomasally with 60 g/d of OA (n = 6) or vehicle (VEH, n = 6) from 1 to 15 d postpartum. Subcutaneous AT (SCAT) explants were collected at −14, +6, and +12 d relative to parturition and incubated for 3 h at 37°C with 0 μM (CON) or 1 μM of β adrenergic agonist isoproterenol (ISO) to induce lipolysis. The anti-lipolytic effect of insulin at low (0.2 μg/L, INL) and high concentration (1 μg/L, INH) was determined during ISO stimulation. Lipolytic responses were evaluated by quantification of glycerol release. ISO responses are reported as % increase over CON. Insulin responses are reported as % decrease over ISO. Statistical analysis was performed using a mixed linear model, considering as fixed effects OA infusion, time, treatments and their interaction. Across treatments and sample time points, ISO increased the lipolytic responses by 300 ± 25% compared with CON ($P < 0.001$). Lipolytic response to ISO was not different between VEH and OA groups before infusions (−14 d). Compared with CON, OA infusion reduced lipolytic response to ISO at +6 d (VEH = 177 ± 6.5% and OA = 167 ± 6.5%; $P = 0.06$) and at +12 d (VEH = 258 ± 7.8% and OA = 157 ± 7.8%; $P = 0.02$). Compared with ISO, INL and INH inhibited lipolysis by −170 ± 2.5% and −231 ± 2.5%, respectively ($P < 0.001$). The anti-lipolytic effect of INL and INH did not differ between VEH or OA groups before infusions (−14 d). At +6 and +12 d, INH had a stronger anti-lipolytic response in OA (−222 ± 5.5% and −238 ± 7.2%) compared with VEH (−106 ± 5.5% and −128 ± 7.2%; $P < 0.05$), respectively. Results demonstrate that oleic acid supplementation immediately postpartum reduces lipolytic responses and improves insulin sensitivity of AT in early lactation dairy cows.

Key Words: lipolysis, oleic acid

W126 Impact of feeding a palmitic enriched supplement on production responses of mid-lactating Jersey and Holstein cows.

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A study was conducted to evaluate the effects of feeding a palmitic enriched supplement on production responses of mid-lactating Jersey and Holstein cows. Eighty cows (40 Holstein and 40 Jersey) were used in a randomized complete block design with a split-plot arrangement, where the main plot was breed and subplot treatment. Cows within each breed were assigned to 1 of 2 treatments: 1) control diet without fat supplement, or 2) control diet plus a palmitic acid enriched supplement fed at 1.5% of diet dry matter. Cows were milked in 2 robotic milking units and treatments were offered through the robots. The diets contained 18.0% forage NDF, 29% NDF, 22.4% starch and 17.3% CP. Treatment period was 6 weeks with the final 3 weeks used for data and sample collection. The statistical model included the random effect of block and cow nested within breed, and the fixed effect of treatment, breed, time and their interactions. Preliminary milk yield was used as a covariate. Differences were declared at $P \leq 0.05$ and tendencies at $P \leq 0.10$. Compared with control, palmitic acid increased milk fat yield (1.36 vs. 1.27 kg/d; SEM = 0.06; $P = 0.03$), and tended to increase FCM (35.6 vs. 33.8 kg/d; SEM = 1.86, $P = 0.09$) as well as ECM (35.8 vs. 34.2 kg/d; SEM = 1.82, $P = 0.10$). There were no effects of palmitic acid on milk yield ($P = 0.68$), milk protein yield ($P = 0.22$), milk lactose yield ($P = 0.74$), BCS ($P = 0.76$) or BW change ($P = 0.91$). Compared with Holstein cows, Jersey cows had lower milk production (29.6 vs. 32.7 kg/d; SEM = 1.95, $P = 0.02$) and milk lactose yield (1.58 vs. 1.44 kg/d; SEM = 0.10, $P = 0.04$). Jersey cows tended to have greater milk fat yield (1.37 vs. 1.27 kg/d; SEM = 0.06, $P = 0.10$) compared with Holstein cows. There was a breed effect on BW change ($P = 0.03$); Holstein cows gained 0.385 kg/d during the experimental period, while Jersey cows gained 0.145 kg/d. There were no effects of breed on milk protein yield ($P = 0.85$), FCM ($P = 0.97$), ECM ($P = 0.99$), and BCS ($P = 0.59$). No interaction treatment × breed was detected for the variables analyzed. Overall, feeding palmitic acid enriched supplement increased milk fat yield on both Jersey and Holstein cows, and the response tended to be better in Jersey than Holstein cows.

Key Words: body weight, fat supplementation, milk fat

W127 Heat treatment from pelleting or extrusion did not impact performance of *Saccharomyces cerevisiae* fermentation products on volatile fatty acid production in an in vitro rumen model. C. Reedy*, I. Yoon, J. Butler, and T. Werner, *Diamond V, Cedar Rapids, IA.*

The objective of this study was to evaluate the effect of *Saccharomyces cerevisiae* fermentation products, Diamond V Original XPC (XPC), XPC Ultra (XPC Ultra), and NutriTek (NutriTek) on in vitro volatile fatty acid (VFA) production before and after going through either pelleting or extrusion. XPC Ultra was pelleted at 80°C for 60 s. XPC and NutriTek were extruded at 135°C for 30 s and dried at 145°C for 30 min. Test products were added to each serum bottle relative to their recommended feeding rates - XPC (14 g/d), XPC Ultra (7 g/d), and NutriTek (19 g/d) - along with 0.3 g of cellulose as substrate. Each serum bottle was inoculated with 40 mL of buffered rumen fluid from cows on a diet consisting of 70% forage and 30% concentrate and was incubated for 24 h. Ten replicates were run per treatment. Data was analyzed using the GLM model of JMP. Means were compared using Tukey test and significance was defined as $P = 0.05$. After incubation, VFA production was measured and reported in Table 1.Results showed that both heat-treated and untreated products significantly increased ruminal VFA production over Control while showing no difference between heat-treated and untreated products. In conclusion, heat-treatment had little effect on the performance of the products tested in an in vitro rumen model, which suggests that *Saccharomyces cerevisiae* fermentation products could go through pelleting or extrusion and fully express their potentials in processed feeds requiring heat treatment.

Key Words: Diamond V Original XPC, pelleting, extrusion

W128 Nutrient digestibility and production responses of lactating dairy cows when commercially available saturated fatty acid supplements are included in diets: A meta-analysis. J. M. dos Santos Neto*[1,2], J. de Souza[1], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*University of São Paulo, Piracicaba, São Paulo, Brazil.*

We evaluated the effects of commercially available saturated fatty acid (SFA) supplements on nutrient digestibility and production responses of lactating dairy cows. The database was formed from 30 peer-reviewed publications in which SFA supplements were fed at $\leq$3% diet DM. Supplements were classified as mixed SFA (MIX: $\geq$80% C16:0+C18:0) or palmitic acid-enriched (PALM: $\geq$80% C16:0) supplements and compared with nonfat supplemented diets used as control. Meta-analysis was performed using PROC MIXED of SAS, including fixed effects of fat source, and random effects of study, and its interaction with treatment. Studies were weighted based on the inverse of the sum of both the within and among study variance. There was no interaction between treatments and experimental design (randomized vs. crossover/Latin square; $P > 0.93$). Overall, SFA supplementation did not affect DMI ($P = 0.68$), increased milk (1.36 kg/d, $P < 0.01$) and fat (0.07 kg/d, $P < 0.01$) yields, tended to increase protein yield (0.03 kg/d, $P = 0.06$), increased milk fat content (0.12%, $P = 0.01$), decreased milk protein content (0.04%, $P = 0.03$), tended to increase DM digestibility (1.19%, $P = 0.07$), increased NDF digestibility (3.19%, $P < 0.01$), and did not affect FA digestibility ($P = 0.29$). Compared with control, MIX did not affect DMI ($P = 0.64$), increased milk (1.19 kg/d, $P < 0.01$) and fat (0.05 kg/d, $P = 0.05$) yields, did not affect protein yield ($P = 0.34$) or fat content ($P = 0.34$), tended to decrease protein content (0.06%, $P = 0.06$), and had no effect on nutrient digestibility (all $P > 0.30$). Compared with control, PALM did not affect DMI ($P = 0.19$), increased milk (1.54 kg/d, $P = 0.04$) and fat (0.10 kg/d, $P < 0.01$) yields, tended to increase protein yield (0.04 kg/d, $P = 0.09$), increased fat content (0.17%; $P < 0.01$), had no effect on protein content ($P = 0.22$), and increased DM and NDF digestibility by 1.56% ($P = 0.02$) and 4.79% ($P < 0.01$) units, respectively. PALM had no effect on FA digestibility ($P = 0.53$). Overall, SFA supplementation increased yields of milk and milk fat without affecting DMI, with differences for each SFA supplement type. PALM increased DM and NDF digestibility but MIX did not impact nutrient digestibility.

Key Words: digestibility, fat supplementation, meta-analysis

W129 The effect of saturated fatty acid supplements in dairy cow diets on odd- and branched-chain fatty acids in milk fat: A meta-analysis and meta-regression. J. M. dos Santos Neto*[1,2], J. de Souza[1], and A. L. Lock[1], [1]*Michigan State University, East Lansing, MI,* [2]*University of São Paulo, Piracicaba, São Paulo, Brazil.*

There are few studies reporting the effects of saturated fatty acid (FA) supplementation on odd- and branched-chain fatty acids (OBCFA)

Table 1 (Abstr. W127). Ruminal VFA production (mM) of untreated and heat-treated products

Product	Treatment	Acetate	Propionate	Butyrate	Total VFA
XPC Ultra	Control	8.55^{a}	1.91^{a}	0.77^{a}	11.28^{a}
	Untreated	11.26^{b}	2.91^{b}	0.94^{b}	15.20^{b}
	Pelleted	11.28^{b}	2.95^{b}	0.95^{b}	15.24^{b}
	SEM	0.13	0.03	0.02	0.18
XPC	Control	11.34^{a}	2.51^{a}	1.61^{a}	15.56^{a}
	Untreated	12.84^{b}	3.00^{b}	1.78^{b}	17.75^{b}
	Extruded	12.94^{b}	3.02^{b}	1.73^{b}	17.80^{b}
	SEM	0.19	0.03	0.02	0.24
NutriTek	Control	10.77^{a}	2.28^{a}	1.14^{a}	14.29^{a}
	Untreated	14.78^{b}	3.67^{b}	1.51^{b}	20.12^{b}
	Extruded	14.71^{b}	3.54^{b}	1.45^{b}	19.86^{b}
	SEM	0.34	0.06	0.02	0.43

^{a,b}Means within a column within product without a common letter differ by $P = 0.05$.

in milk fat or the relationship of nutrient intake and digestibility on OBCFA in milk fat. We used a meta-analysis to evaluate the effects of dietary inclusion of mixed FA (MIX: ~34% C16:0 and ~50% C18:0) or palmitic acid-enriched (PALM: ≥80% C16:0) supplements on OBCFA in milk fat of dairy cows. The meta-regression was used to evaluate the relationships of DMI and NDF digestibility (NDFd) with yields OBCFA in milk fat. OBCFA were grouped as follows: odd linear FA (C13:0 + C15:0 + C17:0); iso FA (iso C13:0 + iso C14:0 + iso C15:0), and anteiso FA (anteiso C13:0 + anteiso C15:0). The data set was assembled with individual cow data from 3 studies at Michigan State University, including individual observations of 88 Holstein cows (74 multiparous and 14 primiparous). PALM (n = 107) and MIX (n = 47) were compared with non-FA supplemented diets as control (n = 105). Statistical analyses were performed using the PROC MIXED of SAS, including study as random effect. PALM reduced the yield of odd linear, iso, and anteiso FA by 1.58 ($P = 0.05$), 0.27 ($P = 0.05$) and 0.50 g/d ($P < 0.01$) respectively; a total decrease in OBCFA of 2.26 g/d ($P = 0.03$). MIX did not affect odd linear FA ($P = 0.41$), decreased iso FA (0.36 g/d; $P = 0.04$), tended to decrease anteiso FA (0.41 g/d; $P = 0.07$), and had no effect on total OBCFA ($P = 0.71$). We observed a similar pattern of results for OBCFA content compared with yield of OBCFA in milk fat. There was a positive linear relationship between DMI (kg/d) and the yield of total OBCFA (R2 = 0.19; $P < 0.01$). There was a tendency for a quadratic relationship between NDFd and yield of iso FA (R2 = 0.04; $P = 0.06$), with the highest value for iso FA yield obtained at 44.8% NDFd. There was no relationship among the other OBCFA groups and NDFd. In conclusion, PALM supplementation decreased the content and yield of all OBCFA groups. MIX supplementation only decreased the yield of iso FA and the content of anteiso FA. DMI increased OBCFA yield.

Key Words: fat supplementation, meta-analysis, odd- and branched-chain fatty acid

W130 Effect of betaine supplementation on total serum fatty acids profile in mid-lactating Holstein dairy cows.

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Betaine is a product of choline oxidation in the body and an ingredient of wheat and sugar beets. Betaine can donate one methyl group to transfer homocysteine into methionine, which is involved in the phosphatidylethanolamine *N*-methyltransferase (PEMT) pathway. We hypothesized that betaine supplementation affects the serum fatty acids (FA) profile in mid-lactation dairy cows. There were 21 mid-lactation dairy cows assigned to a 3 × 3 Latin square design with 3 periods of 28 d each and 3 treatments of betaine (0, 100, and 200 g/d). Milk samples collected on d 21 and d 28 and blood samples obtained on d 26 to 28 of each period were used for FA analysis via gas chromatography with flame ionization detector and an Agilent HP-88 column (100 m × 0.25 mm with 0.2-µm film thickness, Agilent Technologies). Individual FA was identified by comparison to the standard mixture Supelco 37 FAME (Supelco, Bellefonte, PA). Data were analyzed using the Proc Mixed of SAS with significance declared at $P \leq 0.05$ and trends at $P \leq 0.1$. Results showed that no change was observed in the content of total serum saturated FA (40.5, 40.4, and 40.3 ± 0.6% for 0, 100, and 200 g betaine, respectively; $P = 0.96$). The total serum monounsaturated FA decreased with betaine supplementation (16.2, 15.2, and 14.9 ± 0.32%, for 0, 100, and 200 g betaine, respectively; $P = 0.01$). Serum FA profile showed a decline in the n-6 to n-3 ratio (6.80, 7.07, and 6.50 ± 0.16%, for 0, 100, and 200 g betaine, respectively; $P = 0.04$). Results showed however, that milk FA profile did not differ among treatments (0, 100, and 200g betaine/d per cow, respectively). Overall, our study demonstrated that betaine supplementation affected the total serum FA profile in mid-lactation dairy cows without affecting the milk FA profile.

Key Words: lactating dairy cow, dietary betaine, serum fatty acid

W131 Supplementing rumen-protected linseed oil: ovarian dynamics.

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The objective of this study was to evaluate the effect of supplementing calcium salts of linseed oil (AG-Ca; rich in linolenic acid (18:3 (n-3)) on the ovarian dynamics in dairy cows. In a 10-week assay, 38 primiparous and multiparous Holstein dairy cows (with 55 ± 18.1 DIM, 594 ± 96.8 kg of BW, 2.71 ± 0.4 of BCS, 2 ± 1.4 lactation number and milk production of 38 ± 9.4 kg/d) were used in a complete randomized block design. Cows were assigned to one of 2 treatments (O3: AG-Ca supplementation (0.85 kg/d AG-Ca) or CON: no fat) and were blocked by DIM, number of lactations, BW, milk production and corpus luteum presence. Isoenergetic diets were formulated to meet requirements of a Holstein dairy cow of 650 kg BW with 40 DIM, producing 40.0 kg/d of milk with 3.6% fat (NRC, 2001). A timed artificial insemination (TAI) at 80 DIM preceded by a PreSynch (14/12) + OvSynch protocol was implemented for the first service and ovarian dynamics were studied during the OvSynch and TAI. A portable ultrasound Aquila Pro (Pie medical) B mode in real time, with lineal transducer of 8 MHz, was used to determine the presence and sizes of ovarian structures (follicles (FOL) and corpora lutea (CL)), and the gynecological condition. Supplementation does not change milk production and energy status of the cows (Iorio, 2018). After PreSynch, 72.22% of cows of O3 and 75% of CON, presented CL. Linseed oil supplementation had neither effect on the emergence of a new follicular wave (O3: 2.22 ± 1.20 d vs. CON: 2.78 ± 1.79 d; $P = 0.217$) nor the size of preovulatory follicles (O3: 16.66 mm vs. CON: 16.27 mm; $P = 0.707$). Conversely, linseed oil supplementation had effect on the volume of the main CL 6 d after TAI (O3: 17.60 mm vs. CON: 13.67 mm; $P = 0.0218$). Data were analyzed using the MIXED of SAS with significance declared at $P \leq 0.05$. These results probably highlight the importance of linseed oil supplementation on FOL and CL development, which may lead to better pregnancy indexes.

Key Words: linolenic acid, ovarian dynamics, dairy cow

W132 Assessing recovery of ^{13}C-enriched branched-chain VFA and branched-chain AA into rumen bacterial fatty acids.

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To test if incorporation profile of branched-chain VFA (BCVFA) or AA (BCAA) influenced elongation into bacterial FA, we dosed ^{13}C-labeled BCVFA and BCAA into batch cultures of mixed rumen microbes. Treatments were 1) Control, 2) ^{13}C-enriched isovalerate, isobutyrate, 2-methylbutyrate, and valerate (1 mM final concentration each), 3) ^{13}C-enriched Ile, Leu, and Val (1 mM final concentration each), or 4) a 50:50 mix of BCVFA and BCAA (MIX). In 2 replicate tubes, 30 mL of a 1:4 dilution of blended rumen fluid in artificial saliva buffer was added anaerobically to 50-mL tubes with either 0.25 g of corn + 0.25 g of alfalfa hay (AHC) or 0.25 g of corn + 0.25 g of orchardgrass hay (OHC). Total FA in bacteria harvested at 24 h of incubation was not affected by treatment or treatment x feed interaction ($P > 0.55$). Adding BCVFA or BCAA increased ($P < 0.05$) 13:0, *iso* 14:0, and total odd chain FA (OCFA; g/ 100 g total FA). Total dose recovered in FA was not affected ($P > 0.15$) by BCAA (0.169%), MIX (0.193%), or BCVFA (0.206%). Linear (L) and quadratic (Q) contrasts were evaluated with PROC MIXED in SAS (2 runs as random effect) for 1) BCAA, 2) MIX, and 3) BCVFA. For dose recovery in 13:0, *iso* 16:0, 17:0, and *iso* 17:0, there was a L increase ($P < 0.05$) in enrichment for BCVFA substitution over BCAA (Q was $P > 0.15$). There was a treatment x feed interaction ($P < 0.02$) for *anteiso* 14:0, 15:0, *anteiso* 17:0, and OCFA enrichment. With AHC, *anteiso* 14:0 increased enrichment linearly ($P < 0.03$) at a decreasing rate ($P = 0.12$ for Q) with BCVFA substituting for BCAA. For 15:0 and total OCFA, L and Q were both $P < 0.01$, with enrichment increasing linearly at a decreasing rate with BCVFA substituting for BCAA. With OHC, 15:0, *anteiso* 17:0, and total OCFA enrichment increased linearly ($P < 0.03$; $P > 0.15$ for Q) as BCVFA replaced BCAA. Enrichment of *anteiso* 14:0 was highest with MIX ($P > 0.15$ for L; $P < 0.01$ for Q). The bacterial FA profile was similar among treatments, but the enrichment of the OCFA and several BCFA was greatly increased by dosing BCVFA over dosing BCAA, or a MIX, supporting a potential benefit from higher concentration of elongation primers regardless of substrate.

Key Words: branched-chain VFA, branched-chain AA

W133 Nutrient digestibility and production responses of lactating dairy cows when calcium salts of palm fatty acids are included in diets: A meta-analysis.

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We performed a meta-analysis to compare the effects of feeding calcium salts of palm fatty acids (PFAD; ~50% C16:0 and ~50% unsaturated 18-carbon fatty acids) to a nonfat supplemented diet used as control on nutrient digestibility (n = 35) and production responses (n = 121) of lactating dairy cows. Furthermore, the interaction between PFAD supplementation and experimental design (crossover/Latin square [CL] vs. randomized as block or completely randomized [RD]) were examined. The database was formed from 35 peer-reviewed publications with PFAD supplemented at ≤3% diet DM. These publications comprised 13 CL and 22 RD studies. For the 35 digestibility observations, 18 were from CL and 17 from RD; for the 121 production observations, 41 were from CL and 80 from RD. The meta-analysis was performed using PROC MIXED of SAS, including fixed effects of treatment (PFAD and CONTROL), design, and the interaction between treatment and design. Studies were weighted based on the inverse of the sum of both the within and among study variance. We did not observe interactions between treatment and study design for any variable (all $P > 0.30$). Compared with control, PFAD reduced DMI (0.66 kg/d, $P = 0.03$) and milk protein content (0.05%, $P = 0.04$), and increased milk yield (1.42 kg/d, $P = 0.02$) and fat yield (0.08 kg/d, $P < 0.01$). There were no treatment differences for protein yield ($P = 0.95$) or milk fat content ($P = 0.66$). Although PFAD did not affect DM digestibility ($P = 0.91$), PFAD increased NDF digestibility (2.45% units; $P < 0.01$) compared with control. Compared with RD, CL increased DMI (2.14 kg/d, $P = 0.05$) and tended to increase fat yield (0.29 kg/d, $P = 0.06$). Study design did not affect any other variables (all $P > 0.14$). In conclusion, PFAD reduced DMI, increased the yields of milk and milk fat, decreased protein content, and increased NDF digestibility. We observed no interactions between study design and dietary treatment, indicating no reason for the restrictive use of crossover/Latin square designs in fat supplementation studies and meta-analysis.

Key Words: calcium salt, experimental design, meta-analysis

W134 Screening unsaturated fat sources included to low- and high-forage diets with different fat dietary concentration using an in vitro gas production system.

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Fat inclusion can increase energy density of diets fed to ruminants, but detrimental effects to nutrient digestion have been reported. There is evidence that not all fat sources have this negative response and this effect can vary depending on the forage concentration in the diet. The objective of this experiment was to determine the effects of including different types of unsaturated fats to high and low forage diets in vitro digestibility and fermentation. An experiment was conducted using an in vitro gas production (GP) system. Treatments included either low forage (LF; 35%) or high forage (HF; 70%) with 2 dietary fat concentrations (6 or 9%) and of 7 different fat source treatments (control + 6 different types of unsaturated fat sources). The control diet had a basal level of fat in the diet [3% fat (0% fat inclusion); and fat sources were added to attain 6% or 9% fat and consisted of (Coconut oil, CO; Poultry fat, PF; Palm oil, PO; Palm kernel oil, PKO; Ca Salts, MEG; Soybean oil, SOY)]. Modules (GP) were randomly assigned to treatments in a 2 × 2 × 7 factorial design and incubated for 2 24 h runs. A randomized complete block design with 4 replicates per treatment and 2 runs was used. Run was the blocking factor. Data were analyzed using the MIXED procedure of SAS. Apparent digestibility (AD) for DM, OM, NDF, ADF and true dry matter digestibility (TDMD) were higher in LF-treatment. Cumulative gas produced in mL was greater in LF ($P = 0.01$). Fat concentration had no effect on AD, but the 6% fat had a higher gas production ($P = 0.03$; 109.6 vs. 103.5 mL ± 2.44). The CO had the highest DM AD followed by SOY and PF (54.5, 51.8, and 50.6% ± 0.48) and cumulative gas production followed same pattern. The TDMD and OM AD were higher in CO; however, the NDF and ADF AD were higher in MEG-fed modules. Final pH was not affected by treatments. Final NH_3N concentration was greater in HF and 9% fat. These results suggest that LF diets with high dietary fat concentration can be utilized and different types of fat sources may improve DM and fiber rumen digestibility.

Key Words: gas production system, coconut oil, poultry fat

Ruminant Nutrition: Forages

W135 Effects of replacing alfalfa hay and whole corn silage with paper mulberry (*Broussonetia papyrifera* L.) silage in high lactating cow diets on dry matter intake, milk yield, and milk composition. Z. H. Wu, C. Y. Liang, R. C. Huang, J. L. Ouyang, L. Ma, L. S. Zhao, and D. P. Bu*, *Institute of Animal Science, State Key Laboratory of Animal Nutrition, Chinese Academy of Agricultural Sciences, Beijing, Beijing, China.*

The lack of high-quality roughage resources has gradually limited the development of China dairy industry. The objective of this study was to evaluate the possible application of paper mulberry (*Broussonetia papyrifera* L.) silage (PMS) in high lactating cow diets. Sixty multiparous Holstein dairy cows were enrolled into this study based on days in milk, milk yield, and parity. The cows were then randomly assigned to 4 treatments of no PMS added in the diet (CON), 15.7% of alfalfa hay and whole corn silage replaced by 7% PMS (PMS1), 31.4% of alfalfa hay and whole corn silage replaced by 14% PMS (PMS2), and 47.1% of alfalfa hay and whole corn silage replaced by 21% PMS (PMS3). The pre-feeding period for the trial was 14 d, while the trial period was 84 d. Dry matter intake (DMI) was measured for 3 consecutive days every week and condensed into weekly means. Milk yield was recorded daily, and milk composition was analyzed every other week. A mixed-effects model with repeated measures and generalized linear model that included the effect of treatment at each sampling point was used for the data analysis. The results showed that DMI was affected by treatment ($P < 0.05$) and decreased linearly with the increase in PMS feeding ($P < 0.05$). Milk yield of CON was numerically higher than PMS3, and lower than PMS1 and PMS2, but no treatment effects or treatment × time interaction was detected. The effects of feeding PMS on milk components and somatic cell count were also not observed in the current study. In conclusion, paper mulberry silage can be used in high lactating cow diets, while high feeding ratio should be used with caution, as it may decrease the DMI and milk yield.

Key Words: diet, high lactating cow, paper mulberry silage

W136 Effects of feeding floury and brown midrib whole-plant corn silage varieties from corn treated with foliar fungicide to lactating Holstein cows on nutrient digestibility. L. J. Wente*[1], M. S. Akins[2], and F. C. Cardoso[1], [1]*Department of Animal Sciences, University of Illinois, Urbana, IL,* [2]*Department of Dairy Science, University of Wisconsin-Madison, Marshfield, WI.*

It is important to feed high-quality feedstuffs to lactating dairy cows to maximize productivity and economic return. Nutrient intake and digestibility are ways to assess the quality and production potential of these feeds. The purpose of this experiment was to determine the effects of feeding floury and brown midrib (BMR) whole-plant corn silage (WPCS) varieties from corn treated with foliar fungicide on nutrient digestibility in lactating Holstein cows. Thirty-two lactating cows [BW = 649 ± 74 kg; DIM = 189 ± 49 d] were randomized in a 4 × 4 Latin square design balanced to measure carryover effects. Treatments were floury WPCS with fungicide application (FF), floury WPCS with no fungicide application (FC), BMR WPCS with fungicide application (BF), and BMR WPCS with no fungicide application (BC). Corn was sprayed with fungicide (pyraclostrobin, $C_{19}H_{18}ClN_3O_4$ + metconazole, $C_{17}H_{22}ClN_3O$; Headline AMP, BASF, Florham Park, NJ) at the vegetative tassel stage (VT). WPCS was inoculated (Silo-King; Agri-King, Fulton, IL) and allowed to ensile in Ag Bags (Ag Bag Systems, St. Nazianz, WI) for 295 d before feeding. Statistical analysis was conducted using the MIXED procedure of SAS (SAS Institute Inc., Cary, NC). Cows in non-fungicide treatments had greater DM digestibility (74.35 ± 0.37%) than those in fungicide treatments (72.57 ± 0.37%; $P < 0.01$). Cows in non-fungicide treatments also had greater DMI (23.41 ± 0.72 kg/d) than cows in fungicide treatments (22.35 ± 0.72 kg/d; $P < 0.01$). Cows in BMR treatments had greater starch digestibility (93.16 ± 0.10%) than those in floury treatments (92.81 ± 0.10%; $P < 0.01$); cows in fungicide treatments had greater starch digestibility (93.21 ± 0.10%) than those in non-fungicide treatments (92.75 ± 0.10%; $P < 0.01$). Starch intake was greater for FC cows (6.24 ± 0.18 kg/d) than for FF cows (5.89 ± 0.18 kg/d; $P < 0.01$). Crude protein intake was greater for FC cows (4.21 ± 0.12 kg/d) compared with FF cows (3.63 ± 0.12 kg/d; $P < 0.01$). In conclusion, non-fungicide treatments had greater DM and starch digestibility compared with fungicide treatments.

Key Words: foliar fungicide, corn silage, digestibility

W137 Corn silage treated with the spent substrate from white rot mushroom culture to lactating goats. B. C. Agustinho*[1,2], E. Machado[1], J. M. Bragatto[1], C. R. Alcalde[1], P. T. M. Pintro[3], C. R. Schneider[1], B. R. Saraiva[3], and L. M. Zeoula[1], [1]*Programa de Pós Graduação em Zootecnia, Universidade Estadual de Maringá, Maringá, PR, Brazil,* [2]*Department of Animal Sciences, University of Florida, Gainesville, FL,* [3]*Programa de Pós Graduação em Ciência de Alimentos, Universidade Estadual de Maringá, Maringá, PR, Brazil.*

We aimed to evaluate intake, milk yield, and composition of lactating goats using corn silage with the addition of spent substrate from white rot mushroom culture (SSWRMC). We based our treatments on the enzymatic activity: 0; 1100 and 3300 U lignocellulolytic enzymes (LE)/min/kg of fresh material (FM). The enzymatic activity (U/min/g of SSWRMC) was determined to laccase (390.28), ligninperoxidase (176.27), Mnperoxidase (12.56) and cellulase (2,893.98) enzymes. The diets were formulated to meet the nutritional requirements of lactating goats producing 3 kg milk/day (crude protein: 14.6% and TDN: 74.3%) and body weight of 60 kg and the silage corresponded 60% of the diets. The experiment was designed in a triple Latin square (3 × 3) with periods of 22 d (19 d of adaptation and 3 d of sample collection). The milk composition was determined by infrared spectroscopy and total polyphenol by colorimetric method (expressed gallic acid - EqGA). Results were analyzed using the MIXED procedure SAS for linear and nonlinear regression. There were no changes ($P > 0.05$) on corn silage chemical composition (DM,CP, NDF, NFC) with different level of SSWRMC addition. There were no effect of treatments ($P > 0.05$) on dry matter intake (2.9 ± 0.36 kg/d), milk production (2.81 ± 0.68 kg/d) and composition (average: total solid = 10.92%; protein = 2.94%; fat = 2.85%; lactose = 4.22%; milk urea nitrogen = 12.18 mg/dL). However, the total polyphenol concentration in the milk linearly increased ($P = 0.01$) (10,401.78; 10,695.00; 12,694.55 mg EqGA/L, level 0; 1100 and 3300 U/min of LE/kg FM, respectively). This increase is likely associated with the enzymes activities in SSWMC that act in phenolic structures during the lignin cleavage. The increased capacity to transfer polyphenols to the milk can be positive to human health, due to its effects to reduce free radicals. In conclusion, the addition of SSWMC in corn silage increased polyphenols concentration in milk, without changing milk composition, which can be beneficial to human and animal health.

Key Words: laccase, lignin peroxidase, manganese peroxidase

W138 Effects of alfalfa silage treated using different varieties of waste date on feed intake, nutrient digestibility and performance of Holstein lactating dairy cows. M. Ghorbani[1], A. Naserian[1], R. Valizadeh[1], S. H. Ebrahimi[2], B. Kim*[1], and A. Rahimi[1], [1]*Faculty of Agriculture, Animal Science Department, Ferdowsi University of Mashhad, Mashhad, Iran,* [2]*College of Animal Life Sciences, Kangwon National University, Chuncheon, Republic of Korea.*

Waste dates are all damaged dates containing core that don't use for human consumption and have usability as a by-product. The goal of this study was to evaluate the effects of using alfalfa silage treated with different varieties of waste date on feed intake, nutrient digestibility, milk yield and composition of Holstein lactating dairy cows. Eight Holstein lactating dairy cows with days in milk 100 ± 4 d and average milk yield of 41 ± 1 kg were used. Treatment were 1) alfalfa silage without waste date (control), 2) alfalfa silage containing 6% Brin waste date, 3) alfalfa silage containing 6% Ghybani waste date and 4) alfalfa silage containing 6% Amobahri waste date. Data were analyzed as 4 × 4 change over design by the MIXED procedure of SAS ($P < 0.05$). The dry matter intake in cows fed with control was significantly lower ($P < 0.05$) than other treatments (21.89, 23.41, 23.49 and 23.85 kg in control and T2, T3 and T4, respectively). The apparent digestibility of dry matter, organic matter, crude protein, neutral detergent fiber and acid detergent fiber was not affected ($P > 0.05$) by dietary treatments. Milk yield in cows fed with all alfalfa silages treated with waste date were increased significantly ($P < 0.05$) than control diet (35.76, 37.69, 37.54 and 37.48 kg in control, T2, T3 and T4, respectively); however, 3.5% fat-corrected milk yield and energy-corrected milk yield were not affected ($P > 0.05$) among treatments. Percentage of milk components like fat, protein, lactose, nonfat solids and total solids were not affected ($P > 0.05$) by dietary treatments, but amount of milk lactose (kg) was significantly higher ($P < 0.05$) than control in cows fed with alfalfa silages treated with all different varieties of waste dates (1.68, 1.78, 1.75 and 1.79 kg/d in control, T2, T3 and T4, respectively). Rumen PH was significantly lower ($P < 0.05$) in cows fed with alfalfa silage treated with all varieties of waste dates. However, the ruminal ammonia nitrogen concentration in lactating cows was not affected ($P > 0.05$) by experimental diets, but in cows fed with alfalfa silage treated with all varieties of waste dates was shown a higher tendency. Results indicated that using different varieties Brin, Ghybani and Amobahri of waste date in alfalfa silage in level of 6% can improve milk yield and performance of dairy cows.

Key Words: alfalfa silage, waste dates, dairy cow performance

W139 Estimation of activity energy requirement for grazing dairy cows fed with different strategies. D. Talmón*[1], M. Garcia-Roche[1], A. Mendoza[2], D. A. Mattiauda[1], and M. Carriquiry[1], [1]*School of Agronomy, UdelaR, Montevideo, Uruguay,* [2]*National Agricultural Research Institute, La Estanzuela, Colonia, Uruguay.*

The aim of the study was to estimate the activity energy requirement of grazing dairy cows through the difference between the metabolizable energy intake (MEI) estimated measuring heat production (HP) + energy retained (ER) and the predicted MEI estimated by the NRC (2001) model. Twenty mid-lactation (101 ± 11 d in milk) multiparous Holstein cows (fall calving; 579 ± 53 kgBW and 2.6 ± 0.BCS) were assigned to 2 feeding strategies: cows grazing a *Medicago sativa* and *Dactylis glomerata* mix (21 kgDM/d/c herbage allowance above 5cm) in 2 grazing sessions between milkings (18 h/d) and supplemented with 7.5 kgDM/d/c of concentrate (18.5% CP; 13 MJ of ME/kgDM) (PMAX; n = 10) and cows grazing a herbage allowance of 9.7 kgDM/d/c of the same pasture after the afternoon milking (12 h/d) and supplemented with 15 kgDM/d/c of a mixed ration (50:50 forage to concentrate; 18% CP; 10.6 MJ of ME/kgDM) (P30; n = 10). The HP was measured using the O_2pulse technique. Predicted HP was calculated with the NRC (2001) model using $BW^{0.75}$, milk yield and composition (retained energy (RE) in milk), changes in BW and BCS (RE body tissue), and efficiencies (k) for maintenance, milk production, and loss/gain of body reserves. Data were analyzed with a mixed model. Milk production (33 ± 3 kg/d), RE in milk (924 ± 100 $kJ/BW^{0.75}/d$) and MEI (2025 ± 49$kJ/BW^{0.75}$) did not differ between feeding strategies but measured HP was greater ($P < 0.01$) for PMAX than P30 cows (1212 *vs* 1091 ± 36 $kJ/BW^{0.75}/d$). Residual HP was greater ($P < 0.01$) for PMAX than P30 cows (173 *vs* 43 ± 32 $kJ/BW^{0.75}/d$) and represented 8.4 and 2.1 ± 1.5% MEI, respectively. Residual HP was 2.4 fold greater than the estimated NRC requirement for activity of grazing cows for PMAX while it did not differ for P30 cows. Increased maintenance energy requirement associated with the inclusion of grazed forage in the diet may be related not only to activity but also to energy requirements associated with the gastrointestinal tract and excess nitrogen excretion. The NRC model underestimates the energy requirements of grazing dairy cows when pasture forage represents a large proportion of the diet (60% for PMAX cows).

Key Words: indirect calorimetry, dairy cattle, pasture

W140 Comparison of the energy expenditure between grazing and idling activities in Holstein dairy cows during mid-lactation. D. Talmón*[1], M. Garcia-Roche[1], A. Mendoza[2], D. A. Mattiauda[1], and M. Carriquiry[1], [1]*School of Agronomy, UdelaR, Montevideo, Uruguay,* [2]*National Agricultural Research Institute, La Estanzuela, Colonia, Uruguay.*

Energy is the first-limiting nutrient of grazing production systems and efficiency in metabolizable energy (ME) utilization needs to be improved, this demands more information about energy cost of grazing. The aim of this study was to estimate the increment above idling in energy expenditure (EE) when Holstein cows were grazing. Multiparous cows (n = 15; fall calving, 2.6 ± 0.1 BCS and 568 ± 55 kg BW) were assigned to 2 feeding strategies: cows grazing a *Medicago sativa* and *Dactylis glomerata* mix (21 kg DM/d herbage allowance above 5 cm, 20 h/d) and supplemented with 7.6 kg DM/d of concentrate (18.5% CP; 13 MJ of ME/kg DM) (PMAX; n = 7) and cows grazing a herbage allowance of 9.7 kg DM/d of the same pasture (12h/d) and supplemented with 15.1 kg DM/d of a mixed ration (50:50 ratio forage:concentrate; 18% CP; 10.6 MJ of ME/kg DM) (P30; n = 8). At 98 ± 7 d postpartum, cow EE (O_2 pulse technique) and animal activity (MOOnitor collars, ISR) were recorded simultaneously during 4 consecutive days. The EE for each activity was calculated and compared within each hour of access to the pasture to decrease the effect of DM intake on heat production. Data were analyzed using a mixed model that included feeding strategy as fixed effect and cow genotype as random effect. During access time to pasture, PMAX cows presented a longer ($P < 0.01$) grazing time (456 *vs* 202 ± 38 min/d), but a similar herbage intake rate (1.9 ± 0.4 kg DM/h) than P30 cows. Although the EE during idling (1183 *vs* 1051 ± 44 $kJ/BW^{0.75}/d$) and grazing (1251 *vs* 1117 ± 61 $kJ/BW^{0.75}/d$) were greater ($P < 0.01$) for PMAX than P30 cows, the increase in EE above idling when cows were grazing did not differ ($P = 0.85$) between feeding strategies (63 *vs* 58 ± 17 $kJ/BW^{0.75}/d$ and 5.4 *vs* 5.7 ± 1.5% for PMAX *vs* P30). These results demonstrate that the increment in EE above idling for grazing activity did not depend on the inclusion of grazed forage in the diet (63 *vs* 30% for PMAX and P30) but would probably be associated with bite and intake rate and therefore, to sward structure and mass and animal internal state.

Key Words: dairy cattle, grazing energy requirement, indirect calorimetry

W141 Effect of grazing in energy partitioning of Holstein multiparous cows.

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The study aimed to compare energy partitioning between heat production (HP) and retained energy (RE) and gross energy efficiency of dairy cows fed different strategies during mid-lactation. Twenty-four multiparous spring calving cows (665 ± 65 kg BW and 3.0 ± 0.4 BCS at calving) were used in a randomized complete block design in which during summer (114 to 180 ± 11 d in milk) were assigned to 2 feeding strategies: (G0) 100% total mixed ration (TMR) ad libitum (45% forage:55% concentrate) or (G1) grazing alfalfa from 18:00 to 4:00 h (herbage mass = 1380 ± 328 kg DM/ha, herbage allowance = 20 kg DM/d, above 4 cm) and supplemented with TMR (50% of ad libitum offer) after the morning milking. Cow HP (measured by O2 pulse technique), RE in milk (estimated by daily milk yield and milk composition determined every 14 d) and RE in body tissue (estimated from changes in BW and BCS recorded every 14 d) were used to calculate energy balance from 136 to 164 d in milk. Metabolizable energy (ME) intake was estimated as HP + total RE and residual HP was calculated as the difference between measured and predicted HP (based on NRC 2001 model). Data were analyzed with a mixed model including feeding strategy as fixed effect and block as random effect. Milk yield did not differ between treatments but RE in milk was greater ($P = 0.01$; 93.6 *vs* 83.2 ± 2.6 MJ/d) while RE in tissue was less ($P = 0.01$; −4.9 *vs* 0.23 ± 1.4 MJ/d) for G0 than G1 cows. Although ME intake did not differ between treatments (206.5 ± 7.4 MJ/d), measured HP tended ($P = 0.07$) to be greater and residual HP was greater ($P < 0.01$) for G1 than G0 cows (126.3 *vs* 111.7 ± 5.5MJ/d and 13.5 *vs* −9.5 ± 3.9 MJ/d). Residual HP indicated that the NRC model estimated correctly energy requirements of confined cows (G0) but underestimated them for grazing cows (G1) as maintenance energy cost seems to be increased by 6% of ME intake. Energy efficiency adjusted by changes in body reserves was greater ($P = 0.03$) for G0 than G1 cows (0.44 *vs* 0.40 ± 0.01). Results indicated inclusion of pasture grazing in the diet reduced the efficiency in the use of consumed ME associated with a greater total and residual heat production probably related to increased activity, forage digestion and unbalanced protein to energy ratio.

Key Words: energy expenditure, nutrition, dairy cattle

W142 Relationship between corn silage quality traits and dietary proportions on average yearly milk production and composition of Québec dairy farms: Exploratory research.

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A data set containing 2,124 corn silage (CS) gathered throughout the years 2014 to 2018 (YH) was analyzed by near infrared spectroscopy at the Valacta Forage Laboratory (Sainte-Anne-de-Bellevue, QC). The nutritional value and fermentation profile were used to perform a principal component (PC) analysis using SAS 9.4, (SAS Institute Inc., Cary, NC). Six PC were extracted by adopting criterion eigenvalue higher than 1. The PC would correspond to: PC1) Carbohydrates and maturity of CS; PC2) homolactic fermentation, protein solubilization and starch digestibility (DIG); PC3) fermentation length (FL), heterolactic fermentation or other secondary fermentations; PC4) NDF DIG; PC5) Protein degradation and heating; and PC6) L. buchneri fermentation. The PC subject scores were then annually averaged for CS sampled from the same farm from October 20th to August 31st of each YH (2014 to 2017). Then, each farm was characterized for their annual average milk yield (MY) and composition. Only farms (n = 43) with a minimum of one annual average for each respective YH including PC scores, percentage of CS in rations (% CS), MY and composition were retained and analyzed. Mixed model was used to relate MY and composition to average annual PC scores and % CS where FARM, HY and FARM × HY as random effects, PC1 to 6, % CS and PC1 to 6 × % CS as fixed effects in the model (JMP 13, SAS Institute Inc., Cary, NC.). As main results, Annual average milk yield per cow (kg/cow/year) was affected positively ($P < 0.05$) by %CS, PC2 and by the following significant interactions PC2 × %CS and PC4 × %CS. Annual average milk urea (mg/100 mL) was affected positively ($P < 0.05$) by %CS, PC1, negatively by PC2 and the following interactions PC2, PC3 and PC6 × %CS. Average annual log-transformed somatic cell count was only affected positively ($P < 0.05$) by PC5. This exploratory research shows the potential of using historical data from dairy farms to explore the effects of silage analysis on dairy herd performance and developing better data driven tools.

Key Words: corn silage, principal component analysis, milk yield

W143 Milk production performance and ruminal fermentation in lactating dairy cows fed processed oats grain in comparison with barley grain.

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This study aimed to determine the effect of processed oats grains on DM intake (DMI), milk yield and components, digestibility, and rumen fermentation in lactating dairy cows. Eight lactating cows including 4 ruminally-cannulated cows were used in a double 4 × 4 LSD, with a 21d period. Cows were fed a TMR with a 50:50 forage-to-concentrate ratio with one of the 4 treatments: T1 = rolled barley, T2 = rolled oat, T3 = flaked oat, and T4 = pelleted oat, where rolled barley was used as control. On d18 to d20 of each period, DMI, total fecal collection and milk sampling were performed. D21 was used for ruminal fermentation measurement. The data were analyzed using the SAS Mixed model procedure with the analysis LSD model including treatment, square, period, and cows effects. The treatment differences were compared using Tukey method. The DMI did not differ among treatments ($P = 0.16$). Milk yield was higher ($P < 0.01$) for cows fed rolled oats (49.23 kg/d), while fat yield and FCM were not affected by treatment ($P = 0.11$ and $P = 0.17$, respectively). Protein yield (1.34 kg/d) and lactose yield (1.99 kg/d) were significantly lower ($P < 0.05$) for flaked oats when comparing to the other treatments. Feed efficiency was similar for rolled oats and barley, but higher ($P = 0.02$) for pelleted and flaked oats. Digestibility of DM, OM, NDF and CP were not affected by treatment ($P > 0.05$). Starch digestion was lowest ($P < 0.01$) for rolled barley. Ruminal pH was not affected by treatment averaging 6.16. Total short chain fatty acids in the rumen were significantly higher ($P = 0.01$) for flaked oats (96.71 mM) and barley (100.45 mM). Ruminal acetate concentration was lower ($P < 0.01$) for pelleted oats when compared with rolled oats and barley

(54.84, 58.98 and 61.11 mM, respectively). Propionate concentration did not differ between treatments. These results demonstrate that oat grain can increase milk yield without causing milk fat depression or inducing changes in the ruminal pH. In addition, ruminal VFA's, degradability of DM and CP also showed minor difference between grains. This knowledge could be applied to reduce feeding costs through the greater use of oats in dairy cattle diets.

Key Words: oat feeding to replace barley, lactating dairy cow, feed processing

W144 Midwestern US commercial dairy survey results: corn silage kernel processing, rumen starch digestibility and fecal starch content.

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Corn silage is a staple feed for many commercial dairies. Variation in corn silage kernel processing and ensiling extent may impact rumen and total-tract starch digestibility (TTSD, % of starch), however there is question about the variation in starch digestibility for fermented silage. The objective of this study was to survey Midwestern US dairy farms and evaluate if corn silage kernel processing score (KPS), corn silage rumen starch digestibility and fecal starch content (and TTSD) varied for herds feeding fermented corn silage. Fecal and corn silage samples were collected from 59 commercial dairy farms in Wisconsin during summer 2018. TTSD was calculated using the Fredin et al. (2014) equation. Silages were assumed to have fermented at least 6 mo, sampling from farms feeding 2017 crop. Fecal starch content, corn silage starch, KPS (% starch < 4.75mm) and in situ rumen starch digestibility (% starch; 6mm grind) at 0h (isSD0; washout fraction) and 7h (isSD7) were determined by a commercial laboratory (Rock River Laboratory, Watertown, WI). Corn silage samples were analyzed for nutritive parameters by NIR. Dairies were surveyed for TMR nutrient composition, dry matter intake (DMI, kg) from farm records, milk production (kg), milk fat (MF, %) and protein (MP, %) per cow. Energy corrected milk (ECM, kg) was calculated as (12.95 × milk kg × BF) + (7.65 × milk kg × MP) + (0.327 × milk kg) and feed efficiency (FE) as ECM/DMI. KPS, isSD0 and isSD7 correlations with TTSD, ECM and FE were explored by multivariate methods with JMP Pro v14.0. Independent pairwise correlations were significant ($P < 0.05$) for TTSD and KPS (r = 0.26), KPS and isSD0 (r = 0.33), isSD0 and TTSD (r = 0.52), isSD0 and isSD7 (r = 0.60). Trends were evident ($P < 0.10$) for isSD0 and DMI (r = 0.26), isSD7 and TTSD (r = 0.24), ECM and isSD0 (r = 0.24) and ECM and isSD7 (r = 0.24). Survey population statistics are presented here. The coefficients of variation suggest variation exists in kernel processing and starch digestibility for fermented silages.

Key Words: corn silage, kernel processing, starch digestibility

Table 1 (Abstr. W144).

Parameter	Mean	SD	CV
Corn silage			
DM	33.0	4.4	13.3
Starch	33.3	4.7	14.2
KPS	65.5	7.0	10.7
isSD0	80.9	9.1	11.3
isSD7	88.7	3.7	4.1
Dairy measures			
Fecal Starch	2.1	1.7	78.0
TTSD	97.3	2.1	2.1

W145 Forced air versus microwave oven sample drying does not affect rumen starch digestibility, estimated by in situ rumen technique, for total mixed ration, corn silage, or high-moisture corn grain.

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Laboratory drying technique has been thought to impact rumen starch digestibility however little published data exist to support the discussion. The objective here was to determine if commercial laboratory drying techniques impact rumen starch digestibility measures. Commercial dairy total mixed ration (TMR, n = 5), corn silage (CS, n = 5) and high moisture corn (HMC, n = 5) samples were assessed. Samples were split and treated using different drying techniques; undried (UN), forced air (FA) and microwave (MW) oven. For each FA and MW, 2 additional intensities were applied. With FA and MW, low and medium intensities were defined as 52°C (FAL) and 105°C (FAM) or power setting equal to 3 (MWL) and 6 (MWM) in a 1500W oven, respectively. Approximately 200 g of subsample was dried by each technique until weight loss upon further drying was less than 1 g. Mean minutes to dry were 1200, 380, 24 and 16 for FAL, FAM, MWL and MWM. Dried CS and TMR samples were ground to 6 mm and HMSC and UN samples were not ground before being weighed, 3 g, into Ankom R510 bags, and incubated for 3, 7 and 16 h, in triplicate in 3 ruminally cannulated lactating dairy cows consuming a 60% forage, corn silage based diet. Residue bags were rinsed in a laundry machine, dried at 50°C for 24 h and weighed to determine DM disappearance. Residues were composited and starch (% of DM) assessed. Starch digestibility at 3, 7 and 16 h (SD3, SD7, and SD16; % of starch) were determined as starch lost during incubation. Starch, SD3, SD7 and SD16 (dependent variables) were related to feed, oven type, and drying intensity. Farm was considered a random effect. All parameters were fit to model using backward elimination using SAS JMP Pro v11. Effects were considered trends at $P < 0.10$ and significant at $P < 0.05$. Starch, SD3, SD7 and SD16 were not affected by drying technique but each was related to feed type ($P < 0.05$). Drying technique tended to affect SD3 ($P = 0.09$), with MW numerically less than FA or UN. These observations and results suggest that forced air versus microwave oven drying does not impact rumen starch digestibility measures for commercial dairy feeds.

Key Words: starch digestion, corn silage, drying technique

W146 Effects of drying method on nutrient composition, particle size, and in situ washout for corn silage.

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The objective of Study 1 was to determine the effect of drying method on nutrient composition in corn silage (CS). There were 49 CS selected and split into 4 fractions. Two fractions were dried in an HVLT80 oven (O) developed by Cumberland Valley Analytical Services and the other 2 were dried in a microwave (M). Fractions were ground to 1mm and analyzed via NIR for ADICP, NDF, and starch. There were no differences between O and M in DM ($P = 0.79$), aNDFom ($P = 0.87$), and starch ($P = 0.11$). The ADICP was lower for O vs. M (0.43 vs. 0.79%DM, respectively, $P < 0.0001$), suggesting M causes heat damage. The objective of Study 2 was to determine the effects of drying method on particle size and in situ washout. Three corn silages were split into 2 fractions (O and M), and ground to 4mm. There were no differences between O and M for DM ($P = 0.37$), aNDFom ($P = 0.69$), or starch ($P = 0.81$). Mean particle size (MPS) was measured by shaking for 10 min in a Ro-Tap and 0.5 g was weighed into 5 × 5 cm Dacron bags with a pore size of 50 ± 10 µm in quadruplicate. Dacron bags were soaked in warm water for 10 min, rinsed in cold water, and washed in a washing machine. They were dried overnight at 55°C, weighed, then residues remaining was measured. Although the proportion of particles that passed through the 53-µm screen was not different between O and M (1.36 vs. 0.95%, $P = 0.49$), the MPS was smaller for O vs. M (459 vs. 533µm, respectively, $P = 0.006$). The DM washout was higher for O vs. M (46.1 vs. 27.4%, respectively, $P < 0.001$), and so was starch washout (62.8 vs. 39.7%DM, respectively, $P = 0.01$). Microwave drying corn silage resulted in heat damage and increased particle size, perhaps by gelatinizing starch granules, making it an unsuitable drying method. Both oven and microwave drying samples result in high washout of DM and starch for in situ. More research is necessary to evaluate laboratory methods for starch digestion.

Key Words: heat damage, microwave, oven

W147 Differential responses of brown midrib (BMR) and non-BMR maize hybrids to ensiling, aerobic stability and inoculation with combination of heterofermentative lactic acid bacteria strains *Lactobacillus buchneri* and *Lactobacillus hilgardii*.

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In maize, brown midrib phenotype is associated with 6 loci and bm1 to bm4 are spontaneous mutants, influencing cross-linkages between cell wall and lignin. Although several studies report higher digestibility of bmr mutants, field results often report lower yield and sensitivity to stresses. Few information exists evaluating fermentation and aerobic stability (AS). To test ensiling conditions, we compared bm3 (F2F379 and F2F499), bm1 (PO238XR), and non-BMR hybrids (PO533AM1 and TMF2Q419) grown in a randomized complete block design. Ensiling was performed following inoculation (INO: *Lactobacillus buchneri* NCIMB 40788 (150000 cfu/g) and *Lactobacillus hilgardii* CNCM-I-4785 (150000 cfu/g) compared with control (CON). After 60 and 120 d of incubation in mini-silos (5 repetitions; 7 L; 222 kg DM/m^3), we measured fermentation parameters, VOC, and aerobic stability (AS). Statistical analysis was performed using a 5 × 2 factorial (hybrids x inoculation). The fermentation profile for INO was typical of heterofermentation, with a pH slightly higher to CON due to transformation of lactate to acetate, and synthesis of 1,2-propanediol. INO improved starch by 2.4% units, ADF (0.59% unit), NDF, and reduces fresh matter losses (fermentation + AS). Difference in AS was important between hybrids in CON were bmr hybrids had high intrinsic AS (over 186h to reach +2°C over ambient) versus non-BMR (lower 90 h). In CON, concentration of 1,2-propanediol was similar among hybrids (1.01 ± 0.25%DM, $P = 0.363$) and not related to AS. In INO over CON, 1,2-propanediol was increased for all hybrids at 60 and 120 d ($P = 0.001$ and 0.016). By 120 d, 1,2-propanediol concentration ranged from 0.70 to 2.37% DM and accumulation of propionate was observed in all hybrids. Improvements of AS in BMR hybrids could be linked to higher total VFA and lactate, improved in bm3 over bm1 and non-BMR. Some of the observed differences for lactate, ethanol, propionate, ammonium, and pH would be related to difference in germplast between hybrids, not to the bmr mutation. BMR mutation and inoculation improved ensiling parameters, aerobic stability, and some nutritional parameters.

Key Words: maize, hybrid, silage additive

W186 Effects of forage particle size on intake, growth performance, and ruminal pH of Holstein dairy calves.

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This study aim was to test the effects of supplementing forage with varying particle sizes on performance and ruminal pH of calves. Holstein calves (n = 40; 48.3 ± 3.2 kg of BW) from 15 to 90d of age were randomly assigned to 1 of 4 treatments (n = 10/treat; 5 males and 5 females): 1) starter without forage supplementation (CON), 2) starter with wheat straw chopped at 1mm geometrical mean particle length (GMPL) (FPS), 3) starter with wheat straw at 4 mm GMPL (MPS), 4) starter with wheat straw at 7 mm GMPL (LPS). The calves had ad libitum access to water, forage, and starter throughout the study. All calves were offered milk 7L/d from 15 to 25 old, 6L/d from 26 to 35d old, 5L/day from 36 to 45d old, and 3L/day from 45d old until weaning at 56d old. Starter and forage intake were recorded daily. Calves were weighed every 10d. ADG and feed efficiency was calculated for 3 periods: pre-weaning, post-weaning, and overall. Rumen pH was recorded at 35 and 90d of age at 4, 8 and 24h after morning feeding via stomach tube. Data were analyzed using the MIXED procedure of SAS with time as repeated measures for performance variables. Furthermore, a contrast between the control group and all groups fed forage was examined. During the post-weaning and overall periods starter, forage, and total DM intake were not affected by GMPL of wheat straw, but CON calves had lower starter and DM intake ($P = 0.05$) than calves offered forage. Through all 3 periods, ADG did not differ by forage particle size, but CON calves had lower overall ADG than calves fed wheat straw (0.806 kg/d, CON; 0.896 kg/d, MPS; 0.902 kg/d, LPS and 0.930 kg/d, FPS; $P = 0.05$). Feed efficiency did not differ across treatments. Ruminal pH was lower in CON than calves supplemented with forage at 8h after feeding on d 35 (5.50, CON vs 6.08, FPS; 6.16, MPS and 6.01, LPS; $P = 0.05$), 4h after feeding on d 90 (5.32, CON vs. 5.73, FPS; 5.91, MPS and 5.63, LPS; $P = 0.01$), and 8h after feeding on d 90 (5.48, CON vs 5.97, FPS; 6.02, MPS and 5.77, LPS; $P = 0.02$). In conclusion, supplementing wheat straw increased starter intake, improve calf performance, and modified ruminal pH dynamics of dairy calves but no difference was found for different GMPL treatments.

Key Words: chop length, growth, wheat straw

Ruminant Nutrition: Ruminal Fermentation and Gas Production

W148 Design updates to dual-flow continuous culture fermenters reduce variance of digestibility and bacterial nitrogen flow compared with previous fermenters. E. Kesselring[1], L. Antal[1], T. Henthorne[1], and B. Wenner*[2], [1]*Department of Chemistry, The Ohio State University, Columbus, OH,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH.*

In response to the need for updated and affordable dual-flow continuous culture fermenter systems within the scientific community, modifications were made to the classic design for new construction. These modifications are summarized in 4 categories. First, simplification and performance optimization of the base unit with updated electrical components, modernized gearing, and self-calibrating thermostats. Second, changes to the shape of the fermenter jar to decrease construction cost and improve anaerobic stability. Third, extended reach and angle to the stirring mechanism for improved mixing and solids turnover. And lastly, simplification of a 2-stage filter system to reduce failure rate. Our hypothesis was that fermenter experiments with the updated fermenter system would decrease variance estimates for common dependent variables: NDF digestibility, VFA production, ammonia N flow, and bacterial N flow. To test this hypothesis, data were collected from 3 published fermenter experiments (n = 48) utilizing the old system and 2 fermenter experiments with the updated units (n = 48). Experiments utilized common diets, dilution rates, feeding rates, and sampling times. Variances were calculated within treatment, then pooled to provide a ratio of new versus old system variances for the 4 dependent variables. An *F*-test was conducted on the new:old variance ratio and a significant decrease in experimental variance was declared at $P < 0.05$. Updated fermenter systems decreased ($P < 0.01$) variance of NDFd estimates by 55% and decreased ($P < 0.01$) variance of bacterial N flow estimates by 55% compared with the original fermenter system. However, variance for VFA production was increased ($P < 0.01$) by 279% and variance for ammonia N flow tended to be increased ($P = 0.09$) by 46% in new versus old fermenter units. These data indicate the potential for new, more affordable dual-flow continuous culture systems to improve reliability of fiber digestibility and microbial growth research but more work is needed to reduce variance of concentration-based fermentation parameters.

Key Words: continuous culture fermentation, fiber digestibility, methods

W149 Dose response of caffeine on fermentation and nutrient utilization in continuous culture fermenters. S. M. Hussein*, M. X. Toledo, J. Echesabal, S. Twyman, S. Simmons, J. Sinkevitch, G. Loughlin, and G. J. Lascano, *Department of Animal and Veterinary Sciences, Clemson University, Clemson, SC.*

Plants produce various secondary compounds mainly for protecting themselves from insects, fungi or bacteria. Plant secondary metabolites, namely caffeine (CA), is a powerful metabolic stimulant. Scarce experimentation has been conducted to evaluate the potential of CA to manipulate rumen fermentation. The objective of the current study was to determine the effects of using different doses of CA on fermentation profile and nutrient digestion when fed diets for lactating cows in continuous culture fermenters. We hypothesized that the addition of CA introduced at different doses into continuous cultures of mixed ruminal microorganisms will improve fermentation and nutrient utilization. Four treatments were randomly assigned to 8 continuous culture fermenters for 2 periods of 10 d. The experimental model administered according to a completely randomized block design. Treatments included a diet with a 40:60 forage to concentrate ratio with a control treatment (CON, no CA) and 3 treatments representing different doses of CA [CA-1 (50 ppm); CA-2 (100 ppm); CA-3 (150 ppm)]. Data were analyzed using the MIXED procedure of SAS as a randomized complete block design with blocks of period and fermenters; where linear, and quadratic contrasts where evaluated. Dry matter and OM apparent digestibility (AD) was decreased linearly as CA dose increase ($P = 0.03$). There was a quadratic tendency decrease on NDF and ADF AD as CA decreased ($P = 0.06$). Mean culture pH decreased linearly as CA increased in the diets (6.11, 6.00, 5.99 pH). The NH_3-N concentrations were high in CON and increased linearly with the addition of CA in the diets. The results of the current study suggest that adding CA up to 50 ppm can be beneficial for rumen fermentation and nutrient digestibility.

Key Words: caffeine, continuous culture, rumen fermentation

W150 Additive effect and in vitro gas production of diets based on silage of poultry litter, pig manure, urea with cane molasses, and bakery by-product. D. Trujillo Gutiérrez[1], I. A. Domínguez Vara[1], J. L. Bórquez Gastelum[1], S. S. Gonzalez Muñoz*[2], J. M. Pinos Rodríguez[3], and J. E. Ramírez Bribiesca[2], [1]*Facultad de Medicina Veterinaria y Zootecnia, Nutrición de Rumiantes, Universidad Autónoma del Estado de México,* [2]*IREGEP-Ganadería, Campus Montecillo, Colegio de Postgraduados, México,* [3]*Facultad de Medicina Veterinaria y Zootecnia, Universidad Veracruzana, Veracruz, México.*

The objective of this experiment was to evaluate the additive effect of corn stalk silage (CS), 3 nitrogen sources (pig manure, PM; poultry litter, PL; urea, UR) and 2 energy sources (cane molasses, CM; bakery byproduct, BBP), on a diet (145 g CP/DM). Silages composition (g/kg DM) was: 1) 384.6 CS, 384.6 PL, 230.0 CM or BBP; 2) 294.1 CS, 529.4 p.m., 176.5 CM or BBP; 3) 630.0 CS, 30 UR, 340 CM or BBP. The diet contained (g/kg DM): silage 400:600 supplement (corn grain, soybean meal, wheat bran, fish meal, vitamins and minerals). Each silage and concentrate of each treatment were analyzed separately. A factorial arrangement of 3 N sources (PM, PL, UR) × 2 energy sources (CM, BBP) was used in this experiment. The statistical analysis was performed with 2 methods: 1) Mixed lineal procedure (PROC MIXED) assigning literals of treatments with Macro pdmix800.sas (Tukey, $P < 0.05$); 2) adjustment of gas production with NLMIXED to the model $Y_{ij} = \beta_1 + \mu_{i1}/1 + (\beta_2 + \mu_{i2}) e^{-\beta_3 t} + e_i$, where: maximum asymptote of accumulated gas (mL/g DM) at 72 h, β_2 = Ymax/2 (h) and β_3 = fractional rate of degradation (g DM/h). Diets PL-CM and PM-CM had greater gas production ($P < 0.05$) during 72 h, whereas PL-CM concentrate and PL-BBP silage produced more gas ($P < 0.05$) at 72 h. Regarding β_1, diet (273.65) and concentrate (297.86) of PL-CM were greater than the others ($P < 0.05$), but silage PL-BBP (222.58) produced more gas. Besides, there was an additive effect of the combination silage plus concentrate of PM-CM, regarding β_1, which agrees with gas production (153.12) during all the fermentation. For gas production adjustment to the model, the concentrate and PL-CM diet as well as PL-BBP silage, showed greater β_1 and a higher limit which was higher than the others; besides, β_2 was constant between treatments and β_3 was lower in silages. Thus, it may be concluded that PL-CM and

PM-CM combinations have a larger potential for gas production due to the additive effect of their components.

Key Words: sheep nutrition, waste and by-products, in vitro gas production

W151 In vitro rumen fermentation characteristics of high-grade crystalline versus low-grade liquid betaine products. T. Kelley*[1], G. Chibisa[2], P. Rezamand[2], and M. Chahine[1], [1]*University of Idaho, Twin Falls, ID,* [2]*University of Idaho, Moscow, ID.*

Betaine, a co-product of sugar-beet processing, can be used to feed cattle. Because high-grade betaine (>90% pure; DM basis) is expensive, feed-grade products with lower betaine concentration are typically used in cattle rations. However, there is limited information on the impact of feeding the feed-grade betaine products on rumen fermentation characteristics. Therefore, our objective was to compare in vitro rumen fermentation characteristics of a high-grade betaine (97% purity) to a feed-grade betaine product (32% purity). The Ankom gas production system was used (Ankom Technologies, Macedon, NY) to determine the in vitro fermentation characteristics of both products at the same inclusion level. Three dietary treatments were used: control (CON) with no betaine added, high-grade crystalline betaine (CRYS), and feed-grade liquid betaine (LB50) at 0.50% of diet DM. The study was a completely randomized design and each treatment was added to 2 Ankom modules, which contained 1.5 g of TMR, 15 mL rumen fluid, and 45 mL McDougall's buffer. Two Ankom modules were also used as blank/run. A total of 3 runs were conducted. Data were analyzed using the mixed procedure of SAS. Crystalline betaine had a greater CP content compared with the liquid betaine (72.8 vs 56.7% DM). Total volatile fatty acid production tended to be greater in LB50 vs CRYS (140.23 vs. 109.14 mM respectively, $P = 0.09$) while no differences ($P > 0.1$) were detected in the molar proportions of acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and caproate, which averaged 49.15 ± 0.81, 29.67 ± 0.69, 13.78 ± 0.51, 1.27 ± 0.04, 3.50 ± 0.12, 2.06 ± 0.09, and 0.58 ± 0.07% respectively. Final pH did not differ ($P = 0.27$) among treatments and averaged 6.20 ± 0.02. Similarly, in vitro true DM digestibility and methane production did not differ ($P \geq 0.15$) among treatments. In summary, the lack of differences in in vitro fermentation characteristics between an expensive high-grade and a lower-grade betaine product suggests a similar feeding value when fed at the same dietary inclusion rate.

Key Words: in vitro fermentation, VFA, betaine

W152 Potential of CO_2 measurements for ranking of cows for feed efficiency. A. Bayat[1], A. Guinguina[2], and P. Huhtanen*[2], [1]*Milk Production, Production Systems, Natural Resources Institute Finland (LUKE), Jokioinen, Finland,* [2]*Department of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden.*

There is an increasing interest for genetic selection of dairy cows to improve the efficiency of feed utilization, but the progress has been limited due to difficulties in measuring feed intake in practical dairy farms. Determination of CO_2 production could be an alternative approach to direct DMI measurements for evaluation of feed efficiency (FE), provided that CO_2 production is closely related to heat production (HP) estimated by Brouwer equation. The objective of this study was to evaluate the potential of CO_2 production in estimating HP and consequently FE. The relationships were estimated from respiration chamber data in 100 cows fed a diet based on grass silage and concentrate (55:45 on DM basis). Residual ECM (RECM) yield was calculated from ME intake and requirements for maintenance and production. Residual CO_2 per kg ECM (RCO_2) was calculated by adjusting observed values for differences in ECM yield (−3.75 L/kg ECM) to take into account dilution of maintenance. Feed efficiency was expressed as ECM/GE or ME (g/MJ). PROC MIXED of SAS was used to develop models with period as random factor. There was a close relationship between HP and CO_2 production: HP (MJ/d) = $6.8 \pm 1.74 + 0.0185 \pm 0.00022 \times CO_2$ (L/d); adjusted RMSE = 1.2 MJ/d (0.8% of observed mean). This suggests that accurate measurements of CO_2 production can be a useful tool in ranking the cows according to feed efficiency. Residual ECM yield (kg/d) was negatively related to RCO_2 (L/kg ECM): $RECM = -0.21 \pm 0.80 - 0.173 \pm 0.0236 \times RCO_2$ (RMSE and R^2 adjusted for random period effect = 2.8 kg/d and 0.64, respectively), i.e., cows producing more CO_2 were less efficient. Also, gross FE (g ECM/MJ GE) was closely related to CO_2/ECM: $FE = 441 \pm 11.7 - 0.856 \pm 0.054 \times CO_2/ECM$ (adj. RMSE = 9.6 g/MJ, $R^2 = 0.88$). When the cows are in negative energy balance CO_2 production per unit of ECM can be less compared with cows at zero energy balance due to greater efficiency of converting body energy to milk energy compared with dietary ME. To avoid false ranking of cows for FE, measurements of CO_2 should be made in mid-lactation and/or some proxies (e.g., blood NEFA or milk fatty acids) to identify cows in negative energy balance.

Key Words: CO_2 production, dairy cow, feed efficiency

W153 Multi-kingdom microbial shifts and associated functional variation in the rumen of lactating dairy cows: I. Effect of dietary energy source and level. T. Park*[1], L. Ma[2], Y. Ma[2], X. Zhou[2], D. Bu[2,3], and Z. Yu[1], [1]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [2]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, Beijing, China.*

The effects of dietary treatments on the rumen microbiota can vary among the multi-kingdoms of rumen microbes. This study investigated the effect of the source and level of dietary energy on rumen bacteria, methanogens, protozoa and fungi in the rumen of lactating dairy cows. A 2 × 2 factorial design resulted in 4 dietary treatments: low and high energy levels (LE: 1.52–1.53; and HE: 1.71–1.72 Mcal) and 2 energy sources [finely ground corn (GC) and steam-flaked corn (SFC)]. We used a replicated 4 × 4 Latin square design using 8 primiparous Chinese Holstein cows with each period lasting for 21 d. The rumen microbiota was analyzed through sequencing of amplicons of kingdom-specific phylogenetic markers (16S rRNA gene for bacteria and methanogens, 18S rRNA gene for protozoa, and ITSI for fungi) followed with subsequent sequence analysis using the QIIME2 pipeline. GC resulted in a higher prokaryotic (bacterial and methanogen) species richness and phylogenetic diversity than SFC, and for the eukaryotic (protozoa and fungi) microbiota, LE led to significantly higher values of the above measurements than HE. A large number of phyla and genera in all the kingdoms differed in relative abundance between the 2 dietary energy levels, while only *Dasytricha* and *Cryptococcus* were differentially abundant between the 2 corn processing methods. Based on the prokaryotic amplicon sequencing variants from all the samples, functional profiles predicted using PICRUSt2 differed significantly between LE and HE. FishTaco analysis identified *Selenomonas* and *Ruminococcus* as the potential taxonomic drivers of pyruvate and methane metabolism in LE and HE, respectively, while *Butyrivibrio* and *Coprococcus* exhibited the opposite relationship with those functions, particularly in HE. There were strong co-occurrence patterns among the genera of different kingdoms suggesting possible metabolic interactions in the rumen. These results suggest that dietary energy level can significantly affect rumen microbiota across all the kingdoms.

Key Words: rumen microbiota, energy level, corn processing method

W154 Protozoa inhibition by specific inhibitors of lysozyme and peptidases to enhance nitrogen balance in vitro.

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Defaunation studies have shown that rumen protozoa are one of the main causes of low nitrogen utilization efficiency due to their bacterivory and subsequent intraruminal nitrogen recycling in ruminants. In genomic and transcriptomic studies, we found that rumen protozoa express lysozymes and peptidases at high levels. We hypothesized that specific inhibition of lysozyme and peptidases could reduce the activity and growth of rumen protozoa, which can decrease proteolysis and ammoniagenesis by rumen microbiota. To test the above hypothesis, we evaluated 3 specific inhibitors [imidazole (IMI), phenylmethylsulphonyl fluoride (PMSF) and iodoacetamide (IOD)], both individually and in combinations, of lysozyme and different types of peptidases, with sodium dodecyl sulfate as a positive control. Rumen fluid was collected from 2 Jersey cows fed either a concentrate-based dairy ration or alfalfa hay only. Each protozoa-enriched rumen fluid was incubated for 24 h with or without the aforementioned inhibitors and fed a mixture of ground wheat grain, alfalfa, and grass hays to support microbial growth. Live protozoa cells were morphologically identified and counted at 3, 6, 12 and 24 h of incubation. Fermentation characteristics and prokaryotic composition were determined at 24 h of incubation followed by statistical test using GLIMMIX of SAS. Except for IOD, all the inhibitors reduced all the 9 protozoal genera detected, but to different extents, in a time-dependent manner. IOD was the least inhibitory to protozoa, but it halved ammoniagenesis while not decreasing feed digestibility or VFA. ANCOM analysis identified loss of *Fibrobacter* and overgrowth of *Succinivibrio* and *Treponema* up to 43 and 21%, respectively. Functional prediction using the CowPI database showed that all the inhibitors decreased the relative abundance of the genes encoding lysosome and peptidases. Overall, inhibition of protozoa resulted in changes in prokaryotic microbiota and in vitro fermentation, and peptidases, especially cysteine-peptidase, may be targeted to improve nitrogen balance in the rumen.

Key Words: rumen protozoa, nitrogen utilization efficiency, peptidase inhibitor

W155 The effect of ultrasound processing and exogenous cellulase supplementation on the ruminal degradability of palm date seeds.

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The objective of this study was to evaluate the effects of ultrasound processing (sonication) and 2 exogenous pure cellulase enzyme supplements on the rumen degradability of palm date seeds (PDS) under in vitro condition. The 2 enzymes were derived from Trichoderma Ressei (TR) or from *Aspergillus niger* (AN). The sonicated palm date seeds(SPS) were obtained by placing the PDS into 400 mL metal reactor containing 4% NaOH and then sonicating the seeds for 5 min using an ultrasonic processor (900 W, 20 kHz, 100% power level). The SPS were then recovered and freeze-dried. The PDS and SPS were then placed in Dacron bags (2.5g/bag) and incubated in 250 mL jars containing 120 mL of buffer, 30 mL of rumen fluid and 2 g of a TMR diet (50:50 forage and concentrate). Enzyme supplements were added at the rate of 60 mg/kg of diet DM. Jars were incubated in a water bath at 39°C under anaerobic and treatments were run in quadruplicate. Data were analyzed using 2-way ANOVA model of JMP. After 24 h of incubation, the average NDF_{om} and OM degradability of PDS were 26.4 and 23.4%, respectively, and were not affected ($P > 0.05$) by enzyme supplements. On the other hand, the average NDF_{om} and OM degradability of SPS increased to 72.2 and 67.6%, respectively, and the addition of TR, but not the AN, enzyme supplement slightly but significant ($P < 0.05$) increased the SPS NDF_{om} and OM degradability to 77.0 and 71.9%, respectively. In conclusion, sonicating PDS in NaOH solution increased their rumen degradability. The cellulase enzyme supplements had no effect on the rumen degradability of PDS but TR addition improved the rumen degradability for SPS.

Key Words: palm date seeds, sonication, exogenous cellulase supplements

W156 Effect of method of diet delivery to dairy cows on enteric methane emissions, digestion and milk performance.

C. Benchaar and F. Hassanat*, *Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada.*

This study investigated the effects of method of diet delivery (concentrates and forages separately versus total mixed ration) on enteric methane emissions, digestion, and milk production. In a crossover design (35-d periods), 16 lactating multiparous Holstein cows (BW = 647 ± 84 kg; DIM = 78 ± 39.3 d; milk yield = 46 ± 6.3 kg) were fed (ad libitum; 5% orts on as-fed basis) a diet as concentrates and forages separately (CF), or as a total mixed ration (TMR). The diet was composed of 47% pelleted concentrates and 53% Forages (alfalfa silage, corn silage and timothy hay). Production of CH_4 was determined (5 consecutive days) using air flow-controlled chambers, while DMI, milk performance and DM apparent-total-tract digestibility were determined over 7 consecutive days. Data were analyzed using the MIXED procedure of SAS and

Table 1 (Abstr. W155). The effect of sonication and cellulase enzyme supplement on the in vitro ruminal degradability of palm date seeds[1]

	PDS			SPS				*P*-value		
	PDS	PDS+AN	PDS+TR	SPS	SPS+AN	SPS+TR	MSE	Seeds	Enzymes	Seeds × enzymes
NDF_{om},%	26.44^{c}	25.75^{c}	25.88^{c}	72.38^{b}	72.09^{b}	77.00^{a}	0.763	0.01	0.01	0.01
OM,%	23.41^{d}	22.85^{d}	23.24^{d}	67.56^{b}	65.80^{c}	71.94^{a}	0.507	0.01	0.01	0.01

[a–c]Means with different letters within the same row are significantly different ($P < 0.05$).

[1]PDS = palm date seeds; SPS = sonicated palm date seeds; AN = *Aspergillus niger*; TR = *Trichoderma reesei*.

significance was declared at $P \leq 0.05$. Method of diet delivery had no effect on DMI (26 kg/d), or yields of milk (45.5 kg/d), milk fat (1.41 kg/d) and milk protein (1.46 kg/d). Apparent-total-tract digestibility of DM was higher when cows were fed CF (69.9%) compared with when fed TMR (68.9%). Enteric CH_4 emission expressed as g/d, g/kg DMI and as percentage of gross energy intake averaged 420 g/d, 16.2 g/kg and 4.9%, respectively and was not affected by diet delivery method. Results of this study show that offering the diet as concentrates and forages separately or as total mixed ration did not influence enteric CH_4 emissions or milk performance of dairy cows.

Key Words: methane, concentrate and forage, total mixed ration

W157 Effect of kelp meal on milk yield, methane emission, and thyroid hormones in Jersey cows.

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Kelp meal (KM) is a supplement made from the brown seaweed *Ascophyllum nodosum* known to bioaccumulate iodine (I) in addition to be the richest of phlorotannins (PT). Previous research revealed that up to 5% of KM in the diet DM decreased CH_4 emission in vitro and inhibited ruminal archaea in vivo with these responses likely modulated by PT. However, feeding ≥2% of KM is challenging because high intake of I may negatively affect thyroid health. We designed a study in which 2% of KM (400 g/d) was fed during 3 wk (high phase) followed by 0.5% of the diet DM (100 g/d) for another 3 wk (stepdown phase) and complete removal during the last 3 wk of the experiment (withdraw phase). Our goal was to investigate the effect of KM on yields of milk and milk components, CH_4 emission, and thyroid hormones. Twenty Jersey cows (102 ± 52 DIM) were assigned to treatments in a randomized complete block design. Cows were fed (DM basis) 60% alfalfa hay and 40% concentrate. Milk and blood samples were collected on wk 3, 6, and 9. The GreenFeed system was used to measure CH_4 emission throughout the study. Data were analyzed using the MIXED procedure of SAS with repeated measures over time. Iodine intake from KM averaged 240 mg/d (high phase) and 60 mg/d (stepdown phase). This resulted in I intake 34- (high phase) and 8.5-fold (stepdown phase) greater than required (i.e., 7.1 g/d). No treatment by wk interactions were observed for any variable tested. Likewise, treatments did not affect DMI (mean = 20.4 kg/d) and milk yield (mean = 24 kg/d). Concentrations and yields of milk fat and true protein, as well as CH_4 production were also unchanged. Specifically, CH_4 production averaged 389 g/d (high phase), 394 g/d (stepdown phase), and 380 g/d (withdraw phase). Treatments did not affect plasma concentrations of thyroid stimulating hormone (mean = 0.445 µIU/mL), free triiodothyronine (mean = 4.43 ng/mL), free thyroxine (mean = 0.74 ng/mL), and cortisol (mean = 31 ng/mL). Overall, dietary inclusion of up to 2% KM did not affect CH_4 production or thyroid health, suggesting that greater levels of KM may be required to depress methanogenesis and thyroid function.

Key Words: iodine, methane, seaweed

W158 *Enterobacteriaceae* bacteria counts vary for US commercial dairy total mixed rations fed during summer months.

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Feed *Enterobacteriaceae* colony count per g (cfu/g) can be a bacterial contamination measure however population data for commercial dairy TMR are unknown. The objective here was to determine if a food safety assay (3M Petrifilm *Enterobacteriaceae* count plate) was capable of culturing *Enterobacteriaceae* in TMR and determine if counts varied for commercial TMR. Samples were collected (n = 370), from April through September 2018, by US dairy industry professionals and submitted to Rock River Laboratory (Watertown, WI) for analysis. Samples were processed according to 3M Petrifilm instructions (2017, reference 6420/6421). In brief, 10g of TMR was blended into 90 mL Butterfield buffer, shaken and diluted 10, 100, 1000 and 10000-fold. 1mL of diluted solution was plated and incubated at 30C for 24h. Plates counts were manually counted and TMR cfu/g determined by multiplying plate count by plate dilution factor. A separate subsample (approximately 150 g) was dried, ground and analyzed for nutritive parameters by NIR to evaluate correlations with *Enterobacteriaceae* counts. The bacterial count data were found to be not normally distributed, thus data were transformed using log10 transformation. The log10 transformed data were found to be normal using the continuous fit - normal function in JMP v14.0. The resulting population statistics (log10 cfu/g) in TMR were then as follows: mean = 2.75, standard deviation = 1.18, coefficient of variation (CV) = 42.9%, minimum = zero, maximum = 5.02, and 15th and 85th percentiles = 1.67 and 3.95, respectively. Correlations were evaluated using the response screening and multivariate methods functions in JMP v14.0. Independent pairwise correlations were significant ($P < 0.05$) for 11 NIR predicted nutritive parameters, with the largest r-values being water soluble carbohydrate (r = 0.21), dry matter (r = 0.18), and in situ rumen starch digestibility (3h; r = −0.18). These nutrition and bacterial count correlations warrant further investigation. The 3M Petrifilm proved capable of culturing *Enterobacteriaceae* colonies with TMR samples and $\log_{10}$ transformed data population statistics, with a CV greater than 40%, suggest variation exists in *Enterobacteriaceae* counts in TMR on commercial dairies.

Key Words: feed contamination, enterobacteria

W159 Use of a mass flow meter in headbox-style indirect calorimetry, and the effects of gas recovery on estimated energy partition in lactating dairy cows.

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Headbox-style indirect calorimeters can robustly estimate heat production (HP) of cattle. Volumetric flow meters (VFM), originally designed to measure gas flow in residential homes, have proven to be a cost effective method to measure airflow. Effective operation of these meters requires frequent maintenance and recalibration. Additionally, the rate of airflow of a lactating dairy cow is outside of the meters designed range. The objectives of this study were to test mass flow meters (MFM) and VFM by measuring O_2 consumption and CO_2 production and to quantify the effects of incomplete gas recovery on estimated energy partitioning. Two headboxes were initially equipped with VFM and later replaced with MFM. To determine the effects of type of airflow meter on O_2 consumption and CO_2 production, ethanol (100%) was burned for 2 h. Efficiency was calculated as the proportion of O_2 and CO_2 recovered from the amount of alcohol burned. As airflow was estimated by both methods, a subsample of gas was collected into a bag. Air in each bag was analyzed using gas chromatography. Data were analyzed using a paired *t*-test. Recovery of O_2 tended ($P = 0.12$) to be greater for MFM than VFM (99.7 ± 1.16% vs. 86.8 ± 4.50%). Recovery of CO_2 was greater ($P = 0.04$) when using the MFM than VFM (99.0 ± 1.76% vs. 86.0 ± 2.97%). These results suggest that MFM may yield more precise measures needed for indirect calorimetry. Incomplete gas recovery can result in underestimates of HP, thereby affecting estimates of whole-animal energy use. For example, a typical Jersey cow (assuming the body weight = 450 kg, dry matter intake = 18.5 kg, milk yield = 25.0 kg/d, urinary N excretion = 225 g/d and tissue energy = 0.00 Mcal/d) a 5.0% decrease in gas recovery results in a reduction in HP by 1.30 Mcal/d and increase in tissue energy by 1.45 Mcal/d. This tissue energy translates into an increased net energy of 1.46 Mcal/d. Our results indicate that in striving for estimates of gas recovery of 95.0 ± 5.00%, MFM may be better suited for headbox-style indirect calorimetry to estimate HP in lactating cows.

Key Words: energy partition, gas recovery, indirect calorimetry

W160 Fermentation of total mixed ration using microbial inoculants and its effect on in vitro rumen fermentation and microbial population in Hanwoo cows. M. A. Miguel*[1], L. L. Mamuad[1], C. D. Jeong[1], S. M. Kwon[1], M. J. Ku[1], Y. S. Choi[2], Y. I. Cho[1], and S. S. Lee[1], [1]*Department of Animal Science and Technology, College of Bio-industry Science, Sunchon National University, Suncheon, Jeonnam, Republic of Korea,* [2]*Livestock Research Institute, Jeonnam Agricultural Research and Extension Services, Gangjin, Jeonnam, Republic of Korea.*

This study determined the effect of microbial inoculants in fermenting total mixed ration (TMR) and used for in vitro rumen fermentation and microbial population in Hanwoo cows. Three different TMR were used in the study and each TMR were grouped into 3: non-fermented TMR (no inoculants) and fermented TMR (FTMR) for 7 and 14 d (inoculated with mixed culture of *L. acidophilus* and *B. subtilis*, 1.0×10^6 cfu/mL of each inoculant). After fermentation, the treatments were used for in vitro rumen fermentation using ruminal fluid collected from 3 cannulated Hanwoo cows.VFA was analyzed using HPLC and microbial population was determined by quantitative real-time PCR using target specific primers. The data were analyzed as a 3x3 factorial arrangement in a completely randomized design using the MIXED procedure of SAS. Fermentation quality of FTMR showed higher ($P < 0.05$) contents of lactate, acetate, NH_3-N but lower ($P < 0.05$) pH, propionate and butyrate compared with the non-fermented TMR group. For the in vitro rumen fermentation, higher ($P < 0.05$) total gas production and NH_3-N concentration were observed in the FTMR group. Specifically, FTMR2 and FTMR3 for 14 d had the highest ($P < 0.05$) total gas production at 6 and 24 h, whereas FTMR3 for 14 d had the highest ($P < 0.05$) NH_3-N concentration at 24 h. Acetate, propionate and total VFA concentrations in the FTMR group were higher ($P < 0.05$) compared with non-fermented TMR group while no significant differences were observed in the butyrate production in all TMR groups. No differences were found in the number of total bacteria, *Lactobacillus* spp., *Bacillus* spp., and *Fibrobacter succinogenes* in all TMR groups. However, the number of protozoa increased ($P < 0.05$) whereas general anaerobic fungi and *Ruminococcus flavefaciens* population decreased ($P < 0.05$) in the TMR group fermented for 14 d. Overall, the results suggested that fermentation improved the quality of TMR and fermented total mixed ration can enhance rumen fermentation parameters and enable changes in the population of rumen microbes.

Key Words: Hanwoo cow, rumen fermentation, total mixed ration

W161 In vitro evaluation of rumen fermentation of ground, dry rolled and steam-flaked corn compared with pre-grinding super-conditioning pelleted corn. A. Rahimi[1], A. Naserian[1], R. Valizadeh[1], A. Tahmasebi[1], H. Dehghani[2], K.-i. Sung[3], B. Kim*[3], A. Shahdadi[1], S. Kim[3], J. Ghassemi Nejad[4], and M. Malekkhahi[5], [1]*Faculty of Agriculture, Animal Science Department, Ferdowsi University of Mashhad, Mashhad, Iran,* [2]*The Research Institute of Biotechnology, Ferdowsi University of Mashhad, Mashhad, Iran,* [3]*College of Animal Life Sciences, Kangwon National University, Chuncheon, Republic of Korea,* [4]*College of Animal Bio-Sciences, Konkuk University, Seoul, Republic of Korea,* [5]*Dordaneh Razavi, Mashhad, Iran.*

The objective of this study was to evaluate the pre-grinding super-conditioning pelleted corn in comparison with steam-flaked corn on gas production in vitro. An Iranian corn variety (single crass 702) was processed by 1) grinding (G), 2) dry rolling (DR), 3) steam-flaking (SF; moisture 20%, retention time 60 min, rolling temperature 105á´1/4C) and 4) pre-grinding super-conditioning pelleted corn (PSCP; moisture20%, retention time 6 min and conditioning temperature 95á´1/4C). In gas production test, gas pressure and volume were recorded 2, 4, 6, 8, 12, 24, 36 and 48 h after incubation. Gas production kinetics were estimated based on equations without a lag phase. Data were analyzed by GLM procedure of SAS ($P < 0.05$) using a completely randomized design with 6 replications for every treatment by the model; Y = Î1/4 +Txi+Îµij, were Y is the variable, Txi is the effect due to the treatment, and Îµij is the experimental error. The GP of PSCP in 6 h was significantly increased ($P < 0.05$) (163.53, 98.15, 56.24 and 64.50 with SEM 12.76 mL/gDM in PSCP, SF, DR and G, respectively), but in 48 h GP in PSCP and SF was similar (298.7 vis 297.5 mL/gDM). The pH and N-NH3 in 24 h were significantly lower ($P < 0.05$) in PSCP than other processing methods ($P < 0.05$). Total VFA after 24 h incubation was significantly increased ($P < 0.05$) in PSCP in than other processing methods (73.91, 70.74, 70.21 and 68.42 with SEM 0.756 Mmol/ml in PSCP, SF, DR and G, respectively). The SF corn showed higher ($P < 0.05$) acetate (62.94%), lower ($P < 0.05$) propionate (24.24%) and higher ($P < 0.05$) acetate to propionate ratio (2.60) between treatments. However, lower ($P < 0.05$) acetate (58.23%), higher ($P < 0.05$) propionate (28.88%) and lower ($P < 0.05$) acetate to propionate ratio (2.02) were observed in PSCP between treatments. Asymptote gas production (A) was not affected by different processing methods, but time to produce half of A (K; 5.74 h), time for ferment of 25% (2.45 h) and 75% (13.42 h) of substrate were significantly reduced ($P < 0.05$) by PSCP processing. The in vitro gas production fermentation rate (c) was higher ($P < 0.05$) for PSCP (0.113, 0.056, 0.046 and 0.041 /h with SEM 0.008 in PSCP, SF, DR and G, respectively). It seems that PSCP in comparison to SF increased in gas productivity and starch fermentability. Short time for achieving maximum gas production in PSCP corn indicated that PSCP was an effective way for enhancing of corn fermentability in the rumen.

Key Words: gas production, pre-grinding super-conditioning pellet, steam-flaking

W162 Apple of Sodom (*Calotropis procera*) inhibits rumen protozoa without impairing fermentation *in vitro*. A. G. Ayemele[1], L. Ma[1], T. Park[2], J. C. Xu[1,3], Z. T. Yu[2], and D. P. Bu*[2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Department of Animal Sciences, The Ohio State University, Columbus, OH,* [3]*World Agroforestry Center, East and Central Asia, Kunming, China.*

Rumen protozoa, particularly the most predominant *Entodinium* spp., engulf rumen bacteria and subsequently recycle the microbial protein intraruminally, substantially lowering the nitrogen utilization efficiency in dairy cows. This study aimed to identify plants that can improve nitrogen utilization efficiency by inhibiting rumen protozoa, especially the genus *Entodinium*. Four different plants were first screened for their protozoa-suppressing activity using in vitro cultures of rumen fluid. Apple of Sodom (*Calotropis procera*) was found to be the most anti-protozoal, and it was further evaluated using in vitro cultures of fresh rumen fluid collected from 3 lactating cows, which were fed a typical total mixed ration (TMR). Ground leaves of apple of Sodom at one of the 4 levels (0, 0.007, 0.009, and 0.011 g/mL) were tested in 3 replicate in vitro cultures incubated for 24 h at 39°C. Protozoa were enumerated using microscopic identification and counting, while volatile fatty acid (VFA) concentrations and methane production were determined using gas chromatography. Data were analyzed in a complete randomized design using the PROC GLM procedure of SAS 9.4. Orthogonal polynomial contrasts were done to determine linear and quadratic responses of protozoal cells, individual VFA and methane production. The results showed that apple of Sodom at all the tested doses significantly decreased the counts of *Entodinium* and *Isotricha* linearly ($P < 0.0001$) and had a quadratic effect on the counts of *Eudiplodinium* ($P = 0.0083$). *Dasytricha, Ophryocolex*, and *Eudiplodinium* were not affected ($P > 0.05$). Total VFA concentration was increased. Except for acetate, the concentrations of individual VFA were significantly affected linearly ($P < 0.05$), and butyrate and isobutyrate were also affected quadratically ($P < 0.05$). Acetate:propionate ratio was also significantly affected linearly ($P < 0.0001$). Methane emission was increased ($P < 0.0138$). Overall, *Calotropis procera* leaves can be used as a feed supplement to inhibit *Entodinium* and increase nitrogen utilization and fermentation in ruminants.

Key Words: *Calotropis procera*, *Entodinium* spp., nitrogen efficiency

W163 Dose-response effect of the macroalga *Asparagopsis taxiformis* on enteric methane emission in lactating dairy cows.

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An in vitro and an in vivo study were conducted to determine the antimethanogenic activity of the macroalga *Asparagopsis taxiformis* (AT) in lactating dairy cows. In vitro, 9 treatments were tested in 24-h incubations with ruminal inoculum collected from 2 lactating Holstein cows. Treatments were: control (no additives), AT at 0.25, 0.5, 0.75, 1.0 and 1.5% of feed dry matter (DM), and bromoform ($CHBr_3$), the active compound in AT, equivalent to $CHBr_3$ found in AT dosed at 0.25, 1.0, and 1.5%. The in vivo study was conducted with 6 multiparous Holstein cows [68 ± 4.2 d in milk, 46 ± 2.5 kg/d milk yield (MY)]. All cows received the same AT inclusion rates of 0, [background (BG) period], 0.25, 0.5, and 0.75% of feed DM. Each phase consisted of 7-d diet adaptation and 3-d sampling, followed by a 7-d washout period. Enteric methane (CH_4) emission was measured 8 times over 3 d using the GreenFeed system. The last 4 d of each phase were used for statistical analysis of MY and DM intake (DMI). Data were analyzed using the REG (in vitro) and MIXED (in vivo; the model included treatment) procedures of SAS using contrasts. In the in vitro experiment, AT decreased CH_4 yield linearly [$P < 0.001$; 27.3, 35.4, 29.4, 15.0, 1.42, and 0.26 mL CH_4/g feed DM (SEM = 3.68) for control, 0.25, 0.5, 0.75, 1.0, and 1.5% AT, respectively]. $CHBr_3$ had no effect ($P \geq 0.59$) on CH_4 production. In the in vivo experiment, DMI was decreased by the highest (0.75%) level of AT [$P = 0.03$; 22.7, 27.6, 25.4, and 17.7 kg/d (SEM = 1.7) for BG, 0.25, 0.5, and 0.75% AT, respectively]. MY was also decreased by 0.75% AT ($P = 0.05$; 46.2, 47.5, 46.0, and 39.2 kg/d respectively; SEM = 3.4). Daily CH_4 emission decreased linearly ($P < 0.001$) with AT inclusion rate: 392, 367, 84, and 83 g/d, (SEM = 20) respectively. CH_4 yield was decreased quadratically ($P = 0.02$; from 17.5 to 5.7 g/kg DMI; SEM = 1.3) and CH_4 intensity was decreased linearly ($P < 0.001$; from 8.4 to 2.4 g/kg milk; SEM = 3.2) by AT. In the short term in vivo experiment, inclusion of AT at 0.50% of DMI decreased CH_4 emission in lactating dairy cows by 80% and had no effect on DMI or MY.

Key Words: methane, *Asparagopsis taxiformis*, dairy cattle

W164 Assessing ruminal fermentation using the omasal sampling technique and the dual-flow continuous culture system as models: A meta-analytical approach.

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Our objective was to compare data collected in vivo, using the omasal sampling technique (OST), to in vitro data, using the dual-flow continuous culture system (DFCCS), to assess ruminal fermentation and digestion. A meta-analysis was performed using 75 peer-reviewed articles using DFCCS and 20 using OST. The study inclusion criteria were: 1) it must have reported dietary composition; 2) only DFCCS studies were included in the in vitro data set; 3) it must have used ruminal samples from dairy or beef cattle; 4) it must have reported at least one of the dependent variables of interest. The independent variables were: dietary neutral detergent fiber (dNDF), dietary crude protein (dCP), and efficiency of microbial protein synthesis (EMPS), whereas dependent variables were: total volatile fatty acids (VFA) concentration, molar proportions of acetate, propionate, and butyrate, true ruminal digestibility of organic matter (TROMD), and NDF (RNDFD), ammonia nitrogen (NH_3-N) concentration, EMPS and efficiency of nitrogen use (ENU). Data were analyzed using the MIXED procedure of SAS for linear models and the NLMIXED procedure for nonlinear models. Significance levels for fixed and random effects were $P < 0.05$. The system (DFCCS or OST) was tested on each parameter of all models. Dietary NDF was not related ($P > 0.05$) with molar proportion of butyrate, TROMD, NH_3-N concentration, and EMPS. While, dCP was not related ($P > 0.05$) with total VFA concentration and molar proportion of butyrate. For total VFA concentration, acetate, and propionate as function of dNDF an exponential equation was fit. A quadratic equation was fit for TROMD, RNDFD as function of dNDF. A linear equation was fit for acetate and propionate as function of dCP, and for RTDOM as function of EMPS. Overall we observed a strong correlation between data obtained by OST compared with DFCCS. System did not affect the parameters of all tested equations ($P > 0.05$), demonstrating that the DFCCS provides valuable measurements of ruminal fermentation and nutrient digestibility compared with OST.

Key Words: ruminal digestion, microbial fermentation, meta-analysis

W165 Effect of enzyme extracts from *Aspergillus oryzae* and *Aspergillus niger* on rumen bacterial and fungal diversity and fermentation in vitro.

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The effect of enzyme extracts (ENZ) from *Aspergillus oryzae* and *Aspergillus niger* on rumen bacterial and fungal diversity and fermentation variables was investigated in a batch culture in vitro experiment.

ENZ was tested at 5 inclusion levels, equivalent to 0, 4, 8, 12, and 16 g ENZ/head/d, calculated based on 25 kg/head/d dry matter (DM) intake. Replicated incubations were conducted for 0, 3, 6, 12, 18, and 24 h. Gas production and composition, pH, volatile fatty acids (VFA), ammonia, neutral-detergent fiber (NDF) degradability, and bacterial and fungal quantities and diversity data were collected. Samples were analyzed for fermentation variables using routine procedures, and bacterial and fungal quantities and diversity were assayed by MR DNA Molecular Research (Shallowater, TX) using qPCR and bTEFAP technologies. Data were analyzed with the MIXED procedure of SAS as repeated measures [with ar(1) covariance structure], using orthogonal polynomial contrasts. The model included incubation, treatment, incubation time, and interactions. Gas production (average 30.7 mL/g feed DM; SEM = 1.58), methane concentration (average 4.50%; SEM = 0.522), and pH (average 6.41; SEM = 0.016) were not affected ($P \geq 0.19$) by ENZ. Concentration of total VFA decreased linearly ($P < 0.001$; from 69.8 to 67.4 m*M*, SEM = 0.63) with increasing ENZ dose. Molar proportions of acetate, butyrate, and iso-valerate were also decreased ($P \leq 0.02$) by ENZ. NDF degradability linearly decreased ($P < 0.001$; from 63.7 to 54.8%, SEM = 1.53) with increasing ENZ inclusion rate. ENZ had no effect ($P \geq 0.82$) on bacterial and fungal quantities (qPCR). The proportion of bacterial genera *Treponema* and *Porphyromonas* and fungal genus *Neocallimastix* were linearly decreased ($P \leq 0.03$) by ENZ. No other effects of treatment on bacterial and fungal diversity were observed. In the conditions of this in vitro batch culture experiment, the combination of enzyme extracts from *A. oryzae* and *A. niger* had negligible effect on bacterial and fungal quantities and diversity but appeared to inhibit rumen fermentation and NDF degradability.

Key Words: enzyme extract, rumen fermentation, in vitro

W166 Novel ruminal microbial urease inhibitors screened through molecular docking. Z. Zhang[1,2], S. Zhao[1,2], N. Zheng[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing,China.*

Urease inhibitor is considered as one possible method for increasing the utilization efficiency of dietary urea in ruminant animals. Previous studies suggest that Cys319 located on the Thr308-Arg336 flexible loop covering the active site of *Klebsiella aerogenes* urease could be used as a targeting point to inhibit urease activity. In this study, we employed a structure-based molecular docking of the Chemdiv database against the Thr308-Arg336 flexible loop of *K. aerogenes* urease (PDB:4EP8). The Chemdiv database was prepared by using the Compound Filtering module of sybyl-X2.1 software. Two rounds of virtual screening were implemented. Eventually, 99 compounds were achieved to undergo in vitro screening against rumen bacterial urease, which performed by the Berthelot alkaline phenol–hypochlorite method. The IC50 values were determined by nonlinear curve fitting of gradient concentration of the compound with residual activity of urease. Sixteen compounds were found to be relatively potent urease inhibitors with the inhibition rate over 40% at the concentration of 0.5mM. The compound 3-[1-[(aminocarbonyl)amino]-5-(4-methoxyphenyl)-1H-pyrrol-2-yl] propanoic acid (compound 22) was found to be superior agent in the series with an IC50 = 68.42μM. The binding mode of compound 22 was revealed by molecular docking study. Three hydrogen bonds are formed between compound 22 and important active site residues; Asp221, Arg336 and Asp220. Two hydrogen bonds are also formed between the carboxyl group of compound 22 and important active site residues; Ala363 and Asp360. Relatively strong hydrophobic interaction is formed between compound 22 and hydrophobic residues; His320, Cys319, Met364 and Ala167. This study reaffirmed the viability of using the Thr308-Arg336 flexible loop covering the active site of urease as a target to screen chemical databases. These compounds can be used as starting points for lead optimization.

Key Words: molecular docking, urease inhibitor, propanoic acid

W167 Effects of feeding barley grain on intake, rumen pool size and apparent total tract digestibility in lactating dairy cattle fed pasture-based diets. M. Dineen*[1,2], B. McCarthy[2], P. Dillon[2], S. W. Fessenden[1], P. A. LaPierre[1], R. A. Molano[1], and M. E. Van Amburgh[1], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*Teagasc, Animal and Grassland Research and Innovation Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

The objective of this study was to evaluate the effects of barley grain supplementation on intake, rumen pool size (PS) and apparent total-tract digestibility (TTD) of dry matter (DM), Neutral Detergent Fiber (aNDFom) and undigested Neutral Detergent Fiber (uNDFom) in lactating dairy cattle fed mechanically cut fresh perennial ryegrass indoors. Ten ruminally cannulated Holstein cows averaging 49 ± 23 DIM and 513 ± 36 kg of BW were assigned to one of 2 treatments in a switchback design. Diets contained (DM basis) 100% perennial ryegrass (PRG; DM = 18%, CP = 16%, Starch = 2%, aNDFom = 36%) or 80% PRG and 20% rolled barley (PRG+B; DM = 33%, CP = 15%, Starch = 14%, aNDFom = 33%). The trial consisted of 3 29 d periods where each period consisted of 21 d of diet adaptation and 8 d of data and sample collection. To quantify rumen PS, rumen contents were evacuated on d 28 and 29 of each period, weighed, mixed, and sampled. To estimate TTD, fecal samples were collected during 3 8 h intervals on d 24, 26 and 27 to encompass every 2 h of a 24 h cycle. All data were analyzed using the Proc Mixed procedure in SAS. The inclusion of barley grain increased the rumen PS of DM, aNDFom and uNDFom however reduced pasture intake and apparent TTD of DM and aNDFom (Table 1). Energy corrected milk was not effected by treatment (24.5 vs 24.1 ± 0.8 kg day^{-1} for PRG and PRG+B, respectively).

Table 1 (Abstr. W167). Effects of barley grain on intake, rumen pool size (PS) and apparent total tract digestibility (TTD) in lactating dairy cattle fed pasture-based diets

Item	PRG	PRG + B	SEM	*P*-value
Pasture intake (kg DM day^{-1})	15.94	13.02	0.57	<0.0001
Barley intake (kg DM day^{-1})	—	3.32	—	—
Total intake (kg DM day^{-1})	15.94	16.34	0.57	0.12
aNDFom intake (kg day^{-1})	5.76	5.34	0.20	<0.0001
uNDFom intake (kg day^{-1})	0.58	0.68	0.02	<0.0001
Rumen DM PS (kg day^{-1})	9.26	10.08	0.55	0.02
Rumen aNDFom PS (kg day^{-1})	4.81	5.53	0.34	<0.001
Rumen uNDFom PS (kg day^{-1})	1.54	1.75	0.09	<0.0001
Apparent TTDMD[1] (g g^{-1})	0.83	0.80	0.003	<0.0001
Apparent TTaNDFomD[2] (g g^{-1})	0.83	0.75	0.007	<0.0001

[1] TTDMD = total tract dry matter digestibility.

[2] TTaNDFomD = total tract aNDFom digestibility.

Key Words: rumen, pool size, total-tract digestibility

W168 Comparison of ruminal digestibility of *Origanum onites* L. leaves in dairy buffalo and cows. E. Gultepe*[1,2], C. Uyarlar[1], I. Cetingul[1], A. Iqbal[1], U. Ozcinar[1], I. Bayram[1], and B. Bradford[2], [1]*Department of Animal Nutrition and Nutritional Diseases, Afyon Kocatepe University, Afyonkarahisar, Turkey,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

This experiment evaluated the ruminal digestibility of Turkish oregano leaves in dairy buffalo and cows. Ruminally-cannulated, multiparous Brown Swiss cows (n = 3) and water buffalo (*Bubalus bubalis*; n = 3) were used in the experiment. The ad libitum basal diet was balanced to NRC requirements for a dry, nonpregnant multiparous dairy cow (680 kg) and consuming 12.8 kg of DM/d. Air-dried, ground, weighted oregano leaves were inserted in the rumen of all animals before the morning feeding with heat-sealed nylon bags (× 4 replicates). After incubation (4, 8, 24, 48, 72 h), dry matter (DM), crude protein (CP), aNDFom, and ADFom concentrations were determined and compared with initial leaf composition. In situ disappearance (ISD) parameters were evaluated using PROC MIXED of SAS. Degradability kinetic parameters were fit with an exponential model using PROC NLIN and differences in parameters between the species were determined by *t* test. The means for ISD (%) of DM, CP, aNDFom, and ADFom were 50.9 ± 1.9 vs. 61.0 ± 2.3; 31.7 ± 3.5 vs. 62.6 ± 2.7; 41.0 ± 4.2 vs. 41.2 ± 3.5; and 49.4 ± 2.2 vs. 60.5 ± 2.4 for cows and buffalo, respectively, after 24 h incubation. Mean ruminal disappearance of DM, aNDFom and ADFom did not differ between the species, but CP degradation showed a species effect ($P = 0.04$) and a species × time interaction ($P = 0.04$). The degradation rate ($\%\ h^{-1}$) constants of DM, aNDFom, ADFom, and CP were 5.24 ± 0.17 vs. 4.83 ± 0.80; 4.90 ± 0.69 vs. 3.63 ± 1.29; 4.43 ± 0.68 vs. 4.22 ± 0.79; and 2.40 ± 0.80 vs. 3.35 ± 0.97 for cows and buffalo, respectively. The immediately-degraded fractions of DM and CP were significantly greater in buffalo than the cows (27.1 ± 1.5 vs. 17.8 ± 2.1% of DM, $P = 0.02$ and 32.1 ± 0.9 vs. 13.6 ± 6.3% of CP, $P = 0.04$, respectively), but the other model parameters showed no differences between the species. In conclusion, CP of oregano leaves is degraded more quickly in the rumen of dairy buffalo than cows, although extent of digestion did not differ after 72 h of incubation. The ruminal digestibility of *Origanum onites* L. leaves was similar to that of forages commonly fed to dairy cattle.

Key Words: oregano, in situ, Mediterranean buffalo

W169 Comparison of two sampling techniques for evaluating ruminal fermentation in dairy cows. C. F. A. Lage*[1,2], S. E. Räisänen[1], A. Melgar[1], K. Nedelkov[1,3], X. Chen[1,4], J. Oh[1], J. Bender[5], B. Vecchiarelli[5], D. Pitta[5], M. E. Young[1], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil,* [3]*Faculty of Veterinary Medicine Trakia University, Stara Zagora, Bulgaria,* [4]*College of Pastoral Agriculture Science and Technology, Lanzhou University, China,* [5]*School of Veterinary Medicine, University of Pennsylvania, New Bolton Center, PA*

Sampling and analyses of ruminal contents are important tools in ruminant nutrition research. The use of rumen-cannulated cows is the standard method to obtain representative samples of ruminal contents, but the use of an oral stomach tube can be a non-invasive option to collect rumen samples in intact animals. The objective of this study was to compare rumen fluid samples collected through a rumen cannula (CS) or using an oral stomach tube (ST) for measurement of pH, protozoa counts, dissolved hydrogen and volatile fatty acids (VFA). Six rumen-cannulated lactating Holstein cows were fed a standard diet for an adaptation period of 2 wks followed by sampling during the third experimental week. Rumen samples were collected at 0, 2, 4, 6, 8, and 12 h after the morning feeding in 2 consecutive days. CS samples were taken from the ventral sac, the atrium/reticulum, and 2 samples from the feed mat; composited samples were filtered through 2 layers of cheesecloth for further analyses. In parallel, ST samples were collected by inserting the stomach tube via the esophagus into the rumen to an approximately 120 to 150 cm depth. The first 100 to 200 mL of the ST rumen fluid samples were discarded. Data were analyzed using the MIXED procedure of SAS including treatment, sampling time, and their interaction in the model; cow was random effect. Compared with CS, ST samples had or tended to have greater ($P < 0.01$) pH (6.26 and 6.73, respectively; SEM = 0.13), lower ($P < 0.01$) protozoal counts (26.7 and 8.70×10^4, respectively; SEM = 5.10×10^4), and lower total ($P < 0.01$; 142 and 109 m*M*, respectively; SEM = 5.44) and individual VFA concentrations ($P < 0.01$), except for isobutyrate ($P = 0.30$). Molar proportions of individual VFA tended to be lower ($P \leq 0.07$) for ST compared with CS, except for butyrate and propionate ($P \geq 0.17$). There was no difference ($P = 0.51$) in dissolved hydrogen concentration between sampling methods. In conclusion, rumen fluid samples collected through an oral stomach tube are not representative of protozoa counts and fermentation variables as measured in samples of ruminal fluid collected through the rumen cannula.

Key Words: rumen fermentation, sampling method, dairy cow

W170 Mutagenesis of UreG to probe nickel binding and interaction with UreE in predominant urease of ruminal uncultured bacteria. X. Zhang[1,3], S. Zhao[1,2], X. Li[1,2], N. Zhen[1,2], and J. Wang*[1,2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agricultural Science, Beijing, China,* [2]*Key Laboratory of Quality & Safety Control for Milk and Dairy Products of Ministry of Agriculture and Rural Affairs, Institute of Animal Sciences, Chinese Academy of Agricultural Sciences, Beijing, China,* [3]*College of Animal Science and Technology, Huazhong Agricultural University, Wuhan, China.*

Urease, a nickel-dependent enzyme, catalyzes the hydrolysis of urea into ammonia and carbon dioxide. Maturation of urease involves the proper insertion of nickel to its active site and requires at least 4 accessory proteins, namely, UreE, UreF, UreG, and UreD. In addition, it was also demonstrated that both metallochaperones UreE and UreG play a critical role in the maturation of apo-urease. Previously, we obtained the predominant urease gene cluster from ruminal uncultured bacteria BIN257 by metagenomic sequencing and binning. In this study, we overexpressed and purified UreG and UreE from BIN257, nickel binding to UreG and its interactions with UreE as well as their roles in Ni^{2+} transfer were studied. We demonstrated by pull-down assays that UreE could combine with UreG in vitro. The properties of UreE-UreG interactions were examined using isothermal titration calorimetry (ITC) and analytical ultracentrifuge (AUC). The ITC data revealed that the reaction of UreE and UreG was an endothermic process dominated by hydrophobic action. Moreover, the stoichiometry of this band was 1:1, and AUC experiments showed that molecule masses of UreE and UreG were ~51.5 and 25.2 kDa, respectively, corresponding to UreE dimer and an UreG monomer, indicating that each dimer of UreE binds 2 molar eq of UreG monomer to format a complex $(UreE)_2$-$(UreG)_2$. That conformational changes are essential for UreG to change protein partners were further detected by mutagenesis and AUC studies. We identified that UreG residues Glu-23, Asp-41, Glu-46, Glu-66, Cys-70, His-72, Asp-78, and Asp-118 played crucial roles in the conformational changes upon nickel and UreE binding. ITC experiments revealed that

Glu-23, Asp-41, Glu-46, Glu-66, Cys-70, His-72, and Asp-78 on UreG were critical for nickel transfer from UreE to UreG, and Glu-66, Cys-70, His-72, and Asp-78 were also necessary to combine with UreE for UreG. Based on our results, interactions and nickel transfer between UreE and UreG play an important role in urease maturation process.

Key Words: UreG, UreE, nickel

W171 Genomic survey of the rumen microbiome of Argentinian dairy cows provides insights into farm-dependent influences on microbial community structure.

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The rumen microbiome has a direct impact on animal health and performance and as such is an important metric for the comparison of cows across regions and diets. The impact of geography, farm management, and other environmental factors on the rumen microbiome of dairy cows remains uncharacterized to date. The objective of this study was to survey the microbial composition of dairy cows across a range of farms in diverse geographies of Argentina and relate the taxonomic compositions and diversity metrics to farm-specific factors such as diet and feed management regimen. In total, 299 samples from 198 dairy cows across 10 farms in Argentina were sampled to identify trends and factors that influence rumen microbial composition. Bacterial and fungal populations were characterized via 16S rRNA and ITS gene amplicon sequencing, respectively, of rumen and fecal samples. Sequences were processed using the USEARCH pipeline and classified against the RDP database. High level trends identified a core rumen microbiome composed of 795 OTUs shared across all farms. However, distinct microbial compositions and unique taxa were also identified for each farm, resulting in strong clustering of β diversity by farm as determined by principal coordinate analysis with and without the core microbiome included. The forage content of the diet was also shown to have an important role in defining the microbiome of the host animal. Taken together, these findings represent a key step toward identifying the link between environmental factors and farm management on the rumen microbiome.

Key Words: rumen microbiome, sequencing, genomics

W172 Effect of substrate to inoculum ratio on outcomes of in vitro rumen fermentation.

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As a popular method for to predict in vivo rumen function, in vitro fermentation has been utilized for many years. However, little is published regarding the ideal methodology used in in vitro systems. Furthermore, as new and improved technology is applied to in vitro rumen fermentation models, these methods need to be updated to improve model accuracy. The objective of this study was to identify the effects of altering the rumen fluid: substrate ratio on the outcomes of in vitro rumen fermentation. In this study, rumen fluid was collected from 3 ruminally cannulated, mature Holstein heifers. Buffered rumen fluid was incubated with either 0, 1, 1.2, 1.4, 1.6, 1.8, 2, 4, or 8 g dry, ground substrate. The fermentation units were kept at 39°C for 24 h, under constant agitation (60 rpm). Gas pressure and temperature were measured every 15 min using the Ankom Gas Monitoring System (Ankom, cat no #7056; Macedon, NY). Total gas production was calculated according to the ideal gas law. Fermentation was stopped by placing bottles on ice and a sample of rumen fluid was frozen for VFA analysis (HPLC) at Dairyland Laboratories Inc. (Arcadia, WI). Gas production was modeled using the Gompertz Gas model. Increasing the ratio of substrate to inoculum caused an increase in total VFA production, propionic, valeric, acetic, and butyric acids ($P < 0.0001$; all measured as mmol/L). pH decreased as the inclusion rate increased ($P < 0.0001$), as did the ratio of acetic to propionic acid ($P < 0.0001$) and production of NH_3 (mmol/L; $P < 0.0001$). The lag time (h) of fermentation was inversely correlated with the inclusion rate ($P < 0.0001$). No significant change was observed in the fractional rate of fermentation (mmol/ h); $P > 0.05$), and maximum gas production (mmol) was only significantly decreased in the 8.0 g treatment ($P < 0.0001$). It was established that between the ratios of 1.2, and 1.6 g dry substrate: 100 mL inoculum, none of the measured outcomes were statistically different ($P < 0.05$), and the environment of the fermenter units was comparable to that of the functioning rumen. Future studies should conserve this ratio to maximize quality of in vitro fermentation data before in vivo use.

Key Words: VFA, in vitro fermentation, nutrition methods

W173 Biological or chemical modification of soybean meal alters in situ ruminal degradation kinetics.

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Soybean meal (SBM) has been used as a most important source of dietary proteins in ruminant diets, whereas relatively greater ruminal degradation of SBM limits nutritive merits on digestion, absorption, and utilization of its protein by ruminants. Biologically or chemically modified SBM such as fermented SBM (FSBM) or soy protein concentrate (SPC), respectively, are natural feeds that become protected SBM. The objective of this study was to assess in situ dry matter (DM) and crude protein (CP) degradation kinetics for SBM (control), FSBM (*Bacillus* fermentation), and SPC (alcohol extraction) prepared for by CJ CheilJedang (Seoul, South Korea). Three Holstein steers surgically fitted with ruminal cannula were used for in situ measurements for ruminal degradation kinetics. The 3 dietary treatments were compared according to a completely randomized design (n = 3). Kinetics of DM and CP degradation in situ was estimated by the nonlinear regression procedure of SAS. The wash fraction of DM was greater with SBM and FSBM than SPC ($P < 0.05$), but the potentially degradable fraction of DM was greater with SPC compared with SBM and FSBM ($P < 0.05$). The soluble fraction of CP was greater with FSBC than SBM and SPC ($P < 0.05$), while the potentially degradable fraction of CP was lower with FSBM than SBM and SPC ($P < 0.05$). The ruminal degradation rate was the greatest with SBM followed by FSBM and SPC, leading to the reduced effective ruminal degradability with SPC (36.0%) compared with SBM (56.3%) and FSBM (54.1%). Similarly, in situ DM disappearance at 48 h was the greatest with SBM (93.1%) followed by FSBM (79.9%) and SPC (67.1%), resulting in the same pattern of CP disappearance at 48 h (93.4, 78.2, and 60.7% from SBM, FSBM, and SPC, respectively). The overall results indicate that biological or chemical modification of SBM improved ruminal degradation characteristics by reducing DM and CP degradation rate and content with greater improvement achieved by alcohol extraction. With the consideration of sizably elevated CP concentration of SPC (67.2%) and FSBM (60.6%) relative to SBM (55.9%), SPC and FSBM hold potential for greatly valued sources of protein supplement in ruminant diets.

Key Words: soybean meal, fermented soybean meal, soy protein concentrate

W174 Evaluating strategies to reduce ruminal protozoa and their impacts on nutrient utilization and animal performance in ruminants: A meta-analysis. X. Dai* and A. Faciola, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

This study was to evaluate the effects of 2 strategies (complete or partial ruminal protozoa (RP) elimination) on nutrient utilization and animal performance in the past 20 years. This study compared 66 published articles (N_{study} = 15 for complete and N_{study} = 51 for partial RP elimination) that used supplemental phytochemicals and lipids to reduce RP in vivo. Data were analyzed using PROC MIXED in SAS. Significance levels for fixed and random effects were $P \leq 0.05$. Compared with control, both complete and partial RP elimination had no effect on ruminal methanogens and bacteria. Supplemental phytochemicals and lipids decreased RP 6.25% ($P < 0.01$) and 12.2% ($P = 0.01$), respectively. Compared with control, complete RP elimination decreased methane production 18.3% ($P < 0.01$), and supplemental phytochemicals and lipids decreased 17.9% ($P = 0.02$) and 16.7% ($P < 0.01$), respectively. Compared with control, complete RP elimination increased molar proportion of acetate 2.68% ($P = 0.03$) while decreased butyrate 25.7% ($P < 0.01$); supplemental phytochemicals and lipids decreased molar proportion of acetate 2% ($P < 0.01$) and 1.59% ($P = 0.03$) while increased propionate 5.39% ($P < 0.01$) and 4.76% ($P < 0.01$), respectively. Compared with control, complete RP elimination decreased total-tract digestibility of OM 3.77% ($P < 0.01$) and NDF 9.87% ($P < 0.01$); supplemental phytochemicals and lipids decreased total-tract digestibility of DM 14.3% ($P = 0.05$) and 3.7% ($P = 0.04$), respectively. Supplemental lipids also decreased total-tract digestibility OM 3.06% ($P < 0.01$) and DMI 7.2% ($P < 0.01$). Positive linear relationships were found among RP and methane production, NDF, total VFA, molar proportion of acetate, NH_3-N, and DMI; negative linear relationships were found with pH and molar proportion of propionate. In conclusion, RP plays important roles on methane production regardless of diets, and partial elimination RP would be beneficial to ruminants in terms of energy and the environment, but supplemental lipids may have a negative effect on DMI.

Key Words: methane, plant extract, lipid

Ruminant Nutrition: Vitamins and Mineral Nutrition

W175 Responses to ruminally protected choline in transition cows do not depend on body condition. J. M. Bollatti*[1], M. G. Zenobi[1], B. A. Barton[2], J. E. P. Santos[1], and C. R. Staples[1], [1]*University of Florida, Gainesville, FL,* [2]*Balchem Corporation, New Hampton, NY.*

A question often asked is whether responses to supplemental choline during the transition period depend upon the degree of fatness of prepartum cows. Objectives were to evaluate the effect of rumen-protected choline supplemented from 21 d pre- to 21 d postpartum on performance of parous Holstein cows according to BCS at experiment enrollment on d 21 d prepartum. Data from 2 experiments at University of Florida (Zenobi et al. JDS 2018; Bollatti et al. JDS 2018) were combined. Pre- and postpartum diets were supplemented with methionine and the content in diets ranged from 2.24 to 2.35% of MP prepartum and 2.04 to 2.30% of MP postpartum. A total of 192 parous Holsteins cows at 255 d of gestation were blocked by parity and assigned randomly to receive 0 g/d (control) or 12.9 g/d of choline ion as choline chloride in a rumen-protected form (RPC). Cows remained in the experiment until 105 DIM. The body condition of cows was scored at experiment enrollment and cows were categorized as either moderate body condition (Mo; $BCS \leq 3.50$) or overconditioned (Ov; $BCS > 3.50$). Data were analyzed with mixed models for repeated measures and 2 models were used; the first included the fixed effects of treatment, week, BCS linear covariate, and interactions, and the random effects of block, experiment, and cow nested within treatment. The second model used BCS as category (Mo vs. Ov). Supplementing RPC increased yields of milk, ECM, fat, and lactose, improved feed efficiency, and tended to increase milk protein yield. Responses to supplemental RPC were observed regardless of BCS in the prepartum period.

Key Words: body condition, choline, transition

W176 Effect of dietary calcium nitrate on dry matter intake, milk production and ruminal parameters in dairy cows. K. V. Almeida*[1,2], J. A. C. Osorio[1], F. E. Marchi[1], M. R. Sippert[1], M. Figueiredo[1], R. C. Araújo[3], J. A. Horst[4], F. S. Santos[1], J. L. P. Daniel[1], J. C. Damasceno[1], and G. T. Santos[1], [1]*Universidade Estadual de Maringá, Maringá, PR, Brazil,* [2]*University of Florida, Gainesville, FL,* [3]*GRASP EW Nutrition, Curitiba, PR, Brazil,* [4]*Associação Paranaense dos Criadores de Bovinos da raça Holandesa, Curitiba, PR, Brazil.*

Objectives were to evaluate the effects of calcium nitrate (CN) feed for dairy cows on dry matter intake (DMI), milk yield, milk composition, and ruminal fermentation parameters. The hypothesis was that CN could be feed for dairy cows without affecting rumen fermentation parameters and milk composition. Six multiparous lactating dairy cows (106.3 ± 14.8 d in milk; 550.7 ± 21.8 kg-body weight; mean ± SD) were enrolled in a replicated 3 × 3 Latin square, each period was 21 d, with 14 of adaptation and 7 d of collection. Cows were fed ad libitum a TMR composed of 53% corn silage and 47% concentrate on dry matter (DM) basis. The diets were formulated to meet the NRC requirements for a 600 kg Holstein cow in mid-lactation, the diets were isocaloric and isonitrogenous with 16% of crude protein and 11% of rumen degradable protein, nitrogen content was manipulated by adding CN (66.9% NO_3^-). Treatments were: T1: a diet without CN; T2: a diet with 1.5% CN on DM basis; T3: a diet with 3% of CN on DM basis. Milk samples were collected on d 15–16 of each period and analyzed for milk components. Ruminal fluid was collected 2 and 8 h after feeding the pH was determined, and the volatile fatty acids (VFA) profile was determined by gas chromatography. Data were analyzed by ANOVA with mixed models using the Mixed procedure in SAS. DMI and milk true protein were reduced ($P < 0.05$) by T3. However, the treatments had no effect on milk yield ($P > 0.10$). There was a tendency ($P = 0.09$) to decrease energy-corrected milk (ECM) and fat corrected milk (FCM). Lactose and milk urea nitrogen did not differ between treatments. The concentration of isovaleric had a linear reduction ($P < 0.04$) with the increase of CN in the diet, there was a tendency ($P = 0.06$) to increase the acetate:proprionate ratio. The results demonstrate that CN reduces milk true protein and tended to reduce ECM and FCM, however milk efficiency was not affected by feeding CN. Calcium nitrate can be used up to 1,5% of DM basis on dairy cows in corn-based diets without affecting milk composition and without affecting the rumen fermentation parameters.

Key Words: lactating cow, rumen degradable protein, true milk protein

Table 1 (Abstr. W175).

	Control		RPC[1]			*P*-value	
Item	Mo	Ov	Mo	Ov	SEM	RPC	RPC × BCS
DMI, kg/d							
Prepartum	11.2	10.8	10.8	11.3	0.5	0.72	0.07
Postpartum	22.5	22.5	22.7	22.6	0.8	0.60	0.93
Yield, kg/d							
Milk	41.4	43.1	43.7	44.3	1.4	0.05	0.50
ECM	41.9	43.7	44.1	45.3	1.2	0.03	0.72
Fat	1.50	1.58	1.59	1.66	0.04	0.02	0.82
True protein	1.23	1.26	1.28	1.29	0.04	0.10	0.72
Lactose	1.97	2.06	2.09	2.11	0.06	0.06	0.42
ECM/DMI	1.92	2.04	2.02	2.12	0.1	0.02	0.85
Energy balance, Mcal/d							
Prepartum	0.99	−1.21	0.71	0.02	1.1	0.40	0.20
Postpartum	−1.2	−3.1	−2.5	−3.8	2.2	0.05	0.55

[1]ReaShure (Balchem Corp., New Hampton, NY).

W177 Evaluation of cobalt source and level for lactating dairy cows.

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Rumen microbes convert Co to vitamin B_{12}, a coenzyme in energy metabolism and important for milk production; but B_{12} synthesis and milk production outcomes vary by dietary Co source and level. Two studies were conducted on commercial dairies in the Midwest to determine the optimum source and level of Co in diets of lactating dairy cows. In study 1 and 2, Holstein cows (n = 46, 50 respectively), were blocked at calving by parity and calving date and randomly assigned to one of 2 treatments fed through 49 and 50 DIM, respectively. Cows were fed a basal lactating diet supplemented with 80 mg/d folic acid. Robotic milking systems individually fed cows the pelleted treatments and recorded milk production, milk composition and BW daily. Data were analyzed using MIXED procedures of SAS, with repeated-measures ANOVA for milk, components, and BW. Treatment, week, and interactions were fixed effects. Cow within treatment was a random effect. Study 1, cows were fed 1 ppm Co from COPRO (Co glucoheptonate, Zinpro Corp., Eden Prairie, MN) or a new Co source (CoPectin, Zinpro Corp.). There were no treatment differences ($P \geq 0.24$) for fat yield (1.45, 1.51 kg), fat percent (3.61, 3.48%), BW (696, 700 kg) or BW change (−0.9, −0.1 kg) for cows fed COPRO or CoPectin, respectively. Cows fed CoPectin tended ($P = 0.07$) to produce more ECM than COPRO (43.5, 41.1 kg, respectively). Milk production (44.1, 40.9 kg) and protein percent (3.09, 3.01%) were greater ($P \leq 0.05$) for cows fed CoPectin than COPRO, respectively. Study 2, cows were fed 1.0 or 1.5 ppm Co (DM Basis) from CoPectin. As prescribed by farm protocol, multiparous cows received a 20 mL B vitamin injection, d 2 postpartum. Milk (42.6, 41.6 kg), ECM (43.7, 42.8 kg), fat (1.57, 1.54 kg) and protein (1.31, 1.29 kg) were similar between Co levels ($P \geq 0.4$), but numerically greater for cows fed 1.0 vs. 1.5 ppm Co, respectively. Concentrations of fat (3.78, 3.79%) and protein (3.12, 3.15%) and cow BW (653, 667 lb) were similar ($P \geq 0.10$) for cows fed 1.0 or 1.5 ppm Co from CoPectin, respectively. Results demonstrate CoPectin is an improved Co source and can be effectively supplemented in lactation diets at 1.0 ppm DM.

Key Words: cobalt glucoheptonate, cobalt pectin, lactating cow

W178 Assessment of magnesium availability from magnesium oxide sources.

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Four sources (A, B, C, D) of magnesium oxide were assessed for both in vivo and in vitro estimates of ruminal availability. Samples were selected from a population of 41 samples based on laboratory screening. In vivo assessment consisted of 4 lactating cows (250 ± 48 DIM, producing 34 ± 3 kg/day of milk) in a 4 × 4 Latin square design of cows and periods. Cows were individually fed a common diet restricted to 90% of pre-experiment ad libitum intake to maintain consistent intakes throughout the experiment (overall average DM intake 26.1 kg/cow/day). Cows were dosed intraruminally with 40 g Mg per day delivered in 2 equal doses at a.m. and p.m. feedings for 3 d before sampling blood and ruminal fluid. Serum Mg concentrations 3 h post-dosing (overall average 1.35 mM) were unaffected ($P = 0.35$) by source. Blank-corrected weighted mean ruminal fluid Mg concentrations (sampled at various times up to 12 h post-dosing) differed ($P = 0.029$) among magnesium oxide sources, averaging 2.29 mM (A), 3.04 mM (B), 2.37 mM (C), and 4.19 mM (D). In vitro assessment consisted of a pH-stat titration procedure whereby the acid (HCl) consumed due to magnesium oxide solubilization to maintain pH 6.5 in a suspension of magnesium oxide in distilled water was measured with continuous stirring over 12 h. Time-course titration curves (mmol acid consumed/g of magnesium oxide) were described using a generalized Michaelis-Menten equation, $Y_t = Y_{max} \times t^c/(K^c + t^c)$, where Y_t is the acid consumed (mmol acid/g magnesium oxide) at any time (t, hours), Y_{max} is the asymptotic value of Y at infinite time, K is the time for one-half maximal solubilization and c is a dimensionless shape parameter determining the time to reach near asymptotic acid consumption. Both K ($P = 0.006$) and c ($P = 0.035$) but not Y_{max} (average = 105.3, $P = 0.22$) differed due to source. Values of K and c were 17.2 and 1.10 (A), 6.51 and 1.05 (B), 29.2 and 1.26 (C), and 0.83 and 1.44 (D), respectively. Results indicated that the kinetics of in vitro magnesium oxide solubilization may be reflective of in vivo ruminal magnesium oxide availability.

Key Words: magnesium oxide, bioavailability

W179 Effects of additional bioavailable chromium on dry matter intake, milk yield, and component production: A meta-analysis.

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The effect of chromium on production variables in lactating dairy cows has not been characterized. The objective of this study was to perform a meta-analysis to examine dry matter intake (DMI), milk yield (MY) and component production responses when a bioavailable form of chromium (CR) was fed to lactating dairy cows. Data were obtained from 16 peer-reviewed publications. The final data set included 45 diet treatments, with 29 treatments supplementing CR and 16 non-supplemented control treatments (CON). A random-effects model with the effect of study set as random was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between CR and CON treatments as well as standard errors of the differences between means are reported. Across studies, the average amount of additional elemental chromium fed to cows was 8.44 ± 4.19 mg/d. All means are reported as CON vs CR ± standard error of the difference; associated P – value, respectively. Chromium increased DMI (17.69 vs 18.67 ± 0.24 kg/d; $P < 0.001$) and milk yield (34.25 vs 35.8 ± 0.34 kg/d; $P < 0.001$). Milk fat percentage (4.11 vs 4.06 ± 0.06%; $P = 0.432$) and lactose percentage (4.71 vs 4.72% ± 0.01; $P = 0.609$) did not differ between treatments, while milk protein percentage decreased with CR feeding (3.08 vs 3.04 ± 0.02%; $P = 0.033$). Yields of milk fat (1.32 vs 1.39 ± 0.01 kg/d; $P < 0.001$), milk protein (1.17 vs 1.21 ± 0.02 kg/d; $P < 0.001$), and milk lactose (1.72 vs 1.87 ± 0.02 kg/d; $P < 0.001$) increased with CR feeding. In a subset of 5 publications where CR was supplemented to cows in early lactation, body weight loss decreased (−10.34 vs −8.11 ± 0.92% of starting BW; $P = 0.02$). In summary, supplementing CR increased DMI, milk yield, and milk component yield in lactating cows. In addition, BW loss decreased when CR was supplemented to cows in early lactation.

Key Words: meta-analysis, chromium, production

W180 Calcidiol increased milk yield and reduced somatic cell count of late-lactation dairy cows.

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The oral supplementation of dairy cows with calcidiol (25-OH-D_3) can have positive effects on Ca metabolism around calving and in bone and energy metabolism during lactation. The objective of this

experiment was to evaluate oral calcidiol supplementation of cows in late lactation. Thirty Holstein cows (230 ± 137 DIM, 8 primiparous) were individually fed a standard TMR for 14 d and were randomly allocated within blocks (15) to an oral dose (3 × /d) of 1 mg/d of calcidiol (HyD. Rovimix HyD, DSM Nutritional Products) or placebo for 84 d. The basal diet contained 1033 IU/kg of DM of cholecalciferol and 0.77% Ca and 0.46% P in DM. The average intake of cholecalciferol was 23,800 IU/d or 35.7 IU/kg of BW. Data were analyzed with Proc Mixed of SAS with a repeated measures approach. The HyD treatment increased milk yield (31.7 vs 30.9 kg/d. $P < 0.01$, SEM 0.49) and had no effect on DMI (23.0 kg/d. $P = 0.73$). The 4% FCM was increased by HyD (28.8 vs 27.5 kg/d. $P = 0.03$, SEM 0.66). Fat % tended ($P = 0.07$) to be increased (+ 0.10%-units) and protein % tended ($P = 0.09$) to be reduced (- 0.06%-units) by HyD. The HyD reduced milk SCC from 105,000 to 83,500 cells/mL ($P = 0.02$, SEM 12.7) suggesting positive action on mammary gland immunity. Feed efficiencies, BCS, and BW gain did not differ ($P \geq 0.41$). The concentration of calcidiol in plasma was increased by HyD supplementation (117.5 vs 52.6 ng/mL. $P < 0.01$, SEM 3.28) and the increase was of larger magnitude on d 84 than on d 28 ($P < 0.01$ for the interaction of treatment and day). None of the cows had plasma calcidiol below 40 ng/mL, suggesting that the NRC (2001) recommendation of cholecalciferol supplementation (~20,000 UI/d) associated with exposure to sunlight during the second milking of the day was sufficient to maintain adequate calcidiol concentration in plasma. The concentrations of ionized Ca ($P = 0.01$) and total Ca ($P = 0.03$) in serum were increased by HyD, but there were significant interactions ($P < 0.05$) between treatment and day of measurement. The increase in Ca concentrations induced by HyD was pronounced on d 56 and was not detected on d 84. The supplementation of late lactation dairy cows with HyD increased plasma calcidiol and milk yield and reduced milk SCC, at similar DMI.

Key Words: calcidiol, vitamin D, calcium

W181 Meta-analysis of the effects of supplemental rumen-protected choline during the transition period on performance and health of dairy cows.

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Objectives were to use meta-analytic methods to determine the effects of supplementing rumen-protected choline (RPC) starting prepartum on production and health of dairy cows. The literature was systematically reviewed and 23 experiments, including up to 74 treatment means and 1,938 cows, were used. All 23 experiments had a non-supplemented treatment (0 g choline/d). For supplemented cows, the mean ± SD (range) amount of choline ion fed was 12.7 ± 4.3 g/d (3.2 to 25.2). Data collected included number of cows, parity, days on treatment pre- and postpartum, choline ion fed (g/d), ingredients and nutrient content of prepartum diets, and LSM and respective SEM for DMI, production, and incidence of diseases. The contents of NE_L (Mcal/kg), metabolizable protein (MP) as % of DMI (MPDMI), and metabolizable methionine as % of MP (METMP) were estimated for each prepartum diet using NRC (2001). Mixed models were fitted using the MIXED and GLIMMIX procedures of SAS. Models contained the random effect of experiment and data were weighted by the inverse of the SEM squared. Models included the linear and quadratic effects of choline ion, NE_L, MPDMI, METMP, days on treatment prepartum, and interactions between choline and NE_L, and choline and METMP. Additional meta-analytical statistics were used to estimate the effect size using the METAN and METAREG procedures of STATA. Increasing prepartum intake of choline ion linearly ($P < 0.001$) increased pre- ($\beta = 0.0187 \pm 0.0045$; weighted mean difference [WMD] = 0.28 kg/d) and postpartum DMI ($\beta = 0.0438 \pm 0.0125$; WMD = 0.47 kg/d), ECM ($\beta = 0.1773 \pm 0.0219$; WMD = 1.61 kg/d), fat yield ($\beta = 0.0063 \pm 0.0009$; WMD = 0.08 kg/d), and protein yield ($\beta = 0.0048 \pm 0.0007$; WMD = 0.06 kg/d) in cows. Supplementing RPC reduced ($P = 0.05$) the risk of retained placenta (relative risk, RR = 0.69; 95% CI = 0.47–1.02) and tended ($P = 0.07$) to reduce the risk of mastitis (RR = 0.78; 95% CI = 0.58–1.04), but it had no effect on displaced abomasum, ketosis, or metritis. These results support the recommendation of feeding RPC starting prepartum to dairy cows. Responses were linear up to 25 g/d of choline ion.

Key Words: choline, production, transition cow

W183 Effects of soy lecithin on circulating choline metabolite concentrations and phosphatidylcholine profile in Holstein cows.

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Soy lecithin is a source of polyunsaturated fatty acid-enriched phosphatidylcholine (PC) that is known to increase choline supply in non-ruminants. Our objectives were to evaluate the effects of lecithin feeding on circulating choline metabolites including the PC profile in dairy cows fed palmitic acid (PA). Using a split-plot Latin Square design, 16 Holstein cows (160 ± 7 DIM) were randomly allocated to a main plot receiving a corn silage and alfalfa haylage-based diet with palm fat containing either moderate or high PA content at 1.75% of ration DM (MPA and HPA, respectively; BergaFat F-100 or F-100 HP containing 87 or 98% PA, respectively; Berg + Schmidt, Hamburg, Germany; n = 8/group). On each palm fat diet, lecithin (BergaPur, Berg + Schmidt) was top-dressed at 0, 0.12, 0.24, or 0.36% of ration DM in a replicated 4 × 4 Latin Square design. Following a 14-d covariate period, lecithin treatment spanned 14 d with milk and plasma collected during the final 3 d. Choline metabolites were measured using liquid chromatography and mass spectrometry. Untargeted lipidomics was employed for lipid profiling. Univariate and multivariate analyses were conducted that included the fixed effects of palm fat type and lecithin level. While no effects of treatments were detected for plasma choline or methionine, lecithin feeding increased the plasma choline metabolites trimethylamine N-oxide and dimethyl-glycine (24 and 11%, respectively; $P < 0.05$). Plasma PC and sphingomyelin levels increased with lecithin feeding (e.g., PC 35:1 and SM 42:0; $P < 0.05$). Lecithin also increased plasma lysophosphatidylcholine levels (e.g., LPC 20:0; $P < 0.05$) while reducing plasma phosphatidylethanolamine and triacylglycerol levels (e.g., PE 38:6 and TAG 15:0/16:1/18:1; $P < 0.05$). Ceramides increased with lecithin levels being higher in cows fed HPA, relative to MPA (e.g., Cer 16:0; $P < 0.05$). Although increases in microbial-derived trimethylamine N-oxide suggest gastrointestinal lecithin degradation, elevations in plasma dimethyl-glycine, PC, LPC, and SM support choline absorption with high lecithin feeding.

Key Words: dairy cow, soy lecithin, phosphatidylcholine

W184 MegAnion as an anionic salt source in prepartum negative dietary cation-anion difference diets for multiparous dairy cows.

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Incidence of subclinical hypocalcemia in early postpartum dairy cows continues to be an animal welfare concern and an economic burden for producers. Feeding negative dietary cation-anion difference (DCAD)

diets produces metabolic acidosis which mobilizes bone calcium and reduces the incidence of hypocalcemia. Achieving a sufficient degree of metabolic acidosis without reducing DMI is a challenge. This study compared the ability of MegAnion (MA), a new DCAD supplement designed to be more palatable than typical anionic salts, and another palatable commercial chloride (CC) source to create metabolic acidosis without reducing DMI. Prepartum Holstein (HO) and crossbred (XX) cows were blocked by breed and expected calving date and randomly assigned within breed to TMR formulated with MA or CC to have DCAD = −215 mEq/kg DM. Cows (N = 56; 15 MA-HO, 12 CC-HO, 15 MA-XX, 14 CC-XX) consumed the TMR for at least 19 d and completed the 28 d DIM phase of the study. Urine and blood samples were collected weekly and at 1, 2 and 3 DIM. Data were analyzed as a randomized block design by repeated measures (PROC MIXED) with week or DIM as the repeated effect. Prepartum urine pH decreased from 8.2 ± 0.27 prior to treatment to 6.1 ± 0.13 while treated, was not affected by diet ($P = 0.60$) and increased immediately after calving when all cows consumed the same TMR. Pre- ($P > 0.17$) and post- ($P > 0.40$) partum production variables (BW, BCS, DMI, energy balance, milk yield) were not affected by treatment but 3.5% FCM yield was greater in HO than XX cows (45.7 vs. 41.0 ± 1.9 kg/d; $P = 0.037$). Prepartum serum calcium was not affected (9.3 mg/dL, $P = 0.59$) by treatment while non-esterified fatty acids were lower (86 vs. 120 ± 10 μEq/L, $P = 0.015$) and insulin greater (31 vs. 25 ± 1.5 ng/mL, $P = 0.002$) in MA than CC. These differences are supported by the numerical increases in prepartum DMI (1.2 kg/d, $P = 0.17$) and energy balance (1.8 Mcal/d, $P = 0.19$) of cows fed MA. Results indicate MA is a palatable and effective option for producers to induce prepartum metabolic acidosis to minimize hypocalcemia in dairy cows.

Key Words: subclinical hypocalcemia, anionic salts, DCAD diet

W185 Assessment of the capacity of certain mycotoxin binders to adsorb vitamins. A. Kahil, M. Rodriguez-Prado, C. Godoy, C. Cristofol, and S. Calsamiglia*, *Universitat Autonoma de Barcelona, Bellaterra, Barcelona, Spain.*

The objective of the study was to evaluate the capacity of 6 mycotoxin binders to adsorb 2 groups of vitamins: water-soluble vitamins (WSV) and fat-soluble vitamins (FSV). The experiment was conducted in in vitro condition to simulate post ruminal digestion model in 2 phases: the first phase to simulate the gastric digestion with pepsin, malic acid, citric acid, acetic acid and lactic acid at pH 3.0; and the second phase to simulate intestinal digestion with bile salts and pancreatin extract at pH 6.5. The experimental design was a factorial 6 × 5 with main factors being mycotoxin binders (bentonite, clinoptiolite, sepiolite, montmorillonite, active carbon and yeast cell wall) and vitamins (WSV: B2, B3, B6; and FSV: D and E). Vitamins were incubated separately (Study 1) and together (Study 2) with each mycotoxin binder. Each treatment was incubated in triplicate and 2 consecutive periods. Results were analyzed with PROC MIXED of SAS and significance declared at $P < 0.05$. In the 2 studies the adsorption average was highest with montmorillonite for both WSV (47%) and FSV (27%) and lowest with active carbon (17%) for WSV and sepiolite (12%) for FSV. For the adsorption average of vitamins in the 2 studies, the B6 (55%), B2 (33%) and E (35%) were adsorbed the highest, and B3 (5%) and D (3%) were adsorbed the lowest. The adsorption of vitamin B6, B2 and E was highest with montmorillonite (68%, 67% and 48%, respectively), and lowest with clinoptiolite (50%) for B6, with active carbon (4%) for B2 and with sepiolite (15%) for E in both studies. For vitamin D, the adsorption was highest with yeast cell wall (20%) and lowest with bentonite (0%) in Study 1; however, in Study 2, the adsorption for vitamin D was highest with montmorillonite (7%) and lowest for all other adsorbents (0%). Mycotoxin binders may affect bioavailability of some water- and fat- soluble vitamins at different degree depending on the mycotoxin binders and the substrate.

Key Words: mycotoxin binder, vitamin

Teagasc-Moorepark Symposium: Animal Diet, Dairy Product Quality, and Advances in Dairy Foods Nutrition and Health

432 Introduction. P. Kindstedt*, *University of Vermont, Burlington, VT.*

This symposium is the second event to be hosted by the ADSA Dairy Foods Division International Partnership Program (IPP). The IPP arose out of a 2015 strategic initiative of the ADSA board of directors to strengthen the ADSA community for our international members. The Program seeks to accomplish this by partnering with outstanding international dairy research organizations to provide them with a venue to showcase a concentration of their work at our North American Annual Meetings. An equally important objective is to give our North American dairy scientists direct access to these overseas colleagues to facilitate personal connections, collaborations, the sharing of ideas, and a genuine sense of community and collegiality across international lines. The first international organization to collaborate with ADSA under the IPP has been the Teagasc-Moorepark Food Research Center in Ireland. In 2017, the IPP sponsored the Teagasc-Moorepark/University College Cork Cheese Symposium, which was held at the ADSA Annual Meeting and funded jointly by ADSA, Teagasc-Moorepark and University College Cork. Building on the success of the 2017 Cheese Symposium, ADSA and Teagasc-Moorepark are now strengthening their partnership by again jointly funding a day long Symposium which moves beyond cheese science into areas of animal diet and feeding regimen and how they affect dairy product quality, and nutrition and health aspects of dairy foods, areas that are of intense interest to dairy scientists worldwide. As this partnership continues to grow, new opportunities to cooperate, such as through IPP support of short-term PhD student international exchange visits, are beginning to be explored. Beyond this, the resounding success of this first partner experience has provided the IPP with a solid foundation to seek a second outstanding international research organization to join Teagasc-Moorepark to become a founding member of the IPP. Toward that end, efforts are underway to recruit a new partner with the goal of co-sponsoring an IPP Symposium event at the 2020 ADSA Annual Meeting.

Key Words: dairy, research, partnership

433 Impact of cow feeding system on the composition and quality of milk and dairy products. T. F. O'Callaghan*, *Teagasc Moorepark Food Research Center, Fermoy, Co. Cork, Ireland.*

The "Profiling Milk From Grass study" carried out in Teagasc Moorepark, investigated the effects of pasture versus indoor TMR feeding systems on a variety of milk production, composition and quality attributes. Including, the chemical composition, quality characteristics and sensory properties of raw milk, sweet cream butter and full fat cheddar cheese along with the raw milk and rumen metabolome. The experiment had 3 treatments. Group 1 was housed indoors and fed a TMR diet of grass silage, maize silage and concentrates, Group 2 was maintained outdoors on perennial ryegrass (*Lolium perenne* L.) only pasture (GRS), while Group 3 was also maintained outdoors on perennial ryegrass/white clover (*Trifolium repens* L.) pasture (CLV). Feeding system was demonstrated to have a significant effect on the composition and quality of milk and subsequent products. Milk from pasture-based systems had greater fat and protein contents, and improved protein quality compared with milk from TMR. Feeding system was demonstrated to have a significant effect on the vitamin profile of milks. Pasture feeding had a beneficial impact on the fatty acid profile of milk and dairy products with increased concentrations of beneficial nutrients such as n-3 (omega-3) fatty acids, conjugated linoleic acid, vaccenic acid and reduced levels of palmitic acid, n-6 fatty acids and thrombogenecity index score than TMR. Alterations to the fatty acid content resulted in significant rheological differences of butter and Cheddar cheeses including textural properties and color. Pasture derived products were shown to have significantly greater contents of β-carotene, imparting a yellow color on products characteristic of Irish dairy products. Sensory analysis revealed a preference for dairy products derived from the pasture-based systems compared with the TMR-based system. The application of ^{1}H-NMR to raw milks identified significant diet induced alterations to the milk metabolome and identified a variety of potential biomarkers of pasture derived milks. Furthermore, fatty acid profiling and ^{1}H-NMR metabolomics coupled with multivariate analysis demonstrated being capable of distinguishing both rumen-fluid and milk derived from cows on different feeding systems, specifically between indoor TMR and pasture-based diets used in this study.

434 Factors influencing the flavor of bovine milk and cheese from grass-based versus TMR-based milk production systems. K. Kilcawley*, *Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

The impact of diet on the sensory properties of bovine milk and dairy products is complex due to the wide range of on farm and production factors that are potentially involved. It is well established that pasture affects the color of milk and dairy products due to β-carotene content, and that nutritional and potentially textural properties are altered due to dietary factors influencing fat synthesis. Very little research has been undertaken on aromatic volatile compounds derived from diet that may affect sensory perception. It is obvious that any potential effect depends upon their concentration and odor activity. We have found evidence of direct transfer (digestion/absorption or inhalation), and indirect transfer (rumen metabolism) of volatiles and via secondary mechanisms (lipid oxidation, Maillard reactions, de novo systhesis) for volatile incorporation into bovine milk. We found 3 volatile compounds present at higher levels in milk derived from pasture; toluene, dimethyl sulfone and p-cresol, that may be potential biomarkers for pasture feeding based on concentration. In our studies only p-cresol affected flavor, which has a 'barnyard' or 'cowy' attribute and is derived from the rumen metabolism of β-carotene and aromatic acids which are higher in pasture diets, but also from the rumen metabolism of isoflavones present in clover. We are also aware that US consumers have a lower threshold for this 'barnyard' 'cowy' attribute than Irish and Chinese consumers.

435 Influence of herd diet (pasture-based vs. TMR) on the composition, ripening and metabolome of continental-type cheeses. J. J. Sheehan*, *Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

In response to growing consumer interest in pasture fed dairy products, research was conducted on the characteristics of Continental type-cheese derived from milks produced from cows fed indoors on total mixed ration (TMR), or pasture fed cows on grass only (GRA) or grass mixed with clover (CLO). A preliminary study showed that the curd moisture loss rate constant (k/min) was similar for curds from protein-standardised TMR, CLO and GRA milks, showing minimal feed-induced variations in syneresis. Maasdam cheeses manufactured from standardized milks derived from the feeding systems showed that pasture-derived cheeses had significantly lower L* (whiteness) and higher b* values (yellowness) compared to TMR-derived cheeses. Acetate levels were significantly lower in CLO and butyrate levels significantly higher in TMR compared to the other cheeses. Grass-fed cheese had significantly higher scores for smooth texture, ivory color and shiny appearance compared to TMR. The influence of feed type was minimal on cheese yield, composition and on indices of glycolysis, lipolysis and proteolysis during ripening. The untargeted metabolic profiles of the ripened Maasdam cheese samples were profiled using high-resolution nuclear magnetic resonance (^{1}H NMR), high-resolution magic angle spinning NMR (^{1}H HRMAS NMR) and headspace (HS) gas chromatography mass spectrometry (GC-MS). On comparing the ^{1}H NMR metabolic profiles, TMR-derived cheese had higher levels of citrate compared to GRA-derived cheese. The major differences between outdoor or indoor feeding system on cheese metabolites were detected in the lipid phase, as indicated by ^{1}H HRMAS NMR. The toluene content of cheese was significantly higher in GRA or CLO compared with TMR cheeses and dimethyl sulfide was identified only in CLO-derived cheese samples as detected using HS GC-MS. Overall, this study shows that once milk is standardized, cheese manufacture, composition and yield properties are not influenced by herd diet while certain sensory characteristics and a small number of compounds and lipid derived metabolites differ.

436 Developments in dairy-based nutritional beverages: Examples from the Teagasc Research Programme. M. A. Fenelon*, *Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

Milk is an ideal nutritional base for lifestyle beverages, providing both functionality and basic nutrition. It is a complete food source, containing all the major macro- and micro-nutrients, i.e., protein, fat, carbohydrate, minerals and vitamins needed to formulate nutritional, therapeutic and medical and/or sports beverages. Researchers at Teagasc have studied the effect of the interaction between composition and processing parameters on in-process and finished product functionality, and identified the key constraints to beverage manufacture. Studies have demonstrated that feed type (e.g., grass fed), stage of lactation and milk quality influence the formulation dynamics of dairy-based beverages. Selection of protein ingredients, and knowledge of their thermal history, is key to optimising functionality in the final reconstituted formulation. For example, increasing the α-lactalbumin to β-lactoglobulin ratio of a first stage infant formulation (i.e., 1.3% protein (60:40 casein:whey) 7.4% lactose and 3.4% fat) resulted in a significant ($P < 0.05$) reduction in powder particle PSD D(4,3) size and viscosity of the reconstituted formulation. In addition, increasing α-lactalbumin content reduced the rate of thermal gelation on addition of calcium and magnesium. Other strategic priorities at Teagasc include adaption of technology to create a "digital dairy" platform of research within the food program. This area includes food biomechanics, digital mapping and virtual / augmented reality applications for visualization of internal powder structure. When coupled with formulation strategies, the continued integration of digital technology is seen as a significant development for the dairy-based nutritional beverage sector.

437 Milk oligosaccharides: The influence of the milk glycome on human health. R. Hickey*, *Teagasc Food Research Centre, Moorepark, Fermoy, Co. Cork, Ireland.*

Oligosaccharides are the third most abundant component in human milk. It is widely accepted that they play several important protective, physiological, and biological roles including selective growth stimulation for beneficial gut microbiota, inhibition of pathogen adhesion and immune-modulation. However, until recently, very few commercial products on the market capitalized on these functions. This is mainly because the quantities of human milk oligosaccharides required for clinical trials have been unavailable. Recently, clinical studies have tested the potential beneficial effects of feeding infants formula containing 2'-fucosylactose, the most abundant HMO in human milk. These studies have opened this field for further well-designed studies which are required to fully understand the role of HMO. However, one of the most striking features of human milk is its diversity of oligosaccharides with over 150 identified to date. It may be that a mixture of oligosaccharides is even more beneficial to the infant than a single structure. For this reason, the milk of domestic animals has become a focal point in recent years as an alternative source of complex oligosaccharides with associated biological activity. Bovine milk is an ideal candidate, given its wide availability and its use in so many regularly consumed dairy products. The carbohydrate fraction of bovine milk is divided into lactose, free oligosaccharides and bound glycans or glycoconjugates. This presentation will focus specifically on free oligosaccharides and glycosylated bovine milk proteins and the biological roles associated with such structures. A major area of interest is the effect of milk glycans on host-microbial interactions in the gut. For instance, glycosylated components in milk are known to alter intestinal glycosylation, which in turn contributes to early immune development and maturation of the newborn intestinal tract. Strategies to increase the colonisation of infant-associated bifidobacteria have been extensively investigated in recent years with promising results.

438 Relationships between structures of dairy-based matrices and digestibility within the gastrointestinal tract. A. Brodkorb*, *Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

Milk proteins are the main structural components of dairy and dairy-containing foods. Their molecular structure and aggregation behavior can be tuned by targeted, "smart" processing. Whey proteins, previously considered a low-value by-product from cheese production, exhibit a globular molecular structure, and are prone to unfolding and self-aggregation or association with caseins or casein micelles. This can be either detrimental or advantageous for food processing. In this talk, several examples are given on how to understand, control and modify the structure of milk proteins, thereby adding value. Rapid pilot scale, pre-heat treatments of whey proteins can improve the standard processability and/or efficiency of the enzymatic hydrolysis of protein products. This will benefit down-stream production of nutritional products such as infant formula, sports nutrition, and nutrition for the elderly. Changes in processing conditions can also affect the gastro-intestinal transit of dairy proteins and accelerate or delay the bioaccessibility of nutrients, as demonstrated by static (Brodkorb et al., Nature Protocols 2019) or semi-dynamic in vitro digestion methods (Mulet-Cabero et al., Food Hydrocolloids, 2018). In particular, heat treatment and homogenization can have a profound effect on the mechanism and kinetics of in vitro and in vivo gastro-intestinal digestion of dairy proteins, due to gastric restructuring. Dairy proteins can also act as carriers for labile, bioactive components of food to protect or encapsulate them during food production, storage or the harsh environment of the gastrointestinal tract (in vivo and in vitro, Doherty et al., International Dairy Journal 2012).

439 Metagenomic and metabolomic analysis of the impact of exercise or whey protein supplementation on the gut microbiome. W. Barton*, *Teagasc Food Research Centre Moorepark, Fermoy, Co. Cork, Ireland.*

The intestinal microbiome has become intensely investigated as its role in health has become increasingly evident. An important factor of understanding the gut microbiome lies in unravelling the means by which it may be modulated. Although parameters such as diet, medication use, and living environment are well established as modifiers of the microbiome, recent evidence suggests a role of physical activity (i.e., exercise) as one such factor. In conjunction with novel insights on dietary contributions, the session will present evidence for physical activity and whey protein supplementation as influencing agents of the structure and function of the human gut microbiome.

440 Dairy matrix effects: Response to consumption of dairy fat differs when eaten within the cheese matrix. E. Gibney*, *University College Dublin, Belfield, Dublin, Ireland.*

Evidence suggests the association between intake of saturated fat and risk of heart disease is dependent on the food sources of the dietary fat, with much work focused on the impact of saturated fat from dairy and in particular cheese on markers of metabolic health and risk of CVD. Several published randomized controlled trials (RCT) have demonstrated a beneficial effect of cheese consumption on markers of metabolic health and CVD risk. Research conducted within UCD has both supported and added to the existing evidence. A 6-wk randomized parallel intervention involving 164 volunteers who received ~40 g of dairy fat/d, in 1 of 4 treatments: (A) 120 g full-fat Irish cheddar cheese (n = 46); (B) 120 g reduced-fat Irish cheddar cheese + butter (21 g) (n = 45); (C) butter (49 g), calcium caseinate powder (30 g), and Ca supplement (CaCO3) (500 mg) (n = 42); or (D) 120 g full-fat Irish cheddar cheese, for 6 weeks following completion of a 6-wk “run-in” period, where this excluded all dietary cheese before commencing the intervention, was undertaken–delayed intervention group. This study found a stepwise-matrix effect was observed between the groups for total cholesterol (TC) ($P = 0.033$) and LDL cholesterol (LDL-C) ($P = 0.026$), with significantly lower post intervention TC (mean ± SD) (5.23 ± 0.88 mmol/L) and LDL-C (2.97 ± 0.67 mmol/L) when all of the fat was contained within the cheese matrix (Group A), compared with Group C, when it was not (TC: 5.57 ± 0.86 mmol/L; LDL-C: 3.43 ± 0.78 mmol/L). These findings suggest that dairy fat, when eaten in the form of cheese, appears to differently affect blood lipids compared with the same constituents eaten in different matrices, with significantly lower total cholesterol observed when all nutrients are consumed within a cheese matrix. There is a need to further understand this research in the context of both public health and industry needs, and to elucidate the components of the cheese matrix causing these effects.

Animal Health 3: Gastrointestinal Health

441 Guiding antimicrobial therapy: Prevalence of bacteremia in dairy calves with diarrhea. J. Garcia*[1], J. Pempek[1], A. Hinds[2], D. Diaz-Campos[1], and G. Habing[1], [1]*The Ohio State University, College of Veterinary Medicine, Columbus, OH,* [2]*University of Missouri College of Veterinary, Columbia, MO.*

Calfhood diarrhea is the most common cause of mortality in dairy calves. Septicemia is an important sequela of diarrhea, and the primary justification for antimicrobial treatment for diarrhea. Farm workers make routine decisions to initiate antimicrobial therapy based on clinical signs, yet there is a lack of criteria associated with bacteremia. The prevalence of bacteremia in diarrheic calves has been estimated to be 30%; however, this estimate included calves presented to a veterinary hospital or raised for veal, and may not reflect the prevalence in calves on commercial dairy operations. Thus, the objective of this study was to determine the prevalence of bacteremia in diarrheic dairy calves and identify clinical signs associated with bacteremia. We hypothesized that the prevalence of bacteremia would be less than 30% in calves with diarrhea, and that clinical signs, such as temperature and respiratory rate, would be accurate predictors of bacteremia. Calves (≤21 d of age) were enrolled across 2 dairy farms into a diarrheic or clinically healthy group (control to assess aseptic technique). Diarrheic calves enrolled presented with loose to watery stool, dehydration (assessed by skin tent) or depression (assessed by suckle reflex and ability to stand), and were not previously treated with antibiotics. Health assessments were performed at enrollment, and included respiratory signs, joint inflammation, navel score, temperature, and heart and respiratory rate. Following the health assessment, one aseptic blood sample was collected from each calf and cultured to determine bacterial species present using mass spectrometry. Associations between bacteremia and dichotomized health outcomes were analyzed using Fisher's exact tests and continuous outcomes were compared using Student's *t*-tests. The prevalence of bacteremia in diarrheic calves was 15.3% (17/111) and 18.5% (5/27) in clinically healthy calves. There was no association between clinical signs and bacteremia. The prevalence of bacteremia in the diarrheic group was significantly lower than previous estimates, indicating there may be opportunity to reduce antimicrobial use in calves with diarrhea that are not septicemic.

Key Words: calf, diarrhea, bacteremia

442 Climatic conditions at birth associate with serum total proteins and diarrhea occurrence in pre-weaned organic dairy heifers in northern Colorado. A. Velasquez-Munoz*, D. Manriquez, and P. Pinedo, *Department of Animal Sciences, Colorado State University, Fort Collins, CO.*

Climatic conditions may impact health, performance, and well-being of dairy cattle, especially during early life. The objective was to assess the effects of temperature-humidity index (THI) at birth on serum total proteins (STP) concentrations and on the time to the first diarrhea event during the initial 80 d of life of organic certified Holstein heifers. A retrospective analysis was performed on calves born between June 2017 and June 2018, with a total of 420 heifers included. STP concentrations (mg/mL) and diarrhea events from birth to weaning were obtained from farm records. Temperature and humidity were continuously measured during the study period using automated loggers (Onset Computer Corporation, Bourne, MA). STP concentrations were categorized in 3 levels (1 [<5.5; n = 11]; 2 [5.5–7.5; n = 323]; and 3 [>7.5; n = 86]). In addition, calves were grouped considering the maximum THI at their day of birth into THI1 (<55; n = 122); THI2 (56–72; n = 168); and THI3 (>72; n = 130). Data were examined using survival analysis for the time to the first diarrhea up to 80 d of life. Wilcoxon test was used to determine significant differences and hazard ratios (HR) by STP and THI categories were calculated. In addition, ANOVA was performed to detect differences in STP for calves born at different THI categories. Overall, THI at birth had a significant effect on the time to the first diarrhea episode ($P < 0.0001$) with a median survival time for calves in THI3 of 12 d, contrasting with THI1 and THI2 (37 d and 33 d, respectively). The HR for diarrhea presentation for calves born in THI3 was 1.76 times the HR of calves born with THI1 ($P = 0.0008$). Contrary, STP was not affected by THI in the study animals ($P = 0.44$). The presented results are part of a project to determine STP measurements and THI linked to early disease and culling of dairy calves for northern Colorado. From this initial analysis, we conclude that THI > 72 at birth had an effect on the health of calves, increasing the hazard of diarrhea presentation.

Key Words: calf, total serum protein, temperature-humidity index (THI)

443 Bacterial composition and short-chain fatty acids profiles in the gastrointestinal tract of neonate calves. C. Villot*[1,3], L. L. Guan[1], E. Chevaux[3], and M. A. Steele[2], [1]*Department of Agricultural, Food and Nutritional Science. University of Alberta, Edmonton, AB, Canada,* [2]*Department of Animal Biosciences, University of Guelph, Guelph, ON, Canada,* [3]*Lallemand SAS, Blagnac, France.*

Microbial colonization can influence the early development and health of dairy calves. To better understand early life bacterial colonization in the calf gastrointestinal tract (GIT), this study investigated (1) the shift in bacterial groups present at the colon mucosa between birth and 5 d old, and (2) the relationship between short chain fatty acid (SCFA) and bacterial composition at 7 d of age in GIT digesta. Holstein bull calves (n = 20) were natural delivered, directly removed from the dam, and housed in individual straw-bedded pens. Calves received 2 meals of a standardized colostrum at 2 h (7% of BW; 180g of IgG fed) and 12 h (3% of BW; 120g of IgG fed) postnatal. Milk replacer (7.5% of BW; 260 g/kg CP; 160 g/kg crude fat, at 150g/L) was bottle-fed twice daily. Colonic endoscopic biopsies were performed within 2 h and at 5 d postnatal. Calves were euthanized at 7 d of age to obtain jejunal, ileal and colonic digesta. Data from colon biopsies were compared between d0 and d5 and data collected at d7 were compared among digesta (jejunum, ileum, and colon). Calf was used as random effect in the models. Real-time quantitative PCR (qPCR) revealed that the total bacterial density in the colon-mucosa decreased between birth and 5 d old (respectively, 9.18 ± 0.16 *vs* 8.57 ± 0.07 $\log_{10}$ 16S rRNA genes copy number/g, $P < 0.01$). The relative abundances of *Bifidobacterium* spp., *Lactobacillus* spp., and *F. prausnitzii* were increased from birth to d 5 ($P < 0.01$), whereas *E. coli* significantly decreased ($P < 0.01$) at d 5 (0.37 ± 0.09% of total bacteria) compared with birth (10.60 ± 1.90% of total bacteria). Total bacterial density was higher ($P < 0.01$) in colon compared with the ileum and jejunum (colon: 9.37 ± 0.07; ileum: 8.81 ± 0.11; jejunum: 8.12 ± 0.10 $\log_{10}$/g). Total SCFA concentration was higher ($P = 0.03$) in the colon (12.73 ± 1.12 µmol/mL) compared with the ileum (6.68 ± 0.40 µmol/mL) and jejunum (4.92 ± 0.30 µmol/mL). Acetate was the predominant SCFA throughout the GIT (>90% of total SCFA). In calves, there is an early shift of bacterial colonization in the colon-mucosa and major fermentation activity in the colon with a high density of bacteria in digesta.

Key Words: colon-mucosa, microbial colonization, neonate calves

444 *Lactobacillus animalis* LA51 confers protection from the damaging effects of pathogens on the intestinal barrier.

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Maintaining a healthy gut environment is a prerequisite for sustainable animal production. The gut plays a key role in the digestion and absorption of nutrients and constitutes an initial organ exposed to external factors influencing the health of animals. *Lactobacillus animalis* (LA51) is a probiotic strain proven to improve gastrointestinal (GI) health in cattle, in part by reducing the burden of *E. coli* and *Salmonella*. Intestinal dysbiosis can promote overgrowth of theses pathogens, which in neonate cattle results in intestinal barrier damage (leaky gut) and systemic infections. The objective of this study was to evaluate in vitro beneficial effects of LA51 on GI health in the presence of pathogens. Two assays were performed. For the adhesion assay, *E. coli* O157 (DSM17076) was added to intestinal Caco-2 cell monolayers (5×10^7 cfu/well) pre-incubated or not with LA51 (2×10^8 cfu/well). *E. coli* adhesion was quantified by cfu enumeration using MacConkey agar plates incubated 16h at 37°C. For the "leaky gut" assay, transepithelial electrical resistance (TEER) was measured across Caco-2 monolayers exposed to LA51 (1.5×10^8 cfu/transwell) with or without *S. Typhimurium* (DSM 19587, 0.4–1.5×10^8 cfu/transwell) and incubated at 37°C, 5% CO_2. FITC-dextran (FD) was added to the apical side of the Caco-2 cells after 9h of TEER measurements. The amount of FD translocated to the basolateral side was quantified after 2h by measuring the fluorescent signal. LA51 reduced the binding of *E. coli* O157 to the cells by 50% (12.3×10^5 cfu/mL vs. 5.9×10^5 cfu/mL, $P < 0.01$). *S. Typhimurium* caused a dose-dependent TEER decrease, inversely correlated with increasing FD translocation. At each of the 3 *S*. Typhimurium doses tested, LA51 significantly reduced the TEER decrease (41% vs. 84% at lowest infection dose, $P < 0.01$) and the amount of FD translocation (0.14% vs. 0.03% at lowest infection dose, $P < 0.01$). In conclusion, LA51 confers protection against intestinal pathogens by reducing their adherence and by counteracting their damaging effect on the intestinal integrity.

Key Words: leaky gut, *Lactobacillus animalis*, pathogen

445 Withdrawn

446 Investigating the dynamics of Johne's disease in Ontario dairy herds.

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Johne's disease (JD) is a progressive disease that causes economic losses due to decreased milk production, reproductive difficulties, and early culling. The true prevalence of disease is unknown and estimates across Canada vary based on province and method of testing. Much of JD control is based on the culling of positive animals and the adoption of management practices that minimize exposure of the pathogen (*Mycobacterium avium* ssp. *paratuberculosis*) to young stock. Between the years of 2010 and 2013, the province of Ontario instituted a voluntary Johne's control program consisting of whole herd testing and risk assessment. The long-term effects of these agricultural extension programs in the control of diseases such as JD is unknown; our research hopes to describe these benefits and challenges. Using a modified milk ELISA technique (IDEXX) with an optical density cut-off of 0.089, province wide bulk tank (BT) milk testing has been used to assess the prevalence of Johne's high-risk herds at the end of the control program and again 4 years after its completion. The prevalence of JD seems to have increased based on these BT samples. In 2013, 46.8% of bulk tanks tested positive (1828/3908), while in 2017, this increased to 71.4% (2557/3581). There were 3207 farms with matched test results (BT milk tests from 2013 and 2017). Of these farms with matched results, 1828 farms had participated in the voluntary Johne's control program. When the results of the BT testing are dichotomized based on participation in the control program, 34% of participant herds remained positive compared with 40% of non-participants. 19.4% of participant herds remain negative compared with 18.6% of non-participant herds, and 36.2% of participant herds went from negative to positive status compared with 31.8% of non-participants. Both groups had a similar proportion of positive to negative status (10%, 9.9%). Preliminary results suggest additional factors are at play in the increase in JD prevalence in Ontario. In-depth investigations on specific management changes and producer barriers should assist us in developing effective agricultural extension programs for diseases such as Johne's.

Key Words: dairy, Johne's, bulk tank

447 Occurrence of mycotoxins in 2018 US corn grain and corn by-product feeds.

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Mycotoxins are harmful secondary fungal metabolites which are detrimental to animal health and productivity. This study investigated the occurrence and contamination levels of mycotoxins in 2018 US corn grain and corn by-product feeds (distillers dried grains, gluten feed, etc.). Samples from mid-August 2018 through January 2019 were screened via LC-MS/MS technique for the presence of 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN) and ochratoxin A. Parameters of main toxins found were compared with the 2 prior harvest years using Kruskal-Wallis Test (Prism7, GraphPad, La Jolla, CA) and are presented in Table 1. Mean toxin count per sample has increased in 2018 corn grain versus 2017, returning to more than 2 toxins per sample as in 2016. Mean B-Trich level (ppb, parts per billion) in corn grain is elevated in 2018 versus 2017, returning to 2016 levels. Although ZEN contamination does not differ from prior years, prevalence increased to 43% of samples, up from 25% in 2017. Co-contamination in corn by-product feeds is steady, averaging nearly 3 toxins per sample. Mean B-Trich level did not differ from prior harvest years while FUM contamination is similar to 2017 crop, but significantly lower than 2016. ZEN levels in 2018 by-products are the highest seen in the past 3 years. Preliminary results of the 2018 corn grain and corn by-product feeds survey suggest mycotoxin occurrence and contamination levels are approaching those observed in the challenging 2016 crop with continued concerns for co-contamination.

Key Words: mycotoxin, corn, by-product

448 Occurrence of mycotoxins in 2018 US corn silage and total mixed ration samples.

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Mycotoxins are harmful secondary fungal metabolites which contaminate a variety of feedstuffs worldwide and are detrimental to animal

Table 1 (Abstr. 447). *Fusarium* toxin contamination in US corn grain and corn by-product feeds

	Grain			By-Product Feeds		
Parameter	2016	2017	2018	2016	2017	2018
N	353	403	352	126	81	55
Mean toxin count	2.07^{b}	1.51^{a}	2.07^{b}	2.88	2.80	2.67
B-Trich						
Prevalence, %	80	70	74	100	99	93
Mean±SEM, ppb	1207^{b} ± 90	803^{a} ± 201	1132^{b} ± 117	2395 ± 141	1997 ± 192	2670 ± 297
ZEN						
Prevalence, %	42	25	43	79	80	84
Mean±SEM, ppb	438 ± 146	321 ± 73	298 ± 28	246^{b} ± 14	186^{a} ± 16	499^{c} ± 71
FUM						
Prevalence, %	80	52	78	91	91	87
Mean±SEM, ppb	4091 ± 615	2695 ± 386	3760 ± 415	4410^{b} ± 559	1414^{a} ± 1949	1135^{a} ± 1418

$^{a–c}$Values across rows are different, $P < 0.05$.

health and productivity. This study investigated the occurrence and contamination levels of mycotoxins in 2018 US corn silage and total mixed ration (TMR) samples. Corn silage harvested in 2018 as well as TMR samples submitted for analysis since January 2018 were screened via LC-MS/MS technique for the presence of 6 major mycotoxin groups: aflatoxins, type A trichothecenes, type B trichothecenes (B-Trich), fumonisins (FUM), zearalenone (ZEN) and ochratoxin A. Parameters of the main mycotoxins detected are presented (as-received) in Table 1. Corn silage contamination was compared with the prior harvest year using Kruskal-Wallis Test (Prism7, GraphPad, La Jolla, CA). Mean toxin count per sample has increased from 1.2 to 1.7 in 2018 corn silage versus 2017. The prevalence of B-Trich was comparable between years while the percentage of 2018 corn silage samples positive for ZEN was more than 2 times that of 2017 and FUM prevalence was more than 2.5 times that observed in 2017. Mean contamination levels (parts per billion, ppb) for B-Trich and ZEN in 2018 silage samples were significantly greater than 2017 toxin concentrations. Mycotoxin contamination was high in TMR with B-Trich detected in 95% of samples. ZEN and FUM were less frequently detected and at lower average concentrations in TMR samples. Preliminary 2018 corn silage survey results suggest increased contamination levels of B-Trich and ZEN, but consistent FUM contamination when compared with 2017 crop year samples. Although formulations of TMR vary widely across the US, B-Trich contamination was common in these complex rations.

Table 1 (Abstr. 448). *Fusarium* toxin contamination in US corn silage and TMR samples

	Corn silage		
Parameter	2017	2018	TMR
N	208	76	236
Mean toxin count	1.2a	1.7b	1.4
B-Trich			
Prevalence, %	85	83	95
Mean ± SEM, ppb	643^{a} ±34	1162^{b} ± 157	601 ± 25
ZEN			
Prevalence, %	26	55	31
Mean ± SEM, ppb	202^{a} ± 34	387^{b} ± 70	176 ± 18
FUM			
Prevalence, %	12	32	9
Mean ± SEM, ppb	612 ± 144	763 ± 267	443 ± 140

^{a,b}Values across rows are different $P < 0.05$.

Key Words: mycotoxin, corn silage, TMR

449 Effect of high-concentrate feeding and a clay mineral-based mix on liver health and fecal pH in dairy cows. N. Reisinger*[1], A. Stauder[2], E. Humer[2], J. Faas[1], V. Neubauer[2], and Q. Zebeli[2], [1]*BIOMIN Research Center, Tulln, Austria,* [2]*Institute of Animal Nutrition and Functional Plant Compounds, Department for Farm Animals and Veterinary Public Health, Vetmeduni Vienna, Vienna, Austria.*

To fulfill the high-energy requirements of early lactating cows, concentrate-rich diets are fed. However, high amounts of concentrate can negatively affect rumen as well as liver health. Previously, clay minerals have shown potential to counteract the negative effects of high-concentrate feeding. Therefore, the aim of this study was to investigate if a clay mineral-based mix (CM) can counteract negative effects of a concentrate-rich diet in dairy cows. Twenty-four lactating Simmental cows (51 ± 23.8 DMI, 8 primiparous, 16 multiparous) were fed a baseline diet for 2 weeks (BASE1–2, 40% concentrate, 60% roughage; DM base) followed by a high-concentrate diet for 4 weeks (HC1–4, 60% concentrate, 40% roughage; DM base). Animals were either assigned to the group receiving the diet with CM addition (n = 12 cows) from BASE2 onwards, or to the group with no additive (CON; n = 12 cows). Fecal as well as blood samples were collected weekly during the whole trial. Fecal pH was measured by using a hand-held pH meter. The liver enzymes activity of aspartate-amino-transferase (AST), glutamate-dehydrogenase (GLDH), and gamma-glutamyl-transferase (GGT) was measured in the plasma with a fully automated analyzer for clinical chemistry. Haptoglobin (Hp) concentration in the plasma was measured with a commercially available ELISA kit. Statistical analysis was performed by using the MIXED procedure of SAS, including diet, group and parity, as well as a possible interaction between them as fixed effects. HC caused a decrease in fecal pH and an increase in liver enzymes in all cows ($P < 0.05$). Multiparous cows receiving CM had a higher fecal pH during HC4 ($P < 0.05$). Furthermore, primiparous cows receiving CM showed a decrease of GLDH during HC1 and GGT during HC1 and HC4 ($P < 0.05$). In addition, supplementation of CM resulted in a decreased Hp concentration in primiparous cows during BASE2 ($P < 0.05$). Data suggest that HC-feeding negatively affected fecal pH as well as liver health. Furthermore, CM may alleviate the severity of high-concentrate feeding by increasing the fecal pH as well as reducing the activity of liver enzymes.

Key Words: dairy cow, clay minerals, liver enzyme

Animal Health Platform Session: A Summary of Discover Conference 35

450 Discover Conference 35 recap—Stress effects on health and production. M. A. Ballou*[1] and B. J. Bradford[2], [1]*Department of Veterinary Sciences, Texas Tech University, Lubbock, TX,* [2]*Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS.*

Many factors, including stress, contribute to the increased risk for disease during certain periods of life in dairy cattle, and factors that contribute to disease vary dramatically between herds. The immune system of cattle may become dysfunctional and increase the likelihood of infectious diseases, many of which are caused by environmental microorganisms. The stress response is essential for the physiological adaptation to change; however, prolonged stress or a maladaptation is in the etiology of immune dysfunction and increased disease. Stress affects the immune system of animals both through direct and indirect mechanisms. Direct impacts are mediated through catecholamine and glucocorticoid pathways, whereas indirect mechanisms are largely directed through altered digestive and metabolic functions. A few examples of indirect mechanisms that were presented include (1) dietary changes, either during weaning or the transition period, alters microbial ecology and/or increases total gastrointestinal permeability, (2) excessive lipolysis during early lactation causes macrophage infiltration into adipose tissue that further exacerbates the negative effects of body condition loss, (3) maladaptation of the homeorhetic calcium system can impair leukocyte function and increase the risk for disease, and (4) long-term, even trans-generational, consequences of disease sequelae on reproduction. Inflammation was discussed in many contexts, both positive and negative. Inflammation is an essential adaptation mechanism, but also contributes to pathological disruption of normal tissue function. An activated immune response, initiated through an inflammatory response, is metabolically expensive and diverts nutrients and energy away from milk synthesis and reproduction toward immune defense and tissue repair. Further, the severity of an infectious disease in many cases is directly related to the degree of inflammation in a tissue. Improved ability of animals to adapt to physiological changes, tolerance to pathogens, early diagnosis, and quick interventions were discussed as key strategies to improve health and limit the negative impacts of disease.

Key Words: health, inflammation, stress

451 Male dairy calf morbidity and mortality after long-distance transportation. D. Wilson*, J. Stojkov, and D. Fraser, *University of British Columbia, Vancouver, BC, Canada.*

Many male dairy calves are sold and transported to grower facilities at a young age when they are vulnerable to health and welfare problems. Our aim was to describe the age, weight, and health of male dairy calves before long distance transport. Second, we evaluated whether these factors related to early morbidity and mortality at a grower facility. From October 2017 to March 2018, calves (n = 373) from 11 dairy farms in British Columbia were assessed by a veterinarian within 24 h before shipping. Health measures included calf attitude (based on depression, willingness to rise), respiratory and enteric health, navel and joint inflammation, and body temperature. Serum total protein was measured, taking a cut-off value of <5.2 g/dL as an indicator of failure of transfer of passive immunity (FTPI). Chest girth circumference was used to estimate weight, and calf age was recorded. Calves were then transported for approximately 1,100 km to one of 2 calf growers and monitored for 2 weeks during which time disease treatments and mortality were recorded. Mixed logistic regression models, with dairy farm as a random effect and calf grower as a fixed effect, were used to assess calf condition measurements associated with treatment for diarrhea or pneumonia, or mortality. Before transport, some calves were experiencing pneumonia (2%), diarrhea (19%), fever (5%), navel disease (11%), or a depressed attitude (4%). FTPI was identified in 13% of calves and mean (±SD) age and chest girth were 4.6 (±2.6) days and 81.1 (±3.9) cm, respectively. In the first 2 weeks after arrival at the calf grower, 23% and 44% of calves were treated for diarrhea and pneumonia, respectively, and 4% died. Diarrhea treatment was negatively associated with chest girth (OR 0.90; 95% CI: 0.83–0.97; $P = 0.004$), and higher mortality was associated with calves that had a depressed attitude (OR: 13.2; 95% CI: 2.9–59.7; $P < 0.001$). These results suggest calves shipped at a light weight with depressed attitude are at increased risk for early morbidity and mortality after long distance transportation.

Key Words: calf, transportation, morbidity

452 Assessing the utility of leukocyte differential cell counts for predicting mortality risk in neonatal Holstein calves upon arrival and 72 hours post-arrival at calf rearing facilities. T. E. von Konigslow*, D. L. Renaud, T. F. Duffield, C. B. Winder, V. Higginson, and D. F. Kelton, *University of Guelph, Guelph, ON, Canada.*

Advances in the understanding of risk factors and biomarkers in calves entering rearing facilities show promise for identifying high-risk calves upon arrival at veal and dairy beef operations. Rapid, on farm machine leukocyte differential cell counts (DCC) may be a good addition to calf risk identification protocols for implementing selective antimicrobial therapy strategies upon arrival. The objective of this study was to assess the utility of DCC taken at the time of arrival to a calf rearing facility and 72-h post arrival for determining mortality risk during the production cycle. From June to July 2018, blood samples collected in EDTA anticoagulant from 233 calves upon arrival and a subset of 158 calves 72 h post arrival to a veal research facility in Ontario, Canada were evaluated by the QScout BLD test for leukocyte differential cell counts (Advanced Animal Diagnostic, Morrisville, NC). Over the production cycle, 39/233 calves died including 30/158 calves in the 72-h post arrival subset. All calves received a risk assessment upon arrival to the facility using a standardized screening protocol and a blood sample was collected to evaluate serum total protein (TP). Preliminary univariable survival analysis using Cox proportional hazards models suggest that on the day of arrival every 1 g/dL increase in TP lowers the risk of mortality (hazard ratio (HR) = 0.38*;* $P < 0.001$). At 72 h post-arrival, it was found that for every 10^9 cells/L increase in neutrophils the risk of mortality increased (HR = 1.12*;* $P = 0.007$). Of the 30 calves that died from the 72 h post arrival subset, only 15 received antibiotic treatment in the first 3 d of life. Machine DCC taken 72 h after the stress of transport has potential for use in selective antimicrobial therapy protocols with the purpose of reducing antimicrobial use without sacrifice to animal health and welfare in veal and dairy beef facilities.

Key Words: male dairy calf, leukocyte, biomarker

453 Effects of a bovine nonspecific immune stimulant on health of Jersey and Jersey-cross heifer calves in the first month of life. B. Omontese*[1], M. Celestino[2], D. Paiva[1], A. Garcia-Muñoz[1,3], A. Masic[4], V. Machado[2], and L. Caixeta[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN,* [2]*Department of*

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Health and performance of pre-weaned dairy calves have great influence on adult life performance. Among the diseases that affect young dairy calves, diarrhea and pneumonia are the most prevalent and economically important. This study evaluated the effects of a non-specific immune stimulant (IS; Amplimune, NovaVive Inc., Canada) on the health of Jersey and Jersey-cross heifer calves within their first month of life. We hypothesized that calves receiving IS would have lower weekly health scores (HS) and decreased disease treatment hazard. Newborn heifer calves (5 ± 2d) in a large well-managed dairy were randomly allocated to receive either 1mL of saline (CON; n = 458) or 1mL of IS (BTIS; n = 449) at nursery before transport to grower (~18h from Minnesota to New Mexico), or 1mL of IS immediately after transport to grower (ATIS; n = 453). All treatments were administered subcutaneously and blood samples were collected for analysis of serum total solids. Calves were health scored weekly based on nasal/ocular discharge, coughing, ear position, temperature, attitude and fecal consistency. Interval to disease treatment was analyzed using Cox proportional hazards regression and HS by logistic regression. For all analysis, CON was set as reference and non-significant variables were removed from the models using backward stepwise elimination. The number of disease treatment events was lower than the national average. A total of 155 calves were treated at least once and 14 calves died within the first month of life. The proportion of calves treated for any disease was 9.8, 11.2 and 13.1%; whereas mortality was 1.1, 1.6 and 2.4% for BTIS, ATIS and CON groups, respectively. Weekly HS did not differ between BTIS and ATIS calves versus CON calves. Calves that received IS before transport had reduced hazard of treatment for pneumonia when compared with CON (HR: 0.54; 95% CI: 0.31–0.94; $P = 0.03$). In conclusion, the administration of IS did not influence weekly HS but IS administration before transport reduced the number of calves treated for pneumonia within the first 30 d of life.

Key Words: calf, health, immune stimulant

454 Withdrawn

455 Association between hoof lesions and milk yield in dairy cows.

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The objective of this study was to evaluate the association between specific hoof lesion (HL) and milk yield in dairy cows. Jerseys were enrolled at 20 ± 3 DIM (d20), evaluated for HL and body condition (BCS). At 120 ± 3 DIM (d120), cows were reexamined for HL and BCS. At the end of lactation, test day milk yield data for each cow was retrieved. According to HL category at d20, cows were grouped as healthy (n = 1,171), hemorrhage (n = 278), noninfectious (n = 103), and infectious HL (n = 34). To assess the relationship of HL development with milk yield, cows were grouped as healthy (no HL at d20 and d120; n = 308), cured (with HL at d20 and no HL at d120; n = 72), new (no HL at d20 but with HL at d120; n = 587), and chronic (with HL at d20 and d120; n = 208). Separate repeated measures linear mixed models were built using test day milk yield as outcome. Variables of interest offered to the model included the fixed effects of HL status, test day number (1–10+), interaction between HL status and test number, parity (1, 2, >3), BCS and calving season. A total of 1,584 cows comprising 13,655 test day milk yield records were used in the final analyses. Of all the HL diagnosed at d20, hemorrhage (69% of HL) was the most common, followed by other noninfectious HL (24%) and infectious HL (7%). Overall, the average test day milk yield was 20.8 kg (95% CI: 20.2 to 21.4 kg). Cows with HL at d20 had reduced milk yield beginning from the 3rd test day with the greatest losses of up to 1.78 kg (95% Confidence Interval (CI); −2.7 to −0.9 kg) by the eighth test day. Cows with noninfectious HL at d20 had reduced milk yield with the greatest losses of up to 4.7 kg at the eighth test day compared with healthy herdmates (95% Confidence Interval (CI); −6.3 to −3.1 kg). Cows that developed new HL produced more milk from the second to fifth test days compared with healthy cows with the greatest milk yield of 1.8 kg (95% CI: 0.9 to 2.8 kg) at the fourth test day of lactation. We conclude that having a lesion in early lactation has a negative impact on productivity and that higher milk production was risk factor for HL development.

Key Words: lameness, milk yield, Jersey

456 Predicting the next life event including disease by applying deep learning on sequential and pictorial data.

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The common currency in developing solutions to sustainable dairy production are often focusing on increased animal production efficiency through improved animal health. In particular, the use of data driven technologies for early disease detection has shown a lot of promise. In this study, we show how systematically recording fertility and disease events from herd management systems can be helpful in improving existing animal monitoring systems. Moreover, while previous research focused primarily on detecting single outcomes such as mastitis lameness and insemination outcomes, we propose a multiclass prediction model which forecasts a probability distribution over 12 possible life events. Traditional techniques such as Markov for discrimination models can use historical sequences of disease and fertility records to predict a cow's future state. Additionally, we investigated whether more advanced recurrent neural network algorithms are better able to uncover the complex data patterns hidden in the event sequences. Finally, we examined if augmenting a cow's history of events with pictures take can enhance the predictive performance even further by making use of convolutional neural network models. While most picture studies are conducted as experimental designs using complex video camera setups and expensive equipment such as thermal scanners, we worked with pictures taken by the farmers and personel their smartphones, which to our most recent knowledge, has not yet been applied in the context of animal monitoring systems. While the Markov for discrimination models their percentage correctly classified (PCC) and Top-3 PCC ranged from 67% to 68% and from 87% to 88% respectively, the neural network models achieved a PCC of 75% and a Top-3 PCC of 95%. Results show that an ensemble model incorporating sequential as well pictorial information performs best and that the model is able to accurately predict future states such as calving, mastitis, pregnancy and death with an accuracy of 97%. The framework presented in this research can be used to enhance current animal monitoring systems with better animal health and higher sustainability for the dairy industry as a result.

Key Words: artificial intelligence, disease classification, animal monitoring

457 Impact of metabolic, digestive and postpartum disorders on milk yield.

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Milk yield (MY) is influenced by DIM, calving season, weather, cow age, physiological state, dry period length, nutrition and health status. A lactation curve model is a useful tool to separate the continuous components of environmental changes and estimate real MY. This study aimed at quantifying the impact of the postpartum incidence of metabolic and digestive disorders on subsequent MY. Daily individual records of MY and health disorders during all lactation were recorded on a commercial dairy farm in the northern region of Mexico. The data set was comprised of 93,580 daily records of 220 Holstein cows representing 382 lactations. Data were obtained from the AFIMILK software from January 2016 to July 2017. The health disorders evaluated during the complete lactation were diarrhea, hypocalcemia, ketosis, laminitis and mastitis. The test-date records of each cow included in the analysis were expanded using the EXPAND procedure of SAS to homogenize the length of lactation to 305 d. To describe the lactation curves of individual cows, Legendre polynomials were fitted using the SAS MIXED procedure. The fitted model included the fixed effects of a high order interaction (test-date, year and month of calving, lactation number and sex of the offspring generated the modeled lactation), health disorder, as well as random and fixed ninth degree Legendre polynomials of DIM. Prevalence disorder was obtained with PROC UNIVARIATE procedure. The proposed modeling estimated losses in daily MY on the day of diagnosis and prevalence for, diarrhea (1.68 kg/d; 0.18), hypocalcemia (6.42 kg/d; 0.05), ketosis (0.67 kg/d; 0.20), laminitis (2.60 kg/d; 0.06) and mastitis (0.73 kg/d; 0.28). There were cumulative decreases in MY during the whole lactation ranging from 219 to 1927 kg per cow after the incidence of diarrhea (504 kg/cow), low calcium concentration (1927 kg/cow), ketosis (202 kg/cow), hock injuries (781 kg/cow), and udder damages (219 kg/cow). These results demonstrate that reduction in milk yield brought on by health challenges may be substantial throughout the lactation and, that country- or region-specific models are needed to better characterize the impact on metabolic disorders on cow productivity and farm profitability.

Key Words: random regression model, lactation curve, Legendre polynomial

458 Impaired blood neutrophil function under ketotic conditions in peripartal dairy cows revealed through an ex vivo LPS challenge.

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Ketosis is an important metabolic disease associated with the negative energy balance commonly experienced by dairy cows soon after calving, and such condition can have ramifications in the immune system of dairy cows in early lactation. The objective of this study was to evaluate blood neutrophil function under ketotic conditions in early lactation cows via an ex vivo LPS challenge. Fourteen Holstein dairy cows (n = 7/group) were monitored during the peripartal period. Blood BHB was measured with the Precision Xtra at 1, 3, 5, 7, 9, and 11 d postpartum. Cows were classified as subclinically ketotic (KET; > 1.4 mmol/L) or non-ketotic (NONKET; < 1.4 mmol/L). Blood samples were collected on d 5 postpartum for an ex vivo LPS challenge conducted at 0 (control), 0.01 (low dose), and 5 μg of LPS/mL (high dose) for 3.5h. Immediately after oxidative burst, neutrophil %, and selectin L were measured via flow cytometry. Additional blood on d 5 was collected for phagocytosis and neutrophil isolation for gene expression. The MIXED procedure of SAS was used to analyze the data. A priori contrasts statements were used to determine a linear effect based on the LPS challenge. As expected, a Group × Day ($P < 0.01$) was observed in BHB, where BHB increased at a greater rate in KET cows and was reflected in greater ($P < 0.01$) BHB in KET cows than NONKET. A trend ($P = 0.06$) for a Group × LPS interaction was observed in oxidative burst increasing linearly ($P < 0.01$) in NONKET cows as LPS was increased from 0 to 5 μg, while no change ($P = 0.89$) was observed in KET cows. Selectin L decreased linearly ($P = 0.02$) in NONKET cows while no change ($P = 0.22$) was observed in KET cows. Blood neutrophil concentration was not affected by either ketosis ($P = 0.27$) or LPS challenge ($P = 0.31$). These results suggest that neutrophils from cows undergoing a ketosis condition will have a lower ability to detect inflammation sites and lower killing capacity based on the lack of response in oxidative burst and selectin L, respectively when facing a further inflammatory or stress conditions.

Key Words: ketosis, lipopolysaccharide, neutrophils

459 Plasma alpha-1-acid glycoprotein is negatively associated with dry matter intake in postpartum dairy cows.

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Alpha-1-acid glycoprotein (AGP) is an acute-phase protein that may suppress DMI by acting on the leptin receptor in the hypothalamus. Our objective was to characterize plasma AGP concentration and associations with DMI during the transition period. Plasma samples (n = 2,086) from 434 Holstein cows in 6 studies were analyzed on d −21, −12 ± 3, −3, 1, 3, 7 ± 1, 14 ± 1, and 21 ± 1 relative to parturition. Bivariate analysis was used to assess the relationship between AGP and DMI. For significant associations, treatment(study) was added to the model and quadratic associations were included in the model, if significant. Mean plasma AGP concentration (±SEM) was 273 ± 70 μg/mL prepartum, and postpartum was 468 ± 66 μg/mL (d 3), 568 ± 66 μg/mL (d 7), 532 ± 65 μg/mL (d 14), and 457 ± 66 μg/mL (d 21). On d −12, AGP was negatively associated with wk −2 DMI ($P < 0.05$) and wk 1 DMI ($P < 0.05$). On d 3, AGP was associated negatively with DMI in a quadratic manner for wk 1 ($P < 0.001$) and wk 2 ($P < 0.05$) and linearly for wk 3 ($P < 0.001$). Day 7 AGP was associated negatively with DMI in a quadratic manner for wk 2 ($P < 0.05$) and linearly for wk 3 ($P < 0.001$). Similarly, d 14 AGP was negatively associated with DMI for wk 3 ($P < 0.001$) and wk 4 ($P < 0.01$). As d 3 AGP concentration increased over the interquartile range, there was a calculated 1.4 (7.8%), 0.5 (2.8%) and 0.4 (1.9%) kg/d reduction in predicted DMI during wk 1, 2, and 3, respectively. Using bivariate analysis, d 3 AGP explained 15.4% of the variation in DMI during wk 1. Finally, we explored the clinical utility of d 3 AGP to diagnose low DMI, defined as wk 1 DMI more than 1 standard deviation below the mean. Receiver operating characteristic analysis identified a threshold of 480.9 μg/mL providing 76% specificity and 48% sensitivity (AUC = 0.60). These results demonstrate a negative association between plasma AGP concentration and DMI in early postpartum dairy cows, although its diagnostic performance was marginal. Further investigation into whether AGP directly suppresses DMI in dairy cattle is warranted.

Key Words: acute-phase protein

460 Effect of meloxicam on systemic inflammation, immune function, and endometrial health in postpartum dairy cows. O. Bogado Pascotinni*, S. J. Van Schyndel, J. W. Spricigo, M. R. Carvalho, B. Mion, E. R. Ribeiro, and S. J. LeBlanc, *University of Guelph, Guelph, ON, Canada.*

This proof-of-concept study evaluated the effects of the anti-inflammatory drug meloxicam on markers of systemic inflammation and energy metabolism, neutrophil function, and endometritis. Cows received meloxicam (0.5 mg/kg SC (MEL) n = 20) once/d for 4 d (10–13 DIM) or were untreated (CON; n = 22). Blood samples were collected −7, 1, 3, 5, 7, 10, 11, 12, 13, 14, 18, 21, 28, and 35 DIM to measure serum haptoglobin (Hp), albumin, total protein, urea, hepatic enzymes (AST, GGT, GLDH), BHB, NEFA, Ca, glucose, and IGF-1. Neutrophil phagocytosis and oxidative burst were measured at 5, 10, 14, and 21, and endometrial cytology at 5, 10, 14, 21, 28 and 35 DIM. The effect of treatment was assessed with mixed linear regression models. MEL had lower Hp at 11, 12, and 13 DIM (0.2 ± 0.2, 0.3 ± 0.2, and 0.4 ± 0.2 g/L vs. CON 0.8 ± 0.3, 1.2 ± 0.4, and 1.1 ± 0.3 g/L, respectively; $P < 0.05$). BHB was lower in MEL at 11, 12, 13, and 14 DIM (0.6 ± 0.1, 0.7 ± 0.1, 0.5 ± 0.1, and 0.6 ± 0.1 mmol/L vs. CON 0.9 ± 0.2, 0.9 ± 0.2, 1.0 ± 0.4, and 0.8 ± 0.2 mmol/; $P < 0.05$). Serum IGF-1 was greater in MEL during treatment (0.84 ± 0.09, 0.81 ± 0.10, 0.83 ± 0.08, and 0.83 ± 0.09 µg/L vs. 0.76 ± 0.10, 0.67 ± 0.09, 0.65 ± 0.08, and 0.72 ± 0.07 µg/L; $P < 0.03$) and glucose was greater in MEL at 13 DIM (3.50 ± 0.10 vs. 3.01 ± 0.17 mmol/L; $P = 0.04$). Phagocytic activity (fluorescence intensity) was 27% greater ($P = 0.04$) in MEL at 14 DIM. Other metabolites and markers of inflammation were not different between treatments. The proportion of endometrial neutrophils was not different at 5, 10, 14, 21, 28, or 35 DIM (MEL 21 ± 5, 40 ± 6, 50 ± 6, 40 ± 7, 26 ± 8, and 15 ± 5% vs. CON 15 ± 5, 46 ± 7, 52 ± 6, 45 ± 6, 22 ± 5, and 14 ± 5%; $P > 0.3$). MEL attenuated systemic inflammation and improved indicators of energy balance but did not affect uterine inflammation.

Key Words: transition period, neutrophil function, endometritis

Breeding and Genetics: Genomic Methods and GWAS

461 Exact *P*-values for large-scale single-step genome-wide association using the BLUPF90 software suite. D. Lourenco*[1], I. Aguilar[2], Y. Masuda[1], I. Misztal[1], and A. Legarra[3], [1]*University of Georgia, Athens, GA,* [2]*INIA, Las Brujas, Canelones, Uruguay,* [3]*INRA, Castanet Tolosan, France.*

Single-step genomic BLUP (ssGBLUP) is a method that combines all sources of information in a single analysis to compute genomic EBV (GEBV). For single-step genome-wide association studies (ssGWAS), GEBV are backsolved to SNP effects, and those effects are converted to proportion of explained additive genetic variance. Thus far, no formal framework for hypothesis test is currently present in ssGWAS from the BLUPF90 software suite. Our objective was to implement *P*-values for ssGWAS and to apply the method to a large dairy cattle population. *P*-values were obtained based on the prediction error (co)variance for SNP, which uses the inverse of the coefficient matrix for genotyped animals and formulas to compute SNP effects. Six steps are needed for the calculation of *P*-values: 1) factorize and invert the LHS of ssGBLUP; 2) solve MME using sparse Cholesky factor; 3) extract the LHS^{-1} for genotyped animals; 4) backsolve GEBV to SNP effects; 5) obtain the prediction error covariance for SNP effects; 6) calculate *P*-values using the cumulative standard normal function of SNP effect divided by standard deviation of SNP effect. The US Holstein data used in this study consisted of almost 800k udder depth records for 500k cows. Pedigree information was available for 1.3M animals, of which 8,802 sires were genotyped. The model contained the same effects as the official model used for linear type trait evaluation in the US; however, in a single-trait setup. Computation of P required 20Gb of memory and no inflation was observed. The SNP passing the Bonferroni threshold of 6.1 in the −log10 scale were the same as those that explained the highest proportion of additive genetic variance. The exact *P*-value for ssGWAS is a very general and efficient strategy for QTL detection and test. It can be used in complex data sets such as the ones used in animal breeding, where only a proportion of pedigreed animals are genotyped. The BLUPF90 software suite is now equipped with the *P*-value calculation tool.

Key Words: genome-wide association studies (GWAS), single-step genomic BLUP (ssGBLUP), significance test

462 Genomic predictions using more markers and gene tests. G. R. Wiggans[2], P. M. VanRaden[1], D. J. Null[1], and J. B. Cole*[1], [1]*USDA Animal Genomics and Improvement Laboratory, Beltsville, MD,* [2]*Council on Dairy Cattle Breeding, Bowie, MD.*

The number of markers used in US genomic predictions increased to 79,276 (or 80K) in December 2018 from the previous 60,671 (60K) used since 2014. The revised list includes more exact gene tests, removes poorer-quality markers, adds new variants from DNA sequence or high-density chips with larger effects on traits, and improves marker order using the new ARS-UCD1.2 reference map. Missing alleles were imputed for Holsteins by (1) imputing all bulls and their ancestors and (2) using those haplotypes as priors to impute the remaining 2 million females, which required 9 d to compute with 25 processors and 270 GB of memory. The 80K list increased computing times for other key programs by about 30%. Important variants now included directly (e.g., in *DGAT1*, *ABCG2*, β-casein, and β-lactoglobulin) had large effects on yield traits and the net merit index. Of the top 5 effects for each of 41 traits, only 34% were from the original 50,000-marker list for Holsteins, 55% for Jerseys, 53% for Brown Swiss, 33% for Ayrshires, and 38% for Guernseys. For Holsteins, a new sequence marker on chromosome 3 had the largest effect on final score, foot angle, feet-and-legs score, and rear legs (rear view). Gene tests for cholesterol deficiency, complex vertebral malformation, brachyspina, and calpain had large effects on somatic cell score, udder cleft, protein yield, and gestation length, respectively. For Jerseys, *bGHR* had a large effect on productive life. Genomic predictions improved more for breeds with larger reference populations. Individual predictions changed most for animals less related to the US population, with less complete pedigrees, or genotyped with lowest density chips. After excluding older cows genotyped using 3,000 markers and imputed dams, correlations of 80K with 60K predictions were about 0.99 for Jersey, Holstein, and Guernsey yield traits but higher for Ayrshires and Brown Swiss. Correlations for many other traits were lower. For Holsteins, correlations averaged a little less than 0.99 for the 6 new health traits and slightly less than 0.98 for type and calving traits. Reliability is expected to increase by 1 to 3 percentage points.

Key Words: genomic prediction, gene test, sequence

463 Validation of genomic predictions for linear type traits in US Holsteins using over 2 million genotyped animals. S. Tsuruta*[1], D. A. L. Lourenco[1], Y. Masuda[1], I. Misztal[1], and T. J. Lawlor[2], [1]*University of Georgia, Athens, GA,* [2]*Holstein Association USA, Brattleboro, VT.*

As accumulating more genotypes, the validation of genomic predictions can be more reliable. In our previous studies, 0.5 million genotyped Holsteins were used to predict genomic (G)PTA. Today, genotypes for over 2 million Holsteins are available, meaning that more genotyped young animals can be used for validation. The objective of this study was to investigate biases in GPTA for young genotyped bulls and verify that the method with a single-step genomic BLUP that has been used in our studies is reliable. Phenotypes for 18 linear type traits used in 2018 genetic evaluation were provided by Holstein Association USA, and genotypes in 2018 were provided by the Council on Dairy Cattle Breeding. The full data set consisted of 10,946,264 records up to 2018 calving, 13,591,145 animals in the pedigree, and 2,334,951 genotyped animals with 80K SNP. Young genotyped bulls with no daughters in 2014 and with at least 50 daughters in 2018 were used to calculate regression coefficients (b_1) of GPTA in 2014 on daughter yield deviations in 2018 as an indicator of inflation or deflation of GPTA. Coefficients of determination (R^2) were used to compare accuracies in parent averages and GPTA. The BLUP90IOD2 program was used to predict GPTA in 2014 and in 2018 with the single-step genomic BLUP using the algorithm of proven and young animals. Genetic trends were also calculated to detect the bias in GPTA. All results from this study were compared with the results from our previous study that used phenotypes and genotypes as of 2014. The model included inbreeding in the pedigree-based relationships and estimable unknown parent groups to reduce inflation and minimize biases in GPTA as suggested in our previous studies. Inflation or deflation (b_1) in GTPA was within 1.0 ± 0.1 on average, depending on the trait, but more stable than the previous results. Genetic trends verified that the model used in this study was unbiased. More young genotyped bulls could provide more stable and reliable results for the validation of GPTA. Convergence via BLUP90IOD2 was reached in 3 d using >300GB memory. The program can be optimized to accelerate the computing speed in the near future.

Key Words: single-step genomic BLUP, linear type trait, US Holsteins

464 Comparing deep learning methods versus parametric and ensemble methods for the prediction of complex phenotypes.

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Transforming large amount of genomic data into valuable knowledge for predicting complex traits has been one of the most important challenges for animal and plant breeders. The prediction of complex traits does not escape the current excitement around machine learning, including a renewed interest in "deep learning" techniques such as multilayer perceptrons (MLPs) and convolutional neural networks (CNNs). The main goal of this study was to compare the performance of 2 deep learning methods including MLP and CNN, 2 ensemble learning methods including random forest (RF) and gradient boosting (GB), and 2 parametric methods including genomic best linear unbiased prediction (GBLUP) and Bayes B for predicting a complex phenotype, namely sire conception rate (SCR). A data set consisting of 11,790 Holstein bulls with SCR records and 55k SNP markers was used. Model predictive ability was measured as the Pearson correlation between predicted and observed values and mean squared error of prediction using 5-fold cross-validation. The best predictive correlation was obtained with GB (0.36), followed by BayesB (0.35), GBLUP (0.34), RF (0.29), CNN (0.29) and MLP (0.27). The same trend was observed for mean squared error of prediction. To provide a better evaluation of deep learning methods, different simulation studies were conducted based on the observed genotype data, assuming a heritability of 0.30, and 100 QTNs with either additive or non-additive genetic effects. When the trait architecture was purely additive, BayesB (0.88) and GB (0.79) outperformed other methods. When the genetic architecture of the simulated trait was a combination of additive, dominance and epistasis, the best predictive ability was obtained by GB (0.81), followed by BayesB (0.71), GBLUP (0.67), RF (0.61), MLP (0.60) and CNN (0.59). Overall, GB is a robust method for predicting complex traits. The effective use of deep learning approaches for genomic prediction needs further research.

Key Words: convolutional neural networks, genomic prediction, machine learning

465 Changes in predictions when using different core animals in the APY algorithm.

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For populations with small effective population size, a genomic relationship matrix constructed for a large number of animals is singular. The APY algorithm exploits the reduced dimensionality of the matrix for lower computing cost, by splitting animals into core and noncore, and using recursion to predict noncore animals from core animals. Typically, the core animals are randomly selected, and their number is approximately equal to the number of eigenvalues explaining 98% variance in the matrix. While correlations in GEBV obtained when using 2 random cores is >0.99, some animals rerank. The purpose of this study was find the extent and origins of reranking using simulated and field data sets across species, and propose methods to reduce the reranking. In general, changes in GEBV obtained with 2 random cores are small but for some animals can be as large as 0.5 additive SD but predictivity and genetic merit of top 20 or 100 animals are nearly identical. Large changes occur nearly always for lower accuracy animals. Animals with low residual in the recursion do not show large changes. The changes are smaller for core than noncore animals. Some changes originate from blending. The changes can be minimized by increasing the number of core animals, and by treating important animals as core. Keeping same core animals over time reduces the changes for existing animals. GEBV generated with the APY algorithm exhibit some variations with the choice of core animals without affecting accuracy of selection.

Key Words: single-step genomic BLUP (ssGBLUP), APY, genomic selection

466 Cattle variant-detection modelling using selective-sequencing experimental design and statistical learning.

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The objective of the current study is to generate a gold standard variant data set specific for the Holstein breed to train mixture models used in SNP variant identification from whole genome sequence data. It is now plausible to comprehensively and economically catalog genetic variations using whole genome DNA sequencing data. Nevertheless, the data still suffers from a low signal-to-noise ratio, which results in a high degree of false positive variant site detections. To accurately distinguish rare variant sites from the noise in sequencing data, the Genome Analysis Toolkit (GATK) implements a statistical learning method that uses a previously developed training set of validated variant sites to identify true positive variants in a data set. Currently, there is no such validated set of variant sites for use in model-training for cattle variant surveys. We used an inverse weight algorithm to prioritize Holstein bulls for sequencing based on the rarity of their homozygous SNP haplotype segments identified in the US national dairy evaluation database. The final list of 172 prioritized Holstein bulls, which represented approximately 85% of the homozygous haplotypes found in the database, were sequenced to at least 20X coverage on an Illumina HiSeqX. Raw reads were aligned to the reference genome ARS-UCDv1.2 using BWA MEM, and 23,912,824 SNPs were called using the SAMtools workflow. By exploiting the expected homozygous nature of haplotype sequence from these individuals, we were able to curate a list of ~200K high quality, lower-frequency variant sites for use in variant-detection modeling. We used these variant sites as training data for the GATK Variant Quality Score Recalibration module to assess the improvement in accuracy of SNP calling and identified 1.1% more rare variants (frequency <5%) in a cut-off study using several different model training parameters. By establishing a high confidence variant site data set for Holstein cattle, we enable more accurate prediction of low-frequency variants in the population for future whole-genome sequence surveys.

Key Words: SNP, variant detection, whole-genome sequencing

467 Genomic prediction with unknown-parent groups and metafounders for production traits in US Holsteins.

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Single-step GBLUP (ssGBLUP) is a genomic prediction method combining phenotypes, pedigree information, and genotypes in a system of equations. This method needs the inverse of unified relationships ($\mathbf{H}^{-1}$) as a function of numerator relationship matrix for all animals in pedigre ($\mathbf{A}^{-1}$), genomic relationship matrix ($\mathbf{G}^{-1}$), and numerator relationship matrix for genotyped animals ($\mathbf{A}_{22}{}^{-1}$). Although these matrices should be compatible in scale, it is rarely true in dairy-cattle data because the pedigree is long but partially missing and the genotypes are mainly available for the last few generations. Unknown parent-groups (UPG) and metafounders (MF) can be used to fill the missing relationships in the relationship matrices and to make them compatible in scale. The objectives of this study were to show a reasonable relationship matrix with UPG or MF in ssGBLUP, to implement this matrix in genetic prediction software, and to apply this technique to production traits in US Holstein. We have derived $\mathbf{H}^{-1}$ in which UPG are considered only for pedigree relationships. We also derived an alternative $\mathbf{H}^{-1}$ from a joint density function. This alternative form is equivalent to one derived from the MF theory. Although UPG and MF are equivalent under some assumptions, MF seems to be more flexible to keep the compatibility among the relationship matrices. The alternative $\mathbf{H}^{-1}$ showed that the computation of $\mathbf{A}_{22}{}^{-1}$ with MF is greatly simplified with sparse matrix techniques. The newly-derived $\mathbf{H}^{-1}$ was implemented in the BLUPF90 programs. The genotypes, pedigrees, and phenotypes of 305-d milk, fat, and protein yield were provided by the Council of Dairy Cattle Breeding (CDCB). The data included more than 72 million phenotypes for each trait, 80 million pedigree animals, and 2.3 million genotyped animals. The validation predictability and inflation of genomic predictions with UPG or MF will be presented.

Key Words: genomic prediction, metafounder, unknown-parent group

468 Alternative input parameters for Wood's curve within best prediction used by USDA-AGIL for genetic evaluation of production traits in the United States.

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Best Prediction (BP) was developed by USDA-AGIL to predict 305-d lactational production for genetic evaluation in the US. Mean and SD of test days for all production traits are used by BP to predict either primiparous or multiparous 305-d production based on a Wood's lactation curve. Fixed Holstein (HO) breed parameters for Wood's curve (a, b, c, and SD of a, b, c) currently used by BP since 2009 were compared with alternative parameters that were newly estimated. The new parameters were implemented to determine if increased production of cows and reduced use of rBST over the past decade has impacted the effectiveness of BP to predict 305-d production of milk, fat, and protein. Test-day observations of HO cows from 7 high-production dairies in Minnesota that have participated in research with the University of Minnesota were used to estimate the alternative BP parameters. The primiparous cows calved from 2011 to 2016, and the multiparous cows calved from 2012 to 2016. All lactations of cows were required to have at least 250 d in milk and were also required to have at least 6 test days by 265 d in milk. Shapes of Wood's lactation curves for these 3 production traits from BP were noticeably different for the currently used versus alternative fixed breed parameters. However, when the 305-d production records of cows were deviated within months of calving according to procedures used for genetic evaluation in the US, the differences between the 2 alternative predictions of 305-d production were of no practical consequence.

Key Words: best prediction, lactation curve

469 A genome-wide analysis using runs of homozygosity in Italian Holstein cattle.

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Current dairy cattle populations are the result of years of selection that lead to high production levels, but also to a reduction of the within breed genetic diversity. This genomic similarity can be investigated using runs of homozygosity (ROH). Apart from being indicators of inbreeding, ROH are also useful for detecting selection sweeps. In the present study, ROHs were used for GWAS study in dairy cattle production traits. A total of 2,818 Italian Holstein bulls were genotyped with the Illumina HD bead chip. After edits, 609,074 SNPs were retained. Minimum number of SNP and length for a ROH were fixed at 50 and 1Mb, respectively. To account for genotyping/imputation errors one heterozygote was allowed into a ROH. Phenotypes were EBVs for milk yield, fat yield and percentage, protein yield and somatic cell score. A linear model was used to test the effects of ROH presence/absence and year of birth (5 levels according to 7-years interval from 1979 to 2014: 1 = 1979–1986; … 5 = 2007–2014). In the model, only ROHs shared by at least 20 animals were considered. Year of birth affected almost all investigated traits (at least $P < 0.00001$). The significance threshold was fixed as the negative logarithm of the ratio between 0.05 and the number of ROH tested, which was 1,083. Several genes found to be associated with dairy traits in previous studies have been found in the present work. Examples are the *ARHGAP39*, *CYHR1*, *CPSF1*, *DGAT1* and *GRINA* loci. Results of the present study conforms the usefulness of ROH as indicators of selection sweeps.

Key Words: runs of homozygosity, milk production trait, cattle

Table 1 (Abstr. 468). Mean currently used and alternative Holstein parameters

	Currently used parameters			Alternative parameters			
	a	b	c	n	a	b	c
Primiparous				1922			
Milk	13.010	0.267	0.00262		13.376	0.300	0.00238
Fat	0.784	0.120	0.00130		0.762	0.172	0.00125
Protein	0.463	0.203	0.00161		0.462	0.247	0.00158
Multiparous				1730			
Milk	22.009	0.216	0.00357		21.391	0.278	0.00370
Fat	1.287	0.073	0.00213		1.173	0.139	0.00228
Protein	0.854	0.132	0.00232		0.801	0.197	0.00257

470 Association between lipid-related genes implicated in conceptus elongation and female fertility traits in dairy cattle.

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Elongation of the preimplantation conceptus is a requirement for pregnancy success in ruminants, and failures in this developmental phase seem to be highly associated with subfertility in dairy cattle. Identifying genetic markers that are related to early conceptus development and survival, and utilizing these markers in selective breeding can improve reproductive efficiency of dairy herds. The objective of this study was to evaluate the association of 1,679 single nucleotide polymorphisms (SNPs) within or close to 183 candidate genes involved in lipid metabolism of the elongating bovine conceptus with different female fertility traits in US Holstein dairy cattle. A total of 27,371 Holstein bulls with predicted transmitting ability (PTA) records for daughter pregnancy rate (DPR), cow conception rate (CCR), and heifer conception rate (HCR) were used as discovery population. The associations found in the discovery population were validated using 2 female Holstein populations (1,122 heifers and 2,138 cows) each with 4 fertility traits, including success to first insemination (SF), number of services per conception (NSC), fertility categorization (FertC), and age at first conception (AFC) for heifers or days open (DO) for cows. Marker effects were estimated using a linear mixed model with SNP genotype as a linear covariate and a random polygenic effect. After multiple testing correction, 39 SNPs flagging 27 candidate genes were associated with at least one fertility trait in the discovery population. Of these 39 markers, 3 SNPs were validated in the heifer population and 4 SNPs were validated in the cow population. The 3 SNPs validated in heifers are located within or near genes CAT, MYOF, and RBP4 while the 4 SNPs validated in lactating cows are located within or close to genes CHKA, GNAI1, and HMOX2. These validated genes seem to be relevant for reducing pregnancy losses, and the SNPs within these genes are excellent candidates to be included into genomic tests for improving reproductive performance in dairy cattle. Our findings emphasize the importance of lipid metabolism for bovine conceptus development.

Key Words: conceptus development, reproductive trait, validation study

471 Genomic prediction and marker selection using high-density genotypes from 5 dairy breeds.

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Genotypes for 314,765 Jerseys (JE), 9,077 Ayrshires (AY; including Scandinavian Red), 39,191 Brown Swiss, 4,440 Guernsey, and 351,666 Holsteins (HO) were imputed to high density (HD). The separate HD reference populations included Illumina BovineHD genotypes for 410 JE, 527 AY, 181 BS, 147 GU, and 4,015 HO. The 641,459 variants included the HD SNPs and all 79,276 (80K) genetic markers and QTLs used in routine evaluations. The variants were not pruned for high linkage disequilibrium as in previous HD studies of only HO. Chromosome locations used the ARS-UCD1 map after removing some incorrectly placed regions. Imputation using findhap version 3 with 24 processors took <2 d for each breed. Before imputation, 90–97% of genotypes in each breed were unknown, and after imputation, 2.1% of JE, 3.6% of AY, 4.7% of BS, 2.8% of GU, and 0.7% of HO alleles remained unknown. Segments in chromosomes 1 and 2 had the most haplotypes in each breed, indicating possible map issues not discovered in earlier tests with fewer SNPs. Prediction and SNP selection results focus only on JE as an example. Allele effects for 26 traits were estimated using phenotypic reference populations that included up to 6,121 JE males and 99,426 JE females. Convergence took 4–8 d using 1 processor per trait and up to 800 iterations. Correlations of HD with 80K genomic predictions for young animals averaged 0.988; yield traits were highest with correlations of about 0.995; cow conception rate and dairy form were lowest at 0.982. Some HD effects were more than twice as large as the largest 80K SNP. On chromosome 11 at 104 Mbase, HD SNPs had the largest effects for fore udder attachment, front teat placement, rump width, and rump angle. For udder cleft and teat length, HD SNPs had higher effects than the highest SNP already in the 80K list. Large new effects were also discovered from HD for daughter pregnancy rate and cow conception rate. Surprisingly for protein, the β-Lactoglobulin gene test had smaller effects than 3 nearby HD SNPs. Previous studies selected and included markers with large effects for HO traits; adding these newly selected HD markers should also improve JE, AY, BS, GU, and crossbred genomic predictions.

Key Words: variant selection, imputation, minor breeds

Physiology and Endocrinology 4

472 Effects of rumen-protected methionine fed to lactating Holstein cows during a heat stress challenge on mammary explant response to lipopolysaccharide. D. N. Coleman*[1], M. Vailati-Riboni[1], R. T. Pate[1], D. Luchini[2], F. C. Cardoso[1], and J. J. Loor[1], [1]*University of Illinois, Urbana, IL,* [2]*Adisseo, Alpharetta, GA.*

The objective was to investigate the effects of rumen-protected Met (RPM) during a heat stress (HS) challenge on the response of mammary gland explants to lipopolysaccharide (LPS). Thirty-two multiparous, lactating Holstein cows (184 ± 59 DIM) were randomly assigned to 1 of 2 environmental treatment groups, and 1 of 2 dietary treatments [TMR with RPM (Smartamine M; Adisseo Inc., France; 0.105% DM of TMR as top dress) or TMR without RPM] in a crossover design. The study was divided into 2 periods with 2 phases per period. During phase 1 (9d), all cows were in thermoneutral conditions (TN; THI = 60 ± 3) and fed ad libitum. During phase 2 (9d), group 1 (n = 16) was exposed to HS using electric heat blankets. Group 2 (n = 16) remained in TN (THI = 61 ± 4) but was pair-fed to HS counterparts. After a 14d washout and 7d adaptation period, the study was repeated (period 2). Environmental treatments were inverted relative to phase 2 in period 1 (sequence), while the dietary treatments remained the same. Mammary tissue was harvested at the end of phase 2. Twenty-five mg of tissue per cow was incubated with 0 or 3 μg/mL of LPS for 2h. Statistical analysis was performed using the MIXED procedure of SAS (Cary, NC.). Regardless of Met supplementation, incubation with LPS increased mRNA abundance of interleukin-8, 6, 1β, C-X-C motif chemokine ligand 2 (*CXCL2*), tumor necrosis factor α, nuclear factor kappa B subunit 1 (*NFKB1*) and toll-like receptor 2 ($P < 0.001$). An environment × LPS interaction was observed for *NFKB1* ($P = 0.03$); expression was greater in LPS-treated explants from non-HS compared with HS cows. mRNA abundance of *CXCL2*, *NFKB1*, inducible nitric oxide, nitric oxide synthase 2, and superoxide dismutase 2 ($P < 0.05$) decreased with HS. While LPS did not alter abundance of genes in NFE2L2 signaling ($P > 0.10$), explants from HS cows had lower abundance of *NFE2L2* ($P < 0.001$) and cullin 3 ($P = 0.04$) (an inhibitor of NFE2L2). Overall, preliminary evaluation indicates that HS reduced immune and antioxidant responses while RPM did not attenuate the inflammatory response induced by LPS in vitro.

Key Words: amino acid, immune response, mammary gland

473 Dairy cows that are less resilient to metabolic stress have increased markers of subacute inflammation, oxidative stress and endo-toxemia when calving during climatic heat stress. N. Nemes-Navon[1,2], G. Kra[1], N. Ben-Aharon[1], S. Yakoby[1], and M. Zachut*[1], [1]*Department of Ruminant Science, Institute of Animal Science, Volcani Center, Rishon LeZion, Israel,* [2]*Bar Ilan University, Ramat Gan, Israel.*

Cows that are less resilient to metabolic stress exhibit increased adipose tissue (AT) lipolysis and oxidative stress postpartum (PP). We hypothesized that increased AT lipolysis will be related to elevated subacute inflammation and higher release of endotoxins from AT to blood PP, and that seasonal heat stress may exacerbate these effects. The study included 24 multiparous dairy cows calving during winter (W, n = 12) or summer heat stress (S, n = 12) at the Volcani research farm (Israel). Cows were categorized retrospectively to those with low (LWL) or high weight loss (HWL) during the first month PP, indicating on metabolic resilience. Blood samples were obtained twice a week during the transition period for tumor necrotizing factor α (TNF-α), oxidative stress marker (malondialdehyde, MDA) and LPS-binding protein (LBP). Subcutaneous AT biopsies were collected at 7 d PP during S for immunoblots of LBP and TNF-α. Data were analyzed by PROC MIXED (SAS). Blood TNF-α was 6.7-fold higher in S vs. W ($P < 0.0009$), and was 1.8-fold higher in HWL than in LWL cows during S ($P < 0.05$), but not between HWL and LWL cows at W. Blood MDA was 5-fold higher in S than in W ($P < 0.0001$), and was 2-fold higher in HWL than in LWL ($P < 0.05$) during S, and tended to be higher in HWL vs. LWL at W ($P < 0.1$). Across seasons, blood LBP was 1.7-fold higher in HWL than in LWL at 7 d PP ($P < 0.05$). In AT of S cows, the abundances of LBP ($P < 0.05$) and TNF-α ($P < 0.001$) were higher in HWL than in LWL. Together, cows that were less resilient to metabolic stress had a higher inflammatory response and increased signs of endo-toxemia in blood and AT specifically during heat stress. Seasonal heat stress has a dramatic effect on the degree of oxidative stress, subacute inflammation and immune function in transition cows.

Key Words: metabolic stress, heat stress, inflammation

474 Effects of intralipid infusion on metabolism and inflammation in immune-challenged lactating cows. E. A. Horst*, L. M. van den Brink, E. J. Mayorga, M. Al-Qaisi, S. Rodriguez-Jimenez, B. M. Goetz, M. A. Abeyta, and L. H. Baumgard, *Department of Animal Science, Iowa State University, Ames, IA.*

Objectives were to evaluate the effects of Intralipid (IL; 20% i.v. fat emulsion; Fresenius Kabi, Uppsala, Sweeden) infusion on metabolism, inflammation, and liver lipid content following an i.v. lipopolysaccharide (LPS) challenge in lactating cows. Cows (765 ± 32 kg BW; 273 ± 35 DIM) were enrolled in 2 experimental periods (P); during P1 (5d) baseline data were obtained. At the start of P2 (2d), cows were assigned to 1 of 2 treatments: 1) control + IL (CONIL; 3 mL of sterile saline; n = 5) or 2) LPS + IL (LPSIL; 0.375 μg/kg of BW LPS; n = 5). Directly following i.v. bolus (saline or LPS) administration, IL was i.v. infused continuously (200 mL/h) for 16h. Cows were fasted for 16h during P2. Liver biopsies were obtained on d1 of P1 and at 16 and 48h postbolus. Effects of treatment, time, and treatment × time interactions were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Mild pyrexia (0.8°C) was observed for 5.5h postbolus in LPSIL relative to CONIL cows ($P = 0.01$). LPS increased circulating insulin (4-fold) during the IL infusion period relative to CONIL cows ($P < 0.01$). Circulating glucagon increased 3-fold in LPSIL cows from 8 to 12h post-LPS relative to CONIL ($P = 0.03$). NEFA concentrations gradually increased in both treatments (3-fold, relative to baseline; $P \leq 0.04$), but peaked (43%) higher in CONIL compared with LPSIL cows ($P = 0.01$). Circulating BHB decreased in both treatments for the first 8h of P2, after which it gradually increased. Infusing IL increased (36%) liver TG content in CONIL cows at 16 h relative to baseline ($P = 0.05$), but it had no effect on lipid content of LPSIL cows. No treatment differences in liver lipid content were observed at 48h. Relative to CONIL, circulating LPS-binding protein in LPSIL cows increased 2-fold at 8h postbolus then markedly decreased (5-fold; $P < 0.01$). Serum amyloid A concentrations progressively increased in LPSIL cows during P2 (3-fold, relative to CONIL; $P < 0.01$). In summary, IL infusion altered the characteristic patterns of insulin and LBP in response to LPS, but did not cause fatty liver.

Key Words: lipid infusion, LPS

475 Relationships between fecal pH and milk production, metabolism, and acute phase protein response in periparturient dairy cows.

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Objectives were to evaluate the relationship between fecal pH, milk yield (MY) and components, DMI, and blood parameters during the transition period. From d −21 to 28 relative to calving, DMI and MY were recorded daily, whereas fecal pH and milk composition were determined weekly from 52 multiparous cows. Based on antepartum pH, cows were retrospectively categorized into 1 of 2 groups: 1) High (AH; top 1/3 of cows, pH >7.3; n = 17) or 2) Low (AL; bottom 1/3 cows, pH <7.1; n = 17); likewise for postpartum pH (PH, pH >6.8 or PL, pH <6.5), and pH change pre- to postpartum (ΔH or ΔL). Data were analyzed using the MIXED and CORR procedures of SAS. Although magnitude of ΔpH did not affect MY or DMI, these response variables from PL cows were less than those of PH cows 1 wk postpartum ($P \leq 0.03$). Decreased antepartum DMI was associated with lower postpartum fecal pH ($r = -0.28$; $P = 0.05$). A large fecal pH change from wk-1 to 1 was associated with decreased DMI on wk 3 postpartum ($r = -0.33$; $P = 0.05$). Antepartum fecal pH was negatively associated with the rate of MY increase from wk 1–2 ($r = -0.31$; $P = 0.03$). The rate of change in fecal pH from wk 1–2 was positively correlated with the rate of increase in MY from wk 1–4 ($r = 0.38$; $P = 0.03$). Fecal pH was negatively associated with wk 1–4 SCC ($r = -0.28$; $P = 0.05$). Relative to PH cows, those with lower fecal pH postpartum had decreased ECM and concentration of milk fat and lactose 1 wk postpartum ($P \leq 0.04$). Postpartum fecal pH was positively correlated with contents of milk fat, lactose, MUN, and ECM for wk 3 postpartum (r = 0.33, 0.39, 0.29, and 0.28, respectively; $P \leq 0.07$). Fecal ΔpH from wk −1 to 1 was positively associated with the slopes of circulating LBP and Hp concentrations from wk 1–2 postpartum (r = 0.73 and 0.55, respectively; $P \leq 0.03$). Change in pH from wk −1 to 1 was positively correlated with NEFA and BHB concentrations postpartum (r = 0.48 and 0.51, respectively; $P \leq 0.04$). In summary, fecal pH appears to be moderately associated with production parameters, metabolism and inflammation during the transition period in dairy cows.

Key Words: hindgut acidosis, inflammation

476 Effects of hindgut acidosis on metabolism, inflammation, and production in dairy cows acclimated to a low-starch diet.

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Study objectives were to evaluate effects of hindgut acidosis in cows acclimated to a low-starch diet. Ten rumen-cannulated Holstein cows (243 ± 20 DIM; 663 ± 18 kg BW) acclimated to a low-starch diet (15% starch) for 17d were enrolled in a study with 2 experimental periods (P). During P1 (4d), baseline data were collected. During P2 (7d) cows were randomly assigned to 1 of 2 treatments: 1) control (CON; 1.5 L H_2O/infusion) or 2) 4 kg/d of starch (ST; 1 kg corn starch + 1.5 L H_2O/infusion) and abomasally infused every 6 h daily. Milk, feces, and blood samples were collected daily. Effects of treatment, time, and treatment by time interaction were assessed using PROC MIXED (SAS Inst. Inc., Cary, NC). Starch infusions markedly reduced fecal pH compared with CON (5.8 vs. 7.2; $P < 0.01$). Rectal temperature of ST cows progressively increased from d 1–3 of P2 ($P = 0.04$). Milk yield and DMI were unaffected by treatment. Milk protein content of ST cows gradually increased from d 2–5 of P2, while it remained unchanged in CON ($P < 0.01$). No treatment differences were observed in milk fat, although, it decreased in ST cows compared with P1 (6%; $P = 0.10$). Milk urea nitrogen decreased in ST cows from d 1–4 of P2 and then plateaued ($P < 0.01$). No overall treatment differences were observed for SCC during P2, but it decreased in ST cows relative to P1 (29%; $P = 0.04$). During P2, circulating insulin increased (47%; $P = 0.08$) in ST relative to CON, however, glucose and BHB concentrations were unaffected by treatment. Starch infusions decreased BUN concentrations from d 1–5 of P2 (38%; $P < 0.01$) relative to CON. Similarly, NEFA decreased in ST cows (44%; $P = 0.07$) while they were unchanged in CON cows. Relative to CON, ST infusions increased circulating monocytes on d 3 (77%; $P = 0.04$) and decreased basophils on d 0.5 and 7 (both 31%; $P = 0.01$) of P2. Circulating serum amyloid A and lipopolysaccharide binding protein concentrations were unaffected by starch infusion. In summary, although abomasal ST infusion reduced fecal pH and altered energetic metabolism, little to no effects were observed on inflammation and production in cows consuming a low-starch diet.

Key Words: endotoxin, LPS, immune system

477 Oxidative stress pathway components in adipose tissue of Holstein cows during the periparturient period differ by body condition score.

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The periparturient period is characterized by increased oxidative stress status in dairy cows. In non-ruminants, a chronic state of excessive fat deposition is accompanied by inflammation and oxidative stress. The objective was to investigate if prepartal body condition score (BCS) is associated with plasma and adipose tissue biomarkers of oxidative stress in Holstein cows. Twenty 2 multiparous Holstein cows were divided by BCS before parturition (−30 d prepartum) into a BCS ≤ 3.25 (LoBCS, n = 11) or BCS ≥ 3.50 (HiBCS, n = 11) group. Blood sampled from the coccygeal vessel at −30, −15, 7, 10, and 30 d relative to calving date was used for oxidative stress and inflammation biomarkers analysis. Adipose tissue obtained from each group on d −15, 7 and 30 relative to calving date was used for RT-PCR analysis. The statistical model in SAS included the fixed effect of treatment, time and its interaction. There was no difference in prepartal DMI and milk yield between groups ($P > 0.05$). A treatment × time interaction ($P < 0.05$) was observed for postpartal DMI due to greater responses in LoBCS cows. Although there was no overall treatment or interaction effect ($P > 0.05$) for plasma myeloperoxidase and haptoglobin, the fact that ferric-reducing ability (antioxidant capacity) increased between −10 and 7 d (interaction $P = 0.01$) and reactive oxygen metabolites between −10 and 15 to 30 d (interaction $P < 0.01$) in HiBCS cows suggested a chronic inflammatory and oxidative stress state. The greater ($P < 0.05$) overall concentration of carotene in LoBCS cows suggested they had better antioxidant status. Cows with HiBCS had greater abundance of cullin 3 (*CUL3*) ($P < 0.05$) and lower overall abundance of factor erythroid 2-like 2 (*NFE2L2*). However, HiBCS cows had greater ($P < 0.05$) abundance of genes associated with glutathione metabolism including glutathione peroxidase 1 (*GXP1*) and glutathione reductase (*GSR*). Regardless of BCS, mRNA abundance of *NEF2L2* decreased and *KEAP1* increased from 7 to 30 d after parturition. Overall, the data suggest a more pronounced systemic and localized oxidative stress status in cows with prepartal BCS ≥ 3.50.

Key Words: body condition score, dairy cow, oxidative stress

Production, Management, and the Environment 3

478 Late-gestation heat stress impairs performance of daughters and granddaughters.

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We hypothesized that late-gestation hyperthermia impairs performance of daughters and granddaughters of dams heat stressed when dry. To test this hypothesis, we used lactation records from experiments conducted during 10 summers at the University of Florida (2008–2018; THI > 68). A total of 158 daughters (F1; CL_{F1} n = 78, HT_{F1} n = 80) and 45 granddaughters (F2; CL_{F2} n = 24, HT_{F2} n = 21) of multiparous dams that were actively cooled (CL, shade, fans and soakers, n = 78) or not (HT, shade only, n = 80) during a 46-d dry period were used. Milk yield, fat and protein records of the F_1 and F_2 were used to estimate energy corrected milk (ECM), and life events were also assessed (i.e., culling and longevity). We evaluated ECM from calving to 35 weeks in milk (WIM) for 3 consecutive lactations. Data were analyzed using MIXED procedure of SAS with HT or CL, WIM (repeated measure) and their interaction as fixed effects, and dam(treatment) as random effect. Culling percentage before breeding was analyzed by Chi-squared contingency test using FREQ procedure of SAS. In this regard, 30% of HT_{F1} were culled before breeding, whereas 18% CL_{F1} were culled before breeding ($P = 0.07$). The longevity of HT_{F1} was 32% lower than CL_{F1} (2.53 vs. 3.35 ± 0.34 yr respectively; $P = 0.02$). Moreover, HT_{F1} produced less ECM milk than CL_{F1} in their first (29.2 *vs*. 31.6 ± 0.07 kg, respectively; $P < 0.01$), second (36.2 *vs*. 34.3 ± 0.11 kg, respectively; $P < 0.01$) and third lactations (40.1 *vs*. 33.4 ± 0.19 kg, respectively; $P < 0.01$). The probability of culling before breeding (CL_{F2} = 16.7 vs. HT_{F2} = 38.1%; $P = 0.11$) and longevity (CL_{F2} = 3.13 and HT_{F2} = 2.16 ± 0.48 years; $P = 0.16$) was similar between CL_{F2} and HT_{F2}. Granddaughters born to HT_{F1} produced less ECM milk than granddaughters born to CL_{F1} in their first (HT_{F2} = 29.1 *vs*. CL_{F2} = 32.8 ± 0.12 kg; $P < 0.01$) and second lactations (HT_{F2} = 35.3 *vs*. CL_{F2} = 40.0 ± 0.29 kg; $P < 0.01$). Dry period heat stress exerts transgenerational effects on at least 2 generations and 2 lactations of daughters and granddaughters that never experienced HT nor CL treatments directly.

Key Words: dry cow, heat stress, transgenerational

479 Heat stress alters expression of stress and immune response genes in leukocytes of lactating cows.

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Heat stress (HS) is thought to impair immune function of dairy cows via effects on leukocytes but mechanisms are not understood. We hypothesized that HS would elicit the unfolded protein response (UPR), an ubiquitous cellular stress response pathway, and concomitantly downregulate expression of pro-inflammatory immune genes. Expression of the UPR-associated genes, *IRE1, XBP1s, ATF6, PERK*, and *CHOP* was quantified to evaluate the 3 branches of the UPR. Expression of *TNFα* and *IL-8* were determined as markers of immune function. Six mid-lactation Holstein cows were housed in tie stalls in environmental chambers and were exposed to 5d of thermoneutral (TN) conditions (THI~65), followed by HS for 16d (THI~76), and 9d of recovery (REC) under TN conditions. Blood was sampled on d −5, 5, 15, and 25 relative to onset of HS, every 4h during the first 32h of HS, and every 6h during the first 24h of REC. Rectal temperatures increased during HS compared with TN (39.8 vs 38.5 ± 0.4°C; $P < 0.01$), and returned to TN levels during REC. Milk yield and DMI decreased ($P < 0.01$) during HS (32.2 vs 26.4 ± 0.5 kg/d and 24.1 vs 15.9 ± 0.4 kg/d, respectively). Overall, expression of UPR and immune genes differed across the study ($P < 0.05$); all UPR genes declined from d −5 to d 5 ($P < 0.05$). Expression of *IRE1*, *XBP1s*, *CHOP*, and *IL8* returned to pretreatment levels, or higher, during REC ($P < 0.05$). Expression of *PERK* and *ATF6* followed a different pattern and did not change from d5 to d15 nor from d15 to REC ($P > 0.10$), remaining lower than pretreatment through REC. Onset of HS did not alter ($P > 0.10$) UPR gene expression during the first 32h, except for *XBP1s* ($P < 0.01$). Cessation of HS did not alter expression during the first 24h of REC, except for *XBP1s* and *ATF6* ($P < 0.01$). Results demonstrate that HS downregulated expression of immune genes and, contrary to our hypothesis, UPR-associated genes. Expression was not altered acutely after changes in THI. We conclude the UPR is suppressed transiently during HS and expression of genes associated with UPR and immune function in leukocytes did not respond acutely to moderate HS in dairy cows.

Key Words: hyperthermia, unfolded protein response, health

480 When do dry cows get heat stressed? Correlations of rectal temperature, respiration rate, and performance.

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Previous studies have documented the negative effects of heat stress during the dry period (DP) on dairy cow performance during the next lactation. Analysis of 6 different studies (n = 144 cows) was performed to summarize the correlations of rectal temperature (RT), respiration rate (RR) during the DP and milk production during the first 8 wks of lactation (MILK) with calf weight (CW), body weight at calving (BW), gestation length (GL) and DMI pre and postpartum. Studies were conducted in Florida during the summer and dry cows were assigned into 2 treatments. Heat stressed cows (HT; n = 75) were kept under shade but cooled cows (CL; n = 69) had shade with soakers and fans. Average RT and RR of HT increased ($P < 0.01$) vs CL (39.4 ± 0.05 vs 39.1 ± 0.05°C and 77 ± 1.8 vs 51 ± 1.9 bpm, respectively). In addition, CL produced more milk during the first 8 wks of the subsequent lactation relative to HT (36.6 ± 0.9 vs 31.4 ± 0.9 kg; $P < 0.01$). Correlation analysis indicated that RR of HT were negatively correlated with MILK (R = −033; $P < 0.01$) and GL (R = −0.33; $P = 0.01$). In HT, RT was also negatively correlated with MILK (R = −0.25; $P = 0.03$) and GL (R = −0.48; $P < 0.01$) and tended to be negatively correlated with CW (R = −0.20; $P = 0.09$). In addition to being negatively correlated with RT and RR, HT MILK was positively correlated with CW (R = 0.36; $P < 0.01$), GL (R = 0.28; $P = 0.04$) and DMI postpartum (R = 0.32; $P = 0.01$). In CL, no correlations were observed for RR, but RT was positively correlated with BW (R = 0.25; $P = 0.03$) and MILK was positively correlated with DMI postpartum (R = 0.33; $P = 0.01$) and tended to be positively correlated with GL (R = 0.24; $P = 0.08$).Moreover, the overall RT was 39.1 ± 0.48°Cand 61 ± 19.5 bpm, which indicates that RR over 61 bpm is an indicator of heat stress in cows during the dry period. In summary, the more heat load a cow carries in the dry period, the greater are the negative effects on subsequent milk, which may suggest a threshold for the effects of heat stress. And, variation among cows within the HT group indicates that cows with longer gestation length have heavier calves, eat more postpartum, and produce more milk, but not as much as CL.

Key Words: heat stress, dry period, milk production

481 Progesterone based synchronization following Quick-resynch protocol improves cumulative pregnancy rate in lactating dairy cows. A. H. Shahzad*[1], A. Abbas[2], S. Abbas[1], I. Anwar[2], and R. Safdar[2], [1]*University of Veterinary and Animal Sciences, Lahore, Pakistan,* [2]*Livestock and Dairy Development Department, Punjab, Pakistan.*

Objectives of the present study were to evaluate CIDR-estradiol benzoate (CIDR-EB) synchronization protocol followed by Quick-resynch through pregnancy rate (PR). Postpartum cows (n = 396) were assigned to receive standard CIDR-EB protocol. Cows were subjected to timed AI (TAI) and randomly assigned to 1) Quick-resynch group (n = 198), received autoclaved CIDR for d14-d21 following 100 µg GnRH on d 23rd post TAI. Pregnancy was diagnosed on d30 through ultrasonography. Open cows received $PGF_{2\alpha}$ and GnRH of Quick-resynch protocol. 2) Control group (n = 198), subjected to AI after detected estrus from d18–30 post TAI. Pregnancy was confirmed on d60 and d90 in both groups. For progesterone (P4) profile, blood sampling was done on d14, d16 and d30 post TAI and Luteal tissue cross-sectional area was measured on d30 in pregnant animals in both Quick-resynch and Control groups (n = 60). Chi-squared analysis in PROC FREQ of SAS was used to determine the treatment effect on pregnancy rate. Treatment effect on luteal tissue cross-sectional area and P4 profile were analyzed by GLM procedures of SAS ($P < 0.05$). On d30 post TAI, PR was 51 and 43.40% ($P = 0.37$) in both Quick-resynch and Control groups, respectively. On d60 and 90, cumulative PR was 72.20 vs. 50% ($P = 0.03$) and 70 vs. 47.50% ($P = 0.02$) in Quick-resynch and Control groups, respectively. On d60 pregnancy loss in Quick-resynch group was 4.80% in comparison with 9.30% in Control group ($P = 0.34$). Overall pregnancy loss was 4.10 and 12.96% in Quick-resynch and Control groups ($P = 0.02$), respectively. Progesterone profile (ng/mL) was statistically non-significant on d14 ($P > 0.05$) while on d16 and 30 post TAI, P4 was significantly higher ($P < 0.05$) in Quick-resynch group as compared with Control group. Luteal tissue area was significantly higher ($P < 0.05$) in Control group. Parity, cyclicity and BCS did not have any effect on pregnancy neither independently nor in interaction with treatment. In conclusion, cumulative PR was significantly higher in Quick-resynch group and has also been resulted in significantly higher ($P < 0.05$) P4 profile despite smaller luteal tissue as compared with Control group on d30 post TAI.

Key Words: lactating cows, Quick-resynch, pregnancy rate

482 The effect of milk predicted transmitting ability and concentrate supplementation level on milk production and composition in late-lactation spring-calving grazing dairy cows. M. J. Doran*[1], M. B. Lynch[1], F. J. Mulligan[2], A. G. Fahey[1], E. L. Brady[2], C. Grace[1], and K. M. Pierce[1], [1]*School of Agriculture and Food Science, University College Dublin Lyons Farm, Naas, Co. Kildare, Ireland,* [2]*School of Veterinary Medicine, University College Dublin, Veterinary Science Centre, Belfield, Dublin, Ireland.*

In Ireland, spring calving grazing systems utilize CS mainly in the spring and autumn when there is a shortage of grass. Studies show there is a better response to CS in cows with a higher genetic merit for milk production. The objective of this study was to investigate the effect of PTA, CS level and their interaction on milk production and composition in late lactation (+200 DIM) spring calving grazing dairy cows. A factorial experiment, with 2 milk PTA groups (high (+191 kg) versus low (−53 kg)) and 2 CS levels (0 (GO) versus 2.7 kg dry matter (DM) CS) was carried out over a 51-d period. Forty-eight Holstein Friesian (24 low milk (LM) and 24 high milk (HM) PTA) dairy cows were blocked on parity and balanced on DIM, BCS and Economic Breeding Index. Cows within PTA groups were randomly assigned to one of 2 CS levels, resulting in 4 treatment groups (n = 12): 1) LM PTA offered GO (LG); 2) LM PTA plus 2.7 kg DM CS (LC); 3) HM PTA offered GO (HG) and 4) HM PTA plus 2.7 kg DM CS (HC). All cows were grazing full time and were offered 17 kg DM grass/cow per d. HC had higher milk yield (+3.53 kg, $P < 0.05$), fat kg (+0.14 kg, $P < 0.05$) and lactose kg (+0.17 kg, $P < 0.05$) compared with H0. HC tended to have higher milk yield (+3.06 kg, $P = 0.09$) and lactose kg (+0.14 kg, $P = 0.09$) compared with LC. HM PTA increased milk yield (+2.13 kg, $P < 0.05$) and lactose kg (+0.10 kg, $P < 0.05$), while CS increased milk yield (+2.68 kg, $P < 0.01$), fat kg (+0.10 kg, $P < 0.01$), protein kg (+0.08 kg, $P < 0.05$), casein kg (+0.06 kg, $P < 0.05$), fat and protein (milk solids) kg (+0.18 kg, $P < 0.05$) and lactose kg (+0.13 kg, $P < 0.01$). However, HM PTA reduced fat (−0.21%, $P < 0.05$), protein (−0.16%, $P = 0.02$) and casein % (−0.14% $P < 0.05$), while CS decreased protein (−0.14%, $P < 0.05$) and increased lactose % (+0.08%, $P = 0.01$). HM PTA cows had a higher response to CS compared with LM PTA cows (1.31 versus 0.67 kg milk/kg DM CS). In conclusion, HM PTA increased milk yield and lactose kg but decreased composition. HM PTA cows had a better response to CS than LM PTA cows.

Key Words: dairy cow, late lactation, grazing

483 Perception of sustainability of dairy producers in Chile. P. Toro-Mujica*[1,2], L. Robles[3], and E. Vargas-Bello-Pérez[4], [1]*Instituto de Ciencias Agronómicas y Veterinarias, Universidad de O'Higgins, San Fernando, Chile,* [2]*Departamento de Ciencias Animales, Facultad de Agronomía e Ingeniería Forestal, Pontificia Universidad Católica de Chile, Santiago, Chile,* [3]*Departamento de Nutrición Animal, Universidad Autónoma del Estado de México, Toluca, México,* [4]*Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark.*

In many countries, the word sustainability is used at the governmental and commercial level with a positive meaning about environmental care and social and animal welfare. The objective of this study was to evaluate the degree of knowledge that dairy producers have about sustainability and its association with milk production practices. The study was performed in the Southern region of Chile. Twenty-six farmers were surveyed by phone or email. The farms were selected through associations of milk producers and their willingness to participate. The questionnaire consisted of 39 questions, of which only one was an open-ended question. The survey considered general information on the characterization of the producer and his farm. Surveys were subjected to an exploratory descriptive analysis. Statistical tests on the data were performed by 2-dimensional contingency tables and using the Chi-squared test. The average age of the producers was 52 years old. Concerning the educational level, 48% of the producers have university studies and 52% technical studies. In 68% of the cases, those studies were related to the agricultural area. 92% of the producers grew in the field. 41.6% of the farms have less than 200 cows. The average area was of 276 ha, with a stocking rate of 1.3 cows·ha^{-1}. All producers declared to have heard the word sustainability; however, 12% said that they did not know a definition. Among the definitions of sustainability that cover most of the concepts mentioned are "resource use in an efficient way that ensures profitability and does not compromise resources in the future" and "that cycles can be replicated without harming the environment." About knowledge of the management associated with sustainable production there was no relationship between the level of knowledge and education; besides, of 19 sustainable practices mentioned, about 90% of the answers were correct. 84% of producers indicated that the focus of their exploitation was production and 79% gave greater importance to profitability than to the animal welfare. This study can be helpful for public policymakers due to the need to improve strategies to transfer knowledge of sustainable practices. This need is reflected in the lack of implementing sustainability through a set of practices.

Key Words: sustainability, dairy, survey

484 Antibiotic use in cubicle housing and freewalk barn systems.

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Antibiotics use in animals has become part of societal discussion. Therefore, this topic was included in the www.freewalk.eu project studying the use in different housing systems. Antibiotic use was examined in an earlier stage on 94 cubicle housing farms (no ecological) during 2005–2012 (JDS 99:1632–1648). Level of use (in no. of daily dosages-ADDD) was rising in period 2005–2007, followed by a period of growing societal interest in animal antibiotic use resulting in a reduction in use from 2010/11 on. On average, 68% was applied to the udder: mastitis 25% and dry-cow therapy 43%; other drugs tended to decrease the most, while farmers were reluctant to lower use of dry-cow therapy tubes. Use of sensitive drugs (also human used) minimized from 18% of ADDD in period 2005–2010 to 1% in 2012. The data have been recently updated to 2017. ADDD dropped in this period on average from 6.41 in 2007 (top year in use) to 3.12 in 2017 (50% reduction). Antibiotic use and treatment classes on these farms are compared with 24 freewalk (bedded pack) barn systems and 10 ecological farms, which function in this study as indicator for a minimum use level, including a few artificial floor housing systems. In total about 45 freewalk systems are operating in the Netherlands, of which 10–15 with ecological farming and/or human care agriculture. The freewalk and cubicle farm groups had in period 2012 to 2017 comparable average herd sizes (95 to 125 cows), although the freewalk farms were slightly faster growing. In this period, the group of freewalk farms used significantly less antibiotics than the cubicle farms. Presently the historic use of the freewalk farms in period 2005 to 2011 is assessed to better explain the noted difference in use. These farms changed from cubicles to the freewalk system around 2012 or later. Besides housing system, also farm, farmer and herd indicators are studied over 2005 to 2017 to help explaining the difference in use. Research on part of this data indicates significant correlations between calving interval, cell count and overall use of antibiotics and dry cow therapy tubes. The role of the farmer will be addressed as well.

Key Words: antibiotic use, housing system, herd factors

485 Electrical energy consumption in four commercial Midwest dairy barns.

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Consumers are demanding reduced carbon emissions and increased sustainability within food production systems. However, fossil energy consumption data within dairy production are scarce. Therefore, the objective of this study was to measure electricity use to determine areas of high consumption in commercial dairies. Data were collected from freestall barns representative of typical Midwest dairies and located in west central Minnesota: one 9,500 head, cross-ventilated barn with a rotary parlor (A), one 300 head, naturally-ventilated barn with stirring fans and an automatic milking system (B), one 200 head, naturally-ventilated barn with stirring fans and a parabone parlor (C), and one 400 head, naturally-ventilated barn with stirring fans and a parallel parlor (D). Electricity was monitored from July 2018 to January 2019. Electric loads were monitored on the farm side of the electric meter at the circuits to reveal areas of highest usage. Electrical use was calculated on an electrical load basis (Table 1). Despite barn design and capacity differences, ventilation was the largest user of electricity across farms A, B, and C. Ventilation use ranged from 17% to 41% of total electricity used across all farms. Other large electric loads varied across the farms. Electricity for lighting ranged from 7 to 20% of total electricity. Manure handling ranged from 0 to 24% of total electricity. Milk cooling components ranged from 5 to 21% of total electricity. Approximately 14% of the electricity in the automatic dairy was used to operate the automatic milking system. Improving the efficiency of electrical components could provide opportunities to improve the carbon footprint of dairy production systems.

Table 1 (Abstr. 485). Percent of total electricity used by various loads on each farm

	Farm			
	A	B	C	D
Mean kWh per month	650,615	30,613	9,774	26,399
Electricity use, % of total kWh[1]				
Ventilation, %	41.0	27.0	20.0	17.0
Lights, %	14.0	7.0	11.0	20.0
Manure handling, %	10.0	1.0	0.0	24.0
Milk cooling, %	5.0	21.0	14.0	19.0
Automatic milking system, %	NA[2]	14.0	NA	NA

[1] Includes the highest consuming electrical loads on each farm.

[2] Not applicable.

Key Words: dairy, electricity, sustainability

486 Completeness of reporting of experiments: REFLECTing on a year of animal trials in the *Journal of Dairy Science*.

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Reproducibility is an essential element of the scientific process, and requires clear and complete reporting of study design, conduct, and analysis. In human and animal health literature, incomplete reporting is associated with biased effect estimates. Moreover, incomplete reporting precludes knowledge synthesis and undervalues the resources allocated to the primary research. The Reporting Guidelines for Randomized Controlled Trials for Livestock and Food Safety (REFLECT) statement, published in 2010, is a checklist developed by expert consensus to provide guidance on what study elements should be reported in any intervention trial (designed experiment) involving livestock. The *Journal of Dairy Science* has recently endorsed the use of reporting guidelines. To assess the status of reporting of controlled experiments in the journal and to provide a baseline for future comparison, we evaluated the reporting of 18 items from the REFLECT statement checklist in a sample of 137 controlled trials published in the journal in 2017. Two reviewers independently screened titles and abstracts for relevance, and then evaluated a sample of 120 papers reporting controlled trials (experimental studies involving at least one intervention and one comparison or control group), using yes or no questions. While some items, such as treatment details

and statistical analysis, were well-reported, other areas including sample size justification, allocation concealment, blinding, study flow, baseline data, and ancillary analyses were often not reported or incompletely described. This work highlights the need for authors and reviewers to take advantage of guidelines and checklists for reporting. Adherence to reporting guidelines can help improve the completeness of reporting of research, expedite and better inform the peer-review process, increase clarity for the reader, and allow for knowledge synthesis, such as meta-analysis, all of which serve to increase the value of the work conducted.

Key Words: reporting guidelines, transparency, reproducibility

Ruminant Nutrition 6: Fiber and Fermentation

487 Effects on fermentation and aerobic stability of a dual-strain inoculant during the early stage of alfalfa silage fermentation. G. Copani*[1], K. Witt[1], I. Eisner[1], and A. Gallo[2], [1]*Chr. Hansen Animal Health & Nutrition, Hørsholm, Denmark,* [2]*Department of Animal Science, Food and Nutrition (DIANA), Università Cattolica del Sacro Cuore, Piacenza, Italy.*

Alfalfa crop is largely used worldwide in ruminant nutrition. Preserving this crop via ensiling and obtaining a high-quality stable silage, especially after a short period of fermentation, could be difficult due to the high buffering capacity and low content of water-soluble carbohydrates. Silage inoculants can be used for enhancing fermentation and aerobic stability of silage when exposed to air during feed out. The objective of this study was to evaluate the effects of a silage inoculant on fermentation parameters and aerobic stability of alfalfa silage after very short periods of fermentation. Three mini-silos (20 l volume) for each treatment were filled with wilted alfalfa crop (181 ± 6.6 kg DM/m^3, 50.5% DM). Control forage (C) was treated with no inoculant and experimental treatment was inoculated with SiloSolve FC (FC) containing 50:50 of *Lactobacillus buchneri* (DSM22501) and *Lactococcus lactis* (DSM11037). The target application rate was 150,000 cfu/g of forage. The wilted crop was analyzed for composition before ensiling (DM 50.5% as fed; CP 18.2%DM; pH 6.15; NDF 47.9%DM; ADF 36.7%DM). The mini-silos were stored for 2, 4, 8, 16, and 32 d at 20°C. After fermentation, each mini-silo followed an aerobic stability (AS) test for 15 d for each point of fermentation. The AS test was performed by aerobically challenging the mini-silos and monitoring the temperature increase inside the forage mass. The data were analyzed by SAS 9.3 with a completely randomized design and a factorial arrangement of treatments (main effects inoculum, ensiling time and their main interaction). Means were post-hoc compared by Tukey's test. The use of SiloSolve FC significantly enhanced the acetate levels at all ensiling time points ($P < 0.05$). Aerobic stability was increased on average by 4 d in FC inoculated alfalfa silages compared with C ($P < 0.05$) at all ensiling periods ($P < 0.05$) and was especially clear after 4 and 8 d of fermentation, where AS was increased by 6 d ($P < 0.05$) in the inoculated mini-silos. These results demonstrate that the use of SiloSolve FC improves aerobic stability and fermentation of a legume crop that is difficult to ensile even after a very short period of fermentation.

Key Words: aerobic stability, silage inoculant, alfalfa

488 Dissociation of CoEDTA and CrEDTA digesta markers under reducing conditions. P. J. Van Soest[1] and M. B. Hall*[2], [1]*Department of Animal Science, Cornell University, Ithaca, NY,* [2]*U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.*

It is unknown whether the ionic liquid markers CoEDTA and CrEDTA dissociate under ruminal conditions. Conditions in the rumen could reduce Co(III)- to Co(II)EDTA which is a less stable marker. As colored compounds, absorbances (ABS) of Co- and CrEDTA can be evaluated spectrophotometrically to determine if the molecules are intact, dissociated, or reduced. Wavelengths (λ) of peak ABS are 535, 465, and 560 nm for Co(III)EDTA, Co(II)EDTA, and Cr(III)EDTA, respectively, at pH 6.9. The objective of this study was to evaluate whether Co(III)- and Cr(III)EDTA markers dissociate or become reduced in vitro in simulated ruminal reducing conditions. In a complete block design with 2 replicate analytical runs and samples in duplicate within run, approximately 26 mg/L of Cr from Cr(III)EDTA or Co from Co(III)EDTA were incubated in Goering and Van Soest medium prepared without tryptone and with or without resazurin. In a 26 mL reaction volume were 20 mL of medium, 3 mL of Co(III)EDTA, or Cr(III)EDTA solutions, or water (reagent blanks), and 0, 0.25, 0.50, 0.75, or 1.00 mL reducing solution (RedSol) with water added to bring to 3.0 mL. Reduction status was determined with samples with resazurin, and ABS measures made on those without resazurin. Samples were maintained anaerobically. After incubation for 0.5 h at 39°C, ABS were read at λ 535, 465, and 560 nm. Mean reagent blank values were subtracted from Co- and CrEDTA data. Data were analyzed by marker with the MIXED procedure of SAS to detect differences in ABS at peak λ. The model included RedSol with analytical run as a random variable; contrasts were used to detect linear through quartic effects of RedSol. Resazurin color was clear for samples with > 0.25 mL RedSol, slightly pink with 0.25 mL, and remained blue with 0 mL. As RedSol increased, at 535 nm CoEDTA showed a cubic decline in ABS of 75% ($P = 0.03$), and a quadratic increase then decline ($P < 0.01$) at 465 nm. ABS of CrEDTA at 560 nm was unaffected by RedSol (linear $P = 0.14$). Co(III)EDTA apparently became reduced and dissociated to some extent under the in vitro conditions. This suggests that Co(III)EDTA is not a stable marker for use in studies under reducing ruminal conditions.

Key Words: kinetics, liquid, marker

489 Unveiling the relationships between diet composition and fermentation parameters response in dual-flow continuous culture system: A meta-analytical approach. V. Brandao* and A. Faciola, *University of Florida, Gainesville, FL.*

Our objective was to investigate the relationship between diet composition and amount of substrate with microbial fermentation end products in a dual-flow continuous culture system. A meta-analysis was performed using data from 75 peer-reviewed studies. To derive the linear models, the MIXED procedure was used, and for nonlinear models the NLMIXED procedure was used. Significance levels to fit the model assumed for fixed and random effects were $P \leq 0.05$. Independent variables were: dietary neutral detergent fiber (NDF) and crude protein (CP), and fermenter dry matter intake (FDMI), whereas dependent variables were: total volatile fatty acids (VFA) concentration, molar proportions of acetate, propionate, and butyrate, true ruminal digestibility (%) of organic matter (OM), CP, and NDF, ammonia nitrogen (NH_3-N) concentration and flows of non-ammonia nitrogen (NAN), bacterial-N, dietary-N, and efficiency of microbial protein synthesis (EMPS). Ruminal digestibilities of OM, NDF, and CP decreased as FDMI increased ($P < 0.04$). Total VFA linearly increased as FDMI increased ($P < 0.01$); exponentially decreased as dietary NDF increased ($P < 0.01$); and was quadratically associated with dietary CP ($P < 0.01$), in which total VFA concentration was maximized at 18% dietary CP. Molar proportion of acetate exponentially increased ($P < 0.01$) as dietary NDF increased. Molar proportion of propionate exponentially decreased as dietary NDF increased ($P < 0.01$). Bacterial-N quadratically increased and dietary-N exponentially increased as FDMI increased ($P < 0.01$). Flows of bacterial-N and dietary-N linearly decreased as dietary NDF increased ($P < 0.02$), and dietary-N flow was maximized at 18% CP. The EMPS linearly increased as dietary CP increased ($P < 0.02$). Overall, the analysis of this data set demonstrates evidences that the dual-flow continuous culture system provides valuable estimates of ruminal digestibility, VFA concentration, and nitrogen metabolism.

Key Words: ruminal digestibility, in vitro, microbial fermentation

490 Lipopolysaccharide stimulates growth of bacteria that contribute to rumen acidosis.

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Lipopolysaccharide (LPS) may participate in ruminal acidosis by affecting ruminal bacteria.This study determined how LPS affects the growth of pure cultures of rumen bacteria, including those that contribute to acidosis. Concentration of LPS in this study was 200,000 EU. Optical density (OD, 600 nm) measurements were collected until bacterial growth reached plateau. Once bacterial growth reached plateau, culture media was collected to measure pH and determine fermentation end products. Logistic function was used to predict the growth rate and lag time based on OD_{600}. Cellulolytic bacteria (*Ruminococcus albus* 7, *Ruminococcus flavefaciens* FD-1, *Fibrobacter succinogenes* S85), starch-utilizing bacteria (*Streptococcus bovis* JB1, *Succinivibrio dextrinosolvens* 24, *Lactobacillus ruminis* RF1, *Selenomonas ruminantium* HD4), and lactate-utilizing bacteria (*Megasphaera elsdenii* T81) were first tested. Growth rate and lag time of cellulolytic and lactate-utilizing bacteria were not affected by LPS dosing ($P > 0.05$). However, LPS dosing increased growth rate of starch-utilizing bacteria by at least 5% ($P < 0.05$). Except for *L. ruminis* RF1, lag time of other tested starch-utilizing bacteria was decreased by LPS dosing ($P < 0.05$). Then a defined media with LPS as the only carbon source was used to culture *S. bovis* JB1 and it was observed that *S. bovis* JB1 could not grow on it. Then glucose was sequentially added to these defined media and the growth rate of *S. bovis* JB1 was increased by 15% ($P < 0.01$) and lag time was decreased by 12% ($P < 0.01$) on LPS media compared with the control. Finally, detoxified LPS (200,000 EU, lipid A delipidated) was dosed to test toxicity of LPS to *S. bovis* JB1 and *M. elsdenii* T81. *M. elsdenii* T81 was not affected by detoxified LPS. Growth rate of *S. bovis* JB1 was increased by 8% in detoxified LPS ($P = 0.02$) and 16% in normal LPS ($P < 0.01$); lag time was decreased by 3% in detoxified LPS ($P = 0.28$) and 14% in normal LPS ($P < 0.01$). In sum, LPS stimulated growth of rumen bacteria that use starch and produce lactate. Our results suggest that LPS is not only produced during acidosis, but it may also contribute to acidosis' development.

Key Words: detoxified LPS, lag time

491 Comparison of noninvasive ruminal sampling techniques to standard cannula sampling method for ruminal microbial analysis.

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Rumen microbes play an important role in the conversion of indigestible plant material to energy and protein in dairy cows. Sampling for ruminal contents via cannula is considered the gold standard technique for microbial analysis, but this technique requires ruminal cannulated animals, and specialized animal facilities. The purpose of this study was to determine if other sampling methods and locations along the digestive tract may serve as a non-invasive proxy to the cannula method. Six ruminal-cannulated lactating Holstein cows were fed a standard total mixed ration for 2 wk to allow for adaptation followed by samplings during the third week. Sampling locations and method included salivary content, bolus, rumen content via stomach tube, sampling through the rumen cannula, and sampling of feces. Samples were collected at 0 (pre-feeding), 2, 4, 6, 8 and 12 h post-feeding over 2 d. Samples were extracted for genomic DNA, PCR-amplified for the V1V2 region of the 16S rDNA bacterial gene for bacterial diversity analysis using QIIME pipeline followed by statistical analysis in R. At the community level, saliva, bolus and fecal samples clustered in separate groups ($P \leq 0.05$) whereas stomach tube samples and cannulated samples clustered together ($P \geq 0.05$), indicating that stomach tube and cannula samples were homogeneous. Both saliva and feces had certain bacteria that were not previously reported in the rumen, suggesting these sample types have distinct microbial fingerprints. Notably, bolus samples at 6, 8 and 12 h had similar compositions with the corresponding cannula samples. There were several bacterial lineages in bolus samples also detected in saliva samples, but not in other sample types, indicating possible contamination of the bolus sample with saliva. It can be concluded the stomach tube method may serve as a proxy to cannula method for microbial investigations. Bolus samples may also serve as a proxy; however, sampling time and removal of bacterial contaminants from saliva will be needed for microbial analysis.

Key Words: rumen bacteria, dairy cow, ruminal sampling

492 Effects of simple monosaccharides on in vitro NDF digestibility, pH, and volatile fatty acids.

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Sugars are known to improve neutral detergent fiber (NDF) digestibility in lactating dairy cows. However, not much information is known about specific monosaccharides and inclusion levels. A study was conducted to investigate the effects of glucose, fructose, xylose and rhamnose on in vitro NDF digestibility (NDFd), pH and VFA concentration. The sugars were selected from a preliminary trial. Either oat hay (OH), alfalfa hay (AH) or a alfalfa hay-based TMR were separately fermented in vitro with each sugar for 12 and 24 h using different inclusion levels: 0% (control), 10%, 15% or 20% of the NDF content of the feed samples used. Combinations of the samples and the sugars were run in triplicates, across 3 runs. Data were analyzed according to a randomized complete block design with a factorial arrangement of treatments. Each sugar was analyzed separately, with level, feed sample and their interaction as fixed effects and run as random factor. The addition of fructose and rhamnose increased NDFd at the 15% and 20% inclusion levels ($P < 0.05$), respectively, while glucose and xylose were significant only at 10% of the NDF ($P = 0.035$ and $P = 0.022$, respectively). Overall, the sugars were more effective in increasing NDFd when added to the TMR ($P < 0.05$), followed by LH and OH. Independently of the sugar and sample used, the in vitro system was able to maintain the pH above 6.8 ($P > 0.05$) across sugars and inclusion levels. The main VFA quantified increased when sugars were added ($P < 0.05$), especially with the highest inclusion level ($P < 0.05$). Interestingly, while acetic acid was variable, both propionate and butyrate were highest with 20% inclusion level for all sugars ($P < 0.05$). This preliminary work demonstrates the need to explore the effects of specific monosaccharides addition on diets of dairy cows as a way of improving the rumen microenvironment.

Key Words: glucose, fructose, rhamnose

493 Assessing different branched-chain VFA combinations on NDF degradation and VFA production in vitro. Y. Roman-Garcia*[1], B. L. Denton[1], C. Lee[1,2], M. Socha[3], and J. L. Firkins[1], [1]*Department of Animal Science, The Ohio State University, Columbus, OH,* [2]*Ohio Agricultural Research and Development Center, Wooster, OH,* [3]*Zinpro Corporation, Eden Prairie, MN.*

To test if branched-chain VFA (BCVFA) supplements can be reduced to as few as one BCVFA, combinations of BCVFA + valerate were replicated with different substrates in batch cultures of mixed rumen microbes. In Experiment 1, treatments consisted of 1) control, 2) isovalerate (Ival) + isobutyrate (Ibut) + 2-methylbutyrate (2MB) + valerate (Val; 1 mM each), 3) Ival + Ibut + 2MB (1.33 mM each), 4) Ival + Ibut (2 mM each), 5) Ival + 2MB (2 mM each), 6) Ibut + 2MB (2 mM each), 7) Ival (4 mM), 8) Ibut (4 mM), or 9) 2MB (4 mM). In 4 runs with 3 replicates, 30 mL of a 1:4 dilution of rumen fluid (blended to enrich particle-associated bacteria) in artificial saliva were anaerobically added to 50-mL tubes with either: 1) 0.5 g of alfalfa hay, 2) 0.25 g of alfalfa hay + 0.25 g of ground corn, 3) 0.5 g of grass hay, or 4) 0.25 g of grass hay + 0.25 g of ground corn in a 2 × 2 factorial arrangement of treatments. Model had the fixed effect of BCVFA dose, substrate, their interaction and the random effect of run. There was no feed by treatment interaction for NDF degradation ($P = 0.31$). All treatments increased ($P < 0.01$) NDF degradation compared with control, with no difference between any of the acid treatments ($P > 0.10$). Total VFA net production was not affected by treatment. In Experiment 2, treatments were the same, but 1mM of each acid was added (total BCVFA concentration varied among treatments) to study if compensation of dose affected results of Experiment 1. Grass + corn or alfalfa + corn were the sole substrate sources. There was a feed by treatment interaction for NDF degradation. When alfalfa + corn was the substrate, adding BCVFA did not improve NDF degradation ($P = 0.14$). When grass + corn was the substrate, adding 2MB alone, the combination of Ibut + Ival + 2MB + Val, Ibut + 2MB, or Ival + Ibut increased ($P < 0.05$) NDF degradation. Adding valerate was not beneficial. Number of BCVFA supplemented could be reduced to any combination if 4 mM of total acid was added, and the amount supplemented could be reduced when grass was the substrate; however, BCVFA were not as effective when alfalfa was the substrate, likely because of its higher protein content.

Key Words: branched-chain VFA (BCVFA), NDF

494 Increased high-moisture alfalfa hay preservation, in vitro ruminal degradability, fermentability, and decreased proteolysis with the novel use of paper mill byproducts. D. C. Reyes*[1], S. L. Annis[2], A. Y. Leon-Tinoco[1], S. A. Rivera[1], H. M. Dubuc[1], L. B. Perkins[3], J. J. Perry[3], R. J. Kersbergen[4], C. Wu[5], C. W. Knight[4], M. S. Castillo[6], and J. J. Romero[1], [1]*Animal and Veterinary Sciences, School of Food and Agriculture, University of Maine, Orono, ME,* [2]*School of Biology and Ecology, University of Maine, Orono, ME,* [3]*Food Science and Human Nutrition, School of Food and Agriculture, University of Maine, Orono, ME,* [4]*University of Maine Cooperative Extension, Orono, ME,* [5]*Department of Animal and Food Sciences, University of Delaware, Newark, DE,* [6]*Department of Crop and Soil Science, North Carolina State University, Raleigh, NC.*

We evaluated the effect of 2 previously optimized technical lignins, sodium and magnesium lignosulfonate (NaL and MgL, respectively), on the preservation and nutritive value of high-moisture alfalfa hay. Lignins and propionic acid (PRP; positive control) were added to 36 fresh g of sterile alfalfa hay (69.3 ± 0.6% DM), inoculated with a mixture of previously isolated spoilage fungi (5.6 log cfu/fresh g), and aerobically incubated for 15 d (25°C). Treatments were the factorial combination of 3 preservatives (NaL, MgL, and PRP) and 4 concentrations (0, 0.5, 1, and 3% wt/wt fresh basis). Data were analyzed as a randomized complete block design replicated 5 times and differences were declared at $P \leq 0.05$. At d 0, the untreated hay had 47.8 ± 1.2, 11.1 ± 0.59, 66.2 ± 1.5, 30 ± 1.2 of NDF, sugars, and 24 h in vitro ruminal DM (DMD, % of DM) and NDF digestibility (NDFD, % of NDF), respectively. At d 15, relative to untreated (14.9 ± 0.77%), DM losses were lessened by doses as low as 1% for NaL (3.39) and 0.5% for PRP (0.81). This was explained by a reduced mold count for both NaL at 3% (3.92 ± 0.55 log cfu/fresh g) and PRP as low as 0.5% (3.94) vs. untreated (7.76). Consequently, sugars were best preserved by NaL at 3% (10.1 ± 0.283% DM) and PRP as low as 0.5% (10.5) vs. untreated (7.99), while keeping NDF values lower in NaL (45.9 ± 0.66% DM) and PRP-treated (45.1) hays at the same doses, respectively, vs. untreated (49.7 ± 0.66% DM). Hay DMD was increased by doses as low as 3% for NaL (67.5 ± 0.77%), 1% MgL (67.0), and 0.5% PRP (68.5) vs. untreated (61.8). In the case of NDFD, 0.5% for MgL and PRP (30.5 and 30.1 ± 1.09% DM, respectively) and 1% for NaL (30.7) were the lowest effective doses vs. untreated (23.3). At 3%, NaL decreased ruminal NH_3-N (49.6 vs. 58.2 ± 1.50 mg/dL) and increased total volatile fatty acids (111.9 vs. 86.7 ± 1.3 mM) to the greatest extent vs. untreated, respectively. NaL was the best lignin-based preservative, but its effects were lesser than PRP. MgL stimulated digestibility independently of nutrient preservation.

Key Words: hay preservation, technical lignins, ruminal digestibility

495 Effects of branched-chain VFA and branched-chain AA supplementation on NDF degradation and VFA production in vitro. Y. Roman-Garcia*[1], B. L. Denton[1], C. Lee[1,2], M. Socha[3], and J. L. Firkins[1], [1]*The Ohio State University Department of Animal Science, Columbus, OH,* [2]*Ohio Agricultural Research and Development Center, Wooster, OH,* [3]*Zinpro Corporation, Eden Prairie, MN.*

We studied the effects of branched-chain VFA or branched-chain AA on NDF degradation and VFA production in batch cultures of mixed rumen microbes. In Experiment 1, treatments were 1) Control; 2) isovalerate, isobutyrate, 2-methylbutyrate, and valerate (BCVFA; 1mM final concentration each); 3) Ile, Leu, and Val (BCAA; 1mM final concentration each); or 4) a 50:50 mix of BCVFA and BCAA (MIX). In 3 runs with 4 replicate tubes, 30 mL of a 1:4 dilution of blended rumen fluid in artificial saliva buffer was incubated anaerobically in 50-mL tubes in a 4 × 4 arrangement with either 1) 0.5 g of alfalfa hay (AH), 2) 0.25 g of corn + 0.25 g of AH, 3) 0.5 g of orchardgrass hay (OH), or 4) 0.25 g of corn + 0.25 g of OH. Linear (L) and quadratic (Q) contrasts were evaluated for 1) BCAA, 2) MIX, and 3) BCVFA. There was no treatment x feed interaction for NDF degradation, pH, or net VFA production after 24 h incubation. BCVFA tended to increase ($P = 0.13$) NDF degradation compared with control (35.4% vs 33.4%), but BCVFA substituting for BCAA tended to increase NDF degradation (L was $P = 0.15$, Q was $P = 0.78$). BCVFA substituting for BCAA decreased branched chain VFA net production (L was $P < 0.01$, Q was $P = 0.57$). There was a linear decrease (L was $P = 0.02$, Q was $P = 0.71$) in NH_3-N at 24 h with BCVFA substituting for BCAA. In Experiment 2, 2 runs with 3 replicate tubes with the same treatment arrangement as Experiment 1 were made, but doses were made isonitrogenous with $(NH_4)_2SO_4$. Substrates were either 0.25 g of AH + 0.25 g of corn or 0.25 g of OH + 0.25 g of corn. There was no treatment effect or treatment x feed interaction for NDF degradation, pH, or total net VFA production. All branched chain FA (individual and total) had a linear decrease (L was $P < 0.01$, Q was $P > 0.37$ for all) in net production with BCVFA substituting for BCAA, suggesting more cell synthesis from degraded OM. Treatment did not affect NH_3-N concentration at 24 h. When BCVFA were added, net production of BCVFA went down, whereas providing BCAA increased BCVFA production, potentially suggesting BCVFA were incorporated more rapidly into cell constituents such as membrane FA than BCAA.

Key Words: branched-chain VFA (BCVFA), branched-chain AA (BCAA)

496 Factors associated with the digestibility of alfalfa and corn silage on dairy farms.

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A survey was conducted to assess factors associated with the dry matter digestibility (DMD) of silages on Manitoba dairy farms. A total of 20 farms were visited during the summer of 2018 to collect samples of silages (1 to 5 per farm) and to record herd size, silage cut, and storage method. Information on the silage production and feeding methods, including use of fertilizer and manure, seed variety, stage at harvest, wilting time, days between cuts, fill method, amount of wet mass ensiled per day, inoculants and enzymes, cover material, feed out rate, and age of stands were collected. The DMD of silage samples were determined in duplicate using the ANKOM Daisy II incubator with a NDF ending. Regression models were developed using the MIXED procedure of SAS 9.4 to test the relationships between the DMD and herd size (55–120 cows = small, 7 farms; 130–312 cows = average, 6 farms; 312–1100 cows = large, 7 farms), type of forage (corn or alfalfa), silage cut (direct for corn, first or later for alfalfa and alfalfa/grass mixes) and storage method (bunker, tower, pile or bale). The R-square, CV, and root MSE of the final model were 0.22, 3.70, and 3.02, respectively. Summary statistics of the DMD are given in Table 1. The effect of herd size on DMD was significant ($P < 0.01$) and the effect of cut of alfalfa on DMD tended to be significant ($P = 0.09$). The effects of type of forage, storage method, and the interactions between type of forage, herd size, and storage method were not significant. Farms with average and large herd sizes had higher silage DMD compared with farms with small herd sizes (83.9 vs 81.4%, $P < 0.05$). First cut alfalfa silages tended to have higher DMD than later cuts (83.8 vs. 81.9%, $P = 0.10$). Results show that farmers produced alfalfa and corn silages with a high DMD, but that the variation in silage DMD among farms was substantial. On average, the small size farms with the relatively low silage DMD used less synthetic fertilizer, had younger stands of alfalfa, were less aware of the alfalfa seed variety, ensiled less wet mass per day and had lower feed out rates compared with the average and large farms.

Table 1 (Abstr. 496). Summary statistics of DMD (%) of forages

Forage	Average	SD	Maximum	Minimum	N
Alfalfa	82.2	3.28	74.4	87.8	28
Corn	81.1	3.06	72.9	84.9	17

Key Words: silage, digestability, management

497 Simulating precision feeding of high and low forage diets with increasing poultry fat inclusion alter fermentation in continuous culture.

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Including poultry fat (PF) at different dietary concentrations can increase caloric intake and reduce dry matter intake (DMI) under precision feeding conditions where diets are isocaloric and isonitrogenous. High fat intake may lead to reduction in fiber digestibility and inadequate fermentation. However, reduction of DMI as PF is incorporated into diets can provide similar energy to the animal limiting the negative effects associated with fat inclusion. We hypothesized that including PF at increasing dietary concentrations would reduce intake without compromising rumen fermentation and nutrient digestibility in continuous cultures. Treatments included 2 forage combinations, low (LF; 35% forage), and high (HF; 70% forage) and 4 diet with PF dietary concentration starting with a basal level of fat in the diet [3% fat (0% PF inclusion); 5% fat (2% PF inclusion); 7% fat (4% PF inclusion); and 9% fat (6% PF inclusion)]. Fermenters were randomly assigned to treatments in a 2 × 4 factorial design and ran for 4, 10 d periods. Data were analyzed using the MIXED procedure of SAS with repeated measures. The LF-group had higher DM, OM, and NDF digestibility coefficients (dC; $P < 0.01$). The DM, OM, NDF, and ADF dC increased linearly with PF inclusion. Mean culture pH and NH_3-N were lower in the LF vs HF. The inclusion of PF resulted in a linear increase on NH_3-N and a quadratic increase on pH. The total VFA concentration was higher in the LF. There was a reduction in acetate and butyrate and an increase in propionate concentrations with LF. The PF inclusion had a quadratic increase on total VFA ($P = 0.02$). There was a linear interaction effect on acetate, propionate, and butyrate. Acetate and butyrate increased in HF as PF increased and decreased in LF. Propionate decreased in HF as PF increased and increased in LF. The HF yielded higher A:P. There was a linear interaction effect on A:P ($P = 0.05$). The A:P increased in HF as PF increased and decreased in LF. These results suggest that increasing PF in precision fed LF or HF can alter rumen fermentation and improve digestibility.

Key Words: precision feeding, poultry fat, continuous culture

498 Relation of branched-chain VFA supplementation with solids passage rate and pH on NDF degradation and microbial function in continuous culture.

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We studied the effects of branched chain VFA supplementation, pH, and solids passage rate (K_p) on microbial activity in continuous culture using 8 fermenters in 4 periods. Treatments were: 1) control or BCVFA supplementation (2 mmol each/d), 2) high pH (ranging from 6.3 to 6.8) or low pH (5.7 to 6.2), and 3) either low (2.5%/h) or high (5.0%/h) solids $K_p$were assigned in a 2 × 2 x 2 factorial arrangement of treatments. Liquid dilution was maintained at 10.0%/h. Fermenters were fed 50 g of a 50:50 concentrate:forage diet twice daily. Apparent OM degradation was not affected by any treatment. BCVFA treatment tended ($P = 0.08$, main effect) to increase NDF degradation, which also tended ($P = 0.11$) to be increased with higher pH but was not affected by K_p. Interaction among treatments for NDF degradation was $P > 0.15$. Apparent N degradation increased ($P = 0.05$) with high pH and decreased ($P < 0.01$) with high K_p. On low pH, BCVFA did not affect redox, but BCVFA increased ($P = 0.02$) redox when pH was high (interaction $P = 0.09$). Flows of bacterial N, nonammonia-nonbacterial N, and the efficiencies of bacterial N flow per kg of OM and NDF degraded were not affected by treatment ($P > 0.15$). BCVFA increased ($P < 0.01$) CH_4 emission rate

from 1.5 to 10.5 h post feeding. Total VFA concentration in the fermenters tended ($P = 0.06$) to increase with high pH and decreased ($P < 0.01$) with High K_p. Total daily net production of VFA (subtracting doses) was not affected by treatment or treatment × feed. The net production of BCVFA was numerically lower when BCVFA was dosed, most likely due to use of the feed BC components directly. High pH increased ($P < 0.05$) isobutyrate and isovalerate production but decreased ($P < 0.05$) 2-methylbutyrate and valerate net production suggesting increased use by microbes. The *anteiso* FA precursor, 2-methylbutyrate, seems to be important for increasing membrane fluidity with increasing pH, and this BCVFA (unfortunately typically not separated from isovalerate in evaluation of nutrition experiments) appears to support a more critical role among the BCVFA to improve NDF digestibility with higher K_p and higher pH.

Key Words: branched-chain VFA (BCVFA), pH

Ruminant Nutrition Symposium: From Field to Feed Bunk—Application of Remote Sensing and New Age Technology to Improve Forage Production and Nutrient Utilization

499 Farming data—The new agriculture. R. Johnson*, *Cainthus, Ottawa, ON, Canada.*

The objective of any model is to test a hypothesis and determine if it can describe reality. The traditional research approach has been one of developing a hypothesis and designing of an experiment to prove or disprove the idea given a known set of parameters. The artificial intelligence (Ai) approach, on the other hand, is one of rapid iteration that augments analytical capabilities. Given that this computational approach lends itself to the use of vast amounts of unbiased data, its adoption is transforming the research landscape and offering insights that were often overlooked in the past. This presentation will provide an overview of Ai techniques, their purpose, uses and data collection with the goal of offering clarity on present and future possibilities. With the development of Ai, machine learning and deep learning technologies, we are coming ever closer to describing and simulating reality. The direction and limitations of Ai as they apply to the future of animal agriculture will be discussed.

500 Field remote sensing and its relationship to forage and crop yield and quality. J. K. Ward*, *North Carolina State University Biological and Agricultural Engineering, Raleigh, NC.*

Non-contact sensing is becoming more common in forage applications. Portable near-infrared (NIR) spectroscopy has been used to estimate forage quality factors and to make forage management decisions. Current solutions focus on harvested material often removed from its field context or on discrete samples. The purpose of this analysis is to leverage off-the-shelf remote sensing platforms on unmanned aerial vehicles (UAVs) to detect field scale variation in forage production and quality. Remotely sensed values were compared with traditional lab analyses, manual production estimates, and animal performance. Final analyses are ongoing. Long-term goals of this project are to create field scale in situ forage crop quality estimates to predict bale-scale forage quality

Key Words: non-contact sensing, forage applications, near-infrared spectroscopy

501 Forage harvest logistics and optimization. B. Luck*, *University of Wisconsin-Madison, Madison, WI.*

Harvesting forage crops for silage utilizes multiple pieces of equipment to ensure rapid and economical production of feed. Machinery involved in the forage harvest process were instrumented to identify working states during 2 harvest seasons including 4 alfalfa (*Medicago sativa* L.) and one corn (*Zea mays* L.) silage harvest events per year. Self-propelled forage harvester utilization was found to be 61%. During working hours, idle time was found to be 18% to 23% for self-propelled forage harvesters and 10% to 20% for crop transportation machines. Crop transportation efficiency was defined and found to be dependent on transport capacity. Semi-tractor trailer transport vehicles were found to be the most efficient transporting 125 Mg km h^{-1}. A model of corn harvest for silage production, capable of predicting machine working status and total harvest time for a field, using a single harvester, and any number of user defined transport vehicles was developed. Three forage harvesting systems were observed using Global Positioning System (GPS) equipment and the collected data used for the TruckSim model validation. The harvest model predicted harvest times within 10% of observed data and yielded similar results to a previously assessed harvest system. Model scenarios were used to explore the effect of differently sized transport vehicles on harvest time and it was found that placing transport vehicles with longer cycle times at the end of the rotation has the potential to reduce harvest time. The TruckSim model can be used to determine the optimal number of transport vehicles and their dispatch order to minimize total harvest time. Ongoing research aims to improve machine state definition accuracy and develop a machinery movement optimization model to maximize forage harvest efficiency and feed quality.

Key Words: silage, machinery, optimization

502 Determination of the relationships between rumination time, milk fat production, and milk fatty acid profile using real-time rumination data. D. M. Andreen*[1], M. M. Haan[2], C. D. Dechow[1], and K. J. Harvatine[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*Penn State Extension, Berks County, PA.*

Milk fat production is highly influenced by nutrition and rumen fermentation. Rumination is an essential part of the ruminant digestive process and can serve as an indicator of rumen fermentation. The objective of this research was to quantify variation in rumination time between and within dairy herds and to determine relationships between rumination time and milk fat production and fatty acid (FA) profile as a proxy of rumen fermentation. Our hypothesis was that rumination may indicate disruptions to rumen fermentation and that lower-rumination cows would have lower milk fat due to these rumen disruptions. Data were collected from 1733 Holstein cows on 5 commercial dairy farms (4 in PA and 1 in NY) of 200 to 700 head using one of 2 commercially-available rumination sensing systems (RSS). Rumination data were collected for 7 consecutive days leading up to a DHIA test, summed within day, then averaged to obtain average daily minutes of rumination time. Milk samples from the DHIA test were analyzed for fat content by mid infrared spectrum and for milk FA profile by gas chromatography. Rumination data were analyzed using multiple linear regression models in JMP Pro v13.0 and R v3.4.4 (SAS Institute, Cary NC; R Core Team, Vienna, Austria). Rumination time was related to concentration of specific odd and branched-chain and *trans*-FA in milk but was not directly related to milk fat concentration. Rumination also did not contribute to models predicting milk fat concentration. The relationship between *trans*-10 C18:1 and rumination was significant ($P < 0.001$) and positive after accounting for the effect of farm, with a partial R^2 of 2.97%, 4.24%, and 2.22% across all data, RSS 1, and RSS 2 data respectively. While rumination was not related directly to milk fat, it was associated with differences in *trans* and odd and branched-chain FA that also change during subacute ruminal acidosis or milk fat depression, which may impact milk fat and other production variables. Further investigation using rumination data from commercial systems to predict or identify the presence of these conditions is warranted.

Key Words: rumination, fatty acid

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503 Acoustic pulse therapy at dry-off period. G. Leitner*, E. Papirov, and S. Sheft, *Armenta, Kefar-Sava, Israel.*

The dry period, an important resting period between lactations, is used in many dairies for long-term antibiotic treatment of intra-mammary infections and protection against new infections. However, there are concerns regarding the use of antibiotics due to the risk of developing antimicrobial resistance bacteria. Acoustic pulse therapy (APT) device developed to produce low power, deep penetration acoustic pulses distributed over a large treatment area was specifically designed for treating dairy cows. Previous experience with APT on cows with clinical and subclinical mastitis has shown bacterial elimination, increased milk yield and lower SCC. The aim of this study was to evaluate the effect of APT on cows during the dry period as an alternative to current dry cow therapies. Fifty cows, 21 APT and 29 control at 5 repetitions were treated during dry-off, 4–5 weeks before parturition (400 pulses/quarter for 3.5 min, frequency ~1.9Hz, energy density 0.041 mJ/mm^2). All cows were intramammary treated by antibiotics. Cows were paired according to lactation, daily milk yield, DIM, SCC, history of infection, bacteriology and number of infected quarters. In each pair, one cow was assigned to APT and the second to control. Due to management, 1 repetition was not APT treated and all these cows were signed to control. When comparing the first 100 d following the dry-period to the first 100 d in the previous lactation period, an overall increase of 10% in milk yield was observed in the APT treated group compared with 1% in the control group (χ^2). The differences were more notable in the 3rd lactation (11.6%), and the 4th lactation (13.7%). Average SCC in the first 100d was significantly lower in APT compared with control (101,000 cells/mL vs. 427,000 cells/mL). Previously we reported that APT was found more effective than antibiotics in treating clinical mastitis or no-intervention in sub-clinical mastitis in dairy cows. The current report suggests that APT treatment during the dry period can result in increasing angiogenesis which stimulates growth of new blood vessels that supply and oxygenate the treated area, support faster healing of the infected tissue and stimulates secretory cells and lobule.

504 Automated detection of lameness using activity monitoring. S. Paudyal*[1,2] and G. Solano[1], P. Melendez[3], I. Roman-Muniz[1], R. Callan[4], F. Maunsell[5], J. Velez[6], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Texas A&M University, College Station, TX,* [3]*Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA,* [4]*Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, CO,* [5]*Department of Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL,* [6]*Aurora Organic Dairy, Platteville, CO.*

Monitoring of locomotion disorders is traditionally performed by visual observation, using locomotion scoring (LS), which is subjective, time-consuming, and prone to individual bias. Recently developed activity systems could potentially replace visual assessment. Our objective was to characterize the dynamics of activity parameters and milk yield (MY) in animals submitted for LS at multiple points during lactation. Multiparous Holstein cows (n = 310) from a dairy in northern Colorado, USA were afixed with a pedometer (IceQube, IceRobotics, Edinburgh, UK) on one rear leg at 12 ± 8 DIM and monitored for 7 mo for lying time (LT; min/d), number of steps (ST; n/d), lying bouts (LB; n/d), and motion index (MOI; units/d). MY (kg/d) and an estimated probability of lameness (%) provided by the system were also available. Cows were maintained in a free stall barn and were allowed access to grazing during part of the study period. Cows were evaluated for lameness biweekly using a 1 to 5 numerical LS system (1 = sound, 5 = severely lame). Mixed linear models were used to calculate LSM for activity parameters and MY by LS, including parity, DIM, access to pasture, and presence of estrus in the models. As presented in Table 1, activity parameters varied depending on LS. LS5 was associated with greater values for LT, LB, and probability of lameness, while ST and MOI were smaller. The variables in study evidenced significant differences associated with high LS, indicating that activity parameters have the potential to be used for the detection of locomotion disorders.

Key Words: activity, locomotion score

Table 1 (Abstr. 504). Least squares means (± SE) for activity variables and MY across 5 locomotion scores (LS)

Parameter	LS1 (n = 3,876)	LS2 (n = 104)	LS3 (n = 44)	LS4 (n = 24)	LS5 (n = 1)
Lying time (min/d)	575 ± 2.0^{a}	570 ± 13^{a}	585 ± 20^{a}	675 ± 27^{b}	894 ± 121^{b}
Lying bouts (n/d)	18.3 ± 0.11^{a}	19.2 ± 0.64^{ab}	19.0 ± 0.96^{ab}	21.8 ± 1.34^{b}	27.2 ± 5.83^{c}
Steps (n/d)	2,760 ± 27^{c}	2,985 ± 161^{c}	2,977 ± 245^{c}	2,079 ± 342^{b}	700 ± 1,489^{a}
Motion index (units/d)	9,838 ± 106^{c}	10,902 ± 624^{c}	10,480 ± 947^{c}	7,157 ± 1321^{b}	2,194 ± 5,752^{a}
Probability of lameness (%)	15.2 ± 0.2^{a}	15.2 ± 0.9^{a}	15.2 ± 1.5^{a}	25.4 ± 2.1^{b}	60.5 ± 7.9^{c}
Milk yield (kg/d)	35.9 ± 0.18^{a}	34.8 ± 1.06^{a}	36.5 ± 1.57^{a}	34.9 ± 2.16^{a}	38.4 ± 9.60^{a}

$^{a–c}$Different superscripts within a row represent significant differences at $P < 0.05$.

505 Dynamics of activity around corrective and therapeutic hoof trimming. S. Paudyal*[1,2] and G. Solano[1], P. Melendez[3], I. Roman-Muniz[1], R. Callan[4], F. Maunsell[5], J. Velez[6], and P. Pinedo[1], [1]*Department of Animal Sciences, Colorado State University, Fort Collins, CO,* [2]*Texas A&M University, College Station, TX,* [3]*Department of Population Health, College of Veterinary Medicine, University of Georgia, Athens, GA,* [4]*Department of Clinical Sciences, College of Veterinary Medicine, Colorado State University, Fort Collins, CO,* [5]*Department of Clinical Sciences, College of Veterinary Medicine, University of Florida, Florida, Gainesville, FL,* [6]*Aurora Organic Dairy, Platteville, CO.*

Hoof trimming (HT) in dairy cattle is associated with stress and may result in temporary alterations in behavior and production. Our objective was to characterize the dynamics of activity and milk yield (MY) in animals submitted to HT during early lactation. HT (n = 265) was classified into corrective (CHT; n = 190) and therapeutic (THT; n = 75), when a foot disorder was concurrently diagnosed. A total of 310 multiparous Holstein cows from a dairy herd in northern Colorado were afixed with a pedometer (IceQube, IceRobotics, Edinburgh, UK) in one rear leg at 12 ± 8 DIM and monitored for 7 mo for lying time (LY; min/d), number of steps (ST; n/d), lying bouts (LB; n/d), and MY (kg/d). Cohort cows not submitted to HT were considered as healthy controls (HCT). Average daily values for each activity variable and MY were compared with controls within ± 7-d relative to HT. In addition, activity data and MY were categorized into low and high, relative to the median to evaluate their ability on predicting HT categorization. Associations between behavior, MY, and HT were tested by use of mixed linear models and logistic regression analyses. Parity, DIM, access to pasture, and presence of estrus were included in the models. Overall, 21, 21, 10, and 23 cows were diagnosed with foot injury, digital dermatitis, foot rot, and non-specific lameness, respectively. Daily LY was greater in THT, followed by CHT and HCT (626 ± 24; 573 ± 12; 563 ± 8.2 respectively; $P < 0.001$). LB was greater in THT, followed by HCT and CHT (19.6 ± 1.17; 18.7 ± 0.02, and 18.5 ± 0.49; $P < 0.01$). ST and MY were smaller in THT (2,016 ± 121; $P < 0.001$; 36.0 ± 1.3; $P = 0.2$) compared with CHT (2,610 ± 121 and 37.9 ± 0.8) and HCT (2,664 ± 7.9 and 36.2 ± 0.04), respectively. High LY and high LB categories were associated with greater odds (CI) of THT (OR [95%CI] = 2.60 [2.14–3.19] and 1.80 [1.50–2.19] respectively), whereas high ST category was associated with reduced odds of THT (0.2 [0.1–0.16]) and CHT (0.4 [0.24–0.77]). The magnitude of the changes in activity behavior in cows subject to HT was greater when locomotion disorders were present, as compared with CHT.

Key Words: hoof trimming, lying time, activity

506 Association between lying behavior and hoof lesions in lactating dairy cows. B. O. Omontese*[1], R. Bellet-Elias[1], A. M. Argüello[1], G. D. Catandi[1], R. Casagrande[1], Z. Rodriguez[1], R. S. Bisinotto[2], and G. Cramer[1], [1]*Department of Veterinary Population Medicine, University of Minnesota, St. Paul, MN,* [2]*Department of Large Animal Clinical Sciences, University of Florida, Gainesville, FL.*

Lying behavior is a vital indicator of cow welfare and may play significant roles in hoof lesion (HL) development. Objectives were to evaluate the association between lying behavior and HL in lactating dairy cows. Jersey cows (n = 435) without any visible HL were enrolled in early lactation (20 ± 3 DIM; d20), evaluated for body condition score (BCS) and fitted with pedometers (AfiTag II, Afikim, Israel). At mid-lactation (120 ± 3 DIM; d120), cows were reevaluated for the presence of HL and pedometers were removed. Data were analyzed using restricted maximum linear growth mixed measures model with an autoregressive covariance structure. Variables offered to the model included the fixed effects of HL status at d120, DIM, interaction between HL status at d120 and DIM, parity, change in BCS, and calving season. In each model, cow was included as random effect. Lesion status and parity was forced into the final model. Due to data backup failures, only data from 344 cows with a minimum of 6 d of data were used. This resulted in a total of 24,813 d of data were available for analysis. At d120, 56.5% of cows were diagnosed with a HL, of which 88.5% were sole hemorrhage. Fifteen (8.2%) cows were diagnosed with other non-infectious lesions, whereas 8 (4.4%) cows had infectious lesion at d120. Our results show that at enrollment, the cows that developed HL had reduced lying time duration −0.5 h (95% CI: −0.78 to −0.28) compared with cows that remained healthy. This difference in lying time for cows that developed HL gradually became smaller (Coef.: 0.003; 95% CI: 0.002 to 0.005) from early to mid-lactation. Irrespective of lesion status at d120, average lying duration increased progressively from early to mid-lactation. Our results suggest that cow lying behavior is changed before HL development and that the causative mechanism might already have occurred before enrollment in our study.

Key Words: behavior, lying time, lameness

Growth and Development 2

507 Maternal copper and zinc alter epigenetic markers in muscle of offspring. H. Tucker*, J. Chen, S. Bettis, S. Herbstreit, T. Freitas, and M. Vázquez-Añón, *Novus International, St. Charles, MO.*

Trace minerals, Cu and Zn, play a key role in fetal development, being required for immune system and muscle development. However, little is known about the effects of maternal Cu and Zn nutrition on muscle and immune system development of ruminant offspring. Therefore, the objective of this trial was to evaluate effects of trace mineral source on ewe performance during gestation and epigenetic changes to muscle and immune function of their offspring. Ewes were allocated by body weight to receive: no additional Cu or Zn (CON); Cu and Zn sulfates supplying 300% of NRC requirement (ITM); or Cu and Zn as chelates (MINTREX trace mineral, Novus International) supplying 300% of NRC requirement (MMHAC). Treatments were fed 7 d before conception and through parturition. Following parturition, one lamb from every ewe was weighed and harvested at d 0 of life followed by a second lamb at d 60. Jejunum, longissimus dorsi (LD), and thymus were weighed and sub-sampled from lambs for epigenetic and gene expression measures. Treatment and lamb sex were fixed effects in the model. Lambs harvested on d 0 tended to weigh greater ($P < 0.10$) when ewes were fed MMHAC compared with ITM. Weight of LD on d 0 tended ($P < 0.09$) to be greater for lambs from ewes fed MMHAC compared with ITM, with no other differences in organ weights observed on d 0 or d 60. Global histone acetylation tended ($P < 0.10$) to be greater in LD of lambs from ewes fed MMHAC compared with ITM or CON on d 0. Expression of FoxO3 on d 0 was significantly ($P < 0.05$) lower in LD of lambs from ewes fed MMHAC and CON compared with ITM. Expression of IκBKB tended ($P < 0.09$) to be greatest, while TNFα tended ($P < 0.09$) to be lowest, in jejunal tissue of lambs from ewes fed ITM or MMHAC compared with CON on d 0. Expression of TLR2 tended ($P < 0.09$) to be greatest in jejunal tissue of lambs from ewes fed CON compared with ITM or MMHAC on d 60. Villi width and muscular thickness tended ($P < 0.06$) to be lower in jejunum of lambs from ewes fed ITM or MMHAC compared with CON on d 60. These data suggest trace mineral supplementation throughout gestation affects progeny muscle development and immune function.

Key Words: trace mineral, epigenetic, muscle

508 Milk consumption differs by breed and lung consolidation score in automated feeding systems. T. S. Steckler*, M. A. Erasmus, and J. P. Boerman, *Purdue University Department of Animal Sciences, West Lafayette, IN.*

Dairy farmers are increasingly using automation in their operations and a larger number of farms are breeding at least a portion of their cows to beef semen to increase the price received for calves. However, little information is available about best management practices for beef × Holstein F1 crosses and Holsteins in automated feeding systems. The objective of this study was to compare milk consumption, health, and growth of Holstein calves (H) and Angus × Holstein F1 calves (F1) during the pre-weaning phase. Data from calves (n = 212) including daily milk consumption (birth-weaning), serum total protein, pneumonia incidence, and lung scores were collected on a commercial farm from Nov. 9, 2018–Jan. 27, 2019. Calves were fed pasteurized waste milk with 20 g/L of a 30% protein, 5% fat milk enhancer through a Förster-Technik automated calf feeding system (pens = 4) for 63.4 d (range 55–72 d). Calves were weighed at birth and weaning, and the average birth weight was 41.2 ± 5.6 kg (mean ± SD). Thoracic ultrasounds were performed on each calf at 57 ± 4.8 d (mean ± SD) using a 1–3 scale for lung consolidation score (LCS, 1 = no lung consolidation, 2 = consolidation in one lung, and 3 = consolidation in both lungs). Effects of birth weight, health incidences, serum total protein, breed, sex, pen, and LCS on milk consumption and ADG were evaluated using a multiple regression model. Birth weight, LCS (both $P < 0.0001$) and LCS by breed ($P = 0.02$) all affected milk consumption at 60 d ($R^2 = 0.33$). The interaction between LCS and breed resulted in F1 with LCS = 1 consuming more milk than H with LCS = 1 (452 vs 432 L), however, F1 with LCS = 3 consumed less milk than H with LCS = 3 (353 vs 390 L). Factors that impacted ADG included days on feeder and birth weight (both $P < 0.01$) and milk consumption up to 60 d ($P < 0.0001$; entire model ($R^2 = 0.41$)). LCS proved to be a better predictor of milk consumption and ADG than incidence rates of pneumonia. Overall, F1 and H consumed different amounts of milk in an automated feeding system dependent on lung consolidation.

Key Words: milk consumption, average daily gain, lung consolidation

MILK Symposium: Improving Milk Production, Quality, and Safety in Developing Countries

509 The importance of milk in the diets of infants, pregnant women, adolescents and adults. D. I. Givens*, *University of Reading, Reading, UK.*

The different life stages give rise to important nutritional challenges some of which are related to increasingly aged populations in many Western societies. There is however, increasing evidence that diets during childhood and adolescence can affect health in later adulthood. For example, undernutrition in childhood can lead to stunted growth, which is associated with reduced cognitive ability and increased risk of chronic diseases and co-morbidities in adulthood. Despite recent worldwide improvements, stunting in sub-Saharan Africa remains about 40% and some countries have an even higher prevalence. Milk is a key food for reducing stunting with milk proteins having a crucial role. In some Western societies recent reductions in milk consumption have led to sub-optimal intakes of calcium and magnesium by teenage females in particular, at a time when bone growth is at its maximum and of iodine during pregnancy needed to ensure that supply/production of thyroid hormones to the fetus is adequate. It is of note that the concentration of some key nutrients, particularly iodine is influenced by the the iodine intake of the dairy cow. Low intakes of calcium is a particular concern since many populations are also of sub-optimal vitamin D status. This may already have had serious consequences in terms of bone development, which may not be apparent until later life. Recent meta-analyses show no evidence of increased risk of cardiovascular diseases from high consumption of milk and dairy foods but increasing evidence of a reduction in the risk of type 2 diabetes associated with fermented dairy foods, yogurt in particular. The recently updated reports from the World Cancer Research Fund International / American Institute for Cancer Research on the associations between dairy foods and various cancers provide further confidence that total dairy products and milk, are associated with a reduced risk of colorectal cancer and high intakes of milk/dairy are not associated with increased risk of breast cancer. This session aims to examine the role of dairy foods at key life stages in terms of their ability to moderate chronic disease risk.

Key Words: milk, dairy, health

510 Foodborne diseases from dairy products in developing countries: hazards and health implications. A. H. Havelaar*[1], D. Grace-Randolph[2], and F. Wu[3], [1]*University of Florida, Gainesville, FL,* [2]*International Livestock Research Institute, Nairobi, Kenya,* [3]*Michigan State University, East Lansing, MI.*

Dairy products are an important source of animal proteins in developing countries, and increased consumption of these products by pregnant women and young children is advocated to reduce malnutrition and child stunting. However, the nutritional benefits of dairy products can be compromised by the presence of contaminants causing foodborne disease. These food safety risks are increased by frequent consumption of raw or inadequately heated dairy products. The World Health Organization published estimates of the global burden of foodborne disease in 2015, and attribution of this disease burden to specific food groups in 2017. It is estimated that each year, 600 million people fall ill because of foodborne disease, resulting in 435,000 deaths and a disease burden of 33 million disability adjusted life years (DALYs; equivalent to one healthy life year lost). Of this burden, 38% is attributed to animal-source foods (ASF), with 12% of the burden of ASF attributed to dairy products. The average global burden of dairy products is 20 DALYs per 100,000 population. The major contaminants in dairy are *Mycobacterium bovis* (highest burden in Africa), *Campylobacter* spp. (highest burden in Eastern Mediterranean), nontyphoidal *Salmonella enterica* (highest burden in Africa) and *Brucella* spp. (highest burden in Eastern Mediterranean). The burdens of *Cryptosporidium* spp., Shiga-toxin producing *Escherichia coli* and *Toxoplasma gondii* are low. Proper heating of dairy products would be effective in reducing these burdens substantially. The burden of chemical contaminants is less well documented. Adulteration is a potential problem, as illustrated by the melamine contamination incident in Chinese infant formula. Aflatoxin M_1 (AFM_1) is sometimes detected in milk in concentrations higher than maximum tolerable limits in the USA and Europe. AFM_1, which cannot be destroyed by heating milk, is a metabolite of aflatoxin B_1 (AFB_1), a mycotoxin (fungal toxin) frequently found in corn, nuts, and the feed of dairy animals. However, the carcinogenic potential of AFM_1 is significantly lower than that of AFB_1 and the risk of liver cancer from current exposure levels to AFM_1 is likely to be extremely low. There is limited evidence of an association between AFM_1 and stunting, which requires further study. Dioxins cause a high disease burden specifically in Southeast Asia and several metals (lead, arsenic, methylmercury) each cause a global burden of 20–70 DALYs per 100,000. The contribution of dairy products to human exposure to these chemicals is unknown.

Key Words: dairy, foodborne disease, pathogen

511 Subclinical mastitis prevalence, causative pathogens and risk factors in small dairy holders linked to milk collection centers in Rwanda. J. B. Ndahetuye*[1,2], J. Twambazimana[2], A. Nyman[4], Y. Persson[3], and R. Båge[2], [1]*Division of Reproduction, Department of Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden,* [2]*College of Agriculture, Animal Sciences and Veterinary Medicine, University of Rwanda, Busogo, Rwanda,* [3]*National Veterinary Institute, Uppsala, Sweden,* [4]*Vaxa Sverige, Stockholm, Sweden.*

Milk production is prioritized in Rwanda as it represents an essential food source for many families, therefore, higher milk yields are desired. Subclinical mastitis (SCM) is one reason for low milk yield but SCM has not been well studied in Rwanda. The objective of this study was to evaluate prevalence, causative udder pathogens, and cow and herd risk factors associated with SCM in dairy cows linked to milk collection centers (MCC) in Rwanda. Screening with the California Mastitis Test (CMT) was done on 572 cows from 404 herds from 2 MCCs in each of 4 provinces. Milk from udder quarters with CMT score ≥ 3 (scale 1–5) was sampled for bacteriological analyses by culture and a final identification with matrix-assisted laser desorption ionization time of flight mass spectrometry. The prevalence of SCM was 37.3% at the quarter level and 62.0% at cow level. There was a significant ($P \leq 0.05$) difference in SCM prevalence among the MCCs, with highest prevalence in the 2 MCCs in the northern province. Bacteria were isolated in 66.3% of the cultured milk samples, whereas culture-negative and contaminated samples were 30.7% and 2.99%, respectively. Non-aureus staphylococci (NAS) and *S. aureus* were the most prevalent pathogens, representing more than half of all bacteriological findings. *Staphylococcus chromogenes* and *S. epidermidis* were the most prevalent NAS identified. Beta lactamase production was present in 65.7%

of all staphylococci isolates. The risk factor analysis indicated that an increased stage of lactation, dirty udder and legs, no calf suckling the dam and not feeding concentrates were associated with increased odd ratio of SCM in cows. Absence of foremilk stripping was the only factor associated with *S. aureus* infection. High SCM prevalence could affect yield and milk quality at the MCC level. Identified pathogens suggest a possible high rate of within quarter or cow infection transmission and low cure rate against penicillin. Control of the identified risks factors and biosecurity by adjustments in practices, and farmer training could contribute to lowering SCM in Rwanda

Key Words: subclinical mastitis, California Mastitis Test (CMT)

512 A technology package for the control of mastitis in dairy animals at smallholder farmer level. K. Sah*[1], P. Karki[1], R. Shrestha[2], A. Adesogan[3], and G. Dahl[3], [1]*Heifer International Nepal, Kathmandu, Nepal,* [2]*Himalayan College of Agricultural Sciences and Technology, Kathmandu, Nepal,* [3]*Department of Animal Sciences, University of Florida, Gainesville, FL.*

Dairy animals are an important source of income, food and nutritional security at the household level, and improvement in the production and productivity of dairy animals substantially improves the well-being of smallholder farmers. Like other developing countries, dairy animals are key for rural livelihoods in Nepal but often suffer from mastitis, a production disease causing economic losses to farmers, challenges to the dairy processing industry and possible health hazards to consumers. Studies show that the prevalence of sub-clinical mastitis in Africa and Asia exceeds 50%, threatening farmers, dairy processors and consumers. A study was done in Nepal by Heifer International Nepal to develop a technology package to control mastitis in dairy animals. This project was carried out with financial and technical support from the Livestock Systems Innovation Lab at the University of Florida, and in partnership with the Department of Livestock Services and Himalayan College of Agricultural Sciences and Technology. The technology package consisted of (1) identifying knowledge gaps; (2) developing good husbandry practices, including mastitis detection and control technologies; and (3) training of technicians and farmers. The package was applied in the mid-western region of Nepal. Six months after implementation, a reduction in sub-clinical mastitis prevalence was observed in dairy cows (55% at baseline to 28%, n = 432) and buffalo (78% at baseline to 18%, n = 216). These positive study outcomes strongly suggest that the mastitis technology package can be scaled among smallholder farmers across and beyond Nepal to control mastitis in dairy animals.

Key Words: dairy animal, subclinical mastitis, technology package

513 Interventions towards improving the microbiological quality of traditional yogurt in Borana pastoral communities, Ethiopia. K. Amenu*[1], W. Tiki[2], K. Amdhun[1], H. Desta[3], G. Agga[4], B. Wieland[3], O. Kerro Dego[5], D. Grace[6], D. Hunduma[7,1], H. Muhi El-Dine[8], and S. Alonso[3], [1]*Department of Microbiology, Immunology and Veterinary Public Health, College of Veterinary Medicine and Agriculture, Addis Ababa University, Bishoftu, Ethiopia,* [2]*Institute of Leadership and Good Governance, Ethiopian Civil Service University, Addis Ababa, Ethiopia,* [3]*International Livestock Research Institute (ILRI), Addis Ababa, Ethiopia,* [4]*USDA-ARS, Food Animal Environmental Systems Research Unit, Bowling Green, KY,* [5]*Department of Animal Science, University of Tennessee, Knoxville, TN,* [6]*International Livestock Research Institute (ILRI), Nairobi, Kenya,* [7]*Department of Animal Sciences, College of Agriculture and Environmental Sciences, Arsi University, Asella, Ethiopia,* [8]*International Centre for Agricultural Research in the Dry Areas (ICARDA), Amman, Jordan.*

Dairy plays an important role in the diet of Borana pastoral communities of Ethiopia, but poor hygiene during milk production, handling and processing coupled with raw milk consumption are significant health risks for consumers. We conducted experiments to assess the effect of different practices on bacterial quality of traditional yogurt. In a field trial, we investigated the impact of using stainless-steel containers instead of traditional containers made of interwoven fibers on bacterial load and the cultural acceptability of the steel container for yogurt preparation. In a laboratory experiment, we assessed the effect of sanitizing milk containers with smoke from 3 tree species (*Olea europaea* ssp. *africana*, *Faurea speciosa* and *Terminalia brownii*), 2 milk containers (traditional vs. stainless steel) and 2 smoking methods (introducing the burning wood in the container vs. fumigation) on bacterial loads. The field trial showed that the bacterial loads of the yogurt prepared using traditional and stainless-steel milk containers did not differ ($P < 0.05$). Most of the informants appreciated the stainless-steel container because it could be easily sealed with a lid. However, they reported no desire to use it as substitute to the traditional container because (1) it becomes hot during the day and cold during the night, which accelerates souring of milk and (2) it does not have the traditional decorations. Moreover, 55.8% of the respondents indicated that yoghourt prepared using the traditional container had a better taste. The lab experiment showed that the traditional container ($P < 0.001$) and smoking by putting the burning wood inside the container ($P = 0.013$) reduced bacteria loads compared with the alternatives. Similarly, Olea tree species was more effective than others at reducing bacterial loads. Our results suggest that the use of stainless steel containers did not add value for the Borana pastoral communities as it did not reduce bacterial load of yogurt and the container acceptability among end-users was low.

Key Words: milk container sanitation, participatory experiment, traditional dairy processing

514 Effects of ration formulation on the performance of dairy animals in Nepal. B. Shrestha*[1] and A. Adesogan[2], [1]*Heifer International Nepal, Hattiban, Lalitpur, Nepal,* [2]*Florida University, Gainesville, FL.*

Dairy is the most important livestock sub sector contributing almost 2-thirds of the livestock gross domestic product in Nepal. The prevalent dairy animal feeding approach is dependent primarily on locally available feed resources and it aims to fill the guts of animals without proper consideration of their nutrient requirements for various functions. The consequences are suboptimal performance and common occurrence of reproductive disorders in high yielding dairy cows and buffalo, with overall poor lifetime production. A study was carried out to examine effects of least cost ration formulation on the performance of dairy cattle and buffaloes. Twenty-six Jersey and Holstein cattle in early lactation of parity 1–4 and 30 Murrah buffaloes (parity 1–6) were randomly allocated to 2 groups after matching them for breed, parity, and stage of lactation in separate experiments. One group of cows or buffalos in each experiment was fed a balanced ration formulated using a feeding support tool (phone app developed based on nutrient requirements of dairy animals from ICAR and the dairy NRC) for 8 weeks and the control group was fed a ration that typified farmers' practice. The average initial milk yield of cattle in both groups was 9.30 kg/d. This increased to 9.96 kg/d during the experimental period in the treatment group, while the average yield for the control group remained unchanged (9.08 kg/d). The fitted ANCOVA model was significant with an estimated difference in yield of 0.994 kg/d. Similarly in buffaloes, the initial milk yields in treatment and control groups were 5.53 and 5.56 kg/d and these changed to 5.31 and 4.92 kg/d, respectively at the end of the experiment. The estimated increase in milk yield of buffaloes in the treatment versus control group was 0.415 kg/d. A survey of 100 farmers who participated in on-farm testing revealed that 94% of those who used the feeding support tool reported a 0.5 to 2 kg/d increase in milk yield on average with similar or reduced feeding costs. Therefore, using the feeding support tool is an effective strategy for enhancing milk production in Nepal.

Key Words: feeding support tool, dairy animal feeding system, least cost ration

515 Improving milk production on market-oriented dairy farms in Sri Lanka.

D. Vyas*, J. J. Bromfield, C. D. Nelson, and G. E. Dahl, *University of Florida, Gainesville, FL.*

Dairy is the most important sub-sector in the Sri Lankan livestock industry, because of the need to address a growing demand for fresh milk and milk products, and because of its potential influence on the rural economy. The USDA's Food for Progress program awarded a 4.5-year Market-Oriented Dairy (MOD) project to International Executive Service Corps (IESC), a Washington, DC, not-for-profit organization. The objective of MOD is to support Sri Lanka's dairy sector and catalyze sustainable growth by strengthening the dairy sector through better technological, financial, and management practices benefitting all stakeholders and consumers along the dairy value chain. The University of Florida (UF) is working with IESC as technical experts in conducting dairy value chain assessments, identifying gaps and challenges in dairy management practices, extension services, milk quality management standards and artificial insemination (AI) services. Assessment of the dairy value chain in 2018 identified a lack of good quality and quantity of feed, along with poor dairy management practices and ineffective extension services as major constraints in improving dairy productivity in Sri Lanka. In addition, a lack of national milk quality standards that are consistent with international benchmarks and inadequate cooling facilities are significant challenges to improving milk quality. The nutritional status of cows is not suitable for optimal reproductive performance, compromising the success of AI in Sri Lanka. Based on these findings we developed a dairy assessment tool and provided comprehensive training sessions targeting extension agents, veterinarians, and farmers to promote best practices in dairy management. Beyond training, however, industry support for standardization and monitoring of milk and feed quality are needed; providing opportunities for private investment to support the dairy industry. Similar opportunities are available for forage production and delivery to producers. The broader impact of the MOD project intervention is to reduce Sri Lanka's dependency on imported milk and contribute toward the goal of safe, self-sufficient fresh milk.

Key Words: dairy, Sri Lanka

516 "More milk, please!" Does increased milk consumption lead to better child growth and development in rural Nepal?

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Child undernutrition afflicts >150 million children worldwide, contributing to poor child growth, increased risk of infections, and loss of developmental potential. Animal source foods (ASFs) can ameliorate these problems by providing high-quality, high-density, bioavailable protein and micronutrients. However, many children in developing countries lack ASFs in their diet. The objective was to assess the impact of milk on child growth and development in rural Nepal. A 4-year longitudinal randomized trial examined the impact of a Heifer Nepal intervention on child diet, growth, and development. The 3 treatment arms compared were: community development plus training on human nutrition/livestock production vs. this training alone vs. control. Child 24-h diet recall and anthropometry was obtained at 6 household visits (5854 observations, children ages 6–60 mo); developmental assessments (Ages & Stages Questionnaire, ASQ) were performed on a subset of children at midline and endline. Few ASFs were consumed by children in the project area; milk most frequently (29%). The full intervention significantly increased diet diversity but milk consumption remained relatively static despite many households having dairy animals. At endline, 15% drank milk 1–4 times/week, and 64% drank none. However, compared with milk non-consumers, children who consumed milk had significantly higher weight-for-height and head circumference z scores (−0.75 vs −0.66, $P = 0.005$; −1.14 vs −1.04, $P < 0.001$). Additionally, milk consumers had significantly higher ASQ scores (midline 232 vs 204, $P < 0.0001$; endline 211 vs 196, $P < 0.001$) than non-consumers. In this rural Nepali population, milk consumption was strongly linked to child growth and developmental performance. However, an educational intervention promoting ASFs did not increase milk consumption. Milk availability, cultural practices, and preference for sale over child intake may account for this finding. Further investigation of milk consumption practices in this setting are warranted, as the results indicate the fundamental importance of dietary milk to child well-being.

Key Words: milk, child, growth and development

517 Sustainability of dairy production in developing countries.

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Sustainable milk production in developing countries must address food security and climate change mitigation simultaneously. Socioeconomic sustainability is paramount in developing countries where milk production and consumption represent a vehicle to improve human nutrition and health, as well as the potential for increased income, leading to improved livelihoods by subsistence farmers with limited access to markets. These benefits can only be achieved with judicious use of animal stocks and agricultural practices that do not exhaust available natural resources, which are often shared by regional farming communities. Milk and dairy foods provide balance and variety to the diet and make significant contributions to meeting the needs for calcium, magnesium, selenium, riboflavin, vitamin B_{12} and pantothenic acid (vitamin B_5) in undernourished populations and especially children. Milk production in developing countries largely occurs in smallholder mixed crop-livestock systems where the animals may suffer from malnutrition leading to negligible or no milk production during several months of the year. Livestock are also valued for non-food functions such as draft, fuel (manure), store of capital and insurance against crop failure incentivizing the maintenance of large unproductive herds that place stress on feed (land) and water resources. Under these circumstances, sustainable intensification–increasing milk production from currently available resources–represents the single most important and practical strategy for improving the sustainability of milk production and consumption in developing countries. Improving the genetic potential of the animals, the availability of quality feed, and providing balanced nutrition are the most promising strategies to improve milk production and sustainability in developing countries. For example, the productivity gap for milk

in Ethiopia is estimated at 4.5 billion L/year, which can be closed, in part, with balanced nutrition. Milk production in developing countries will be sustainable if it supplies more essential nutrients to under and malnourished populations while utilizing the natural resources available.

Key Words: sustainability, dairy, developing countries

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518 Feeding a diet with high corn distillers grain with solubles alters manure characteristics and decreases ammonia emission from manure in dairy cows. C. Lee*[1], D. L. Morris[1,2], and K. M. Lefever[1], [1]*Department of Animal Sciences, OARDC, The Ohio State University, Wooster, OH,* [2]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE.*

The objective of the experiment was to examine effects of a diet containing high corn distillers grain with solubles on manure characteristics and NH_3 emission in dairy cows. Eighteen cows were blocked by parity and DIM and cows in each block were assigned to the following treatments: CON, control diet; 2) DG, CON with distillers grain with solubles at 28.8% (DM basis) replacing mainly soybean meal. The experiment was conducted for 11 wk and feces and urine from individual cows were collected over 3 d in wk 11 (total 8 spot samples per cow). Fecal or urine samples were composited based on an equal volume basis and frozen at −20°C. Immediately before the manure incubation, composited feces and urine were thawed and sampled to determine manure characteristics. Manure was reconstituted according to daily fecal and urine excretion estimated using markers (indigestible NDF and creatinine, respectively) for individual cows. The manures (2 kg of each) were incubated using a multiple chamber system over 10 d to measure NH_3 and H_2S emissions. Due to the limited number of incubation chambers, 6 manures (3 per treatment) have been finished for incubation and sample analysis, and the data from 6 manures were analyzed using the MIXED procedure of SAS where block and incubation chamber within block were random effects and diet was a fixed effect. The ratio of feces to urine and the contents of manure total and volatile solids were not different among treatments. Manure from DG had lower pH (6.64 vs. 7.37; $P < 0.01$), tended to have lower N (5.2 vs. 5.9 g/kg manure; $P = 0.06$), and had greater S (1.0 vs. 0.5 g/kg manure; $P < 0.01$) compared with CON. During the 10-d incubation, NH_3 emission was considerably lower (353 vs. 639 mg/kg manure; $P < 0.01$) for DG vs. CON. However, H_2S emission over 10 d for DG was similar (5.4 vs. 4.4 mg/kg manure; $P = 0.23$) with that for CON. After the incubation, manure N concentration was greater (4.3 vs. 3.8 g/kg manure; $P = 0.01$) for DG vs. CON. In conclusion, manure from cows fed a high-DG diet decreased NH_3 emission mainly due to lower pH of manure.

Key Words: corn distillers grain with solubles, manure, ammonia emission

519 The effects of concentrate feeding strategy and dairy cow genotype on milk production and metabolic status under restricted grazing conditions during the breeding season. E. L. Brady*[1], M. B. Lynch[2], K. M. Pierce[2], A. G. Fahey[2], E. Kelly[1], M. J. Doran[2], C. Grace[2], C. McDonnell[2], and F. J. Mulligan[1], [1]*School of Veterinary Medicine, University College Dublin, Belfield, Dublin, Ireland,* [2]*School of Agriculture and Food Science, University College Dublin, Belfield, Dublin, Ireland.*

Concentrate supplementation is used in grass-based production systems to increase overall dry matter intake and milk production and to manage deficits in grass supply. Different genotypes may respond differently to concentrate supplementation. Therefore, the aim of this study was to compare the effects of contrasting feeding strategies and dairy cow genotypes on milk production and metabolic status under restricted grazing conditions during the breeding season. Fifty-eight Holstein-Friesian cows were blocked on calving date, previous 305-d milk yield, BCS and genotype. Cows of high fertility low milk (HFLM) and low fertility high milk (LFHM) genotype were randomly assigned to 1 of 2 treatments in a 2 × 2 factorial, randomised block design. The dietary treatments were (1) a low flat rate concentrate allowance of 2 kg (1.76 kg of DM) per cow (FR, n= 29); (2) a feed to yield concentrate allowance (FY, n = 29). The FY treatment included feeding a base of 2 kg plus 0.5 kg (0.44 kg of DM) of concentrates per kg of milk above the base milk yield. All cows were allocated 13 kg of grass DM. These diets were offered for the duration of the 11-week experimental period. Feed to yield resulted in higher milk yield (+1.76 kg, $P < 0.05$) and a higher fat and protein yield (+0.11 kg, $P < 0.05$). There was a tendency towards an interaction between feeding strategy and genotype ($P = 0.06$) for milk yield. For LFHM genotype cows, FY increased milk production and fat and protein by 3.35 kg ($P < 0.05$) and 0.20 kg ($P < 0.05$). In comparison, milk yield or yield of fat and protein was not increased by FY for the HFLM genotype. Feeding strategy had an effect on metabolic status as FY had lower BHB ($P < 0.05$) and NEFA ($P < 0.10$). The FY treatment increased BCS (2.77, $P < 0.01$) in comparison to FR (2.65, $P < 0.01$). In conclusion, the FY concentrate feeding strategy improved milk yield and milk component yield for the cows of LFHM genotype. Feed to yield improved BCS and metabolic status compared with FR under restricted grazing conditions.

Key Words: concentrate feeding strategy, restricted grazing, genotype

520 Effects of milk replacer feeding rate and frequency on performance of preweaning dairy calves during summer. R. M. Orellana Rivas*, T. Rodrigues, J. Silveira, T. N. Marins, J. K. Bernard, and S. Tao, *University of Georgia, Tifton, GA.*

To evaluate the effect of milk replacer (MR) feeding rate and frequency (FF) on performance during the summer, 48 Holstein calves (BW = 40.8 ± 4.3 kg) were randomly assigned at 7 d of age to 1 of 4 treatments in a 2 × 2 factorial arrangement. Treatments included 2 rates of MR allowance (0.68 [LOW] or 0.79 kg/d [HIGH] of a 26% CP and 17% fat MR) fed either 2× (0700 and 1600 h) or 3× (0700, 1600, and 2200 h) daily. Calves were housed in polyethylene hutches and managed similarly throughout the trial. Milk replacer (12.5% solids) was fed to calves based on their respective treatments until d 42 when MR allowance was reduced by 50% and offered 1×/d (0700 h) for the following 7 d until weaning. Calves remained on trial until d 63. Calf starter and water were offered ad libitum. Ambient temperature and relative humidity inside and outside hutches were measured hourly. Starter and MR intake were recorded daily. Respiration rate and rectal temperature were recorded 3×/wk. Structural growth and BW were measured weekly. Data were analyzed using the mixed procedure of SAS. The average temperature-humidity index was 77 in and outside hutches during the experiment. Feeding 3× reduced respiration rate from 3 to 6 wk of age by 10–18% (FF × time, $P < 0.05$) and tended to decrease rectal temperature compared with 2× only for calves fed LOW (39.27 vs. 39.44°C for 3× and 2× fed LOW, FF × MR, $P = 0.06$). Treatment did not affect gain in BW, hip height, wither height and body length ($P > 0.42$). On wk 2, calves fed LOW and HIGH had similar MR intake due to diseases (e.g., scour) and then increased to the projected intake by design (MR × time, $P < 0.01$). Feeding 3× tended to increase MR intake compared with 2× fed LOW (FF × MR, $P = 0.07$). Calves fed 3× consumed more starter during wk 8 and 9 than calves fed 2× (FF × time, $P = 0.05$). Starter intake was higher for LOW during wk 7 compared with HIGH (MR × time, $P =$

0.05). Compared with 2×, feeding calves 3× increased total DMI during wk 4, 5, 8 and 9 (FF × time, $P < 0.05$). In conclusion, feeding MR 3× during summer may reduce heat strain on calves and increase intake, but did not alter growth.

Key Words: summer, feeding frequency, calf

521 Management strategies to optimize milk fatty acid composition and component production.

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The objective of this study was to determine the relationship between management strategies and fatty acid composition of dairy herds. Nine NC dairy herds (n = 6 Jersey, n = 3 Holstein) were enrolled. Bulk tank samples were collected monthly during milk pickup of enrolled herds for 7 collections per month. Samples were analyzed for milk components and fatty acids. Herds were evaluated every 3 mo for stocking density, body condition, locomotion, and feed particle size. Particle size was determined using the Penn State Particle Separator. Farms were classified as high de novo (HDN), low de novo (LDN), high mixed origin (HMO), low mixed origin (LMO), high preformed (HPF), and low preformed (LPF) fatty acid composition each season. Data were analyzed using the GLIMMIX procedure of SAS, where herd classification was considered the fixed effect. Means were separated using Fisher's Least Significant Difference, and significance was declared when $P \leq 0.05$. Tendencies were discussed when $0.05 < P \leq 0.10$. Jersey herds classified as HDN and HMO had greater milk protein (%) than LDN and LMO herds (3.62 and 3.65 v. 3.51 and 3.48 ± 0.013%, respective, $P < 0.001$). Holstein herds classified as HDN had greater milk protein (%) than LDN (3.13 v. 3.00 ± 0.016% $P < 0.001$). Nonesterified fatty acids (uEq/L) were less in HMO and LPF compared with LMO and HPF Jersey herds (187.7 and 198.0 v. 245.2 and 234.9 ± 8.75 uEq/L, respective, $P < 0.001$). Locomotion score was unaffected by classification. Herds classified as HMO had less feed retained in the first sieve (19 mm, $P = 0.008$), more feed retained in the second sieve (8 mm, $P = 0.029$), more feed retained in the third sieve (1.18 mm, $P = 0.001$), and tended to have less feed retained in the bottom compartment ($P = 0.053$) compared with LMO herds. Average body condition tended to be less in HPF herds compared with LPF herds (3.00 v. 2.93 ± 0.025, $P = 0.055$). Stocking density tended to be greater in HPF herds compared with LPF herds (120.34 v. 98.84 ± 8.88%, $P = 0.055$). Management of feed particle distribution may result in greater fatty acid composition and more optimal milk composition.

Key Words: milk fatty acids, feed particle size, management

522 Withdrawn

523 Pasture and supplement intake, milk production, and partial economic profit in commercial dairy systems: Effects of two contrasting productive strategies.

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The objective of this work was to determine the factors that have major impact on pasture dry matter intake (DMI) by cows in commercial dairy farms. Fortnightly visits to commercial dairies were carried out between June 2016 and May 2017 to record supplement on offer, pasture access time (PAT), herbage mass (HM) and allowance (HA), milk production per cow, and feed and milk prices. Dairy farms were categorized in high (>8.5 kg DM/cow; HPI, n = 8) or low (<7.0 kg DM/cow; LPI, n = 8) daily pasture DMI per cow, estimated by energy balance (NRC, 2001). Potential pasture DMI per cow was estimated from recorded HA using the equation of Baudracco et al. (2010) for dairy cows grazing without supplementation. The 2 groups were compared with the MIXED procedure and considered different when $P \leq 0.05$. This study reveals 2 contrasting productive strategies, with dairy systems (LPI) that aimed for a higher yield per cow (23.3 ± 0.7 L/cow/day, $P \leq 0.01$) based on greater supplementation (12.0 ± 0.3 kg DM/cow/day, $P \leq 0.01$) and lower pasture DMI (5.5 ± 0.3 kg DM/cow/day, $P \leq 0.01$) versus systems (HPI) that aimed to produce under a limited amount of supplement (5.8 ± 0.3 kg DM/cow/day) and high pasture inclusion (10.1 ± 0.3 kg DM/cow/day), albeit achieving lower milk production (19.0 ± 0.7 L/cow/day). Although to a lesser extent in HPI, pasture DMI was conditioned by supplementation level and not by pasture management in both groups, since cows could have harvested higher amounts of pasture according to HM (2303 ± 178 kg DM/ha, $P = 0.16$), PAT (12.7 ± 0.6 h, $P = 0.14$) and HA (29.4 *vs* 23.2 ± 3.1 kg DM/cow/d for HPI and LPI respectively, $P \leq 0.01$). Even though HPI offered greater HA, pasture DMI was closer to potential DMI than in LPI (differences between potential and actual pasture DMI: 3.6 *vs* 6.9 ± 0.3 kg DM/cow/day respectively, $P \leq 0.01$). Therefore, disconnection between grazing and supplementation management was more accentuated in the LPI systems. Both strategies resulted in similar margin over feeding costs (3.00 ± 0.15 US$/cow/day). These results reinforce the importance of an efficient grazing management, applying supplementation as a complementary tool to balance the diet and not as an isolated food, disconnected from the offered forage.

Production, Management, and the Environment Symposium: Future of Housing for Dairy Cattle

524 Housing and management systems for dairy cows. P. J. Galama*, A. Kuipers, and H. J. van Dooren, *Wageningen Livestock Research, Wageningen, Gelderland, the Netherlands.*

The Dutch dairy chain works intensively on achieving a set of goals on climate neutral development, improvement of animal welfare and health, maintain grazing and biodiversity and environment. These goals are yearly monitored by Wageningen Economic Institute. All 16.500 dairy farmers in Netherlands use a management tool, the Annual Nutrient Cycling Assessment (ANCA) to give insight in the mineral cycle (N, P, C) at cow, soil and farm level and in pollution of soil, water and air. Housing and manure management play an important role in achieving these goals. The development of new techniques, especially in housing, aim to improve animal welfare, reduce emissions of ammonia and green house gasses, increase manure quality for use as fertiliser or soil improver, and have public support. Two Freewalk housing systems will be demonstrated, a bedding of organic material like wood chips and an artificial floor which separates urine from feces. Different floor types and handling systems of manure, like aerating the slurry, are studied at the Climate Measurement Units on Research Station Dairy Campus. Results of low emission techniques will be shown. For example: the ammonia emission per cow is 30% lower in a Freewalk housing system with wood chips compared with cubicle housing, despite more than twice as much space per cow. A new impressive development to collect urine in the concentrate feeder is the Cowtoilet. The idea is to lower the emission of ammonia and upgrade the urine fraction. Also examples to increase capital efficiency by keeping other species or horticulture on the compost bedding during the grazing season will be shown. To maintain grazing, new grazing systems are researched by using sensor data to predict grass growth, grass intake and to save labor by using virtual fencing. The floating farm in the harbour of Rotterdam is in development to get closer contact with the society and local stakeholders by using by-products and waste.

Key Words: dairy, housing, innovations

525 Comparing cattle welfare in compost barns and freestalls in six European countries. I. Blanco-Penedo*[1], A. Kuipers[2], M. Klopcic[3], and U. Emanuelson[1], [1]*SLU, Department of Clinical Sciences, Uppsala, Sweden,* [2]*WUR, Wageningen Livestock Research, Wageningen, the Netherlands,* [3]*UL, Department of Animal Science, Groblje, Slovenia.*

The ERA-Net Freewalk project aims to study animal health and welfare, milk quality, environmental and socioeconomic impacts in dairy farms that use compost pack bedding (CPB) versus freestalls (FS). The specific aim here was to assess the welfare of dairy cows and to test the hypothesis that dairy cows in CPB systems have a better welfare than those kept in FS. Forty commercial dairy farms (20 CPB and 20 FS) in 6 European countries were selected for evaluation, using mainly animal-based measures (ABM), from an adaptation of the Welfare Quality protocol. Farms were visited during winter 2017-summer 2018, where 4036 dairy cows were scored by the same observer. The average within-farm prevalence of "dirtiness," graded as "very dirty," was 62% of the lower hind legs and 43% of the hindquarters, being lower in FS. Cows with at least one hairless area on the body differed significantly, with 52% and 80% in CPB and FS, respectively. This was most pronounced on lower hind legs. Lesions were present in 9% of CPB vs 24% in FS and swellings in 4% vs 10%. BCS was normal (acceptable) in 92% of the cows but slightly higher in CPB. Light and severe lameness was lower on CPB (22%) vs FS (26%) but varied according to the seasons. Other health parameters were within the safe range according to thresholds of the Welfare Quality, except ocular discharge that was too common in CPB. Time for lying down varied between systems, with 5.33 in CPB vs 6 s in FS, reaching warning thresholds in 24% (CPB) vs 43% (FS). Colliding with housing equipment or cows was also less common in CPB (9%) than in FS (35%). Scores for rising up showed significantly easier movement in CPB (2.31) than FS (2.92); colliding less in CPB (18.3%) than FS (76.7%). The results showed a large influence by the housing system on ABM and comfort around resting. Further analysis (welfare criteria and principles level) are in progress to find the most advantageous system to improve animal welfare.

Key Words: dairy cow, animal welfare, compost bedded pack

526 Current and future of compost bedded pack barns in North America. J. L. Taraba*, *University of Kentucky, Lexington, KY.*

A successful compost bedded dairy barn is the result of combining the management of the composting resting bed and the function of the structure enclosing the cow resting area to achieve an environment for excellent cow welfare and comfort while maintaining efficient milk production. The housing structure must provide enough air exchanges for removal of bed and cow produced moisture, heat and gases (e.g., CO_2, NH_3) and air velocity over the cows for heat dissipation and the compost bed for moisture drying. The system has additional values: reduced environmental impacts on air and water quality. Quality of water and air resources benefit from the resting area composting processes. Odors are reduced through aerobic decomposition, while these same processes bind fertilizer N and P in the organic form, which allow slow release of these plant nutrients after land application. Further, manure storage occurs under roof which allows storage times of one year or longer depending on bedding usage or area allowed per cow. Storage flexibility improves field application timing of the compost to meet field conditions and cropping nutrient needs. Challenges to economic success come from the cost and availability of bedding materials as well as a housing envelope whose structural components can respond to climatic conditions to meet cow comfort in the region of the world where the dairy is located. Bedding materials may be expensive, limited in availability when needed (particularly cold and wet weather). In the United States, wood sawdust is primarily used, but alternate biomass from crop plants need to be assessed so that bedding supplies can be increased. Understanding the impacts that alternate materials exhibit in the compost bed and the housing envelope is needed to adjust the bed and structure management recommendations. An example would be low C/N ratio biomass, e.g., soybean straw, which create composting C/N ratios where high rates of NH3 are released. Various housing strategies for the compost bedded dairies have been implemented throughout the United States in different climatic conditions and will be discussed. This presentation will also summarize various challenges to cow welfare from recent research findings.

Key Words: compost bedded pack barn, housing structure, comfort

527 The future of dairy cattle housing: Societal and animal welfare implications. A. Beaver*, C. Ritter, and M. A. G. von Keyserlingk, *Animal Welfare Program, University of British Columbia, Vancouver, BC, Canada.*

The animal welfare paradigm developed by Fraser et al. in 1997 suggests that welfare is optimized at the intersection of 3 key domains: health and biological functioning, affective state, and natural living. These domains are differentially prioritized by stakeholders, with those not familiar with dairy farming tending to emphasize natural living and producers often focusing on optimizing health. The intensification of housing systems for dairy cattle has catered to this health focus, permitting increased capability to provide individualized care. For example, cattle on approximately 39% of US dairy herds are housed in tie stalls, which permit individual rationing and reduce competitive interactions. Similarly, dairy calves are typically separated from the dam at parturition and housed individually over concerns of disease transmission. However, such housing systems come at the expense of natural behavior: in tie stalls, cattle movement is restricted and the opportunity to engage in natural social behavior, pasture grazing, or estrus expression is compromised. Compared with group-housed calves, calves reared individually have impaired cognition, increased fear responses, and reduced solid feed intake before weaning. Moreover, calves permitted to suckle the dam show a diverse repertoire of social behaviors paired with a reduction in oral stereotypies. Particularly given the societal concern that animals lead a natural life, future housing for dairy cattle could be reimagined to incorporate naturalness while maintaining a commitment to animal health and biological functioning. In particular, the distinction between "natural living" and "natural behavior" can be harnessed to incorporate opportunities for animals to express their natural behavior without rendering the system non-operational or antiquated. Preference and motivation testing are also useful tools to determine which natural behaviors are actually of importance to the animal; housing systems may then be modified to accommodate the realization of these behaviors. Examples include improving floor design to facilitate estrus expression, providing pasture access in appropriate weather conditions, and implementing group housing for calves.

Key Words: natural behavior, dairy cattle housing, animal welfare

528 Transition period and calving housing: Latest information and where are we heading? K. Proudfoot*, *Ohio State University, Columbus, OH.*

Many advancements in transition cow management and housing have been made over the last decade. The objectives of this presentation are to describe: 1) research to date on the housing of transition dairy cows before giving birth, and 2) opportunities for future research in this area. A focus of transition cow housing research has been on the effect of the cow's social environment during the 3 weeks before calving on behavior and health. Specifically, researchers have measured the impacts of overcrowding and regrouping on behavior, physiological biomarkers, and clinical signs of disease. The results of these studies are variable, especially when assessing one factor alone. However, cows likely experience cumulative stressors during transition, which may have a greater effect on their health. For example, cows that experienced a combination of overstocking, unpredictable feeding times, and social instability were more likely to develop endometritis after calving compared with those housed in more predictable environments. A second area of research has focused on understanding cows' natural behavior during labor and preferences for a calving environment. For example, cows provided free access to pasture and a barn sought areas with natural tree cover, or manufactured cover (barn) when giving birth. Similarly, when kept indoors, individually housed dairy cows sought a secluded area to give birth, especially if they calved during the day. Providing cows with the opportunity to seclude in group pens may be more complicated, especially when stocking density is high. For example, cows provided with a physical barrier in a group pen were more likely to calve next to the barrier, but this tended to occur only when stocking density was low. Future research should focus on: 1) developing a better understanding of how individual cows cope with transition housing practices, 2) using novel measurements of cow welfare during transition, including their affective state and ability to maintain sufficient sleep, and 3) determining the impacts of housing on cows during the short period after calving when cows are at the highest risk of disease.

Key Words: behavior, welfare, maternity

ADSA Foundation Scholar Lecture and Ruminant Nutrition Symposium: Dietary Methyl Donor Supplementation and Hepatic Health in Transition Dairy Cows

529 ADSA Foundation Scholar Presentation (Production): Influencing hepatic metabolism: Can fatty acids and methyl donors modulate nutrient partitioning to support metabolic health in the transition dairy cow? H. White*, *University of Wisconsin Madison, Madison, WI.*

Hepatic de novo production of glucose and oxidation of fatty acids (FA) are critical in supporting milk production during the transition to lactation period. During this period of metabolic challenge, there is an increase in FA taken up by the liver. Although the optimal fate for these FA is complete oxidation through the TCA cycle, alternative fates include incomplete oxidation via ketogenesis or storage within the liver as triglycerides (TG). Influencing the relative capacity of these pathways may prevent ketosis and fatty liver and improve hepatic efficiency. Hepatic nutrient partitioning reflects complex regulation of key enzymatic steps modulated by FA, substrates, and methyl donors. Fatty acids mobilized from adipose tissue have regulatory effects on genes such as pyruvate carboxylase; however, both in vivo and vitro work suggests there may be other influences present which can result in differential regulation between cows that subsequently develop sub-clinical ketosis and that some FA may be preferentially stored or oxidized. Supporting TG export or subsequent lipolysis is also important for minimizing liver TG accumulation. Although there is biochemical overlap between choline and methionine within both methyl donor metabolism and cellular lipid packaging, there is evidence that each of these nutrients have their own metabolic priorities. Increased FA oxidation and TG export with choline treatment may support both decreased cellular TG and ketone body secretion. Together, these shifts in pathway flux may also support the increased cellular glycogen observed in vitro and give reason to further examine gluconeogenic capacity in cows supplemented with choline. While further research is needed to continue refining our understanding of the intricate balance that regulates hepatic metabolism, shifting nutrient partitioning via oxidative pathways may be key in supporting both efficiency and metabolic health.

Key Words: gluconeogenesis, oxidation, choline

530 Methyl donor metabolism and nutrition in the transition dairy cow: Should we consider fatty acid nutrition simultaneously? J. W. McFadden*, *Cornell University, Ithaca, NY.*

One-carbon metabolism involves the folate and methionine (Met) cycles that work in unison to support lipid, nucleotide, and protein synthesis, as well as methylation reactions and the maintenance of redox status. Methyltetrahydrofolate is utilized by Met synthase to couple the folate and Met cycles. Methionine may also be formed by the transformation of choline-derived betaine by betaine hydroxymethyltransferase. Within the Met cycle, S-adenosylmethionine is formed and used to synthesize phosphatidylcholine. Such actions are considered important for hepatic very-low density lipoprotein assembly and triglyceride secretion. Following methylation, homocysteine is formed and utilized to synthesize glutathione within the transsulfuration pathway, which scavenges reactive oxygen species. These biochemical complexities play a major role in the transition dairy cow that experiences a demand for compounds with a labile methyl group. Indeed, dietary methyl donor supplementation has merit when we consider research demonstrating improved lactation performance, and prevention of hepatic triglyceride deposition, inflammation, and oxidative stress with this feeding strategy. However, our understanding of methyl donor utilization and efficacy to maintain peripartal health deserves consideration within the context of fatty acid supply and metabolism. In non-ruminants, transmethylation-dependent phosphatidylcholine synthesis favors phosphatidylethanolamine enriched in very-long chain polyunsaturated fatty acids (PUFA). Because hepatic PUFA depletion is a feature of fatty liver in transition cows, inadequate hepatic PUFA concentrations may limit the ability of methyl donors to stimulate phosphatidylcholine synthesis and prevent steatosis. Moreover, fatty acid oversupply or composition may counteract the potential benefits of dietary methyl donor supplementation on redox homeostasis and immune status. Although work has focused on methyl donor co-supplementation (e.g., choline and Met), future studies should determine whether specific dietary fatty acid feeding regimens optimize methyl donor efficacy in the transition cow.

Key Words: liver, methyl donor, transition cow

531 Potential impacts of betaine supplementation on dairy cattle during the transition period and under heat stress. S. Tao*, J. K. Bernard, R. M. Orellana Rivas, T. N. Marins, and Y. Chen, *University of Georgia, Tifton, GA.*

Betaine (tri-methylglycine) is a natural compound present in bacteria, plant and animal cells. It is an important nutrient in human and animal diets and exerts functions in osmolality regulation, maintenance of cell function as a chaperone and one-carbon metabolism as a methyl donor. Betaine in the animal body can be synthesized endogenously by choline oxidation or absorbed from the diet. As a methyl donor, betaine is catalyzed by betaine-homocysteine methyltransferase and donates a methyl group to homocysteine for re-synthesizing methionine, primarily in liver. This potentially spares choline or methionine as methyl donors, and promotes the production of S-adenosylmethionine, a universal methyl donor. As an organic osmolyte, betaine regulates cell volume and organizes water structure in a cell, stabilizing cellular proteins, especially under stress conditions. Therefore, betaine has numerous functions in an animal's body. In laboratory animals, betaine supplementation is reported to alleviate hepatic fat accumulation and other symptoms associated with alcohol or non-alcohol related liver injury. In coccdia-infected poultry, betaine stabilizes intestinal structure and maintains gut health and function. In swine and poultry, betaine is used as a "carcass modifier" to increase lean tissue synthesis. In ruminants, limited research suggest that supplementation of betaine influences rumen fermentation, improves nutrient digestibility, and increases milk yield during lactation. Because it functions as a methyl donor and an organic osmolyte, betaine has the potential to be utilized in dairy cattle diet during the transition period and summer to improve hepatic health and improve stress responses. However, convincing evidence is still limited and extensive research is warranted.

Key Words: betaine, transition period, heat stress

532 Folic acid and vitamin B_{12} requirements of mature cows: Importance of endogenous production of methyl donors from the

one-carbon pool. C. L. Girard* and M. Duplessis, *Agriculture and Agri-Food Canada, Sherbrooke, QC, Canada.*

Methionine (Met) is the precursor of S-adenosylmethionine (SAM), donor of methyl groups in more than 50 metabolic reactions, among them synthesis of creatine and phosphatidylcholine and DNA methylation. In the transmethylation pathway (TM), after giving its methyl group, SAM is converted into S-adenosylhomocysteine (SAH) and then into homocysteine (Hcy). The latter can be catabolized into cysteine or remethylated into Met. The role of the remethylation cycle is to ensure a constant supply of SAM, even when Met supply is low. Remethylation of Met can be achieved using preformed labile methyl groups provided by choline and betaine or by methylneogenesis. For endogenous production of methyl groups, tetrahydrofolate (THF) accepts a 1-carbon unit, mostly provided by catabolism of serine, glycine or formate, to form 5,10-methylene-THF which can be irreversibly converted into 5-methyl-THF. The latter transfers its methyl group to the vitamin B_{12} coenzyme which acts as an intermediary for the transfer of the methyl group to Hcy to regenerate Met and THF. In multiparous dairy cows, when Met supply is above 2.2% MP, a supplement of folic acid and vitamin B12 has no effect on TM but when Met supply is lower than 1.9% MP, the vitamin supplement increases TM. In the latter, the supplement of vitamins also increases *AHCY* (SAH hydrolase) supporting the previous observation that, when Met supply is low, methylneogenesis allows maintaining TM. The fate of Hcy flowing TM, however, varies according to plasma Met concentration. When plasma Met is low, plasma Hcy and Cys are decreased by the vitamin supplement; methylneogenesis reduces the need for Met by increasing the number of times that a molecule of Hcy is remethylated within hepatic cells before being catabolized. However, when plasma Met is high, this effect is reduced and plasma Hcy and Cys increased. Providing an adequate supply in folic acid and vitamin B12 allows for sufficient endogenous production of methyl groups to support TM, which is likely to be especially of critical importance when methionine supply is low.

Key Words: dairy cow, B vitamin, S-adenosylmethionine

533 Methionine supplementation during the transition period: Fine-tuning immunometabolism. Z. Zhou*, *Michigan State University, East Lansing, MI.*

Around parturition in dairy cows, the increased demand for nutrients to sustain fetal growth and lactation, coupled with depressed feed intake, impose tremendous metabolic stress. Consequently, the metabolic challenge and inflammation incurred during this period have been shown to lead to health problems and compromised lactation performance. Various nutrients are known to promote a smooth transition from gestation to lactation by fine-tuning immunometabolism. Methionine, an essential amino acid (AA) engaged in various key physiologic events, has been underscored as a limiting AA for milk protein synthesis. Apart from its apparent key role in mammary gland and liver protein synthesis, methionine also serves as a substrate for sulfur-containing antioxidants, namely glutathione and taurine. Additionally, as a key component of one-carbon metabolism, hundreds of methylation reactions acquire methyl groups from methionine via S-adenosyl methionine. Recent research has highlighted the milk production and health benefits from methionine supplementation during the transition period. This presentation will provide a brief review of the impact of methionine on transition dairy cow health and productivity as well as the underlying alterations in immunometabolism. Specifically, this presentation will discuss results from studies showing the role of methionine supplementation in inducing metabolic changes in (1) plasma AA profile and utilization; (2) liver and skeletal muscle metabolome; (3) hepatic sulfur-containing antioxidant pool and metabolism; and (4) hepatic one-carbon metabolism in vivo and in vitro. In the future, the opportunity and challenge will be to continue to improve our understanding of how functional nutrients affect immunometabolism in dairy cows and effectively apply this knowledge to feeding and management in the dairy industry.

Key Words: methionine, transition period, immunometabolism

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534 The effects of isoenergetic high-starch or high-fat diets on energy and nitrogen partitioning and utilization in late-lactation Jersey cows. D. L. Morris*[1], T. M. Brown-Brandl[2], K. E. Hales[3], K. J. Harvatine[4], and P. J. Kononoff[1], [1]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE,* [2]*Department of Biological Systems Engineering, University of Nebraska-Lincoln, Lincoln, NE,* [3]*USDA Agricultural Research Service, US Meat Animal Research Center, Clay Center, NE,* [4]*Department of Animal Science, The Pennsylvania State University, University Park, PA.*

The objectives were to determine the effects of an isoenergetic high-starch or high-fat diet on energy and N partitioning and utilization. Twelve multiparous Jersey cows (192 ± 11 d in milk) in a crossover design with 28-d periods (24-d adaptation and 4-d collection) were used to compare a high-starch (STA; 30.8% starch, 31.8% NDF, 2.5% crude fat) or high-fat (HFA; 16.8% starch, 41.7% NDF, 4.3% crude fat) diet. Nutrient composition was varied primarily by replacing corn grain in STA with a rumen-inert fat source and cottonseed hulls in HFA. Data were analyzed with a model that included the fixed effect of treatment and the random effect of period, cow, and error. Gross energy content was lower ($P < 0.02$) for STA compared with HFA (4.43 vs. 4.54 ± 0.01 Mcal/kg of DM); while, digestible (2.93 vs. 2.74 ± 0.04 Mcal/kg of DM), metabolizable (2.60 vs. 2.41 ± 0.03 Mcal/kg of DM) and net (1.89 vs. 1.70 ± 0.04 Mcal/kg of DM) energy content were all greater. Tissue energy deposit as body fat tended ($P = 0.12$) to be greater for STA compared with HFA (5.41 vs. 2.51 ± 1.16 Mcal/d). Compared with HFA, STA increased ($P = 0.03$) milk N secretion (141 vs. 131 ± 10.5 g/d) and decreased ($P < 0.01$) urinary N excretion (123 vs. 150 ± 6.4 g/d). Compared with HFA, STA increased ($P < 0.01$) apparent total-tract digestibility of DM (66.7 vs. 61.7 ± 1.06%), energy (66.0 vs. 60.4 ± 0.92%), and 18 carbon fatty acids (67.9 vs. 61.2 ± 1.60%), and decreased ($P < 0.01$) starch digestibility (97.0 to 94.5 ± 0.48%). Compared with HFA, STA tended ($P < 0.15$) to increase milk yield (19.7 vs. 18.9 ± 1.38 kg/d), milk protein content (4.03 vs. 3.93 ± 0.10%), and milk protein yield (0.79 vs. 0.74 ± 0.05 kg/d. In addition, STA compared with HFA decreased ($P < 0.01$) milk fat content (5.93 vs 6.37 ± 0.15%) but did not affect ($P > 0.29$) milk fat yield (1.19 ± 0.09 kg/d) or energy-corrected milk yield (27.2 ± 1.99 kg/d). Our results suggest that STA had a greater net energy content, increased partitioning of energy toward tissue energy deposit as fat, and partitioning of N toward milk secretion and away from urinary excretion.

Key Words: starch, fat, energy

535 Characterization of daily patterns within the rumen metaproteome of Holstein dairy cattle. M. Honan* and S. Greenwood, *The University of Vermont, Burlington, VT.*

Our understanding of rumen microbial diversity and the biochemical pathways performed by specific microbe populations is expanding; however, there is a distinct lack in the use of proteomic techniques to progress our knowledge of shifts in magnitude and pattern of these protein-mediated pathways. It was hypothesized that within a 24-h period, there would be fluctuations of rumen microbial protein abundances due to feed intake-mediated nutrient availability. This study investigated the fluctuations of bovine rumen metaproteome over a 24-h period utilizing 3 mid to late-lactation Holsteins (DIM = 219.3 ± 77.7) that were fed the same TMR *ad libitum*. Rumen fluid was collected on 3 d within a 5-d period at 3 time points throughout the day relative to their first offering of TMR (0h, 4h, and 6h). Samples were pooled within time point within cow across day, analyzed using LC-MS/MS techniques, and analyzed for variations across hour of sampling using PROC MIXED of SAS. A total of 256 proteins were characterized across 12 microbial species, with 21 proteins identified from a variety of 7 species affected by time of collection. From 4 of these species, 8 50S ribosomal protein subunits were affected by hour of sampling, with 7 out of 8 of these subunits increased in abundance over the 3 time points. *Ruminococcus flavefaciens 007c* had lower abundance of a nitrogen fixing protein ($P = 0.04$) as hour of sampling increased, while 3 other species expressed a decrease in abundance of gluconeogenesis-involved proteins GAPDH ($P = 0.04$) and PPDK ($P = 0.003$ and $P = 0.026$) as hour of sampling increased. Protein abundances of cysteine synthase ($P = 0.033$; *F. succinogenes*) and O-acetylhomoserine sulfhydrolase ($P = 0.037$; *T. saccharophilum*), 2 proteins involved in amino acid synthesis, were negatively correlated with hour of sampling. Results suggest that as nutrients become more readily available, microbes shift from conversion-focused biosynthetic routes to more encompassing DNA-driven pathways.

Key Words: proteomics, liquid chromatography-tandem MS (LC-MS/MS)

536 Postpartum ammoniated lactate supplementation altered gene expression to support hepatic metabolism. R. Caputo Oliveira*, S. J. Erb, C. R. Seely, J. L. Woolf, and H. M. White, *University of Wisconsin-Madison, Madison, WI.*

The objective of this study was to examine the effects of fermented ammoniated condensed whey (FACW) supplementation on hepatic gene expression and plasma metabolites related to liver function and inflammation, and on liver triglyceride content (lvTG). Individually fed multiparous Holstein cows were blocked by calving date and randomly assigned to postpartum (1 to 45 d in milk, DIM) isonitrogenous treatments: control (CTL; n = 20) or FACW (2.9% DM of diet as GlucoBoost, Fermented Nutrition, Luxemburg, WI, replacing soybean meal; n = 19). Liver biopsies were performed at 14 and 28 DIM for analysis of mRNA expression by quantitative real-time PCR and lvTG per unit of liver DNA. Blood samples were collected at 3, 7, 14, and 28 DIM for metabolite analysis. Models were fitted using the GLIMMIX procedure of SAS (SAS 9.4); linear predictors included the fixed effects of treatment, DRTC, and interaction, and the random effects of block and cow(treatment). Cows supplemented with FACW had increased ($P < 0.05$) mRNA expression of *G6PC* at 14, and *PC* at 14 and 28 DIM, relative to CTL. Supplementation with FACW did not alter ($P > 0.31$) mRNA expression of the *PCK1,* resulting in a 1.9-fold greater ($P = 0.04$) *PC:PCK1* for FACW cows compared with CTL. There was no evidence ($P > 0.26$) that FACW altered *LDHA* or *LDHB*, although post-transcriptional regulation was not examined. There was no evidence ($P > 0.26$) for altered expression of inflammatory genes. There was marginal evidence ($P = 0.09$) for a 34.8% lower lvTG at 14 DIM, and 10% lower ($P = 0.05$) plasma urea nitrogen with FACW supplementation compared with CTL. There was no evidence ($P > 0.21$) for differences between treatments on serum haptoglobin and plasma alanine aminotransferase, aspartate aminotransferase, or albumin. Increased *G6PC,* together with previously reported increased blood glucose and similar milk lactose output, suggests that FACW provided gluconeogenic precursors. Increased hepatic *PC:PCK1*, together with previously reported decreased plasma β-hydroxybutyrate and the marginal evidence for lower lvTG, suggests greater complete hepatic oxidative capacity of fatty acids in FACW cows.

Key Words: transition cow, steatosis, fatty liver

537 Effects of rumen-protected capsicum alone or in a combination with an artificial sweetener on productivity and fat mobilization in early lactation dairy cows. J. Oh[1], M. T. Harper[1], A. Melgar*[1], S. Räisänen[1], X. Chen[1,2], K. Nedelkov[1,3], E. H. Wall[4], and A. N. Hristov[1], [1]*The Pennsylvania State University, University Park, PA,* [2]*College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou, Gansu, China,* [3]*Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria,* [4]*Pancosma, Geneva, Switzerland.*

The objective of this study was to investigate the effects of rumen-protected capsicum (RPC; Nexulin, Pancosma, Switzerland) alone or a combination with an artificial sweetener (Sucram, Pancosma) on productivity and fat mobilization in early lactation dairy cows. The study involved 15 primi- and 30 multiparous Holstein cows in a randomized complete block design experiment. Cows were blocked based on parity, predicted calving date, and predicted milk yield. Treatments were (15 cows/treatment): (1) control (no additive), (2) 100 mg/cow/d of RPC (RPC), and (3) RPC plus Sucram at 2 g/cow/d (RPCSUC). Three wks before calving, RPC and RPCSUC cows received 100 mg/cow/d RPC. Production data were collected for 8 wks after calving. Dry matter intake (DMI) was restricted from d 8 to 11 post-calving to 70% of prior DMI to experimentally induce subclinical ketosis. Blood samples were collected on d 10 and 11 during feed restriction and analyzed for concentrations of nonesterified fatty acids (NEFA), BHB, and glucose. Data were analyzed using the MIXED procedure of SAS as repeated measures (where applicable); production data were analyzed by week. Treatment did not affect DMI ($P \geq 0.24$; average of 21.6 kg/d; SEM = 1.33). Compared with the control alone, milk yield tended to be increased ($P = 0.06$) by RPC (45.5 vs 42.3 kg/d) in wk 3 (i.e., post-feed restriction), and was or tended to be increased for wk 4 (49.4 vs 44.6 kg/d; $P = 0.02$) and 5 (50.5 vs 46.8 kg/d; $P = 0.11$). Feed efficiency also tended to be greater ($P = 0.08$) for RPC vs the control (2.21 vs 1.99 kg/kg, respectively) in wk 3. Milk composition, body weight change, and body condition score were not affected ($P \geq 0.16$) by treatment. Treatment had no effect ($P \geq 0.64$) on blood NEFA, BHB, and glucose. In this experiment, dietary supplementation of RPC, but not artificial sweetener, appeared to increase milk production and feed efficiency in dairy cows following feed restriction to induce sub-clinical ketosis and had no effect on fat mobilization.

Key Words: capsicum, artificial sweetener, fat mobilization

538 Lactation performance of dairy cows fed rehydrated and ensiled corn grain differing in particle size and concentration in the diet. L. P. Castro[1], J. D. L. Dias[1], D. V. D. Lage[1], E. F. Barbosa[1], R. P. Melo[1], K. Ferreira[1], J. T. R. Carvalho[1], F. F. Cardoso[1], R. A. N. Pereira[3,2], and M. N. Pereira*[1,2], [1]*Universidade Federal de Lavras, Lavras, MG, Brazil,* [2]*Better Nature Research Center, Ijaci, MG, Brazil,* [3]*Empresa de Pesquisa Agropecuaria de Minas Gerais, Lavras, MG, Brazil.*

Rehydrated and ensiled ground corn (REC) has high ruminal starch digestibility, but particle size (PS) and dietary starch concentration (S) can affect starch digestion. An 84% vitreousness hybrid was ground to geometric mean particle sizes (GMPS) of 1,591 (Fine) and 2,185 (Coarse) µm for rehydration (60% DM) and ensiling in 200 L buckets for at least 205 d. The rate of grinding (ton/h) was 3.9 for Fine and 11.7 for Coarse ($P < 0.01$). Ensiling increased ($P < 0.01$) ruminal in situ DM degradation (63.7 vs 34.1%), regardless of particle size. Sixteen Holsteins (152 ± 96 DIM) in 4 × 4 Latin squares (21-d periods) were individually fed a 2 × 2 factorial combination of low or high starch with Fine or Coarse. Variation in the concentration of starch in the diet (29.2 vs 23.5% of DM) was achieved by partial replacement of REC for citrus pulp. Milk yield (31.0 kg/d) and solids secretion did not differ ($P \geq 0.19$). Fine REC tended to reduce DMI and to increase feed efficiency only with the high starch diet ($P \leq 0.10$ for the interaction of S and PS). Total-tract starch digestibility tended ($P = 0.06$) to be reduced by Coarse (96.4 vs 97.2% of intake) and the daily secretion of urinary allantoin tended to be increased ($P = 0.09$). The molar proportions of acetate and butyrate in ruminal fluid were reduced and propionate and isoacids were increased by high starch ($P < 0.01$). Coarse reduced plasma D-lactate concentration when fed in the high starch diet ($P < 0.08$ for the interaction of S and PS). High starch reduced the proportion of daily intake from 1900 to 0700 h ($P = 0.02$) and induced preferential intake of feed particles <8 mm ($P = 0.06$) and greater refusal of particles >19 mm ($P = 0.01$) in the morning. The preferential intake of <8 mm particles tended ($P = 0.06$) to be increased by Fine in the morning. Coarse grinding of REC ensiled for more than 200 d had no effect on lactation performance and intake when starch in the diet was 23.5% of DM. When dietary starch was increased to 29.2%, fine grinding induced short-term intake depression, gain in feed efficiency, and increased plasma D-lactate concentration, suggestive of ruminal acidosis. Funded by FAPEMIG/CNPq.

Key Words: starch digestibility, corn grain particle size, corn grain silage

539 Effects of lecithin supplementation on milk production and circulating markers of metabolic health in Holstein cows. A. B. P. Fontoura*[1], J. E. Rico[1], K. M. Keller[1], A. N. Davis[1], W. A. Myers[1], J. T. Siegel[1], R. Gervais[2], and J. W. McFadden[1], [1]*Cornell University, Ithaca, NY,* [2]*Université Laval, Quebec City, QC, Canada.*

Our objectives were to evaluate the effects of lecithin feeding on milk production and circulating markers of metabolic health in dairy cows fed palmitic acid (PA). In a split-plot Latin square design, 16 Holstein cows (160 ± 7 DIM) were randomly allocated to a main plot receiving a corn silage and alfalfa haylage-based diet with palm fat containing either moderate or high PA content at 1.75% of ration DM (MPA and HPA, respectively; BergaFat F-100 or F-100 HP containing 87 or 98% PA, respectively; Berg + Schmidt, Hamburg, Germany; n = 8/group). On each palm fat diet, deoiled lecithin was top-dressed at 0, 0.12, 0.24, or 0.36% of ration DM in a replicated 4 × 4 Latin Square design. Following a 14 d covariate period, lecithin treatment spanned 14 d with milk and blood collected during the final 3 d. Milk composition and pooled serum markers were measured. The statistical model included the fixed effects of PA type, lecithin level, period, and their interactions as well as the random effect of cow. Lecithin linearly decreased DMI (29.2, 28.7, 27.0 and 27.3 kg/d, $P = 0.01$). In cows fed HPA, lecithin feeding reduced milk fat content (interaction, $P < 0.01$) and tended to decrease milk fat yield (interaction, $P = 0.10$). Although no changes in milk yield were observed, a quadratic reduction in 3.5% FCM was observed with increasing lecithin feeding ($P = 0.001$). Lecithin linearly increased efficiency to produce ECM in cows fed MPA ($P < 0.05$). The proportion of 16C fatty acids (FA) in milk fat decreased linearly with lecithin level, whereas 18C FA increased linearly (e.g., 18:0; $P < 0.01$). De novo FA (<16C) tended to be reduced by lecithin ($P = 0.08$). Lecithin feeding increased MUN, relative to unsupplemented cows (0 vs rest, $P = 0.01$) and linearly increased serum FA concentrations ($P =$

0.01). Although increasing lecithin did not modify liver enzyme levels, several interactions were observed between palm fat type and lecithin level but were not of clinical importance. We conclude that lecithin feeding decreased DMI, increased MUN, and lowered milk fat content; therefore, rumen biohydrogenation was likely modified.

Key Words: lecithin, milk production, palm fat

540 Effects of 3-nitrooxypropanol on enteric methane emission and lactational performance of dairy cows.

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This study examined the effect of 3-nitrooxypropanol (3NOP), an investigational substance, on enteric methane (CH_4) emission and lactational performance of Holstein dairy cows housed in a free-stall barn. Following a 3-wk covariate period, 40 multi- and primiparous cows averaging (±SD) 118 ± 28 d in milk (DIM), 43.4 ± 8 kg/d milk yield, and 594 ± 57 kg body weight (BW) were blocked based on covariate DIM, milk yield, and enteric CH_4 emission and randomly assigned to one of 2 treatments: (1) control, no 3NOP, and (2) 3NOP applied at 60 mg/kg feed dry matter. 3NOP was incorporated in the total mixed ration and fed for 15 consecutive wks. Enteric gaseous emissions (CH_4, carbon dioxide, and hydrogen) were measured using 3 GreenFeed units. Data were analyzed using PROC MIXED of SAS as repeated measures [ar(1) covariance structure] and with block and block × treatment as random effects. Dry matter intake (DMI, 25.6 kg/d; SEM = 0.38), cow BW (617 kg; SEM = 2.89), and BW change (458 g/d; SEM = 50.9) were not affected ($P \geq 0.45$) by 3NOP. Compared with the control, 3NOP decreased ($P < 0.001$) CH_4 daily emission by 26% (302 vs. 411 g/d; SEM = 6.16); decreased ($P < 0.001$) CH_4 emission yield (11.9 vs. 16.4 g/kg DMI; SEM = 0.25), and emission intensity [8.2 vs. 11.5 g/kg energy corrected milk (ECM); SEM = 0.28]. Hydrogen emission increased ($P < 0.001$) by 3NOP from 0.44 g/cow/d (control) to 2.55 g/cow/d (SEM = 0.119). Carbon dioxide emission was not affected by treatment (average of 13.3 kg/cow/d; SEM = 1.16; $P = 0.27$). Treatment had no effect ($P \geq 0.45$) on milk and ECM yields, averaging 38.4 kg (SEM = 0.63) and 37.2 kg/d, (SEM = 0.59), respectively. ECM feed efficiency was also not affected ($P \geq 0.94$) by 3NOP. Milk protein and lactose concentration and yield were not affected ($P \geq 0.26$) by treatment. 3NOP increased ($P = 0.01$) milk fat concentration (4.08 vs. 3.83%, respectively; SEM = 0.064) and tended to increase ($P = 0.07$) fat yield. In this experiment, 3NOP decreased enteric CH_4 daily emission, yield, and intensity without affecting DMI and milk yield, but increased milk fat in lactating dairy cows.

Key Words: 3-nitrooxypropanol, enteric methane, dairy cattle

541 Body condition score prior to parturition is associated with abundance of ruminal bacteria during the peripartal period in Holstein dairy cows.

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The objective of the current study was to evaluate the influence of prepartal body condition score (BCS) on ruminal bacteria during the peripartal period in dairy cows. Twenty-six Holstein dairy cows individually fed were retrospectively classified by BCS at d −30 before parturition into BCS ≤3.25 (LoBCS) and BCS ≥3.50 (HiBCS) groups (n = 13/group). Ruminal contents were collected from each cow 4 h postfeeding via stomach tubing at d −30 and d −15 before expected calving date and at +15 and +30 d in milk (DIM). Ruminal contents were immediately frozen in liquid nitrogen and stored at −80°C. The MIXED procedure of SAS was used for repeated measures analysis of dry matter intake (DMI), milk yield, and bacterial abundance. The fixed effects in the model were BCS and time (week or day), and the random effect was cow. Significance was determined at $P \leq 0.05$. Although DMI did not differ prepartum (BCS $P = 0.65$), HiBCS cows had ~1.5 kg/d greater DMI postpartum ($P = 0.04$). A BCS × day interaction ($P = 0.03$) was observed for postpartum DMI due to greater DMI in HiBCS cows during the last wk of the study. The HiBCS cows produced more milk overall (BCS $P = 0.02$; 42.5 vs. 37.1 kg/d). Results of 16S rRNA copy number of the total ruminal bacterial community indicated that HiBCS cows had greater overall ($P < 0.01$) bacterial density. However, HiBCS cows had lower ($P < 0.05$) overall relative abundance of ruminal bacteria with key roles in cellulose (*Eubacterium ruminantium*, *Fibrobacter succinogenes*), xylan (*Butyrivibrio proteoclasticus*), and starch degradation (*Streptococcus bovis*, *Rumicoccus flavefaciens, Prevotella ruminicola*). Cows in HiBCS also had lower overall abundance ($P < 0.01$) of lactate utilizers (*Selenomonas ruminantium*) and lipolytic bacteria (*Anaerovibrio lipolytica*). Overall, data suggest the existence of some associations between prepartal BCS, postpartal dry matter intake, and ruminal bacteria profiles during the peripartal period.

Key Words: BCS, rumen, bacteria

542 Hindgut microbiome and metabolome in neonatal Holstein heifer calves with divergent residual feed intake during the preweaning period.

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The objective was to determine differences in hindgut microbiome and metabolome in neonatal heifer calves retrospectively grouped as most feed-efficient (M-eff) or least feed-efficient (L-eff). Immediately after birth, 26 Holstein heifer calves were selected randomly for the study. Calves received 3.8 L of first-milking colostrum from the respective dam within 8 h after birth. Calves were housed in individual outdoor hutches bedded with straw, fed twice daily with a milk replacer, and had ad libitum access to a starter grain mix from birth to weaning at 42 d of age. Calves were classified into M-eff (n = 13; RFI coefficient = −0.17 +/−0.03 kg daily solid feed intake (FI)/d) and L-eff (n = 13; RFI coefficient = 0.17 +/−0.03 kg FI/d) based on a linear regression model involving FI, average daily gain (ADG), and metabolic body weight. Fecal samples were collected at d 0 (i.e., at birth before colostrum feeding), 14, 28, and 42 (before weaning) for microbiome and untargeted metabolome analyses using 16s rRNA gene sequencing and LC-MS. The MIXED procedure of SAS 9.4 was used for repeated measures analysis of body measurements, FI, and ADG. Both RFI groups and time (day or week) were considered as fixed factors in the model. Fecal microbiome data were analyzed with QIIME 2 and metabolome data with MetaboAnalyst 4.0. During the preweaning period, M-eff heifers had lower FI ($P < 0.01$) and cumulative fiber intake ($P < 0.01$) compared with L-eff heifers. However, no differences between groups were detected ($P >$

0.05) for body weight, ADG, wither height, hip height, and hip width. Fecal microbiome analysis revealed that M-eff calves had greater ($P < 0.05$) *Proteobacteria*, *Succinivibrio*, and *Aeromonadales* and lower ($P < 0.05$) *Fusobacteria*, *Spirochaetia*, and *Lachnospiraceae*. Furthermore, higher efficiency calves had favorable alterations in hindgut metabolome as indicated by upregulation (fold change > 2.0) of pathways associated with energy production (citric acid cycle), amino acid metabolism (tryptophan and phenylalanine) and vitamin metabolism (folate and biotin). Overall, results indicate greater feed efficiency in preweaned dairy heifers is associated with unique hindgut microbiome and metabolome profiles.

Key Words: residual feed intake (RFI), microbiota, metabolomics

543 Evaluating heat stress response in lactating Holstein cows with supplementation of a feed additive during mid lactation.

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Heat stress (HS) has detrimental effects on lactating cattle especially when they are in a negative energy balance. Feed additives have been shown to mitigate the effects of HS by improving metabolic and immune function. The objective of this study was to evaluate the effect of feeding a dietary supplement (PMI Nutritional Additives, Arden Hills, MN) on the HS response in multi-parturient dairy cows in mid lactation. Two pens of cows at a commercial dairy were fed either control (CON) or additive (FA) at 113 g/cow per d for 2 weeks before arrival. Study cows (n = 12) were balanced in days in milk (DIM), milk production, and parity (111.91 ± 4.85 d, 33.67 ± 0.96 kg/d, and 2.25 ± 0.18). Cows were randomly selected from both groups (6 TRT and 6 CON) and housed in environmentally controlled chambers for 18 d and fed appropriate diet. Cows were subjected to 7 of thermoneutral (TN) conditions, 7 d of HS, and 4 d of recovery (REC) under TN conditions. Dry matter intake (DMI), milk production, and milk composition were measured daily. Rectal temperature (RT) and respiration rate (RR) were measured at peak temperature daily. Blood samples were collected once daily at 1500 h following catheterization on d 4 of TN to d 4 of REC. Serum samples were analyzed for glucose, insulin, blood urea nitrogen (BUN), β-hydroxybutyrate (BHB), and nonesterified fatty acids (NEFA). Results were analyzed using repeated measures in the PROC MIXED of SAS. HS increased RT ($P < 0.0001$), RR ($P < 0.0001$), BUN ($P < 0.0001$), insulin ($P = 0.04$), neutrophil ($P = 0.009$), and water intake ($P = 0.0005$). HS decreased lymphocyte ($P = 0.0008$), DMI ($P = 0.0007$), energy corrected milk (ECM, $P = 0.01$), and 4% fat corrected milk (FCM, $P = 0.02$). FA decreased the feed efficiency ratio ($P = 0.03$). FA had no effect on blood parameters. There was a treatment x environment interaction with cows fed FA having lower feed efficiency ($P = 0.02$) during peak thermal loads than CON. Results of this study suggest that HS exposure had performance and metabolic impacts in mid lactation cows. Supplementation with FA alleviated some of the performance effects associated with HS.

Key Words: heat stress, feed additive, dairy cow

544 Growth performance, metabolic and rumen profile, and health of calves fed condensed whey solubles with starter pellets.

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Our objective was to evaluate growth, metabolic and rumen profile, and health of calves supplemented with condensed whey solubles (CWS) on starter pellets. Forty-eight 2 d-old calves in huts were used in a 12-wk randomized complete block design study. Calves were blocked by breed (33 Holstein, 15 Brown Swiss), sex (30 female; 18 male), and birth date. Treatments were: 1) control (CON) with no supplement, 2) 40 mL/d CWS (CWSL), and 3) 80 mL/d CWS (CWSH) top-dressed on ad libitum-fed starter pellets. Calves were fed 2.83 L of pasteurized milk 2 × /d during wk 1 to 5, 1 × /d in wk 6, and weaned at d 42. Fecal scores (0 = firm, 3 = watery) and respiratory scores from the sum of rectal temperature, cough, ocular and nasal discharge scores were observed daily. Body weights (BW), frame measures, and jugular blood samples were taken 1 d/wk at 3 h post feeding. Rumen samples were taken wk 8 and 12 via esophageal tubing. Data were analyzed using MIXED procedures of SAS 9.4 with repeated measures. Significance was declared at $P < 0.05$ and tendencies were $0.05 \leq P < 0.10$. Total DMI (1.53, 1.64, and 1.67 kg/d; SEM = 0.73 for CON, CWSL, and CWSH, respectively) were similar, but had a treatment by time interaction, tending to increase with CWS post-weaning. Average daily gains (0.68, 0.72, 0.75 kg/d; SEM 0.06) were similar, but BW (69.5, 70.8, 73.2 kg; SEM = 1.59), withers heights (82.9, 84.2, and 84.1 cm; SEM = 0.82), and heart girth (92.7, 93.6, 93.8 cm; SEM = 0.66) tended to increase post-weaning with CWS. Other frame measures and BCS were similar. Health scores were not different, but post-weaning fecal scores (0.11, 0.09, and 0.13; SEM = 0.03) were firmer ($P < 0.01$) for CWSL. Rumen VFA profiles, PUN (18.2, 17.7, and 17.6 mg/dL; SEM = 0.63), glucose (96.4, 100.5, and 104.8 mg/dL; SEM = 1.63), cholesterol (47.1, 49.3, and 51.5 mg/dL; SEM = 2.34), triglycerides (15.8, 15.8, and 16.4 mg/dL; SEM = 1.17) and BHB (35.4, 35.1, and 36.0 mg/dL; SEM = 0.63) were similar. Supplementing CWS improved calf post-weaning intakes, growth, and fecal scores with maintained rumen VFA, and metabolic profile.

Key Words: condensed whey solubles, dairy calf, growth performance

Author Index

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Key Word Index

Numbers following names refer to abstract numbers. A number alone indicates an oral presentation; an M preceding the number indicates a Monday poster, a T indicates a Tuesday poster, and a W indicates a Wednesday poster. Orals are listed first, followed by Monday, Tuesday, and Wednesday posters in numeric order.

The index is created directly and automatically from the submitted abstracts. Efforts have been made to make this index consistent; however, error from author entry contributes to inaccuracies.