W366 Determination of the metabolizable methionine contributions of three different sources of lipid coated methionine. E. Devillard[1], F. Rouffineau[1], and B. Sloan*[2], *[1]Adisseo France, Commentry, France, [2]Adisseo North and Central America, Alpharetta, GA.*

Lysine and methionine (Met) are the most limiting amino acids (AA) for dairy cow production. To supply the required quantities of metabolizable lysine and Met, dairy rations often need to be supplemented with rumen-protected AA. This study aims at quantifying the metabolizable Met contribution of 3 different rumen-protected products: MethioPlus (Soda Feed Ingredients, protection by encapsulation), Mepron M85 (Evonik Degussa, protection by encapsulation) and Smartamine M (Adisseo, protection by pH-sensitive coating) and 2 experimental products that will not be discussed here. The methodology based on mathematically integrating the increases in blood plasma Met levels following a spot dose of product (50 g of Met), was as described by Graulet et al. (2005). The area under the curve (AUC) was thus used to determine the proportion of Met reaching the blood stream. Eight nonlactating Jersey cows were used in a replicated incomplete Latin square design (Cochran and Cox 1962), with 5 periods of one week. The total quantity of product was introduced in the rumen at 2 p.m. via the rumen cannula of cows receiving a diet composed of 75% hay and 25% concentrate. Blood samples were taken before and after the spot dose (at −22, −6, −3, 0, +6, +10, +14, +20, +24, +28, +32, +38, +48, +72 h) for quantification of Met concentration. An ANOVA with repeated measures was performed on the data using the PROC MIXED of SAS/STAT software. The maximum Met concentrations in plasma were observed 24h after the spot dose for Smartamine M (183 µmol/L) or Mepron M85 (49 µmol/L), and after only 14h (43 µmol/L) for MethioPlus, suggesting that product formulation characteristics influence rumen residence time, rate of release in the rumen and postruminally and Met absorption. From the AUC, the proportion of Met reaching the blood stream was calculated at 81% for Smartamine M, which was significantly higher ($P < 0.001$) than those of Mepron M85 and Methioplus, respectively 30% and 21%. These results suggest that products vary greatly in their ability to deliver metabolizable Met to meet requirements.

Key words: metabolizable methionine, rumen-protected amino acids

W367 In vitro degradation of melamine in rumen liquor. T. Calitz and C. W. Cruywagen*, *Stellenbosch University, Stellenbosch, South Africa.*

Melamine contains 667 g/kg N, which makes it an attractive protein adulterant, as it has the ability to inflate the crude protein content of feed- and foodstuffs artificially. Although melamine has been found to be a poor source of nitrogen for ruminants, its rumen degradability has not been determined previously. The current study was done to measure the in vitro degradability of melamine over time. For each of 5 repetitions, melamine (100 mg) was placed in Erlenmeyer flasks (n = 4/treatment) in a water bath at 39°C. Rumen liquor was collected from 4 ruminally cannulated dairy cows and volumes of 100 mL per cow were transferred to the respective flasks. Initial melamine concentrations were thus 1000 mg/L. Flasks were purged with CO2 and fitted with rubber stoppers equipped with one-way gas release valves. The flasks were then transferred to an incubator and samples were incubated for 6, 24 or 48 h at 39°C. Two control treatments were included where rumen fermentation was inhibited by either killing the microbes with the addition of 1 mL of 10% formaldehyde per 100 mL rumen liquor, or by exposing it to air while placing it on ice for 2 h. Erlenmeyer flasks in the control treatments were not incubated and served as a 0 h treatment and also to calculate the recovery rate of melamine after analysis. The total experimental sequence was repeated 5 times in different weeks in a randomized block design. A main effects ANOVA was done on the data with the aid of Statistica version 10. Main effects were treatment, cow and block. Melamine recovery from the control treatments was 91%. When the control treatments were adjusted to 100% recovery (1000 mg/L), melamine concentrations in the incubated samples were 993 mg/L (6 h), 1003 mg/L (24 h) and 1007 mg/L (48 h). Treatment means did not differ ($P = 0.981$). It was concluded that melamine is not degraded in rumen liquor up to 48 h of incubation.

Key words: degradation, melamine, rumen

W368 Characterization of lipase-producing bacteria in the presence of varying energy substrates in vitro. H. D. Edwards*[1], R. C. Anderson[2], R. K. Miller[1], T. M. Taylor[1], M. D. Hardin[3], S. B. Smith[1], N. A. Krueger[2], and D. J. Nisbet[2], *[1]Texas A&M University, College Station, [2]United States Department of Agriculture/Agricultural Research Service, Southern Plains Agricultural Research Center, College Station, TX, [3]IEH Laboratories & Consulting Group, Lake Forest Park, WA.*

Ruminal lipolysis has long been attributed mainly to *Anaerovibrio lipolyticus* and *Butyrivibrio fibrisolvens*. Conversely, *Propionibacterium* species *avidum* and *acnes* are also known to express lipase activity but little is known regarding the contribution of these prominent anaerobes to rumen lipolysis. To further characterize and understand the lipase activity of these 4 different bacteria, each was grown with 4 different energy substrates: olive oil, corn oil, flax seed oil, and glycerol. The bacteria were cultured in triplicate in tubes containing glass beads (which served as a solid support matrix), 6 mL of anaerobic medium containing minerals, vitamins, yeast extract, trypticase, with or without added glucose and with 0.2 mL of the respective triacylglyceride-derived energy substrates. Tubes were incubated horizontally and agitated at 39°C and growth and enzyme activity was stopped when cells reached early log and stationary phase (based from growth curves done before the study). Free fatty acid accumulation was measured colormetrically with the glycerol treatment acting as the negative control in this study. Results were analyzed using a general ANOVA with Tukey's separation of means. Because findings from studies conducted with or without added glucose supported the same conclusions, we present results from studies conducted with added glucose only. Olive oil and flax seed oil promoted the highest ($P < 0.05$) rates of free fatty acid accumulation for all bacteria, averaging 213.84 ± 37.94 and 245.76 ± 34.82 nmol/ml per h, respectively, when compared with corn oil (76.72 ± 36.93 nmol/ml per h). Compared with the other bacteria, *P. avidum* demonstrated the most rapid rates ($P < 0.05$) of lipolysis, which were 649.99 ± 77.86 and 700.02 ± 69.64 nmol/ml per h for cultures grown with olive oil and flaxseed oil, respectively. The results suggest that diets containing a high content of oleic acid and linolenic acid promote high rate of lipolysis in the rumen and *P. avidum* may contribute to a higher amount of lipolysis than previously considered.

Key words: rumen, lipolysis, energy substrates

W369 Exogenous fibrolytic enzymes: Unlocking nutrients from fiber for ruminant production. W. F. J. van de Vyver* and C. W.

Cruywagen, *Stellenbosch University, Stellenbosch, Western Cape, South Africa.*

Exogenous fibrolytic enzymes (EFE) as additives in ruminant feeds are being researched worldwide, but there is a need for a better understanding of the mode-of-action thereof. Four forages, treated with EFE, were evaluated in vitro and at microscopic level, in an attempt to determine the effect of EFE on tissue degradation. For the histological evaluation, weeping love grass and kikuyu leaf material and alfalfa and wheat straw stem material were used. Simultaneously, the forages were incubated in rumen fluid inoculated media for the determination of the in vitro digestibility. The main focus, however, was a quantitative assessment of the degradation of the plant tissue at histological level. The section to slide technique was used to mount plant tissues on microscope slides for incubation in buffered rumen fluid media. Degradation of cell wall components were quantified using image analysis software. The in vitro digestibility data were subjected to a Factorial ANOVA whereas histology data were analyzed with either a Bonferroni or Newman-Keuls multifactorial test, using Statistica 8.1 (2008). In vitro digestibility was significantly higher for EFE treated alfalfa and kikuyu at 24h of incubation ($P < 0.05$). Clear histological differences were observed for all tissue types over the incubation period. Cell wall of the metaxylem of leaf material were thinner for the EFE treated samples at 12h of incubation ($P < 0.05$). There was also a significant thinning effect of EFE on the cell wall of phloem at 12h of incubation for kikuyu as well as the adaxial epidermis at 24h. The abaxial epidermis at 12h was thinner for weeping love grass due to EFE treatment. Excluding the thinner epidermis of EFE treated alfalfa (at 12h incubation, $P < 0.05$), no significant effects of EFE on stem material was observed. It was concluded that image analysis can be useful to quantify changes in cell wall over an incubation period and that the addition of exogenous enzymes could be quantified by this system. There was a definite, subtle thinning effect of EFE on cell wall thickness of plant material which could be indicative of the mode-of-action of EFE.

Key words: fibrolytic enzymes, plant histology, digestibility

W370 Comparison rumen degradability of *Sedilitzia rosmarinus*, *Halocnemum strobilaceum* and *Kochia scoparia* with wheat straw and alfalfa hay. M. Mahmoodi-Abyane*, R. Valizadeh, A. A. Naserian, and A. Koocheki, *Ferdowsi University of Mashhad.*

Rumen degradability of 3 halophyte species including *Sedilitzia rosmarinus*, *Halocnemum strobilaceum* and *Kochia scoparia* were determined and compared with the measure parameters for alfalfa hay and wheat straw samples. In situ rumen degradability was determined at 0, 2, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h after ruminal incubation. The results of ruminal degradability demonstrated that the "a" fraction (rapidly degradable) of *Halocnemum strobilaceum* was significantly highest ($P < 0.05$) among the halophytes and alfalfa hay or wheat straw, whereas the value "b" fraction (slowly degradable) for wheat straw was higher ($P < 0.05$) than other treatments. The "c" fraction (rate of degradation) of *Sedilitzia rosmarinus* and *Halocnemum strobilaceum* was significantly ($P < 0.05$) higher than that for other treatments whereas lowest level of "c" fraction was absorbed in wheat straw sample. Potential degradability (PD) level of *Sedilitzia rosmarinus* (76%) was significantly ($P < 0.05$) higher than other forage whereas lowest of this factor was observed in wheat straw (60%). Also effective degradability (ED) level in *Sedilitzia rosmarinus* (70%) was significantly ($P < 0.05$) highest among the other treatments whereas lowest level of ED was observed in wheat straw (60%). It was concluded that *Sedilitzia rosmarinus* and *Halocnemum strobilaceum* could be relatively a suitable forage for the dry area of many part of the Iranian wilderness.

Key words: rumen degradability, *Sedilitzia rosmarinus*, *Halocnemum strobilaceum*

W371 Comparison rumen degradability of *Phragmites australis*, *Nitraria schoberi* and *Atriplex canescens* species with wheat straw and alfalfa hay. M. Mahmoodi-Abyane*, R. Valizadeh, A. A. Naserian, and A. Koocheki, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran.*

Ruminal degradability of 3 halophyte species including *Phragmites australis*, *Nitraria schoberi* and *Atriplex canescens* were determined and compared with the measure parameters for alfalfa hay and wheat straw samples. In situ ruminal degradability was determined at 0, 2, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h after ruminal incubation. The results of ruminal degradability indicated that the a fraction (rapidly degradable) of *Atriplex canescens* was significantly ($P < 0.05$) highest among the halophytes and alfalfa hay or wheat straw whereas lowest of this fraction was observed in *Phragmites australis*. The value b fraction (slowly degradable) for wheat straw and *Phragmites australis* was significantly ($P < 0.05$) higher than other treatments, respectively. The c fraction (rate of degradation) of *Nitraria schoberi* was significantly ($P < 0.05$) higher than that for other treatments whereas lowest level of c was absorbed in *Phragmites australis* and wheat straw samples. Potential degradability (PD) level of alfalfa (0.6357) and *Atriplex canescens* (0.6065) were significantly ($P < 0.05$) higher than other forage whereas lowest of this factor was observed in *Phragmites australis* (0.4639). Also effective degradability (ED) level in alfalfa (0.5621) and *Atriplex canescens* (0.5366) were significantly ($P < 0.05$) highest among the other treatments whereas lowest level of ED was observed in *Phragmites australis* (0.2663) and wheat straw (0.5995). It was concluded that ruminal degradability of *Atriplex canescens* similar to alfalfa hay and *Phragmites australis* had similar value with wheat straw.

Key words: alfalfa, ruminal degradability, wheat straw

W372 The comparison of chemical composition of *Pragmates australis* ensiled forage by various feed additives. R. Valizadeh, M. Mahmoodi-Abyane*, and A. Salahi, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran.*

The chemical composition of whole plant of *Pragmates australis* silage treated with different feed additives were investigated. The applied treatments were: 1) *Pragmates australis* (Pa) ensiled without additive, 2) Pa + 4% NaOH, 3) Pa + 4% urea, 4) Pa + 10% molasses, 5) Pa + 4% urea + 10% molasses and 6) Pa + 4% urea + 10% molasses + 4% NaOH (on DM basis). The NDF, ADF, CP, and ash content of initial sample and not ensiled forage were 78, 47, 14, and 12.7%, respectively, that significantly ($P < 0.05$) changed while ensiling by the various feed additives. The NDF content of ensiled sample with urea was highest (72.5%) whereas it was lowest in the NaOH treated forage(62.0%). ADF percent of urea treated forage (44.4%) and as well as ensiled sample without additive (44.8%) were higher than other treatment with various feed additives while NaOH treated forage and molasses ensiled forage decreased to 40.1 and 41.6%, respectively. CP content of the urea treated forage (14.1%) was also higher than that for other samples and lowest was observed in NaOH treated forage (9.7%). Ash content of the NaOH treated forage (19.0%) was significantly ($P < 0.05$) higher in caparison with other treatments

whereas lowest one was seen in ensiled sample with urea (12.3%). It concluded that molasses additive had good efficacy on chemical composition of this forage and higher level of this additive supplemented must be beneficial.

Key words: chemical composition, silage, *Pragmates australis*

W373 The comparison of qualitative characteristics of *Pragmates australis* ensiled forage by various feed additives. R. Valizadeh, M. Mahmoodi-Abyane*, and A. Salahi, *Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran.*

The qualitative characteristics of whole plant of *Pragmates australis* silage treated with different feed additives were investigated. The applied treatments were: 1) *Pragmates australis* (Pa) ensiled without additive, 2) Pa + 4% NaOH, 3) Pa + 4% urea, 4) Pa + 10% molasses, 5) Pa + 4% urea + 10% molasses, and 6) Pa + 4% urea + 10% molasses + 4% NaOH (DM basis). Initial sample of forage with 25% DM ensiled and silages opened after 60 d. Comparison of DM content silages after ensiling indicated that treatment without additive had lowest level of DM (26.7%) while forage supplemented with tree additives had highest one (32.3%). pH level was significantly ($P < 0.05$) different among the treatment. Lowest level of pH observed in ensiled sample was with molasses (4.2) whereas urea treated forage (8.3) had highest of pH. Highest level of acidity also observed in sample with molasses (12.6%) whereas the NaOH treated forage (4.1%) had lowest of acidity. Ammonia-N (mg/dl) content of the urea treated forage (63.8) was also higher than that for other samples and lowest was observed in molasses treated forage (4,0). It concluded that fresh *Pragmates australis* could be harvested and treated with the appropriate supplement (molasses) and ensiled for subsequent utilization when the availability of forages is limited.

Key words: qualitative characteristics, *Pragmates australis*, silage

W374 A comparison of methods to analyze physical effectiveness fiber. R. S. Goulart*, L. G. Nussio, A. V. Pirez, J. L. P. Daniel, R. C. do Amaral, and V. P. Santos, *University of Sao Paulo/ESALQ, Piracicaba, Sao Paulo, Brazil.*

The physical effectiveness factor (pef) or effectiveness factor (ef) from fiber sources were evaluated in a 6 × 6 Latin square trial using 6 Nellore steers in a bioassay methods (BM) as recommended by Armentano and Pereira (1997). Laboratory methods (LM) were also performed according to Mertens (1997) and Lammers et al. (1996) to estimate the pef of the fiber sources (pef ≥ 1.18 or pef ≥ 8.0mm, respectively). Six diets were formulated with different fiber contents and sources: negative control (NC) (10% of the NDF from corn silage – CS – in TMR), positive control (PC) (CS with 20% of the NDF in TMR) and 4 diets containing 10% of NDF from CS added with 10% of the NDF from each the following sources: sugarcane (SC), sugarcane bagasse (SCB), soybean hulls (SH) and low oil–cottonseed meal (LOCM). By using the BM, differences in pef were observed ($P \geq 0.05$) between the standard fiber source, CS (pef = 100%) and the following fiber sources: SCB, SC, SH and LOCM (116, 106, zero and 68%, respectively) considering chewing time in min/day. When chewing time in min/kg of DMI was considered as target trait higher values of pef were observed for the SCB (250%), following SC (120%), SH (zero) and LOCM (68%). Ruminal mat consistency calculated by method of Welch (1982) showed the following values of pef: 100, 135, 150, 0, and 61% (CS, SCB, SC, SH and LOCM, respectively). The mean ruminal pH values were utilized to estimate the pe values: 100, 162, 145, 66 and 166% (CS, SCB, SC, SH and LOCM, respectively). Estimated values from the LM showed a larger range when compared with the BM. The pef ≥ 1.18 values were: 95, 60, 88, 71, 87% and the pef ≥ 8.0mm values were: 87, 63, 77, 20, 72% for CS, SCB, SC, SH and LOCM, respectively. The LM showed low or no correlation ($P \geq 0.05$) with any animal response parameter utilized in this study. Values of effectiveness can vary significantly within the same method and across methods (bioassay and laboratory). This study demonstrated that particle size analyses were affected by LM. There is a need to achieve standardization and validation of the method for measuring pef and consequently establish its requirements for beef cattle.

Key words: roughage, byproducts, Nellore

W375 Rumen degradability of sugarcane (*Saccharum* spp.) treated with different hydrolysis agents used in Brazilian farms. S. L. S. Cabral Filho*[1,2], D. C. Pinto[1], and R. A. Mandarino[1], *[1]Universidade de Brasília, Brasília, Distrito Federal, Brasil, [2]Fazenda Experimental Agua Limpa, Brasilis, Distrito Federal, Brasil.*

The feeding sugarcane to cattle has been an alternative for Brazilian farmers in Brazil lowering the costs of production. The processing of sugarcane with hydrolysis agents has been promoted as a way to improve the fiber degradability of sugarcane. The aim of this study was to evaluated changes in effective ruminal degradability of the fiber fraction (ENDFD and EDADF) of non-treated sugar cane (SCNT) and sugar cane submitted to treatments of 5% of NaOH (SCT1), 1.5% of CaO (SCT2) and 5% of urea (SCT3). A dose of treatment for SCT1 was based in the recommendations of commercial products used in Brazil, for SCT2 and SCT3 were based in research results. The ruminal degradability was evaluated by in situ using nylon bags incubated in 2 fistulated cattle. The means were compared with Tukey test ($P < 0.05$) in a randomized design scheme with 3 replicates per treatment. The treatments SCT1 and SCT2, promoted a significant improvement in degradability of the fiber of sugarcane, compared with SCNT ($P < 0.05$). The means were 37, 42, 63 and 37% of ENDFD and 35, 39, 61 and 35% of EADFD, for SCNT, SCT1, SCT2 and SCT3, respectively. The treatment SCT3 resulted in no improvements in degradability ($P > 0.05$). The experiment suggests the adoption of treatment with 5% NaOH or 1.5% CaO, since they improved the degradability of the fiber. However, care should be used because they are, especially NaOH, corrosive compound. More experiments are necessary to evaluate the economic advantages of those treatments.

Key words: forage, fiber, in situ

W376 Effect of dietary fish oil level on selected strains of rumen bacteria in continuous culture fermenters. A. Ishlak*, A. A. AbuGhazaleh, P. Gudla, and D. Hastings, *Southern Illinois University, Carbondale.*

Previous studies have shown that adding fish oil (FO) to cows diet increased vaccenic acid (VA) accumulation in the rumen. Therefore, the objective of this study was to evaluate the effects of FO level on selected strains of rumen bacteria involved in trans fatty acids formation. A single-flow continuous culture system consisting of 4 fermenters was used in a 4 × 4 Latin square design with 4 9 d consecutive periods. Treatment diets were: 1) control diet (53:47 forage to concentrate; CON), 2) CON + FO at 0.50% (DM basis; FOL), 3) CON + FO at 2% (FOM), and 4) CON + FO at 3.5% (FOH). Alfalfa hay and grass hay (4:1 DM basis) were used as forage source. Fermenters were fed treatment diets 3 times daily at 120 g/d. Samples were collected

from each fermenter on d 9 of each period at 1.5, 3 and 6 h post morning feeding and then composited into one sample per fermenter. Data were analyzed as a Latin square using the PROC MIXED of SAS. Preplanned comparisons were linear, quadratic, and FO versus control. Increasing dietary FO level resulted in a linear decrease ($P < 0.01$) in acetate and isobutyrate concentrations. Propionate, butyrate, valerate and isovalerate concentrations were not affected ($P > 0.05$) by treatment diets. Concentrations of C18:0 in fermenters linearly decreased ($P < 0.01$) while concentrations of trans-10 C18:1 and VA linearly increased ($P < 0.01$) as dietary FO level increased. The DNA abundance for *Butyrivibrio fibrisolvens* (64.63, 32.20, 18.54, and 27.04 pg/36ng of total DNA for treatment diets 1 to 4, respectively), *Butyrivibrio* VA subgroup (1.54, 1.34, 0.50 and 0.70 pg/24 ng of total DNA), *Butyrivibrio* SA subgroup (39.79, 38.97, 19.16, and 18.89 pg/18 ng of total DNA) and *Butyrivibrio proteoclasticum* (1.46, 1.25, 0.40 and 0.60 pg/18ng of total DNA) linearly decreased ($P < 0.01$) as dietary FO level increased. Fish oil had no effect ($P > 0.05$) on the DNA abundance for *Anaerovibrio lipolytica* and *Ruminococcus flavefaciens*. In conclusion, FO effects on VA accumulation in the rumen may be explained in part by FO influence on *Butyrivibrio* species.

Key words: fish oil, fatty acids, bacteria

W377 Effects of rumen-protected niacin on lipid metabolism, oxidative stress and production of transition dairy cows during summer in Wisconsin. K. Yuan*[1], R. Shaver[1], S. Bertics[1], M. Espineira[1], and R. Grummer[2], *[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, [2]Balchem Corporation, New Hampton, NY.*

The objective of this study was to evaluate the effects of a rumen-protected niacin product (RPN; NiaShure, Balchem Corp., New Hampton, NY) on lipid metabolism, oxidative stress and performance of transition dairy cows during the summer in Wisconsin. Thirty multiparous Holstein cows were paired according to expected calving date and randomly assigned to either RPN at 12/g/cow/d or control (C) un-supplemented diets. Treatment diets were fed from 21 d before expected calving through 21 DIM. Ambient temperature and humidity were monitored weekly to calculate temperature-humidity index, and individual cow rectal temperatures were measured weekly to characterize heat stress conditions during the experiment. Blood samples were taken on d −21, 1, 7, 14, and 21 relative to calving for analyses. Data were analyzed for a randomized complete block design using Proc Mixed of SAS with repeated measures. Pre- (10.2 vs. 11.7 kg/d) and postpartum (15.5 vs. 15.9 kg/d) DMI, milk yield (33.4 vs. 33.3 kg/d), milk fat percent (4.87 vs. 4.54%), milk protein percent (3.19 vs. 3.08%), linear somatic cell score (3.40 vs. 2.34) and rectal temperature (38.7 vs. 38.8°C) were unaffected ($P > 0.05$) by treatment. While body weight and body condition score decreased ($P < 0.01$) during the experimental period, no treatment effects ($P > 0.05$) were observed. Time ($P < 0.01$) and time × treatment ($P < 0.05$) effects were observed for plasma NEFA. On d 1 postpartum, NEFA reached 1138 ± 80 μEq/L for control cows compared with 698 ± 80 μEq/L for RPN cows. Cows in RPN group tended to ($P < 0.10$) have lower plasma NEFA concentrations than control cows on d 7 and 14 postpartum. Plasma glucose concentrations were similar ($P > 0.05$) for RPN and C. Plasma SOD was unaffected ($P > 0.05$) by treatment; a trend for a time effect ($P < 0.10$) on SOD was observed. In conclusion, under summer conditions in Wisconsin, dietary supplementation with 12g/d per cow RPN decreased plasma NEFA concentrations, but did not affect the anti-oxidant enzyme SOD, lactation performance or body temperature of transition dairy cows.

Key words: transition cows, rumen-protected niacin, oxidative stress

W378 Using rumen microbes for consolidated bioprocessing to convert plant fiber to ethanol or other biofuels. R. A. Kohn* and S.-W. Kim, *University of Maryland, College Park.*

Microorganisms that live in the cow's rumen orchestrate the fastest biological degradation of biomass on earth. For example, a strain of *Ruminococcus albus* readily degrades ligno-cellulose without pretreatment to produce acetate and ethanol. The objective of this study was to isolate fiber-digesting microorganisms from the rumen that produce ethanol to a high concentration. Rumen fluid was collected from a fistulated cow and enriched for fiber digesting microbes by incubating in media with timothy grass hay as the main substrate. Every 3 to 5 d a portion of the culture was transferred to new media. For some enrichments, the media contained 6% or 10% ethanol by volume. Individual strains that grew on Avicel, filter paper or cellobiose were isolated on agar from diluted enrichments. Several isolates could digest various types of biomass (e.g., cellulose, hemicellulose, grass) and convert it directly to ethanol. Microbial cultures from the rumen converted 1% cellobiose to 0.5% ethanol increasing ethanol concentration from initial 6.0% to more than 6.5%. A second addition of cellobiose further increased ethanol to more than 7.0%. The 16s rDNA sequences of 20 strains that converted cellobiose to ethanol were >97% homologous with at least one of *Clostridium bifermentans*, *C. sordelli*, *C. sporogenes*, *Enterococcus casselflavus*, *E. muntii*, *E. sangunicola*, *E. faecium*, *E. lactis*, *Pediococcus acidilactici*, *Lactobacillus mucosae*, or *Staphylococcus epidermidis*. Some of these bacteria also produced 1-butanol or 1-propanol from plant fiber. Additional bacteria (*Enterococcus avis*) produced high concentrations of alcohols from H_2 and CO_2 or CO. Microorganisms like these can produce ethanol directly or indirectly from waste biomass or grass (patent pending). The biomass is sterilized with heat and pressure and then simultaneously digested and fermented to ethanol.

Key words: biofuel, fiber digestion, cellulosic ethanol

W379 Fiber-digesting rumen bacteria that predominantly produce propionate or butyrate. S.-W. Kim* and R. A. Kohn, *University of Maryland, College Park.*

Most known fiber-digesting bacteria from the rumen of cattle produce acetic acid as a major end product. The present investigators isolated 2 new species of rumen bacteria that primarily produce propionate or butyrate directly from cellulose. When one strain was incubated with Avicel, 45% of the NDF disappeared within 4 d incubation (n = 2). A control culture of *Ruminococcus albus* (strain 7) resulted in 66% NDF disappearance in the same time frame (n = 2). The molar percentages of VFA produced by the new strain were 45% acetate, 55% propionate and 0% butyrate. A second bacterial strain digested 65% of Avicel NDF in 4 d (n = 2), and produced the molar percentages of VFA of 1.3% acetate, 3.5% propionate, and 86.2% butyrate. Both strains were gram-positive rods and no spores were observed. The propionate-producing strain was 96.08% homologous with *Selenomonas ruminantium*, and the butyrate-producing strain was 94.31% homologous with *Clostridium bifermentans* on the basis of 16S rDNA sequence. Neither closest-related species is known to digest cellulose and homology of 16S rDNA less than 97% suggests the isolates are members of unique species. Further characterization is needed. We propose that such microbes may be useful as probiotics or for conversion of plant fiber to VFA to be used as feeds or to produce other bioproducts.

Key words: fiber digestion, butyrate, propionate

W380 The combination of garlic oil and cinnamaldehyde modify rumen fermentation profile reducing methane production. P. W. Cardozo*[1], M. Blanch[1], M. D. Carro[2], and M. J. Ranilla[2], *[1]Novus International Inc., St. Charles, MO, [2]Deptartamento de Producción Animal, Universidad de León, León, Spain.*

The objective of this study was to analyze the effects of 3 doses (200, 300 and 400 mg/L of product) of NEXT Enhance 300 (NE300; containing cinnamaldehyde and diallyl disulfide) on in vitro ruminal fermentation. Batch cultures (120 mL serum bottles) of mixed ruminal microorganisms (BCRM) were used to test the effects of the additive. Three hundred mg of 60:40 alfalfa hay:concentrate diet was used as a basal substrate. The rumen fluid inoculum was obtained from 4 rumen-cannulated Merino sheep fed the same diet incubated in BCRM, mixed and strained through 4 layers of cheesecloth into an Erlenmeyer flask with an O_2-free headspace. Particle-free fluid was mixed with the buffer solution (no trypticase added) in a proportion 1:4 (vol/vol) at 39°C under continuous flushing with CO_2. Thirty mL of buffered rumen fluid were added into each bottle under CO_2 flushing and were sealed with rubber stoppers and aluminum caps, and incubated at 39°C for 24 h. After incubation total gas production was measured, and a gas sample was removed for methane production. Bottles were then uncapped, the pH was measured immediately, and samples for volatile fatty acid (VFA) and ammonia-N analyses were taken. Incubations were repeated on 4 different days to allow statistical analysis of results. Differences were declared at $P < 0.05$. Doses of 400 mg/L of NE300 decreased total VFA production and apparently fermented organic matter compared with control (CTR, no additive), thus indicating some inhibition of ruminal fermentation. NE300 at 200 mg/L reduced acetate:propionate ratio, methane production and methane/VFA ratio compared with CTR. NE300 at 300 mg/L reduced ammonia-N concentrations, methane production, acetate proportion, and acetate:propionate ratio, and increased propionate proportion compared with CTR. In conclusion, NE300 at 300 mg/L decreased methane production and increased propionate proportion without affecting total VFA production, and this would indicate a higher supply of energy for the host animal.

Key words: cinnamaldehyde, garlic oil, methane

W381 Ruminal kinetics of the diets with increasing levels of crude propane-1,2,3-triol. R. Mello*[1], C. M. M. Bittar[2], L. A. M. A. da Costa[3], R. C. de Araújo[2], and A. L. Abdalla[2], *[1]Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil, [2]Universidade de São Paulo - Escola Superior de Agricultura 'Luiz de Queiroz', Piracicaba, São Paulo, Brazil, [3]Universidade Federal de Roraima, Boa Vista, Roraima, Brazil.*

The objective was to assess the effect of different levels of propane-1,2,3-triol in the diets on rumen fermentation kinetics. Treatments were 0, 3, 6, 9, 12, 15, 18, 21 and 24% addition levels of crude propane-1,2,3-triol, co-product derived from the production of biodiesel fuels with palm oil (*Attalea maripa*), replacing corn on dry matter of the diets. A randomized block experimental design with 9 treatments and 3 replications was used, considering the incubation as blocking criterion. The ruminal kinetics was analyzed by in vitro gas production technique. Substrates were incubated with ruminal fluid buffered in triplicate. Gas production was followed over time (72h) in an automated system by radiofrequency (Ankom). Data were fitted by dual-pool logistic model and parameters estimated through the Gauss-Newton algorithm implemented in the NLIN procedure of SAS® software. The increase of propane-1,2,3-triol resulted in longest lag time: Lag (h) = 0.220+0.203x; $r^2 = 0.91$; i.e., a delay of 12 min in the latency period to each 1% of propane-1,2,3-triol. The maximum volume of gas produced (mL per gram of incubated organic matter) by degradation of soluble fraction ($V_1 = 84.71–2.415x$; $r^2 = 0.88$) and insoluble potentially degradable fraction ($V_2 = 78.62–2.264x$; $r^2 = 0.87$) reduced, while the specific rates of gas production by degradation of soluble fraction ($k_1 = 0.106$) and insoluble potentially degradable fraction ($k_2 = 0.031$) were constant as the propane-1,2,3-triol level raised in the diets. Total volume of gas produced also reduced, can be represented by equation: VT (V_1+V_2) = 163.34–4.680x ($r^2 = 0.99$). This suggests that propane-1,2,3-triol has an effect in vivo glycogen to be absorbed directly (intact) or indirectly (as propionate) by the ruminal epithelium. Volume of methane produced (mL/g OM) also decreased with increase of propane-1,2,3-triol in the diets ($CH_4 = 43.8633–0.3514x$; $r^2 = 0.56$). Thereby, addition of propane-1,2,3-triol can help in mitigation enteric methane and improve energy supply. Thus, the inclusion of propane-1,2,3-triol in ruminant diets may be an important alternative to mitigate greenhouse gas emissions.

Key words: 1,2,3-propanetriol, glycerol, glycerin

W382 Effect of various semi-arid medicinal plant essential oils on in vitro ruminal methane emission and feed fermentation efficiency. H. Jahani-Azizabadi*[1], M. Danesh Mesgaran[1], A. R. Vakili[1], and K. Rezayazdi[2], *[1]Dept. of Animal Science, Excellence Center for Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran, [2]Dept. of Animal Science, Faculty of Agriculture, University of Tehran, Karaj, Tehran, Iran.*

The objective of the present study was to investigate the in vitro effect of some semi-arid medicinal plant essential oils on ruminal methane emission and feed fermentation efficiency (FFE). A mixed diet of alfalfa hay: concentrate (50:50, based on DM) was provided. It was then ground to pass through a 1- mm screen. Approximately, 500 mg of the diet alone (as control) or plus essential oil of cinnamon, dill, oregano or peppermint (100 µL/ g DM) were placed into a 125 mL serum bottle (n = 6) containing 50 mL of buffered-rumen fluid (2:1). Rumen fluid was obtained from 3 ruminally fistulated sheep (49.5 ± 2.5 kg, body weight), before the morning feeding. Bottles were placed in shaking water bath for 24 h at 38.5°C. Gas produced of each bottle was recorded using a pressure transducer and then sampled. Gas pressure was converted into volume using an experimentally calibrated curve. Then, bottle content was filtered (42 µm) and residual was dried (60°C, 48 h) to determine dry matter disappearance (DMD). Data were statistically analyzed using SAS (V. 9/1) and Dunnett's test was used to compare the means ($P < 0.05$). Feed fermentation efficiency was estimated as FFE = DMD (g/kg)/cumulative gas (ml) produced at 24 h. Methane content of the produced gas was determined using gas chromatography procedure. Results indicated that these essential oils caused a significant ($P < 0.05$) decrease in methane and total gas produced over 24 incubation compared with those of the control (Table 1). The essential oil of Dill enhanced FFE ($P < 0.05$) compared with that of the control. Present results demonstrated a positive effect of the essential oils on ruminal fermentation pattern.

Table 1. In vitro effect of medicinal plant essential oils on total gas produced, methane emission and feed fermentation efficiency

Item	Control	Cinnamon	Dill	Oregano	Peppermint	SEM
Total gas (ml/ g DMD)	276.1	115.4 *	253.1 *	186.5 *	187.8 *	0.61
Methane (ml/ g DMD)	41.28	13.97 *	36.89 *	24.28 *	23.61 *	4.8
FFE	6.1	10.6 *	5.9	6.0	6.5	0.5

*Within a row, means with an asterisk differ significantly from the control (P< 0.05).

Key words: methane, essential oil, fermentation efficiency

W383 Rumen parameters and digestibility of diets with different levels of crude propane-1,2,3-triol. R. Mello*[1], C. M. M. Bittar[2], L. A. M. A. da Costa[3], P. B. Costa[4], J. K. Kirinus[1], and J. L. Nörnberg[1], *[1]Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul, Brazil, [2]Universidade de São Paulo - Escola Superior de Agricultura 'Luiz de Queiroz', Piracicaba, São Paulo, Brazil, [3]Universidade Federal de Roraima, Boa Vista, Roraima, Brazil, [4]Universidade Estadual do Oeste do Paraná, Marechal Cândido Rondon, Paraná, Brazil.*

The objective was to assess the effect of increasing levels of propane-1,2,3-triol in the diets on rumen fermentation parameters and digestibility. Treatments were 0, 3, 6, 9, 12, 15, 18, 21 and 24% addition levels of crude propane-1,2,3-triol, co-product derived from biodiesel production with palm oil (*Attalea maripa*), replacing corn on DM of the diets. A randomized block experimental design with 9 treatments and 3 replications was used, considering the incubation as blocking criterion. Substrates were incubated with ruminal fluid buffered in triplicate. The digestibility was evaluated after 48 h and ruminal parameters were measured after 72 h of in vitro incubation. Data were analyzed in the SAS software. The table below shows the least squares means of dependent variables. The acetate and butyrate concentrations, acetate: propionate ratio, in vitro true digestibility (IVTD) of DM, organic matter (OM) and NDF coefficients decreased ($P < 0.05$); and the pH values increased ($P < 0.05$) as the propane-1,2,3-triol level raised in the diets. Thus, the inclusion of propane-1,2,3-triol with 35.6% purity in substitution of non-fibrous carbohydrates on DM of ruminant diets negatively affect the ruminal fermentation parameters and digestibility, but can be an important alternative of destination to the surplus generated from biodiesel chain.

Table 1. Least squares means

Variables	*P*-value	Equation	r^2
pH	0.0016	5.7953+0.0144x	0.92
NH3-N, mg/dL	0.7898	17.7	-
Microbial protein, mg/L	0.0859	273.0	-
C2, mM/mL	0.0106	37.6280-0.5702x	0.51
C3, mM/mL	0.9017	24.0	-
C4, mM/mL	0.0288	7.9102-0.0893x	0.93
VFA, mM/mL	0.0699	64.8	-
C2:C3	0.0003	1.6237-0.0272x	0.66
IVTD-DM, %	0.0001	77.7867-0.2322x	0.91
IVTD-OM, %	0.0001	75.3363-0.3033x	0.93
IVTD-NDF, %	0.0001	22.0815-0.6083x	0.80

Key words: 1,2,3-propanetriol, glycerol, glycerin

W384 Dose response effects of a garlic oil chemical compound propyl-propyl thiosulfate (PTSO) on rumen microbial fermentation in a dual flow continuous culture system. A. Foskolos*[1], A. F. De Souza[1], M. Rodriguez-Prado[1], A. Ferret[1], D. Bravo[2], and S. Calsamiglia[1], *[1]Animal Nutrition, Management and Welfare Research Group, Universitat Autònoma de Barcelona, Bellaterra, Spain, [2]Pancosma, Geneva, Switzerland.*

Oxy-propyl-thiosulphate (PTSO) is an active molecule purified from garlic bulb. The objective of this experiment was to investigate the effects of increasing doses of PTSO on ruminal fermentation in vitro. Eight dual flow continuous culture fermentors inoculated with rumen liquid from a dairy cow were used in 2 replicated periods (blocks). Temperature (39 ◦C), pH (6.4), and liquid (0.10/h) and solid (0.05/h) dilution rates were maintained constant. Fermenters were fed 95 g DM of a diet (21.6% corn silage, 43.6% dehydrated alfalfa, 11.4% soybean meal, 31.6% corn grain and 0.83% vitamin-mineral mix, DM basis) in 3 equal portions daily and treatments were no additive (CTR) and 50, 100 and 150 mg/L of PTSO. Each experimental period consisted of 5 d for adaptation and 3 d for sampling. Samples were collected 2 h after the morning feeding and from the 24-h effluent of the 3 sampling days. Results were analyzed with PROC MIXED and significance was declared at $P < 0.05$. Contrasts were used to analyze for linear, quadratic and cubic responses. Total VFA and molar proportions of acetic and propionic acids concentrations and the acetate to propionic acid ratio responded quadratically with higher total VFA and propionic acid and lower acetic acid concentrations and acetic to propionic ratio in the intermediate doses. Branch-chained VFA decreased linearly by increasing doses of PTSO and ammonia-N concentration was not affected by treatments. In the samples from the 24-h incubations, only the total VFA and BCVFA concentrations responded quadratically and linearly by increasing dose of PTSO, respectively. Results suggest the potential of PTSO to modify rumen fermentation in a direction consistent with better energy utilization.

Key words: essential oils, garlic, fermenters

W385 Estimation of protein fractions of tropical grasses by near infrared reflectance spectroscopy. R. G. Basurto[1], G. Buendia-Rodriguez[1], E. R. Ramírez[1], M. A. Barron[2], J. J. G. Bustamante[3], R. E. Santos[4], J. J. M. Maldonado[5], and S. S. Gonzalez-Muñoz*[6], *[1]CENID Fisiologia Animal-INIFAP, Queretaro, Mexico, [2]CE Huimanguillo-INIFAP, Tabasco, Mexico, [3]CE Santiago Ixcuintla-INIFAP, Nayarit, Mexico, [4]CE Iguala-INIFAP, Guerrero, Mexico, [5]CE Rosario Izapa-INIFAP, Chiapas, Mexico, [6]Colegio de Postgraduados, Montecillo, Estado de Mexico, Mexico.*

The aim of the study was to investigate the use of near infrared reflectance spectroscopy (NIRS) as an alternative method to estimate crude protein (CP), degradable (DIP) and undegradable (UIP) intake protein of tropical grasses. A total of 945 samples of 13 species were collected by clipping at different ages of re-growth (28, 42, 56, 70 and 84 d) in plots in 4 states (Chiapas, Guerrero, Nayarit and Tabasco) in Mexico. The grass species included were *D. aristatum*, *C. dactylon*, *H. altissima*, *U. brizantha*, *D swazilandensis*, *C. plectostachyus*, *U. maximum*, *B. humidicola*, *C. echinatus*, *A. gayanus*, *B. brizantha* x *ruziziensens*, *D. eriantha* and. DIP was estimated as the protein fraction that disappeared after incubation with a protease ofand UIP was the remaining protein in the sample. Samples were scanned using a spectrophotometer Nicolet FT-IR 6700 (Thermo Fisher Scientific, Inc.) over a wavelength range of 1000 to 2500 nm in reflectance. Data were stored as log (1/R) at intervals of 4 nm. Calibration equations were developed using modified partial least squares with the TQ Analyst

program (v8.0). The selection of the equations was based on: the coefficient of determination of calibration (R^2cal), the minimization of the standard error of calibration (SEC), the standard error of cross validation (SECV), and the ratio SECV/SD. If the ratio was >0.33, then calibration had a low predictive power. The best NIRS models obtained are shown in Table 1. It is concluded that NIRS calibration for PC has good precision, but DIP and UIP calibrations are less precise.

Table 1. Laboratory data and calibration and validation statistics of NIRS models

ITEM (% of DM)	N	Mean	Range	SD[1]	R^2cal	SEC	SEP	SECV	Ratio
CP cal[2]	675	7.98	2.7 - 20.7	3.01	0.94	0.79			
CP val[3]	244	7.91	3.6 - 18.7	2.85			0.90	0.94	0.31
DIP cal	688	4.49	1.1 - 11.2	1.93	0.86	0.71			
DIP val	216	4.63	1.1 - 11.5	1.92			0.83	0.84	0.43
UIP cal	680	3.44	1.0 - 8.6	1.32	0.90	0.42			
UIP val	241	3.39	1.2 - 7.5	1.20			0.48	0.45	0.35

[1]SD = standard deviation.

[2]CPcal=Calibration set.

[3]CPval=Validation set.

Key words: NIRS, protein degradation, tropical grasses

W386 Commodity blood meal variation: digestible RUP and amino acids. R. Brown*[1], D. Stucker[1], J. R. Knapp[2], and N. R. St-Pierre[3], *[1]Venture Milling, Salisbury, MD, [2]Fox Hollow Consulting, LLC, Columbus, OH, [3]The Ohio State University, Columbus.*

The objective was to determine the variation in nutrient availability of commodity blood meal as a source of rumen undegradable protein (RUP). The nutritional value of RUP sources is based on their ability to deliver amino acids for absorption in the small intestine and is affected by the protein content, the rumen degradability of the protein, and the post-ruminal digestibility of the RUP. To date, a modification of the procedure of Calsamiglia and Stern (1995) appears to be the best in predicting the nutritional value of ingredients rich in RUP. This 3-step procedure consists of an in sacco incubation in rumen-fistulated cows followed by sequential in vitro protease digestions with pepsin and pancreatin. The residues from the incubation and digestions can be analyzed for amino acids, and amino acid digestibilities determined. The procedure has been modified in our laboratory by partial standardization of enzymes, use of fuzzy standards, and Bayesian statistics to adjust for inter-assay variation. Amino acid analyses were performed by the University of Missouri Agricultural Experiment Station Chemical Laboratories. Commodity blood meal samples (n = 265, porcine and bovine) were obtained over the past 5 years from commercial sources across the US The results using this procedure showed reasonably consistent crude protein contents (90.1 ± 3.7%, mean ± S.D) on an as fed basis. However, RUP (% of CP) was more variable (76.8 ± 14.8%CP) and RUP digestibility was highly variable (64.6 ± 23.1%RUP). No relationship between RUP and RUP digestibilities was observed. Also, average RUP digestibility was lower than the 80% reported for ring-dried blood meal in the 2001 NRC Requirements for Dairy Cattle. Amino acid digestibilities were generally similar to RUP digestibility (65 ± 24%), with the exception of lysine digestibility, which was consistently lower (56 ± 27%). Lysine digestibility approached 0% at RUP digestibility of 20%. These data provide a significant improvement in our knowledge regarding the digestibility and nutritional value of commodity blood meal and should improve feed library values found in ration formulation software currently used throughout the dairy industry.

Key words: blood meal, lysine, RUP

W387 Tannin content and rate of ruminal protein degradation of legume hays. S. Colombini*[1], G. A. Broderick[2], J. H. Grabber[2], and W. K. Coblentz[3], *[1]University of Milan, Milan, Italy, [2]U.S. Dairy Forage Research Center, Madison, WI, [3]U.S. Dairy Forage Research Center, Marshfield, WI.*

This study evaluated ruminal protein degradation rates of legume hays that varied in tannin content. Two cuttings of 5 varieties of birdsfoot trefoil (*Lotus corniculatus*) that were selected for different tannin contents, but similar NDF and CP, and Spredor-4 alfalfa (control) were conserved as hay. Samples were ground (1 mm) and analyzed for chemical composition and tannin content. Protein degradation rate was determined using ruminal inocula in the Michaelis-Menten inhibitor in vitro method; 3 incubations were performed. Extent of degradation was estimated from net release of N in ammonia (phenol-hypochlorite colorimetry) plus amino acids and small peptides (o-phtaldialdehyde colorimetry). Samples also were incubated in situ to estimate RDP. Data were analyzed using mixed procedure of SAS and are in the table. There were differences among trefoils in tannin content with the greatest values for Dewey and Goldie and lowest for Exact. Within cut, alfalfa had the most rapid degradation rate and Exact had the numerically greatest rate among trefoils. Rate was significantly affected ($P < 0.001$) by variety and cutting; there also was a variety*cutting interaction ($P = 0.017$). Regression between degradation rate and tannin content was: $y = -0.0027 (\pm 0.0007) x + 0.231 (\pm 0.019)$ ($r^2 = 0.61$). Regression between in situ RDP and tannin content was: $y = -0.194 (\pm 0.024) x + 84.0 (\pm 0.68)$ ($r^2 = 0.87$). Soluble N content must be determined to compute RDP within the in vitro method. Degradation rates were more rapid for second cutting hays; this may be explained partly by greater NDIN content in hays from first vs. second cutting (21 vs. 18% of total N).

Table 1. Results

Variety	Cut	Tannin (g/kg DM)	NDF (% DM)	Total N (% DM)	NDIN (% total N)	In vitro rate(/h)
Dewey	1	36.8	36.7	3.05	26.9	0.126
Georgia	1	23.0	37.1	3.07	20.8	0.140
Exact	1	15.6	38.2	3.13	18.2	0.150
Lotanova	1	27.8	38.2	3.00	20.7	0.134
Goldie	1	36.7	38.1	2.91	23.2	0.137
Alfalfa	1	0	42.0	3.11	16.6	0.207
Dewey	2	40.2	39.6	2.88	21.5	0.141
Georgia	2	27.0	38.8	3.23	15.2	0.149
Exact	2	20.1	38.3	3.30	18.8	0.173
Lotanova	2	31.6	36.8	3.30	16.3	0.172
Goldie	2	38.8	37.9	3.20	20.5	0.150
Alfalfa	2	0	38.7	3.16	15.4	0.290

Key words: protein degradation, birdsfoot trefoil, tannins

W388 Evaluation of acid-insoluble ash and indigestible neutral-detergent fiber as total tract digestibility markers. C. Lee*, A. N. Hristov, T. Cassidy, and K. Heyler, *Pennsylvania State University, University Park.*

The objective of this experiment was to evaluate acid insoluble ash (AIA) and indigestible NDF (INDF) as intrinsic digestibility markers in comparison with total fecal collection (TC). The experiment was part of a larger experiment, which involved 8 Holstein cows (102 ± 28 DIM; 26.0 ± 0.79 kg/d DMI; 40.9 ± 1.46 kg/d milk yield). The experimental design was a replicated 4 × 4 Latin square with the following treatments: 15.6% crude protein (CP) diet (HighCP), 14.0% CP diet (LowCP), 14.0% CP diet supplemented with a ruminally protected Lys (AminoShure-L, 100 g/cow/d; LowCPLys), and 14.0% CP diet supplemented with ruminally–protected Lys plus a ruminally–protected Met (Mepron, 24 g/cow/d; LowCPLysMet). Each period consisted of 14 d of adaptation and 7 d of sample collections. Total feces were collected for 5 consecutive days during each period. Composite TMR and fecal samples were analyzed for nutrients, AIA (digestion with 2 N HCl) and INDF (12 d ruminal incubation in 25μm pore size bags). Apparent total tract digestibilities of all nutrients were greater for AIA compared with INDF and TC (Table 1). Digestibility estimated using INDF or TC were not different. There was method × treatment interaction (TC and INDF) for all nutrients (P = 0.02 to 0.07), except CP. Examination of the method × treatment mean comparisons revealed no significant differences between TC and INDF. In conclusion, using AIA as a digestibility marker yielded lower fecal output estimates and consequently greater apparent digestibility values for all dietary nutrients compared with TC or INDF. Apparent total tract digestibility of nutrients did not differ between TC and INDF. In the conditions of this experiment, INDF was a more appropriate intrinsic digestibility marker than AIA.

Table 1. Comparisons of methods for determining total tract apparent digestibility

	Method				
Item	TC	INDF	AIA	SEM	*P*-value
DM	61.0[b]	59.4[b]	67.9[a]	1.10	<0.001
OM	62.2[b]	60.8[b]	69.0[a]	1.13	<0.001
NDF	40.4[b]	38.2[b]	51.1[a]	1.21	<0.001
ADF	35.5[b]	33.0[b]	47.0[a]	1.35	<0.001
CP	52.4[b]	50.4[b]	60.8[a]	1.66	0.002

Key words: dairy cow, digestibility, intrinsic marker

W389 Nutritional value of *Smallanthus sonchifolius* and *Moringa oleifera* tropic forage as alternative in ruminant feeding. L. C. Bernal Bechara*, *Universidad de La Salle, Bogotá, Colombia.*

The aim objective was to evaluate the nutritional quality of *Smallanthus sonchifolius* and *Moringa oleifera* to determinate their physical and chemical properties as a tool to predict its nutritive value. Two treatments were evaluated: a. *Smallanthus sonchifolius* leaves and b. *Moringa oleifera* leaves; with 4 replications per forage type. Physical properties evaluated were density (g/ml), solubility (%), the water holding capacity (g/g) and buffering capacity (meq). The samples were reduced to a particle size of 1 mm. Chemical fractions were analyzed for dry matter (DM, %), crude protein (CP, %), neutral detergent fiber (NDF, %), acid detergent fiber (ADF, %), ether extract (EE; %), total digestible nutrients (TDN,%), net energy NE of lactation, maintenance and gain (NEl, NEm, NEg; Mcal/kg) and in vitro digestibility of dry matter IVDDM (%). A completely randomized was evaluated experimental design. The data of the selected variables was analyzed using by procedure a GLM model and differences between means treatments were analyzed using Tukey in the SAS Statistical package. Density, water absorption capacity and buffering capacity did not difference (P > 0.05) between forages types, but solubility was greater (P < 0.001) in *Smallanthus sonchifolius* (14%) than *Moringa oleifera* (7%). There was no difference in DM, CP and EE between forages, but NDF and ADF were greater in *Moringa oleifera* than *Smallanthus sonchifolius* (61.24% vs. 42.77% FDN; 38.96 vs. 26.33% FDA, respectively). TDN, net energy and IVDDM was in *Smallanthus sonchifolius* than *Moringa oleifera* (69.47 vs. 60.56% TDN; 57.6 vs. 48% IVDDM; 1.5 vs. 1.3 Mcal/kg NEl; 1.7 vs. 1.4 Mcal/kg NEm; 1.0 vs. 0.7 Mcal/kg NEg). These physical and chemical characterization results suggest that leaves of *Smallanthus sonchifolius* are potential forage to be used in cattle feeding, because of its low fiber content and greater IVDDM than *Moringa oleifera*.

Key words: *Moringa oleifera*, nutritional quality, *Smallanthus sonchifolius*

W390 Postprandial hypoglycemia after feeding of alcohol-fermented apple pomace silage. M. Kondo, H. Moriuchi, J. Fang, H. Suzuki, and M. Matsuzaki*, *Hirosaki University, Hirosaki, Aomori, Japan.*

Four Suffolk ewes were used to study the effect of feeding apple pomace silage (APS) with different ethanol content on postprandial glucose homeostasis in a 4 × 4 Latin square design trial. Ewes were fed alfalfa hay cube and either a concentrate (Ctrl), low-ethanol APS (L-APS), high-ethanol APS (H-APS) or the concentrate supplemented with equal amount of ethanol in H-APS (+EtOH). Alfalfa hay cube and the concentrate or APS provided half of TDN requirement for maintenance. The 70% apple pomace, 6% soybean meal, 12% wheat bran and 12% beet pulp (as FM basis) were mixed for APS preparation to contain equal amount of TDN and CP as the concentrate. Ethanol content of APS was manipulated by ensiling with either fresh apple pomace, which has slight amount of ethanol, or alcohol-fermented apple pomace, which left on 2-mo after production. The resultant L-APS and H-APS contained 1.4 and 3.3% of ethanol, respectively. At the end of each 14-d period, blood samples were collected via catheters before and after the morning feed for 120 min and then another 120 min after intravenous insulin challenge (0.1Î1/4U/kg BW). Plasma glucose was assayed for all samples and the concentrations of Insulin, glucagon, ethanol and D-3-Hydroxybutyric acid (DHBA) were assayed for samples for the first 120 min. The area under or upper the curve (AUC) of each plasma measurement was calculated. Plasma glucose was decreased and ethanol level increased after ingesting ethanol in the L-APS, H-APS and +EtOH treatments (P < 0.05). The AUCs of plasma glucose and ethanol in H-APS treatment were greater (P < 0.05) than the Ctrl, with the L-APS and +EtOH intermediate. Plasma insulin level was elevated only in the L-APS after feeding (P < 0.05) while glucagon level was unchanged by feeding in all treatments. Plasma DHBA was gradually increased after feeding and that in the L-APS was higher than other treatments (P < 0.05). The glucose AUC after insulin challenge was greater (P < 0.05) in the Ctrl than other treatments. A significant correlation between the AUCs of plasma glucose and ethanol (P < 0.05) suggest that postprandial hypoglycemia after APS feeding is due primarily to ingestion of ethanol.

Key words: apple pomace, glucose, ethanol

W391 Inclusion of substrate of *Pleurotus ostreatus* on kinetics of in vitro fermentation of *Brachiaria* hay. S. L. S. Cabral Filho*[1,2], R. S. Oliveira[1], R. A. Mandarino[1], and C. A. Lobo[1], *[1]Universidade de Brasília, Brasilia, Distrito Federal, Brasil, [2]Fazenda Experimental Agua Limpa, Brasilia, Distrito Federal, Brasil.*

The demand for increased and faster production of food in the world makes the production of mushrooms an important alternative. However, each 80g of mushrooms produced resulted in 100g of substrate residue. The aim of this study was to assess the inclusion of the substrate production of *Pleurotus ostreatus* on in vitro fermentation of hay-based diet of *Brachiaria brizantha* for ruminants, as well as measuring gas production and rumen kinetics for different levels of inclusion of substrate. The experiment was conducted using a semi-automatic gas production technique, the rumen inocula were collected from 3 fistulated bovines, grazing *Brachiaria* pasture. We used a *Brachiaria brizantha* hay and exhausted substrate for *Pleurotus* production, for the composition of the treatments, which were defined as: ES (100% of exhausted substrate), BH (100% *Brachiaria* hay), ES5 (5%ES +95% BH), ES20 (20%ES +80%BH) and SE30 (30%ES +70%BH). The experiment was conducted in a 5 × 3 factorial with 5 treatments and 3 inocula (3 donor animals). The dry matter degradability (DMD) was determined after 96h of fermentation. No significant differences among treatments were observed on the potential gas productions (A) BH, ES5, ES20 and ES30. The averages were 262.6, 284.3, 256.6 and 261.7 mL, respectively ($P > 0.05$), showing that the inclusion of the substrate did not affect the fermentation of *Brachiaria* hay. The ES treatment presented a lower potential gas production and DMD (165.9 mL and 52%, $P < 0.05$) demonstrated the lower quality of the exhausted substrate compared with hay, despite the presence of enzymes that degrade the cellulose in the mycelium of *Pleurotus*. The ES treatment had a shorter lag phase (L = 2.6 h, $P < 0.05$), which can be attributed to higher concentration of soluble carbohydrates due to higher proportion of wheat meal and mycelium in this treatment. The inclusion of the exhausted substrate did not impair microbial degradation of hay and it by-product can be used in ruminant feed.

Key words: gas production, roughages, mushrooms

W392 Evaluation of protein fractions of tropical grasses by near infrared reflectance spectroscopy. R. G. Basurto[1], G. Buendía-Rodríguez[1], S. S. González-Muñoz*[6], R. E. Ramirez[1], M. A. Barrón[2], G. J. J. Bustamante[3], R. E. Santos[4], M. J. J. Maldonado[5], and C. J. A. Bonilla[3], *[1]CENID Fisiología y Mejoramiento Animal, Ajuchitlán, Querétaro, [2]CE Huimanguillo–CIRG, Huimanguillo, Tabasco, [3]CE Santiago Ixcuintla–CIRPAS, Nayarit, [4]CE Iguala–CIRPAS, Iguala, Guerrero, [5]CE Rosario Izapa–CIRPAS, Tapachula. INIFAP–México, [6]Colegio de Postgraduados, Montecillo, Estado de México, México.*

The aim of study was to evaluate the use of near infrared reflectance spectroscopy as alternative method to determine CP, and degradable (DIP) and undegradable (UIP) intake protein of tropical grasses. Nine hundred and 23 samples of 13 species were collected by clipping sampling at different ages of re-growth (28, 42, 56, 70 and 84 d) in plots in the states of Chiapas, Guerrero, Nayarit and Tabasco, México. The species were *D. aristatum*, *C. dactylon*, *H. altissima*, *U. brizantha*, *D swazilandensis*, *C. plectostachyus*, *U. maximum*, *B. humidicola*, *C. echinatus*, *A. gayanus*, *B. brizantha* × *ruziziensens*, *D. eriantha*, and *U. mutica*. DIP was estimated as the protein fraction that disappeared after incubation with a protease, and UIP was equal to the remaining protein in the samples. The samples were scanned at a spectrophotometer Nicolet FT-IR 6700 (Thermo Fisher Scientific, Inc.) over a wavelength range from 1,000 to 2,500 nm in reflectance; data were stored as log (1/R) at intervals of 4 nm. Calibrations equations were developed using modified partial least squares with TQ Analyst program (v8.0). The selection of the equations was based on: coefficient of determination of calibration (R^2 cal), the minimization of standard error of calibration (SEC) and standard error of cross validation (SECV) and ratio SECV/SD; if the ratio is >0.33, then calibration had a low predictive power. It is concluded that NIRS calibration for PC showed good accuracy, but calibrations were less accurate for DIP and UIP.

Table 1.

		Chemical analysis			Statistics				
Item	N	Mean	Range	SD	R^2 cal	SEC	SEP	SECV	Ratio
Crude protein (DM)									
Calibration	677	7.98	1.0–8.6	3.01	0.94	0.79			
Validation	244	3.01	1.2–7.5	2.05			0.90	0.94	0.31
DIP (% DM)									
Calibration	681	3.44	1.0–8.6	1.32	0.91	0.39			
Validation	242	3.39	1.2–7.5	1.20			0.46	0.44	0.34
UIP (% DM)									
Calibration	689	4.57	1.1–13.0	2.06	0.86	0.76			
Validation	212	4.61	1.1–11.5	1.93			0.87	0.82	0.41

SD = Standard deviation.

Key words: NIR, protein fractions, tropical grasses

W393 The effect of storage structure on haylage and corn silage fermentation. C. Rasmussen*, D. Petri, S. Jens, and A. H. Smith, *Danisco USA, Waukesha, WI.*

Decreasing nutritional and dry matter losses of ensiled forages has always been an important topic of research and debate. Many factors contribute to silage quality (time of harvest, packing density, aerobic exposure, etc.), but one factor often overlooked is storage structure. The objective of this study was to determine if certain storage structures produce better quality silage and if certain storage structures are better suited for specific crops. Samples used in this study included 170 haylage and 242 corn silage stored in bunkers, bags, upright silos, or piles received from 2009 and 2010 and originating from commercial farms within the United States, primarily the upper Midwest. Samples were analyzed for pH, dry matter, lactate, acetate, ethanol, yeast, and mold. Data was analyzed as a 2 × 4 factorial Anova using Proc Mixed procedure as well as Proc corr procedure for correlation analysis (SAS 9.1.3). The mean pH for corn silage and haylage at 3.96 ± 0.031 and 4.76 ± 0.036 respectively would be considered high for well-ensiled crops reflecting the fact that samples received for analysis were generally those of questionable quality. All other measured parameters were within normal ranges. The pH was significantly higher for silos ($P \leq 0.05$), which was correlated with significantly higher levels of spoilage organisms, both yeast and mold ($P \leq 0.05$). Further investigation is needed to determine whether fermentation is more effective in bunkers, piles and bags and if the samples obtained for analysis from these structures are from deeper regions with less oxygen exposure than silo samples.

Key words: silage, corn, haylage

W394 The effect of direct fed lactic acid bacteria combined with monensin. R. C. de Souza*[1], R. B. Reis[1], J. Holliday[2], E. Rabelo[4], and R. A. Filho[3], *[1]Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brasil, [2]Chr. Hansen - Animal Health and Nutrition, Hørsholm, Denmark, [3]Chr. Hansen - Animal, Valinhos, São Paulo, Brasil, [4]Rehagro Team Consultation, Belo Horizonte, Minas Gerais, Brasil.*

Monensin is widely accepted in the American cattle industry, but alternatives to ionophores such as direct fed microbials (DFM) are receiving increased awareness. The question is, if the 2 combined can have a synergistic effect. To address this issue, both a continuous culture experiment and an in vivo study were conducted. The in vivo study included 40 multiparous Holstein cows (milk yield between 8.000 and 10.000 kg) which were supplemented with monensin and a DFM (contaning *E. faecium* and *S. cerevisiae*, Probios TC, Chr. Hansen). The continuous culture trial included a diet with a forage:concentrate ratio of 50:50 combined with four treatments (No additive, DFM, Monensin, and DFM+Monensin). The in vivo trial tested the effect of the DFM on milk yield and milk composition following three alternative applications: included solely pre-partum, solely post-partum or both pre and post-partum. Post-partum, the cows were fed a diet containing corn silage (forage:concentrate ratio of 50:50) and sufficient monensin to supply 16 ppm in the diet DM. The results from the continuous culture showed that monensin significantly ($P < 0.05$) reduced NDF digestibility compared to control, while adding the DFM diminished this effect. There were no treatment effects on the molar proportion of the VFA of any of the treatments. In the in vivo study, supplementing the DFM on diets with monensin, resulted in an additional increase ($P < 0.01$) in milk yield (32.27, 28.37, 35.65 and 36.20, respectively for treatments no additive, DFM pre-partum, DFM solely postpartum and DFM pre and post-partum), milk fat and protein production in both the post-partum and the post and pre-partum period. A negative effect on milk yield ($P < 0.01$) was observed when feeding DFM solely pre-partum. The DFM supplementation only after parturition was better for the increasing milk production and its quality according to composition compared to treatments with no additive and DFM pre-partum.

Key words: transition, DFM, monensin

W395 Morphological response of the ruminal and omasal mucosae to the variation in the energy of the diet. R. F. de Lima, J. C. de Resende Júnior*, J. L. P. Daniel, S. de F. Costa, M. B. Moreira, and M. G. Cardoso, *Universidade Federal de Lavras.*

The rumen capacity to absorb volatile fatty acids (VFA) is proportional to the extent of their absorptive surface which responds positively to the direct and indirect stimulation of VFA. There are indications that the wall of the omasum, which in dairy cows is responsible for about 40% of absorption of VFA, also could respond to the same stimuli of proliferation of the rumen wall. Aiming to test this hypothesis we compared the morphology of fragments of rumen and omasum obtained by biopsy. Four nonlactating cows and not pregnant, not defined breeds and unknown ages were sequentially fed 2 diets. One diet only of chopped grass (CG) and other with concentrate plus chopped grass (CCG). In the first 18 d of the experiment the animals were fed CG diet. In subsequent 18 d the cows received the CCG diet. Then were fasted for 72 h. Biopsies of the ventral sac of the rumen were made at the end of the period of the CG diet, at 4 and 18 d of the CCG diet and at end of fasting period. After fasting the cows were fed with the CCG diet again by 18 d with biopsies on d 4, 12 and 18. Data were analyzed as repeated measures considering the effect of cow, compartment, diet, possible interactions and error. The DMI and the TDN intake were higher ($P < 0.001$) in periods in which the animals were fed with the CCG diet. The mitotic index (MI) of the basal layer in the epithelium of the rumen and of the omasum were higher ($P = 0.01$ for interaction between compartment and diet) in the fourth days of CCG diet and the same occurred with the VFA concentration in the rumen ($P <$ 0,001). There was positive correlation between MI of the rumen and the omasum ($R^2 = 0.66$; $P < 0,01$) . The width of the rumen papillae varied among treatments and was greater at 18 d of CCG diet ($P < 0.001$), which revealed proliferative stimulus of the rumen wall. The similarity ($P = 0.67$) in thickness of the not keratinized layers of the epithelium and the positive correlation between the MI of the rumen and of the omasum, indicate that the stimulation of cell division caused by the energy content of the diet has simultaneous effect in both compartments. However the omasum seems respond more quickly to the stimulus.

Key words: acidosis, ruminant stomach morphology, transition diet

W396 Determination of solubility of alternate magnesium sources and their impact on pH with an optimized in vitro rumen fermentation protocol. S. J. Taylor*[1], J. Apajalahti[2], E. Pennala[2], C. Murphy[1], and T. Rinttilä[2], *[1]Celtic Sea Minerals Ltd., Cork, Ireland, [2]Alimetrics Ltd., Espoo, Finland.*

Absorption of Mg from the rumen of mature ruminants is dependent on its concentration in the liquid phase. An in vitro model of rumen function modified to investigate the mode of action of materials known to affect pH (Taylor et al. 2011) was used to investigate the solubility of different sources of Mg. Simulation protocol. The simulation used 1 g (DM) feed (50% grass silage, 40% barley meal, 10% soy). The bicarbonate and phosphate buffer (Agriculture Handbook, Vol 379, USDA 1970) was diluted 1:4 with 0.9% NaCl. The simulation was inoculated with 5% of fresh, strained rumen fluid from a fistulated cow on a high energy diet and continued for 9 h in triplicate. Anaerobic techniques were applied throughout. Ten treatments included negative control, Acid Buf alone (50 mg/40mL) and Acid Buf plus 8 different sources of Mg oxide (15mg/40mL). Products referred to as country codes in the table were obtained from international feed industry. Analyses. Mg and pH were determined at indicated time points from inoculation. Soluble Mg was analyzed by ICP. Results were compared with the volume of 0.1N HCl consumed by 0.25 g of product in 2 h at pH 5.5 using Titrino Automated pH meter. Inclusion of Acid Buf reduced the drop of pH. Mg was released into solution at a rate dependent on the source and correlated with the titration. Release of Mg significantly suppressed the decline of pH.

Table 1. Results

	2 h	9 h	9 h		Titration with 0.1 *N* HCl
Treatment	Mg (mg/L)	Mg (mg/L)	% Mg solubility	pH	ml*
Control	29^{d}	44^{c}		5.18^{d}	
Acid Buf (AB)	70^{c}	123^{b}	99	5.44^{c}	
AB & Norway	173^{b}	313^{a}	101	5.85^{a}	110.7
AB &S Africa	90^{c}	150^{b}	14	5.62^{b}	16.5
AB & Aus	81^{c}	130^{b}	4	5.61^{bc}	3.7
AB & CNA	81^{c}	177^{b}	28	5.68^{ab}	8.8
AB & UK	84^{c}	160^{b}	20	5.61^{bc}	9.04
AB & Spain	73^{c}	140^{b}	9	5.59^{bc}	16.74
AB & US	92^{c}	173^{b}	27	5.69^{ab}	86.8
AB & Ire	197^{a}	297^{a}	92	5.82^{a}	110.8

*HCl consumed by products (excluding Acid Buf) Numbers with the same suffix are not significantly different ($P = 0.05$).

Key words: magnesium, rumen, solubility