T205 Effects of prophylactic subcutaneous calcium treatment at calving on macro mineral status and health in Holstein cows. Hamid Amanlou[1], Ahmad Pourakbari[1], Noelia Silva del Rio*[2], and Najme Eslamian Farsuni[1], *[1]Zanjan University, Zanjan, Zanjan, Iran, [2]University of California, Davis, CA.*

The objective of this study was to evaluate the effects of prophylactic subcutaneous (SC) Ca treatment at calving on DMI at 1 DIM, serum concentrations of Ca, P, Mg and subclinical hypocalcemia (SHC) and metritis incidence on a commercial HO herd. Cows were blocked based on parity [first (n = 143), second (n = 108) and third or greater (n = 124)], and randomly assigned to treatments after calving. Treatments were no treatment (Control; n = 190); 250 ml of 40% Ca borogluconate (CB) by SC injection at calving ($1SC_{250}$; n = 72); 500 ml of 40% CB by SC injection at calving ($1SC_{500;}$ n = 63); 250 ml of 40% CB by SC injection at calving and at 12 to 18 h after the first injection ($2SC_{250;}$ n = 50). Blood samples were collected immediately after calving and at 1, 2, 4, and 7 DIM. Dry matter intake was measured for 24 h after calving in individual calving pens. Intake was analyzed with GLM procedure, including treatment, parity, and their interactions in the model. Serum Ca, P and Mg were analyzed as repeated measures with mixed procedure of SAS. Metritis (foul-smelling uterine discharge with fever) and SHC (Ca ≤8.5 mg/dl) were analyzed with PROC GENMOD with binomial distributions and logit link functions including treatment, parity, Ca status (≤8.5 vs >8.5 mg/dl) and their interactions. At 1 DIM, intake was greater ($P = 0.004$) for $1SC_{250}$ (13.5 kg), $1SC_{500}$ (15.0 kg) and $2SC_{250}$ (15.6 kg) relative to control (12.4 kg). Compared with control (8.4 mg/dl), postpartum serum Ca was greater for $1SC_{250}$, $1SC_{500}$ and $2SC_{250}$ with 8.9, 9.2 and 9.0 mg/dl respectively. No treatment effect was found on serum P and Mg. The odds of SHC were 3.7, 3.0 and 14.0 times greater ($P = 0.01$) for control than $1SC_{250}$, $1SC_{500}$ and $2SC_{250}$ respectively. The odds to develop metritis tended to be 1.7 times higher for control than $2SC_{250}$ ($P = 0.07$; 95% CI = 0.9- 3.3). These results suggest that prophylactic SC injections of Ca at calving can improve postpartum Ca status in HO cows and DMI at 1 DIM. Given the reduction on metritis and SHC with treatment, the evaluation of immune status warrant further investigation.

Key Words: calcium status, subcutaneous injection, metritis

T458 Effect of time of gestation on fatty acid transporter and receptor mRNA concentration in bovine placenta. Ramiro Desantadina[1], Silvina Quntana[2], Mariana Recavarren[2], Luis Fazzio[1], and Alejandro Relling*[1,3], *[1]Fc Cs Veterinarias, UNLP, La Plata, Argentina, [2]Lab. Farestaie, Mar del Plata, Argentina, [3]IGEVET CCT La Plata, CONICET, Argentina.*

The aim of the study was to evaluate the effect of time of gestation on fatty acid transporter and receptor mRNA concentration in maternal and fetal bovine placenta. Placentas from 12 cows at different thirds of gestation (n = 4 per third) were sampled at slaughter to measure FATP-1, FATP-4, FABP-1 mRNA concentration in maternal (caruncles) and fetal (cotyledons) side. Once the placenta was removed, 1cm2 was dissected and, divided into caruncles and cotyledons, stored in sterile tubes, dropped into liquid nitrogen and kept at −80°C until rtPCR analysis. Extraction of RNA was performed with TRIzol. Fetal and maternal placenta cDNA was subjected to qPCR assays using EvaGreen as intercalating dye (KAPA FAST, Biosystems, Woburn). Quantitative PCR was performed in a Rotor Gene Q thermocycler (Qiagen). Relative mRNA concentration was calculated by ddCt method using β actin as housekeeping gene. Data were analyzed as a complete randomized design with a 3 × 2 factorial arrangement, using the mixed procedure (SAS 9.3) with repeated measurements on space. Time of gestation, size of the placenta and their interaction were fixed factors, whereas animal was a random factor. There was a time by treatment interaction ($P < 0.01$) on FATP-1 mRNA expression of due to a greater mRNA expression in cotyledons on the first third of gestation as compared with the concentration in caruncles (Table 1). On the second and third thirds of gestation, the mRNA concentration in cotyledons decreased, reaching a similar concentration to that observed in caruncles (Table 1). FATP-4 and FABP-1 mRNA concentration were not different ($P > 0.1$, Table 1). We conclude that FATP-1 would play an important role in fatty acid transport during early fetal development.

Table 1 (Abstr. T458). Relative mRNA concentration of FATP-1, FATP-4, FABP-1 mRNA in different thirds of gestation on maternal (caruncles-M) and fetal (cotyledons-F) side on bovine placenta

	M			F				*P*-value		
Item	1	2	3	1	2	3	SEM	Side	Third	Side × Third
FATP-1	0.63	0.30	0.94	2.14	1.26	0.92	0.29	0.01	0.15	0.07
FATP-4	1.85	0.90	1.94	1.94	0.99	0.78	0.78	0.55	0.42	0.53
FABP-1	3.07	2.01	2.62	3.29	4.09	3.77	1.42	0.35	0.99	0.80

Key Words: fatty acid transporter, fatty acid receptor, bovine placenta

T459 Ensiling carinata meal with forages to decrease glucosinolate concentrations. Karla Rodriguez-Hernandez*[1,2], Jill L. Anderson[1], and Mark A. Berhow[3], *[1]Dairy Science Department, South Dakota State University, Brookings, SD, [2]CIRNOC, INIFAP, Matamoros, Mexico, [3]USDA, ARS, NCAUR, Peoria, IL.*

Carinata meal (CM) has high quality protein, but it also has high concentration of sinigrin, a glucosinolate, which limits its use as a feedstuff. Our objective was to determine if ensiling CM with forages would decrease sinigrin concentration without compromising fermentation. Two trials were conducted, one on ensiling CM with alfalfa haylage (AH) and one with corn silage (CS). For both trials 3 blends of CM and forage were made of 0:100, 25:27, and 50:50 on a DM basis. In both approximately 637 g of DM for each the 3 blends were packed in 942 cm^3 microsilos in quadruplicate for 0, 7, 21 and 60 d of ensiling. Sinigrin was greatest ($P < 0.01$) in the 50:50 and decreased over time ($P < 0.01$) in the 25:75 and 50:50 in both trials. There was no treatment by d interaction for AH blends, but there was a treatment by d interaction for the CS blends for sinigrin. The pH decreased in all blends over time, but was greater in the 50:50 compared with the other blends. Acetic acid and lactic acid increased over time in all blends. Acetic acid was less in the AH blends with increased CM. There was no treatment effect on acetic acid for the CS blends. Lactic acid was less in both trials with increased inclusion of CM. In both trials, CP increased with inclusion of CM. The CP was similar over d of ensiling in AH blends, but tended ($P = 0.05$) to decrease over d in CS blends. In both trials, NDF was less with the addition of the CM and there was a treatment by d interaction ($P < 0.01$) in CS, and a tendency ($P = 0.08$) for interaction in the AH trial. Ensiling CM with forage decreases sinigrin concentration, without major detriment to silage fermentation.

Table 1 (Abstr. T459).

Item, % DM	Blend	0:100	25:75	50:50	SEM	Treatment	Day	Trt × Day
Sinigrin, mg/g	AH	—	2.24	5.78	0.13	<0.01	<0.01	0.65
	CS	—	3.67	7.12	0.17	<0.01	<0.01	<0.01
pH	AH	4.89	4.83	5.0	0.02	<0.01	<0.01	<0.01
	CS	3.96	4.23	4.47	0.04	<0.01	<0.01	0.90
Acetic acid	AH	1.32	1.05	0.75	0.04	<0.01	<0.01	<0.01
	CS	0.89	0.96	0.94	0.03	0.22	<0.01	0.61
Lactic acid	AH	3.71	2.57	1.77	0.07	<0.01	<0.01	<0.01
	CS	2.64	2.35	2.16	0.05	<0.01	<0.01	<0.01
CP	AH	24.6	29.6	32.6	0.96	<0.01	0.20	0.09
	CS	6.2	15.6	25.0	0.28	<0.01	0.05	0.19
NDF	AH	38.5	34.8	31.8	0.25	<0.01	<0.01	0.08
	CS	33.9	30.4	28.7	0.22	<0.01	0.05	<0.01

Key Words: carinata meal, glucosinolate, ensiling

T460 Double-layered S/O/W emulsions as rumen delivery system for potential site-selective delivery of lysine in cows. Yongguang Guan and Qixin Zhong*, *Department of Food Science and Technology, University of Tennessee, Knoxville, TN.*

Essential amino acids such as lysine are deficient in most ingredients fed to cows. Direct supplementation of lysine in cow diets however is questionable because the residence time of diets in the rumen is as long as 12 h and lysine can be hydrolyzed by some rumen microorganisms. Safe rumen delivery systems are therefore highly demanded to utilize essential amino acids. The objective of the present work was to study solid/oil/water (S/O/W) emulsions as a potential rumen delivery system that can release most lysine post-rumen. Spray-dried lysine solid particles were prepared and were suspended in soybean oil. The S/O suspension was emulsified in neutral aqueous suspensions with 2, 10, and 30% whey protein concentration (WPC), which was then acidified to pH 3.0 and mixed with a 0.5% sodium alginate solution at pH 2.0 to prepare double-layered emulsion droplets. The emulsion was then spray-dried. The dimension and morphology of droplets were studied using a laser diffraction particle size analyzer, optical microscopy, and scanning electron microscopy. The amount of unencapsulated lysine and the amount of lysine released from spray-dried powder after incubation in simulated rumen, abomasum, and intestinal juices at 37°C were determined using high performance liquid chromatography. All experiments were carried out at least in triplicate. ANOVA was carried out to determine significant differences between treatments at a significance level of 0.05. Results showed that increasing WPC concentration significantly ($P < 0.05$) improved the encapsulation efficiency of lysine, up to 78.5% for the 30% WPC treatment. The particle dimension was around 10 µm based on particle size analysis and microscopy. About 10% lysine was released after 12-h incubation in the simulated rumen juice containing proteases. After an additional 3-h incubation in the simulated abomasum juice at pH 2 with pepsin, the cumulatively released lysine was less than 18%. After further in vitro digestion in the simulated intestinal juice at pH 7.0 with pancreatin, pepsin, and bile salt, the cumulatively released lysine was up to 56%. Therefore, the S/O/W emulsion can be developed as delivery systems to release most lysine post-rumen to supply important amino acids for cows.

Key Words: rumen delivery system, lysine, solid/lipid/water emulsions

T461 Effects of dietary n-6 and n-3 fatty acid sources on intake, digestibility, rumen microbes and fatty acid profile in sheep. Sardar M. Amanullah[1,3], Sam Churl Kim*[1], Dong Hyeon Kim[1], Hyuk Jun lee[1], Young Ho Joo[1], and Eun Tae Kim[2], *[1]Division of Applied Life Science (BK21Plus, Insti. of Agric. & Life Sci.), Gyeongsang National University, Jinju, Gyeongsangnam-do, South Korea, [2]Dairy Science Division, National Institute of Animal Science, RDA, Cheonan, Chungcheongnam-do, South Korea, [3]Bangladesh Livestock Research Institute, Dhaka, Bangladesh.*

A study was conducted to know the effects of dietary oil sources rich in n-6 and n-3 FA on nutrient intake, digestibility, rumen microbial population and fatty acid (FA) profile in sheep. Four pre-pubertal female sheep (48.03 ± 3.69 kg) were housed individually in digestion crates in 4 × 4 Latin square design. Timothy hay and concentrate mixture (3:7 ratio) was the basal diet which was supplied at 2% of live weight on DM basis. Treatments were control (CON), corn oil (CO: n-6), linseed oil (LSO; n-3), and calcium salt of FA (Ca-Salt; protected n-6). Oil sources were pre-mixed with concentrate at 3% of fresh weight. Each period consisted with 10 d of adaptation and 5 d of sample collection. The orts, feces and urine were collected daily before morning feed during the collection period. Blood sample was collected on collection d 4 after 1 h of morning feed, while rumen fluid sample was collected on d 5 at 3 h after morning feed by stomach tube. Data were analyzed using the GLM procedure of SAS. It was observed that intake and digestibility of nutrients were not affected by the supplementing oil sources ($P > 0.05$). Real Time PCR revealed no differences in DNA concentration of methanogenic archaea ($P = 0.047$), *Fibrobacter succinogens* ($P = 0.307$) and *Ruminococcus falvefacience* ($P = 0.327$) among treatments. On the other hand, DNA concentration of rumen ciliate (*Entodinium*) was reduced ($P = 0.018$) by Ca-Salt, while *Ruminococcus albus* was reduced ($P = 0.028$) by LSO compared with the others. The ruminal concentration of C18:0 was highest ($P = 0.012$), but C16:0 ($P = 0.001$) and C16:1n-9 ($P = 0.018$) were lowest in LSO treatment. Other major 18-carbon FAs remained unaffected ($P > 0.05$) in rumen contents. Plasma FA profile showed that, not only C18:3n-3 ($P < 0.001$), but also C20:5n-3 ($P = 0.033$) were increased by LSO supplementation. Consequently, n-6 to n-3 FA ratio ($P = 0.007$) was decreased by LSO treatment. Results indicated that, increased concentration of n-3 FA and decreased n-6 to n-3 FA ratio in plasma can be achieved by supplementing linseed oil in ruminant's diet without affecting intake and digestibility of nutrients.

Key Words: fatty acid profile, oil source, rumen microbe

T462 Approaches to confidence intervals for the energy requirements of beef cattle. Hugo Colombarolli Bonfá*, Edenio Detmann, Paulo Roberto Cecon, Sebastião de Campos Valadares Filho, and José Gilson Louzada Regadas Filho, *Universidade Federal de Minas Gerais, Viçosa, Minas Gerais, Brazil.*

The objective of this study was to propose approaches to the confidence intervals for the net and metabolizable energy requirements for maintenance and for the efficiency of utilization of metabolizable energy for maintenance and weight gain in beef cattle. A simulated population of 100,000 animals was used to demonstrate the distributional properties of the energy requirements. One hundred random samples (n = 100) were taken from a simulated population (n = 100,000). From those samples it was obtained through the Qui-Square and Kolmogorov-Smirnoff tests that net and metabolizable energy requirements for maintenance can be studied by using the properties of the normal distribution ($P > 0.05$). This condition can be reinforced by the sigmoid pattern showed by the upper limits of the confidence intervals when plotted in a scatter graph. The confidence intervals approaches were proposed and demonstrated

using the properties of the normal distribution, and using approaches based on anamorphosis techniques and on utilization of a Taylor's series. A data set of 158 animals was used to demonstrate and validate the proposed approaches. The methods that were developed in this study allow obtaining the variance information and confidence intervals for the energy requirements of cattle more affordable by ruminant nutrition researchers, who can obtain confidence intervals for both energy requirements and efficiency of energy utilization based on information from one single experiment. The results demonstrated the feasibility of use of such approaches, which are relevant tools for the practice of inductive statistics and for the inter- and intra-experimental comparisons.

Key Words: efficiency of use of metabolizable energy, nutrient requirements of cattle, statistical inference

T463 Predicting ruminal methane inhibition by condensed tannins using nonlinear exponential decay regression analysis. Harley D. Naumann[1], Mozart A. Fonseca*[2], and Luis O. Tedeschi[2], *[1]University of Missouri, Columbia, MO, [2]Texas A&M University, College Station, TX.*

Methane (CH_4) is a potent greenhouse gas that is normally produced by microbial fermentation in the rumen and released to the environment during the eructation process. Prediction of ruminal CH_4 is important for ruminant nutrition, especially for determination of ME intake. Equations have been developed to predict ruminal CH_4 production based on dietary constituents, but none have considered condensed tannins (CT). Our objective was to develop an equation to predict ruminal CH_4 inhibition by CT. We gathered CH_4 production data from 24- to 48-h in vitro fermentation of diverse forages containing different concentrations of CT over the course of 3 years. Our analysis included 113 observations. The predictor variable CT was regressed on the response variable CH_4 using PROC NLIN of SAS and the Gauss-Newton method to converge the parameters of the nonlinear regression. We used the following exponential decay model to express the relationship between CT and CH_4: $Y = span \times e^{(-KxX)} + plateau$, where Y is CH_4, g/kg FOM; *span* is the difference between Y when CT equals zero and the *plateau* (Y value at infinite), g/kg FOM; K is the fractional rate of decline, 1/% DM; and X is CT concentration, % DM. The following nonlinear exponential decay regression equation was developed: $CH_4 = 113.6 \times e^{-0.1751 \times CT} - 2.18$ ($r^2 = 0.52$; $P < 0.0001$). This equation predicted that CH_4 production could be reduced by 50% when CT is about 3.85% DM. We used several statistics to evaluate the adequacy of this equation, including precision and accuracy. We determined that this equation is more accurate when screening CT-containing forages for their potential ability to mitigate CH_4 production by ruminants when the CT concentration is greater than 5% DM. We concluded that despite the large degree of variability in ruminal CH_4 production, this equation can be used as a tool for predicting potential ruminal CH_4 inhibition that occurs when feeding CT-containing forages to ruminants. Future research should focus on the development of predictive equations when other potential reducers of ruminal CH_4 are used in conjunction with CT.

Key Words: forage, greenhouse gas, modeling

T464 Effect of lipid sources with different fatty acid profiles on intake and nutrient digestion of feedlot Nellore steers. Juliana Duarte Messana*, Giovani Fiorentini, Isabela P. C. Carvalho, Pablo S. Castagnino, and Telma T. Berchielli, *UNESP - Univ. Estadual Paulista, Jaboticabal, São Paulo, Brazil.*

The present study was conducted to determine the effect of lipid sources with different fatty acid profiles on intake and nutrient digestion. Ten rumen and duodenal fistulated Nellore steers (268 body weight ± 27 kg) were distributed in a double 5 × 5 Latin square (5 periods of 20 d, including 15 d for diet adjustment and 5 d for sample collection). 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). Throughout the entire experimental period, the allowance was adjusted to allow refusals of approximately 100 g/kg in relation to the total amount consumed on the previous day. Feed refusals were collected and weighed before feeding for the first 5 d of each experimental period. Feces were collected for 5 d to estimate the digestibility of dietary constituents. Data were analyzed using the PROC MIXED of SAS. The higher intake ($P < 0.001$) of DM, OM was found in animals on the diet with PF and WF. Animals fed with WS had intermediate intake, whereas the diet PO had the lowest intake. The intake of DM and OM of animals fed with LO did not differ from PO and WS diets. The higher EE intake ($P < 0.001$) occurred in animals receiving PF. However, animals fed PO, showed intake similar to that of animals fed WF. There were no differences for the NDF intake ($P > 0.05$). The treatments with PO and LO decreased total digestibility of DM ($P = 0.021$), OM ($P = 0.048$) and EE ($P < 0.001$). Animals fed with the WF diet had lower ($P < 0.001$) EE digestibility than other diets. The addition of LO resulted in the lowest NDF digestibility ($P = 0.047$; 0.40 kg/kg), and the NDF digestibility was not different among the other diets. These observations may be linked to the number of double bonds in the fatty acid molecules and to the high availability of this source (LO), which tends to reduce cellulolytic bacteria proportion. Diets with lipid sources with different fatty acid profiles affected intake and nutrient digestion.

Key Words: beef cattle, lipid, metabolism ruminant

T465 Experimental design and data-reporting needs to help support the advancement of nutrition research and nutrient requirement models. C. Roselina Angel[7], Mark Hanigan[2], Ermias Kebreab[3], Brian Kerr[4], John P. McNamara[5], Nathalie Trottier[1], Luis O. Tedeschi[6], Mike J. Vandehaar[1], and Robin R. White*[2], *[1]Michigan State University, East Lansing, MI, [2]Virginia Tech University, Blacksburg, VA [3]University of California Davis, Davis, CA, [4]ARS USDA, Ames, IA, [5]Washington State University, Pullman, WA. [6]Texas A&M, College Station, TX, [7]University of Maryland, College Park, MD.*

The National Animal Nutrition Program, National Research Support Project 9, supports efforts in livestock nutrition, including the National Research Council's committees on the Nutrient Requirements of Animals. Our objective was to review the current status of experimentation and data reporting in animal nutrition literature and to provide suggestions for the advancement of animal nutrition research and the ongoing improvement of field-applied nutrient requirement models. Improved data reporting consistency and completeness represent a substantial opportunity to improve nutrition-related mathematical models. A body of nutrition research was reviewed and common phrases used to describe diets, animals, housing and environmental conditions were recorded and equivalent numerical data that could be reported were proposed. With the increasing availability of online supplementary material sections available in journals, a comprehensive checklist of data that should be included in publications was developed. To continue to improve our research effectiveness, studies utilizing multiple research methodologies to address complex systems and measure multiple variables will be necessary. From the current body of animal nutrition literature, a series of opportunities to integrate research focuses (nutrition, reproduction and

genetics) to advance the development of nutrient requirement models were identified. Examples of possibilities to integrate research methodologies include analysis of the energy cost of ionic gradients in cells and protein turnover in tissues. From our survey of experimentation and data reporting in animal nutrition, 4 key opportunities to advance animal nutrition knowledge were identified: 1) coordinated experiments must be designed to employ multiple research methodologies; 2) publication guidelines and restrictions should be updated to allow more complete data sets to be made available; 3) systems-oriented research approaches should be encouraged and supported; and 4) such new data should be more rapidly be integrated into our knowledge bases, research programs and practical applications

Key Words: experimental design, systems biology, NANP

T466 Oxygen uptake by splanchnic tissues of sheep infused with different N compounds into the mesenteric vein. Simone Stefanello, Gilberto V. Kozloski*, Mariana P. Mezzomo, Alsiane S. Capelesso, Tiago Orlandi, Fernanda Hentz, and Diego Zeni, *Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*

Gluconeogenesis and ureagenesis might be integrated and energy expensive processes and, thus, a trial was conducted to measure the impact of the mesenteric infusion of different N compounds on oxygen uptake by splanchnic tissues of wethers. The trial was conducted with 4 multicatheterized wethers as a 4 × 4 Latin square with 210-min daily periods. The blood flow through portal-drained viscera (PDV) and liver was determined by downstream dilution of 15 g/L *p*-aminohippurate (PAH) infused continuously (1.5 mL/min) into the mesenteric vein. In parallel, wethers were continually infused into the mesenteric vein with a saline (0.15 *M* NaCl) solution during 90 min followed by the infusion, during more 120 min, of either: saline (control), 0.25 *M* NH_4HCO_3, 0.25 *M* L-alanine or 0.125 *M* L-arginine, all of them infused at a rate of 1.5 mL/min to provide 375 µmol N/min. Throughout infusion periods simultaneous arterial, portal and hepatic blood samples were taken at 30 min interval and analyzed for PAH and oxygen concentration. The PROC MIXED of SAS was used for variance analysis, which generated a residual error. The oxygen uptake during treatment infusion (i.e., 90 to 210 min) was compared with that of control period (i.e., first 90 min) within each treatment by F test. Oxygen uptake by PDV during treatment infusion was not different from that observed during saline infusion for any treatment, whereas only NH_4HCO_3 infusion increased oxygen uptake by liver (Table 1). In conclusion, increased ammonia load increased energy expenditure by liver of wethers.

Table 1 (Abstr. T466). Oxygen uptake (mL/h) by portal-drained viscera (PDV) and liver of wethers infused with saline (NaCl) or with 375 µmol N/min of different N compounds into the mesenteric vein

		Infusion treatments				
Item	Time (min)[1]	NaCl	Ammonia	Alanine	Arginine	SEM
PDV	0-90	2871	2692	3210	3532	580.2
	90-210	2857	2579	3115	3583	280.5
*P**		0.981	0.822	0.880	0.948	
Liver	0-90	1600	1617	2164	3154	316.6
	90-210	1917	2583	2460	2548	246.5
*P**		0.453	0.045	0.385	0.157	

[1]Saline solution was used through the first 90 minutes in all treatments.
*Probability of the difference between 90-210 vs. 0-90 min means.

Key Words: ammonia, amino acids, ureagenesis

T467 Net flux of metabolites by liver of sheep infused with different N compounds into the mesenteric vein. Simone Stefanello, Gilberto V. Kozloski*, Renato N. Libardoni, Gabriela P. Coradini, Sabrina Bäumer, Marta L. R. Leal, and André V. Soares, *Universidade Federal de Santa Maria, Santa Maria, RS, Brazil.*

Ureagenesis and gluconeogenesis might be integrated processes and, thus, a trial with 4 multicatheterized wethers was conducted to measure the impact of the mesenteric infusion of different N compounds on liver net flux of urea and glucose. The trial was conducted as a 4 × 4 Latin square with 210-min daily periods. The blood flow through portal-drained viscera (PDV) and total splanchnic tissues (ST) was determined by downstream dilution of 15 g/L *p*-aminohippurate (PAH) infused continuously (1.5 mL/min) into the mesenteric vein. In parallel, wethers were continually infused into the mesenteric vein with a saline (0.15 *M* NaCl) solution during 90 min followed by the infusion, during more 120 min, of either: saline (control), 0.25 *M* NH_4HCO_3, 0.25 *M* L-alanine or 0.125 *M* L-arginine, all of them infused at a rate of 1.5 mL/min to provide 375 µmol N/min. Throughout infusion periods simultaneous arterial, portal and hepatic blood samples were taken at 30 min interval and analyzed for PAH, urea and glucose. Liver net flux was the difference between TS and PDV values. The PROC MIXED of SAS was used for variance analysis, which generated a residual error. The hepatic net flux obtained during treatment infusion (i.e., 90 to 210 min) was compared with that of control period (i.e., first 90 min) within each treatment by F test. Hepatic net flux of urea was increased only for NH_4HCO_3 whereas glucose net flux was increased only when alanine was infused into the mesenteric vein (Table 1). In conclusion, no clear relationship between ureagenesis and gluconeogenesis was observed in wethers.

Table 1 (Abstr. T467). Hepatic net flux (mg/h) of metabolites by wethers infused with saline (NaCl) or with 375 µmol N/min of different N compounds into the mesenteric vein

		Infusion treatments				
Item	Time (min)[1]	NaCl	Ammonia	Alanine	Arginine	SEM
Urea	0-90	431	437	585	538	68.9
	90-210	450	752	602	519	89.1
*P**		0.796	0.044	0.888	0.889	
Glucose	0-90	2817	4220	3042	3799	540.5
	90-210	3825	4648	5502	4306	395.5
*P**		0.194	0.757	0.018	0.556	

[1]Saline solution was used through the first 90 minutes in all treatments.
*Probability of the difference between 90-210 *vs.* 0-90 min means.

Key Words: amino acids, gluconeogenesis, ureagenesis

T468 Evaluation of dairy and beef farm greenhouse gas emissions in different areas of Spain. Ibidhi Ridha and Sergio Calsamiglia*, *Animal Nutrition and Welfare Service, Universitat Autonoma de Barcelona, Bellaterra, Spain.*

Ruminants are recognized sources of greenhouse gas (GHG) emissions. The objective of this paper was to estimate the carbon footprint of dairy and beef farms in different production systems in Spain. Component models for predicting all important sources of CH4, N_2O, and CO_2 from primary and secondary sources in dairy and beef production were estimated with the Integrated Farm System Model (IFSM) and reported in CO_2 equivalent (eCO_2/kg of energy-corrected milk (ECM) or kg body weight (BW)). The IFSM and Cornell Net Carbohydrate and Protein System (CNCPS) were used to evaluate dairy and beef farms in Spain for

GHG emissions and diet evaluation on methane production, respectively. Three dairy farms from each of 3 regions were selected: Mediterranean (Catalonia, Valencia and Murcia), Cantabric area (Galicia, Asturias and Cantabria) and Central zone (Castilla-La Mancha, Castilla-Leon and Madrid). The average carbon footprint (kg eCO_2/kg of ECM) of all dairy farms was 0.83, with the Mediterranean farms being highest ($P <$ 0.01); 0.98) compared with the Central Zone (0.84) and the Cantabric area (0.67). Two extreme farms were selected for further simulations: the first one had the highest carbon footprint and non-enteric methane, while the second had the lowest carbon footprint and the highest enteric methane. These farms were simulated by the IFSM model using different management change scenarios (higher productivity, manure type collection, bedding type, anaerobic digester and storage type of manure) and dietary changes (modification of the ratio forage:concentrate, improved forages quality, inclusion of fat, use of ionophore). Management changes reduced methane emission up to 30% while dietary change reduced it up to 5%. Two beef farms fed 90:10 concentrate:straw and one fed with corn silage were used to simulate GHG emissions using the same models. The carbon footprint (eCO_2/kg BW) was 6.98 in beef fed corn silage and 6.90 in beef fed without corn silage. Management strategies provided a greater potential to reduce methane emissions compared with dietary scenarios changes.

Key Words: carbon footprint, dairy and beef farm, Integrated Farm System Model (IFSM)

T469 Detect the association of protein structures to protein nutrient utilization and availability of co-products from bio-fuel and bio-brewing processing. Xuewei Zhang[1], Limei Chen[2,1], Yajing Ban[2], and Peiqiang Yu*[2,1], *[1]Department of Animal Science, Tianjin Agricultural University, Tianjin, China, [2]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada.*

The objective of this study was to identify the correlation between protein molecular structures and protein nutritional profiles of co-products from cereal grains after bio-fuel and bio-brewing processing in terms of (1) protein chemical profiles; (2) protein subfractions; (3) total digestible protein; (4) protein degradation and estimated intestinal CP digestibility. Five kinds of corn DDGS and 2 kinds of barley DDGS were collected from different manufactures in the north of China from 2012 to 2013. The protein molecular structure spectral feature were analyzed using advanced molecular spectroscopy technique at University of Saskatchewan. The protein subfractions were partitioned using CNCPS system. Total digestible protein, protein rumen degradation and intestinal digestion were determined using dry Holstein dry cows at University of Saskatchewan. Statistical analysis was performed using the PROC MIXED of SAS 9.3. The results showed that the co-products from corn and barley grains differed in both protein nutritional profiles and protein molecular structures in terms of α-helix, β-sheet spectral intensity and their ratio and amide I, amide II spectral intensity and their ratio. Protein amide II height had a positive correlation with ($P < 0.05$) PB2 fraction with R = 0.53, but that other protein amide parameters had no correlation with ($P > 0.05$) PA, PB1, PB3 and PC fractions. Protein amide II height had a positive correlation with ($P < 0.05$) TDN with R = 0.74. Protein amide II height has a negative correlation with ($P <$ 0.05) protein degradability (R_DCP) with R = −0.67, and a positive correlation with ($P < 0.05$) intestinal protein digestibility (I_DCP) with R = 0.60 and total-tract available protein (T_ACP) with R = 0.58. For protein secondary structure, the α-helix to β-sheet ratio was negatively correlated with ($P < 0.05$) total protein digestibility (T_DCP) with R = −0.56 and positively correlated with ($P < 0.05$) total digestible crude protein (tdCP) with R = 0.55. In conclusion, protein molecular spectral parameters in the co-products are association with protein nutritional profiles and protein degradation and digestion.

Key Words: protein nutritive value, protein molecular structure, molecular spectroscopy

T470 Effects of essential oils from wormwood hybrids on *in vitro* digestibility, microbial diversity and rumen fermentation of bermudagrass hay and soybean meal . Seong Shin Lee*[1], Hee Yoon[1], Hyuk Jun Lee[1], Dong Hyeon Kim[1,3], Sardar M. Amanullah[1], Young Ho Joo[1], Eun Tae Kim[2], Adegbola T. Adesogan[3], and Sam Churl Kim[1], *[1]Division of Applied Life Science (BK21Plus, Insti. of Agri. & Life Sci.), Gyeongsang National University, Jinju, Gyeongsangnam-do, South Korea, [2]Dairy Science Division, National Institute of Animal Science, RDA, Cheonan, Chungcheongnam-do, South Korea, [3]Department of Animal Science, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, FL.*

Effects of wormwood essential oil (EO) supplementations on in vitro digestibility and rumen fermentation characteristics of soybean meal (SBM) or bermudagrass hay (BH) were examined. Rumen fluid was collected from 2 cannulated Holstein cows, filtered through 2 layers of cheese cloth and mixed with Van Soest medium (1:2 ratio). Exactly 0.5 g of SBM or BH was treated with nothing or with 200 mL of wormwood EO from Ganghwa (GA), Injin (IN) or San (SA) wormwood hybrids grown at the Research Unit, Gyeongsang National University, South Korea. Three replicates of each treatment with 3 blanks were incubated at 39°C for 72 h in buffered-rumen fluid (40 mL). In vitro DM digestibility (DMD), pH, ammonia-N and volatile fatty acids (VFA) were measured. The population of certain rumen microbes was measured by Real-time PCR. Data were analyzed using the GLM procedure of SAS (SAS Inst., Cary, NC). Total VFA concentration (m*M*) of SBM was decreased ($P < 0.05$) by GA and SA (157 vs. 143, and 146) but not IN (149). Other fermentation or digestibility measures and the methanogenic archaea population were unaffected by EO. However, the fold change of *Fibrobacter succinogens* was reduced ($P < 0.05$) by GA, IN and SA (18.8 vs. 9.13, 2.36, 2.63), that of *Ruminococcus albus* was increased ($P <$ 0.05) by SA (10.8 vs. 24.5), that of *Streptococcus bovis* was increased ($P < 0.05$) by GA and SA though greatest by SA ($P < 0.05$) and that of *R. flavefaciens* was increased ($P < 0.05$) by GA and IN. The DMD of BH was not increased by EO but GA decreased NDF digestibility. Ammonia-N concentration was increased ($P < 0.05$) by SA (17.7 vs. 19.9 mg N/dl). Total VFA concentration was reduced ($P < 0.05$) by GA (136.1 vs. 119.8 m*M*) but pH and VFA molar proportion did not differ among treatments ($P > 0.05$). Adding the EO to SBM or BH had no beneficial effects on the fermentation or digestibility despite altering the ruminal bacteria population.

Key Words: essential oil, fermentation indices, rumen microbe

T471 Dry matter intake and feeding behavior of cattle fed cottonseed and vitamin E. Ricardo Galbiatti Sandoval Nogueira*, Flavio Perna Jr, Eduardo Cuellar Orlandi Cassiano, Lizbeth Collazo Paucar, Mariane Cheschin Ernandes, Diana Carolina Zapata Vasquez, Adrielle Matias Ferrinho, Romulo Germano de Resende, Felipe Bispo Mendonça, Renata Gardenalli, Angélica Simone Cravo Pereira, and Paulo Henrique Mazza Rodrigues, *University of São Paulo, Pirassununga, São Paulo, Brazil.*

The objective of this study was to evaluate the dry matter intake and feeding behavior of cattle fed cottonseed and vitamin E. The experi-

ment was conducted University of São Paulo, Campus Pirassununga. Six cannulated non-pregnant, nonlactating cows were distributed in a replicated 3 × 3 Latin square design. Feed was offered ad libitum twice daily. Feeding behavior was observed during 24 h 5 by 5 min. Treatments were: Control; Cottonseed (CS): 30.47% cottonseed included; vitamin E (VitE): 30.47% cottonseed plus 500 IU VitE included. Diets were isoenergetic and isonitrogenous. The ether extract content was 3.74, 8.32 and 8.32% for the Control, CS and VitE diets. Data were analyzed by SAS (v9.3) and significance declared at $P < 0.05$. Dry matter intake, dry matter intake in relation to body weight and per unit of metabolic size, total time and average time per event eating, ruminating, free and chewing (eating plus ruminating) were compared through orthogonal contrasts, where contrast 1 = CS and VitE vs. control, and contrast 2 = CS vs. VitE. Both diets provided ($P > 0.05$) similar amounts of dry matter intake (15.44 and 15.40 vs. 14.64 kg animal day^{-1}), dry matter intake in relation to body weight (1.74 and 1.74 vs. 1.66) and per unit of metabolic size (95.10 and 95.25 vs. 90.69). Treatments CS and VitE had greater time eating (219.1 and 215.0 vs. 190.8 min), ruminating (437.5 and 430.0 vs. 291.6 min), chewing (656.6 and 645.0 vs. 482.5 min) but less time free (775.0 and 786.9 vs. 947.5) compared with control. The average eating time per event was not different among treatments (33.1 and 36.9 vs. 32.4 min), but the average ruminating time per event (29.12 and 24.58 vs. 20.62 min) and chewing (30.0 and 27.3 vs. 24.16 min) was greater for CS and VitE compared with control. The average free time per event was less for CS and VitE than control (40.5 and 38.4 vs. 49.5 min). Including cottonseed in a diet at 30% did not decrease dry matter intake and the animals spent more time eating, ruminating, and chewing with less free time. Vitamin E did not affect dry matter intake parameters and feeding behavior.

Key Words: dry matter intake, feeding behavior, lipid

T472 In situ degradability, rumen bacteria population, and in vitro gas production in cannulated steers fed diets with and without HMTBa. Y. Liang, S. E. Bettis, M. Wehmeyer, G. I. Zanton, H. A. Tucker*, and M. Vazquez-Anon, *Novus International Inc., St. Charles, MO.*

The objective of this study was to investigate changes in rumen environment when animals are fed a diet containing 2-hydroxy-4-(methylthio) butanoate (HMTBa). Six cannulated Holstein steers were utilized in a crossover design trial with two 42-d periods (28 d adaptation, 14 d sampling) and were fed a ration with hay, corn, and soyhulls with HMTBa (0.1%, DM basis) or without (CON). Whole rumen contents (WRC), rumen solids (RS) and rumen liquid (RL) samples were taken 3 h pre- and post-feeding on d 29 of each period. Samples were analyzed for bacteria sequencing. Additional rumen fluid, collected 3 h post-feeding was used to measure gas production using the ANKOM gas production system and alfalfa hay as substrate. On d 36 of each period, the effect of HMTBa supplementation on dry matter and NDF digestibility of alfalfa and grass hays were evaluated using in situ methods. In vitro gas production at 24 h was greater ($P = 0.002$) in rumen fluid from steers fed HMTBa than CON. Supplementing HMTBa increased DM digestibility of alfalfa hay at 4 ($P = 0.001$) and 12 h ($P = 0.025$) of incubation and increased ($P = 0.013$) that of grass hay at 24 h of incubation. In situ NDF digestibility was greater at 12 h of incubation for alfalfa hay ($P = 0.005$) and 24 h of incubation for grass hay ($P = 0.006$) with HMTBa supplementation. Feeding steers HMTBa tended ($P = 0.074$) to increase Proteobacteria in RS and ($P = 0.039$) Actinomycetales in RL. Supplementing HMTBa resulted in *Ruminococcaceae* accounting for a greater proportion of bacteria at the family level in RL ($P = 0.048$), RS ($P = 0.054$) and WRC ($P = 0.037$). Furthermore, feeding steers HMTBa increased ($P = 0.028$) *Ruminococcus* in WRC, and tended to increase in RL ($P = 0.054$) and RS ($P = 0.052$). Greater population of *Ruminococcus* sp. was detected in WRC ($P = 0.022$), RL ($P = 0.031$), and RS ($P = 0.025$) in HMTBa fed steers. In conclusion, supplementing HMTBa increases fibrolytic bacterial species, resulting in a greater gas production in vitro, and greater in situ DM and NDF digestibility of selected hays.

Key Words: 2-hydroxy-4-(methylthio) butanoate (HMTBa), rumen bacteria, NDF digestibility

T473 Effect of sustained-release mineral dietetic feed bolus on plasma trace minerals status in grazing heifers. J. M. Beguin*[1], R. P. Dagorne[1], and R. Lecrubier[2], *[1]NEOLAIT, Yffiniac, France, [2]ESA, Angers, France.*

The objective of this study was to evaluate, in grazing dairy heifers, the effect of an oral bolus on plasma trace mineral status. Heifers (n = 74, initial BW = 427 kg, age = 445 d) from 4 dairy farms in Western France, were randomly assigned to a control group (CONTROL) and a trial group (TEST). Heifers in TEST received 2 boluses (Dietevit Excell, Néolait, Yffiniac, France), at the start of grazing season. Boluses supplies zinc, manganese, copper, iodine, cobalt, selenium, vitamin A, vitamin D3 and vitamin E. Blood samples were taken before turn out in April (d 0), in July (d 106), and at the end of the grazing season in November (d 245). Plasma trace minerals analysis was carried out by ICP-MS (LEA Vendée, France). Glutathione peroxidase was also measured (LDH, Oniris, France). Metabolic profiles were compared using Repeated Measures ANOVA (SPSS v18). Samples of grass were taken from the grazing plots and were analyzed by ICP-MS (Sciantec Analytical Services Ltd., United Kingdom). Grass trace mineral content per kg DM was zinc (25.4 mg), manganese (84 mg), iron (72 mg), copper (5.3 mg), cobalt (0.10 mg) and selenium (0.03 mg). Compared with CONTROL, heifers in TEST had the same trace minerals levels in d 0, higher selenium levels (42.6 vs. 25.7 µg/L in d 106 and 36.4 vs. 26.6 µg/L in d 245, $P = 0.01$ and $P = 0.03$), higher glutathione peroxidase levels (205 vs. 114 µg/L in d 106 and 205 vs. 129 µg/L in d 245, $P = 0.01$ and $P = 0.02$) and higher iodine levels (79 vs. 48 µg/L in d 106 and 62 vs. 56 µg/L in d 245, $P = 0.01$ and $P = 0.07$). There was no difference in plasma level for zinc (1064 vs. 999 µg/L in d 106 and 954 vs. 922 µg/L in d 245), manganese (2.8 vs. 2.8 µg/L in d 106 and 3.3 vs. 3.4 µg/L in d 245), copper (892 vs. 916 µg/L in d 106 and 995 vs. 1017 µg/L in d 245) and cobalt (1.4 vs. 1.2 µg/L in d 106 and 1.1 vs. 0.9 µg/L in d 245). Administration of 2 Dietevit Excell boluses to dairy heifers before turn out maintains healthy selenium, glutathione peroxidase and iodine plasma levels over a period of at least 245 d. Results show that Dietevit Excell gradually dissolves providing a sustained release of minerals over the grazing season.

Key Words: heifer, trace mineral, bolus

T474 Crambe meal (*Crambe abyssinca*) inclusion in feed of Santa Inês crossbred lambs on blood serum urea. Kariny Ferreira Moreira, Darcilene Maria Figueiredo*, Adriano Oliveira Cruz, Ronald Matos dos Santos, Juscilene Aparecida Silva Pacheco, Cassiane Gomes dos Santos, Daniela Cordeiro Rocha, Marianne Schorer, and Aldrin Vieira Pires, *Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil.*

The objective of this study was to evaluate the effect of increasing levels of crude protein of crambe meal (*Crambe abyssinca*) (0, 25, 50, and 75%) on blood serum urea levels of lambs. Twenty-four Santa

Inês crossbred male lambs, with an average weight and age of 18 kg of body weight, and 4 mo, respectively. The experiment was conducted in a completely randomized design with 4 treatment and 6 replicates each. Animals remained 7 d for adaptation, and 3 periods of 28 d for data collection. Lambs were kept in individual pens (1.5 m x 1.0 m), equipped with trough and drinker. Animals received diets with 19% crude protein (% DM), and 65.4% total digestible nutrients (% DM), based on average daily gain of 200 g head^{-1} with forage: concentrate ratio of 50:50. Lambs were fed ad libitum, twice a day, always at 0700 and 1500 h, allowing leftovers of approximately 20%. Sampling of urea serum in the lambs were through blood samples taken by puncture of the jugular vein at 13th day of the second experimental period immediately at 0, 3, 6 and 9 h after the morning feeding. Data were subjected to ANOVA and regression, at 5% probability. The blood serum urea level did not differ ($P > 0.05$) among treatments and 25.96, 25.65, 24.23, and 21.70 g day^{-1}, respectively, for diets with 0, 25, 50, and 75% crude protein inclusion of crambe meal. This result demonstrates the appropriateness of the use of nitrogen compounds in the rumen due to the availability of degradable organic matter. Therefore the use of crambe meal on diet has satisfactory effect with regard in lamb blood serum urea.

Key Words: alternative feed, protein feed, sheep breeding

T475 Crambe meal (*Crambe abyssinica*) inclusion in food of Santa Inês crossbred lambs on urea nitrogen. Kariny Ferreira Moreira, Darcilene Maria Figueiredo*, Adriano Oliveira Cruz, Ronald Matos dos Santos, Juscilene Aparecida Silva Pacheco, Cassiane Gomes dos Santos, Daniela Cordeiro Barbosa, Marianne Schorer, and Aldrin Vieira Pires, *Universidade Federal dos Vales do Jequitinhonha e Mucuri, Diamantina, MG, Brazil.*

The objective of this study was to evaluate the increasing levels of crude protein of crambe meal (*Crambe abyssinica*) (0, 25, 50, and 75% DM) in lambs diets on the urinary excretion of urea nitrogen (UEUN). Twenty-four Santa Ines crossbred male sheep, with an average weight and age of 18 kg of body weight, and 4 mo, respectively. The experiment was conducted in a completely randomized design with 4 treatment and 6 replicates each. Animals remained 7 d for adaptation, and 3 periods of 28 d for data collection. Lambs were kept in individual pens (1.5 m x 1.0 m), equipped with trough and drinker. Animals received diets with 19% crude protein (% DM), and 65.4% total digestible nutrients (% DM), based on average daily gain of 200 g head^{-1} with forage: concentrate ratio of 50:50. Lambs were fed ad libitum, twice a day, always at 0700 to 1500 h, allowing leftovers of approximately 20%. For determination of UEUN spot urine samples were collected by spontaneous urination on the 13th day of the second experimental period immediately at 0h, 3h, 6h and 9h after morning feeding. Data were subjected to ANOVA and regression, at 5% probability. There was no effect ($P > 0.05$) between diets on the UEUN, and 2.49, 2.41, 2.47 and 2.66 g day^{-1}, respectively for diets containing 0, 25, 50 and 75% of crude protein inclusion of crambe meal indicating low amount of nitrogen without being fully utilized by the animal. Therefore the inclusion of crude protein from the crambe meal for lambs feeding had a beneficial effect in relation to UEUN.

Key Words: alternative food, protein food, sheep breeding

T476 Induction of subacute ruminal acidosis affects gene expression in rumen epithelial tissue. J. C. McCann*, S. Alqarni, S. Luan, F. C. Cardoso, and J. J. Loor, *University of Illinois at Urbana-Champaign, Urbana, IL.*

Subacute ruminal acidosis (SARA) negatively affects the dairy industry by decreasing dry matter intake, milk production, profitability, and increasing culling rate and death loss. These consequences are related to a loss of barrier function in the rumen epithelial tissue associated with a reduction in ruminal pH. Six lactating Holstein cows were used in a replicated 2 × 2 Latin square design to determine the effects of SARA induction on the rumen epithelial tissue. Experimental periods were 10 d with d 1 to 3 for ad libitum intake of control diet, followed by 50% feed restriction on d 4, and ad libitum access on d 5 of the control diet (control) or control diet + 4.6 kg of a 50:50 wheat/barley pellet (challenge). Ruminal papillae biopsies were collected on d 1 and 6 of each period and stored at −80 C. Quantitative RT-PCR was used to determine expression genes related to barrier function with all reactions run in triplicate. Three reference genes (*CMTM6*, *ERC1*, and *MRPL39*) were selected due to stable expression across animals and times. Data were analyzed using the MIXED procedure of SAS with day, treatment, period, and square as fixed effects and cow as a random effect. Of the evaluated barrier function genes, the greatest relative abundance was observed for *CLDN1* followed by *CLDN4*. Expression of *CLDN1*, *CLDN4*, and *JAM2* was upregulated on d 6 ($P < 0.1$). Greater expression of *TJP1*/*ZO-1* was observed for the control treatment ($P = 0.086$). A treatment × day effect was observed for *OCLN* ($P = 0.07$) as expression was upregulated on d 6 for the challenge treatment but downregulated for the control. No treatment effect ($P > 0.1$) was observed for *CLDN1*, *CLDN4*, *JAM2*, *CAR*, *TLR2*, *TLR4*, and *IGFBP3*. Toll-like receptors recognize bacterial components and are capable of upregulating barrier function of epithelial tissue. Both *TLR2* and *TLR4* were downregulated on d 6 ($P = 0.05$ and $P = 0.1$, respectively). Collectively, results suggest feed restriction and subsequent refeeding caused a greater effect on expression of barrier function genes than the additional starch in the challenge treatment. However the results may still be indicative of the rumen epithelium tissue response to SARA.

Key Words: acidosis, rumen, epithelium

T477 Effects of monensin and essential oils from some Nigerian spices on methane production and ruminal fermentation in vitro. Musibau A. Bamikole[1,2], Ibukun M. Ogunade*[1], Felipe Amaro[1], Yun Jiang[1], Thiago F. Bernardes[1], Darren D. Henry[3], F. O. Ugiagbe[2], U. J. Ikhatua[2], Nicolas DiLorenzo[3], and Adegbola T. Adesogan[1], *[1]University of Florida, Gainesville, FL, [2]University of Benin, Benin city, Nigeria, [3]North Florida Research and Education Center, University of Florida, Marianna, FL.*

Plant extracts may be potential replacements for antibiotic feed additives used in livestock production and they are considered safer. Effects of essential oils (EO) extracted from rosemary (*Rosimarinus officcinalis*; REO) leaves and clove (*Syzgium aromaticum*; COE) flower on in vitro rumen fermentation parameters including methane production were examined. A corn silage-based TMR (0.5 g; CP 16.6%; NDF 35.9%) was treated with CEO or REO at rates of 0 (Control), 10 (Low), 20 (Med) and 30 (High) μL/50 mL of rumen fluid-buffer inoculum (ratio 1:2) and with monensin (0.6 mg/50 mL). Each suspension was incubated in a 120-mL gas-tight culture bottle in triplicate at 39oC for 24 h in each of 2 runs. Fermentation parameters, gas and methane production, in vitro DM digestibility (DMD), and fermentation efficiency (FE; DMD g kg-1/gas volume) were measured. Data for each EO were separately analyzed with the Glimmix procedure of SAS. The DMD was reduced ($P < 0.05$) by monensin, Med REO and High CEO (526, 531 and 513 vs. 555 g/kg). Gas volume (mL/g DM) was increased ($P < 0.05$) by Low REO or CEO (84.5 vs. 92.4 and 96.2) and decreased ($P < 0.05$) by their High rates or monensin (76.7, 63.6 and 66.8), respectively. The

FE was increased ($P < 0.05$) by High REO or CEO or monensin (7.27, 8.69 and 7.92 vs. 6.58) and reduced ($P < 0.05$) by Low REO (5.85), respectively. Methane production (mg/g DM digested) was reduced ($P < 0.05$) by High REO and CEO and monensin (8.65, 8.02, and 7.10 vs. 11.44). The pH was increased ($P < 0.05$) by monensin (5.75 vs. 5.66) but not by EO. Ammonia-N and VFA concentrations were unaffected by treatment except that monensin reduced ($P < 0.05$) acetate concentration and increased valerate concentration. Also, monensin and Med and high CEO increased ($P < 0.05$) butyrate concentration. High doses of essential oils from clove and rosemary decreased methane production and increased fermentation efficiency in a manner comparable to monensin

Key Words: essential oil, spice, in vitro fermentation

T478 Effect of heating method on alteration of protein molecular structure in flaxseed: Relationship with changes in protein subfraction profile and digestion in dairy cows. Nazir A. Khan[1], Helen Booker[2], Yajing Ban[1], and Peiqiang Yu*[1], *[1]Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, SK, Canada, [2]Department of Plant Sciences, University of Saskatchewan, Department of Plant Sciences, University of Saskatchewan, Saskatoon, SK, Canada.*

This study evaluated the effect of heating methods on alteration of protein structure in flaxseed (*Linum usitatissimum* L.) in relation to changes in protein subfraction profile and digestion in dairy cows. Seeds from 2 flaxseed varieties, sampled from 2 replicate plots at 2 locations, were evaluated. The plots (n = 4) were used as replicates. The seeds were either maintained in their raw state or heated in an air-draft oven (dry heating) or autoclave (moist heating) for 60 min at 120°C or by microwave irradiation (MIR) for 5 min. Compared with raw seeds, moist heating decreased ($P < 0.05$) soluble protein (SP) content (56.5 ± 5.55 to 25.9 ± 6.16% crude protein) and increased ($P < 0.05$) rumen undegraded protein (RUP) content (36.0 ± 5.19 to 46.9 ± 2.72% CP) and intestinal digestibility of RUP (61.0 ± 2.28 to 63.8 ± 2.67% RUP). Dry heating did not alter ($P > 0.05$) the protein subfraction profile and rumen degradation kinetics, whereas MIR increased ($P < 0.05$) the RUP content from 36.0 ± 5.19 to 40.4 ± 4.67% CP. The MIR and dry heating did not alter ($P > 0.05$) the amide I to amide II ratio, but moist heating decreased ($P < 0.05$) both the amide I to amide II ratio and α-helix-to-β-sheet ratio. Regression equations based on protein molecular spectral intensities provided high prediction power for estimation of heat-induced changes in SP ($R^2 = 0.62$), RUP ($R^2 = 0.71$), and intestinal digestibility of RUP ($R^2 = 0.72$). Overall, heat-induced changes in protein nutritive value and digestion were strongly associated with heat-induced alteration in protein molecular structures.

Key Words: heat processing method, protein molecular structure, protein subfraction

T479 Investigation of protein digestion kinetics in vitro using ^{15}N-labeled timothy and red clover. Merko Vaga*, Kerstin Huss-Danell, Mårten Hetta, and Pekka Huhtanen, *Dept. of Agricultural Research for Northern Sweden, Swedish University of Agricultural Sciences, Umeå, Sweden.*

An in vitro method based on ^{15}N-labeled forage N was developed to study ruminal protein degradation of timothy and red clover. Timothy and red clover were grown on the same field with 2 replicate plots, 2 m^2 each. One replicate plot was fertilized with ^{15}N-enriched NH_4NO_3 while the other received non-enriched fertilizer. Labeled timothy and red clover had average enrichment of 2.37 and 1.23 atom % excess ^{15}N, respectively. Forages from the first-cut were preserved either as hay (TH, RCH), untreated (T, RC) or formic acid-treated silage (TF, RCF). Rumen fluid was collected from 2 Swedish red cows fed on grass silage:concentrate diet (60:40 DM basis). Samples of 1 g were incubated in 60 mL of buffered rumen fluid at 39°C for 48 h. For non-labeled forages NH_3-N in the inoculum was labeled with ^{15}N enriched $(NH_4)_2SO_4$. Vessels containing labeled forages received the same amount of non-enriched $(NH_4)_2SO_4$. Liquid samples were collected every hour at 0 to 10 h, after 12, 16, 24, 30 and 48 h, and later analyzed for NH_3-N and ^{15}N. Degraded CP was calculated as a sum of appearance of ^{15}N from labeled forages and disappearance of ^{15}N from labeled NH_3-N. Degradation parameters were estimated by the 2 pool exponential model using the SAS NLIN procedure. Timothy forages had higher rapidly degradable fraction (b1). Slowly degradable fraction (b2) was smaller in formic acid treated silages and in red clover silages. Hay had slower degradation rates (kd) than silages. Total degraded CP (a+b) was higher for timothy and for hay. It is concluded that incubating ^{15}N labeled feeds or feed fractions simultaneously with corresponding unlabeled feeds by labeling ammonia pool with ^{15}N can be a useful tool in investigating kinetics of ruminal protein degradation.

Table 1 (Asbtr. T479). CP concentration and degradation parameters of timothy (T) and red clover (RC)

	TF	RCF	T	RC	TH	RCH
CP, g/kg DM	155	147	166	133	144	137
CP degradation[1]						
b1, g/kg	495	418	335	334	473	391
b2, g/kg	380	362	594	507	490	452
kd1, 1/h	0.696	1.250	1.263	1.200	0.815	0.818
kd2, 1/h	0.189	0.132	0.139	0.161	0.055	0.091
a+b, g/kg	875	780	929	841	963	843

[1]b1 = rapidly degradable, b2 = slowly degradable, kd1 = degradation rate of b1, kd2 = degradation rate of b2.

Key Words: in vitro, ^{15}N, protein

T480 Effects of yam (*Dioscorea opposite*) supplementation on *in vitro* digestibility and rumen fermentation characteristics of ground corn and perennial ryegrass. Jin Yeon Park*[1], Tea Hyeon Kim[1], Hyuk Jun Lee[2], Young Ho Joo[2], Sardar M. Amanullah[2], Dong Hyeon Kim[2], In Hak Choi[3], and Sam Churl Kim[1,2], *[1]Department of Animal Science, Gyungsang National University, Jinju, Gyeongsangnam-do, South Korea, [2]Division of Applied Life Science (BK21Plus, Insti. of Agric. & Life Sci.), Gyungsang National University, Jinju, Gyeongsangnam-do, South Korea, [3]Department of Companion Animal & Animal Resources Sciences, Joongbu University, Geumsan, Chungcheongnam-do, South Korea.*

This study was carried out to investigate the effect of yam (*Dioscorea opposite*) supplement levels on in vitro digestibility, gas production and rumen fermentation characteristics of ground corn and perennial ryegrass using a novel wireless automated gas production system. Rumen fluid was collected from 2 cannulated Hanwoo heifers, filtered by 2 layers of cheese cloth and mixed with Van Soest medium at 1:2 ratio. The rumen fluid mixture (40 mL) added to the incubation bottles containing ground corn or perennial ryegrass (0.5 g) with 4 levels of Yam supplement (0, 5, 25 and 50 mg). Four replicates in each treatment with 2 blanks were incubated at 39°C for 48 h and 72 h for ground corn and perennial ryegrass, respectively. After incubation, the bottle content was used for the analyses of in vitro digestibilities of dry matter (IVDMD) and neutral detergent fiber (IVNDFD), pH, ammonia-N and

volatile fatty acid (VFA). Data were analyzed using the GLM procedure of SAS (SAS Inst., Cary, NC). The pH (Linear, $P < 0.001$) and the concentrations of iso-butyrate (Quadratic, $P = 0.025$) and iso-valerate (Quadratic, $P < 0.001$) of ground corn decreased with increasing yam supplementation level, while the concentrations of total VFA (Linear, $P = 0.01$) and butyrate (Quadratic, $P = 0.001$) increased. However, the fermentation kinetic of ground corn was not affected by Yam supplementation level. With increasing yam supplementation level, IVDMD (Linear, $P < 0.001$), IVNDFD (Linear, $P = 0.002$), the potentially fermentable fraction (Linear, $P = 0.057$) and the total fermentable fraction (Linear, $P = 0.018$) of perennial ryegrass increased. Results indicated that yam (*Dioscorea opposite*) supplementation can increase not only total VFA concentration of ground corn, but also in vitro digestibilities of perennial ryegrass.

Key Words: digestibility, rumen fermentation, yam (*Dioscorea opposite*)

T481 In vitro study of yeast cell-wall b-glucans behavior in ruminal fluid. Nadia Yacoubi[1], Jean Philippe Marden[3], and Corine Bayourthe*[2], *[1]INRA UR1268 Biopolymers Interactions Assemblies, Nantes, France, [2]Université de Toulouse, INRA, UMR 1388 INRA-INPT GenPhySE, Castanet-Tolosan, France, [3]Phileo Lesaffre Animal Care, Marcq en Baroeul, France.*

Behavior of β-(1,3/1,6)-glucans (BG) of yeast cell-wall (*Saccharomyces cerevisiae*) in ruminal milieu was evaluated in vitro. The solubility of BG, pH, and production of VFA were measured after 4, 8, and 16 h of incubation in ruminal fluid. Two yeast cell-wall products were used: brewer's yeast (BrYBG) and baker's yeast (BaYBG), containing 15.1 and 26.3% DM of BG, respectively. Ruminal fluid was collected 3h post-feeding from a ruminally fistulated Holstein dairy cow fed a hay-based diet and strained through a metal sieve (2 mm). Within 45 min of sampling, 60 mL of ruminal fluid and 60 mL of degassed buffer solution (pH 7, 39°C) were added to 250-mL flasks, containing 5 g of yeast cell-wall product, flushed with O_2-free CO_2, and capped. For each incubation time, 5 flasks were prepared: 2 replicates each for BrYBG, and BaYBG, and one without product, used as control. Flasks were kept from light and air at 39°C in a waterbath rotary shaker. The pH was recorded at the start of incubation (0 h), 4 h, 8 h, and 16 h. Once the pH was measured, batch culture was centrifuged (4,500 × *g* for 10 min). Supernatants were taken to determine VFA contents. Total VFA produced was calculated by subtracting the initial total VFA concentration from the final concentration. The sediment BG content was determined (enzymatic kit, Megazyme). BG solubility was calculated by subtracting residual BG in sediment at the end of each incubation time from the starting concentration at 0 h. This batch culture experiment was repeated on 3 different days. At 4 h, the percentage of soluble BG was 3 times higher ($P < 0.0001$) for BaYBG than for BrYBG. At 16 h, it reached 96.4% and 92.5 for BaYBG and BrYBG, respectively. Amount of soluble BG increased ($P < 0.0001$) linearly with time ($r^2 = 0.916$ for BrYBG and 0.993 for BaYBG). Total VFA contents at 16 h differ ($P = 0.0007$): 95.2 vs. 110.7 m*M,* for BaYBG and BrYBG, respectively. The pH fall was less important ($P < 0.0001$) for BaYBG than for BrYBG. The decrease in pH and increase in total VFA production suggested that ruminal microflora would be potentially capable of degrading some soluble components in the yeast cell-wall products. But at 16h, 70% of DM BaYBG and 56% of DM BrYBG escape the ruminal degradation suggesting bioavailability in post-ruminal digestive tract.

Key Words: beta-glucan, yeast, in vitro

T482 Effect of different sources of glycerol on in vitro fermentation parameters of corn silage. E. H. C. B. van Cleef*[1,2], E. S. Castro Filho[1], M. T. C. Almeida[1], J. R. Paschoaloto[1], I. Monsignati[1], S. F. B. Buzinaro[3], and J. M. B. Ezequiel[1], *[1]São Paulo State University, Jaboticabal, São Paulo, Brazil, [2]FAPESP, São Paulo, Brazil, [3]University of São Paulo, Pirassununga, São Paulo, Brazil.*

Two studies were conducted to evaluate the effect of sources of glycerol on in vitro fermentation, and dry matter and neutral detergent fiber digestibilities of corn silage. In the first trial, rumen content was collected from 2 ruminally cannulated male sheep (68.5 kg BW) fed diet composed of 40% corn silage and 60% concentrate (corn, soybean hulls, soybean meal, urea, and minerals). Substrates tested were (1) corn silage, (2) corn silage + 20% crude glycerin (86% glycerol), (3) corn silage + dry glycerin (68% glycerol), and (4) corn silage + pure glycerin (99% glycerol). Substrates (200 mg) and buffered rumen fluid (20 mL McDougall's buffer and 10 mL rumen fluid) were placed into 60-mL bottles (n = 20), purged with helium gas and sealed. Gas production, pH, ammonia nitrogen (NH_3-N) and DM disappearance were evaluated after 24 h of incubation at 39°C. In the second trial, Ankom Daisy^{II} fermenter was used to evaluate in vitro digestibility of DM and NDF. Filter bags (n = 25) were filled with substrates, and incubated for 48 h (39°C) into vessels containing a solution composed of 400 mL rumen fluid, 1330 mL buffer A and 266 mL buffer B. After 48-h incubation, 40 mL of 6 *N* HCl and 8 g of pepsin was added to each digestion vessel, and incubated for another 24 h. Substrates and residues were evaluated for DM and NDF content. All the procedures were replicated for both trials. There was no effect of treatments on 24-h pH (6.1) and NH_3-N (38.4 mg/dL), nor for production of total gas (51.2 mL), CH_4 (8.9 mL), and CO_2 (39.7 mL). When DM disappearance. When the dry matter disappearance was taken into account, corn silage produced more gas (total gas) and more CO_2 than glycerol treatments ($P = 0.01$ and $P = 0.003$, respectively), and no difference was observed among treatments for CH_4, and among glycerol treatments for CO_2. Glycerol, regardless the source, increased IVDMD of corn silage ($P < 0.0001$), and no changes were observed among glycerol treatments (71.7%). IVDMD was unaffected by treatments (41.7%). All sources of glycerol (crude, dry or pure) do not alter neither rumen fermentation nor in vitro digestibility, when inoculated at 20% with corn silage, thus any of these sources are suitable to improve IVDMD of corn silage.

Key Words: glycerol, in vitro dry matter digestibility, methane

T483 Quality evaluation of corn silage prepared with microbial inoculants. Luiz Keller*[1,4], Marcos Aronovich[3], Christiane Perali[2,4], Eliane Rodrigues[3], Airton Castagna[3], and Carlos Rosa[2,4], *[1]Universidade Federal Fluminese (UFF), Rio de Janeiro, RJ, Brazil, [2]Universidade Federal Rural do Rio de Janeiro (UFRRJ), Rio de Janeiro, RJ, Brazil, [3]Empresa de Desenvolvimento Agropecuário do Estado do Rio de Janeiro (PESAGRO-RJ), Rio de Janeiro, RJ, Brazil, [4]Conselho Nacional de Pesquisas Científicas (CNPq), Belo Horizonte, MG, Brazil.*

Silage is a common widespread practice to preserve forages in Brazil. One of the main problems encountered is the high value of rations and raw materials, so use mainly corn, sorghum and agro-industrial sub products. Being reviewed mycobiota present in the ensiled material and mycotoxins levels are an indicator of quality, because the poor storage conditions can lead contamination and toxins production. The research evaluated the quality of silages preserved with biological inoculants on mycobiota, mycotoxins levels and nutritional assessment parameters. Two dosages of commercial inoculant product, with 10 replications was used on corn silage and evaluated at 0, 30, 60 and 90 d. Fungal

counts were done by surface-spread method and toxigenic ability of isolates strains was evaluated with in vitro conditions. Aflatoxins (AFs), ochratoxin A (OTA), fumonisin B1 (FB1) and deoxinivalenol (DON) natural contamination was determined with immunoaffinity columns extracts in HPLC. Total fungal counts were generally high (range 1.0×10^6 to 1.0×10^2 cfu g^{-1}). *Aspergillus flavus, Penicillium citrinum,* and *Fusarium verticillioides* were the prevalent toxigenic strains isolated. Mycotoxins levels differed ($P < 0.001$) from pre and post-fermentation samples, probably due to mold growth. Dry matter, carbohydrates, lipids, protein, volatile fatty acids, and fiber content not differed ($P < 0.001$) from pre and post-fermentation samples and were not different from literature. The inoculants does not helped in reducing the count of fungal propagules but decreased the biodiversity of the toxigenic strains presents in the treated silos. So, the use of microbial inoculant on silage production should be recommended to reduce some toxigenic strains contamination. However, each product must be evaluated and the applying process must be carefully conducted. The mycotoxin binding and nutritional quality increase related on literature was not observed on the present study.

Key Words: aflatoxin, corn silage, feedstuffs

T484 Utilization of equations to predict carbohydrate fractions in some tropical grasses. Romualdo S. Fukushima*, Carolina B. Bacha, Adriana P. Fuzeto, Ana C. R. Port, Valdo R. Herling, and Alejandro V. Velasquez, *Sao Paulo University, Pirassununga, SP, Brazil.*

The chemical composition of 5 tropical grasses, divided into stalk and leaf, at 3 maturity stages, was used to predict carbohydrate fractions by equations of the Cornell Net Carbohydrate and Protein System (CNCPS) or equations from our research group. Carbohydrate fraction A is a rapidly fermented pool that is primarily composed of sugars, some organic acids and short oligosaccharides. Fraction B1 is constituted mainly of starch and pectin. Fraction B2 has a slower rate of degradation and is available cell wall carbohydrates. The C pool is unavailable cell wall, which includes lignin. These carbohydrates fractions are estimated based on NDF analysis. However, NDF does not recover pectic substances and other ND soluble oligosaccharides such as β-glucans, fructans, galactans, etc. that are part of the cell wall matrix. Structurally, NDF is not complete plant cell wall. Then, a crude cell wall (CW) preparation, which represents plant cell wall matrix more completely because it preserves those carbohydrates that otherwise would be solubilized by the ND solution, was used in equations to predict carbohydrate fractions. By substituting NDF for CW, it was found that pectin and other ND soluble oligosaccharides (soluble fiber - SF) actually appeared in the fraction A of CNCPS, the rapidly degradable carbohydrate pool, instead of fraction B1, as proposed in the original CNCPS model. However, location of SF in the fraction A seems inadequate because degradation rate of SF is lower than fraction A components; thus, an alternative could be to place SF in a specific carbohydrate fraction (B2). This B2 fraction, soluble fiber, can be estimated by subtracting NDF from CW preparation. Because in the original CNCPS model the slowly degradable cell wall carbohydrates were assigned as fraction B2, we suggest naming this carbohydrate pool a new fraction, B3. With this arrangement, the fraction B1 would be constituted only by starch. These fractions are expressed on total carbohydrate basis, here suggested as: CHO = 100 - (CP + EE + MM + Lignin). This equation excludes lignin from the CHO compartment.

Key Words: cell wall, Cornell, soluble fiber

T485 Analysis of microbial populations in Rusitec fermenters fed diets of variable composition. Ivan Mateos[2], Maria Jose Ranilla*[2,3], Cristina Saro[2], Alexey Díaz[2], Maria Gracia De Garnica[2], Jairo Garcia[2], and Maria Dolores Carro[1], *[1]Technical University of Madrid, Madrid, Spain, [2]University of León, León, Spain, [3]IGM (CSIC-ULE), Grulleros, León, Spain.*

Fermenters are widely used to study ruminal fermentation, but information on microbial populations developing in fermenters over the incubation period is limited. Four Rusitec fermenters were fed 2 diets representative of those administered to dairy sheep (DAI; 50:50 alfalfa hay:concentrate) and fattening lambs (FAT; 15:85 barley straw:concentrate) in a crossover design with 2 14-d incubation periods to assess the evolution of the microbial populations. There were 4 fermenters per diet. The fermenters received daily 30 g of diet DM and samples from liquid (LIQ) and solid (SOL) digesta were taken on d 3, 8 and 14, and stored frozen at −80ºC until DNA extraction. Concentrations of bacterial and protozoal DNA and relative abundance of fungi and methanogenic archaea to total bacterial DNA concentration were determined by real time PCR using previously validated primers and DNA from bacteria and protozoa isolated from sheep rumen as standards. Data were analyzed as a mixed model with repeated measures using the PROC MIXED of SAS. The model included diet, incubation run, time, and diet × time as fixed effects, and fermenter as a random effect. Diet x sampling time interactions ($P > 0.05$) were detected for bacterial and protozoal DNA concentrations in both digesta phases. The bacterial DNA concentrations in SOL did not change ($P = 0.002$) over the incubation period, whereas concentrations in LIQ increased ($P < 0.001$) by 1.5 and 1.8 times for DAI and FAT diets by the end of the incubation, respectively. Protozoal DNA concentrations on d 14 were 37.8 and 8.0 times lower ($P < 0.001$; means across diets) than those on d 3 for SOL and LIQ phases, respectively. Relative abundance of fungi decreased ($P < 0.05$) with time in both phases, and that of methanogenic archaea remain unchanged in LIQ and increased ($P = 0.021$) in SOL. Concentration of bacterial and protozoal DNA and the relative abundance of methanogenic archaea were greater in the fermenters fed the DAI diet ($P < 0.05$) compared with FAT diet. The results show that microbial populations in Rusitec fermenters are affected by the incubated diet and change over the incubation period.

Key Words: Rusitec fermenter, microbial populations, real-time PCR

T486 Influence of inoculum preparation method on in vitro methane production by ruminal microorganisms. Mireia Ramos[1], Ivan Mateos[2], Cristina Saro[2], Alexey Díaz[2], Maria Jose Ranilla*[2,3], and Maria Dolores Carro[1], *[1]Technical University of Madrid, Madrid, Spain, [2]University of León, León, Spain, [3]IGM (CSIC-ULE), Grulleros, León, Spain.*

The characteristics of the inoculum are recognized as one of the most relevant factors influencing the results of in vitro fermentations in batch cultures of ruminal microorganisms. Four rumen-fistulated sheep fed a 66:34 alfalfa hay:concentrate diet were used as donors to investigate the effect of rumen contents' processing on in vitro methane (CH_4) and volatile fatty acid (VFA) production from 3 substrates of variable composition. Rumen contents were sampled from each individual sheep and subjected to the following treatments: SQ) squeezed through 4 layers of cheesecloth; FIL) SQ treatment and further filtration through a 100-µm nylon cloth; STO) treated with a Stomacher for 3 min at 230 rev min^{-1} and followed by SQ. The resulting fluids were used as inoculum for batch cultures containing alfalfa hay, concentrate, or a 50:50 mixture of both feeds. Cultures were incubated at 39°C for 8 and 24 h, and CH_4 and VFA production was measured. There were no treatment × substrate

interactions ($P > 0.05$) for any variable at any incubation time, excepting for the molar proportion of acetate at 24 h ($P = 0.019$). The method of processing the rumen contents did not affect ($P > 0.05$) either total VFA and CH_4 production or molar proportions of individual VFA at any time. At both incubation times, increasing the amount of concentrate in the substrate increased CH_4 production ($P < 0.001$, quadratic) and molar proportion of butyrate ($P < 0.001$, linear), but decreased acetate proportion ($P < 0.001$, quadratic) without affecting ($P > 0.05$) proportions of propionate. Whereas total VFA production was linearly decreased ($P = 0.007$) by increased amounts of concentrate in the substrate at 8 h of incubation, it was quadratically increased ($P < 0.001$) after 24 h of incubation. There were clear differences in CH_4 and VFA production among inocula from different sheep, which persisted across substrates. The results show that the tested methods of processing rumen contents did not affect in vitro fermentation characteristics of good quality substrates, but studies analyzing their possible influence on fermentation of low-quality substrates are required.

Key Words: Rumen content treatment, methane, volatile fatty acids

T487 Microbial rDNA sequences as markers to determine microbial synthesis in Rusitec fermenters: A comparison with ^{15}N. Cristina Saro[2], Maria Jose Ranilla*[2,3], Ivan Mateos[2], Alexey Díaz[2], Jairo Garcia[2], Maria Gracia de Garnica[2], and Maria Dolores Carro[1], *[1]Technical University of Madrid, Madrid, Spain, [2]University of León, León, Spain, [3]IGM (CSIC-ULE), Grulleros, León, Spain.*

Microbial rDNA sequences have been proposed as potential internal markers to determine microbial synthesis in the rumen. The objective of this experiment was to compare values of microbial growth determined using ^{15}N as external marker with concentrations of microbial DNA in fermenters, and to assess if both procedures detected similar differences between diets and solid (SOL) and liquid (LIQ) digesta phases. Four Rusitec vessels were used in a crossover trial with 2 14-d periods. In each period, 2 fermenters received a 50:50 alfalfa hay:concentrate diet (MC) and 2 were fed a 15:85 barley straw:concentrate diet (HC). A solution of $^{15}NH_4Cl$ was infused for 5 d before taken samples of SOL and LIQ digesta and isolation of bacterial pellets from both digesta phases to estimate microbial protein synthesis. Samples of SOL and LIQ digesta were simultaneously taken for DNA extraction and analysis of concentrations of total bacterial and protozoal DNA by quantitative PCR. Total microbial N (TMN) was calculated from the ^{15}N enrichment in digesta and isolated bacterial pellets, and total microbial DNA (TMDNA) was calculated as the sum of bacterial DNA and protozoal DNA in both digesta fractions. There were no diet × digesta phase interactions ($P > 0.05$) with any marker. Both TMN and TMDNA were greater ($P < 0.001$) in MC-fermenters than in HC-fermenters (1.5 and 2.0 times greater for TMN and TMDNA, respectively). Values of TMN were greater ($P = 0.004$) in SOL than in LIQ digesta (108 and 89.7 mg N, respectively), whereas the opposite was found for TMDNA (3.37 and 13.1 mg DNA, respectively). There was no difference between diets ($P > 0.05$) in the contribution of SOL digesta to TMN (53.8 and 55.7% for MC and HC diets, respectively), but contribution of SOL digesta to TMDNA was greater in MC than in HC diet ($P = 0.039$; 24.5 and 11.5%, respectively). There was no relationship ($P > 0.05$) between TMN and TMDNA values, but a significant relationship was observed when only values in the LIQ digesta were considered ($P = 0.024$; r = 0.821). In summary, both markers detected similar differences between diets, but not between digesta phases.

Key Words: microbial growth, ^{15}N, qPCR

T488 Comparison of Roche 454 and Ion Torrent Personal Genome Machine sequencing on the rumen bacterial profiles of dairy cows. Nagaraju Indugu*, Sanjay Kumar, Bonnie Vecchiarelli, and Dipti Pitta, *Department of Clinical Studies, School of Veterinary Medicine, New Bolton Center, University of Pennsylvania, Kennett Square, PA.*

Next generation sequencing (NGS) is a widely accepted technology used by microbial ecologists for metagenomic analysis of complex microbial communities. As technologies continue to improve, it is necessary to compare data sets obtained from different platforms and analyze their effect on community structure. In the present study, we compared the 454 data set with that of Ion Torrent Personal Genome Machine (PGM) on the same DNA samples (n = 14) obtained from the rumen of dairy cows during their transition period. Despite the substantial difference in number of reads and length of reads, the platforms provided a similar community structure. The weighted UniFrac distances between the samples that were sequenced on both 454 and PGM were significantly correlated, as determined by Procrustes analysis of principal coordinate matrices (M2 = 0.319; Monte Carlo $P < 0.001$). Though similar major abundant phyla were detected by both platforms, PGM recovered 4 additional phyla. At the genus level, there was no substantial variation between the 454 and PGM data sets for each animal except for *Prevotella*, *Cyanobacteria* YS2 and *Succiniliclasticum* ($P < 0.05$; chi-squared test). However, there was variation in the abundance of genera between different animals, irrespective of the platform used. Collectively, the present study will be useful for microbiologists/ecologists to compare the microbial community structure obtained from different platforms; particularly with the expectation 454 will be completely replaced by PGM and/ or Illumina.

Key Words: Ion Torrent PGM, microbial community, 454-Roche

T489 Effect of chitosan in dairy cows diets on ruminal fermentation and milk yield and composition. Carlos Eduardo Cardoso Consentini[1], Elmeson Ferreira de Jesus[2], Pablo Gomes de Paiva*[2], Tiago Antonio Del Valle[1], Gustavo Ferreira de Almeida[1], Artur Gabriel Brao Vilas Boas Costa[1], Fernanda Carolina Ramos dos Santos[1], Victor Chiaroni Galvão[1], and Francisco Palma Rennó[1], *[1]School of Veterinary Medicine and Animal Science of USP, Pirassununga, São Paulo, Brazil, [2]School of Agricultural and Veterinary Sciences of UNESP, Jaboticabal, São Paulo, Brazil.*

In this study, we evaluate the effects of chitosan level for cows in late lactation on ruminal fermentation and milk yield and composition. Eight Holstein cows cannulated in the rumen (215.4 ± 60.9 DIM, 22.07 ± 5.32 kg of milk yield and 641.6 ± 41.06 kg of BW) in replicated 4 × 4 Latin squares, were fed the following diets: (CTR) control diet without addition of chitosan, CHI75 mg/kg, CHI150 mg/kg and 225 mg/ kg of chitosan addition per kg BW. Each period had 14 d adaptation and 7 for collection data. Ruminal fluid was collected on 20th day of each period at 7 times to evaluate the effect of the diets on ruminal fermentation. Sampling of milk was done on 16 to 18th day of each period to evaluate the composition. The results of milk composition was subjected to ANOVA, while ruminal fermentation data were analyzed as repeated measures by PROC MIXED of SAS. Chitosan changed ruminal fermentation profile, increasing rumen propionate ($P < 0.05$) without shift acetate and total VFA concentrations ($P > 0.05$). However, chitosan decreased linearly acetate:propionate ratio ($P < 0.05$) similarly to butyrate and AGCR concentrations ($P < 0.05$). Chitosan increased milk yield ($P < 0.05$). Furthermore, dietary chitosan linear increased protein and lactose milk production ($P < 0.05$). Chitosan as feed additive

improve animal performance and ruminal fermentation with increased ruminal propionate concentration.

Key Words: additive, antimicrobial, performance

T490 Method to measure production of volatile fatty acids and gases in vitro. Latisha M. Judd* and Richard A. Kohn, *University of Maryland, College Park, MD.*

In vitro methods have been developed to measure digestibility, but such methods may not accurately estimate methane or volatile fatty acid (VFA) production. Methane emissions are stoichiometrically linked with VFA profiles. For example, a shift from acetate to propionate may decrease CO_2 and H_2 production, and in turn decrease conversion of CO_2 and H_2 to methane. The objective of this study was to determine the effect of different conditions of in vitro procedures on VFA and gas profiles in comparison with in vivo measurements. Experimental design was a 4 × 2 × 2 factorial CRD with 4 replicates. Treatments were 4 ratios of rumen fluid to buffer by volume (95/5, 75/25, 50/50, 25/75), 2 concentrations (w/v) of added timothy hay (0.5% or 1%), and with or without sodium acetate addition (50 mmol final concentration). Statistical analysis was conducted using a mixed model that included all fixed effects and interactions. Total volume of broth (rumen fluid and buffer) was 10 mL per tube. Measurements of gas production and VFAs were recorded at 0, 4, 16, 24, and 48 h. Total gas was proportioned into CO_2 and non-CO_2 after collection at 48 h. Higher hay concentration averaged more ($P < 0.0001$) total gas than the lower hay concentration (12.1 vs. 6.7 mL gas/tube; SE = ± 0.23), and more ($P < 0.0001$) non-CO_2 gas (0.28 vs. 0.15 mL; SE = ± 0.046). Total gas production increased ($P < 0.001$) with higher buffer concentration, and averaged 6.1, 9.1, 11.2 and 11.2 mL per tube (SE = ± 0.32 mL) as buffer concentration increased. The acetate/propionate (A/P) concentration decreased over time ($P < 0.0001$). The initial rumen fluid A/P ratio was 3.7 but the A/P ratio of produced VFA averaged 2.5 (SE = ± 0.14). The addition of acetate did not affect gas production or A/P ratio of produced VFA. This could mean that adding acetate to a system does not necessarily shift production away from acetate. A/P ratio differed for VFA produced in vitro compared with initial rumen fluid, but no tested treatments were identified as a cause of the difference.

Key Words: in vitro digestion, volatile fatty acids, methane

T491 A rapid mold and yeast enumeration technique is comparable to a conventional technique for animal feedstuffs. Lauren Meyer*[1] and John Goeser[1,2], *[1]Rock River Laboratory, Watertown, WI, [2]University of Wisconsin-Madison, Madison, WI.*

Feedstuff and TMR yeast and mold enumerations (cfu/g) have grown in popularity to diagnose opportunities on farm. Turnaround time with conventional enumeration (CON) limits utility, requiring 5 d incubation, extending total time from sampling to reporting to 7d or more. More recent human food grade, rapid yeast and mold enumeration techniques (RAP) offer faster turnaround and may have utility for production agriculture. The objective here was to determine if RAP, tested under 2 incubation lengths, was equivalent to CON. Corn silage (n = 17), TMR (n = 3), alfalfa silage (n = 15), high moisture corn or snaplage (n = 6), small grain silages (n = 6), and concentrate (n = 6) samples submitted for CON in late February 2015 were further assayed using RAP, with both 48-h and 5-d incubation. When samples arrived, roughly 5g feed was blended and stored at 1C for later plating. At plating, 1g wet feed was subsampled and diluted to 100mL in sterile buffer, shaken and then serially diluted 1:1000, 1:10,000 and 1:100,000 for most probable number enumeration. For CON, subsamples of each dilution were taken with sterile glass pipette and plated on potato-dextrose agar using spread-plate method. For RAP, subsamples of each dilution taken with an electronic pipette were plated on Petrifilm, using a Petrifilm flat spreader (3M, St. Paul, MN). For RAP plates were aerobically incubated at 28C for both 48h and 5d and CON for 5d. Post incubation, enumeration was done by direct microscopy. Yeast and mold count mean/median across feeds and techniques were $1.69 \times 10^6/1 \times 10^3$ and $2.53 \times 10^5/1 \times 10^4$, respectively. Raw and log-transformed data were determined not normally distributed, hence data were fit using one-way analysis option of SAS JMPv11.0. Technique (CON, RAP-48h, and RAP-5d) and feed main effects were compared using non-parametric Wilcoxon/Kruskal-Wallis test. Significance was declared if resulting Chi-squared statistic p-value was < 0.05. For both mold and yeast enumeration, techniques did not differ ($P > 0.05$) while feed types differed ($P < 0.01$). Our results suggest both yeast and mold enumeration results are comparable across the techniques tested here.

Key Words: feed, mold, yeast

T492 Comparison of acetyl bromide lignin with acid detergent lignin and relationship with in vitro forage degradability. Romualdo S. Fukushima*[1,2], Monty Kerley[2], Marcelo H. Ramos[2], James H. Porter[2], and Robert L. Kallenbach[2], *[1]Sao Paulo University, Pirassununga, SP, Brazil, [2]University of Missouri, Columbia, MO.*

The spectroscopic acetyl bromide lignin (ABL) and the gravimetric acid detergent lignin (ADL) methods were compared with in vitro forage dry matter degradability (IVDMD) and neutral detergent fiber degradability (IVNDFD) assays of 73 grass and legume samples, and a conjecture was made with the lignin component of the Cornell Net Carbohydrate and Protein System (CNCPS) equations. The slopes and intercepts of regressions were declared different when there was an interaction effect among forages within each lignin method (MIXED procedure of SAS). Regression curves of ADL values with forage IVDMD (grass: y = −7.929x + 901.8; legume: y = −3.663x + 853.6) and IVNDFD (grass: y = −3.289x + 916.0; legume: y = −1.051x + 697.8) revealed different slopes, with steeper curves for grasses. Grass and legume samples assayed with the ABL procedure, exhibited similar slopes, with parallel lines for both IVDMD (grass: y = −3.847x + 886.7; legume: y = −3.638x + 789.8) and IVNDFD (grass: y = −3.636x + 1117.7; legume: y = −3.454x + 889.9) assays. Steeper inclination of curve for grasses relative to legumes in the ADL method has been attributed to grass lignin being more inhibitory to degradation than legume lignin. Similar and parallel curves of ABL method suggests that grass lignin is no more inhibitory than legume lignin. However, the steeper inclination may be attributed to partial loss of grass lignin during the ADL procedure. We hypothesize that this loss is around 2.4, that is, the residual ADL multiplied by 2.4 would yield the actual lignin content. This number is the same used in the Cornell equations to estimate the B2 and C carbohydrate fractions (NDF x Lignin x 2.4). When we multiplied the grass ADL values by 2.4, forage IVDMD regressions were: (grass: y = −3.690x + 934.7; legume: y = −3.663x + 853.6), which originated parallel lines and were strikingly similar to the ones obtained with the ABL method. After correcting IVNDFD, the regressions were: (grass: y = −1.744x + 962.9; legume: y = −1.737x + 805.8), also yielding parallel curves. At this moment we can speculate that grass and legume lignins have the same effect on cell wall degradation and that ABL method seems a promising procedure for lignin quantification.

Key Words: lignin methods, Cornell carbohydrate equations

T493 What roles do viruses play in the rumen? Christopher Anderson, Galen Erickson, and Samodha Fernando*, *University of Nebraska, Lincoln, NE.*

Viruses are the most abundant biological entity on earth. However, the role of viruses within ecosystems are poorly understood. As an attempt to better understand the role and functional relationships of viruses and their influence on rumen bacterial communities, we investigated viral and bacterial community relationships using culture independent metagenomic approaches under 4 different dietary conditions (55% corn silage, 27% condensed distillers plus solubles (CDS), 40% modified distillers grains plus solubles (MDGS), corn based control diet) in a Latin-square design with 5 fistulated steers. Rumen samples were collected after total rumen evacuation and mixing following a 21-d adaptation period. Tangential flow filtration was performed to enrich for viral particles from the rumen sample. The enriched viral metagenome and the total metagenome were sequenced using the Ion Torrent Personnel Genome Machine (PGM) to identify species composition, interactions between viruses and bacteria, and to identify the role of virus auxiliary genes within rumen metabolism. The metagenome analysis displayed the total metagenome contained 118 differentially abundant genes and the viral metagenome 309 differentially abundant genes based on diet. Interestingly, the metagenomes and metaviromes contained different metabolic profiles. To better understand the role of the virome in ecosystem function, we mapped the genes identified to a community metabolic network. Using the metabolic networks we compared topological features of enzyme nodes to identify the roles of differentially abundant genes. The nodes that were differentially abundant in the total metagenome and virome had significantly higher betweenness centrality ($P < 0.05$) and a lower average shortest path length compared with non-differential genes in the network ($P < 0.05$). In addition, differential viral genes had a significantly higher total degree and in-degree compared with non-differential genes and the differential genes in the total metagenome ($P < 0.05$). Currently, we are applying network approaches to understand the ecological roles of viruses within energy metabolism in the rumen environment.

Key Words: metagenome, virome, metabolic network