Ruminant Nutrition: Ruminal Metabolism

M373 Evaluation of algae as livestock feed. C. P. Payne*, J. E. Sawyer, and T. A. Wickersham, *Texas A&M University.*

Cultivation of algae for biofuel would result in the production of significant amounts of post-extraction algal residue (PEAR). The economic viability of algae as source of biofuel is dependent on deriving value from PEAR. Livestock feed is an attractive option for PEAR because of the successful utilization of other co-products by ruminants. While sufficient quantities of PEAR were not available for analysis, we evaluated 2 strains of algae that, based on their lipid content and growth characteristics, possess potential as a source of biofuel. Algae samples, unknown wild algae (WA) and *Neochloris oleoabundans* (NO), were analyzed for ash, crude protein, amino acid profile, total fat content (ether extract), fatty acid profile, fat soluble vitamins, macro- and micro-minerals, and heavy metals (n = 1). Samples were observed to have relatively high levels of ash, 30.5 and 43.2% for WA and NO, respectively. As expected, Na content of each sample was high, 10.3 (WA) and 10.8% (NO). The Ca and P contents were 0.67 and 0.43% for WA and 1.02 and 0.26% for NO, accordingly. Algae samples WA and NO contained 34.2 and 48.6 ppm Cu, 848 and 756 ppm Fe, and 20.1 and 62.3 ppm Zn, respectively. WA and NO contained significant quantities of aluminum at 840 and 858 ppm, accordingly. Analysis of algal CP content, 17.4 WA and 20.6% NO, indicates that PEAR may serve as a source of N in ruminant diets. Samples contained similar amounts of methionine (0.31 and 0.32%; WA and NO, respectively) and lysine (1.00 and 1.18%; WA and NO, respectively). Total fat content was 9.1 and 11.8% WA and NO, respectively. Fatty acids (% total fat) were profiled for WA and NO, correspondingly: Palmitic, 21.3 and 32.0; Oleic, 34.1 and 15.6; Trans-vaccenic, 7.7 and 5.6; Linoleic, 11.3 and 19.8; α-Linolenic, 16.3 and15.4%. Vitamin A content was high for both samples, with WA measuring 6614 and 5203 IU/kg for NO. Vitamin E content was 56.5 for WA and 87.7 IU/kg for NO. Future nutritive evaluations of algae and the resulting PEAR should focus on its value as a source of N in ruminant diets.

Key words: algae, coproduct

M374 Hourly changes in fatty acid profile of ruminal contents in continuous cultures as soybean oil is added and removed from the diet. C. M. Klein*, S. K. Thurmond, P. H. Morris, and T. C. Jenkins, *Clemson University, Clemson, SC.*

The objective of this experiment was to determine how quickly biohydrogenation patterns change in ruminal contents in response to the addition and removal of unsaturated fat from the diet. Four dual-flow continuous fermenters were fed 60 g/d of 1:1 forage (alfalfa hay) to concentrate mix in 2 equal portions at 0800 and 1600 h. Diets were fed to flasks for 4 12-d periods each divided into 3 phases; 1) a control diet for d 1–4 (CON1), 2) a diet with 4% added soybean oil for d 5–8 (SBO), and 3) the control diet for d 9–12(CON2). Samples of culture contents were taken at 0 (just before feeding), 2, and 4 h after the morning feeding on all days except d 5 and d 9. On d 5 and d 9, samples were taken hourly for 12 h starting just before the morning feeding. Results are expressed as g fatty acid/100 g total fatty acids. Differences among sampling times were declared if $P < 0.05$ and determined in SAS using single degree of freedom contrasts. During the CON1 phase, changes in fatty acid profile occurred daily after each feeding that were characterized by increased ($P < 0.05$) proportions of C18:2 and *cis*-9, *trans*-11 CLA, and decreased ($P < 0.05$) proportions of stearic acid, *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA. Linoleic acid averaged 11.5 ± 1.4% during CON1 and peaked at 33.3 ± 1.1% immediately after the addition of soybean oil (d4h1). Linoleic acid averaged 26.1 ± 1.1% and 16.4 ± 2.6% during SBO and CON2 phases, respectively. Stearic acid showed little change from SBO to CON2 phases (averaging 9.6 ± 1.7 and 9.5 ± 1.9%, respectively). *Trans*-10, *cis*-12 CLA proportions averaged 0.61 ± 0.08% during CON1, increased gradually over the SBO phase, and peaked at 5.38 ± 1.51% at the start of CON2 (d 8h 0). *Trans*-10 C18:1 averaged 0.62 ± 0.12% over CON1 and peaked at 6.12 ± 1.62% during CON2 (d9h0). The results of this study show that the introduction of soybean oil into the diet causes an immediate increase in linoleic acid concentration in ruminal contents that is accompanied by little change in stearic acid and slow-developing increases in *trans*-10, *cis*-12 CLA and *trans*-10 C18:1.

Key words: biohydrogenation, soybean oil, conjugated linoleic acid

M375 Effects of tannin extracts on in vitro growth of selected food-borne pathogenic bacteria. B. J. Min[1], B. R. Min[1], J. M. Sieg[2], J.-S. Eun*[2], D. R. ZoBell[2], and D. C. Tice[1], [1]*Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL,* [2]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan.*

An in vitro study was conducted to assess the growth inhibition of tannin extracts (TE) against *Escherichia coli* O157:H7, *Salmonella typhimurium*, *Listeria monocytogenes*, and *Staphylococcus aureus* in pure culture. Commercially available quebracho (QT; mainly condensed tannins), chestnut (CNT; containing 80% hydrolysable tannins), and mimosa tannins (MT; containing about 70% condensed tannins) (Chemtan, Exter, NH) were tested. An agar diffusion assay was used to evaluate the antimicrobial activity of TE at 1 mg TE/4 mL ethanol solution (1:5 dilution) and 1 mg TE/49 mL ethanol solution (1:50 dilution) against the bacteria. The in vitro experiment was performed in a 3 (source of TE) × 4 (pathogenic bacteria) × 2 (dilution rate) factorial design (n = 3). An aliquot of 10 mL hard tryptic soy broth was used to inoculate *E. coli* O157:H7 and *S. typhimurium*, whereas 10 mL hard brain heart infusion broth was used to inoculate *L. monocytogenes* and *S. aureus* at $10^{5–6}$ cfu/mL. Plates were incubated at 37°C for 48 h, and the disc with ethanol only was used as a control. After incubation, the diameter of inhibition zones was measured at least 3 cross-section points, and mean value was used for inhibition zone. At 1:5 dilution, CNT depicted the most inhibitory response (2.05 mm) followed by MT (1.44 mm) ($P < 0.05$). The CNT elicited an inhibitory effect across all pathogenic bacteria (*E. coli* O157:H7 = *S. typhimurium* > *L. monocytogenes* > *S. aureus*) ($P < 0.05$). Mimosa tannins had less inhibitory effects compared with CNT, while QT did not affect bacterial growth. At 1:50 dilution, only the CNT inhibited bacterial growth (*E. coli* O157:H7 = *S. typhimurium* > *L. momcytogenes*), but the overall response was lower than that in 1:5 dilution (1.10 vs. 2.05 mm). Results from this in vitro experiment showed that the CNT exerted a greater inhibition of pathogenic bacterial growth than the MT and the QT.

Key words: pathogenic bacteria, tannin extracts, growth inhibition

M376 Tannin extracts decrease in vitro growth of ruminal acidosis-causing bacteria in pure culture. J.-S. Eun*[1], B. R. Min[2], J. M. Sieg[1], D. R. ZoBell[1], and A. J. Young[1], [1]*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan,* [2]*Department*

of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL.

Antimicrobial activity of tannins has been well documented. However, there is lack of detailed knowledge to explore potential effects of tannin extracts (TE) on the growth of ruminal acidosis-causing bacteria (RACB) in beef steers. Two strains of RACB were used in a 2 (strain of RACB) × 4 (source of TE) factorial designed experiment (n = 3) to determine the effects of sources of TE on growth of RACB. Two strains of RACB, *Selenomonas ruminantium* JY35 (SR) and *Streptococcus bovis* S81 A Xy2 (SB), were tested in pure culture, as the 2 bacteria have been considered as main microbes causing ruminal acidosis in finishing beef steers. The bacterial growth was measured by OD_{550} readings during 24-h incubation at 39°C in Hungate tubes under CO_2 with a typical beef steer finishing TMR extracted using an artificial saliva in the growth medium containing soluble protein and carbohydrate. Commercially available quebracho (QT; mainly condensed tannins), chestnut (CNT; containing 80% hydrolysable tannins), and mimosa tannins (MT; containing about 70% condensed tannins) (Chemtan, Exter, NH) were used as sources of TE. Overall growth pattern of the RACB differed in response to TE ($P < 0.05$ for RACB × TE interactions). Adding TE decreased growth of SR starting at 2 h, and CNT was most effective to decrease growth of SR at 12 and 24 h followed by MT and QT ($P < 0.05$). At 24 h, the CNT decreased growth of SR at 48%. Growth of SB was inhibited by adding TE beginning at 4 h. At 12 and 24 h, the CNT elicited the least growth of SB followed by the MT and the QT ($P < 0.05$). The CNT decreased growth of SB at 73% at 24 h. Results of this study indicate that supplementing TE in a beef steer finishing diet decreased in vitro growth of SR and SB, and the CNT was the most effective in inhibiting the growth of RACB.

Key words: ruminal acidosis-causing bacteria, tannin extracts, bacterial growth

M377 Effects of wheat dried distillers grains with solubles (DDGS) and cinnamaldehyde (CIN) on fermentation and protein degradation in Rusitec. Y. L. Li[1,2], M. L. He[1], K. A. Beauchemin[1], and W. Z. Yang*[1], [1]*Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada,* [2]*Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing, China.*

A study was conducted to evaluate the effect of wheat DDGS and CIN on in vitro fermentation using the Rusitec. The experiment was designed as a completely randomized block with a 2x2 factorial arrangement of treatment with 4 replications in each treatment. The control diet (10% barley silage, 90% barley concentrate, DM basis) and wheat DDGS diet (10% silage, 60% barley concentrate, and 30% wheat DDGS) were combined with 0 and 300 mg CIN/L of culture fluid. Experiment consisted of 10 d of adaptation and 7 d of data collection. Interactions of DDGS with CIN on fermentation and nutrient disappearances were not significant. Replaced barley grain with DDGS increased the concentration of total VFA (45 vs. 38 mM; $P < 0.04$), and molar proportions of acetate (46 vs. 41%; $P < 0.01$), propionate (19 vs. 17%; $P < 0.02$), and NH_3-N (15 vs. 10 mg/100 mL; $P < 0.01$) without altering ratio of acetate to propionate and CH_4. Disappearance of DM (48 vs. 45%; $P < 0.03$) and bacterial protein production (72 vs. 51 g; $P < 0.01$) were greater, whereas the disappearances of CP (46 vs. 50%) and NDF (17 vs. 24%) were less ($P < 0.01$) with DDGS than with control diet. With addition of CIN, concentration of total VFA decreased (33 vs. 49 mM; $P < 0.01$) and fermentation pattern changed to greater ($P < 0.01$) acetate and less ($P < 0.01$) propionate molar proportions. Supplementation of CIN overall reduced ($P < 0.05$) the nutrient disappearance by 10 to 15% depending on the nutrient studied. Consequently, the production of bacterial N reduced ($P < 0.01$) by 14%. The results indicate that substitution of wheat DDGS for barley grain or supplementation of CIN in finishing diet potentially increased protein in the intestine as a result of increased CP supply and RUP. However, decreased NDF disappearance with DDGS or CIN may reduce feeding value of the diet especially DDGS is high in NDF.

Key words: cinnamaldehyde, fermentation, wheat DDGS

M378 In vitro digestion and gas production of wheat grain varying processing. W. Z. Yang*[1], T. A. McAllister[1], and M. Oba[2], [1]*Research Centre, Agriculture and Agri-Food Canada, Lethbridge, AB, Canada,* [2]*Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada.*

Rapid starch digestion in the rumen may lower rumen pH, depress fiber digestion and cause digestive disturbances. The rate and extent of DM digestion varies among wheat sources and with the extent of processing, but seldom have both of these properties been studied in the same experiment. Eight wheat samples collected from various location in Alberta were either ground (1-mm) or dry-rolled, and fermentability was assessed by measuring in vitro gas production (GP) and DM disappearances (DMD) at 0, 4, 8, 14, 24 and 48 h of incubation. The DMD increased ($P < 0.01$) from 21 to 81%, and from 12 to 53%, respectively, for ground and rolled wheat with increasing incubation time from 4 to 48 h. There was no interaction between wheat source and processing on GP and DMD at 24 h of incubation, whereas it was significant ($P < 0.01$) at 48 h of incubation. Variations in GP and DMD among wheat samples were substantial. The GP (ml/g OM) varied from 226 to 311 for ground wheat and from 95 to 194 for rolled wheat, and the DMD ranged from 60 to 81% for ground wheat, and from 30 to 50% for rolled wheat. As expected, GP and DMD were greater ($P < 0.01$) for ground wheat (279 and 64%) than for rolled wheat (141 and 34%) after 24 h of incubation. Similarly, VFA concentration in the culture fluid was higher ($P < 0.01$) for ground (102 m*M*) than for rolled wheat (90 mM). However, ratio of acetate to propionate was lower ($P < 0.01$) for ground (2.3) than for rolled wheat (3.4), indicate that more starch is available to be fermented for ground wheat. This work demonstrates that there is substantial variation in the digestive value of commercially available wheat grain and emphasize the need to have an accurate and rapid means of quality assessment at the point of sale.

Key words: wheat grain, DM digestion, batch culture

M379 The effect of DDGS when replacing corn or soybean meal on rumen microbial growth in vitro as measured using real-time PCR. E. Castillo-Lopez* and P. J. Kononoff, *University of Nebraska-Lincoln, Lincoln.*

Ethanol byproducts are a good source of energy and protein in ruminant diets. The aims were to evaluate the effect of dried distillers grains and solubles (DDGS) and in vitro fermentation time on the growth of rumen bacteria and protozoa, and to measure the contribution of yeast originating from DDGS to total microbial crude protein (MCP). Treatments were: 1) CONT, control with no DDGS, but with alfalfa hay, corn silage (CS), ground corn (GC) and soybean meal (SBM) included at 25% (DM basis); 2) RC, 20% (DM Basis) DDGS replacing GC; 3) RS, 20% (DM basis) DDGS replacing SBM; 4) RCS, 20% DDGS replacing 10% GC and 10% SBM (DM basis). For each treatment, 1 g of substrate was incubated in vitro in 100 mL of inoculum in duplicate. At 0, 4, 16, 32, 48 and 96 h of fermentation DNA was extracted from

each treatment and MCP was measured by real-time PCR. Microbial markers used are from the 16S rRNA gene, 18S rRNA gene and the II chromosome; for bacteria, protozoa and yeast, respectively. Data were analyzed as a completely randomized design with repeated measures to test the effects of treatments and fermentation time. Treatment did not affect ($P = 0.18$) mean bacterial CP which was observed to be 157.22 ± 16.53 mg/g DM across treatment. However, a treatment by time interaction was observed ($P < 0.05$). Specifically, at 16 h the RCS diet yielded higher ($P < 0.01$) bacterial CP than CONT (306.32 and 141.37 ± 49.97 mg/g DM for RCS and CONT respectively). However, at 32 h only the RS yielded higher ($P < 0.01$) bacterial CP than the CONT (393.08 and 251.15 ± 49.97 mg/g DM for RS and CONT respectively). In addition, compared with the CONT, bacterial CP of RCS tended ($P = 0.07$) to increase (343.67 and 251.15 ± 49.97 mg/g DM for RCS and CONT respectively). At 32 h the RS and RCS diet yielded higher ($P < 0.01$) protozoa CP when compared with the CONT (209.31, 165.38 and 117.64 ± 15.01 mg/g DM for RS, RCS and CONT respectively). Treatment did not affect ($P = 0.51$) yeast CP and averaged 0.03 ± 0.02 mg/g DM. Overall, bacterial and protozoal growth was improved when DDGS replaced SBM and it was maintained when DDGS replaced GC.

Key words: DDGS, microbial crude protein, real-time PCR

M380 Effects of semi-arid medicinal herb essential oils on growth of pure culture of *Butyrivibrio fibrisolvens* SH13. 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 effect of some semi-arid medicinal herb essential oils (EO) on Butyrivibrio fibrisolvens SH13 growth characteristics. The liquid version of Hobson's M2 medium (Hobson, 1969) in Hungate tubes was used to estimate sensitivity of *Butyrivibrio fibrisolvens* SH13 to semi-arid native cinnamon, thyme and coriander essential oils. *Butyrivibrio fibrisolvens* SH13 stock culture was grown anaerobically in M2 medium in 125-mL bottles for 16 h at 38.6°C before testing. *Butyrivibrio fibrisolvens* SH13 was obtained from the Rowett Research Institute (Aberdeen, UK) culture collection. After the medium was autoclaved, each essential oil was applied to give a concentration ranging from 0.0 (as control) to 10, 20, 40, 80, 120, 180, 240, and 360 ppm (4replicates). Essential oils were previously dissolved in equal volume of ethanol. All cultures were grown anaerobically at 38.6°C using an inoculum from stationary phase of stock culture (5% of v/v) for 24 h. The concentration of a EO at which the *Butyrivibrio fibrisolvens* SH13 growth was half of that measured in the control (IC50) was recorded during 24 h of incubation. *Butyrivibrio fibrisolvens* SH13 growth was measured by hourly reading optical density of the medium at 650 nm (OD650). As presented in Table 1, when each EO applied to the culture medium inhibited the growth of *Butyrivibrio fibrisolvens* SH13 at the concentration of higher than 240 ppm. An increase in the concentration of coriander EO (UP to 240 ppm) led to increase *Butyrivibrio fibrisolvens* SH13 OD650 compared with those of the control ($P < 0.05$). Results of the present study demonstrated that the essential oils might alter growth pattern of *Butyrivibrio fibrisolvens* SH13.

Table 1. The concentration of semi-arid medicinal plant essential oils at which the *Butyrivibrio fibrisolvens* SH13 growth was half of that measured in the control (IC50) during 24 h of incubation

	IC50 of EO (ppm)
Cinnamon	≥360
Coriander	>360
Thyme	≥240

Key words: *Butyrivibrio fibrisolvens*, coriander, essential oil

M381 Effects of microbial contamination on in situ estimates of ruminal degradability of fiber fractions. J. M. Arroyo, J. Guevara-González, F. Díaz-Royon*, and J. González, *Universidad Politécnica de Madrid, Madrid, Spain.*

Measures of ruminal digestibility of fiber constituents are usually considered as truly estimates. However, vegetable feeds are subjected, during its rumen residence, to a microbial contamination, which is especially high in rich fibrous feeds. Therefore, errors may occur if the fiber determination methods are not able to remove this contamination, as it is normally assumed. The microbial contamination of the neutral and acid detergent fractions (NDF and ADF) and of their N components (NDIN and ADIN, respectively) of in situ incubated residues of a fibrous Italian ryegrass (*Lolium multiflorum*) hay was determined as well as the associated effects on the ruminal degradation estimates. Hay samples (ground to pass a 2-mm screen) were incubated for 72 h in nylon bags (46 µm pore size) on 3 ruminally cannulated wethers fed with 75 g /$Kg^{0.75}$ of a 40:60 Italian ryegrass hay to concentrate diet. Incubations were performed in stable conditions of ^{15}N infusion (30 mg ^{15}N per day) and solid-associated bacteria were isolated and used as reference sample to control contamination. Analyses of NDF, ADF, as well as of N and ^{15}N abundance of both fiber fractions were performed. Effects of microbial contamination were determined by one-way variance analysis. Microbial contribution to NDF, ADF, NDIN and ADIN in the tested sample was 4.14, 0.45, 65.1 and 15.9%, respectively. The lack of contamination correction led to underevaluations of ruminal degradation: 22.4% (89.8 vs. 69.7%) for NDIN, 4.7% (79.4 vs. 75.7%) for ADIN, 2.3% (65.0 vs. 63.5%) for NDF and 0.3% (63.6 vs. 63.4%) for ADF ($P < 0.001$). The procedures of fiber fractioning with detergent solutions do not promote the total detachment of microorganisms adhered to fiber residues of rumen incubated samples leading to large errors for the concentration and degradation of NDIN and ADIN. The associated errors are moderate for NDF and very low for ADF.

Key words: fiber, microbial contamination, ruminal degradation

M382 Measurement of dry matter degradation of sugar cane molasses in rumen of bovine using nylon bag technique. J. J. Lomeli*[1], L. R. Flores[1], R. H. Ley[1], J. E. Guerra[2], I. Quintero[1], J. E. Borbolla[1], and R. Barajas[1], *[1]FMVZ-Universidad Autonóma de Sinaloa, Culiacán, Sinaloa, México, [2]FA-Universidad Autónoma de Sinaloa, Culiacán, Sinaloa, México.*

With the objective of determine the degradation of dry matter of sugar cane molasses in the rumen of bovine using nylon bag technique 2 experiments were performed. Two cows fitted with 10 cm ID cannula and fed a 70% concentrate diet (14.7% CP; NEm 1.73 Mcal/kg) containing 10% of sugar cane molasses were used. Exp. 1: Nylon bags (10 x15 cm) were filled with a combination of ground corn and rewashed oven-dried river-sand in proportions of 100, 90, 80, 70, 60, 50, 40, 30,

20, 10, and 0% of corn and complete to 100% with sand. Bags were incubated during 24 h in rumen. Compared by orthogonal contrasts, corn-DM disappearance was not affected ($P = 0.98$) by sand level, with values of 61.7% vs. 61.1% for only corn, and all other levels, respectively. Exp 2: Ground corn and cane molasses were mixed in proportion of 0, 20, 40, 60, and 100% molasses. To prevent the outflow of molasses from the bags, it was blend with dried river-sand. Nylon bags (10 x15 cm) bags were filled to contain the equivalent of 5 g of sample once discounted river-sand. Then were placed in rumen during 3, 6, 9, 12, 18 or 24 h. Data of rumen DM degradation for treatments containing from 0 to 60% of molasses at each time were used to calculate by linear regression the corresponding DM of molasses as 100% of DM. Calculated values were contrasted by regression against observed DM disappearance of 100% molasses treatment. The kinetics of degradation of molasses-DM was calculated by exponential regression. Molasses DM solubility was 69% and calculated by regression was 72%. Molasses DM effectively degraded in rumen was 98.5% at degradation rate of 84%/h ($r = 0.97$; $P < 0.0001$), and requires proximately 2 h. Predicted by regression and observed values shown a highly linear relationship ($r = 0.99$; $P < 0.01$). It is concluded that almost totality of molasses DM is degraded in rumen in the first 2 h after feeding

Key words: cane molasses, ruminal degradation, nylon bag

M383 Ruminal degradation of the dry matter of the sugar cane silage. J. A. Reyes-Gutierez[1,2], O. D. Montañez-Valdez*[1], R. Rodríguez Macías[2], E. Salcedo Pérez[2], M. A. Ruiz López[2], and M. R. Rodriguez-Ramirez[3], *[1]Centro Universitario del Sur de la Universidad de Guadalajara, Ciudad Guzmán, Jalisco, México, [2]Centro Universitario de Ciencias Biológicas y Agropecuarias de la Universidad de Guadalajara, Las Agujas, Zapopan, Jalisco, México, [3]Instituto Nacional de Investigaciones Agricolas y Pecuarias, Tecomán, Colima,México.*

The objetive of this work was to study the rumen degradability of dry matter (DM) of sugarcane (*Saccharum officinarum*) in 2 forms for use in ruminant nutrition: T1) fresh sugar cane (FSC) and T2) sugar cane silage (SCS). The digestibility was determined in situ using the technique of nylon bag with 4 Holstein cows fitted with ruminal cannula, which were fed only with each, FSC or SCS and supplemented with 1 kg of commercial dairy concentrate. Five grams of ground sample of FSC and SCS were incubated in nylon bags for periods of 0, 8, 12, 24, 36, 48, 72 and 96 h. Treatments were distributed in a completely randomized design with 6 replicates per treatment. It was found that in situ digestibility of dry matter was higher ($P \leq 0.05$) for FSC in most incubation periods with respect to SCS, except at 24 h of incubation ($P \geq 0.05$, Table 1). Ruminal pH showed no significant differences ($P \geq 0.05$) between treatments. We hypothesized that the degradation of the DM and OM in SCS was higher by the additive and inoculum use, although FSC showed the higher values on this study, the major problem is the daily harvest, sugarcane sours rapidly and becomes unpalatable if left unattended after chopping. Ensiling of sugarcane may solve these problems. However, knowledge on the feeding value of ensiled sugarcane is limited and most studies have been conducted with this forage.

Table 1. Coefficients of digestibility in situ of experimental materials (%)

Component	FSC	SCS	EE
Dry Matter			
96[1]	61.93[a]	56.60[b]	1.15
72	60.75[a]	52.29[b]	0.89
48	56.80[a]	51.45[b]	1.08
36	47.21[a]	44.08[b]	0.66
Organic Matter			
96	57.88[a]	47.43[b]	2.35
72	56.66[a]	54.66[a]	0.90
48	50.70[a]	45.87[b]	1.50
36	52.29[a]	47.50[b]	1.06
Ruminal pH			
Average	7.02	7.15	0.14

[a,b]Different letters in the same row differ ($P \leq 0.05$). [1]Hours of incubation.

Key words: degradation, sugar cane, ruminant

M384 A novel method to measure rumen stability of three rumen protected products. M. Sakkers*[1], P. H. Robinson[2], L. J. Erasmus[1], J. Garrett[3], and R. Meeske[4], *[1]University of Pretoria, Pretoria, South Africa, [2]University of California, Davis, Davis, [3]Quali Tech Inc., Chaska, MN, [4]Western Cape Department of Agriculture, Western Cape, South Africa.*

There are currently a large number of rumen protected products (RPP) on the market, designed to achieve precision delivery of key nutrients post-ruminally. There is however no method to assess the quantitative stability of these products in the rumen. The objective was to determine the stability of 3 RPP using a novel in vivo dual fluid phase marker technique. Three RPP were evaluated, being ascorbic acid, lysine (L) and niacin, composed of 62.3% nutrient (51.87% for L), 8.9% Co-EDTA (8.65% for L) and 28.8% fat matrix (39.48% for L) (specific gravity approximately 1.21). Four ruminally cannulated Jersey cows were fed a common total mixed ration composed of chopped lucerne hay, maize stover, maize meal, soybean oilcake, hominy chop, molasses, urea, Megalac and a vitamin/mineral premix containing 18% CP, 31.7% NDF and 21.3% starch on a dry matter basis. The experiment was a 4 × 4 Latin Square design with 4 14-d periods. Cows were ruminally dosed on d 11 with Cr-EDTA and one of the 3 RPP to deliver 2.4 g of Co and 2.4 g of Cr. Rumen fluid samples were collected before dosing, at 2 h intervals through 25 h, and then every 4 h until 49 h post-dosing. These samples were analyzed for Co, Cr and pH. Ruminal pH was unaffected by treatment and averaged 5.88, with diurnal variation between 5.65 and 6.40. Animal performance was unaffected by treatment with average milk production of 24.6 L/day, milk fat of 4.18% and milk protein of 3.56%. The stability of the RPP within the rumen was measured as the proportion of the area under the curve of rumen clearance of Co (in the RPP as Co-EDTA) relative to the clearance of the Cr (as free Cr-EDTA). The rumen stability of RP Niacin was the highest ($P = 0.06$) at 66.7% relative to RP Lysine at 55.0%, but only tended ($P = 0.14$) to differ from RP Ascorbic acid at 58.7%. Simultaneous in sacco incubations of the RPP showed that the appropriate incubation time to estimate the in vivo rumen stability was approximately 24 h. Results show that this in vivo method can be utilized to quantitate rumen stability of RPP, and indicate the most appropriate rumen in sacco incubation time to reflect that measurement.

Key words: area under curve, clearance rate, stability

M385 Biohydrogenation of docosahexaenoic acid into unsaturated 22-carbon fatty acid intermediates in ruminal batch cultures. C. M. Klein*, W. C. Bridges, and T. C. Jenkins, *Clemson University, Clemson, SC.*

Docosahexaenoic acid (DHA) disappears from the rumen indicating that it is converted into other compounds; however, there is limited information on what these compounds are. In this study batch cultures of mixed ruminal microorganisms were injected with 0, 0.5, or 1% DHA and incubated for 0, 6, 24, or 48h to determine if pathways similar to linoleic acid biohydrogenation are responsible for the decrease in DHA. Triplicate cultures were lyophilized and fatty acids were analyzed by GC. Molecular weights were determined by GC/MS using chemical ionization. Statistical analysis was completed using SAS 9.2. A LSmeans ANOVA in proc GLM was used to test time and treatment effects, and proc GLIMMIX was used to test changes in fatty acid profile. At 48h, trans-11 18:1 increased with increasing DHA supplementation with levels of 0.85, 1.59, and 1.86mg for 0, 0.5, and 1% DHA ($P < 0.05$). Stearic acid levels at 48h decreased when DHA was supplemented from 7.91mg with 0% DHA to 4.26 and 4.23mg at 0.5 and 1% DHA ($P < 0.05$). By 48h, 95 and 92% of DHA had disappeared from cultures for the 0.5 and 1% treatment groups respectively indicating biohydrogenation of DHA was occurring as seen in vivo. At 48h, ketostearate increased from 0.10mg with 0% DHA to 0.50 and 0.54mg at 0.5, and 1% DHA respectively ($P < 0.05$). In cultures supplemented with 0.5, 1, 2, or 3% U-13C DHA there was no label in ketostearate at 48h indicating that although ketostearate increases with DHA supplementation it is not produced from DHA ($P > 0.05$). In DHA cultures, up to 5 isomers of C22:5, 6 isomers of C22:4, 5 isomers of C22:3, and 5 isomers of C22:1 were isolated in 6, 24 or 48h cultures. No unsaturated 22 carbon fatty acids were isolated from cultures when DHA was not added. Over time, the isotope profiles changed from 55% C22:5 and 23% C22:4 at 6h to 35% C22:3 and 29% 22:1 at 48h ($P < 0.05$). The time course appearance of unsaturated 22 carbon fatty acids indicates that biohydrogenation of DHA in ruminal batch cultures occurs by pathways of isomerization and hydrogenation resulting in a variety of unsaturated 22 carbon intermediates.

Key words: rumen, docosahexaenoic acid (DHA), biohydrogenation

M386 Effect of a handmade inoculum and additive on in vitro dry matter digestibility of sugar cane silage. O. D. Montañez-Valdez*[1], J. A. Reyes-Gutierrez[1], G. Rocha-Chavez[1], J. M. Tapia-Gonzalez[1], J. A. Martinez-Ibarra[1], C. E. Guerra-Medina[2], J. J. Tinajero-Martinez[4], J. H. Avellaneda-Cevallos[3], and R. Santibañez-Escobar[1], [1]*Centro Universitario del Sur, Ciudad Guzmán, Jalisco, México.,* [2]*Centro Universitario del la Costa Sur, Autlán de la Grana, Jalsico, México.,* [3]*Universidad Técnica Estatal de Quevedo, Los Ríos, Ecuador.,* [4]*Facultad de Ciencias Agrícolas, Universidad Autónoma de Chiapas, México.*

The objective of this study was to evaluate the effect of adding inoculum and an additive handmade in sugar cane silage (SCS) on the in vitro digestibility of DM and OM. The treatments were: T1) sugar cane silage with 1% inoculum and 1% additive; T2) sugar cane silage with 3% inoculum and 3% additive were incubated for 48 h incubations. Data were analyzed by mean comparison using a T Student test. The inoculum consists of 10% molasses, 1.0% of yogurt, 5.0% chicken manure, 0.5% urea and 83.0% water and the additive was formulated with 1.0% urea, 0.1% ammonium sulfate and 0.25% phosphorus on DM basis. There were differences ($P \leq 0.05$) between treatments on the in vitro digestibility of DM. T1 shows higher percentages of DM (41.93 ± 0.024 vs. 35.06 ± 0.030), but we do not found change in OM (84.18 ± 0.48 vs. 85.28 ± 0.39); these in vitro results were not reflected improved of inclusion of higher levels of inoculum and additive in sugar cane silage. These results possibly demonstrate that although the primary objective of the addition of inoculums and additives to the sugar cane silages is for avoiding an alcoholic fermentation, these additives can affect negatively the ruminal environment and decrease the animal production.

Key words: additive, inoculum, sugar cane

M387 Effects of dietary probiotics on growth performance, nutrient digestibility, blood profiles, fecal gas emission, fecal microflora and diarrhea index in weanling pigs. S. M. Hong*[1], T. X. Zhou[1], I. H. Kim[1], and Y. H. Park[2], [1]*Dankook University, Cheonan, Choongnam, South Korea,* [2]*Yeungnam university, Daedong, Gyeongsang, South Korea.*

This study was conducted to investigate the effects of dietary probiotics on growth performance, nutrient digestibility, blood profiles, fecal gas emission, fecal microflora and diarrhea index in weanling pigs. A total of 140 weanling pigs (7.90 ± 0.92kg, initial body weight) were used for 4 weeks. Dietary treatments included: 1) NC (free antibiotics diet), 2) PC (free antibiotics diet + 0.01% tyromix), 3) P1 (NC + 0.1% probiotics), and 4) P2 (NC + 0.2% probiotics). Each treatment had 7 replicates of 5 pigs per pen in a randomized complete block design. From 0 to 2 weeks, the ADG was higher ($P < 0.05$) in PC treatment than NC treatment. PC and P1 treatments were higher than NC treatment in ADG through the entire experiment period.PC treatment was higher than other treatments in nitrogen digestibility ($P < 0.05$) and P1 treatment was higher than NC treatment in gross energy digestibility ($P < 0.05$) at the end of 2 weeks. At the end of 4 weeks, nitrogen digestibility was higher in PC, P1 and P2 treatments ($P < 0.05$) than those in NC treatment. And gross digestibility was higher ($P < 0.05$) in P1 and P2 treatments than NC and PC treatments. P1 treatment had a higher blood lymphocyte percentage than NC treatment. IgG concentration was higher in PC, P1 and P2 treatments than NC treatment ($P < 0.05$). Dietary probiotics supplementation decreased ammonia, total mercaptan and hydrogen sulfide of fecal gas emission ($P < 0.05$). Lactobacillus of fecal microflora was higher in P1 treatment than those in NC and PC treatments ($P < 0.05$) No significant difference was noted in *Escherichia coli* among treatments ($P > 0.05$). Diarrhea index was lower in P1 treatment than that in NC treatment ($P < 0.05$). In conclusion, dietary probiotics supplementation can improve growth performance, nutrient digestibility, blood profiles, fecal gas emission, Lactobacillus in fecal microflora and prevent diarrhea.

Key words: growth performance, nutrient digestibility, probiotics

M388 The response of urea-N^{15} in ruminal content influenced by essential oils. S. Zhao, J. Wang*, D. Bu, and Y. Zhang, *State Key Laboratory of Animal Nutrition, Institute of Animal Sciences, Chinese Academy of Agriculture Sciences, Beijing, China.*

Urea-N is an important and cost-less nitrogen source for ruminant, because rumen microorganisms have the ability to hydrolyze urea to ammonia which is used for microbial protein synthesis. Essential oils were found that they regulated nitrogen metabolism and fermentation in rumen. However the effect of essential oils on urea-N metabolism in rumen is limited. The purpose of this study is to reveal the distribution of urea-N in rumen and its changes influenced by the essential oils in vitro. Garlic oil, tea tree oil, and eucalyptus oil were added

into different serum bottles (containing 1.5 g TMR diet, 100 mL McDougall′s buffer, 50 mL strained ruminal fluid and 0.015 g urea-N^{15}) to the final concentrations of 300 mg/L respectively. All bottles were inoculated in a 39°C shaking water bath. Blanks without essential oils were included. All treatments were incubated in triplicate. The content of each bottle was collected at 0, 6, 12 and 24 h of incubation. The microorganisms were isolated by differential centrifugation, and the abundance of N^{15} was analyzed by mass spectrometer. The results showed that the abundance of urea-N^{15} in fermentation fluid decreased following the incubation time, but which in solid-associated and liquid-associated microorganisms increased with time. The 3 kinds of essential oils had no significant effect on urea-N^{15} distribution in solid-associated and liquid-associated microorganisms from 0 to 12 h. However, the abundance of urea-N^{15} from garlic oil treatment decreased significantly compared with tea tree and eucalyptus oil treatment in solid-associated and liquid-associated microorganisms, at 24 h. The concentration of ammonia nitrogen from garlic oil treatment in fermentation fluid decreased significantly at 24 h. DGGE revealed that bacterial population can be changed by 3 kinds of essential oils. The Shannon′s diversity index of bacteria was 2.86, 3.66, 3.82 and 2.78 for blank, garlic oil, tea tree oil and eucalyptus oil treatment. In conclusion, urea-N is accumulated by microorganism and can be influenced by garlic oil with the increase of incubation time.

Key words: essential oils, urea-N^{15}, rumen

M389 Effects of polyclonal antibody against urease on ruminal fermentation and microbiota diversity in vitro. S. Zhao, J. Wang*, D. Bu, and Y. Zhang, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Microbial urease plays an important role in the nitrogen metabolism in rumen for ruminants. However the problem is the hydrolysis rate of urea exceeds the utilization of ammonia. Therefore, many nutritionists are eager to look for inhibitors of urea hydrolysis in the rumen. The purpose of this study is to evaluate the effect of urease antibody on ruminal fermentation and bacterial diversity. Urease gene was linked to pET-30 vector and expressed in *E.coli* BL21 (DE3). The purified urease protein was injected into rabbits to prepare antibody against urease from blood. Rabbit antibodies were dosed (0, 0.5 and 1 mL/bottle) into serum bottles containing 0.25 g TMR diet, 20 mL McDougall′s buffer, 10 mL strained ruminal fluid, 30 mg urea and inoculated at 39°C. The contents of each bottle were collected at 0, 1, 2, 4, 8 and 12 h of incubation. The total microbial DNA in content was extracted by the method of Repeated Bead Beating Plus Column (RBB+C), and analyzed by DGGE to reveal bacterial diversity. The results showed that about 10 mg purified urease protein was obtained. The titer of polyclonal antibody against urease was about 1: 51200 by ELISA analysis. Antibody had a significant effect on the concentration of urea and ammonia. Compared with that of 0 mL/bottle of antibodies, the rates of urea disappearance and subsequent ammonia formation from 1 mL/bottle of antibodies descended 70.3% and 17.9% respectively ($P < 0.05$). In addition, there were 10.9%, 9.4%, 11.8%, 6.5%, 21.4%, 45.2% increases in total VFA, acetate, propionate, butyrate, valerate, isovalerate between 0 and 1 mL/bottle of antibodies ($P < 0.05$). DGGE revealed that the microbial spices were changed, with 51% identity, after added with antibody. Shannon diversity index in 0.5 mL/bottle of antibodies (3.04) and 1 mL/bottle of antibodies (2.92) differed significantly from non-addition of antibody (3.24). In conclusion, antibody against urease could slow down the rate of urea hydrolysis, which may be caused by changes of microorganism.

Key words: microbiota diversity, rumen, urease

M390 Effects of nitrate on microbial communities and rumen fermentation characteristic by using consecutive culture system. Z. Zhou*[1], Z. Yu[2], and Q. Meng[1], [1]*College of Animal Science and Technology and State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, 100193, China,* [2]*The MAPLE Research Initiative, Department of Animal Sciences, The Ohio State University, Columbus.*

The primary objective of the study was to investigate the effect of sodium nitrate on population shift of methanogen and 3 cellulolytic species (*Ruminococcus albus*, *Fibrobacter succinogenes*, and *Ruminococcus flavefaciens*), methane production and fermentative characteristic in consecutive culture system. The effects of nitrate on rumen fermentation were compared during 6 24 h consecutive cultures of ruminal microbes. When consecutive culture inoculated with 12 mM nitrate, the cumulative CH_4 production was drastically increased ($P > 0.05$) in the 3rd and 4th series culture, and decreased in the 5th and 6th series culture. Analysis of volatile fatty acids at the end of the consecutive incubation revealed no ($P > 0.05$) or minor effects of nitrate treatment on acetate accumulations, no effect ($P > 0.05$) on propionate accumulations and ratio of acetate/propionate. Real-time polymerase chain reaction (PCR) was used to quantify for mean values of relative population size (RPS, the percent of bacterial 16S rRNA copy number) of methanogens and 3 cellulolytic species. In the consecutive bath culture, methanogens distinctly decrease (RPS > 88.08%) was shown from the 2nd incubation series. The abundance of *R. flavefaciens* was also decreased to nearly undetermined level from the 2nd incubation series. The mean RPS values of *R. albus* were not significantly decreased by nitrate addition consecutive incubation series. *F. succinogens* showed a general trend to increase in the consecutive culture. These data suggest that nitrate inhibited ruminal methane production in our in vitro system but their effects on fermentation differed. Nitrate inhibit populations of methanogens, *F. succinogenes* and *R. flavefaciens*, but have hardly effect on total bacteria and *R. albus*.

Key words: cellulolytic species, methanogene, nitrate

M391 Effects of lipid sources on performance and carcass traits of beef cattle finished at pasture. T. T. Berchielli*[1,2], I. P. C. Carvalho[1,2], G. Fiorentini[1,2], and J. F. Lage[1,2], [1]*São Paulo State University, Jaboticabal, São Paulo, Brazil,* [2]*FAPESP– Fundação de Amparo á Pesquisa do Estado de São Paulo, São Paulo, SÃo Paulo, Brazil.*

This study was carried out to evaluate the effects of lipid sources added to protein-energy supplements on performance and carcass traits of finishing beef steers kept at pasture. Forty-five Nellore steers (initial average body weight of 440 ± 14 kg) were assigned to 5 treatments of a completely randomized design. The animals were divided in to 10 paddocks (2 paddocks per treatment) of *Brachiaria brizantha* ‘Xaraés’. The lipid sources: linseed oil, palm oil, soybean grain and by-pass fat (Lactoplus) were added to a supplement offered to the animals once a day (amount of 1,0% of the body weight) trying to complete 6% of ether extract on the total diet. The control treatment was composed of an energy-protein supplement with no additional fat. The supplements were based on corn and soybean meal. All the concentrate containing 20% CP and 10% EE (except the control supplement, which contained 3% EE). The experimental period was 90 d and the animals were weighed every 28 d and slaughtered at 495.6 kg. The treatments were compared by analyzing variables using the GLM procedure (SAS

9.1, SAS Institute, Inc., Cary, NC). Average daily gain (ADG) was not affected ($P > 0.05$) by the lipid sources, with mean of 0.601 kg/d. The hot carcass yield (HCY), hindquarter yield (HY), spare ribs yield (SRY) and forequarter yield (FY) were also not affected ($P > 0.05$) by the lipid source on the supplement (57.0, 48.3, 12.1 and 39.7% respectively). There was no effect ($P > 0.05$) on fat thickness, loin eye area (LEA) and LEA/100 kg of BW. The average values obtained for these traits were 7.78mm, 73.40 cm^2 and 14.83 cm^2 respectively. These results suggest that the addition of lipid sources on supplements for grazing beef cattle do not influence the performance and carcass traits when the fat level on the total diet is above 6% of ether extract.

Key words: carcass yield, fat thickness, loin eye area

M392 Effect of the different lipid sources on the carcass traits of the steers finished in a feedlot. T. T. Berchielli*[1,2], G. Fiorentini[1,2], I. P. C. Carvalho[1,2], J. F. Lage[1,2], and R. C. Canesin[1,2], *[1]São Paulo State University, Jaboticabal, São Paulo, Brazil, [2]FAPESP– Fundação de Amparo á Pesquisa do Estado de São Paulo, São Paulo, São Paulo, Brazil.*

The objective of this study was to evaluate the effect of the different lipidic sources on the carcass traits of the steers finished in feedlot. Forty-five Nellore steers (average initial body weight of 423 ± 15 kg, 16 mo of age) were fed with 60% of roughage basis of corn silage and 40% concentrate, with 7.0% of ether extract level. The fat sources were: soybean grain, protected fat (Lactoplus), linseed oil and palm oil plus a control, without additional fat. The supplements were based on corn and soybean meal. The animals were housed in individual stalls, for 90 d and slaughtered at 497.96 kg. The study was in a completely randomized design, with 5 treatments and 9 replications, and the averages were compared by the Tukey test at 5%. The carcass traits were evaluated: weight at slaughter (WS, kg), hot carcass yield (HCY, %), cold carcass yield (CCY, %), forequarter yield (FY, %), special hindquarter yield (SHY, %), spare rib performance (SRP, %), pH (24 h post slaughter), loin eye area (LEA, cm^2) and fat thickness (FT, mm). No effects of diets were observed ($P > 0.05$) in relationship to FY, SRP, pH, LEA and FT, with mean of 36.75, 9.39, 5.77, 82.41 and 6.48 respectively. However the other traits were different between treatments ($P < 0.05$) the WS was lower in animals that received the palm oil than the animals fed with linseed oil, protected fat, soybean grain and control (436.44, 494.57, 523.56, 510.22 and 522.67, respectively). Consequently the HCY and CCY were also affected and animals receiving the diet with protected fat had a greater yield than the animals fed with palm oil. The palm oil diet showed an increase of SHY compared with diet control (55.19% versus 53.31%, respectively). These results suggest that the addition of lipid sources at the level of 7.0% in diet influence the carcass traits of feedlot steers.

Key words: fat thickness, loin eye area, protected fat