Hydrogen sulfide (H_2S) release in the rumen depends upon ruminal pH, sulfur availability and its interaction with other minerals. An in vitro rumen fluid incubation was conducted using 2 sources of sulfur and 2 sources of Zn, Cu and Mn in a 2 × 2 factorial arrangement of treatments during 2 consecutive 24-h periods. A synthetic diet consisting of 36% cellulose, 32% starch, 19% CP, 5% fat and 2.4% sugar provided substrate for microbial metabolism. Sulfur was added as $NaSO_4$ or sulfur-bound lignosulfonate to a final concentration of 0.75% of DM. Copper, Zn and Mn were added as $CuSO_4$, $ZnSO_4$ and $MnSO_4$ or as protected Cu, Zn and Mn (SQM protected minerals, Quali Tech Inc.) to a final concentration of 16, 56 and 71 ppm of DM, respectively. Rumen fluid was obtained from a ruminal cannulated lactating dairy cow and mixed with McDougall's artificial saliva to a 1:4 ratio. Treatments were assigned in 6 replicates to 120-mL serum bottles containing 40 mL of the inoculum mix and 0.5 g dietary DM. Serum bottles were flushed with N_2, crimp sealed and incubated during 24 h at 39.1°C. At the end of incubations, gas volume was measured, H_2S in the headspace of bottles was analyzed and final pH of incubations was recorded. Results were analyzed as a 2 × 2 factorial design. An interaction between lignosulfonate and mineral source was detected. Addition of SQM minerals and lignosulfonate resulted in lower pH ($P < 0.05$) than that without lignosulfonate (5.87 vs. 5.95, respectively), while absence of SQM minerals resulted in intermediate pH of incubations despite lignosulfonate (5.90 ± 0.04). Addition of lignosulfonate without SQM minerals decreased total gas production ($P < 0.001$) compared with the other treatments (173.1 vs. 175.9 mL/g OM). Lignosulfonate resulted in a lower ($P < 0.001$) production of H_2S (416.2 vs. 475 µg/g OM). In contrast, addition of SQM minerals increased ($P < 0.001$) production of H_2S (469.5 vs. 421.5 µg/g OM). Results indicate that source of trace mineral can influence the dynamics of rumen fermentation.

Key Words: rumen, hydrogen sulfide, in vitro

T378 Comparison of bacterial diversity in the rumen of sheep and in Rusitec fermenters as assessed by ARISA–PCR. M. J. Ranilla*[1,2], M. L. Tejido[1,2], C. Saro[1,2], and M. D. Carro[1,2], [1]*Dpto. Producción Animal, Universidad de León, 24071, León, Spain*, [2]*Instituto de Ganadería de Montaña (CSIC-ULE), Finca Marzanas s/n, 24346 Grulleros, León, Spain*.

This study was designed to compare the effects of 4 diets on bacterial communities in bacterial pellets (BP) isolated from the solid (SAB) and liquid phase (LAB) of the rumen of sheep with those observed in Rusitec fermenters. The 4 experimental diets had forage:concentrate ratios (F:C) of 70:30 (HF) or 30:70 (HC) and alfalfa hay or grass hay as forage (FOR). SAB and LAB were isolated from each sheep (4 per diet) and fermenter (n = 4) immediately before feeding, and bacterial diversity was analyzed by ARISA-PCR of the 16S ribosomal DNA. A total of 170 peaks were detected in the ARISA electropherograms across the full set of 64 BP. The number of peaks (NP) in BP from sheep ranged from 42 to 82 for LAB, and from 31 to 81 for SAB (168 peaks in total). In fermenters, NP ranged from 53 to 79 for LAB, and from 21 to 69 for SAB (162 peaks in total). No effect of F:C ($P > 0.05$) on NP or Shannon index (SI) was observed on LAB in any system. F:C did not affect SAB profile in fermenters, but NP and SI were greater ($P < 0.05$) in SAB from sheep fed HF diets compared with those from HC-sheep. Feeding grass hay diets promoted greater ($P < 0.01$) SAB diversity in both systems compared with alfalfa hay diets. FOR did not ($P > 0.05$) affect LAB profile in sheep, but grass hay-fed fermenters had greater ($P < 0.01$) LAB diversity compared with fermenters fed alfalfa hay diets. The results indicate that bacterial diversity was more markedly affected by FOR than by F:C. There was a positive relationship (P = 0.001) between the NP in LAB and that in SAB in Rusitec, but no relationship ($P = 0.72$) was found in sheep; this would indicate that dietary effects on bacterial diversity were similar in LAB and SAB in fermenters, but contrasting in sheep. When all samples were analyzed together by clustering analysis, 2 distinct clusters were observed for in vivo and in vitro BP, which suggests a different structure of the bacterial communities in sheep and fermenters.

Key Words: rumen, fermenters, bacterial diversity

T379 Effect of supplemented diet by sucrose or starch on fungi populations in rumen fluid as determined by real-time polymerase chain reaction in Holstein steers. A. Vakili*, M. Danesh Mesgaran, H. Jahani Aziz-abadi, F. Rezaii, and S. Ghovvati, *Dept. of Animal Science, Ferdowsi University of Mashhad, Mashhad, Iran*.

The objective of this work was to investigate the effect of diets containing different type of non-fiber carbohydrates (sucrose or starch) on fungi populations in rumen fluid as determined by real-time polymerase chain reaction. Four Holstein steers (BW = 280; SD = 15 kg) were assigned to a 4 × 4 Latin square with 21-d periods. A basal diet was formulated to be contained of alfalfa hay, barley grain, soybean meal and sugar beet pulp (400, 290, 190 and 50 g/ kg, respectively). Starch (St) or sucrose (Su) or a 1:1 mixture of starch and sucrose (St+Su) was added to the basal diet at the rate of 70g/kg DM. Diets were offered as 2–2.5 times of maintenance requirements (7 kg DM/d). Rumen fluid samples were collected before and 4 h after the morning feeding. DNA was extracted from the samples using the QIAamp DNA stool mini kit (Qiagen Ltd, Crawley, West Sussex, UK) following the manufacturer′s instructions. Fungi rDNA concentrations were measured by real time PCR relative to total bacteria amplification (ΔΔCt). The 16s rRNA gene-targeted primer sets used in the present study were forward: GAGGAAGTAAAAGTCGTAACAAGGTTTC and reverse: CAAATTCACAAAGGGTAGGATGATT. Cycling conditions were 95°C for 5 min, 40 cycles of 95°C for 15s, 60°C for 15s and 72°C for 30s; fluorescence readings were taken after each extension step, and a final melting analysis was obtained by heating at 0.1°C/s increment from 60 to 95°C, with fluorescence collection at 0.2°C at intervals. Data are expressed relative to quantification of the total bacterial population. Data were analyzed using mixed procedure of SAS (2003). Statistical model was: $Y_{ijk} = \mu + T_i + C_j + P_k + \varepsilon_{ijk}$, where Y_{ijk} is dependent variable, µ is the overall mean, T_i is treatment effect, C_j is cow effect, P_k is period effect, and ε_{ijk} is error. The results of this experiment showed that different type of non-fiber carbohydrates didn't have any effect on fungi populations before or 4 h after the morning feeding[St = 52 and 44,Su = 48 and 45, St+Su = 47 and 42, SEM = 5 and 3 ($10 \times ^{-7}$) fungi relative to total bacteria, respectively].

Key Words: fungi, real-time PCR, rumen

T380 Sodium acetate/acetic acid as a buffer solution to simulate an acidic in vitro rumen environment. R. C. Araujo*[1], A. V. Pires[1], and A. L. Abdalla[2], [1]*ESALQ, Universidade de São Paulo, Piracicaba, SP, Brazil*, [2]*CENA, Universidade de São Paulo, Piracicaba, SP, Brazil*.

Incubation media based on $NaHCO_3$ and NH_4HCO_3 as buffers have a pH close to 6.8. A low-pH medium would provide a more realistic in vitro rumen simulation of animals fed feedlot diets. Treatments were: CTL6.8 – Theodorou's medium with a pH of 6.8 based on $NaHCO_3$ (7.28 g/L of medium) and NH_4HCO_3 (0.83 g/L of medium) as buffers; CTL5.8 – control acidified with 72% sulfuric acid to achieve a pH of 5.8; NaAc – Theodorou′s medium with Na acetate (56.6 g/L of medium) and glacial acetic acid (2.08 mL/L of medium) as buffers to achieve a pH of 5.8. In each flask (160 mL), 0.5 g of an 80:20 concentrate:forage diet

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