M416 Samples dried with commercial dry matter techniques differ in volatile compound contents. Donald Meyer*[1], Lynn Nagengast[1], Dustin Sawyer[1], and John Goeser[1,2], *[1]Rock River Laboratory, Watertown, WI, [2]University of Wisconsin-Madison, Madison, WI.*

On-farm feed dry matter techniques determine DM by difference between original feed weight and a dried weight. Oven DM techniques, though, have been documented to volatilize more than water, leading to underestimated feed DM measures. The objective of our work was to evaluate if feeds, dried through commercially adopted DM techniques, differ in total volatile compound content relative to undried. Corn (n = 14), grass (n = 5), legume (n = 15) and small grain silages (n = 14) were collected, divided into equal subsamples using a riffle-splitter, vacuum-sealed, and frozen until analyzed. Subsamples were thawed and then handled according to 5 different drying treatments; undried (CTL), on-farm type forced-air oven dry, 60 min (KOS), 50C for 48h forced-air oven dry (OV), freeze-dry (FD) or sequential microwave-NIR (LAB). Following treatment, and to assess DM technique non-water losses, undried and corresponding dried samples were analyzed for volatile fermentation products by HPLC (lactic, acetic, propionic, butyric, succinic, and formic acids, and ethanol). Each constituent was expressed as a % of DM, using sequential microwave-3 h 105°C oven dry as a standard DM. Fermentation products were then summed to determine total volatile compounds (TV). Technique difference from CTL represents significant non-water loss and a DM measure error. The resulting data were not normally distributed and were log-transformed before being evaluated using the Fit Model procedure in SAS JMPv11.0. Feed, drying treatment and their interaction were treated as fixed effects and assessed using backward-elimination. Significance was declared at $P < 0.05$. Feed type and drying treatment were significantly related to TV. Results presented here are converted back to % of DM. The TV means were compared using Tukey's test, finding legume and corn silage (5.1 and 4.9) differed from small grain (3.6), which differed grass silage (1.80). The TV was the greatest for CTL (4.43) and was not significantly different from LAB, KOS, or FD (4.18, 3.67, and 3.13, respectively). The CTL differed ($P < 0.05$) from OV (2.7) while KOS and FD did not differ from OV. Results suggest OV underestimates feed DM.

Key Words: silage, dry matter, volatiles

M417 Comparison of in situ and in vitro methods for predicting in vivo fiber digestion. David E. Cook*[1], John P. Goeser[1,2], Lynn Nagengast[2], and David K. Combs[1], *[1]Department of Dairy Science, University of Wisconsin-Madison, Madison, WI, [2]Rock River Laboratory Inc., Watertown, WI.*

Our objectives were to compare potentially digestible NDF (pdNDF) and pdNDF digestion rate (k_d), using in situ (IS) or traditional in vitro (TR) assays, and compare subsequent estimates of total-tract NDF digestibility (TTNDFD) to in vivo (IV) TTNDFD measurements. High and low digestible corn silages (HDCS and LDCS) and alfalfa silage (AS) previously characterized for pdNDF, k_d and TTNDFD by in vivo analysis were utilized. For the IS and TR analyses, samples were incubated for 6, 12, 24, 30, 48, 72, 96, 120, and 240h. For TR, 0.5g dried, 1mm ground feed was weighed into flasks and digested with Van Soest buffer. Rumen fluid was collected from 2 cannulated, lactating cows consuming a high forage diet. Rumen fluid was held under CO_2, processed and used to immediately inoculate samples. For IS, 0.5g dried, 2mm ground feed was weighed in Ankom F57 bags. TR samples were digested in each of 2 separate runs and IS in each of 3 cannulated, lactating cows consuming a high forage diet. The NDFD data from both methods were modeled using nonlinear option of SAS JMP (v11.0) to determine pdNDF and associated k_d for each digestion method. The k_d (%/h) and concentration of pdNDF (% of NDF), using IS were 2.39 and 88.4 for HDCS, 2.24 and 83.5 for LDCS, and 5.54 and 65.5 for AS, respectively. The k_d and pdNDF using TR were 4.64 and 74.93 for HDCS, 9.82 and 56.29 for LDCS, and 5.28 and 69.84 for AS, respectively. Total-tract NDF digestibility (TTNDFD) was predicted using the following equation: $pdNDF(k_d/(k_d+k_p))$. The k_p (2.67%/h) was derived from a meta analysis of in vivo passage rates for pdNDF. HDCS, LDCS, and AS TTNDFD were 46.4, 42.3, and 49.2 for IS; and 52.8, 51.5, and 49.2 for TR. Weighted averages of the feed TTNDFD values were used to predict TMR TTNDFD for comparison with in vivo observations. Resulting TMR TTNDFD were then compared across techniques using a linear model within JMP. Main effects were IS, TR and IV. Means for each technique were compared using student's *t*-test. The TTNDFD determined from TR (50.7) differed ($P < 0.01$) from IS (46.3) and IV (43.6). Estimates of total-tract NDF digestion based on TR, k_d, and pdNDF overestimated TTNDFD measured in vivo.

Key Words: total tract, NDF, digestion

M418 Response to iso-alpha acids from *Humulus lupulus* (hops) extract on fermentation by rumen microbes in continuous culture fermenters. Isaac J. Salfer*, Samuel W. Fessenden, and Marshall D. Stern, *University of Minnesota, St. Paul, MN.*

Iso-α acids from hops (*Humulus lupulus)* derived from the brewing industry have been shown to exhibit bacteriostatic properties against gram-positive bacteria. Previous research using whole or ground hops has shown promising results for decreasing hyper-ammonia producing bacteria in the rumen. However, hops contain additional fermentable substrate and other metabolites including tannins, β-acids and xanthohumol that confound the direct effects of iso-α-acids on rumen fermentation. Research using strictly iso-α acids in rumen culture is limited. The objective of this study was to examine the direct effects of iso-α acids on fermentation by rumen microbes using a dual-flow continuous culture system. Eight fermenters were used in 2 consecutive 10-d periods consisting of 7 d of adaptation followed by 3 d of sampling. Fermenters were provided with a basal diet consisting of 44% corn silage, 14% alfalfa hay, 13% ground corn, 11% protein mix, 10% corn gluten feed, 5% cottonseed and 3% liquid vitamin and mineral supplements on a DM basis. This diet provided substrate for ruminal microbes maintained in continuous culture at a rate of 75 g of DM/L of fermenter volume/day. Iso-α Extract (IE) solution was added to the artificial saliva buffer to supply 0 (CON), 600 (LOW), 1200 (MED) and 1800 (HIGH) mg of IE/kg of diet DM/day. There was no effect ($P > 0.05$) on DM, OM, NDF or ADF digestion (%). Volatile fatty acid (VFA) metabolism was not affected by IE treatment ($P > 0.05$), with total VFA concentrations of 105.5, 93.4, 87.9 and 103.6 mM for the CON, LOW, MED and HIGH treatments, respectively. Similarly, N metabolism was not affected ($P > 0.05$) by IE level, with the CON, LOW MED, and HIGH treatments resulting in nitrogen concentrations of 7.4, 5.3, 7.6 and 6.8 mg N/dL of rumen fluid, respectively. No significant effects ($P > 0.05$) on fermenter pH were observed. In conclusion, administration of IE had no effects

on measurements of fermentation by ruminal microbes maintained in continuous culture fermenters.

Key Words: rumen, continuous culture, hops

M419 Sodium salicylate depresses fermentation by ruminal microbes in vitro. Abigail J. Carpenter*, Claudio F. Vargas-Rodriguez, Jacob A. B. Jantz, and Barry J. Bradford, *Kansas State University, Manhattan, KS.*

The administration of the anti-inflammatory medication sodium salicylate (SS) after calving has been shown to increase whole-lactation milk production in multiparous cows; however, treatment with SS is associated with hypoglycemia following its administration in some circumstances. We hypothesized that decreased glucogenic substrate supply from fermentation may contribute to decreased blood glucose concentrations in SS-treated cattle. We performed a 24-h batch culture experiment to determine the effects of SS on rumen microorganisms in vitro. Strained and pooled fluid from 3 heifers was combined in a 2:1 ratio with McDougall's buffer, and 150 mL of the inoculum was added to each flask (n = 5/treatment). Blank flasks (n = 5) contained inoculum alone, while each treated and control flask contained 2.5 g of fermentation substrate. Before inoculum was added to the flasks, 1 mL of premixed treatment mixtures were added to achieve the desired final amount of SS (CON = 0 mg, LOW = 125 mg, MED = 250 mg, HI = 375 mg). Gas production was measured with the ANKOM^{RF} Gas Production System. Dry matter disappearance (DMD) was significantly depressed by inclusion of SS ($P < 0.05$), with HI having a lower DMD than LOW ($P < 0.05$), and MED intermediate (CON = 48%, LOW = 37%, MED = 30%, HI = 23% of DM; SEM = 2%). Final pH was not different between LOW and CON, but MED and HI had higher final pH than CON (CON = 6.31, LOW = 6.36, MED = 6.42, HI = 6.45; SEM = 0.01, $P < 0.05$). No differences were observed due to treatment for volume, rate, or lag in gas production ($P \geq 0.28$). These results indicate that SS may have an inhibitory effect on rumen microorganisms in vitro, which is counterintuitive considering previous findings that SS administration in early lactation increases 305-d milk production, but consistent with previous observations of decreased blood glucose concentrations in SS-treated cows.

Key Words: sodium salicylate, fermentation, rumen modification

M420 Comparison of different four methods for determining in vitro digestibility of annual ryegrass. Mariano Alende*[1,2], Louisa Bowen[1], Prabha Ranasinghe[1], Gabriela Volpi-Lagreca[1,2], Gustavo Lascano[1], and John Andrae[1], *[1]Clemson University, Clemson, SC, [2]INTA, Anguil, Argentina.*

Multiple in vitro methods have been developed to assess forage digestibility but little is known regarding agreement in disappearance among them. This study compared 3 different rumen in vitro apparent digestibility (AD) methods (Daisy^{II} [D], Batch Culture [BC] and the Ankom Gas Production System [G]) at 4 incubation times ([IT];12, 24, 36 and 48 h). Additionally, results obtained at 24 h were compared with those obtained from dual-flow continuous fermenters [CF]. Annual ryegrass (*Lolium multiflorum*; 33.8% NDF, 16.6% CP, 91.1% OM, 27.3% WSC) was clipped from an ungrazed pasture, dried (<60°C) and ground in a Wiley mill (1 mm). Three runs of each method were conducted using rumen fluid from a cannulated Holstein cow in mid-lactation fed a 34% corn silage, 6% grass hay and 60% grain mix diet. Ankom F57 acetone pre-rinsed bags containing 0.5 ± 0.01 g of sample were used for D, BC and G. Digestibility coefficients in CF were estimated in 3 periods (7-d adaptation, 3-d collection) ran simultaneously with the other methods. The same buffer was used for all 4 methods. Data were analyzed using Mixed procedure of SAS in a model including method, IT and period as a random factor, with IT as repeated measure. Means within each IT were compared by PDIFF function. Results are presented in Table 1. Results indicate that D predicts higher AD than G and BC at IT greater than 12 h. Digestibility estimated using CF was similar to the obtained with BC and G at 24 h. We conclude that different in vitro digestibility methods could yield different results.

Table 1 (Abstr. M422). Dry matter digestibility (%) data of annual ryegrass samples assessed by 4 in vitro methods at 4 incubation times

	Incubation time (h)			
In vitro method	12	24	36	48
Daisy^{II}	58.35	70.92^{a}	75.93^{a}	79.85^{a}
Batch culture	52.88	61.29^{b}	68.14^{b}	71.82^{b}
Gas Production System	51.23	58.89^{b}	64.01^{b}	66.44^{b}
Continuous fermenter		58.97^{b}		

^{a,b}Means within each column with a different letter indicate significant differences ($P < 0.05$).

Key Words: rumen in vitro DMD, methods comparison

M421 The effect of dietary inclusion of sugar and type of sugar on ruminal short-chain fatty acid and glucose uptake across the ovine ruminal epithelium. Katie M. Wood*[1], Christine L. Rosser[1], Matthew E. Walpole[1], Rodrigo Kanafany Guzmán[1], Beth Mason[2], Timothy Mutsvangwa[1], and Gregory B. Penner[1], *[1]Dept of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada, [2]Saputo Dairy Products Canada Ltd., Saint-Léonard, QC, Canada.*

The objective was to determine whether sugar inclusion and the type of sugar (lactose; LAC or sucrose; SUC) as a replacement for starch influences ruminal glucose and short-chain fatty acid (SCFA) uptake by the rumen epithelium. Eighteen Suffolk × Arcott wethers were randomly assigned to 1 of 3 diets: control (CON), LAC (whey permeate), and SUC (molasses). Sugar content was 6% of DM for LAC and SUC and partially replaced barley grain ensuring NSC was balanced among treatments. After 31d, lambs were killed and the caudal-ventral blind sac was collected and washed using a pre-heated buffer solution. The tissue was then stripped of the underlying muscular layer and mounted in Ussing chambers under short-circuit conditions. The uptake of ^{14}C-butyrate, ^{14}C-propionate and ^{3}H-acetate under non-inhibited conditions and conditions that inhibit protein-mediated uptake were measured. The uptake of ^{14}C-glucose was evaluated without inhibition and when phlorizin (0.5 m*M*; inhibitor of sodium-linked glucose transporter; SGLT-1), or phloretin (0.5 m*M*; inhibitor of facilitated glucose transporters) were included. Data were analyzed using PROC MIXED in SAS and contrasts used to evaluate the effect of sugar (CON vs. sugar) and type (LAC vs. SUC). Acetate uptake was reduced ($P = 0.04$) for lambs fed sugar due to a reduction in passive diffusion ($P = 0.02$), whereas protein-mediated uptake did not differ ($P = 0.24$). Propionate uptake was not affected by sugar inclusion; however, lambs fed LAC tended ($P = 0.098$) to have greater propionate uptake and had greater ($P = 0.043$) protein-mediated propionate uptake than those fed SUC. Lambs fed LAC tended ($P = 0.10$) to have greater protein-mediated and passive uptake of butyrate than those fed SUC. Glucose uptake was increased 1.7 times for lambs fed sugar compared with those fed CON ($P = 0.012$). The SGLT-1 uptake tended to be greater for lambs fed sugar than those fed CON ($P = 0.09$).

The results of this study indicate that the type of sugar included in diets may influence SCFA and glucose uptake by the rumen epithelium.

Key Words: rumen, absorption, glucose

M422 Evaluation of three rumen-protected lysine sources produced in two different batches using a modified three-step in vitro procedure. Haley E. Larson*[1], Izuru Shinzato[2], Makoto Miura[3], Samuel W. Fessenden[1], Isaac J. Salfer[1], and Marshall D. Stern[1], *[1]University of Minnesota, Saint Paul, MN, [2]Ajinomoto Heartland, Inc., Chicago, IL, [3]Research Institute for Bioscience Products & Fine Chemicals, Ajinomoto Co., Inc., Kawasaki, Japan.*

A standardized 3-step in vitro procedure for evaluating rumen-protected lysine (RPL) sources was modified by Ajinomoto Co. Inc. and the University of Minnesota to estimate bioavailability of lysine within RPL products. The objective of this study was to evaluate variation between batch and source of RPL products AjiPro-L (A) and 2 other commercial products (B and C). Each RPL product was replicated 3 times in 3 runs to evaluate consistency of results within and between runs using the procedure. One gram of product from each RPL source, taken from one of 2 batches (2013, 2014) was individually weighed into a Dacron polyester bag. Bags were incubated in media bottles in a shaking water bath at 39°C for 30 h. Simulation of ruminal (20 h), abomasal (2 h) and intestinal (8 h) digestion was accomplished by immersing solutions at 20, 22 and 30 h. At each time point, a 10-mL aliquot of buffer was collected and analyzed for lysine concentration using a Bioflow BF-7 biosensor (Oji Scientific Instruments Co., Ltd., Japan). Statistical differences ($P < 0.05$) were identified using a one-way ANOVA. Differences ($P < 0.001$) were observed between the 3 product sources for rumen insoluble lysine (RIL) and abomasal insoluble lysine (AIL). Intestinally available lysine (IAL) for product A differed from B and C ($P < 0.001$). Results showed variation between 2013 and 2014 batches of product sources A and B for RIL and IAL, and product sources B and C for AIL ($P < 0.001$). The release efficiency (RE) of RIL for each product source differed between years ($P < 0.001$). Within each run of the procedure, there was no variation observed between replicate samples ($P > 0.05$). Variation was observed between runs for RIL ($P < 0.002$), AIL ($P < 0.001$) and RE ($P < 0.03$), with no variation between IAL ($P > 0.05$). The modified 3-step procedure demonstrated differences between product types and batches within the same product source. The procedure was consistent between replicates of a run demonstrating its ability to compare differences in bioavailability of RPL products.

Key Words: in vitro, rumen-protected lysine

M423 Duodenal infusion of casein but not glutamic acid increases nitrogen retention in cattle provided continuous duodenal infusion of cornstarch. Ethan J. Blom*[1], Derek W. Brake[1], and David E. Anderson[2], *[1]South Dakota State University, Brookings, SD, [2]University of Tennessee Knoxville, Knoxville, TN.*

Our objective was to quantify effects of increases among small intestinal starch digestion in response to greater postruminal flows of glutamic acid (Glu) on N retention. Five steers (351 ± 11 kg BW) were fed (5.1 ± 0.06 kg DM/d) a soybean hull-based diet formulated to provide adequate ruminally available N, moderate amounts of RUP, and small amounts of starch (0.8% DM). Cattle were placed in a 5 × 5 Latin square with 12-d periods. Cattle received (DM basis) continuous duodenal infusion of raw cornstarch (1.5 ± 0.08 kg/d), and either 0 (control), 30.9 ± 0.59, 62.4 ± 1.16, 120.4 ± 3.39 g/d Glu or 407 ± 18.3 g/d casein (a positive control). Data were analyzed with Mixed procedures of SAS; linear and quadratic effects of Glu were determined and the positive control was compared with the negative control by a *t* test. Nitrogen intake from feed was not different ($P \geq 0.23$). Infusate N increased from 0 to 13 g/d with greater amounts of Glu (Linear < 0.01) and casein provided 61 g N/d ($P < 0.01$). Similarly, total N intake was greater when cattle were provided casein ($P < 0.01$), but was not affected by Glu (Linear = 0.75). Increases in postruminal Glu did not affect urinary N excretion ($P \geq 0.30$), but casein increased urinary N excretion ($P < 0.01$). Fecal N excretion was not different ($P \geq 0.55$) despite reduced ileal flow of starch in response to greater postruminal flows of Glu (Linear = 0.04) or casein ($P = 0.07$). Glutamic acid had little impact on N retention ($P \geq 0.95$) despite increases in small intestinal starch digestion (Linear = 0.02), but casein increased N retention ($P < 0.01$). It is possible that increases in energy available for gain from increased small intestinal starch digestion in response to greater postruminal flow of Glu are used for purposes other than protein gain; however, it is likely that increases in energy available for gain exceeded capabilities for N deposition under conditions of our experimental model, because N retained as a proportion of N intake was not different when cattle were provided Glu ($P \geq 0.83$) or casein ($P = 0.38$).

Key Words: nitrogen retention, glutamic acid, cattle

M424 Rumen fermentation responses to phytogenic medicinal oils. Barbara J. M. Lemos*, Flavia M. Souza, Edemilson C. Conceição, Victor R. M. Couto, and Juliano J. R. Fernandes, *Universidade Federal de Goias, Goiania, Goias, Brazil.*

The effects of oils extracted from the trunk of *Copaifera langsdorffii* (Copaiba; 21.3% β-caryophyllene, a essential oil), and from the fruit of *Pterodon emarginatus* (Sucupira; 5.3% β-caryophyllene) on rumen fermentation were evaluated in 2 independent in vitro experiments at doses CONTROL, LOW, MEDIUM and HIGH (0, 30, 300, and 3000 mg/L of buffered rumen fluid, respectively). The oils were dissolved in 2.5 mL of ethanol, also added to CONTROL. The incubation was repeated 4 times in each experiment. The diet (50:50 concentrate:roughage ratio, 90% DM, 20% CP, 2.2% EE, 36% NDF) was ground (1 mm) and incubated using filter bags for 3, 6, 12, 24, 48, 72 and 96 h. Rumen fluid was collected from a cannulated Holstein steer fed the same concentrate:roughage ratio diet. Samples of culture fluid collected after 96 h were analyzed for N-NH_3 and VFA concentrations. The disappearance data of DM were used to calculate the degradability of DM (Orskov et al., 1980). The HIGH dose of *C. langsdorffii* oil, showed degradability of parameter b (potential degradability of the DM assumed to be degraded over time) 29% lower than CONTROL (63.4 *vs* 45.3%; $P = 0.002$) and the potential degradability (a + b, amount of DM which can be degraded within the rumen given sufficient time) was 15% lower than CONTROL (87.9 *vs* 75.2%; $P = 0.001$). No responses of *C. langsdorffii* oil was observed ($P > 0.05$) on effective degradability (at rates of passage of 5 and 8%/h) and on constituents of culture fluid (N-NH_3, total VFA, acetate, propionate, isobutirate, butirate, isovalerate, valerate and acetate:propionate ratio). In the *P. emarginatus* oil study, the response on parameter b was 45% lower for HIGH dose compared with CONTROL dose (60.4 *vs* 32.1%; $P < 0.001$). The potential degradability was affected by *P. emarginatus* doses ($P = 0.013$), it was similar to CONTROL and LOW doses (highest values, 83.0 and 83.4%, respectively), intermediate to MEDIUM (73.3%) and lower to HIGH dose (64.8%; 22% lower than CONTROL dose, $P = 0.023$). There were no effects ($P > 0.05$) of *P. emarginatus* oil on effective degradability and constituents of culture fluid. In conclusion, these phytogenic oils were able to modify microbial fermentation in the rumen environment.

Key Words: degradability, feed additive, in vitro

M425 Effect of thyme (*Thymus vulgaris*) and peppermint (*Mentha piperita*) on digestibility of a finishing diet in lambs. H. Khamisabadi[1,2], F. Kafilzadeh[1], B. Charaien[2], and G. Taasoli*[1], *[1]Department of Animal Science, Razi University, Kermanshah, Iran, [2]Research Centre of agriculture and natural resource of Kermanshah, Kermanshah, Iran.*

This experiment was conducted to study the effect of addition of 2 medicinal plants, peppermint or thyme, on digestibility of a finishing diet in lambs. Eighteen male lambs (initial body weight 21.6 ± 1.5 kg) were randomly assigned to one of 3 diets: control (with no additive), peppermint (control diet plus 3% of peppermint) or thyme (control diet plus 3% of thyme), for a 90-d finishing period. Lambs were fed individually and digestibility of nutrients were determined using insoluble ash method during 5 d of fecal sampling from all lambs. The ratio of concentrate:forage was 70:30. Diets were isocaloric and isonitrogenous. Data were analyzed as a completely randomized design with means compared using Duncan's test (SAS v9.2). Addition of peppermint or thyme to diets had no effect on digestibility of CP, ether extract, NDF and ADF but improved DM intake and digestibility of calcium and phosphorus ($P < 0.05$). Supplementation with peppermint or thyme could increase performance of finishing lambs through improved feed intake. Results are given in Table 1.

Table 1 (Abstr. M425). Effect of peppermint and thyme on DMI (g/d) and in vivo nutrient digestibility (%) of a finishing diet in lambs

Item	Peppermint	Thyme	Control	SEM	*P*-value
DM intake	1,494.4[a]	1,488.5[a]	1,185.5[b]	40.6	0.001
Nutrient digestibility					
Crude protein	77.65	75.17	76.96	0.667	1.14
Ether extract	77.93	75.43	76.58	0.074	1.28
NDF	58.04	59.50	65.19	0.424	2.20
ADF	49.76	45.96	50.13	0.183	2.56
Calcium	53.98[a]	44.21[a]	37.30[b]	2.99	0.05
Phosphorus	66.65[a]	53.61[a]	50.66[b]	2.99	0.03

Key Words: Sanjabi sheep, peppermint, thyme

M426 Abomasal infusion of glucose increases intramuscular lipid content and acetate incorporation into fatty acids in subcutaneous adipose tissue relative to ruminal acetate infusion. Terronica L. Blackmon*, Tryon A. Wickersham, Jason E. Sawyer, Rhonda K. Miller, and Stephen B. Smith, *Texas A&M University, College Station, TX.*

We hypothesized that abomasal infusion of glucose, which would provide more glucose for absorption from the small intestine, would promote intramuscular (i.m.) adipose tissue development to a greater extent than ruminal infusion of acetate, propionate, or glucose. At 22 mo of age, Angus crossbred steers (n = 24) were fitted with ruminal cannulas and adapted to a standard, corn/sorghum finishing diet over a 2-wk period. Subsequently, the steers were infused with isocaloric amounts (3.76 Mcal/d) of glucose, propionate, or acetate. Glucose was infused either into the rumen (control group) or into the abomasum, whereas propionate and acetate were infused into the rumen. Relative to abomasal glucose infusion, acetate infusion decreased DM, OM, DE, and GE intake ($P \leq 0.05$). USDA marbling scores were greater in acetate-infused steers than in than in ruminal glucose-infused steers ($P = 0.04$) and abomasal glucose-infused steers ($P = 0.08$). Ruminal propionate-infused steers had lower subcutaneous (s.c.) fat thickness over the 12th thoracic rib ($P \leq 0.10$) and lower USDA yield grades ($P \leq 0.05$) than ruminal acetate-infused steers and ruminal glucose-infused steers ($P = 0.05$). The lowest proportions of palmitic acid and palmitoleic acid in s.c. adipose tissue was observed in ruminal glucose-infused steers. Acetate infusion decreased the lipid content of i.m. adipose tissue ($P = 0.09$) and decreased the in vitro incorporation of acetate into fatty acids in s.c. adipose tissue relative to abomasal glucose infusion, ruminal glucose infusion, or propionate infusion ($P \leq 0.02$). Carcass data, lipogenesis, and fatty acid composition were analyzed using the General linear mixed models of SPSS statistics. The rate of glucose incorporation into fatty acids was greater in i.m. adipose tissue of propionate-infused steers than in abomasal or ruminal glucose-infused steers ($P \leq 0.07$). In summary, abomasal infusion of glucose did not promote higher marbling scores but did cause the greatest amounts of i.m. adipose tissue lipid, whereas propionate infusion promoted the greatest rates of fatty acid synthesis from glucose in i.m. adipose tissue.

Key Words: glucose, beef quality, lipogenesis

M427 Effects of increased inclusion of algae meal with differing fatty acid profiles on lamb total-tract digestibility. Rebecca S. Stokes*, Daniel D. Loy, Megan L. Van Emon, and Stephanie L. Hansen, *Department of Animal Science, Iowa State University,Ames, IA.*

Heterotrophic microalgae can be manipulated to contain specific fatty acids; however, it is unknown how these differences may affect nutrient digestibility in ruminants. Algae meal (ALG) contains delipidated algae (57% DM basis) and soyhulls (43%). To determine the impact of replacing corn with ALG of differing fatty acid profiles, high oleic acid (HE) or high lauric acid (HL), on nutrient digestibility, 10 whiteface wethers (27.9 ± 0.87 kg) were used in a replicated 5 × 5 Latin square. There were 5 periods, with 10 d of adaptation and 5 d of fecal and urine collection. Sheep (n = 2 sheep·diet^{-1}·period^{-1}) received one of 5 finishing diets containing corn, 35% corn silage, 5% hay, 10% soybean meal, and 5% micro ingredients: a corn-based control (CON), 18% HE ALG (18HE), 36% HE ALG (36HE), 18% HL ALG (18HL), and 36% HL ALG (36HL). Both the HE and HL ALG were similar in nutrient composition except for fat (15.8 and 8.19%, respectively) and K (1.6 and 5.4%, respectively). Pooled SEM and LSMEANS are reported. Intake of DM linearly ($P < 0.01$) increased as either ALG increased in the diet; however, DMI was less ($P < 0.01$) for 18HE-fed lambs vs. 18HL-fed lambs. Urine output linearly ($P < 0.001$) increased as ALG increased in the HL-fed lambs and was less ($P < 0.001$) for 36HE-fed lambs vs. 36HL-fed lambs. Digestibility of DM linearly ($P \leq 0.04$) decreased as both HE and HL ALG increased in the diet (75.2, 73.9, 70.1, 73.2, and 72.7 ± 0.82% for CON, 18HE, 36HE, 18HL, and 36HL, respectively). Lambs fed 36HE had less ($P = 0.03$) DM digestibility than those fed 36HL. Digestibility of NDF linearly ($P = 0.04$) increased in lambs fed HL diets (47.7, 51.6, 54.5 ± 2.18 for CON, 18HL, and 36HL, respectively). Fat digestibility linearly ($P \leq 0.02$) increased as either ALG increased in the diet; however, digestibility was less ($P < 0.001$) for 36HE-fed lambs than 36HL-fed lambs. Both ALG are highly digestible and could serve as viable feedstuffs in feedlot diets. Due to varying inclusions of fat and K within the strains of algae it was difficult to ascertain if the fatty acid profile had any effect on nutrient digestibility.

Key Words: algae, digestibility, sheep

M428 Urine pH, serum calcium, and dry matter intake evaluated in Jersey cows fed anionic salts or Animate. Tyler J. Schell*[1], Shelby A. Armstrong[1], Derek J. McLean[1], Ken P. Zanzalari[1], James D. Chapman[1], and Lane O. Ely[2], [1]*Phibro Animal Health Corporation, Quincy, IL,* [2]*University of Georgia, Athens, GA.*

Negative DCAD diets have been proven beneficial for reducing incidence of periparturient diseases in dairy cows, however maintaining dry matter intakes (DMI) have been an issue with these strategies. Twelve non-pregnant, nonlactating Jersey cows (464 kg ± 19 kg BW) were used in a crossover design study to evaluate the effect of diets fully acidified (−15 mEq/100g DM) with either an anionic salt mix (SM) or Animate (AN) on urine pH, serum calcium and DMI when fed continuously for 21 or 24 d. Cows were randomly assigned to diet (6 h/diet) at the onset of the study. The first feeding period was 24 d (P1) and the second was 21 d (P2), with a 7-d wash-out non-anionic (NA) diet period between P1 and P2. In P1, P2 and NA urine pH and DMI were recorded daily. Urine samples were collected mid-stream, 4–6 h post-feeding and pH measured. Anionic diets were offered in grain mixes and adjusted daily to maintain a urine pH between 5.5 and 6.0. Blood samples were taken on d 0 (diet assignment), 7, 14, 21, 24 in P1 and 7, 14 and 21 in P2. Diets were balanced using NDS Professional, fed as TMRs 1x/d in Calan gates and orts recorded. To insure adaptation to diets, data recorded in the last 4 d in P1, P2 and NA were averaged and compared across periods using PROC GLM and significance tested to $P < 0.05$. Urine pH in P1 and P2 for cows fed the SM and AN were 5.8 ± 0.14 and 5.66 ± 0.14, respectively, and were different from the NA (6.52 ± 0.14, $P = 0.001$). Serum calcium (mg/dL) was similar for cows fed SM or AN in P1 (9.32 ± 0.19 vs. 9.37 ± 0.2) and P2 (9.33 ± 0.21 vs. 9.2 ± 0.24) and not different from the NA (9.18 ± 0.12). Prior to start of P1, cows averaged 8.95 ± 0.18 kg DM. During P1 and P2, cows fed AN had greater DMI (+0.79 kg/d) than when fed the SM diets ($P = 0.027$). Comparing the last 4 d of P1 and P2, cows fed the AN diets consumed more DM (+1.16 kg/d, +1.02 kg/d, respectively) than when fed the SM diets ($P = 0.001$) but DMI was not different from the NA period (8.94 ± 0.19kg). In conclusion, Animate was equally effective as anionic salts for reducing urine pH and maintaining serum calcium levels in Jersey cows; however, DMI were significantly improved.

Key Words: dry matter intake, Animate, Jersey cow

M429 Nonlinear models to describe the transit of particles through the ruminant digestive tract: Evaluation of models and theoretical implications. Ricardo Augusto Mendonça Vieira*, Marcelo Cabral da Silva, Tadeu Silva de Oliveira, and Alberto Magno Fernandes, *Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil.*

Fecal profiles (n) of the particulate markers Cr (n = 52) and La (n = 30) and the fluid marker complex Co-EDTA (n = 57) were obtained from digestion kinetic studies with cows, steers, and sheep with the aim of evaluating mathematical models designed to interpret marker excretion profiles in feces. The models evaluated were the multicompartmental model of Dhanoa et al. (1985), the Gamma model described as GN by Pond et al. (1988), and the GNG1 model proposed by Matis (1972). Models were fitted by ordinary nonlinear least squares and evaluated on the basis of the Akaike information criterion and derived measures (information-likelihood criteria). Despite remarkable differences in terms of information-likelihood measures, the models were quite similar in terms of visual adherence to observed time profiles and presented overlapping interval estimates for compartment mean retention times. The major relative numerical discrepancies observed among the models were related to the mean and variance of the transit time for the first appearance of the marker in feces. The model with the best performance in mimicking the marker profiles was the GN model; the GNG1 model and the multicompartmental model were almost equivalent in terms of information-likelihood. Therefore, we derived a mathematical model to account for digestibility (D) and fill (Q) of the ruminoreticular digesta whenever the best model used to interpret marker studies, chosen based on information-likelihood criteria, was the GN model. Therefore, the GN model solutions for D and Q are as follows: $D = [k/(\lambda + k)]\ \Sigma_i[\lambda/(\lambda + k)]^{i-1}$, and $Q = AF/(\lambda + k)\ \Sigma_i[\lambda/(\lambda + k)]^{i-1} + UFN/\lambda$, for i = 1, 2, …, N. In the models, k, λ, A, and U are the fractional rate of the pool of escapable particles in the rumen, the fractional escape of particles from the rumination pool to the escapable pool, the fiber fraction available for digestion, and the unavailable fiber fraction, respectively. F is the average daily intake rate, and N is the order of time dependency. Funded by CNPq, CAPES, and FAPERJ.

Key Words: ruminant digestive tract, kinetics, mathematical model

M430 Supplementation of grazing cow's diet with corn oil and palm kernel oil: ruminal fermentation, milk production and fatty acids profile. Jair Esteban Parales, Martha Lucia Pabón, and Juan Evagenlista Carulla*, *Universidad Nacional de Colombia, Bogotá, Cundinamarca, Colombia.*

The effects of supplementing corn oil (CO) alone or combined with palm kernel oil (PKO) to grazing cows on ruminal fermentation, milk production and its fatty acid (FA) profile were studied. Six multiparous Holstein cows (597 ± 11.5 kg BW, 160 ± 29 d in milk) were assigned to a double Latin square design (3 cows × 3 diets × 3 periods × 2 squares). Three diets were evaluated: a control (C) without oil and 2 with oil addition (720 g/cow/day), one with CO, and the other with CO:PKO (75:25; COKP). Each trial period lasted 28 d (21 for adaptation and 7 for sample collection). Cows strip grazed a *Pennisetum clandestinum* pasture (3 kg DM/100 kg LW/d) and received daily corn silage (0.9 kg DM), concentrate (4.2 kg DM) and chromium oxide (9 g). Rumen fluid was collected on d 28th, milk samples on d 15, 18 and 21 of each period and feces from d 15 to 21. Milk production was recorded daily. Milk FA and ruminal VFA were determined by GC and Cr in feces and concentrate by AA. Methane production was estimated using ruminal VFA concentration. Data were subjected to ANOVA using GLM procedure of SAS and comparisons among means using Tukey's test. Voluntary intake and digestibility of the diet were similar among treatments as well as the molar proportions of acetate, propionate, and butyrate. Total VFA (m/L) was lower (C = 94.7 vs CO = 72.2 and COKP = 74.0, $P < 0.001$) for the diets with oils resulting in lower estimated methane production ($P < 0.01$). Addition of oils increased milk production (l/d) (C = 21.4 vs CO = 23.6 and COKP = 23.9; $P < 0.01$) and milk fat (%) (C = 3.15 vs CO = 3.40 and COKP = 3.40; $P < 0.05$). De novo synthesized FA (g/100 g FA) in milk was higher for the control treatment (C = 68.5 vs. CO = 52.2 and COKP = 58.6; $P < 0.01$). Conjugated linoleic acid (CLA) (g/100g FA) in milk was higher for the oil diets but decreased by addition of CPK (C = 0.68 vs CO = 1.56, and COKP = 1.01; $P < 0.01$). Supplementation with CO or COKP in diets of grazing dairy cows increased milk production without affecting voluntary intake or the digestibility of the diet but CO addition resulted in higher milk CLA.

Key Words: methane, CLA, *Pennisetum clandestinum*

M431 Effect of rumen-protected carbohydrate supplementation on blood and plasma metabolites in finishing steers during heat stress. Juan P. Russi*[1,3], Elias Peruzzo[1], Nicolas DiLorenzo[2], and Alejandro E. Relling[1], *[1]Facultad de Cs Veterinarias, UNLP, Buenos Aires, Argentina, [2]University of Florida, Marianna, FL, [3]RUPCA LLC, Merced, CA.*

Finishing steers during the summer can be challenging due to the effects of high temperatures and humidity on DMI. The objective of this study was to evaluate the inclusion of a rumen-protected carbohydrate (RUPCA) (US Patent # 8,507,025) on blood metabolites of finishing steers during heat stress. Temperature humidity Index average measured every day during the experiment was 72 ± 4.9. Crossbred steers (n = 135; 355 ± 20kg) were used in a 62-d experiment. Steers were blocked by initial BW and placed into 15 pens. Steers within blocks were randomly assigned to 3 treatments. T0) fed 91.4% of a basal diet (% DM), 22.3% corn silage, 65.9% dry corn, 0.6% sunflower meal, 0.5% urea, 2% minerals and vitamins and 8.6% of a supplement containing (% DM) 58.1% soybean meal, 38.9% soluble carbohydrates, 2% urea and 1% minerals salts, T1) fed the basal diet plus 4.3% supplement and 4.3% RUPCA and T2) fed basal diet plus 8.6% RUPCA. The supplement and RUPCA consisted of the same ingredients, differing on the processing of the carbohydrate (i.e., protected or not from ruminal degradation). Blood samples were taken from jugular vein prior morning feeding on d 0, 15, 39, and 62 and analyzed for glucose, insulin, urea and NEFA concentrations. Data were analyzed as a randomized complete block design with repeated measures using a mixed model of SAS. Initial body weight was used as a covariate. Treatment × day interactions were found for insulin ($P = 0.01$) and urea ($P = 0.02$) plasma concentrations. There were no differences on plasma concentration of insulin, NEFA or urea among treatments ($P > 0.10$). T0 showed higher blood glucose concentration ($P = 0.05$). The results suggest that including RUPCA might help to mitigate the negative effects of heat stress on blood metabolites, potentially improving animal performance. Results are shown in Table 1.

Table 1 (Abstr. M431).

					P-value		
Item	T0	T1	T2	SEM	Trt	Day	Trt × day
Glucose, mg/dL	89.4[a]	81.9[b]	83.5[b]	2.73	0.05	<0.0001	0.35
Insulin, µg/dL	0.56	0.73	0.68	0.072	0.35	0.0014	0.01
NEFA, m*M*	184.4	191.3	160.8	17.15	0.43	<0.0001	0.49
UREA, mg/dL	25.8	26.1	22.0	0.029	0.21	<0.0001	0.02

[a,b]Means without common superscript differ ($P < 0.05$).

Key Words: carbohydrate, heat stress, bypass energy

M432 Effect of rumen-protected carbohydrate supplementation on performance, blood and plasma metabolites in growing heifers. Juan P. Russi*[1], Elias Peruzzo[1], Nicolas DiLorenzo[2], and Alejandro E. Relling[1], *[1]Facultad de Cs Veterinarias, UNLP, Buenos Aires, Argentina, [2]University of Florida, Mariana, FL.*

The objective of this study was to evaluate the inclusion of a rumen-protected carbohydrate (RUPCA) (US Patent # 8,507,025) on performance, blood and plasma metabolites in growing heifers. Crossbred heifers (n = 135; 136 ± 14kg) were used in an 84-d experiment. Heifers were blocked by initial BW, placed into 15 pens and fed a diet comprised of (DM basis) 38.8% corn silage, 41.5% dry corn, 2% minerals and vitamins mix, and 17.7% supplement or RUPCA, which varied depending on treatments. The supplement and RUPCA consisted of the same ingredients (58.1% soybean meal, 38.9% soluble carbohydrates, 2% urea and 1% mineral salt), differing in the processing of the carbohydrate (i.e., protected or not from ruminal degradation). Heifers within blocks were randomly assigned to 3 treatments: T0) 17.7% supplement (100% unprotected carbohydrate), T1) 8.85% supplement and 8.85% RUPCA, and T2) 17.7% RUPCA (100% protected carbohydrate). Body weight was measured on d 0, 21, 42, 63, and 84. Pen DMI was measured weekly from d 21 to 84. Blood samples were taken on d 0, 42, 63, and 84 from jugular vein prior morning feeding and analyzed for glucose, insulin, urea and NEFA concentrations. Data were analyzed as a randomized complete block design with repeated measures using a mixed model of SAS. Treatment × day interaction were found for DMI ($P = 0.02$), ADG ($P < 0.0001$) and G: F ($P < 0.0001$) and with T1 having the lowest DMI ($P < 0.05$) and the greatest G:F ($P < 0.05$). No differences were found in the concentrations of blood glucose ($P > 0.91$), plasma insulin ($P = 0.82$), plasma NEFA ($P = 0.802$) or plasma urea ($P = 0.336$). Feeding RUPCA to growing heifers improved G:F through lower DMI without altering ADG, blood or plasma metabolites. Results are shown in Table 1.

Table 1 (Abstr. M432).

					P-value		
Item	T0	T1	T2	SEM	Trt	Day	Trt × day
DMI, kg/d	6.9[a]	5.9[b]	6.8[a]	0.06	<0.0001	<0.0001	0.02
ADG, kg	1.18	1.13	1.19	0.027	0.21	<0.0001	<0.0001
G:F	0.161[b]	0.202[a]	0.177[ab]	0.0103	0.0003	<0.0001	<0.0001
Glucose, mg/dL	90.2	91.6	91.2	3.52	0.91	<0.0001	0.92
Insulin, µg/dL	0.26	0.26	0.24	0.053	0.82	0.016	0.72
NEFA, m*M*	200.8	187.9	181.3	18.74	0.54	0.0025	0.42
Urea, mg/dL	14.9	13.4	14.8	1.03	0.36	<0.0001	0.39

[a,b]Means without common superscript differ ($P < 0.05$).

Key Words: carbohydrate, rumen, bypass energy

M433 Nonlinear parameter estimation in R and SAS: Similarities and discrepancies of both statistical programs based on a case study of digestion kinetics and animal growth curves. Ricardo Augusto Mendonça Vieira*[1], Leonardo Siqueira Glória[2], and Fabyano Fonseca e Silva[2], *[1]Universidade Estadual do Norte Fluminense, Campos dos Goytacazes, RJ, Brazil, [2]Universidade Federal de Viçosa, Viçosa, MG, Brazil.*

Growth, digestion, and passage kinetics in ruminants were studied as reference problems of nonlinear phenomena in animal science to be analyzed using nonlinear models. The statistical packages R and SAS were compared in terms of their nonlinear parameter estimation by ordinary nonlinear least squares and maximum likelihood algorithms. The assessed programs included the nls2, nlsLM, and nlme functions of R, and the NLIN, %NLINMIX macro of SAS. The quality of fit of the models was evaluated through likelihood criteria. The NLIN, nls2, and nlsLM functions yielded nonlinear parameter estimates that were almost equal in terms of scale; nevertheless, the interval estimates obtained with nls2 and nlsLM were within those estimated with PROC NLIN, despite the fact that the approximate confidence intervals are estimated using the same Student's *t*-test in both R and SAS. The degradation of fiber and passage kinetics of particulate markers were predicted with very small

numerical differences both in terms of scale and dispersion estimates. For the nonlinear mixed-effects models used to interpret growth data, the nlme (R) and %NLINMIX macro (SAS) algorithms differed in terms of the value of the likelihood function whenever heterogeneous variances, correlations, and weighting variances were fitted; nonetheless, when assuming independence and homoscedasticity, the results of the log-likelihood function were identical. The number of possible models fitted to the growth profiles was 28 with nlme function of R, whereas with %NLINMIX macro of SAS, only 13 possible models were fitted. Fortunately, the conclusions reached by fitting growth models to lamb growth data with either R or SAS were the same. Nonetheless, because each fitting experience is unique, there is no guarantee that the same conclusions would be achieved because the programs do not behave equally in the case of fitting nonlinear mixed models with different correlation and variance structures combinations. Funded by CNPq, CAPES, and FAPERJ.

Key Words: nonlinear phenomena, R-project, SAS software

M434 Effect of crude glycerin in supplement on rumen microbial profile of Nellore steers consuming low quality pasture during the dry season. Elias San Vito*, Pablo Castagnino, Erick E. Dallantonia, Yury T. Granja-Salcedo, Lutti M. Delevatti, and Telma T. Berchielli, *University Estadual Paulista-UNESP, Jaboticabal, São Paulo, Brazil.*

The effect of crude glycerin (CG) - 80% of glycerol - inclusion as a substitute to corn grain in supplements on rumen fluid protozoa numbers, and relative proportion of cellulolytic bacteria and methanogenic archaea of rumen-cannulated Nellore steers (n = 10; BW = 408.8 ± 38.5 kg) on low quality pasture, in the dry season was studied. Treatments were constituted by 5 levels of CG in the supplement: 0, 7, 14, 21 and 28% DM of CG. Animals were supplemented, daily at 1000 h in a ratio of 700 g/100 kg of BW. Supplement consisted of corn grain, soybean meal, urea, gluten meal and mineral mix. Bacteria and protozoa samples were collected on the d 11 of each experimental period, 3 h after supplementation, in the solid and liquid extracts in different parts of the rumen. Real-time PCR was used to quantify microbial population. Methanogens and cellulolytic bacteria were expressed as a proportion of total rumen bacterial 16S rDNA. Ciliate protozoa species were identified and quantified (#/mL) in a Sedgewick-Rafter chamber. Data of ciliated protozoa were log10-transformed and analyzed in a replicated Latin square design using the MIXED procedure of SAS. Bacterial proportions were analyzed using the software R, with data compared between treatments (with or without 28% DM of CG in the supplement) using the Wilcoxon test, considered significant effects at $P < 0.05$. The inclusion of CG in supplement did not affect ($P > 0.05$) rumen protozoa number (6.52, 4.46, 3.02, 2.95, 1.73, 2.16 and 1.67 for the genera *Entodinium, Dasytricha, Isotricha, Eremoplastron, Eudiplodinium, Elytroplastron*, and *Polyplastron*). The inclusion of CG had no effect in the rumen relative proportion of *Ruminococcus albus* ($P = 0.237$), *Ruminococcus flavefaciens* ($P = 0.129$), and methanogens ($P = 0.151$) with mean values of 0.0046, 0.0044, and 0.0169. However, increased ($P = 0.003$) *Fibrobacter succinogeneses*, with mean values of 0.0033 and 0.0296 for 0% DM and 28% DM of CG in the supplements, respectively. Inclusion up to 28% DM of CG in the supplement did not interfere negatively on rumen microbial profile, increased relative proportion of *F. succinogenes* of Nellore steers in low quality pasture.

Key Words: pasture, cellulolytic, glycerol

M435 Effect of crude glycerin in supplement on rumen microbial profile of Nellore steers grazing tropical grass during the rainy season. Telma T. Berchielli*, Elias San Vito, Pablo Castagnino, Yury T. Granja-Salcedo, and Erick E. Dallantonia, *University Estadual Paulista-UNESP, Jaboticabal, São Paulo, Brazil.*

The effect of crude glycerin (CG) - 80% of glycerol - inclusion as a substitute to corn grain in supplements on rumen fluid protozoa numbers, and relative proportion of cellulolytic bacteria and methanogenic archaea of rumen-cannulated Nellore steers (n = 10; BW = 490 ± 47 kg) grazing tropical grass, during the rainy season was studied. Treatments were constituted by 5 levels of CG in the supplement: 0, 7, 14, 21 and 28% DM of CG. Animals were supplemented daily at 1000 h in a proportion of 300 g/100 kg of BW. Supplement consisted of corn grain, soybean meal, urea, gluten meal and mineral mix. Bacteria and protozoa samples were collected on the d 11 of each experimental period, 3 h after supplementation, in the solid and liquid extracts in different parts of the rumen. Real-time PCR was used to quantify microbial population. Methanogens and cellulolytic bacteria were expressed as a proportion (%) of total rumen bacterial 16S rDNA. Ciliate protozoa species were identified and quantified (#/mL) in a Sedgewick-Rafter chamber. Data of protozoa were log10-transformed and analyzed in a replicated Latin square design using the MIXED procedure of SAS. Bacterial proportions were analyzed using the software R, with data compared between treatments (with or without 28% DM of CG in the supplement) using the Wilcoxon test, the effects of treatments were considered significant at $P < 0.05$. The inclusion of crude glycerin in supplement did not affect ($P > 0.05$) rumen protozoa number (6.8, 5.6, 5.2, 2.4, 2.0, and 3.6 for the genera *Entodinium*, *Dasytricha*, *Isotricha*, *Eremoplastron*, *Diploplastron*, and *Polyplastron*). The inclusion of CG decreased the relative proportion of *Ruminococcus albus* ($P = 0.047$) and *Ruminococcus flavefaciens* ($P = 0.036$), with mean values of 0.0702 and 0.0174; 0.0411 and 0.0073 respectively for 0% DM and 28% DM of CG in the supplements. However, had no effect on *Fibrobacter succinogenes* ($P = 0.420$), and methanogens ($P = 0.150$) with mean values of 0.0199 and 0.1791, respectively. Inclusion of CG at the level of 28% of DM in the supplement negatively affect the rumen cellulolytic bacteria *R. albus* and *R. flavefaciens* of Nellore steers grazing tropical grass.

M436 Improvement in saccharification yield of mixed rumen enzymes by identification of recalcitrant cell wall constituents using enzyme fingerprinting. Ajay Badhan[1], Yuxi Wang*[1], Robert Gruninger[1], Justin Powlowski[2], Adrian Tsang[2], and Tim McAllister[1], *[1]Agriculture and Agri-Food Canada, Lethbridge Research Centre, Lethbridge, AB, Canada, [2]Centre for Structural and Functional Genomics, Concordia Univ, Montreal, QC, Canada.*

Identification of factors that limit plant cell wall digestion of forages and the development of enzymatic approaches that improve hydrolysis could play a key role in improving the production efficiency of ruminants fed these feeds. Enzyme fingerprinting and FTIR analysis of barley silage and total-tract indigestible fiber residue (TIFR) in heifers' feces were used to identify cell wall components resistant to digestion. The results identified acetyl xylan esterases as key to enhance ruminal digestion, and that polysaccharide-lignin complexes are principal components in TIFR. Enzyme pre-treatment to enhance glucose yield from hydrolyses of barley straw and alfalfa hay by mixed rumen enzymes (MRE) was subsequently developed. The enzymes for pre-hydrolysis were recombinant acetyl xylan esterases (AXE16B_ASPNG and AXE16A_ASPNG), polygalacturonase (PGA28A_ASPNG), α-arabinofuranosidase (ABF54B_ASPNG) all from *Aspergillus niger*, feruloyl esterase (FAE1a) from *Anaeromyces mucronatus* (expressed in

E. coli), endoglucanase GH7 (EGL7A_THITE) from *Thielavia terrestris* (produced in *A. niger*). Lyophilized cell-free rumen fluid from cows fed forage diet was used as source of MRE. The study was conducted using micro assays in combination with simplex lattice mixture models that were designed using Design of Experiment statistical software. Fungal hemicellulases and auxiliary enzymes initiated degradation of structural polysaccharides upon application and improved the in vitro saccharification of alfalfa and barley straw by MRE. The analysis model predicted 75% (SEM: 2.8%) higher relative glucose yield from alfalfa pretreated with PGA28A_ASPNG and ABF54B_ASPNG in 1:1 ratio. Whereas, prehydrolysis of barley straw with a mixture of 50% EGL7A_THITE and 50% FAE1a increased glucose release by 100% (SEM: 1.8%) upon incubation with MRE. The results showed that microassays in combination with simplex lattice statistical experimental design can be used to predict effective enzyme pretreatments that can enhance plant cell wall digestion by MRE, and strengthen the rationale of developing specific enzyme pretreatments for forages, depending on their structure and composition.

Key Words: enzyme fingerprinting, saccharification, plant cell wall

M437 Effect of dietary protein level and vigna hay supplementation on production and efficiency of lactating dairy cows. Elmer Edgardo Corea-Guillén[1], J. M. Aguilar-Aguilar[1], N. P. Alas-Avelar[1], E. A. Alas-García[1], J. M. Flores-Tensos[1], and Glen A. Broderick*[2], *[1]Universidad de El Salvador, San Salvador, El Salvador, [2]Broderick Nutrition & Research LLC, Madison, WI.*

This trial assessed whether dietary content of CP or supplementation with vigna hay (*Vigna sinensis*) would improve milk production or efficiency of lactating cows. Thirty-two multiparous Holstein cows were blocked by DIM into 8 squares of 4 cows in a replicated 4 × 4 Latin square with a 2 × 2 arrangement of treatments: 15.5 or 17% CP, and with or without vigna hay (16.6% CP), which replaced 12.5% of dietary DM from sorghum silage (8.4% CP). Soybean meal was fed to adjust dietary CP; diets contained about 50% forage and 50% concentrate, and 38% NDF. Cows within squares were randomly assigned to treatment sequences and fed diets for 3-wk periods; data from the last week were analyzed using the mixed procedures of SAS. Table 1 reports LS-means. Diet did not alter yield of milk or milk components. Higher dietary CP increased DMI but also increased MUN and reduced N efficiency without improving yield. Supplementing dietary sorghum silage with vigna hay maintained production, reduced DMI and MUN, and increased milk/DMI, N efficiency and apparent NDF digestibility. Daily income over feed cost (IOC) averaged $0.85/cow more on the vigna hay diets. Results from this trial indicated that replacing a portion of dietary forage with vigna hay improved nutrient and economic efficiency of milk production.

Contd.

Table 1 (Abstr. M437). Effect of dietary CP (15% and 17%) and vigna hay supplementation (0 and 12.5%) on production

	15.5% CP		17% CP			Probability		
Item	0	12.5	0	12.5	SEM	CP	Vig	CP × Vig
DMI, kg/d	19.5	19.0	20.4	19.8	0.34	<0.01	<0.01	0.73
Milk yield, kg/d	28.0	29.3	29.1	29.1	0.77	0.35	0.19	0.23
Milk/DMI	1.45	1.55	1.43	1.48	0.047	0.08	0.01	0.33
Fat yield, kg/d	0.96	0.99	0.98	1.02	0.035	0.38	0.17	0.78
Protein yield, kg/d	0.88	0.91	0.92	0.92	0.024	0.17	0.31	0.30
MUN, mg/dL	17.4	16.1	19.6	18.6	0.33	<0.01	<0.01	0.54
Milk N/NI, %	28.2	30.0	25.8	27.4	0.88	<0.01	<0.01	0.81
NDF digestibility, %	42.4	52.1	41.5	52.5	0.68	0.68	<0.01	0.31
IOC, USD/cow/d	9.40	10.47	9.23	10.06	0.438	0.28	<0.01	0.67

Key Words: dietary protein, vigna hay, income over feed cost

M438 In vitro effects of a commercial blend of functional oils on rumen fermentation, methane production, and methanogenic archaea. Ahmad Reza Seradj[1], Joan Torrent*[2], Gabriel de la Fuente[1], and Joaquim Balcells[1], *[1]University of Lleida, Lleida, Catalonia, Spain, [2]Oligo Basics, Cary, NC.*

A complete randomized block design trial with 4 in vitro incubation sets were prepared to evaluate the effect of a commercial blend of functional oils (FO) containing cashew nut shell liquid and castor oil as active ingredients (Essential, Oligo Basics Agroind. Ltda., Cascavel, Brazil) on rumen fermentation, methane production and methanogenic archaea. Bottles of 120 mL were filled with 600 mg of concentrate (same as given to 4 rumen liquid donor steers), and 80 mL of an incubation solution including rumen inoculum, mineral, buffer and reducing solutions under a CO_2 stream. Sealed bottles were incubated at 39 ± 1°C for 24 h and either dosed with 500 µg of FO/g DM of basal diet or not (control). The headspace pressure was measured and sampled (0.1 mL) at 2 h intervals, up to 12 h and then 24 h post incubation to determine gas and then methane concentration using GC. The pattern of cumulative gas/methane production (y) was fitted to the model: $y = a(1-e^{-b(t-c)})$, being a the potential cumulative gas/methane production (mL); b the production rate (mL/h) and c the lag time (h). After 12 and 24 h, 2 bottles per treatment per set were sampled for NH_3-N, volatile fatty acid (VFA) concentration and molecular analyses. The DNA was extracted using a QIAamp Kit. Specific primers were used to determine absolute abundance (Log_{10} gene copy number/ g fresh sample) of total bacteria and hydrogenotrophic methanogenic archaea (HMA) and the relative abundance ($2^{(-\Delta Ct)}$) of HMA in relation to total archaea using qPCR (*CFX96* Touch). Bottles supplemented with FO showed a tendency (14.26 vs. 13.50 SEM 0. 284; $P = 0.06$) to decrease methane production (mL/g DM substrate) and reduced the methane ratio (CH_4/gas; v/v; 0.093 vs. 0.089 SEM 0.0011; $P = 0.049$), where the discrete lag time for gas production increased (0.04 vs.0.12 SEM 0.024; $P = 0.047$). Addition of FO improved rumen fermentation, increasing molar proportion of propionate (33.5 vs. 34.3 SEM 0.24; $P = 0.024$) and decreasing NH_3-N concentration (305.3 vs. 284.4 SEM 5.95; $P = 0.022$) and relative abundance of HMA (17.8 vs. 14.7 SEM 0.96; $P = 0.032$).

Key Words: fermentation, functional oil, methanogenic archaea

M439 The influence of feeding oscillating dietary crude protein contents on milk production and nitrogen utilization in lactating dairy cows. Jolet Köhler* and Timothy Mutsvangwa, *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

There is increasing public pressure on intensive dairy operations to reduce nitrogen (N) excretion into the environment, which can be achieved by adopting feeding practices that enhance the efficiency of N utilization. One such feeding strategy is feeding diets with oscillating crude protein (CP) contents, and studies with finishing beef cattle and growing sheep have reported improvements in N retention when oscillating CP diets are fed compared with static CP diets. This experiment was conducted to determine: 1) the effects of feeding oscillating CP diets on N balance and milk production in dairy cows; and 2) the optimum frequency of oscillating dietary CP concentration (i.e., 24, 48, or 72 h). Eight Holstein cows (714 ± 36 kg of BW; 114 ± 15 DIM) were used in a replicated 4 × 4 Latin square design with 30-d periods (consisting of 18 d of dietary adaptation and 12 d of sample and data collection). Treatments were a diet containing 17% CP fed on a continuous basis (designated STATIC), and diets containing 14% and 20% CP that were fed on an oscillating basis at 24 (OSC-24), 48 (OSC-48), or 72 (OSC-72) h. Diets were fed twice per day as TMR. The actual CP contents were 17.8% for the STATIC, and 14.9 and 20.3% for the oscillating CP diets, which deviated from the target CP concentrations due to variations in forage CP content. Dry matter intake (mean = 26.6 kg/d) and milk production (mean = 36.4 kg/d) were not affected ($P \geq 0.57$) by diet. Milk contents and yields of fat, protein, and lactose were unaffected ($P \geq 0.33$) by diet. Although N intakes were similar across dietary treatments, retained N was greater ($P = 0.02$) in cows fed the OSC-48 diet compared with those fed the STATIC and OSC-24 diets. Apparent total-tract DM, organic matter, CP, NDF, and ADF digestibilities did not differ ($P > 0.05$) among diets; however, cows fed the OSC-72 diet had greater ($P = 0.02$) apparent total-tract fat digestibility compared with those fed the STATIC and OSC-24 diets. Our results demonstrate that feeding oscillating dietary CP diets on a 48-h basis improves N efficiency by enhancing N retention when compared with feeding a static CP diet or an oscillating dietary CP on a 24-h basis.

Key Words: milk production, nitrogen utilization, oscillating crude protein

M440 Effect of guanidinoacetic acid on metabolism of cattle. Mehrnaz Ardalan*[1], Erick D. Batista[1,2], Cheryl K. Armendariz[1], and Evan C. Titgemeyer[1], *[1]Department of Animal Sciences and Industry, Kansas State University, Manhattan, KS, [2]Departamento de Zootecnia, Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil.*

Guanidinoacetic acid (GAA) is the precursor of creatine, which is produced by hepatic methylation of GAA. We evaluated the metabolic response of cattle to post-ruminal supplementation of GAA with and without methionine (Met) supplementation as a source of methyl groups. Six ruminally cannulated Holstein heifers (520 ± 49 kg initial BW) were fed twice daily a diet containing 6 kg/d rolled corn, 4 kg/d alfalfa hay, and 50 g/d trace-mineralized salt. The experiment used a split-plot design. All treatments were infused continuously to the abomasum. The main plot treatments were 0 or 12 g/d of L-Met arranged in a completely randomized design; 3 heifers received each treatment throughout the entire experiment. Subplot treatments were 0, 10, 20, 30, and 40 g/d of GAA, with treatments provided in sequence from lowest to highest level using 6-d periods for each level. Blood and urine samples were collected on d 6 of each period. Met supplementation increased plasma Met ($P < 0.01$). GAA supplementation linearly increased plasma Arg (% of total AA), suggesting a sparing of Arg for GAA synthesis. GAA supplementation linearly increased plasma concentrations of GAA and creatinine ($P < 0.001$) and increased plasma creatine at all levels of GAA except when 40 g/d of GAA was supplemented with no Met (GAA quadratic × Met, $P = 0.07$). Plasma homocysteine was not affected by GAA supplementation when heifers received 12 g/d Met, but it was increased in response to 30 or 40 g/d of GAA when no Met was supplemented (GAA linear × Met, $P = 0.003$); increases did not suggest a dangerous hyperhomocysteinemia. Urinary GAA and creatine concentrations were increased by all levels of GAA when 12 g/d Met was provided and by GAA supplementation up to 30 g/d when no Met was provided, but 40 g/d GAA did not increase urinary concentrations of GAA or creatine when no Met was supplemented (GAA quadratic x Met, $P \leq 0.06$). Data suggest that post-ruminal GAA supplementation increased creatine supply to cattle. An apparent methyl group deficiency, demonstrated by increases in plasma homocysteine, developed when 30 or 40 g/d of GAA was supplemented, but it was ameliorated by 12 g/d Met.

Key Words: creatine, guanidinoacetic acid, methionine

M441 Canola meals produced in different years have similar contents of rumen-undegraded protein. Glen A. Broderick*[1], Stefania Colombini[2], and Sara Costa[2], *[1]Broderick Nutrition & Research LLC, Madison, WI, [2]University of Milan, Milan, Italy.*

When evaluating protein degradability in canola meal (CM) produced in 2011 and 2012 we observed that CM from different processing plants differed in rumen-degraded protein (RDP) and rumen-undegraded protein (RUP), estimated using the Michaelis-Menten inhibitor in vitro method (MMIIV). However, these analyses were conducted in 2 different years and may have been influenced by methodological changes over time (e.g., degradative activity of rumen inoculum may have altered between years). Three CM samples were collected from each of 12 Canadian production plants in each of 2 years (2013 and 2014; total = 72). Meals were analyzed chemically and for protein degradation rate, RDP and RUP by MMIIV, assuming passage rates of 0.16 and 0.06/h for soluble and insoluble proteins, respectively (Colombini et al., J. Dairy Sci. 94:1967–1977, 2011). Half of the meals from both years were analyzed in each of 3 MMIIV incubations; a total of 6 incubations were conducted. Differences among plants and years were assessed with the Proc Mixed procedure of SAS, and LSM over all plants for each year are in Table 1. As seen in 2011 and 2012, there were large differences in RUP among plants (data not shown). There were differences by year ($P \leq 0.04$) in DM and protein fraction degraded at $t = 0$ (FD_0); CP in CM from 2013 was 1.8 percentage units greater than 2014 ($P < 0.01$). Theta (unadjusted degradation rate) was not different between years ($P = 0.50$). However, adjusting Theta for FD_0 resulted in differences in degradation rate by year ($P = 0.03$) and trends ($P = 0.06$) for differences in RDP and RUP. The results suggest that apparent differences among production years in RUP content of CM may be due to small differences in protein already degraded before incubation.

Contd.

Table 1 (Abstr. M441). Composition and degradability of canola meals from 2 years

Trait[1]	2013	2014	SEM	*P*-value
DM, %	90.9	90.4	0.15	0.04
CP, % of DM	42.4	40.6	0.22	<0.01
Insoluble-N, % of TN	71.5	72.7	0.55	0.14
Soluble-N, % of TN	28.5	27.3	0.55	0.14
FD_0, % of TN	2.4	1.8	0.13	0.01
Theta, /h	0.154	0.156	0.0078	0.50
Adjusted degradation rate, /h	0.143	0.147	0.0078	0.03
RDP, % of CP	64.5	65.0	1.18	0.06
RUP, % of CP	35.5	35.0	1.18	0.06

[1]TN = total N; FD_0 = protein degraded at t = 0; Theta = unadjusted degradation rate.

Key Words: canola meal, inhibitor in vitro, rumen-undegraded protein

M442 Enteric methane emissions of crossbred heifers fed mixtures of *Pennisetum purpureum* grass and *Leucaena leucocephala*. A.T. Piñeiro-Vázquez*[1], J.R. Canul-Solis[1], J.A. Alayón-Gamboa[2], A.J. Ayala-Burgos[1], F.J. Solorio-Sánchez[1], C.F. Aguilar-Pérez[1], and J.C. Ku-Vera[1], *[1]Faculty of Veterinary Medicine and Animal Science, University of Yucatán, Mérida, Yucatán, México, [2]The College of the Southern Frontier, Campeche, México.*

The aim of the work was to assess feed intake, apparent digestibility and methane (CH_4) emissions of crossbred heifers fed a basal ration of *Pennisetum purpureum* grass mixed with increasing amounts of chopped forage of the tropical legume *Leucaena leucocephala*. Five crossbred (*Bos indicus* × *Bos taurus*) heifers with an average live weight of 295 ± 6 kg were used. Heifers were randomly allotted to 5 treatments (0, 20, 40, 60 and 80% *L. leucocephala* on a DM basis) in a 5 × 5 Latin square design. The DM intake, DM digestibility and CH_4 production (L/day) were determined in 24 h periods, while heifers were housed in open-circuit respiration chambers. Data were analyzed with PROC GLM of SAS. Dry matter intake (DMI) and organic matter intake (OMI) were similar ($P > 0.05$) between treatments registering an average of 7.0 and 6.5 kg/day and an intake of 98.7 g DM/$kg^{0.75}$/day. Dry matter digestibility (DMD) was similar ($P > 0.05$) between treatments registering an average of 492.3 g/kg. Organic matter digestibility (OMD) was similar between the control treatment (without *L. leucocephala*) and treatments with incorporation of 20 and 40% of *L. leucocephala*, however, with 60 and 80% incorporation, OMD was significantly different ($P \leq 0.05$) compared with the control treatment. Molar proportions of acetic and propionic acids in the rumen were significantly ($P \leq 0.05$) affected, observing a difference between treatments with incorporation of *L. leucocephala* and the control treatment. A linear ($P = 0.0005$, 0.0004 and 0.0022) reduction was observed in enteric CH_4 emissions when expressed as DM, OM, and NDF (L/kg) respectively, as the level of *L. leucocephala* was increased, additionally a reduction of 61.3, 61.7 and 53.1% was observed in CH_4 emissions (L/kg) when expressed per unit of DMI, OMI and NDFI respectively, with 80% incorporation of the legume in ration DM compared with the control treatment. These data point out toward the potential of *L. leucocephala* to reduce CH_4 emissions in cattle fed a basal ration of *P. purpureum* grass.

Key Words: methane emissions, respiration chamber, feed intake

M443 Effect of dietary crude protein content on milk yield and composition in dairy cows fed diets based on rehydrated corn silage and sugar cane silage. Marcos André Arcari, Cristian Marlon de Magalhães Rodrigues Martins, Juliano Leonel Gonçalves, Dannylo Oliveira Sousa, Bruna Gomes Alves*, Alessandra Módena Orsi, and Marcos Veiga dos Santos, *Universidade de São Paulo, Pirassununga, SP, Brazil.*

The aim was to evaluate the effect of dietary crude protein (CP) content on milk yield and composition for dairy cows fed with rehydrated corn silage (RCS) and sugar cane silage (SS). Fifteen Holstein cows were distributed in 3 contemporary 3 × 3 Latin square design and diets consisted of 600g / kg dry matter (DM) of SS, 400g / kg DM of RCS and CP levels (135, 161 and 186 g CP / kg DM) contained 89.9, 120.8 and 180.5 g soybean meal; 21.2, 40.4 and 50.5 g corn meal gluten and 6.7, 8.8 and 7.9 g urea / kg DM. The dry matter intake was 18.7 kg, 21.0 kg and 20.5 kg DM respectively. The data were analyzed using the MIXED procedure of SAS, were treatment effect was decomposed into 2 orthogonal polynomial contrasts (linear and quadratic). The CP content linearly increased the milk yield ($P = 0.0005$) (27.65, 29.88 and 30.32 L / day) [Y = 20.6593 (SE = 2.3976) + 0.5509 (SE = 0.1350) × CP] and 3.5% fat-corrected milk ($P = 0.0002$) (27.90, 30.63, 31.46 L / day) [Y = 18.6007 (SE = 2.8809) + 0.7275 (SE = 0.1643) × CP). There was a linear increase in the milk concentrations of fat ($P = 0.045$) (3.53, 3.64 and 3.71g / 100mL) [Y = 3.0605 (SE = 0.2827) + 0.03627 (SE = 0.01675) × CP), protein ($P = 0.025$) (2.82, 2.87 and 2.9g / 100mL) [Y = 2.6042 (SE = 0.1305) + 0.01728 (SE = 0.007107) × CP), casein ($P < 0.0001$) (2.08, 2.17 and 2.32g / 100mL) [Y = 1.4634 (SE = 0.06818) + 0.04644 (SE = 0.003836) × CP), total solids ($P = 0.011$) (11.91, 12.24 and 12.23%) [Y = 11.0888 (SE = 0.4023) + 0.06661 (SE = 0.02284) × CP), milk urea nitrogen (12.71, 18.44 and 18.95 mg / dL) [Y = −3.6595 (SE = 2.4423) + 1.2998 (SE = 0.1440) × CP) and casein percentage:milk protein ratio ($P < 0.0001$) (73.78, 75.61 and 79.88%) [Y = 58.0580 (SE = 3.4810) + 1.1727 (SE = 0.2044) × CP) for cows fed 135, 161 and 186 g CP / kg DM, respectively. And, there was a quadratic effect on the lactose content ($P = 0.005$) [Y = 1.8836 (SE = 0.8580) + 0.3406 (SE = 0.1127) × CP - 0.01091 (SE = 0.003650) × PB^2). The increase in dietary CP content when cows were fed with RCS and SS caused an increase on milk yield and its main compounds solids.

Key Words: milk, protein, rehydrated corn silage

M444 Effects of levels of whole cottonseed and soybean oil on intake and ruminal fermentation in Nellore steers. Vinicius N. Gouvea*[1], Marcos V. Biehl[2], Marcos V. C. Ferraz Junior[1], Jose A. Faleiro Neto[1], Elizangela M. Moreira[1], Marcelo H. Santos[1], Renan G. Silva[1], Mariana F. Westphalen[2], Alexandre A. Miszura[1], Daniel M. Polizel[1], and Alexandre V. Pires[2,1], *[1]University of Sao Paulo, Pirassununga, SP, Brazil, [2]University of Sao Paulo, Piracicaba, SP, Brazil.*

In this study 6 ruminally cannulated Nellore steers (407 ± 24 kg BW) were used in a 6 × 6 Latin square design to evaluate 5 levels of whole cottonseed (0, 8, 16, 24 and 32% DM basis respectively treatments WC0; WC8; WC16; WC24 and WC32) plus a negative control diet – WCO + soybean oil (treatment SO; to reach the WC0 diet at the same fat content of WC32). Isonitrogenous (14% CP) diets containing 20% roughage 80% concentrate were formulated to provide the same amount of ruminally degradable protein. The 26-d experimental periods consisted 21-d for adaptation followed by 5-d for collection. Increasing WC linearly decreased DMI ($P < 0.01$; 7.9; 7.21; 6.77; 6.06; 6.18 kg/d respectively for WC0; WC8; WC16; WC24 and WC32).

No differences were observed between S0 and WC0 ($P = 0.23$; 7.17 *vs* 7.90 kg/d respectively) nether between S0 and WC32 ($P = 0.08$; 7.17 *vs* 6.18 kg respectively). Increasing WC increased quadratically the pH ($P < 0.001$; 6.24; 6.44; 6.54; 6.65; 6.56 respectively for WC0; WC8; WC16; WC24 and WC32). The SO inclusion increased the pH compared with WC0 diet ($P < 0.05$; 6.37 *vs* 6.24 respectively). Even with the same fat content WC32 diet presented higher pH than SO diet ($P < 0.001$; 6.56 *vs* 6.37 respectively). Total volatile fatty acids (VFA) linearly decreased with WC inclusion ($P < 0.001$; 107; 94.4; 87.0; 79.8 and 79.0 m*M* respectively for WC0; WC8; WC16; WC24 and WC32). The SO inclusion did not change total VFA compared with WC0 diet ($P = 0.22$; 102 *vs* 107 m*M* respectively). The SO inclusion increased the total VFA compared with WC32 diet ($P < 0.001$; 102 *vs* 79 m*M* respectively). Acetate:propionate (A:P) ratio linearly decreased with WC inclusion ($P < 0.001$; 2.60; 2.62; 2.22; 2.23; 1.88 respectively for WC0; WC8; WC16; WC24 and WC32). The SO inclusion did not change the A:P ratio compared with WC0 diet ($P = 0.73$; 2.66 *vs* 2.60 respectively) but compared with WC32 the SO increased the A:P ratio ($P < 0.005$; 2.66 *vs* 1.88 respectively). Intake and rumen fermentation parameters were negatively affected by the levels of WC inclusion in the diets but the main reasons for that not seems only related with the increased fat content.

Key Words: beef, fatty acid, feedlot

M445 Effect of lipid sources with different fatty acid profiles on rumen metabolites of feedlot Nellore steers. Juliana Duarte Messana*, Giovani Fiorentini, Pablo S. Castagnino, Roberta C. Canesin, and Telma T. Berchielli, *UNESP - Univ. Estadual Paulista, Jaboticabal, SP, Brazil.*

The aim of this study was to investigate the effect of diets containing lipid sources with different fatty acid profiles on ruminal pH, NH_3-N, volatile fatty acids (VFA) and microbial protein synthesis. Ten Nellore steers with initial body weight of 268 ± 27 kg, ruminally cannulated, were used in a double 5 × 5 Latin square design (20 d of each period). Dietary treatments were: without fat (WF), palm oil (PO), linseed oil (LO), protected fat (PF; Lactoplus), and whole soybeans (WS). The roughage feed was corn silage (600 g/kg on a DM basis) plus concentrate (400 g/kg on a DM basis). To evaluate rumen fermentation parameters, rumen fluid samples (approximately 80 mL) were collected manually on the last day of the experimental period (20 d), both before supplying the diet (time zero) and 1, 2, 4, 6, 8, 10, 12, and 14 h after feeding. Microbial protein synthesis was calculated via urinary total excretion of purine derivatives (allantoin + uric acid). The diet with PF and WF increased the concentration of NH_3-N ($P < 0.001$); however, the diet did not change in VFAs ($P > 0.05$), such as the molar percentage of acetate (ACE), propionate (PROP), butyrate and the ACE:PROP ratio. There was a tendency ($P = 0.06$) to change ruminal pH values and total VFA ($P = 0.092$) with lipid diets. The higher NH_3-N and tendency to reduce pH values in the WF and PF diets could be linked to the higher DMI (nitrogen and organic matter), and consequently higher ruminal fermentation. The higher production of microbial N ($P = 0.030$) was in the animals fed WF, LO and WS, whereas animals of the PO diet had the lowest production. The higher microbial protein synthesis was found in animals on the diet with LO and WS ($P = 0.040$). Diets with some type of protection (PF and WS) caused less disturbance on ruminal fermentation.

Key Words: pH, ruminal fermentation, volatile fatty acid

M446 The effect of carbohydrate source in a urea-based liquid supplement on ruminal fermentation and methane production of wintering beef cows fed low-quality forage. A. C. Conway[1], J. J. Michal[1], J. S. Chang[2], B. Carter[3], M. E. Benson[1], T. Bodine[4], and K. A. Johnson*[1], *[1]Department of Animal Sciences, Pullman, WA,, [2]Korea National Open University, Seoul, Korea, [3]Perfomix Nutrition Systems, Nampa, ID, [4]Northwest Research & Nutrition, LLC, Yakima, WA.*

The objectives of this study were to investigate the effects of 2 readily fermentable carbohydrate (RFC) sources in a urea-based liquid supplement on ruminal fermentation characteristics. Four ruminally cannulated Angus cows were fed 11.8 kg of bluegrass straw (BGS), 0.78 kg chopped triticale (TRIT), and 0.56 kg supplement on a DM basis. The experiment was performed in a Latin square design with repeated measures. Treatments were alfalfa hay (CON), a molasses-urea liquid supplement (MOL), a glycerol-urea liquid supplement (GLY) and a mixture of MOL and GLY (50/50). Ruminal fluid samples were collected at 0, 2, 4, 8, 12, 16, 20, and 24 h after feeding to analyze pH, VFA, and ruminal ammonia (NH_3-N) concentration. Ruminally incubated samples of BGS and TRIT were removed at 0, 6, 12, 24, 48, and 72 h after feeding to assess digestibility. Ruminal fluid was collected for bacterial speciation. Disappearance of BGS DM, OM and CP were unaffected ($P < 0.05$). Disappearance of NDF (48.2, 48.5, 50.3 versus 44.2 ± 2.0, respectively) and ADF (47.1, 47.5, 49.0 versus 43.9 ± 3.4 respectively) in BGS increased 4–6% with MOL, GLY, and 50/50 ($P = 0.03$) compared with CON. There was no treatment effect for digestibility of TRIT ($P < 0.05$). Total VFA concentration, VFA ratios, and ruminal pH were unaffected ($P < 0.05$), but MOL, GLY, and 50/50 increased NH_3-N concentration ($P = 0.007$). Average CH_4 emissions (192.13 ± 25.6 g/d) and methane yield (g CH_4/g gross energy intake; 5.2–5.5%) were unaffected ($P < 0.05$). Microbial species varied over time ($P < 0.01$) but not with RFC source. The improved fiber degradation in BGS indicates a GLY-based supplement is as effective as the standard MOL-based liquid supplement for wintering cows.

Key Words: liquid supplement, glycerol, methane

M447 Effect of varying type of forage and feeding times of rumen degradable nitrogen sources on the production, digestibility, feeding behavior and rumen metabolites of lactating dairy cows. Mustafa Hajilou*[1], Hamid Reza Mirzaei Alamouti[1], Mehdi Ganjkhanlou[2], Hamid Amanlou[1], and Mehdi Dehghan Banadacki[2], *[1]Department of Animal Science, University of Zanjan, Zanjan, Iran, [2]Department of Animal Science, Campus of Agriculture and Natural Resources, University of Tehran, Karaj, Iran.*

This study was designed to evaluate effects of varying type of forage and feeding times of rumen degradable nitrogen sources on milk production, digestibility, feeding behavior and rumen metabolites of lactating dairy cows. Twelve multiparous (100 ± 10 d in milk) Holstein dairy cows were used in incomplete Latin square design (five 21-d periods) with a 2 × 3 factorial arrangement of treatments. All diets had a 50:50 forage-to-concentrate ratio, contained 16% CP, and were formulated to be equal in rumen-degradable protein. Treatments include different alfalfa hay-to-corn-silage ratios (35:15 and 15:35) and different feeding times of the rumen degradable nitrogen sources: 1- total mixed ration (TMR) was offered once daily in the morning (0900); 2- part of soybean meal was offered at 2100; 3- part of urea was offered at 2100. Data were analyzed using the mixed procedures of SAS. Diets based on corn silage and feeding soybean meal at night increased milk and ECM yield ($P < 0.05$) (ALFA1 = 32.06, ALFA2 = 33.95, ALFA3 = 32.25, CORN1 =

32.69, CORN2 = 34.17, CORN3 = 33.91; SEM = 0.55). Dry matter, organic matter and neutral detergent fiber digestibility numerically increased in cows feed corn silage diets ($P < 0.09$) and not affected by feeding times of rumen degradable nitrogen sources ($P < 0.25$). Feeding urea at night increased ruminating and chewing time (min/d and min/kg of NDF intake) in alfalfa hay based diets ($P < 0.05$), but not in corn silage based diets. Standing ruminating (min/d) was higher for alfalfa hay fed cows ($P < 0.01$) and resting ruminating (min/d) was higher for corn silage fed cows ($P < 0.01$). Total volatile fatty acids and molar proportions not affected by treatments ($P > 0.15$). Corn silage based diets improved milk production and digestibility.

Key Words: forage, Holstein dairy cow, nitrogen source

M448 Effects of intravenous infusion of olive oil, safflower oil, and flaxseed oil on milk fatty acid composition in dairy cows. C. Bai*, C. J. Ao, Khas-Erdene, P. Gao, Y. Zhang, F. Y. Mi, T. L. Zhang, J. Wen, and Y. K. Zheng, *College of Animal Science, Inner Mongolia Agricultural University, Hohhot, Inner Mongolia, P. R. China.*

The objective of this study was to determine the effects of intravenous infusion of olive oil, safflower oil and flaxseed oil in various combinations on milk fat composition in lactating dairy cows. Eight Chinese Holstein cows (101 ± 11 DIM) were used in a replicated 4 × 4 Latin square design. Each period lasted 12 d with 5 d of infusion and 7 d of adaptation. Treatments were jugular vein infusion of 1) 170 g/d of oil mixture contained olive and safflower oil (43.10% *cis*-9 C18:1, 42.58% *cis*-6 C18:2 and 1.08% n-3 C18:3, OS); 2) 167 g/d of oil mixture contained olive and flaxseed oil (43.69% *cis*-9 C18:1, 13.89% *cis*-6 C18:2 and 29.94% n-3 C18:3, OF); 3) 161 g/d of oil mixture contained safflower and flaxseed oil (15.12% *cis*-9 C18:1, 44.72% *cis*-6 C18:2 and 30.50% n-3 C18:3, SF); and 4) 224 g/d of oil mixture contained olive, safflower and flaxseed oil (33.02% *cis*-9 C18:1, 32.94% *cis*-6 C18:2 and 22.63% n-3 C18:3, OSF). Treatment emulsions were consisted of water, oil mixture, 10 g/L of soy lecithin and 25 g/L of glycerol. The volume of the emulsion was 2 L/d per cow. Infusion process lasted for 6 h per day. Milk samples were collected twice daily during the last 2 d of each 5 d infusion period. Data were analyzed by using the Mixed procedure of SAS. Significance was declared at $P < 0.05$. Milk fatty acid from OF had greater concentration of *cis*-9 C18:1 (26.29%) compared with 21.80% from SF and 22.25% from OSF (SEM = 1.04). The concentration of *cis*-6 C18:2 from OF (4.36%) had the lowest level ($P < 0.0001$) compared with 6.77%, 7.07% and 7.00% from OS, SF and OSF (SEM = 0.21), respectively. The concentration of n-3 C18:3 in OS (0.66%) was lower ($P < 0.0001$) than in OF (3.57%), SF (3.26%) and OSF (3.34%), SEM = 0.13. The concentrations of C18:0 and short- and medium-chain fatty acids were not changed through treatments, whereas C16:0 was lower ($P = 0.0202$) in treatment OF (30.70%) than in treatment OS (32.62%), SEM = 0.74. Infusion of different oil combinations increased target fatty acids in milk and markedly altered milk fat composition.

Key Words: intravenous infusion, milk fatty acid, dairy cow

M449 Pelleting-induced changes at different conditioning temperatures and times on metabolic characteristics of the proteins and feed milk value of co-products from bio-oil processing. Xuewei Huang, Tom Scott, Colleen Christensen, Yajing Ban, Xinxin Li, and Peiqiang Yu*, *Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The dramatic increase in bio-oil production in Canada has resulted in millions of tonnes of different types of co-products: carinata meal and canola meal. Little research has been conducted to determine pelleting induced changes at different conditioning temperature and time on the metabolic characteristics of the proteins in co-products from bio-oil processing in dairy cattle. The objectives of this study were to investigate the effects of conditioning temperature (70, 80 and 90°C), time (50 and 75 s), and interaction (temperature × time) during the pelleting process on the metabolic characteristics of the proteins, feed milk value, the total truly absorbed protein supply (DVE), and degraded protein balance (DPB) to dairy cattle and to compare between unprocessed mash and pellets in true protein supply to small intestine of dairy cows. The DVE/OEB system was applied in determining metabolic characteristics of the proteins, feed milk value, DVE and DPB values. The data were analyzed with a randomized complete block design (RCBD) with a 3 × 2 factorial arrangement. Statistical analyses were performed through MIXED procedure of SAS 9.3. The results showed that the unprocessed co-product from bio-oil processing is a good source of the truly digested protein in small intestine (DVE: 170 g/kg DM) with DPB of 115 g/kg DM. It was unexpectedly found that the pelleting process under current conditions did not increased but reduced DVE and increased DPB values. Within pelleting processed treatments, there was no interaction ($P = 0.64$) between conditioning temperature and time on the metabolic characteristics of the proteins. However, increasing conditioning temperature tended to decrease DPB ($P = 0.051$) values of co-product pellets. Feed milk value of the co-products was reduced after pelleting process as well (3.4 vs. 3.0 kg milk per kg feed). In conclusion, pelleting with relative low temperature (70–90°C) decreased DVE, feed milk value, and increased DPB of co-products compared with raw co-products. Increasing conditioning temperature during pelleting tended to decrease the potential N loss of pelleted co-products.

Key Words: feed technology, bio-oil processing co-product, protein

M450 Ruminal fermentation of Nellore steers fed different sources of forage in diets with crude glycerin in feedlot. Andressa F. Ribeiro, Antonio Jose Neto*, Luis G. Rossi, Erick. E. Dallantonia, Ana Laura E. G. F. Carvalho, Monaliza. O. Santana, Manuela. B. Abra, Juliana D. Messana, and Telma T. Berchielli, *Sao Paulo State University, Jaboticabal, SP, Brazil.*

This study investigated the effect of alternative forages for corn silage such as sugar cane and sugar cane bagasse included in a similar forage NDF level (fNDF) in diets with crude glycerin (80.64% of glycerol) on ruminal fermentation of Nellore feedlot steers. Nine ruminally cannulated Nellore steers (300.0 ± 30kg of BW and 18 ± 2 mo of age) were used in a 3x3 Latin Square experimental design with 3 treatments and 3 animals in 3 simultaneous triplicates to evaluate the effect of different sources of forage in diets with crude glycerin (80.64% of glycerol) on ruminal pH and ammonia-N concentration. Experimental periods were 15 d (14 d for adaptation and 1 d for ruminal sampling). The treatments were different sources of forage (fixed 15% of NDF from forage; fNDF): corn silage (CS), sugar cane (SC) and sugar cane bagasse (SB), in diets with 10% (DM) of crude glycerin. Ruminal contents were obtained at 0, 2, 4, 6, 8, 10, and 12 h after feeding. Data were analyzed as a triple Latin Square design and repeated measurements on time using the PROC MIXED procedure of SAS. The least squares means were generated and

compared ($P < 0.05$) using Tukey's test. The pH values of animals fed with SB (6.40) were greater compared with CS (6.08; $P < 0.05$), which did not differ from animals fed with SC (6.27; $P > 0.05$). Animals fed with SC presented lower values of NH_3-N (11.54; $P < 0.05$) compared with animals fed with CS (15.42) and SB (15.40). Sugar cane and sugar cane bagasse included in 15% of fNDF in diets with crude glycerin (10% DM) altered ruminal parameters, however, maintained adequate conditions for animal performance.

Key Words: byproduct, corn silage, sugar cane

M451 Comparison of ruminal microbial diversity and richness in whole rumen content, rumen liquid and solid fractions.

Shoukun Ji*, Yajing Wang, Zhijun Cao, Gibson Maswayi Alugongo, Haitao Shi, and Shengli Li, *State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China.*

Whole rumen content, fluid or solid fractions have been used often to assess the rumen microbial diversity and richness; however, the suitability of using each fraction assessing the ruminal microbial diversity and richness has not been evaluated. To compare the bacterial profiles in each fraction, samples of rumen content from 6 cows were collected via fistula and squeezed through 4 layers of cheesecloth to get liquid and solid fractions before analyzing with the next generation sequencing technique. Results showed that the Operational Taxonomic Units (OTUs) numbers (4897 ± 582, 5253 ± 855 and 4860 ± 615 for whole content, liquid and solid fraction, respectively) and Simpson indices (0.99 ± 0.008, 0.99 ± 0.006 and 0.99 ± 0.004 for whole content, liquid and solid fraction, respectively) among whole content, liquid and solid fractions were similar ($P > 0.05$). No statistical difference was found among the inner- or inter-group similarities for each kind of samples using Bray-Curtis metric ($P > 0.05$). At Phylum level, Bacteroidetes and Firmicutes were the predominant bacteria and accounted for more than 90% of the microbes in all samples in our study. However, richness of Bacteroidetes in liquid fraction was 64.29 ± 0.03% which was higher than that in solid fraction as 48.25 ± 0.04% ($P < 0.05$), while that of Firmicutes was 31.72 ± 0.03% in liquid fraction which was lower compared with that of solid fraction as 44.71 ± 0.05% ($P < 0.05$). At genus level, on analyzing the top 9 bacteria (accounting for more than 43% in quantity in all samples), the fold change values of liquid fraction versus whole content (liquid/whole) were 0.51, 0.73, 0.76, 0.82, 1.07, 1.19, 1.20, 1.35, and 1.38 for *Coprococcus*, *Succiniclasticum*, *Butyrivibrio*, *Shuttleworthia*, YRC22, *Prevotella*, *Ruminococcus*, CF231, and *Oscillospira*, respectively. These findings indicate that using whole, fluid, or solid fractions to assess the microbial diversity generate similar results. However, the richness of predominant bacteria in phylum and genus may differ depending on the sample fraction type.

Key Words: rumen microbial diversity, rumen microbial richness, rumen content fraction