

Ruminant Nutrition I

M230 Ratio of dietary forage-to-concentrate affect liver and mammary tissue transcriptome in primiparous Holstein dairy cows. Z. Zhou^{1,2}, L. Ma^{1,4}, J. Q. Wang¹, J. J. Loo², M. Bionaz³, and D. P. Bu^{*1,5}, ¹State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²Department of Animal Sciences and Division of Nutritional Sciences, University of Illinois, Urbana, IL, ³Animal and Rangeland Sciences, Oregon State University, Corvallis, OR, ⁴CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, ⁵Hunan Co-Innovation Center of Safety Animal Production, CICSAP, Changsha, Hunan, China.

A transcriptomics approach was used to evaluate the influence of forage-to-concentrate ratio (F:C) on liver (LV) and mammary (MG) tissue metabolism in mid-lactation dairy cows. Twenty-four primiparous lactating Holstein cows (body weight, 558 ± 10 kg; days in milk, 136 ± 37; daily milk yield, 21.12 ± 2.30 kg) were randomly allocated to 2 groups receiving either a high-forage diet (HF, F:C = 60:40) with Chinese wild rye, alfalfa hay and corn silage as forage source or a low-forage diet (LF, F:C = 40:60) with corn stover as forage source. A subgroup of cows (n = 5/diet) was used for analysis of liver and mammary transcriptome. Biopsies of LV and MG were collected at the end of 8-week feeding to measure mRNA profiles using a 4 × 44K Bovine Agilent microarray chip. Data were analyzed with SAS JMP Genomics using ANOVA with a false discovery rate correction (FDR < 0.05). The Dynamic Impact Approach was used for pathway analysis. The analyses uncovered 759 and 225 differentially expressed genes (DEG) between LF and HF group in MG and LV, respectively. The greater number of DEG in MG underscored that mammary transcriptome was more responsive to changes in F:C compared with LV. Among the LV DEG, more genes were upregulated (135 vs. 90), whereas more DEG were downregulated (315 vs. 444) in MG, suggesting a potentially different response upon changes in F:C. Pathway analysis revealed enhanced amino acid metabolism, such as taurine and hypotaurine metabolism, and decreased protein export in MG of LF-fed cows. Similarly, LF-fed cows also had enhanced lipid metabolism, such as steroid hormone biosynthesis in the MG compared with cows in HF. In contrast, compared with cows in HF, LF cows had overall lower folate biosynthesis which contributed to overall lower cofactor and vitamin metabolism. Although pathway analysis revealed that amino acid, lipid, energy, as well as cofactors and vitamin metabolism were among the most impacted biological processes in MG, similar alterations were not observed in LV. Overall, results indicate that MG and LV transcriptome in primiparous dairy cows was affected to a different extent by forage-to-concentrate ratio in mid-lactation.

Key Words: forage-to-concentrate ratio, liver and mammary tissue, transcriptome

M231 Maternal ethyl-cellulose rumen-protected methionine supplementation affects Holstein heifer calf development and growth. A. S. Alharthi^{*1}, F. Batistel¹, C. Parys², A. Helmbrecht², and J. J. Loo¹, ¹University of Illinois at Urbana-Champaign, Urbana, IL, ²Evonik Nutrition & Care GmbH, Hanau-Wolfgang, Germany.

The objectives were to investigate if increasing supply of methionine during late-pregnancy in Holstein cows affects developmental parameters at birth and subsequent growth, and whether such effect is an utero-placenta or colostrum-dependent response. Thirty-nine Holstein

heifers born to cows fed a basal control [CON; 1.47 Mcal/kg dry matter and 15.3% crude protein] diet with no added methionine or CON plus ethyl-cellulose rumen-protected methionine (MET; Mepron, Evonik Industries AG, Germany) were used. The MET was fed during the last 28 d of pregnancy at a rate of 0.09% of diet DM. Immediately after birth, heifers were randomly allocated considering dam treatment and colostrum as follows: 1) calves from CON cows and colostrum from CON cows (n = 9); 2) calves from CON cows and colostrum from MET cows (n = 9); 3) calves from MET cows and colostrum from MET cows (n = 11); and 4) calves from MET cows and colostrum from CON cows (n = 10). Body weight, hip and wither height, hip width and body length were measured at birth and weekly through weaning (42 d of age) and until 9 weeks of age. Calves from dams fed MET had greater hip height ($P = 0.02$; 81.0 vs 78.5 cm) at birth. However, body weight at birth ($P = 0.67$; 41.1 vs 41.7 kg), hip width ($P = 0.15$; 16.4 vs 15.8 cm), wither height ($P = 0.11$; 75.8 vs 77.3 cm) as well as colostrum quality ($P = 0.95$; 55.4 vs 52.4 IgG) and quantity ($P = 0.80$; 5.9 vs 5.7 kg) were not affected by maternal treatment. Over the first 9 wk of life, there was no colostrum effect for any of the growth variables measured. However, compared with CON, calves from dams fed MET had greater body weight ($P = 0.03$; 60.8 vs 57.3 kg), hip height ($P = 0.02$; 87.7 vs 85.7 cm), wither height ($P = 0.05$; 83.5 vs 81.9 cm), and average daily gain ($P = 0.01$; 0.69 vs 0.60 kg/day). Hip width ($P = 0.73$; 19.7 vs 19.6 cm) and body length ($P = 0.24$; 122.8 vs 124.8 cm) were not affected by maternal MET. Overall, the data indicate that maternal supplementation with MET during late-gestation had a positive effect on neonatal heifer calf growth.

Key Words: fetal programming, amino acid, nutrition

M232 Effect of pasteurized and non-pasteurized colostrum on the growth performance and development of gastrointestinal tract of calves. G. T. Liu^{1,2}, D. P. Bu^{1,5}, S. C. Li³, K. Yang⁴, and Q. E. Zhang^{*2}, ¹State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²Department of Animal Science, Ningxia University, Yinchuan, Ningxia, China, ³Department of Animal Science, University of Manitoba, Winnipeg, Canada, ⁴Dongying Austasia Modern Dairy Farm Co., Ltd., Dongying, Shandong, China, ⁵Hunan Co-Innovation Center of Safety Animal Production, CICSAP, Changsha, Hunan, China.

The effects of colostrum pasteurization on the growth and gastrointestinal tract of calves were studied. Twenty newborn Holstein calves were randomly divided into 2 groups (n = 10) and were raised in individual pens. Calves were either fed non-pasteurized colostrum (NPC) or pasteurized colostrum (PC) processed at 60°C for 60 min using a Dairy Tech colostrum pasteurizer (Dairy Tech Inc., Colorado). All calves were offered 4 L of colostrum within 1 h after birth and given a second feeding of 2 L of colostrum 6 h later. The experiment was carried out over 90 d. Measurements of the body weight, body length and metatarsal circumference of the calves were recorded every 30 d during the trial, while the dietary intake and diarrhea frequency were measured daily. Five calves from each group were randomly selected and slaughtered at 90 d of age. The dorsal sac and ventral sac of the rumen and small intestine were sampled to make paraffin sections and then observed its morphological changes. A repeated measures analysis was conducted by using the MIXED procedure of SAS while Initial body weight was included as a covariate in the model when appropriate. The calves in

Table 1 (abstract M232). Body weight (BW), body length (BL), and metatarsal circumference (MC) data

Item	NPC				PC				SEM	P-value		
	0 d	30 d	60 d	90 d	0 d	30 d	60 d	90 d		Trt	Day	Trt × day
BW (kg)	42.2	58.1	89.8	119.4	42.3	57.6	90.1	121.2	1.4	0.77	<0.0001	0.75
BL (cm)	71.1	78.8	90.0	99.3	69.8	78.3	89.9	100.7	0.6	0.81	<0.0001	0.09
MC (cm)	11.6	12.2	14.0	14.9	11.6	12.2	14.2	15.3	0.1	0.40	<0.0001	0.02

PC group had higher ($P < 0.05$) metatarsal circumference and tended to have longer ($P = 0.09$) body length at 90 d of age (Table 1). Colostrum type had no effect on the intake of starter feed and diarrhea frequency. However, the papilla width of rumen dorsal sac (1.25 vs. 1.02 mm) and rumen ventral sac (1.18 vs. 0.96 mm), the villus height of duodenum (0.55 vs. 0.43 mm) and jejunum (0.64 vs. 0.46 mm) in PC group were greater ($P < 0.05$) than that in NPC group, and the crypt depth of jejunum (0.39 vs. 0.49 mm) were smaller ($P < 0.05$). The results indicate that the pasteurized colostrum has a positive impact on the growth performance and development of gastrointestinal tract for calves in 90 d of age.

Key Words: calf, pasteurized colostrum, growth

M233 Modulation of feeding behavior in lactating dairy cows by sweet sensory additives. M. Blanch^{*1}, F. Bargo¹, G. Tedó¹, I. R. Ipharraguerre^{1,2}, I. Guasch³, and A. Bach^{4,5}, ¹Lucta S.A., Barcelona, Spain, ²University of Kiel, Germany, ³Blanca from the Pyrenees, Spain, ⁴ICREA, Barcelona, Spain, ⁵IRTA, Caldes de Montbui, Spain.

The aim of this study was to evaluate changes in feeding behavior and performance of lactating dairy cows in response to the addition of sensory additives into their TMR. A 90-d experiment was conducted involving 42 lactating cows (15 primiparous, 27 multiparous; BW = 690 ± 63 kg; DIM = 148 ± 73; milk yield = 38 ± 8 kg/d) randomly allocated to 3 treatments and fed a common TMR (15.4% CP, 29.2% NDF, 1.67 Mcal of NEI/kg). Treatments were either no supplementation (CON) or supplementation with sensory additive A (SAA - containing stevia glycosides) or B (SAB - the same formulation without stevia glycosides) at 30 g/d. Dry matter intake (DMI), milk production, milk composition, BW, feeding behavior, and feed efficiency (FE) were determined daily. Data were analyzed with a mixed-effect model that included the fixed effects of treatment, parity, week, and their 2-way interactions. Results are shown in Table 1. Feed intake was reduced ($P < 0.001$) when both sensory additives were added into the TMR;

however, milk yield and composition did not differ among treatments. Regarding feeding behavior, SAB reduced ($P < 0.001$) the number of meals and eating time, and increased ($P < 0.001$) meal size compared with SAA and CON. In contrast, SAA reduced meal size ($P < 0.001$) and eating rate ($P < 0.001$) compared with SAB and CON. The use of sweet sensory additives may modulate feeding behavior in lactating dairy cows; nevertheless, such a response appears to be dictated by their composition (i.e., sweet ingredients).

Key Words: feeding behavior, lactating cows, sensory additives

M234 Metabolic changes in rumen fluid from dairy cows in response to heat stress. L. Ma^{1,2}, Y. X. Yang¹, S. T. Gao^{1,2}, L. S. Zhao^{1,2}, L. Baumgard³, Z. T. Yu⁴, and D. P. Bu^{*1,2}, ¹State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China, ²CAAS-ICRAF Joint Lab on Agroforestry and Sustainable Animal Husbandry, World Agroforestry Centre, East and Central Asia, Beijing, China, ³Iowa State University, Ames, IA, ⁴Department of Animal Sciences, The Ohio State University, Columbus, OH, ⁵Hunan Co-Innovation Center of Safety Animal Production, CICSAP, Changsha, Hunan, China.

Heat stress (HS) decreases milk yield and deleteriously alters milk composition in dairy cows. This study examined the ruminal metabolic response of dairy cows to HS using a combination of LC-MS, GC-MS, and ¹H NMR. Four multiparous Holstein dairy cows (101 ± 10 DIM; 574 ± 36 kg of BW, 38 ± 2 kg of milk/d) were randomly assigned to 4 environment chambers with a crossover design. Cows were either subjected to HS [HS: 36°C with light and 32°C without light; THI = 87.2 and 81.8] or kept under thermal neutral conditions [TN: 20°C; THI = 65.5] for 9 d for adaptation and then for another 9 d of pair-feeding to eliminate confounding effects of dissimilar feed intake. There was a 30-d washout period between periods. Rumen fluid was collected at 1000 h (after feeding) on d 9. All data were analyzed using R, SIMCA-P 13.0,

Table 1 (abstract M233). Performance and feeding behavior results

Item	Treatments			SE	P-value		
	CON	SAA	SAB		T	T × wk	T × lact
Milk, kg/d	34.6	34.5	35.2	2.2	0.96	<0.01	NS
Fat, %	3.97	3.84	3.78	0.08	0.19	0.04	NS
Prot, %	3.32	3.31	3.28	0.02	0.49	NS	NS
DMI, kg	24.7a	23.3b	23.2b	0.29	<0.001	0.01	<0.001
FE, kg/kg	1.42	1.48	1.53	0.08	0.57	0.01	NS
BW, kg	709.0	700.9	688.0	17.0	0.65	NS	NS
N meals, /d	9.28 ^a	9.39 ^a	8.27 ^b	0.21	<0.001	<0.001	0.04
Meal duration, min	22.1	22.0	22.1	0.08	0.79	NS	NS
Meal size, kg	2.74 ^b	2.56 ^c	2.89 ^a	0.05	<0.001	<0.001	NS
Eating time, h/d	3.42 ^a	3.46 ^a	3.04 ^b	0.08	<0.001	<0.001	0.04
Eating rate, g/min	123.9 ^a	115.5 ^b	130.4 ^a	2.62	<0.001	<0.001	NS

and SIEVE software. Metabolites were identified by m/z value matched to the NIST database. A student *t*-test was used to search for changed metabolic profiles with Variable Importance in the Projection (VIP) greater than 1 in an OPLS-DA model and a *P*-value less than 0.05. The OPLS-DA results showed that all the metabolic profiles in the rumen fluid changed were separated into 2 groups in response to HS and TN. Based on LC-MS, GC-MS and ¹H NMR results, the metabolites of glucose, galactose, glycerol, butyrate, glucosamine, heptacosane and hentriacontane were increased by HS (VIP >1, *P* < 0.05, Fold change >0), while fatty acids and amino acids were decreased (VIP >1, *P* < 0.05, Fold change <0). Most of the rumen metabolites affected by HS were related to several metabolic pathways, including urine cycle pathway, metabolism of amino acids, tryptophan metabolism and citrate cycle, which likely affect the precursor supply for milk component synthesis. These findings indicate that the use of multiple metabolomics platforms permits a far more detailed understanding of HS-induced metabolic changes in rumen digestion: potential mechanism by which HS decreases milk production and component synthesis.

Key Words: heat stress, metabolic, rumen fluid

M235 Impact of ad libitum milk feeding and butyrate supplementation on organ and epithelial growth in the gastrointestinal tract of dairy calves. C. Gerbert¹, D. Frieten^{*2}, C. Koch¹, G. Dusel², K. Eder³, R. Zitnan⁴, and H. M. Hammon⁵, ¹*Educational and Research Centre for Animal Husbandry, Hofgut Neumuehle, Muenchweiler an der Alsenz, Germany*, ²*Department of Life Sciences and Engineering, University of Applied Sciences Bingen, Bingen, Germany*, ³*Institute of Animal Nutrition and Nutrition Physiology, Justus-Liebig-University Giessen, Giessen, Germany*, ⁴*National Agricultural and Food Centre, Research Institute for Animal Production, Nitra, Slovakia*, ⁵*Institute of Nutritional Physiology, Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany*.

Ad libitum milk replacer (MR) feeding as well as butyrate (B) have independently shown that both stimulate postnatal growth and development in calves. We hypothesized that the combination of intensive MR feeding and B supplementation expedites the development of the gastrointestinal tract in preweaning calves in a synergistic manner. Holstein male calves were studied from birth until d 80 of age. All calves received colostrum and transition milk until d 3 of age and from d 4 onwards were fed MR (12.5% dry matter) in amounts of either 6 L/d (Res; n = 16) or ad libitum (Adl; n = 16) for 8 wk. In both feeding groups half of the calves were fed MR with 0.24% B (ResB+; AdlB+) or same MR with no B supplement (ResB-; AdlB-). From wk 9 to wk 10 MR was linearly reduced in all calves to 2 L/d. Hay, water, and concentrate were offered ad libitum. At d 80 calves were harvested and mucosa samples of the rumen and small intestine were taken for measurement of rumen papilla and intestinal villus and crypt size. Data (LSM) were analyzed by the Mixed Model of SAS with feeding regimen, B supplementation, and rumen/intestinal segment as fixed effects. Body weight (124 vs. 110 kg) and kidney fat weight (514 vs. 363 g) were greater (*P* < 0.05) but pancreas weight (92 vs. 106 g) was lower (*P* < 0.05) in Adl than in Res. The small intestine was longer (*P* < 0.05) in AdlB- than in ResB- (27.3 vs. 23.6 m). Villus height in duodenum, proximal jejunum and ileum were greater (*P* < 0.05) in Adl than in Res (567, 637, 555 vs. 546, 611, 529 μm, resp.). Villus height was greater (*P* < 0.05) in B+ than in B- (585 vs. 554 μm). Crypt depth was reduced (*P* < 0.01) by B in ileum (262 vs. 253 μm). The villus height/crypt depth ratio increased by ad libitum feeding and by B and was greatest (*P* < 0.05) in AdlB+ throughout the small intestine (2.27, 2.44, 2.24, 2.35 for AdlB-, AdlB+, ResB-, ResB+, respectively). Intensive MR feeding and B increased small intestinal mucosa growth.

These findings indicated a synergistic effect on small intestinal mucosa growth by intensive MR feeding and B supplementation.

Key Words: ad libitum milk replacer feeding, butyrate, intestine

M236 The effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows. R. C. Oliveira^{*1}, K. J. Sailer¹, R. S. Pralle¹, H. T. Holdorf¹, G. D. Poppy², and H. M. White¹, ¹*University of Wisconsin-Madison, Madison, WI*, ²*Fermented Nutrition Corporation, Luxemburg, WI*.

Supplementation of gluconeogenic precursors in postpartum dairy cattle diets may improve production performance and decrease incidence of metabolic disorders related to negative energy balance. The objective of this trial was to examine the effect of fermented ammoniated condensed whey supplementation on hyperketonemia incidence in transition dairy cows. Holstein cows were individually housed in a tie-stall facility from 28 d prepartum to 45 d postpartum. Cows were blocked by expected calving date and randomly assigned to control (ctl; n = 20) or GlucoBoost (GB; Fermented Nutrition; n = 19). All cows received the same high-energy prepartum ration precalving to achieve postpartum hyperketonemia incidence consistent with industry averages. Cows began treatment rations (GB 0.9 kg AF/day replacing DM equivalent in ctl) on the day of calving. Hyperketonemia incidence was calculated from blood samples collected on d 1, 3, 5, 7, 9, 11, 14 and 17 after calving. Categorical data were analyzed by logistic regression (GLIMMIX, SAS) fitting a binary distribution response. Continuous variables were analyzed with the MIXED procedure of SAS. Models containing the fixed effect of treatment (GLIMMIX) or treatment, time, and the interaction of treatment x time (MIXED) and random effects of block and cow(block × treatment). Supplementation did not alter (*P* > 0.54) milk volume (41.1 vs. 42.2 ± 1.53 kg, ctl vs. GB), fat, protein, or lactose yield. Cows supplemented with GB had a numerically reduced hyperketonemia incidence (60.0 vs. 36.8 ± 11.28%, *P* = 0.17). Body weight change (-69.7 vs. -60.4 ± 8.78 kg, ctl vs. GB) and body condition score change (-0.39 vs. -0.30 ± 0.052 units, ctl vs. GB) from calving to 45 d did not differ (*P* > 0.25) by treatment. Concentration of NEFA differed over time but was not affected (*P* = 0.70) by treatment. Supplementation of GB did not alter body tissue mobilization or milk production, but reduced hyperketonemia incidence by 1.6 times. This suggest that GB may have altered hepatic metabolism to improve postpartum metabolic disease incidence.

Key Words: ketosis, GlucoBoost, transition cow

M237 Prepartum conjugated linoleic acid supplementation on lactation performance and metabolic health in dairy cows. R. C. Oliveira^{*1}, R. S. Pralle¹, L. C. de Resende², C. H. P. C. Nova³, V. Caprarulo⁴, J. A. Jendza⁵, A. Troesch⁶, and H. M. White¹, ¹*University of Wisconsin-Madison, Madison, WI*, ²*Federal University of Lavras, Lavras, Brazil*, ³*State University of Northern Rio de Janeiro, RJ, Brazil*, ⁴*University of Milan, Milan, Italy*, ⁵*BASF, Florham, NJ*, ⁶*BASF, Lampertheim, Germany*.

Prepartum supplementation with conjugated linoleic acid (CLA) may influence lipolysis and milk fat synthesis. The objective of this study was to examine the effect of prepartum CLA supplementation on lactation and metabolic health of dairy cows. Multiparous cows were enrolled in the study at -18 d prepartum, and randomly assigned 20 g/d of a mixture of *trans*-10, *cis*-12 and *cis*-9, *trans*-11 CLA (CLA n = 143; 100 g/d of Lutrell Pure; BASF) or an equivalent amount of saturated lipids as Control (Ctl n = 141; 75 g of Energy Booster 100; Milk Specialties

Global). Treatments were top dressed individually to cows daily from enrollment to calving and all cows were offered the same ration. Blood samples were collected on the first day of supplementation, -10 d prepartum, and d 1, 7, 14, and 30 of lactation. Milk yield was recorded daily until 60 DIM and averaged weekly. A milk sample was obtained weekly for component analysis. Categorical data were analyzed by logistic regression (GLIMMIX, SAS) fitting a binary distribution response. Continuous variables were analyzed with the MIXED procedure of SAS. Models containing the fixed effect of treatment (GLIMMIX) or treatment, time, and treatment x time (MIXED) and random effects of block and cow(block x treatment). Prepartum treatment period was 16.1 ± 4.5 and 16.4 ± 4.3 for Ctl and CLA, respectively. Cows supplemented with CLA had increased milk protein yield (1.38 vs. 1.43 ± 0.019 kg, $P = 0.01$), and tended to have increased milk fat (1.94 vs. 2.00 ± 0.025 kg, $P = 0.07$) and milk yield (46.6 vs. 47.6 ± 0.45 kg, $P = 0.09$), that resulted in greater energy content of milk (35.35 vs. 36.36 ± 0.379 Mcal/d, $P = 0.03$). CLA supplemented cows had a tendency for lower serum NEFA (0.28 vs. 0.23 ± 0.016 mEq/L, $P = 0.06$) and serum BHBA (0.96 vs. 0.80 ± 0.067 mM, $P = 0.09$), which resulted in decreased prevalence of hyperketonemia on d 14 postpartum (23.5% vs. 3.5% ± 5.79, $P = 0.05$). Body condition score change was not affected ($P > 0.51$) by treatment. There were no significant differences in other health disorders ($P > 0.20$). Prepartum supplementation of CLA improved lactation performance and metabolic health of dairy cows.

Key Words: CLA, transition period, ketosis

M238 Effect of live yeast and a combination of live yeast and calcified seaweed on rumen fermentation. N. D. Walker^{*1}, O. AlZahal¹, B. Tas², and W. van Straalen², ¹*AB Vista, Marlborough, Wiltshire, UK*, ²*Schothorst Feed Research, Lelystad, the Netherlands*.

The objective of this study was to investigate the effect of active-dry yeast (ADY; Vistacell, Marlborough, UK) and a combination of ADY and a calcified seaweed product (4% Vistacell AB, AB Vista) on rumen fermentation characteristics. Three ruminally-fistulated dairy cows (49 ± 3 DIM) were used in a 3 × 3 Latin square arrangement. The treatments were: T1 (100 g/d limestone), T2 (4 g/d of ADY + 96 g/d of limestone, and T3 (4 g/d of ADY + 88 g/d of AB + 8 g/d of limestone). The cows were fed ad libitum 2×/d a 65:35 (F:C; DM basis). Each period lasted 3 wks, a 2-wk adaptation phase followed by 1 wk of measurements. Throughout the experiment, milk yield and DMI were measured daily. Ruminal pH was recorded every minute from d 15 through d 21 using an indwelling electrode system. Apparent total-tract digestibility was measured during d19 to d21. Rumen samples were collected during d19 to d21 at 0, 2, 4, 6 and 8 h after AM feeding and analyzed for VFA and ammonia content. Milk components were analyzed during d19 to d21 for protein, fat content, and somatic cell count. Statistical analysis was conducted using ANOVA. Results showed that T2 and T3 increased ($P < 0.05$) 4% FPCM yield compared with T1 (37.1 kg/d) by 1.5 and

1.8kg/ day respectively. DMI was increased by T2 by 0.8 kg/day ($P < 0.05$) compared with T1 and T3 (23.4 vs 23.6 kg/d, respectively). There was no significant effect on feed efficiency between the 3 groups (1.59 vs 1.60 vs 1.65; T1, T2, T3, respectively). Mean daily ruminal pH was numerically higher (pH 6.28 ± 0.5) for both T2 and T3 than T1 (pH 6.22 ± 0.7). Time spent below pH 5.8 was 159, 87, and 50 min/d for T1, T2, and T3, respectively. Different fermentation profiles were observed, indicating a shift in acetate:propionate:butyrate (mol/100 mmol) with T3 (63.1: 20.2: 13.6), T1(61.9: 22.2: 13.0) and T2 61.4:22.8: 12.8) with T3 showing an increase in acetate and butyrate ($P < 0.05$) and reduction in propionate ($P < 0.05$). Propionate was increased in T2 ($P < 0.05$). No significant effects were observed on digestibility. The results showed that ADY can positively affect rumen fermentation and animal performance. Different effects are observed when in combination with calcified seaweed

Key Words: live yeast, rumen fermentation, performance

M239 Factors affecting performance responses to supplementation of rumen-protected methionine for dairy cows. G. F. M. Leão², J. R. R. Dórea³, and M. A. C. Danes^{*1}, ¹*University of Lavras, Lavras, MG, Brazil*, ²*Federal University of Paraná, Curitiba, PR, Brazil*, ³*University of Wisconsin, Madison, WI*.

Methionine is often the first limiting amino acid (AA) for milk production. Supplementation of rumen-protected methionine (RPM) is a strategy to improve metabolizable protein (MP) utilization and animal performance. However, current literature shows inconsistent results regarding size of responses to RPM. The objective of this study was to better understand how other factors affect performance responses to RPM and therefore speculate about the feeding situations with greater potential of better responses. Thirty-two published studies yielding 39 mean comparisons between a control diet (CD) and CD + RPM were analyzed using a meta-analytical approach. Studies were classified according to the level of dietary crude protein (CPdiet), days in milk (DIM) of the cows, and duration of the treatment. Study was included as random effect and models were weight by the number of observations in each study. Results (Table 1) indicate that the response of milk protein yield (Mprot) to RPM is influenced by CPdiet and that this relationship changes according to stage of lactation. Furthermore, the interaction between CPdiet and DIM may also affect responses of dry matter intake and milk yield to RPM. The duration of the treatment also affected Mprot response ($P = 0.07$). When cows received the RPM for longer than 60 d, the average increase in Mprot was 54 g/d, while when shorter than 60 d it was only 19 g/d. Overall, the results suggest that better responses to RPM can be expected when cows are past peak of lactation, receiving diets higher than 16% of CP for a period longer than

Table 1 (abstract M239). Size of responses to rumen-protected methionine supplementation (n = 39) according to diet crude protein (CPdiet; > or <16% CP) and DIM

	0-59 DIM		60-109 DIM		110-140 DIM		SEM	<i>P</i> -value		
	>16%	<16%	>16%	<16%	>16%	<16%		CPdiet	DIM	CPdiet × DIM
DMI, kg/d	-0.73	0.15	0.59	-0.60	0.36	0.17	0.45	0.50	0.48	0.01
Milk, kg/d	-0.50	1.26	1.17	-0.67	0.92	-0.26	0.60	0.20	0.97	<0.001
Protein, g/d	21	31	73	-1	77	19	24	<0.001	0.64	0.04
Fat, g/d	4	73	50	48	133	72	51	0.94	0.26	0.18

60 d. Greater responses in Mprot when diet CP is high may challenge the assumption that better AA profile of MP allows for diet CP reduction.

Key Words: meta-analysis, protein, days in milk

M240 The use of H-nuclear magnetic resonance (H-NMR) in ewes suffering milk fat depression: Could blood metabolomic differences explain the individual variations? A. A. K. Salama*¹, P. G. Toral², G. Hervás², G. Caja¹, and P. Frutos², ¹Group of Ruminant Research (G2R), Universitat Autònoma de Barcelona, Bellaterra, Spain, ²Instituto de Ganadería de Montaña (CSIC-ULE), Grulleros, León, Spain.

Dietary supplementation of dairy ewes with marine lipids improves milk fatty acid (FA) profile, but causes milk fat depression (MFD). A large individual variation in the MFD response has been observed, and factors causing this variation are not clear. This study was conducted in dairy sheep to test whether differences in the blood metabolomic profile would clarify causes of individual variations in terms of MFD when marine lipids were added to the diet. Assaf dairy ewes (n = 15) received a total mixed ration supplemented with 0 (control; n = 5) or 20 g of fish-oil/kg DM [n = 10; with animals divided in those showing a strong (RESPO+, n = 5) or slight (RESPO-) MFD]. Blood samples collected before (d 0) and after (d 36) oil supplementation were analyzed by H-NMR spectroscopy operating at 600 MHz. For better metabolite detection, spectral editing was done based on spin-spin relaxation time (CPMG filter) and molecular diffusion (DOSY filter). Multivariate analyses of data were carried out by the ChemoSpec package of R program and the web-based MetaboAnalyst program. Principal component and partial least square–discriminant analyses were used to detect metabolite differences among treatment groups. Both RESPO- and RESPO+ ewes supplemented with fish oil had lower concentrations of proline, valine, isoleucine, keratin, lactate and β -glucose. The reductions in amino acid concentrations were in accordance with lower ($P < 0.05$) milk protein content in lipid-supplemented ewes (4.75%) compared with control ewes (5.03%). Additionally, some monounsaturated FA increased in RESPO- and RESPO+ ewes, which could include some potential antilipogenic FA that are supposed to be able to induce MFD. However, there was no clear discrimination between RESPO+ and RESPO- ewes. In conclusion, lipid supplementation caused significant differences in blood H-NMR metabolomics, but no clear discrimination was observed between animals showing slight and strong MFD. Study funded by Project AGL2014–54587 (Plan Nacional, MINECO, Spain).

Key Words: fish oil supplementation, H-NMR metabolomics, sheep

M241 Nitrogen utilization in dairy cows fed a corn silage based-diet supplemented with increasing amounts of linseed oil. F. Hassanat*, C. Cherif, D. Warner, and C. Benchaar, *Agriculture and Agri-Food Canada, Sherbrooke Research and Development Centre, Sherbrooke, QC, Canada.*

Previously we showed that supplementation of a corn silage-based diet with up to 3% of linseed oil (LO) reduced enteric CH₄ production without adverse effects on DMI and milk production. However, a higher supplementation level (4%) impaired DMI and milk yield. In the same study, we also examined the effects of supplementing increasing amounts LO on N balance of dairy cows fed corn silage-based diet. For this purpose, 12 lactating, multiparous Holstein cows (DIM = 84 ± 28; milk yield = 42 ± 4.6 kg/d) were used in a replicated 4 × 4 Latin square design (35-d period, 14-d adaptation). Cows were fed for ad libitum (5% orts,

on an as-fed basis) a corn silage-based TMR (61:39; forage:concentrate ratio) not supplemented (control) or supplemented with 2, 3 or 4% LO (on DM basis). Intake of N, N excretion (fecal and urinary) and milk N secretion were determined over 6 consecutive days, while ruminal NH₃ concentration was determined over 2 consecutive days. Data were analyzed using the MIXED procedure (SAS) and differences between treatments and the control were declared significant ($P \leq 0.05$) using Dunnett's comparison test. Ruminal NH₃ concentration was unaffected by adding LO at 2 and 3% to the diet, but decreased at 4% LO. A decline in N intake was observed when LO was included at 2, 3 and 4% in the diet (–6, –8 and –19%, respectively). Nitrogen excretion (g/d) in urine and feces also decreased with increasing inclusion of LO in the diet. However, when expressed as a proportion of N intake, excretion of N in feces and urine was not affected by adding LO to the diet. Milk N efficiency (g milk N/g N intake) was higher for cows fed LO-supplemented diets (averaged 31%) compared with cows fed the control diet (26%). Results show that diet supplementation with up to 4% of LO reduced N intake, had no effect on fecal and urinary N excretion (as a proportion of N intake) and improved milk N efficiency of dairy cows. It is concluded that supplementing a corn-silage based diet with up to 3% of LO reduced the amount of N excreted in the manure and increased milk N efficiency without negative effects on DMI or milk yield.

Key Words: linseed oil, corn silage, nitrogen utilization

M242 Effect of *Lactobacillus animalis* LA-51 and *Propionibacterium freudenreichii* PF-24 on the total tract digestibility of protein, starch, NDF and on fecal starch concentrations in high-producing cows. K. E. Nestor Jr.*, S. Lerner, and C. Jamison, *Chr. Hansen Animal Health, Milwaukee, WI.*

The effect of a combination of probiotic strains of *Lactobacillus animalis* (LA-51) and *Propionibacterium freudenreichii* (PF-24) fed at a total concentration of 3×10^9 on total-tract digestibility of NDF, protein, and starch and on the concentration of starch in manure was assessed in a commercial study. Observations were gathered from 25 herds, ranging in total size from 80 to 6,000 cows. High groups within each herd were selected for testing. Groups ranged in size from 20 to 200 cows and were producing ≥ 80 lb average milk per day. Both Jerseys and Holsteins were included in the test. Group samples of manure and total mixed rations (TMR) were collected one week before and 3 weeks after the initiation of supplementation of feed with probiotics. Fecal and TMR samples were sent to Cumberland Valley Analytical Laboratory for analysis. Data were analyzed using 2-tailed paired *t*-test using herd as the experimental unit. There was no effect of probiotic feeding on total-tract digestibility of NDF or protein. Starch digestibility was increased ($P < 0.01$) and fecal starch was decreased ($P < 0.01$) with daily feeding of the combination of LA-51 and PF-24. When the data were limited to those herds where the initial concentration of fecal starch was $\geq 3\%$, total-tract digestibility of protein and starch increased ($P < 0.05$ and $P < 0.0001$, respectively), fecal starch decreased ($P < 0.001$), and total-tract digestibility of NDF tended to increase ($P < 0.12$). The addition of an effective combination of probiotic organisms can improve the apparent digestibility of nutrients when fed to high-producing dairy cows.

Key Words: probiotic, digestibility, *Lactobacillus*

M243 Gaseous emissions from forages and rumen-cannulated steers: Do ruminants emit nitrous oxide? K. Gerlach*¹, A. Sommer¹, A. J. Schmithausen², M. Trimborn², W. Büscher², and K.-H. Südekum¹, ¹Institute of Animal Science, University of Bonn,

Bonn, Germany, ²Institute of Agricultural Engineering, University of Bonn, Bonn, Germany.

This study aimed at assigning gaseous emissions from ruminants to animals or feeds they have consumed. Three adult rumen-cannulated German Holstein steers and 3 forage types were used in a 3x3 Latin square design. Forages were corn silage (CS, 366 g dry matter (DM)/kg, 70.7 g crude protein (CP)/kg DM), alfalfa silage (AS, 411 g DM/kg, 246 g CP/kg DM) and grass hay (GH, 881 g DM/kg, 79 g CP/kg DM). Each period consisted of 10 d where animals received 10 kg DM/d of one of the forages as sole feed and the last 3 d of each period were used for sampling. A defined amount of forage was put in a closed vessel and gas samples were obtained using evacuated headspace vials after 0, 10, 20, 30, and 40 min. Additional samples were taken 3 h after filling following the same procedure. Samples from the gaseous phase of the steers' rumen were taken 3 h after offering feed in the morning. In 10-min intervals, 4 samples were obtained with a syringe through the closed lid of the rumen-cannula and filled into headspace vials. This was repeated on 3 consecutive d. Samples were analyzed for carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) using gas chromatography. Data were analyzed using SAS 9.4 and a mixed model taking forage type and period as fixed effect as well as their interaction. For the rumen samples, animal was added as random effect. Within each period, day was taken as repeated measurement. There were large differences in the amount of CO₂ and N₂O emitting from the forages. Most N₂O came from AS (9.9 µg/kg fresh matter (FM) · h; $P < 0.05$) and only small amounts from GH (0.20 µg/kg FM · h) and CS (0.04 µg/kg FM · h). Highest CO₂ concentrations were measured in CS ($P < 0.05$). After 3 h, emissions from forages were strongly reduced. Methane was not detected in any forage sample. Animals fed CS showed slightly lower ($P < 0.05$) CH₄ concentrations in the rumen gas sample than when fed AS or GH (16.9% vs. 20.6% and 20.3%). Big differences were found for N₂O with 0.857 ppm for AS and 0.246 and 0.171 ppm for CS and GH ($P < 0.001$). Results indicate that fermented forages rich in CP may release climate-relevant N₂O. The N₂O emitting from ruminants seems to originate from the consumed feedstuff and is probably not synthesized in the rumen.

Key Words: emission, forage, ruminant

M244 In vitro post-ruminal digestion of rumen bypass emulsions encapsulated by interfacial crosslinking using polyphenol oxidase from potato tuber peels. F. Gadeyne^{*1}, N. De Neve¹, B. Vlaeminck¹, P. Van der Meeren², and V. Fievez¹, ¹Laboratory for Animal Nutrition and Animal Product Quality, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium, ²Particle and Interfacial Technology Group, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, Ghent, Belgium.

The protection of unsaturated fatty acids by emulsifying oil in a polyphenol oxidase (PPO) rich protein extract and cross-linking of interfacial protein upon 4-methylcatechol (4-MC) addition has been shown before as a promising technology to bypass rumen biohydrogenation (BH). An in vitro experiment was designed to assess the post-ruminal availability of linolenic acid (LNA) in rumen bypass emulsions. Therefore, emulsions ($n = 3$) were made with a protein extract of potato tuber peels, containing 20 mg triacylglycerols (TAG) of linseed oil per ml extract and a final 4-MC concentration of 0, 5, 10 or 20 mM, and were subjected to in vitro ruminal, abomasal and a time series of intestinal incubations. As TAG have to be hydrolyzed to nonesterified fatty acids (NEFA) before BH can occur, it was hypothesized protected LNA remained in the TAG fraction during ruminal incubation, but was released from its protective shell and concomitantly hydrolyzed to bio-available NEFA

post-ruminally. Lipids were extracted from emulsions, after rumen or after the subsequent abomasal and intestinal incubation, separated in lipid fractions, methylated and analyzed using gas chromatography. Results were analyzed with the MIXED procedure of SAS. TAG LNA disappeared from the original emulsions after rumen incubation when no 4-MC was present ($P < 0.001$), but less in case of 5 ($P = 0.006$) or 10 ($P = 0.024$) mM 4-MC and no differences were observed anymore with 20 mM 4-MC ($P = 0.879$). All TAG LNA disappeared after abomasal and intestinal incubation compared with the original emulsions ($P < 0.001$), but LNA appeared partly as NEFA after abomasal and intestinal incubation when 4-MC was present ($P < 0.004$). Time series of intestinal incubations indicated, however, some level of overprotection, as LNA was least released in the NEFA fraction at the highest levels of rumen protection. In conclusion, dietary LNA in PPO-protected emulsions became bio-available for intestinal uptake, as a fraction of 0.223, 0.237, or 0.303 for emulsions with 5, 10 or 20 mM 4-MC, respectively, survived rumen BH and was released as NEFA after abomasal and intestinal incubations.

Key Words: polyphenol oxidase, biohydrogenation, rumen bypass

M245 The effect of concentrate supplementation strategy on milk production and rumen fermentation parameters in late-lactation spring-calving grazing dairy cows. Z. C. McKay^{*}, M. B. Lynch, F. J. Mulligan, G. Rajauria, and K. M. Pierce, UCD Lyons Research Farm, Lyons Estate, Celbridge, Nass, Co. Kildare, Ireland.

The dairy industry in Ireland is based on a spring-calving grazing system with the use of concentrate supplementation in the spring and autumn when there is a scarcity of grass. Problems with milk quality are more pronounced in such a system when the majority of the national herd reach late lactation at the same time. Therefore, the objective of this research was to investigate the effect of concentrate supplementation strategy on milk yield and composition and rumen fermentation parameters in late lactation dairy cows. Thirty-six Holsten Friesian dairy cows were blocked on days in milk (+185DIM) and balanced for parity, pre-experimental milk yield and milk composition, predicted 305-day milk yield and BCS. Cows were randomly assigned to one of 3 dietary treatments in a randomized complete block design ($n = 12$). The dietary treatments (T) were grass only (T1); grass + 2.6kg DM barley based concentrate (T2); grass + 2.6kg DM maize based concentrate (T3). The diets were fed for a 14-day acclimatization period and then for a further 63days. Cows offered T1 had a lower milk yield (14.54 kg) than T2 (17.15kg, $P < 0.001$) and T3 (16.73, $P < 0.001$). Similarly, T1 had lower milk solids (kg fat and protein; 1.47 kg) than T2 and T3 (1.51 and 1.48 kg, respectively; $P < 0.001$). The response to concentrates averaged 0.8:1 kg of milk per kg of concentrate (range of 0.5 to 1.2). Milk urea was higher in T1 (0.041%) than both T2 (0.038%, $P < 0.001$) and T3 (0.039%, $P = 0.004$) and rumen ammonia was significantly higher in T1 (5.63 mmol/L, $P = 0.006$) and T3 (5.77 mmol/L, $P < 0.001$) than T2 (5.28 mmol/L). Mean rumen pH of cows offered T2 (6.32) was lower than T1 (6.42; $P = 0.002$) and T3 (6.42, $P = 0.003$). In conclusion, concentrate supplementation increased milk yield and kilograms of milk solids and altered rumen fermentation parameters. Differences between barley and maize based concentrates were seen for rumen pH and ammonia.

Key Words: late-lactation, grazing, supplementation

M246 Reduction of aflatoxin transfer into milk of lactating dairy cows with the addition of a commercial clay. S. C. Allen^{*1}, Z. A. Mason¹, B. J. Rude¹, R. H. Bailey¹, A. Hoang¹, D. L. Sparks¹,

A. B. Johnson¹, and S. H. Ward², ¹Mississippi State University, Mississippi State, MS, ²North Carolina State University, Raleigh, NC.

Twenty-four Holstein cows were used in a randomized complete block design to test the efficacy of clay, MYCOAD, in reducing aflatoxin M₁ (AFM₁) in milk. Cows were blocked by parity and stage of lactation. Cows were housed in a freestall barn with sand bedding and were fed and milked twice daily. Cows were adapted to individual feed gates (Calan Broadbent Feeding System, American Calan, Northwood, NH) one week before the treatment period. The experiment consisted of a 7 d treatment period. Cows were randomly assigned one of 4 dietary treatments (n = 6): (1) control (CON) TMR; (2) aflatoxin (AF) TMR contaminated with 300ppb aflatoxin B₁ (AFB₁); (3) adsorbent diet (MYC) TMR containing 50 g of clay/cow/day; (4) AF diet with clay (AF+MYC) contaminated with 300ppb AFB₁ containing 50 g of clay/cow/day. All additions to TMR were topdressed. Data were analyzed using the MIXED procedure of SAS (Cary, NC). Main effects were treatment, days in milk, parity, and day. All interactions were tested and backward stepwise elimination was used to remove nonsignificant interactions. Significance was declared at $P < 0.05$. Dry matter intake (DMI) was similar across treatments. Cows consuming CON produced less milk compared with cows on other treatments (29.0 v. 36.1 kg/d; $P < 0.01$). Cows consuming CON were less efficient compared with cows on other treatments (0.81 v. 1.71 kg DMI/kg milk). This decrease in efficiency of CON cows was likely due to the decrease in MY. Although milk components (%) were similar across treatments, yields (kg) of protein and solids were less in cows fed CON diets. Protein yield averaged 0.85, 1.12, 1.08, and 1.09 kg/d ($P < 0.02$), and solids yield averaged 2.53, 3.13, 3.06, and 3.24 kg/d for CON, AF, MYC, and MYC+AF respectively. Milk from cows fed AF had the greatest concentration of AFM₁ ($P < 0.001$), and concentrations averaged 0.24, 2.26, 0.15, and 0.83 for CON, AF, MYC, and MYC+AF respectively. Cows fed AF+MYC averaged 1.43 ± 0.30 ppb AFM₁ less than cows fed AF, resulting in a 63% reduction. Results from this study show that adding MYCOAD to contaminated diets was effective at reducing AFM₁ concentrations in the milk of cows fed AFB₁ without negatively affecting DMI, milk yield or feed efficiency.

Key Words: aflatoxin, lactating cow, adsorbent

M247 Revised representation of urea recycling and ruminal nitrogen metabolism for the Molly cow model. M. Li^{*1}, R. R. White², and M. D. Hanigan¹, ¹Department of Dairy Science, Virginia Tech, Blacksburg, VA, ²Department of Animal and Poultry Sciences, Virginia Tech, Blacksburg, VA.

Accurately predicting nitrogen (N) digestion and utilization will allow diet optimization to achieve improved N efficiency. The objectives of this study were to revise the representation of urea recycling in Molly cow model and evaluate the revisions. The work included 1) modification of the existing urinary urea excretion equation to include BW as a scalar; 2) supplement of urea gut entry rate to derive parameters related to urea return to rumen; and 3) reparameterization of equations related to urea N recycling and ruminal N metabolism. Model parameters were changed from initial values to optimized values. Model predictions were compared with a data set from 12 published studies with 54 treatment means before and after the revisions. Mean squared errors were assessed to characterize model performance. Residual analyses demonstrated that the modifications improved the accuracy of predictions of ruminal N digestion, absorption and recycling. After the modifications, prediction errors for duodenal flows of total N, microbial N, non-ammonia N, and non-ammonia non-microbial N were 14.8, 22.4, 17.8 and 28.2%, respectively, which were all approximately 2% units less than observed with the initial model due primarily to the decreased mean bias. Compared

with the initial model, predictions of ruminal ammonia and blood urea concentrations were greatly improved with substantial decreases in mean and slope bias. Prediction errors for gut entry rate were 19.2% with 0.93% mean bias and 1.73% slope bias, which indicated that urea N recycling mechanisms were properly represented in the model. Although the accuracy of urinary urea flow was improved, it still had 81.7% prediction error, which implies high variation of urinary urea N secretion may exist in collected studies, and therefore the reparameterization is not necessarily more accurate even though it fits the observed data. In summary, the model modifications led to a robust representation of urea N recycling and ruminal N metabolism which enabled more accurate and precise predictions of the effects of feeding and management decisions on N efficiency, thus contributing to sustainability of the dairy industry.

Key Words: model, recycling, urea

M248 Value of pulp from green protein extraction of grass clover as forage for dairy cows. V. K. Damborg^{*}, S. K. Jensen, and M. R. Weisbjerg, Department of Animal Science, Aarhus University, Aarhus, Foulum, Denmark.

The increasing demands for animal protein necessitate alternative feed protein sources e.g., protein from green biomass. Pulp of a grass-clover mixture produced by screw-pressing is macerated to release protein, and potentially improve accessibility of fiber and fiber-bound protein. The object of the study was to compare the pulp to grass-clover as forage for dairy cows. Freshly harvested grass-clover was separated into pulp and juice in an industrial scale Vincent twin-screw press (TSP-12). The juice was acidified by lactic acid fermentation precipitating the protein, followed by a decanting into paste, which was spin flash dried into green protein (GP). Pulp and grass-clover from the same field was separately ensiled without additives. The in vitro organic matter (OM) digestibility was 70.7 and 67.6% (n = 4) and crude protein (CP) concentrations were 16.8 and 13.6% of dry matter (DM), for pulp (P) and grass-clover (GC) silage, respectively. GP had a CP concentration of 33.5%DM. A production trial including 12 primiparous and 24 multiparous lactating cows was designed to compare P and GC, and to compare protein deficient rations (~14% CP of DM) to GP or to soybean meal (S) supplemented rations (~16% CP of DM). The trial design was an incomplete Latin square (4x6) consisting of 4 3-week periods and 6 treatments (GC, P, GC+GP, P+GP, GC+S, P+S) where rations without protein supplement (CG and P) were designed to be protein deficient. The forage:concentrate ratio was 55:45 for protein deficient TMRs and 50:50 for supplemented. The P or GC silage composed 68%DM of the forage. The DM intake (DMI) of P silage TMRs was 23.1 (± 0.3) kg/d, which was higher than GC silage TMRs, 22.6 (± 0.3) kg/d ($P < 0.001$). Milk yield for P silage TMRs was 37.4 (± 0.9) kg/d, and lower for GC silage, 34.6 (± 0.9) kg/d ($P < 0.001$). The results indicate that pulp from protein extraction of green biomass was a valuable forage for dairy cows compared with grass clover, even though part of the digestible soluble matter had been removed.

Key Words: protein extraction, pulp, forage

M249 Ruminal degradability and intestinal digestibility of dry matter and crude protein of wheat and corn dry distillers grains with or without solubles. K. Nedelkov^{*1}, N. Todorov¹, M. T. Harper², D. Girginov¹, and M. Simeonov³, ¹Trakia University, Stara Zagora, Bulgaria, ²The Pennsylvania State University, University Park, PA, ³Agricultural Institute, Stara Zagora, Bulgaria.

The objective of this study was to compare rumen degradation kinetics and intestinal digestibility of wheat and corn dry distillers grains with solubles (DDGS) or without solubles (DDG). Three nonlactating Jersey cows with an average body weight of 436 ± 18 kg fitted with a rumen and T-type duodenal cannulas were used in the experiment. Six DDG products (3 from wheat, DDGSw1, DDGSw2, DDGSw3 and 3 from corn, DDGSc1, DDGSc2 and DDGc4) were collected from 4 ethanol plants. Feed samples were incubated in the rumen of the cows for 0, 2, 4, 8, 16, 24 and 48 h in 6 replications. The effective degradability of DM was higher ($P < 0.05$) for both wheat and corn DDGS products from ethanol plants 1 and 2 compared with DDGSw from plant 3 and DDGc from plant 4. The soluble fraction of CP ranged from 23.9% for DDGSw2 to 12.4% for DDGc4 with higher ($P < 0.05$) values for wheat DDGS than those from corn. Effective degradability of CP at different outflow rates ($k_p = 0.045, 0.06, \text{ and } 0.08$) for DDGSw1 and DDGSw2 were higher ($P < 0.05$) compared with those for corn DDGS. Correlation between color parameters (L – lightness, a- yellowness and b - redness) of the wheat DDGS and their CP effective degradability was high ($r = 0.82 \text{ to } 0.95$). Further research is needed to evaluate the possibility of using these easily estimated color parameters as a proxy of the protein nutritional value of DDGS. The intestinal digestibility of rumen degradable DM, measured by the mobile bag technique, ranged from 52.0% for DDGSw3 to 38.3% for DDGc4 and was higher ($P \leq 0.05$) for wheat DDGS compared with DDGSc1 and DDGc4. Intestinal digestibility of CP was not different ($P > 0.05$) among corn DDGS. Intestinal digestibility of CP from wheat DDGS was also highly variable (79.7% to 93.6%). Although nutrient composition of DDGS from different ethanol plants is highly variable, the protein degradability and digestibility values obtained in this experiment can be used in formulating rations for ruminant animals.

Key Words: dry distillers grains, intestinal digestibility, rumen degradability

M250 Effects of combinations of prilled fatty acids with or without potassium carbonate on fermentation and biohydrogenation intermediates in continuous culture fermenters. L. E. Koch^{*1}, B. M. Koch¹, S. M. Hussein¹, V. R. Trutwin¹, T. C. Jenkins¹, C. Soderholm², J. Linn², J. Albrecht², and G. J. Lascano¹, ¹*Clemson University, Clemson, SC*, ²*Milk Specialties Global, Eden Prairie, MN*.

The addition of buffers such as K_2CO_3 have been investigated in how they alter ruminal fermentation and reduce accumulation of milk fat inhibitors (MFI). Thus, we hypothesized that prilled saturated free fatty acids (FFA; C16:0 and C18:0) combined with K_2CO_3 would provide a slower, more prolonged release of K_2CO_3 than feeding it alone in

reducing production of MFI. Four treatments were randomly assigned to 8 continuous culture fermenters for 2 periods of 10 d. Treatments included 4 combinations of FFA (Supplement A) and K_2CO_3 coated with prilled fatty acids (1:1; Supplement B) representing 1.25:0 (1), 0.83:0.83 (2), 0.42:1.66 (3), and 0:2.5% DM (4) of supplement A to B ratios. All treatments provided 1.25% DM of FA with K_2CO_3 increasing gradually from 0 to 1.25% DM. Data were analyzed using the MIXED procedure of SAS as a randomized complete block design with blocks of period and fermenters; where linear, quadratic and preplanned polynomial contrasts were evaluated. Addition of K_2CO_3 altered pH and biohydrogenation (Table 1). There was a quadratic increase in total VFA ($P < 0.01$) but butyrate was reduced linearly ($P < 0.05$), while valerate and isoacids increased quadratically with increasing K_2CO_3 . Adding K_2CO_3 tended to increase the outflow of C18:1 ($P = 0.09$), C18:2 ($P = 0.06$) and reduce C18:3 ($P = 0.06$) linearly. Biohydrogenation intermediates, trans-10 and trans-12 18:1, were quadratically reduced with K_2CO_3 . These results indicate that combinations of prilled fatty acids and K carbonate increase pH and reduce production of biohydrogenation intermediates linked to milk fat depression exhibiting a quadratic response.

Key Words: lipid, biohydrogenation, continuous culture

M251 Undigested NDF (uNDF240h) excretion in feces of lactating cows: A comparison of multiple time-points, diurnal and 24-hour composite collections. E. Bonfante^{*}, D. Cavallini, A. Palmonari, M. Fustini, L. Mammi, G. Canestrari, and A. Formigoni, *DIMEVET, Dipartimento di Scienze Mediche Veterinarie, Ozzano dell'Emilia, BO, Italy*.

The aim of the study was to evaluate the pattern of undigested NDF (uNDF240h, %DM) excretion in feces and to compare the singular time points with a 24h composite. Four dairy cows, paired for lactation number (2), days in milk (184 ± 38 d), milk production (35 ± 2 kg), weight (664 ± 50 kg), and daily rumination time (564 ± 50 min), were housed in tie-stalls for individual dry matter intake (DMI) determination. The feed was delivered once a day (0800h). After an adaptation period the feces collection started. The feces were collected every 2h (12 time points in total) for 3d; all samples were divided in 3 aliquots: one was kept separate, one was composited to create a 4 time points sample (c08–14h, c16–22, c00–06), representative for the morning, afternoon, and night excretion; the latter aliquot was used to create a composite of the whole day (c24h). During the study, the DMI, rumination time, milk production, were recorded daily, and the total mixed ration (TMR) sampled every day. The chemical composition of feeds and feces were determined. The data were analyzed using JMP-12 (SAS Institute Inc., Cary NC). A mixed model was used; the time points and the day were

Table 1 (abstract M250). Biohydrogenation and pH profile of fermenters fed 4 combinations of supplements A and B

Item	TRT				SE	Contrasts, <i>P</i> -value	
	1	2	3	4		L	Q
pH	6.06	6.26	6.32	6.22	0.06	0.07	0.03
pH, h to 6.0	2.00	2.75	3.75	2.25	0.29	0.21	<0.01
pH, h < 6.0	7.00	4.50	3.00	5.50	0.68	0.08	<0.01
min pH	5.57	5.63	5.69	5.59	0.10	0.69	0.19
max pH	6.83	6.84	6.95	6.85	0.17	0.65	0.49
Biohydrogenation							
18:2	58.00	55.47	43.91	52.45	7.77	0.06	0.09
18:3	68.77	66.14	56.65	64.20	6.30	0.05	0.06

considered as fixed effect, the cow as random. The TMR during the experimental period was uniform in its physical and chemical composition. The DMI was 24.5 ± 2.9 kg, the rumination time 452 ± 87 min, the milk production 34.0 ± 4.0 L. The mean uNDF240h excretion during the day was $32.4 \pm 1.0\%$ DM; the higher excretion was at 18h ($33.9 \pm 0.9\%$ DM) and the lower at 8h (feed delivery; 30.9 ± 0.9). Some significant differences were detected comparing the 12 time points, most of all between the morning (10 and 12h) and the night (18, 20, 22, 2, 4h; $P < 0.05$) samples. No difference were detected comparing the 12 time points with the c08–14h, c16–22, c00–06, and c24h, except for the c24h and 18h (32.1 vs 33.9% DM; $P = 0.048$). We can conclude that the uNDF240h excretion isn't linear, with a min reached at feed delivery and a max reached 10h after. However, except for the difference between max excretion time point and c24h, the multiple time points are not different from the composites.

Key Words: feces collection, undigested NDF (uNDF240h)

M252 miRNA regulation of the neutrophil transcriptome in response to prepartal energy intake in Holstein cows: an in silico approach. M. Vailati Riboni^{*1}, V. Palombo², A. Agrawal¹, M. J. Khan¹, and J. J. Loor¹, ¹Urbana, IL 61801, Urbana, IL, ²Università degli Studi del Molise, Campobasso, Italy.

Overfeeding energy prepartum leads to a chronic and potentially detrimental activation of PMNL during the adaptation to lactation. Using microarray transcriptome data, the present study aimed to examine the role of microRNA (miRNA) in the observed expression profiles in response to prepartal energy intake. Sixteen Holstein cows were fed a high-straw, control diet (NEL = 1.34 Mcal/kg) or overfed a moderate-energy diet (NEL = 1.62 Mcal/kg) during the dry period. PMNL were isolated from blood at -14 and +7 d relative to parturition and isolated RNA was hybridized to the Agilent 44K Bovine (V2) Microarray chip. Data were adjusted for dye and array effects and a MIXED model with repeated measures was then fitted to the normalized \log_2 -transformed adjusted ratios. A list of miRNA families and their predicted target genes for *Bos taurus* were downloaded from the Microcosm targets website (v. 5.0). Results for the main effect of diet were then used to predict miRNA activity from the mRNA expression profiles through 3 approaches: Wilcoxon rank test, ranked ratio, and mean absolute expression. The dynamic impact approach was then used for pathway analysis on the compiled differentially expressed target genes (false discovery rate < 0.05) of the predicted miRNA. Overlapping the results of the 3 approaches, 10 miRNA were predicted to be involved in the observed transcriptome changes: miR-let7a, miR-26a, miR-101, miR-126, miR-191, miR-200a, miR-369, miR-374, miR-450, and miR-545. Pathway analysis revealed an overall impact on amino acid, cofactor, and vitamin metabolism, with a particular involvement in the one-carbon metabolism (e.g., methionine, and folate cycle). 'Translation', 'folding, sorting, and degradation', 'DNA repair' and 'cell growth and death' were among the most-impacted non-metabolic category, together with immune processes such as the 'NOD-like receptor' and 'Fc epsilon RI' signaling pathways, and 'Fc gamma R-mediated phagocytosis'. Overall, data support the role of miRNA in the PMNL response to prepartum overfeeding, particularly in non-metabolic processes.

Key Words: miRNA, PMNL, transition cow

M253 Effects of a rumen-protected lysine product as a source of metabolizable lysine for high-producing dairy compared with porcine blood meal. S. Polukis^{*1}, A. Barnard¹, T. Gressley¹, N.

Lobos², K. Griswold², and L. Kung Jr.¹, ¹University of Delaware, Newark, DE, ²Kemin Industries, Inc., Des Moines, IA.

Research indicates that lactating dairy cows have a requirement for metabolizable lysine (MP Lys). Porcine blood meal (PBM) is a common, but highly inconsistent source of MP Lys in dairy diets. The study objective was to determine the efficacy of a rumen-protected lysine product (USA Lysine, Kemin Industries, Inc., Des Moines, IA) as a source of MP Lys in diets fed to early lactation Holstein cows compared with PBM. Thirty cows (8 primiparous and 22 multiparous; average 121, range 45–175 DIM) were utilized in a trial consisting of a 2-wk covariate period, a 4-wk treatment period (period 1), a 1-wk washout period and a 4-wk treatment period (period 2). At the end of the covariate, cows were blocked by parity, DIM and milk yield, then randomly assigned to 1 of 2 treatment sequences ($n = 15$) according to a crossover design. There were 2 diets: 1) Control (CON) - PBM as the main source of MP Lys; and 2) USA Lysine (USA-L) - USA Lysine with dried distillers grains w/ solubles (DDGS) replacing most the PBM. Cows were individually fed $1 \times /d$ and milked $2 \times /d$. Milk yield and dry matter intake (DMI) were recorded daily and milk components were determined weekly. Data were analyzed with the Mixed procedure of SAS (SAS Institute, Inc. Cary, NC). The model included the fixed effects of treatment, period, week in period (as a repeated measure), sequence, parity, treatment by week in period, treatment by parity and the random effect of cow nested within sequence. Significance was declared at $P < 0.05$ and tendencies at $0.05 < P < 0.10$. There were no differences ($P > 0.25$) between the CON and USA-L treatment groups in DMI (25.90 vs. 25.63 kg/h/d) or milk yield (41.71 vs. 41.19 kg/h/d), respectively. Milk components and component yields, as well as feed efficiency, were nearly identical across treatments. Nitrogen use efficiency in terms of milk urea nitrogen (MUN) was improved with the USA-L diet (9.98 vs. 10.74 mg/dL, $P < 0.01$) compared with the CON diet. In conclusion, USA Lysine in combination with DDGS effectively replaced PBM as the main source of MP Lys in a diet formulated for high-producing Holstein dairy cows.

Key Words: lysine, blood meal, milk yield

M254 In silico prediction of miRNA activity in the hepatic response to prepartum body condition score and plane of nutrition during the transition period in grazing dairy cows. M. Vailati Riboni^{*1}, V. Palombo², M. D. Mitchell³, M. A. Crookenden⁴, A. Heiser⁵, S. L. Rodriguez-Zas¹, J. R. Roche⁴, and J. J. Loor¹, ¹University of Illinois at Urbana-Champaign, Urbana, IL, ²Università degli Studi del Molise, Campobasso, Italy, ³University of Queensland, Herston, Queensland, Australia, ⁴DairyNZ Limited, Hamilton, New Zealand, ⁵AgResearch, Palmerston North, New Zealand.

An in silico approach was applied to investigate the possible role of microRNAs (miRNA) in the liver transcriptome response to prepartum body condition score (BCS) and feeding management in the weeks before calving. Thirty-two mid-lactation grazing dairy cows of mixed age and breed were randomly allocated to 1 of 4 treatment groups in a 2×2 factorial design: 2 prepartum BCS categories [4.0 (thin) and 5.0 (optimal); 10-point scale], and 2 levels of energy intake during the 3 wk preceding calving (75% and 125% of estimated requirements). Liver tissue was harvested by biopsy at -7 and 7 d relative to calving and subsequent RNA was hybridized to the Agilent 44K Bovine V2 Microarray chip. A MIXED model with repeated measures was fitted to the normalized \log_2 -transformed adjusted ratios using Proc MIXED. The transcriptome profile for the comparisons BCS 4 125vs75 and BCS 5 125vs75 was used to predict miRNA activity through 3 approaches: Wilcoxon rank test, ranked ratio, and mean absolute expression (results were overlapped to find common predicted miRNA). The analysis was

performed on a list of miRNA families and their predicted target genes for *B. taurus* downloaded from the Microcosm targets website (v. 5.0). The dynamic impact approach was used for pathway analysis on the target differentially expressed genes (fold change < -1.5 or >1.5, $P < 0.05$) of the predicted miRNA at each time point. For BCS 4 there were 9 and 7 miRNA (pre- and postpartum, respectively) predicted to be involved in the response to prepartum nutrition. Their activity was related to cell proliferation and immune signaling. Postpartum, they also involved pyruvate, nitrogen, glutathione, and glycan metabolism. For BCS 5 only 2 (pre-) and 1 (postpartum) miRNA were common to the 3 approaches, with ubiquitin-mediated proteolysis affected prepartum, and mineral absorption, bile secretion, and tryptophan metabolism potentially regulated by miRNA postpartum. Overall, miRNA seem to be involved in the response to prepartum BCS and nutrition, particularly in thinner cows.

Key Words: BCS, prepartum nutrition, liver miRNA

M255 Pre-weaning and post-weaning performance in dairy calves fed an active dry yeast (*Saccharomyces cerevisiae* CNCM I-1077). A. Faulkner², A. Clay³, L. Waldron², A. Aguilar^{*1}, E. Chevau¹, and A. Turney¹, ¹Lallemand Animal Nutrition, Milwaukee, WI, ²Vitech/Lallemand, Auckland, NZ, ³Nutritech/Lallemand, Auckland, NZ.

A trial was run to examine the influence of feeding a rumen specific live yeast, *Saccharomyces cerevisiae* CNCM I-1077 (SC), on feed intakes and body weights of calves at weaning. Sixty male, Friesian cross calves, aged 4 d old, were randomly allocated to either a control (unsupplemented) creep feed diet or one containing SC at 4×10^9 cfu/kg. All calves were fed a commercial milk replacer (CMR) and offered straw and creep feed ad libitum from entry, and intakes and weight gains were recorded. At 6 weeks of age, the calves were transferred to grazing with ad libitum access to creep feed and monitored for a further 2 weeks, covering the transition period from milk to grazing. The calves fed SC had higher feed intakes at weaning (1.105 kg/yr versus 1.523 kg/d at 6 wk of age; $P = 0.0434$ and 1.179 kg/d versus 1.965 kg/d at 8 wk of age; $P = 0.0272$ for control and SC respectively). Correspondingly, average daily gain (ADG) was improved. At 7 wk of age, ADG was 0.659 kg versus 0.912 kg ($P = 0.039$), and at 8 wk of age ADG was 0.457 kg versus 0.707 kg ($P = 0.0650$) for control and SC respectively. It was concluded that supplying SC via creep feed prevented the drop off in calf performance at weaning, in terms of both feed intake and weight gain. This is important, as early rumen development and pre-weaning growth rates are related to a reduction in gastric upsets at weaning, future growth performance in calves and future lactation performance.

Key Words: calf, live yeast, performance

M256 Evaluation of sample preparation methods for the determination of fecal pH in dairy cows. E. H. Branstad, C. S. Shouse*, B. C. Dooley, A. D. Thomas, L. H. Baumgard, and H. A. Ramirez-Ramirez, Iowa State University, Ames, IA.

A standard procedure for measurement of fecal pH in dairy cows does not currently exist. Consequently, sample preparation may influence the precision of this measurement; thus, limiting comparisons across literature reports. The objectives of this study were to determine if differences exist based on preparation method, and to determine variation across methods. Thirty fresh fecal samples were collected from lactating Holstein cows housed in the same pen and consuming the same diet. Five samples were collected at a time and prepared according to the

following methods: (1) direct measurement (DIR) in which the pH probe was directly inserted into the fecal sample; (2) strained fecal fluid (STR) obtained by squeezing the fecal sample through 4 layers of cheesecloth. Three dilution rates (distilled water:feces) were also tested: (3) 0.5:1 dilution (D1), (4) 1:1 dilution (D2), and (5) 2:1 dilution (D3). Each sample was prepared using all methods, resulting in a total of 150 pH measurements. The UNIVARIATE and GLM procedures of SAS were used to test normality and homogeneity of variance, respectively. The Shapiro-Wilk test confirmed that data were normally distributed ($P = 0.08$). The Levene's test showed heterogeneity of variance ($P = 0.02$), thus the SATTERTHWAITTE approximation of degrees of freedom for denominator was used for the ANOVA via the GLIMMIX procedure. Sample preparation method affected ($P < 0.01$) pH values, resulting in D3 having the highest pH of 6.91 ± 0.04 , followed by D2 with a value of 6.79 ± 0.04 . Measurements of pH by D1 and DIR were similar, and averaged 6.67 ± 0.04 ($P = 0.17$); whereas, STR had the lowest value of 6.60 ± 0.04 . Descriptive statistics showed the standard deviation for the STR method was 0.173 and 0.174 for D2, while that of D1, D3 and DIR was 0.224, 0.226 and 0.296, respectively. These results demonstrate that pH measurements in strained fecal fluid or a 1:1 dilution rate have reduced variability when compared with direct measurements and other dilution rates.

Key Words: hindgut acidosis, gastrointestinal health, colonic fermentation

M257 Bioavailability of AjiPro-L 2G and AjiPro-L 3G using the plasma free lysine dose-response technique. N. Whitehouse^{*1}, A. Brito¹, C. Schwab^{1,2}, I. Shinzato³, and M. Miura⁴, ¹University of New Hampshire, Durham, NH, ²Schwab Consulting LLC, Boscobel, WI, ³Ajinomoto Heartland Inc., Chicago, IL, ⁴Ajinomoto Co. Inc., Tokyo, Japan.

The objective was to compare the bioavailability of 2 rumen-protected supplements (AjiPro-L 2G vs. AjiPro-L 3G) using the plasma free Lys dose-response technique. Seven lactating multiparous Holstein cows (202 \pm 49 DIM) equipped with ruminal cannulas were used in a 7×7 Latin square with 7-d periods. The treatments were (1) 0 g/d Lys, (2) 30 g/d of abomasally infused Lys, (3) 60 g/d of abomasally infused Lys, (4) 30 g/d fed Lys from AjiPro-L 2G, (5) 60 g/d fed Lys from AjiPro-L 2G, (6) 30 g/d fed Lys from AjiPro-L 3G, and (7) 60 g/d fed Lys from AjiPro-L 3G. The infusion treatments consisted of Lys-HCl and were infused continuously into the abomasum via the ruminal cannulas. To ensure complete consumption, the AjiPro-L2G and AjiPro-L 3G were mixed with 1.5 kg of TMR, stored at 4°C for 8 h before feeding and placed in tubs in front of the cows 30 min before each of the 3 daily feedings. Blood samples were obtained from each cow on the last 3 d of each period every 2 h, 4 times daily, from the tail vein, centrifuged, deproteinized, composited into 1 daily sample/cow, and analyzed for AA. Data for plasma AA concentrations (μ M basis) were analyzed using the PROC MIXED and PROC REG procedures of SAS. Data from 2 cows was removed from the data set due to very low or negative response to all treatments. The slope for the AjiPro-L 3G (i.e., 0.01407; $P < 0.001$) was greater than the slope for the AjiPro-L 2G (i.e., 0.01257; $P < 0.04$) resulting in a 12% improvement in bioavailability of Lys from the AjiPro-L 3G based on the ratio of the 2 slopes. Although the bioavailabilities of AjiPro-L 2G and AjiPro-L3G were not significantly different, the 12% numerical increase in bioavailability with feeding AjiPro-L3G results in 1.1 g/d more absorbable Lys when 60 g/d of product are fed.

Key Words: AjiPro, bioavailability, plasma lysine

M258 In vitro investigation of supplementing microalgal protein precipitate material as a source of dietary protein in a dairy diet using continuous cultures. S. Y. Yang¹, J. M. Yang¹, J. Marriott², J.-S. Eun*¹, R. C. Sims², and R. C. Anderson³, ¹*Department of Animal, Dairy, and Veterinary Sciences, Utah State University, Logan, UT*, ²*Department of Biological Engineering, Utah State University, Logan, UT*, ³*USDA-ARS, Southern Plains Agricultural Research Center, Food and Feed Safety Research Unit, College Station, TX*.

The fat-extracted microalgal biomass derived from the biofuel production has been suggested as a promising source of protein supplement in ruminant diets. Consequently, we performed an in vitro experiment to investigate the effects of supplementing microalgal protein precipitate material (APP) obtained via wet lipid extraction procedure. The APP was attained using dairy waste from Caine Dairy Research Center (Wellsville, UT) at Utah State University. The APP used in this study had low bacterial counts on blood agar, approximately 1.8×10^2 cfu/g. In addition, in a 24-h incubation assay with a suspension of bovine fecal contents, we observed neither an inhibitory nor a stimulatory effect of the APP on growth or survivability of *Salmonella enterica* serovar Typhimurium ($8.65 \pm 0.18 \log_{10}$ cfu/mL) or *Escherichia coli* O157:H7 ($8.91 \pm 0.11 \log_{10}$ cfu/mL). The APP contained 30.6% crude protein and 6.60% ether extract on a dry matter (DM) basis. The experiment was performed as a completely randomized design to test 4 dietary treatments in a typical dairy diet (n = 4): 1) no APP (control), 2) 1.2% APP, 3) 2.4% APP, and 3.6% APP in a DM basis. Although increasing APP in the diet tended to linearly decrease culture pH ($P = 0.08$), the culture pH was maintained at least 6.0 throughout culture incubation. Total VFA concentration was similar across dietary treatments. Molar proportion of acetate linearly increased by increasing APP inclusion ($P = 0.03$), while propionate portion did not change, resulting in no effect on acetate-to-propionate ratio. Increasing addition of APP linearly decreased molar proportion of isovalerate ($P < 0.01$). Concentration of ammonia-N averaged 7.32 mg/100 mL, and it was not influenced due to dietary treatments ($P = 0.27$). Additionally, APP supplementation did not affect methane production in the culture regardless of dietary concentration. Overall data in this in vitro study suggest that APP attained from dairy waste did not have any negative impacts when added up to 3.6% DM on ruminal fermentation profiles and show a potential to be a sustainable source of dietary protein in dairy diets.

Key Words: dairy waste, microalgal protein precipitate material, ruminal fermentation

M259 Is a pelleted feed required in an automated milking system (AMS)? K. S. Paddick*, S. B. Menajovsky, and G. B. Penner, *University of Saskatchewan, Saskatoon, SK, Canada*.

It is recommended that a pelleted concentrate is offered to cows milked in an AMS. The previous recommendation is designed to ensure cattle can consume concentrate rapidly to enable precision feeding strategies; however, it is not clear if pelleted feeds are necessary when low quantities of concentrate are offered. The objective of this study was to evaluate the effect of providing barley grain that was either steam-rolled (SR) or pelleted (PEL) on DMI and milk and milk component yield. Five Holstein cows (98 ± 7.8 DIM) housed in a feed-first guided-traffic flow barn were used in a crossover design with 24-d periods. Cows were fed a common partial mixed ration (PMR) containing a 55:45 forage-to-concentrate ratio and were offered sufficient concentrate in the AMS to achieve either 2.5 kg/d of SR or PEL (DM basis). Milking permissions were granted when predicted milk yield exceeded 9 kg or when the

interval from the last milking exceeded 4 h. Dry matter intake (PMR and AMS concentrate), voluntary visits to the AMS, and milk and milk component yield were measured. The form of concentrate offered in the AMS had no effect on total DMI or PMR intake with average values of 29.8 and 27.3 kg/d, respectively ($P \geq 0.79$). Interestingly, cows fed PEL and SR did not differ for concentrate intake in the AMS averaging 2.48 kg/d ($P = 0.16$). The type of concentrate provided in the AMS did not affect variability for AMS or PMR concentrate intake among days ($P \geq 0.59$). Voluntary milking frequency was not affected by form of concentrate offered in the AMS with an average of 3.51 visits/d. Milk yield (41.5 kg/d), and the yield of CP (1.43 kg/d) and fat (1.60 kg/d) did not differ among treatments ($P \geq 0.27$). However, milk fat concentration was reduced for cows fed PEL compared with SR (3.82 vs. 3.92%; $P = 0.03$). Milk urea nitrogen tended to be reduced for cows fed PEL compared with SR (13.8 vs. 15.6 mg/dL; $P = 0.10$). These data indicate that, with a low quantity of concentrate allocated in the AMS, it may be possible to feed steam-rolled barley grain without negatively affecting voluntary visits to the AMS and milk component yield.

Key Words: automatic milking systems, dairy nutrition

M260 Lipidomics reveals phosphatidylcholines as candidate biomarkers for metabolic disease. S. S. Samii*¹, Y. Zang¹, E. Grilli², and J. W. McFadden¹, ¹*West Virginia University, Morgantown, WV*, ²*University of Bologna, Bologna, Italy*.

The discovery of novel biomarkers for metabolic disease can refine nutritional interventions aimed at improving dairy cow health and performance. Therefore, our objective was to identify metabolites associated with common markers of metabolic disease. Thirty multiparous Holstein cows were enrolled -28 d prepartum and fed diets formulated to meet or exceed requirements. Blood and liver samples were routinely collected from enrollment through d 14 postpartum (pp). To characterize the plasma lipidome spanning 9 time points, untargeted lipidomics was performed using quadrupole time-of-flight mass spectrometry. Univariate and multivariate analyses of normalized, auto-scaled lipidomic data were performed. Based on pp metabolic health data, cows were separately categorized into low or high free fatty acid area under the curve (FFA_{AUC}; d 1 - 14 pp; $4,915 \pm 1,369$ vs. $12,501 \pm 2,761$ [$\mu\text{mol/L} \times 14$ d]; n = 18), β -hydroxybutyrate area under the curve (BHB_{AUC}; d 1 - 14 pp; $4,583 \pm 459$ vs. $7,901 \pm 1,206$ [$\mu\text{mol/L} \times 14$ d]; n = 18), or mean pp liver lipid content (d 5 and 14 pp; 5 ± 1 vs. $12 \pm 2\%$ of wet weight; n = 18). Significant variables associated with a specific category were identified based on leverage/squared prediction error plots. Lipidomics revealed 301 plasma lipids including 8 cholesterol esters, 163 phospholipids, and 130 acylglycerols. Independent of category, all cows displayed dramatic decreases in plasma triacylglycerols and monoalkyl-diacylglycerols ($P < 0.01$), and the majority of phospholipids reached a nadir at parturition ($P < 0.01$). Analyses revealed that phosphatidylcholine (PC) 32:3, 35:5, 37:5 were specific for high FFA_{AUC}, PC 31:3, 32:3, 35:5, and 37:5 were specific for high BHB_{AUC}, and PC 31:2, 31:3, and 32:3 were specific for high liver lipid. Notably, PC 32:3 was specific for high FFA_{AUC}, BHB_{AUC}, and liver lipid cows, a metabolite that was lower in abundance relative to the low categories ($P < 0.01$). Other lipids specific for 2 or more categories included phosphatidylglycerol 38:4 and lysophosphatidylcholine 15:0. Nutritional interventions that increase plasma PC 32:3 during the peripartum should be explored.

Key Words: biomarker, lipidomics, metabolic disease

M261 Performance of crossbred Holstein × Zebu cows supplemented with fibrolytic enzyme in diets with different forage levels. A. M. Teixeira¹, L. C. Gonçalves², L. F. Martins¹, A. P. D'Abadia Netto¹, B. O. Silva¹, G. C. Oliveira¹, T. T. Santos³, N. Ferreira Junior³, N. D. Walker⁴, and T. L. Resende⁵, ¹Universidade Federal de Uberlândia, Uberlândia, MG Brazil, ²Universidade Federal de Minas Gerais, Belo Horizonte, MG Brazil, ³AB Vista Brazil, São Paulo, SP Brazil, ⁴AB Vista, Marlborough, Wiltshire, UK, ⁵Auster Nutrição Animal Ltda, Hortolândia, SP Brazil.

The aim of the trial was to evaluate the performance and concentration of ketone bodies in the blood of early lactation cows supplemented with fibrolytic enzymes (750 mL/ T DM; Xylanase 350,000 BXU/g, Cellulase10,000ECU/g, AB Enzymes, Finland) in diets with different forage levels. Twenty Holstein × Zebu cows (46 ± 31 d in milk), blocked according to yield, DIM and parity, were individually fed for 132 d divided into 6 periods of 8 d adaptation and 14 d measurements in a 2 × 2 factorial randomized complete block design. The treatments consisted of 4 groups: (1) low forage (53.8%) without enzyme (LF); (2) low forage with enzyme (LFE); (3) high forage (62.3%) without enzyme (HF); (4) high forage with enzyme (HFE). The LF (33.07 kg/d) and LFE (31.72 kg/d) groups had higher milk yield than the HF group (27.01 kg/d), with the HFE group being intermediate (30.42 kg/d) ($P = 0.02$). 4% FCM ($P = 0.022$) and ECM ($P = 0.044$) showed the same behavior. There was a tendency for LF (1.27 kg/d) and LFE (1.23 kg/d) to present a higher fat yield than the HF group (1.08 kg/d), with the HFE group being intermediate (1.18 kg/d) ($P = 0.068$). LF had a tendency ($P = 0.058$) to present higher solids yield in relation to the HF and HFE groups. The LFE and LF groups had higher lactose content than the HF group, with the HFE group being intermediate ($P = 0.001$). Cows fed the LF diet had higher ($P < 0.001$) protein content (3.30 vs 3.14%) and higher ($P = 0.001$) lactose yield in relation to the cows that received the HF diet, regardless of enzyme addition. The protein yield of the LF group was similar to the LFE group and higher than the others ($P = 0.005$). The HFE group presented higher urea nitrogen content ($P = 0.001$) and tendency of higher blood ketone concentration ($P = 0.059$) compared with the others. To conclude, addition of enzyme improved the performance of cows fed diets containing a higher proportion of forage, approaching a similar level of performance as animals receiving a lower proportion of forage and higher proportion of concentrate.

Key Words: digestibility, enzyme, fiber

M262 Kinetics of *trans*-10,*cis*-12 and *cis*-9,*trans*-11 conjugated linoleic acid (CLA) transfer to plasma and milk following an abomasal bolus in lactating dairy cows. N. L. Urrutia^{1,2}, R. Bomberger¹, M. Baldin¹, M. Toledo¹, and K. J. Harvatine^{*1}, ¹The Pennsylvania State University, University Park, PA, ²Instituto Nacional de Investigaciones Agropecuarias-Remehue, Osorno, X Region de Los Lagos, Chile.

Dietary fatty acids (FA) are directly transferred to milk through chylomicrons and indirectly through tissue recycling. The objective of this study was to characterize the direct and indirect transfer rates of the *cis*-9,*trans*-11 (c9t11) and *trans*-10,*cis*-12 (t10c12) CLA through plasma to milk following a single abomasally infused bolus. Five ruminally cannulated multiparous mid-lactation cows (148 ± 86 DIM; Milk 44.1 ± 11.2 kg/d) received a single abomasal bolus infusion of an enriched CLA mixture providing 15 g of each CLA isomer (c9t11, t10c12) over a 30 min period. Total transfer of CLA was analyzed in a model that included cow as a random effect and CLA isomer as a fixed effect (JMP Pro). Time course data were analyzed as repeated measures in SAS and least squares means were fit to a double exponential decay function by

nonlinear curve fitting (JMP Pro) to characterize direct (fast pool) and indirect (slow pool) transfer of CLA isomers to milk. Plasma CLA concentration peaked at 2 h, reaching 0.32 and 0.31% of plasma FA for c9t11 and t10c12, respectively, and returned to baseline at 72 h. Milk t10c12 concentration peaked at 14 h (0.5% of FA) and returned to baseline at 86 h post infusion. Milk c9t11 concentration initially peaked at 14 h (0.98% of FA), returned to baseline at 86 h post infusion, and then had a second peak between 146 to 158 h (0.56% of FA) post infusion. Total transfer of CLA to milk differed between isomers and was 79.3 and 40.8% of the bolus for c9t11 and t10c12, respectively ($P < 0.001$). Time course of CLA isomers transferred to milk fit a biexponential model ($R^2 = 0.99$). The area (% of total) under the first exponential representing direct transfer was 17 and 73% and the second exponential representing indirect transfer was 83 and 27% of the total CLA isomers transferred for c9t11 and t10c12, respectively. In conclusion, although plasma kinetics of c9t11 and t10c12 were similar, transfer of CLA isomers to milk differed greatly in their transfer efficiency and major pool of transfer.

Key Words: conjugated linoleic acid, plasma, milk

M263 Palmitic acid feeding increases plasma ceramide concentrations in Holstein dairy cows during early lactation. A. N. Davis¹, Z. C. Phipps^{*1}, Q. Zeng¹, J. de Souza², J. E. Rico¹, A. L. Lock², and J. W. McFadden¹, ¹West Virginia University, Morgantown, WV, ²Michigan State University, East Lansing, MI.

Reduced insulin action is an adaptation that develops during early lactation to enhance lipolysis and promote glucose partitioning for the mammary synthesis of milk. The onset of lactation is accompanied by the accumulation of ceramides, antagonists of insulin signaling, which have been reported to be enhanced by palmitic acid (C16:0) feeding in monogastrics. To determine whether C16:0 feeding increases plasma ceramides during early lactation, 37 multiparous cows were assigned to 1 of 3 treatments: CON-CON (control diet with no supplemental fat; 1–67 DIM); CON-PA (control diet fed from 1 to 24 DIM, and a C16:0-supplemented diet fed from 25 to 67 DIM); and PA-PA (C16:0 supplemented diet; 1–67 DIM). Diets were formulated to contain a minimum of 17% CP, 29% NDF, and 24% starch. The C16:0 supplement (85% C16:0), added at 1.5% of diet DM, replaced soyhulls in the CON diets. Blood was collected at 11, 32, and 60 DIM. Plasma ceramide and monohexosylceramide (GlcCer) concentrations were determined using mass spectrometry. Data were analyzed as repeated measures using a mixed model, and Pearson correlations were analyzed. Circulating ceramides increased with the progression of lactation ($P < 0.01$), albeit the magnitude was greater with PA feeding (day by treatment interaction; $P < 0.05$). PA increased total ceramide levels by 39% ($P < 0.01$). The addition of C16:0 in CON-PA increased total ceramides by 60% at d 32, relative to d 11 ($P < 0.01$). Relative to CON-CON, PA-PA increased C24:0-ceramide levels by 26, 48, and 58% at d 11, 32, and 60, respectively ($P < 0.05$). The ratio of C24:0- to C16:0-ceramide decreased over time and was elevated in PA-PA ($P < 0.05$). The majority of GlcCer increased over time, and were elevated in PA-PA, relative to CON-CON ($P < 0.01$). For example, total GlcCer increased 25% from d 11 to 60, and was 25% higher in PA-PA, relative to CON-CON ($P < 0.01$). Plasma total ceramide and C24:0-ceramide levels were positively correlated with milk yield and energy-corrected milk ($r = 0.27$ to 0.52 ; $P < 0.01$). The ability of palmitic acid-induced ceramide to suppress insulin action during early lactation requires further investigation.

Key Words: ceramide, insulin resistance, palmitic acid

M264 Effect of rumen fluid inoculum and substrate on in vitro volatile fatty acid production and fiber digestibility. R. A. Kohn^{*1}, L. M. Judd¹, C. Stoffel², and E. Evans³, ¹University of Maryland, College Park, MD, ²Papillon Agricultural Company, Easton, MD, ³Essi Evans Technical Advisory Services, Bowmanville, Canada.

This in vitro experiment used a 4 × 2 × 2 factorial design with 6 replicates (3 per run) to determine effects of rumen fluid treatment, substrate and probiotics. There were 4 treatments of rumen fluid inoculant, 2 pre-weighed substrates (0.5 g timothy hay or 0.5 g timothy hay with 0.5 g corn grain), each with or without a probiotic supplement containing yeast, lactic acid bacteria, and digestive enzymes. Control (C) rumen fluid (10 mL/flask) was collected 4 h after feeding, and was blended and strained. Other rumen fluid treatments used 0.5 mL rumen fluid: otherwise as control (A), collected before feeding (B), or not blended (D). Samples in 40 mL of medium plus inoculant were incubated at 39°C for 24 h. Results were analyzed by the model: $Y = \mu + T + S + P + R + \text{all interactions} + E$, where Y is the response variable, and T, S, and P are fixed effects of rumen fluid, substrate, and probiotic, and R and E are random effects of run and residual error. Differences were reported at $P < 0.05$. NDF digestibility at 24 h was lower for the treatments with corn (residual NDF 66.2%) compared with treatments with hay alone (residual NDF 57.8%). NDF digestibility was greater for C, and for C and B for treatments without corn. Total gas production in mL per g digested OM was higher for C (316 mL/g) and B (279 mL/g) than A and D (241.5 mL/g). Total VFA were highest for B with corn (178 vs. 171–181 mM), and lowest for D without corn (75 vs. 82–88 mM). Acetate concentration increased most in treatment C with corn (91 mM), compared with other treatments with corn (74–76 mM) and treatments without corn (43–51 mM). Propionate concentration increased most in treatments with low volume of inoculant with corn (91–95 mM), compared with C with corn (38 mM), and without corn (17–22 mM). Butyrate concentration was greatest for treatment C with corn (24.1 mM), compared with other treatments with corn (9.3–11.6 mM), and without corn (4.9–6.9 mM). Probiotic effects were not consistent across runs, but were exhibited within runs for digestion, VFA and gas. Ethanol was detected in low-volume inoculants with corn (9.8–14.7 mM). Low microbial competition shifted in vitro fermentation toward propionate.

Key Words: in vitro rumen fermentation, volatile fatty acids, in vitro fiber digestibility

M265 Effect of feeding two fat sources varying in palmitic and stearic acid content in mid-lactation dairy cows. P. Piantoni^{*}, Y. Sun, A. A. A. Jacobs, and G. F. Schroeder, *Cargill Animal Nutrition Innovation Center, Elk River, MN.*

Twenty-four cows were used in a 3 × 3 Latin square design experiment with 21-d periods to evaluate production responses to 2 highly saturated fatty acid (FA) supplements enriched in either stearic or palmitic acid. Cows (122 ± 39 DIM; 43.5 ± 9 kg/d milk yield) were randomly assigned to squares and treatment sequences within square. Treatments were: control (CTR; base diet with no supplemental FA; 53% forage, 1:1 corn silage to alfalfa ratio), an enriched palmitic acid supplement (PALM; > 80% C16:0), and an enriched stearic acid supplement (STEAR; 65–75% C18:0, 20–25% C16:0). Both free FA supplements were added to the base diet at 2.0% of the diet DM. Milk yield and DMI from d 14 to 20 and milk composition from d 20 of each period were used for the analysis. Contrasts compared CTR vs. PALM and STEAR, and PALM vs. STEAR. FA supplementation decreased dry matter intake (DMI) by 1.3 kg/d (22.0 vs. 23.3 kg/d; $P = 0.03$), but did not affect net energy intake compared with CTR. Treatments did not affect fat or protein concentrations in milk or yields of milk, 3.5% fat-corrected milk, and

milk components. FA supplementation increased components efficiency (yields of milk fat and protein/DMI; 11.3 vs. 10.7%; $P = 0.04$) and feed efficiency (milk yield/DMI) compared with CTR (1.77 vs. 1.66, $P = 0.01$). Treatments did not affect body weight or body condition score. Production responses to PALM were not different from those of STEAR. Overall, FA supplementation decreased concentration of FA from de novo synthesis in milk compared with CTR (24.6 vs. 26.9%; $P < 0.01$), and the effect tended to be more pronounced for PALM than STEAR (24.1 vs. 25.1%; $P = 0.07$). FA supplementation increased mixed-source ($P < 0.01$) but not pre-formed FA in milk compared with CTR. PALM increased concentration of mixed-source FA in milk compared with STEAR (35.3 vs. 30.6%; $P < 0.01$), while STEAR increased that of pre-formed FA compared with PALM (41.2 vs. 37.1%; $P < 0.01$). In conclusion, FA supplements decreased DMI, did not affect milk yield or concentrations of milk fat or protein, and increased feed efficiency compared with a control diet with no supplemental FA.

Key Words: fatty acids, palmitic acid, stearic acid

M266 Estimation of dry matter intake of individual cows fed in a group setting using common on-farm measurements. M. E. Iwaniuk^{*1}, E. E. Connor², and R. A. Erdman¹, ¹University of Maryland, College Park, MD, ²USDA-ARS, Beltsville, MD.

Feed is the single largest expense for producing milk on dairy farms so producers are interested in exploring methods to improve dairy feed efficiency (FE). To calculate FE and to allow for genetic selection of the most feed-efficient cows within a herd, knowing the dry matter intake (DMI; kg/d) of individual cows is required. The vast majority of dairy cows are fed in large groups such that the DMI of an individual cow within a group is unknown. The objective of this study was to develop and validate a system to estimate DMI of individual cows using measurements that are already commonly recorded on dairy farms. The proposed approach is a modification of an original model developed by Jonker et al. (1998; *J. Dairy Sci.* 81:2681–2692). For this study, the data set included 2-wk averages for dietary nitrogen (DietN) concentrations (g/kg DM) as well as DMI and milk production (kg/d), milk protein (g/d), and milk urea N concentrations (MUN; mg/dL) from 167 individual cows (2,157 cow observations) across 52 2-week periods. The data were used to predict N losses in milk, urine, and feces to estimate DMI of individual cows fed the same diet by 2-wk periods using the following equation: $DMI (kg/d) = [MilkN + A + (B \times MUN)] / (0.8 \times DietN + 5 - C)$ where: MilkN = milk N output (g/d), A = intercept for predicted urinary N losses (g/d), B = coefficient used to predict urinary N output based on changes in MUN, 0.8 = availability of DietN digested, DietN = dietary N concentration (g/kg diet DM), 5 = mean metabolic fecal N (MFN; g/kg diet DM), and C = adjustment in differences in diet N availability and MFN. Predicted DMI explained 59% of the total variation in measured DMI ($R^2 = 0.59$; RMSE = 2.15; $P < 0.0001$). Residual plots of the deviations in actual vs. predicted DMI indicated no mean or linear biases. Therefore, it was concluded that DMI can be estimated on an individual cow basis using common on-farm measurements even if the cows are fed in a group setting. These results will enable producers to calculate individual cow DMI as well as FE to allow for the genetic selection of more feed-efficient cows.

Key Words: DMI, dairy cow, prediction model

M267 Effects of a starch binding agent on in vitro rumen degradability of corn and sorghum starch. M. N. T. Shipandeni^{*1,2}, E. Raffrenato¹, and C. W. Cruywagen¹, ¹Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa,

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The objective of the trial was to quantify the potential of a starch binding agent (BioProtect) to reduce in vitro rumen starch degradation of corn and sorghum particles varying in size. Corn and sorghum grain samples were ground through 1 and 2 mm sieves using a Wiley mill and subsequently sieved to obtain the following sizes: <250, 250–500, 500–1180 and 1180–2000 µm. All fractions were separately analyzed for starch. Samples were treated 24 h before fermentation by spraying with BioProtect according to product guidelines. Both, treated and untreated corn and sorghum samples were fermented in vitro for 0, 3, 6, 9, 12, 24 and 48 h to quantify starch digestibility (Sd). Rates of digestion (kd) were calculated using a first order decay model and 48 h fermentation residuals were used to estimate indigestible starch. Data were analyzed according to a randomized complete block design with a factorial arrangement of treatments. The main effects tested were grain, particle size, product, time (for Sd only) and their interactions. Fermentation run (n = 3) was considered a random effect. As particle size decreased, starch increased from 701 to 821 g/kg and from 730 to 810 g/kg for corn and sorghum, respectively. For both grain types, Sd and kd increased linearly with decreased particle size ($P < 0.01$). Sd (kd) increased from 41 (0.10) to 58% (0.26 h⁻¹) and 30 (0.11) to 53% (0.24 h⁻¹) for corn and sorghum, respectively. BioProtect was effective ($P < 0.001$) in decreasing starch digestibility for both grains. The product was more effective with smaller particle size, by reducing Sd 17%-units for the smallest particles vs. 7%-units for the largest particles. A time interaction was observed ($P < 0.0001$), showing that the highest impact of BioProtect occurred after 12 h of fermentation for both grains. The starch binding agent resulted in an effective decrease of in vitro starch digestion, but results would be affected by particle size and fermentation time.

Key Words: BioProtect, starch, in vitro

M268 Effects of oilseed supplementation on performance, methane emission and nitrogen utilization efficiency of lactating dairy cows. C. Muñoz¹, R. C. Sánchez², A. M. T. Peralta¹, S. Espíndola³, T. Yan⁴, R. Morales¹, and E. M. Ungerfeld⁵, ¹Instituto de Investigaciones Agropecuarias, INIA Remehue, Osorno, Chile, ²Facultad de Ciencias Veterinarias, Universidad de Concepción, Concepción, Chile, ³Cooperativa Agrícola y Lechera de La Unión, La Unión, Chile, ⁴Agri-Food and Biosciences Institute (AFBI), Agriculture Branch, Hillsborough, UK, ⁵Instituto de Investigaciones Agropecuarias, INIA Carillanca, Temuco, Chile.

Oilseeds can decrease enteric methane emission and affect nutrient utilization in dairy cows. The objective of this study was to evaluate the effects of oilseed supplementation on milk production and composition, methane emission and efficiency of nitrogen (N) utilization of dairy cows. Eight multiparous Holstein Friesian cows (75.4 ± 15.9 d in milk) were randomly allocated to treatments in a double 4 × 4 Latin square with 4 periods (22 d for adaptation and 6 d for measurements in digestibility units). Diets fed as TMR consisted of: maize silage, grass silage, a concentrate based on ground corn and soybean meal, and 1 of 4 treatments: control (CON; commercial fractionated palm oil protected fat; 3.5% DM basis), whole linseed (LNS; 7.5% DM basis), whole rapeseed (RPS; 6.9% DM basis) and whole cottonseed (CTS; 18.4% DM basis). Measurements of methane emissions were conducted using the SF₆ technique. Data were analyzed using a mixed model including the fixed effects of treatment and period and the random effect of cow. The RPS diet increased ($P < 0.05$) DM intake compared with the other treatments. Treatments had no effect on milk yield. Milk fat content was decreased ($P < 0.001$) by LNS and RPS compared with CON. CTS

tended to increase ($P = 0.07$) milk protein and increased ($P < 0.001$) milk lactose compared with CON, but was not different ($P > 0.05$) from LNS or RPS. Methane emission per cow ($P < 0.001$) and per kg of DM intake ($P < 0.01$), were decreased by CTS compared with RPS and LNS. Methane emission per kg of energy corrected milk ($P = 0.03$) was decreased by CTS compared with RPS, but was not different ($P > 0.05$) than CON and LNS diets. Total N intake was increased ($P < 0.05$) by RPS compared with CON. All oilseeds increased ($P < 0.001$) fecal N output (g/d) compared with CON. Feeding CTS increased ($P < 0.001$) urine N output compared with LNS, RPS and CON, and tended ($P = 0.05$) to decrease retained N compared with RPS. In conclusion, the inclusion of CTS in the diet of lactating dairy tended to increase milk protein and lowered methane emissions compared with LNS and RPS, but increased N excretion in urine.

Key Words: oilseeds, methane, cottonseed

M269 Effect of different physiological stages on plasma adropin, insulin, nonesterified fatty acids, and glucose concentration in lactating dairy cows. H. M. Edvardsson* and A. E. Relling, Department of Animal Sciences, The Ohio State University, Wooster, OH.

The objective of this research was to investigate the effect of physiological stage on plasma concentrations of adropin, nonesterified fatty acids (NEFA), glucose, and insulin in lactating dairy cows. Twenty-three lactating dairy cows were selected based on days in milk (DIM) and daily average milk yield. There were 7 early lactation cows (EL, < 50 DIM), 8 in mid-lactation high producing (HPML) and 8 in mid-lactation low producing (LPML). High and low production mid-lactation (100–200 DIM) were determined by taking an average of all DIM appropriate cows, and using plus or minus a standard deviation to create the minimum milk yield for the HPML cows and the maximum milk yield for the LPML cows. Blood samples from the cows were drawn once via the coccygeal vein before feeding time and the plasma was used for glucose, NEFA, insulin, and adropin assays. Data were analyzed as a complete randomized design with a mixed model (SAS 9.4) considering each treatment as a fixed variable and the cow as a random variable. The option PDIF of SAS was used for mean separation if overall treatment effect has a P value < 0.05. We were able to validate a human adropin assay as a valid method to measure bovine adropin, using parallel displacement and recovery points. Plasma glucose (EL: 72.15 mg/dL, HPML: 73.88 mg/dL) and insulin (EL: 0.25 ng/mL, HPML: 0.33 ng/mL) concentrations of EL and HPML cows were similar ($P > 0.1$) while LPML had greater ($P < 0.05$) concentrations (79.82 mg/dL and 0.5382 ng/mL for glucose and insulin respectively). Average NEFA concentrations of HPML (218 µEq/mL) and LPML (254 µEq/mL) were similar ($P > 0.1$) while EL had much greater concentrations (537 µEq/mL, $P < 0.05$). There was a trend ($P < 0.1$) for adropin to have a lower concentration in EL (0.48 pg/mL) than HPML (0.78 pg/mL), while LPML had similar concentrations to both (0.63 pg/mL, $P > 0.1$). Our results show that different stages of lactation tend to have different concentrations of adropin, insulin NEFA and glucose and the concentration is not dependent of physiological stage or milk yield but the interaction between them.

Key Words: metabolism, endocrinology, milk yield

M270 Effect of different heat processing methods on both morphological changes of starch granules and degradability of barley grain. S. Shirmohammadi*, A. Taghizadeh, G. A. Moghadam, and A. H. Khani, University of Tabriz, Tabriz, East Azerbaijan, Iran.

The aim of this study was to evaluate the effect of different heat-processing methods on both morphological changes of starch granules and degradability of barley grain. Treatments included 1) control: whole barley grain with no processing (WBG), 2) roasted: roasting for 5 min at 130°C (RBG), 3) microwave irradiated: microwaved for 2 min at 1200W (MBG), and 4) steam flaked: moisturized for 30 min on direct steam flow of boiling water and flaked just after moisturizing (SBG). To evaluate degradability by treatments, a gas production technique and an in situ method were utilized. For estimating post-ruminal digestibility, a modified 3-step method was adopted. Scanning electron microscopy (SEM) was used to identify morphological changes and also as a new method for explaining digestion kinetics. Cumulatively produced gas was recorded at 2, 4, 6, 8, 12, 16, 24, 36, 48, 72, and 96 h of incubation. Kinetics of digestion were estimated using the model of $G_p = A(1 - e^{-ct})$. The in situ method used for determination of ruminal digestion kinetics. Two bags were incubated in the rumen of 3 wethers fitted with a rumen cannula. Incubation times were 0, 2, 4, 8, 12, 16, 24, 36, and 48 h. Degradation kinetics of DM were calculated using the model of $y = a + b(1 - e^{-ct})$. In vitro intestinal disappearance of ruminal DM residue after 12 h of incubation was estimated using a modified 3-step procedure. Different heat-processing methods increased ($P < 0.05$) cumulative gas production, being 179.9, 190.2, 200.6, and 211.8 mL/g of DM for WBG, RBG, MBG, and SBG respectively. Disappearance of DM increased ($P < 0.05$) from 70.9% for WBG to 74.8%, 79.5%, and 84.2% for RBG, MBG, and SBG, respectively. Extent of intestinal disappearance of DM was greatest for SBG and least for WBG. All these results were evident by the ratio of holes to starch granule surface using SEM. Heat-processing can be used not only to make greater use of grain in the rumen but also can be used to improve post-ruminal digestibility.

Key Words: barley grain, scanning electron microscope, gas production technique

M271 Effect of parity on grazing behavior of dairy cows grazing oat pastures. J. P. Soutto, P. Pellaton, P. Gauthier, M. Carriquiry, P. Chilibruste*, and A. I. Trujillo, *Facultad de Agronomía, UDELAR, Montevideo, Uruguay.*

Information concerning to grazing pattern of multiparous (MUL) and primiparous (PRIM) cows is especially valuable to understand mechanisms involved in feed intake as well as to improve dairy grazing management. The study was conducted in a randomized block design to assess the effect of parity (3 block; $n = 9$ cows per treatment) of Holstein dairy cows (days in milk = 73 ± 7 ; BW = 521 ± 32 kg; milk yield = 26 ± 3 kg) grazing a vegetative oat pasture (8 h of access to pasture from 8:30 to 16:30 h; pasture allowance = 30 kgDM/cow/day; DM = 14%, CP = 23%, NDF = 46%, dry basis) on grazing behavior (grazing, ruminating and idling times; GT, RT and IT, respectively) and number of prehension bites (NB). Cows were milked twice daily and fed, after the afternoon milking, 6 kg DM/day of a mixture (70:30 forage to concentrate ratio as-fed basis). Individual cows were observed every 5 min on 3 consecutive days and grazing, ruminating or idling and other activities were recorded. The GT, RT and IT were estimated assuming that the recorded activity was maintained between 2 consecutive records. The number of bites was estimated by counting prehension bites/min every 5 min during the grazing activity. All variables were calculated for the 8 h of access to pasture and for 4 intervals of 2 h each during the access time to pasture (INT1 to INT4). Data were analyzed as a repeated measures using a mixed model with treatment (MUL and PRIM), day and INT (when corresponded) as fixed effects and block as a random effect. The NB (14,990 vs 13,670; $P = 0.033$) and GT (320 vs 300 min; $P = 0.075$) were greater for MUL than PRIM while RT and IT did not

differ ($P > 0.10$) between parities during access time to pasture. When INT were evaluated, differences were detected only in INT 3: MUL cows performed 591 more NB ($P = 0.013$) and grazed 14 min more ($P = 0.012$) than PRIM, while PRIM ruminated 7 min more ($P = 0.048$) and idled 7 min more ($P = 0.0543$) than MUL. Results suggest that the different grazing behavior performed by MUL and PRIM could be associated with different pasture intake.

Key Words: ruminant behavior, dairy cow, oat pasture

M272 Changes in rumen bacteria communities in continuous cultures fed high and low levels of unsaturated fatty acids with increasing rates of starch degradability. V. Richards, T. Jenkins, L. Koch, and G. Lascano*, *Clemson University, Clemson, SC.*

Dietary changes can alter the rumen environment and provoke shifts in microbial communities leading to incomplete biohydrogenation (BH). The objective of this study was to compare bacterial diversity in diets previously shown to cause shifts in BH intermediates. Diets containing low (LF) or high (HF) concentrations of unsaturated fatty acids (0 or 3.3% soybean oil added) were modified using corn sources with low (L), medium (M) or high (H) starch degradability (SKd; 48.4 L, 66.2 M, or 84.0% h^{-1} in 7 h in vitro test) and arranged in a 2×3 factorial design. Diets were fed for 4 10 d periods. Bacterial community composition from overflow samples was determined using Illumina MiSeq16S rRNA gene V4 variable region amplicon sequencing. Significant differences in β diversity among sample groupings were determined using a Python script within QIIME to perform a PERMANOVA. For individual relative abundance (rA) of interest, the MIXED procedure of SAS was used. Significance for main effect of fat and linear and quadratic contrasts for SKd were set at $P \leq 0.10$. Results showed 519 species belonging to 248 genera across treatments. Beta diversity was different between LF and HF ($P = 0.10$); and significantly increased with SKd ($P = 0.02$) with a pronounced separation between L and H SKd ($P < 0.01$). Among the 60 species of most frequently detected taxa, 29 showed a progressive decrease ($n = 20$) or increase ($n = 9$) in frequency moving from L to H SKd respectively. The rA of fibrolytic bacteria *Prevotella ruminicola* was increased while *Ruminococcus flavefaciens* was reduced ($P \leq 0.01$) as fat was added. There was a linear decrease in rA of *Butyrvibrio fibrisolvens* and *Butyrvibrio hungatei* (BH enabler; $P \leq 0.10$) as SKd increased. Whereas *Streptococcus bovis* and *Prevotella bryantii* (amylolytic) showed a linear increase as SKd increased ($P \leq 0.05$). Taxa responsible for lipolysis (*Anaerovibrio lipolyticus*) or utilizing lactic acid (*Megasphaera elsdenii*) were not different. These results suggest that fat level affect bacteria diversity and that increasing the SKd with constant starch level causes significant changes in microbial communities.

Key Words: lipid, starch, bacteria diversity

M273 In vitro fermentation of Moringa oleifera leaves supplemented in a ruminant diet. S. Chizona*, J. Allen, and V. Fellner, *North Carolina State University, Raleigh, NC.*

Global population growth continues to drive the need for dairy farm sustainability and improvement of system efficiencies. This has led to the exploration of alternative feed sources. *Moringa oleifera* is a multipurpose tree whose leaves are used as animal feed in many parts of the world and is a potential dairy animal feed. The objective of this study was to explore the potential use of *Moringa* as a dairy feed through analysis of in vitro fermentation properties. A batch culture in vitro digestibility study was carried out to analyze the effect of *Moringa* on rumen fermentation of a corn-based diet. Three levels of *Moringa*

inclusion (50, 75, and 100%) replacing corn, were used and 100% alfalfa was the control. These were incubated with rumen fluid at periods of 0, 12, 24, and 48 h in triplicates plus blanks, making a total of 52 samples. Short-chain fatty acids (SCFA) profiles, pH, neutral detergent fiber (NDF) and methane from rumen samples were analyzed and dry matter disappearance (DMD) was calculated. Analysis indicated that feeds used had a crude protein (CP) content of 22% CP for *Moringa*, 15% CP alfalfa, and 10% CP for corn. The results indicated that methane production was significantly lower ($P < 0.0001$) with *Moringa* inclusion (14.56 ± 1.72 nmol/mL at 100%) than alfalfa (891.88 ± 351.89 nmol/mL). The total SCFA was not significantly different across all treatments at 48 h, with 128.53 ± 4.7 mM for *Moringa* inclusion and 129.64 ± 3.6 mM for the control. *Moringa* inclusion increased digestibility of DM and NDF. Inclusion of *Moringa* increased NDF from $9.1 \pm 0.95\%$ at 50% to 19.4 ± 2.26 at 100%. DMD increased only up to 75% *Moringa* inclusion ($42.3 \pm 0.85\%$) then dropped to $35.2 \pm 1.34\%$ at 100% inclusion and control was at $29.2 \pm 0.31\%$. There was more butyrate produced from the control diet (13.1 ± 1.46 mM versus 7.11 ± 0.61 mM for *Moringa*) but *Moringa* increased propionate levels (47.23 ± 0.97 mM versus 31.28 ± 1.58 mM for control). The pH increased across all treatments, with time. Suppression of methane saves energy that might be wasted as gas production in the ruminant animal. The results suggest *Moringa* performs similarly to alfalfa as a high protein feed ingredient.

Key Words: efficiency, dairy, nutrition

M274 Impact of feed restriction-induced negative energy balance on the fatty acid profile of liver lipid fractions of dairy cows.

C. M. Prom*, L. C. Worden, S. E. Schmidt, K. M. Thelen, G. A. Contreras, and A. L. Lock, Michigan State University, East Lansing, MI.

Our objective was to determine the impact of feed restriction-induced negative energy balance on the fatty acid (FA) profile of lipid fractions in liver. Ten multiparous Holstein cows were randomly assigned to treatments of ad libitum feed intake (CON; $n = 5$) or feed restriction (RES; $n = 5$), with RES resulting in a mean energy balance of -13.1 ± 2.0 Mcal/d following 4 d of treatment. Cows were euthanized on d 4 and liver samples collected. Liver lipids were extracted, fractionated, and FA concentrations determined for triglycerides (TG), phospholipids (PL), cholesterol esters (CE), and free FA (FFA). The statistical model included the fixed effects of treatment, block, fraction, and interactions, as well as the random effect of cow nested within block. Compared with CON, RES altered the distribution of FA in liver lipid fractions. The proportion of FA in each fraction for CON and RES was 5.82% and 35.2% for TG ($P < 0.01$), 3.18% and 3.45% for FFA ($P = 0.96$), 88.1% and 59.0% for PL ($P < 0.01$), and 2.89% and 2.34% for CE ($P = 0.91$), respectively. Compared with CON, RES increased the concentration of C16:0 ($P < 0.01$ for all) and cis-9 18:1 ($P < 0.01$ for all) and decreased

the concentration of C18:0 ($P < 0.05$ for all) in all fractions. The largest fold increase for C16:0 and cis-9 18:1 was in TG and FFA, respectively; the greatest fold decrease for C18:0 was in TG. The concentration (g/100 g FA) of C16:0, C18:0, and cis-9 18:1 for CON vs. RES was 27.2 vs. 40.7, 12.4 vs. 5.93, and 11.1 vs. 25.6 in TG, respectively; 16.1 vs. 21.8, 33.2 vs. 22.8, and 5.24 vs. 17.6 in FFA; 9.17 vs. 13.2, 31.6 vs. 28.2, and 7.09 vs. 14.8 in PL; and 15.6 vs. 21.8, 12.7 vs. 10.6, and 5.76 vs. 13.8 in CE. Compared with CON, RES decreased cis-9, cis-12 C18:2 (g/100 g FA) in TG (8.27 vs. 4.75; $P < 0.01$) but did not alter it in FFA (8.80 vs. 8.07; $P = 0.36$), PL (10.2 vs. 11.7; $P = 0.35$), or CE (25.7 vs. 22.6; $P = 0.16$). RES decreased cis-5, cis-8, cis-11, cis-14 C20:4 in TG ($P < 0.01$), FFA ($P < 0.01$), and CE ($P = 0.08$), but not in PL ($P = 0.74$). In conclusion, feed restriction induced-negative energy balance increased the proportion of FA in TG and increased C16:0 and cis-9 C18:1 in all liver lipid fractions.

Key Words: fatty acids, liver, negative energy balance

M275 Effect of feeding different levels of total undigested NDF and forage on production responses of lactating dairy cows.

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Twenty-four Holstein cows were used in a replicated 4x4 Latin square design experiment with 28-d periods to evaluate the effects of different sources and levels of total undigested neutral-detergent fiber (uNDF; determined after 240 h of in vitro fermentation) on animal performance. Treatments, expressed as % of body weight, were: 1) 0.40% uNDF with 33% forage (high uNDF, low forage: HULF), 2) 0.40% uNDF with 38% forage (high uNDF, medium forage: HUMF), 3) 0.40% uNDF with 48% forage (high uNDF, high forage: HUHf), and 4) 0.30% uNDF with 38% forage (low uNDF, medium forage: LUMF). Linear and quadratic contrasts were used to evaluate the effect of increasing levels of dietary forage at a constant level of uNDF. In addition, a simple contrast was run between treatments HUMF and LUMF to evaluate the effect of 2 levels of uNDF at a similar forage level. There was a quadratic negative effect of increasing forage level on dry matter intake (DMI) and yields of milk, milk fat, and milk protein (Table 1). Feed efficiency (milk yield/DMI) increased as forage in the diet increased at the same level of uNDF. Forage level affected total-tract NDF digestibility (TTNDFD) quadratically: HUMF increased TTNDFD the most, followed by LFHI, and then HFLI. LUMF decreased DMI and increased TTNDFD compared with the HUMF, but did not affect milk yield, resulting in increased feed efficiency. Under the conditions of this study, level of forage was better related to changes in DMI and performance than total uNDF intake.

Key Words: indigestible NDF, control of intake, undigested NDF

Table 1 (abstract M275). Effect of source of undigested uNDF on performance of dairy cows

Item	HULF	HUMF	HUHF	LUMF	SEM	P-value	Contrasts		
							Linear	Quadratic	HUMF vs. LUMF
DMI, kg/d	29.3	25.4	24.3	24.3	0.79	<0.01	<0.01	<0.01	<0.01
Milk yield, kg/d	35.2	31.9	32.4	32.5	2.06	<0.01	<0.01	<0.01	0.31
Milk fat yield, kg/d	1.34	1.21	1.2	1.22	0.07	<0.01	<0.01	0.04	0.71
Milk protein yield, kg/d	1.13	1.01	1.00	1.00	0.05	<0.01	<0.01	<0.01	0.98
Feed efficiency	1.26	1.32	1.38	1.40	0.06	<0.01	<0.01	0.84	0.04
TTNDFD, %	40.4	43.3	42.5	51.1	0.76	<0.01	0.03	0.02	<0.01

M276 Curve-linear relationship between altered carbohydrate traits and rumen and intestinal digestion in dairy cattle in hull-less barley varieties (*Hordeum vulgare* L.). B. Sun^{1,2}, M. Sun^{1,3}, and P. Yu*¹, ¹University of Saskatchewan, Sask, Canada, ²Southchina Agricultural University, Guangzhou, China, ³Northeast Agricultural University, Harbin, China.

The objective of this study was to investigate the curve-linear relationship between altered carbohydrate (CHO) traits (different levels of amylose, amylopectin and β -glucan) and rumen and intestinal digestion in dairy cattle in CDC developed hull-less barley cultivars. Four hull-less barley cultivars (zero-amylose waxy, Fibar; 5%-amylose waxy, Rattan; normal amylose, McGwire and high-amylose, HB08302) were developed at the Crop Development Centre, University of Saskatchewan, with differences in carbohydrates traits on the basis of amylose (1–40% DM) and β -glucan (5–10% DM) content. All cultivars were planted and grown in Saskatoon (Canada) and then harvested in 3 consecutive years for experimental purposes. The digestion in the rumen and intestine was determined used in situ dairy cows with RCBD design with samples year as a random effect. The rumen degradation was carried out with in situ nylon bag technique. The intestinal digestibility was carried out with 3-step in vitro technique with 16 h pre-incubation. The data were analyzed with Proc mixed model. Results showed that: 1) intestinal (IDP: 38 to 69 g/kg DM) and total digestible protein (TDP: 106 to 154 g/kg DM) had cubical ($P < 0.05$) relation and truly digestible neutral detergent fiber (TDNDF: 53 to 105 g/kg DM) had quadratic ($P < 0.05$) relation with the β -glucan level. TDP and TDNDF showed a cubical effect and IDP showed a quadratic effect ($P < 0.05$) with the ratio of amylose to amylopectin (A:AP). There were cubical ($P < 0.05$) relation between intestinal digestible rumen undegraded starch (IDBST: 78–184 g/kg DM) and A:AP, quadratic ($P < 0.05$) relationship between IDBST and β -glucan level. Total digestible starch (TDST) had a quadratic effect with the A:AP and a cubical effect with the β -glucan level ($P < 0.05$). Intestinal digestible rumen undegraded CHO (IDBCHO: 15 to 33 g/kg DM) was cubically related to the A:AP ($P < 0.05$). In conclusion, the alternation of carbohydrate traits in hull-less barley through modern breeding tool results in curve-linear relationship with rumen and intestinal digestion in dairy cows.

Key Words: alteration of carbohydrate traits, ratio of amylose to amylopectin and β -glucan, rumen and intestinal digestion

M277 Interactions between levels of flaxseed oil and corn grain particle size on milk yield and nutrient digestibility in Jersey cows. V. Brossillon¹, A. F. Brito*², S. F. Reis², D. C. Moura³, J. G. B. Galvão Jr.⁴, C. Côrtes¹, and A. S. Oliveira⁵, ¹Ecole Supérieure d'Agricultures, Angers, France, ²University of New Hampshire:

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Organically certified cows in the United States must have year-round access to the outdoors to comply with the National Organic Program rules. Thus, increasing dietary energy via flaxseed oil (FO) may be an attractive strategy to improve milk yield during the winter season. In addition, FO may interact with corn grain of 2 particle sizes [cracked corn (CC) vs. ground corn (GC)] to modulate nutrient digestibility ultimately affecting DMI and milk yield. Sixteen mid-lactation organically certified Jersey cows were randomly assigned to treatments in a replicated factorial 4 × 4 Latin square design with 24-d periods. Treatments were fed as TMR (55:45 forage-to-concentrate ratio) and consisted (DM basis) of (1) 0% FO + 28.3% CC, (2) 0% FO + 27.1% GC, (3) 3% FO + 28.3% CC, and (4) 3% FO + 27.1% GC. Diets averaged 19.5% starch, and 4.9 and 7.6% ether extract for 0 and 3% FO, respectively. Corn grain mean particle size averaged 2,047 μ m (CC) and 580 μ m (GC). Contrasts were used to compare: FO level, corn particle size, and oil × corn interaction. Data are presented in Table 1. DMI and yields of milk fat and protein were not affected by treatments. Cows fed 3% FO produced more milk than those fed no FO. Conversely, concentrations of milk fat and protein, and total-tract aNDFom digestibility decreased with feeding 3% FO vs. 0% FO. Total-tract digestibilities of OM and starch were greater in cows fed GC than CC. Overall, FO appears to be viable strategy to increase milk yield during the winter season when cows are housed outdoors.

Key Words: corn grain, dairy cow, flaxseed oil

M278 Fatty acid challenge increases oxidation and glucose production in a substrate-specific manner in bovine primary hepatocytes. T. L. Chandler*, S. J. Erb, K. J. Sailer, S. J. Bertics, and H. M. White, University of Wisconsin-Madison, Madison, WI.

Adipose tissue mobilization increases circulating fatty acid (FA) concentration, hepatic FA uptake, and influences hepatic metabolism. The objective of this experiment was to examine the effect of FA challenge on complete and incomplete oxidation, glucose output, and oxidative stress in bovine primary hepatocytes. Primary hepatocytes isolated from 3 neonatal Holstein calves were maintained as monolayer cultures for 24 h. At 24 h, media was refreshed with a glucose-free media containing only pyruvate as a gluconeogenic precursor, and cells were exposed to 0 or a 1 mM FA cocktail that reflected the circulating FA profile at calving.

Table 1 (abstract M277). Milk yield and composition and total-tract digestibility of nutrients

Item	Flax oil		Corn grain		SEM	Contrasts ($P > F$)	
	0%	3%	Cracked	Ground		Oil	Corn
DMI, kg/d	16.3	15.9	16.1	16.0	0.44	0.77	0.21
Milk yield, kg/d	17.1	18.2	17.6	17.6	0.55	<0.01	0.98
Milk fat, %	5.26	5.11	5.22	5.15	0.10	0.05	0.33
Milk fat, kg/d	0.87	0.90	0.89	0.88	0.02	0.16	0.56
Milk protein, %	3.63	3.45	3.54	3.54	0.07	<0.001	0.97
Milk protein, kg/d	0.60	0.61	0.60	0.61	0.02	0.73	0.77
OM digestibility, %	62.7	61.8	60.0	64.5	0.89	0.42	<0.001
aNDFom digestibility, %	46.5	42.1	44.5	44.2	1.05	<0.01	0.81
Starch digestibility, %	93.0	93.9	89.0	97.9	0.75	0.29	<0.001

After 21h of treatment, ¹⁴C-labeled palmitate or pyruvate was added to the media and both CO₂ and acid soluble products (ASP) were collected after a 3-h incubation. Media was harvested to quantify glucose and reactive oxygen species (ROS). Cell lysates were collected and DNA quantified to normalize all data. Data were analyzed by PROC MIXED (SAS 9.4) in a model accounting for fixed effect of FA treatment and random effect of calf. Data are reported as least squares means ± SE and differences declared at $P \leq 0.10$ and tendencies at $P < 0.15$. Fatty acid treatment decreased ($P = 0.01$) the relative recovery of pyruvate label as CO₂ (3.8 vs. 1.94 ± 0.31 arbitrary units) and increased ($P = 0.02$) label recovery as ASP (20.0 vs. 27.3 ± 1.4 arbitrary units). Recovery of palmitate label as CO₂ tended to be greater ($P = 0.14$) in FA treated cells (0.87 vs. 1.05 ± 0.15 arbitrary units) but ASP from palmitate was not affected ($P > 0.18$) by FA treatment. Glucose output from cells exposed to FA was increased ($P < 0.007$) by 35%. Treatment with FA increased ($P = 0.07$) ROS in cell culture media. Fatty acid challenge appears to shift oxidative and gluconeogenic capacity in a substrate-specific manner. Decreased complete and increased incomplete oxidation of pyruvate indicates a shift of pyruvate conversion toward ASP or glucose production. Conversely, fatty acid challenge increased complete oxidation of palmitate, consistent with previous indications that FA can upregulate key oxidative enzymes at the time of calving.

Key Words: gluconeogenesis, ketones, flux

M279 Nutritive value of common feedstuffs fed to dairy cows measured using the in vitro gas production technique. K. Mjoun*, L. Shearer, and B. Kubat, *Alltech, Brookings, Sd.*

The fermentation parameters of feedstuffs commonly fed to dairy cows were evaluated using the in vitro gas production technique. Feeds were grouped into different categories; corn silage (n = 15), small grains silages (n = 15), alfalfa hay (n = 11), alfalfa haylage (n = 4), byproducts (n = 20); protein meals (n = 7), energy feeds (n = 17), and lactation TMR (n = 12). Incubations (48h) were completed using rumen fluid from a lactating cow fed a 50:50 forage to concentrate diet. The fermentation kinetics were estimated using a logistic model separating gas production per 1 g of DM truly digested into fast pool (FP), slow pool (SP) and their respective rates of fermentation (FR) and (SR) (Table 1). Grain silages produced the most gas (GP) mainly from the SP. Protein meals had the highest FP:SP and haylage had the highest fermentation rates.

Table 1 (abstract M279)

	Corn silage	Small grains silage	Alfalfa haylage	Alfalfa hay	By-products	Protein meals	Energy feeds	Lactation TMR	SEM
FP, mL	78.4 ^c	115.2 ^a	79.9 ^c	85.2 ^c	100.8 ^b	104.3 ^{ab}	73.5 ^c	74.5 ^c	8.13
FR, %/h	23.6 ^a	18.5 ^c	27.4 ^a	18.7 ^{bc}	18.1 ^c	17.4 ^c	14.9 ^c	22.8 ^{ab}	2.65
SP, mL	236.7 ^a	230.9 ^a	158.4 ^{bc}	151.1 ^c	169.2 ^{bc}	109.4 ^d	174.0 ^b	176.5 ^b	10.9
SR, %/h	4.65 ^{bc}	4.17 ^d	5.51 ^a	4.87 ^b	4.54 ^{bc}	4.41 ^{cd}	3.45 ^e	5.30 ^a	0.24
GP, mL	251.6 ^{ab}	260.5 ^a	209.1 ^{de}	202.8 ^e	239.2 ^{bc}	203.1 ^e	232.4 ^{cd}	214.5 ^{de}	11.6
FP:SP	0.34 ^c	0.51 ^c	0.51 ^c	0.57 ^b	0.62 ^b	0.95 ^a	0.43 ^c	0.43 ^c	0.06
TDMD, %	73.4 ^d	63.7 ^e	81.9 ^{bc}	78.0 ^c	82.5 ^b	90.8 ^a	91.5 ^a	79.4 ^{bc}	2.67
VFA, mM	26.1 ^{bc}	21.4 ^d	25.8 ^{bc}	24.7 ^c	28.3 ^b	29.1 ^{ab}	31.2 ^a	24.1 ^{cd}	1.79
Acetate, %	38.6 ^d	48.4 ^b	56.6 ^a	55.7 ^a	45.4 ^b	43.9 ^{bc}	28.4 ^e	40.2 ^{cd}	2.80
Propionate, %	32.7 ^{cd}	30.6 ^d	23.0 ^e	25.3 ^e	33.3 ^{bc}	31.3 ^{cd}	41.1 ^a	35.5 ^b	1.64
Butyrate, %	23.1 ^a	15.1 ^{cd}	9.9 ^e	11.3 ^e	16.1 ^c	13.7 ^d	22.5 ^a	18.1 ^b	1.11
MPS, mg	138 ^d	162 ^d	273 ^{abc}	275 ^{ab}	212 ^c	320 ^a	249 ^{bc}	270 ^{abc}	31.30
YATP	9.6 ^d	15.2 ^{bc}	20.4 ^{ab}	22.9 ^a	14.1 ^c	23.0 ^a	22.5 ^a	18.1 ^b	2.73
CH ₄ , mL/g of DM	41.1 ^{ab}	42.6 ^a	47.5 ^a	43.6 ^a	36.2 ^{bc}	37.1 ^{abc}	26.8 ^d	31.5 ^{cd}	4.21

^{a-c}Values with different superscripts within the same row differ at $P < 0.05$.

The partitioning of digested DM into gases, VFA, and microbial protein (MPS) suggests that the fermentation efficiency (Y_{ATP}) is highest for protein feeds, alfalfa forages and TMR, intermediate for energy feeds, byproducts and small grains silages, and lowest for corn silages.

Key Words: in vitro, rumen, feedstuffs

M280 Effect of RDP:RUP ratio and corn processing on lactation performance, milk quality and efficiency of nutrients utilization in lactating dairy cows. C. M. M. R. Martins*¹, D. C. M. Fonseca¹, M. A. Arcari¹, B. G. Alves¹, K. C. Welter², F. P. Rennó¹, and M. V. Santos¹, ¹Department of Animal Nutrition and Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil, ²Department of Animal Science, School of Animal Science and Food Engineering, University of São Paulo, Pirassununga, São Paulo, Brazil.

The study aimed to evaluate the effect of RDP:RUP ratio and corn processing on lactation performance and milk heat coagulation time (HCT) at 140°C. Twenty Holstein cows (8 fitted with ruminal cannulas) averaged 162 ± 70 DIM, 666 ± 68 kg of body weight and 36 ± 7.78 kg/d of milk yield, were distributed in a Latin Square design with 5 contemporary squares, 4 periods of 21 d and 4 treatments (factorial arrangement 2×2). Factor 1 was corn processing (ground through 2-mm screen [GC] or steam-flaked [SFC]), and factor 2 was RDP:RUP ratio (High: 11% of RDP and 5.3% of RUP or Low: 9.8% of RDP and 6.5% of RUP; DM basis; NRC, 2001). For diets with High RDP:RUP ratio, 103.4 g/kg of conventional soybean meal (SM) and 7.8 g/kg of urea were included. For diets with Low RDP:RUP ratio, 91.9 of SM, 2.8 of urea and 44.7 g/kg of heated SM (SOYPASS, Brazil) were included. There was a tendency of interaction ($P = 0.06$) between RDP:RUP ratio and corn processing on DMI. Cows fed GC with low RDP:RUP ratio had higher DMI than cows fed GC with high RDP:RUP ratio. No effect of RDP:RUP ratio was found when cows were fed SFC. Cows fed SFC had lower DMI ($P = 0.007$), and higher DM ($P = 0.03$) and starch total apparent digestibility ($P = 0.02$), and higher productive efficiency ($P = 0.002$) than cows fed GC. Rumen pH was not affected by diets. It was observed an interaction between treatments on MY ($P = 0.04$), HCT ($P = 0.029$) and milk lactose content ($P = 0.04$). Cows fed GC had reduced MY by 2.3 kg/d when the diet had high RDP:RUP ratio, than cows fed low RDP:RUP ratio. However, when cows were fed SFC, MY did not

change according to the RDP:RUP ratio. Similar interaction results were found for HCT and lactose content. Cows fed low RDP:RUP ratio had higher fat-corrected milk, milk protein content, and efficiency of N utilization for milk protein yield. Thus, diets with GC and high RDP:RUP ratio reduces MY and milk quality. For SFC corn diets the RDP:RUP ratio does not affect the lactation performance. Overall, feeding cows with low RDP:RUP ratio results in higher efficiency of N utilization to milk protein yield.

Key Words: casein, dairy industry, milk stability

M281 Impact of dietary starch concentration formulated with two types of corn silage on methane and ammonia emissions in dairy cows.

J. I. Sanchez-Duarte^{*1}, K. F. Kalscheur², and J. M. Powell², ¹South Dakota State University, Brookings, SD, ²US Dairy Forage Research Center, USDA, ARS, Madison, WI.

The objective of this study was to evaluate methane (CH₄) and ammonia (NH₃) emissions of lactating dairy cows fed different starch level and corn silage type. After the completion of an 8-wk production study, 48 Holstein cows were allocated to 1 of 4 air-flow controlled chambers (2 cows/chamber) for 6 d in a randomized complete block design. Chamber was the experimental unit. Cows were fed 1 of 4 diets arranged as a 2 × 2 factorial with 2 corn silage hybrids [conventional (CS) and brown midrib (BMR) corn silage] and 2 dietary starch concentrations (19 and 25% of DM). Performance data from the last 6 d and emission measurements last 3 d were recorded and used for analysis. Soyhulls and beet pulp replaced corn grain in the diet to decrease starch concentration. There was no effect of dietary starch concentration and corn silage on DMI, ECM, ECM/DMI, and milk protein percentage, however milk fat percentage was greater ($P < 0.03$) for cows fed diets formulated at 25% starch rather than diets with 19% starch. An interaction of silage × starch ($P < 0.03$) was observed for CH₄ expressed as per unit of DMI and for MUN. Cows fed CS-25% starch had the lowest MUN. Cows fed BMR-25% starch produced 1.3 g CH₄ less per unit of DMI than cows fed CS-25% starch, but were similar to cows fed 21% starch for any silage type. Emissions of CH₄ and NH₃ (g/d), and CH₄/ECM did not differ among treatments. It was concluded that cows fed the BMR-25% starch have the potential to reduce CH₄ emissions per unit of DMI even though productive performance was not improved.

Table 1 (abstract M281).

Item	CS		BMR		SEM	P > F ¹
	19%	25%	19%	25%		
DMI, kg/d	25.4	25.1	24.9	25.9	0.64	NS
ECM, kg/d	44.0	44.7	40.8	44.6	1.42	NS
ECM/DMI	1.74	1.79	1.65	1.74	0.06	NS
Fat, %	3.91	4.07	3.68	4.02	0.11	S
Protein, %	3.04	3.10	3.05	3.08	0.07	NS
MUN, mg/dL	11.9 ^a	10.8 ^b	11.2 ^{ab}	11.8 ^{ab}	0.37	I
CH ₄ , g/d	503.0	515.9	494.0	497.4	13.0	NS
NH ₃ , g/d	22.7	27.6	23.4	23.1	2.65	NS
CH ₄ /DMI	19.8 ^{ab}	20.5 ^a	19.9 ^{ab}	19.2 ^b	0.56	I
CH ₄ /ECM	11.5	11.6	12.1	11.2	0.34	NS

^{ab}Mean differences ($P < 0.05$).

¹S = starch effect; I = silage by starch effect; NS = not significant.

Key Words: BMR corn silage, milk, gas emissions

M282 Forage fiber quality interacts with dietary protein level to determine nitrogen use efficiency. C. S. Malherbe^{*} and E. Raf-frenato, *Department of Animal Sciences, Stellenbosch University, Stellenbosch, South Africa.*

The aim of this study was to improve nitrogen use efficiency (NUE) by optimizing the use of dietary protein and higher quality fiber from forage. Four lactating Holstein cows were used in a 4 × 4 Latin square design balanced for carryover effects with a 2 × 2 factorial arrangement of treatments. Treatments were low CP concentration with high NDF digestibility (LpHd), high CP concentration with high NDF digestibility (HpHd), low CP concentration with low NDF digestibility (LpLd) and high CP concentration with low NDF digestibility (HpLd). Crude protein concentrations for the rations were formulated to be 18 and 15% for the Hp and Lp diets, respectively. Diets were formulated around the LpHd diet to satisfy 105% of metabolizable protein requirements. Data were evaluated using the Mixed procedure in SAS with cow and period as random effects and diet as fixed effect. The indigestible NDF, as % of the NDF, of the 2 oat hays used were 40.80% for Ld hay and 31.54% for the Hd hay and wheat straw was included in the Ld diets to obtain iso-NDF diets. Cows were fed ad libitum for 14 d with data collection over the last 4 d of each period. Dry matter intake (DMI) for 3 of the diets were found to be similar, with the exception of the LpLd diet having lower DMI ($P < 0.001$) than the other 3 diets, showing how protein can counteract the lower forage quality by stimulating fibrolytic bacteria. Energy corrected milk yield decreased ($P < 0.01$) when feeding less CP, specifically of 2.46 kg/d for the Hd forage diet and 3.00 kg/d for Ld forage diet. However, nitrogen use efficiency was the highest ($P < 0.05$) for the lower protein diet in combination with Hd forage (32.3%). We conclude that lowering protein improved NUE significantly, probably due to higher N recycling by the animals, with forage digestibility and iNDF contributing to the level of improvement.

Key Words: nitrogen use efficiency, iNDF, crude protein

M283 Predicting rumen passage rate of NDF fractions in lactating dairy cows. J. R. R. Dórea^{*1}, E.B. Alves², and D. K.

Combs¹, ¹University of Wisconsin, Madison, WI, ²Federal University of Lavras, Lavras, MG, Brazil.

Measures of passage rates (kp) of potentially digestible NDF (pdNDF) and indigestible NDF (iNDF) are needed to model the process of rumen fiber digestion. Our objective was to develop and validate models to predict kp of iNDF and pdNDF. Nineteen flow influx studies with lactating dairy cows were compiled (n = 73, treatment means). Three empirical models to predict kp iNDF were developed. The following parameters were included in all 3 models: milk yield (MY, kg/d), DIM, iNDF incubation time (h), diet NDF (%). The 3 models differed by one parameter: Model 1 (M1) also included diet iNDF:NDF ratio, Model 2 (M2) included diet iNDF(%), and Model 3 (M3) included ratio of diet concentrate proportion to diet NDF. Models were validated with an independent data set from 2 experiments (n = 64, individual animals). M1, M2 and M3 were used to predict kp iNDF of individual animals in the independent data set. The relationship between kp iNDF and kp pdNDF was best described with a segmented regression with kp iNDF as an independent variable and kp pdNDF the dependent variable. When kp iNDF < 2.45%/h, kp of pdNDF was constant, but when kp iNDF > 2.45%/h, a regression: $kp\ pdNDF = -0.011 + 0.942 \times kp\ iNDF\ (\%/h)$ described the passage rates of iNDF and pdNDF. Concordance correlation coefficient (CCC) of kp iNDF predictions and observed kp iNDF was 0.40, 0.44, and 0.35, for M1, M2, and M3, respectively. When kp pdNDF calculated from M1, M2, and M3 was compared with observed, CCC was 0.33, 0.36, and 0.26, respectively. Rumen NDF digestibility

was calculated as $kd/(kd+kp) \times pdNDF$, where kp was predicted by M2 and adjusted by the segmented regression, kd (degradation rate) was estimated by *in vitro* incubation (24, 48 and 72 h), and $pdNDF$ was estimated using 240 h *in situ* incubation. When M2-predicted NDF digestibility was compared with observed, CCC and root mean square error of prediction (RMSEP) was 0.60 and 7.6%, respectively. When an equation using kp *iNDF* was used (Kriszan et al., 2010), CCC and RMSEP were 0.51 and 8.0%, respectively. These data suggest that kp $pdNDF$ can increase the accuracy and precision of rumen NDF digestibility predictions.

Key Words: fiber digestion, fiber passage, *iNDF*

M284 Effect of contrasting predicted residual feed intake on performance and CH₄ emission of dairy cows fed 2 levels of forage neutral detergent fiber. M. Aguerre^{*1}, F. Sun², J. M. Powell³, K. Weigel², A. Pelletier⁴, P. Crump⁵, and M. Wattiaux², ¹*Animal and Veterinary Science Department, Clemson University, Clemson, SC*, ²*Dairy Science Department, University of Wisconsin-Madison, Madison, WI*, ³*U.S. Dairy Forage Research Center, Madison, WI*, ⁴*Soils Science Department, University of Wisconsin-Madison, Madison, WI*, ⁵*Department of Computing and Biometry, University of Wisconsin-Madison, Madison, WI*.

The objective of this study was to determine the effects of selecting cows with contrasting predicted residual feed intake (RFI) on animal performance and CH₄ emission and whether cow responses were affected by dietary forage neutral detergent fiber (NDF) level. Mid-lactation multiparous Holstein cows ($n = 24$) with contrasting predicted RFI were selected from a cohort of 47 cows using genotypic and phenotypic data from previous studies and grouped as low RFI (mean \pm SD; -0.42 ± 0.13 kg/d) and high RFI (0.34 ± 0.11 kg/d), respectively. Half of the cows in each RFI group were randomly assigned to a dietary treatment with either 22% or 30% forage NDF in a randomized complete block design. Following a 2-wk covariate period, cows were fed their assigned treatment diets for 4 weeks. Gas emission measurements were conducted in 4 tie-stall emission chambers during 3 consecutive days the last week of the covariate and experimental periods. Results are covariate-adjusted least squares means (\pm SEM). There was no RFI by forage NDF level interaction ($P \geq 0.05$) for any of the measured variables. A tendency ($0.05 < P < 0.10$) was observed for higher MUN and CH₄/DMI for high RFI-high forage NDF. Except for fat yield (1.74 vs. 1.50 ± 0.06 kg/d for low and high RFI, respectively), predicted RFI did not affect any measured responses. Increasing forage NDF reduced ($P < 0.05$) milk yield (44.3 vs. 40.6 ± 0.74 kg/d), fat-and-protein corrected milk (FPCM; 43.5 vs. 38.0 ± 1.24 kg/d), fat (1.77 vs. 1.51 ± 0.06 kg/d) and milk true protein yield (1.28 vs. 1.10 ± 0.04 kg/d). Greater forage NDF tended ($P = 0.08$) to decrease FPCM/DMI (1.56 vs. 1.43 ± 0.05), but increased MUN (11.2 vs. 12.7 ± 0.54 mg/dl) and CH₄ emission (533 vs. 564 ± 19.7 g/d). Forage NDF level did not affect DMI (27.0 ± 0.74 kg/d), milk/DMI (1.58 ± 0.36), CH₄/DMI (23.5 ± 0.77 g/kg), CH₄/milk (15.4 ± 0.75 g/kg), and CH₄/FPCM (14.9 ± 0.72 g/kg). Under the conditions of this study, selecting cow with lower RFI (presumably higher efficiency) had negligible effects on animal performance and CH₄ emission. Increasing forage NDF level has negative impacts on animal performance and CH₄ emission.

Key Words: greenhouse, forage, residual feed intake

M285 Effects of supplementing active dry yeast, a blend of probiotic bacteria, or a combination of both on rumen fermentation profiles and nutrient digestion in continuous rumen

fermentors. Y. Liang^{*}, E. Davis, and M. A. Ballou, *Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX*.

This study evaluated the effects of supplementing yeast, probiotic bacteria, or a combination on rumen fermentation and nutrient digestion in continuous rumen fermentors. Rumen fluid (10 L) from a mid-lactation fistulated Holstein cow was collected and used to inoculate eight 1-L glass continuous culture fermentors. Fermentors were randomly assigned to 1 of 4 dietary treatments in a 2×2 factorial arrangement, including: Control, no supplement; Yeast, supplemented with 3.6×10^7 cfu/d *Saccharomyces cerevisiae*; Probiotic, supplemented 5.4×10^6 cfu/d of a blend of *Enterococcus faecium* and *Lactobacillus casei*; or Combination, supplemented with both the Yeast and Probiotic treatments. The study was conducted in 2 blocks with 8 fermentors / block ($n = 4$). Fermentors were fed 20 g twice daily. Artificial saliva was added for a 6% dilution/h. Samples were collected from each fermentor on d 5 and 7 at 0, 2, 4, 6, 8, and 12 h relative to the AM feeding and pH recorded and samples collected for volatile fatty acids. On d 5, 6, and 7 total collection of the 24-h effluent was used to determine digestibility of DM and NDF. Data were analyzed as a repeated measures for a 2×2 factorial design using the Mixed procedure of SAS with period included as a random effect. Data are reported as Control, Yeast, Probiotic, and Combination throughout. There were no treatment \times time or treatment effects ($P \geq 0.582$) on pH (6.24, 6.23, 6.19, and 6.21 ± 0.081). Additionally, there were no treatment \times time or treatment effects on concentrations of acetate ($P \geq 0.237$; 62.2, 63.7, 64.6, and 63.8 ± 0.99 mM), propionate ($P \geq 0.263$; 24.6, 25.8, 24.9, and 26.0 ± 1.48 mM), or total volatile fatty acids ($P \geq 0.450$; 109.7, 113.4, 113.1, and 114.6 ± 4.78 mM). There was a tendency for yeast supplementation ($P = 0.067$; 51.5, 56.8, 53.1, and $54.1 \pm 1.54\%$) to increase DM digestion. There was no treatment effects on digestion of NDF ($P \geq 0.715$; 56.9, 59.6, 59.0, and $59.0 \pm 4.07\%$). These data indicate that supplementing active dry yeast may improve DM digestion.

Key Words: probiotic, rumen, yeast

M286 Performance of dairy cows fed conventional sorghum or corn silages compared to brown midrib sorghum silage: A meta-analysis. J. I. Sanchez-Duarte^{*1}, K. F. Kalscheur², A. D. Garcia¹, and F. E. Contreras-Govea³, ¹*South Dakota State University, Brookings, SD*, ²*US Dairy Forage Research Center, USDA, ARS, Madison, WI*, ³*University of Wisconsin, Madison, WI*.

A meta-analysis was conducted to compare the effects of feeding dairy cows conventional sorghum (CSS) or corn silages (CCS) vs. brown midrib sorghum silage (BMRSS) on dry matter intake (DMI), milk yield, and milk composition. Data from 9 published articles (1984–2015) were used to contrast CSS (7 comparisons; 104 cows) or CCS (13 comparisons; 204 cows) vs. BMRSS. Statistical analysis was performed using fixed or random effects models in R. The degree of heterogeneity was measured with I² statistic and publication bias was determined with funnel plots and Egger's regression test. Other sources of heterogeneity of response were analyzed through meta-regression. Estimated effect size was calculated for DMI, milk yield, and milk composition. No evidence of publication bias was observed for all variables tested. DMI and milk yield had the highest (I² = 41.5 [CSS vs. BMRSS]; I² = 72.6% [CCS vs. BMRSS]) and lowest (I² = 0%) degree of heterogeneity, respectively. Compared with CSS, cows fed BMRSS tended to increase DMI (0.83 kg/d; $P = 0.09$), and significantly increased milk yield (1.64 kg/d; $P < 0.001$), milk fat (0.09%; $P = 0.03$), and yields of milk fat (0.08 kg/d; $P < 0.001$), protein (0.04 kg/d, $P < 0.001$), and lactose (0.16 kg/d; $P = 0.02$). No differences were observed for milk protein and lactose percentage ($P > 0.05$). Compared with CCS, cows

fed BMRSS increased milk fat (0.10%; $P = 0.009$), but decreased milk protein (0.06%; $P = 0.03$). There were no effects on DMI, milk yield, yields of milk fat, protein, and lactose, and lactose percentage between CCS and BMRSS. Meta-regression indicated that days in milk affected DMI and milk production when CSS was compared with BMRSS and DMI when CCS was compared with BMRSS. Overall, lactation performance improved when cows were fed diets formulated with BMRSS compared with cows fed diets formulated with CSS; however, performance was not different between cows fed BMRSS and CCS. Future research comparing BMRSS with CSS or CCS needs to consider days in milk because cows respond differently throughout their lactation according to meta-regression analysis.

Key Words: sorghum silage, corn silage, meta-analysis

M287 Effects of experimental design and protein substitution strategy on production responses to feeding different levels of protein to primiparous dairy cows. G. I. Zanton*, *USDA-Agricultural Research Service; Dairy Forage Research Center, Madison, WI.*

The objective of this study was to evaluate the effects of reducing crude protein (CP) and CP substitution strategy on performance when primiparous dairy cows were fed diets continuously or according to a change-over experimental design. Fifty-four primiparous, Holstein cows were randomly assigned to either a randomized complete block design (CONT; $n = 36$, initial mean \pm SD: 129 ± 36 DIM, 580 ± 40 kg BW, 44.1 ± 3.2 kg milk) or to a replicated, 3x3 Latin square design balanced for the effects of previous treatment (CHANGE; $n = 18$, initial mean \pm SD: 129 ± 35 DIM, 583 ± 39 kg BW, 44.4 ± 3.7 kg milk). Experimental designs were run concurrently with three 28-d periods and sampling on d 22–28 of each period. Cows were milked 3x daily and were individually fed once daily a diet that was predicted to be either adequate (ADMP; 16.7%CP, 28.3%NDF, 25.5% starch) or deficient (LOMP) in metabolizable protein (MP). ADMP contained expellers soybean meal, whereas this was removed in LOMP diets and replaced with either dry ground corn (STARCH; 14.9%CP, 28.1%NDF, 28.6% starch) or soyhulls (FIBER; 15.1%CP, 30.7%NDF, 25.4% starch) for a total of 3 diets. Contrasts for the effects of MP (ADMP vs LOMP) and carbohydrate source (CHO: STARCH vs FIBER) were evaluated for both experimental designs, with $P < 0.05$ significant and $P < 0.10$ trends. DMI was greater for ADMP than LOMP in CONT, whereas DMI only tended to be greater in CHANGE. Yield of milk and protein were greater for ADMP in both designs whereas yield of fat was greater for ADMP in CHANGE, but only tended to be greater for ADMP in CONT. Within CONT, CHO did not affect DMI, milk, or component yield; however within CHANGE, cows fed STARCH produced more milk and tended to produce more protein. This discrepancy was not due to statistical power of the designs because the results were directionally opposite from CONT where cows fed FIBER had yields that were numerically, though not significantly, greater. Inferences about MP status were generally similar for production measures in both designs whereas inferences about CHO were affected by experimental design.

Key Words: experimental design, protein, carbohydrates

M288 Direct and indirect causal effects of dietary starch on fiber digestibility. J. R. R. Dórea*, G. J. M. Rosa, and D. K. Combs, *University of Wisconsin, Madison, WI.*

Depression in fiber digestibility in lactating dairy cows has been widely associated with increases in dietary starch (DS) due to reductions in rumen pH. However, greater starch intake (SI) can increase fiber pas-

sage rate (kp) that may also depress NDF digestibility. The objective of this study was to infer if the depression in NDF digestibility associated with DS is due to pH or fiber kp. Two structural equation models were built using 2 research trials ($n = 64$, Latin square design) in which DS, SI, total-tract NDF digestibility (TTNDFD), pH, and kp of potentially digestible NDF (pdNDF) were measured. All variables were adjusted for the fixed effect of diet and random effects of period within square, cow within square, square and trial. The first model (M1) tested the direct effects of DS on TTNDFD and SI on TTNDFD, and the indirect effect of DS on TTNDFD. The indirect effect of DS on TTNDFD was composed by the effects of DS on SI, SI on pH, and then pH on TTNDFD. The same approach was used in the second model (M2), but pH was replaced with kp pdNDF. Results (Table 1) suggest that there is an indirect effect of DS on TTNDFD through the increase in kp pdNDF caused by greater SI ($P < 0.05$). However, there is no evidence of causal effect of DS on TTNDFD due to changes in rumen pH ($P > 0.05$). The chi-squared test (χ^2), comparative fit index (CFI), Akaike information criteria (AIC), and root mean square of the approximation (RMSEA) for M1 were: χ^2 ($P = 0.07$), CFI = 0.89, AIC = 685, and RMSEA = 0.31, while for M2 were: χ^2 ($P = 0.95$), CFI = 1.0, AIC = 660, and RMSEA = < 0.001 . Increase in passage rate is the main factor that depresses TTNDFD when SI increases in diets with DS ranging from 16 to 27%.

Table 1 (abstract M288). Direct and indirect effects of dietary starch (DS, % of DM) on total tract NDF digestibility (TTNDFD, %)

Item ¹	Estimate	SE	P-value
Direct effects			
DS on TTNDFD	-0.18	0.15	0.24
SI on TTNDFD	0.24	0.16	0.13
Indirect effect (M1)			
DS on SI	0.74	0.08	<0.001
SI on pH	-0.31	0.11	0.01
pH on TTNDFD	0.04	0.13	0.72
Indirect effect (M2)			
DS on SI	0.74	0.08	<0.001
SI on kp pdNDF	0.42	0.11	<0.001
kp pdNDF on TTNDFD	-0.56	0.11	<0.001

¹SI = starch intake (kg/d), pH = rumen pH, kp pdNDF = passage rate of potentially digestible NDF (%/h).

Key Words: pH, starch, fiber

M289 Physical characterization of fat supplements highly enriched in palmitic and stearic acid. R. P. Shepardson*, E. Bazileyskaya, and K. J. Harvatine, *Penn State University, University Park, PA.*

Fatty acid (FA) supplements are widely used in lactating cow diets to increase energy intake. Previous published research has reported that supplements with moderate enrichment (~85%) of palmitic acid have expected digestibility, while very high enrichments (~98%) have lower digestibility. Saturated FA have the potential to form organized secondary structures at high purity. Differential scanning calorimetry (DSC) is a thermal technique commonly used in material science to measure the change in heat flow as energy is absorbed or released from a sample during heating. Our hypothesis was that a supplement with a very high enrichment would differ in physical characteristics due to the formation of a secondary structure, which may contribute to a decreased digestibility. A 98% stearic acid (SA; 98.5% C18:0, 0.4% C16:0), 98% palmitic acid (PA; 98.5% C16:0, 0.7% C18:0), and a mixture of palmitic and stearic (PA/SA; 54.5% C16:0, 44.5% C18:0) sample were used for this

characterization. Sample FA profile was determined by gas chromatography with a flame ionization detector. Data from DSC was gathered on a Q600 SDT (TA Instruments). Briefly, ~40 mg of sample was heated at 5°C/min from 25°C to 110°C. TA Universal Analysis software (TA Instruments) was used to determine melting temperature, and total enthalpy of melting (area under the curve). Triplicate runs were highly reproducible. Melting temperature of the PA/SA mixture was 61.2°C, similar to the reported melting temperature of PA (62.9°C). However, the melting temperatures of the high purity PA and SA were increased to 67.8°C and 73.7°C, respectively. The enthalpy of melting was increased 12.5% in the high purity PA compared with the PA/SA blend. Melting enthalpy for the high purity SA supplement was increased 3.5% from the high purity PA and 16.4% from PA/SA mixture. Although melting temperatures are well above body temperature, increasing the energy required to melt the sample is indicative of physical characteristics of the supplement expected to decrease digestibility.

Key Words: fatty acid supplement, lipid, melting point

M290 Circulating blood metabolites in early lactation dairy cows fed canola or soybean meals. S. A. E. Moore*¹ and K. F. Kalscheur², ¹University of Wisconsin, Madison, WI, ²US Dairy Forage Research Center, USDA-ARS, Madison, WI.

A successful transition from pregnancy to lactation is imperative for dairy cows to maximize milk production potential. Altering the dietary protein source can change the availability of energy and protein to the cow. The objective of this experiment was to evaluate the effect of CP source [canola meal (CM) or soybean meal (SBM)] and CP concentration [HI (18.1%) or LO (16.2%) DM basis] on circulating blood metabolites. At calving, multiparous Holstein cows (n = 79) were enrolled into a 2 × 2 factorial arrangement of treatments in a randomized complete block design. Cows were blocked by calving date and individual cow was the experimental unit. Enzymatic colorimetry was used to evaluate circulating concentrations of glucose, nonesterified fatty acids (NEFA), β-hydroxybutyrate (BHB), triglycerides (TG), and plasma urea nitrogen (PUN). Serum and plasma coccygeal vein samples were collected 2 × during wk 1, 2, 3, 4, 6, and 8 postpartum. Samples were pooled by wk for each cow. Data were analyzed using the MIXED procedure of SAS. BCS and BW at calving were used as covariates when appropriate. Total milk yield was greater for CM-fed than SBM-fed cows during wk 1–8 of lactation (mean ± SEM; 53.2 vs 49.2 ± 0.98 kg/d; *P* < 0.001), while there was no difference in DMI (*P* = 0.11) to support additional production. No treatment effect was observed for glucose or BHB. Circulating TG concentration was greater for cows fed CM compared with SBM-fed cows (0.125 vs 0.118 ± 0.002 mM; *P* = 0.02). There was an interaction of source and wk for NEFA concentration (*P* = 0.04). Efficiency of nitrogen utilization favored CM vs SBM-fed cows for both circulating PUN (0.37 vs 0.40 ± 0.01 mM; *P* = 0.02) and concentration of milk urea N (MUN; 10.7 vs 11.4 ± 0.24 mg/dL; *P* = 0.04). HI-fed cows were greater in PUN (0.44 vs 0.33 ± 0.01 mM; *P* < 0.001) and MUN concentration (9.64 vs 12.5 ± 0.24 mg/dL; *P* < 0.001) compared with LO-fed cows. The increase in milk yield can be attributed in part, to an increase in circulating TG and nitrogen utilization. However, further investigation into the CM vs SBM milk disparity in early lactation is needed.

Key Words: canola meal, early lactation, energy

M291 Effect of supplementing rumen-protected methionine pre- and postpartum on milk yield and components of dairy cows during early lactation. M. L. Stangaferro*¹, M. M. Perez¹, M. Masello¹, R. Wijma¹, M. E. Van Amburgh¹, T. R. Overton¹, D.

Luchini³, M. C. Wiltbank², R. D. Shaver², and J. O. Giordano¹, ¹Cornell University, Ithaca, NY, ²University of Wisconsin-Madison, Madison, WI, ³Adisseo USA Inc., Alpharetta, GA.

The objective of this study was to assess the effect of feeding rumen-protected methionine (RPM) pre- and postpartum on milk yield (MY) and composition up to 14 wk of lactation. Three weeks before calving, multiparous Holstein cows (n = 211) were randomly assigned to a control (CON) or RPM group, whereby the only difference in the diets was the inclusion rate of Smartamine M pre- (PreP) and postpartum (PostP). Methionine feeding (MP Met as % of MP) in the diet were 2.76 vs 3.35 PreP, and 2.10 vs 2.68 PostP, for CON and RPM, respectively. Diets were formulated using CNCPS v7 at DMI of 12.7 and 24.4 kg of DM/cow/d PreP and PostP, respectively. Diets were isoenergetic, and provided the same lysine supply (9.73 PreP and 7.10% of MP PostP, respectively), with a Lys:Met ratio of 3.5 vs 2.9 for CON and RPM PreP, and 3.4 vs 2.7 for CON and RPM PostP. Cows were allocated to pens (1 pen per group PreP, and 6 pens of 16 cows per group PostP), and the diets were fed to pens. Milk samples were collected at wk 1 and 2, and then biweekly until 14 weeks PostP. Data were analyzed by ANOVA with repeated measurements using PROC MIXED of SAS, with treatment (trt), pen(trt), week and trt by week as fixed effects and cow(pen trt) as a random effect. Supplementing RPM PreP and PostP increased milk true protein percentage from wk 2 to 14 (2.9 vs. 3.0%; *P* < 0.01), milk fat percentage from wk 8 to 14 (3.5 vs. 3.7%; *P* < 0.01), total solids percentage from wk 6 to 14 (12.2 vs. 12.5%; *P* < 0.01), milk urea nitrogen for all 14 weeks (8.42 vs. 8.82 mg/dl; *P* < 0.01), and tended to increase true protein yield (1.44 vs. 1.48 kg/d; *P* = 0.09). Feeding RPM tended to reduce milk lactose percentage (4.84 vs. 4.80%; *P* = 0.07). No effect was observed on MY (48.5 vs. 47.9 kg/d; *P* = 0.61), energy corrected milk (ECM, 49.9 vs. 50.5 kg/d; *P* = 0.31), milk fat yield (1.85 vs. 1.90 kg/d; *P* = 0.12), milk lactose yield (2.37 vs. 2.33 kg/d; *P* = 0.30), and total solids yield (6.14 vs. 6.17 kg/d; *P* = 0.61) for CON and RPM, respectively. Supplementing RPM pre- and postpartum improved lactation performance by increasing true protein percentage and yield, fat percentage, and total solids percentage, but did not affect total MY or ECM.

Key Words: methionine, milk production, protein

M292 Methane mitigation with corn oil and calcium sulfate, responses on whole animal energy and nitrogen balance in dairy cattle consuming reduced-fat distillers grains plus solubles. J. V. Judy*¹, T. M. Brown-Brandl², S. C. Fernando¹, and P. J. Kononoff¹, ¹University of Nebraska-Lincoln, Lincoln, NE, ²USDA, ARS, US Meat Animal Research Center, Clay Center, NE.

Addition of fat and calcium sulfate to diets fed to ruminants has been shown to reduce methane production, but these factors have not shown effects on energy balance. A study using indirect calorimetry and 16 multiparous (8 Holstein and 8 Jersey) (78 ± 15 DIM; mean ± SD) lactating dairy cows was conducted to determine how mitigating methane by adding corn oil or calcium sulfate to diets containing reduced-fat distillers grains, affect energy and nitrogen balance in dairy cattle. A replicated 4 × 4 Latin square design with 35 d periods (28 d adaption and 7 d collections) was used to compare 4 different dietary treatments. Treatments were composed of a control (CON) diet which did not contain reduced-fat distillers grain plus solubles (RFDDGS), and treatment diets containing 20% (DM basis) RFDDGS (DG), 20% RFDDGS with 1.38% (DM basis) added corn oil (CO), and 20% RFDDGS with 0.93% (DM basis) added calcium sulfate (CaS). Addition of CaS reduced (*P* = 0.02) and addition of CO tended (*P* = 0.17) to reduce methane production compared with CON diet (421.6, 429.5, 394.7, and 381.4 ± 14.41

L/d for CON, DG, CO, and CaS respectively). Digestible energy was greater ($P < 0.01$) for DG and CO treatments compared with CON and CaS treatments (57.2, 61.5, 61.4, and 58.5 ± 1.13 for CON, DG, CO, and CaS, respectively). Metabolizable energy was greater ($P < 0.01$) in treatments containing RFDDGS compared with CON (50.5, 54.8, 55.0, and 52.3 ± 1.07 for CON, DG, CO, and CaS, respectively). Net energy of lactation per unit of DMI was greater in DG and CO than CON (1.44, 1.52, and 1.33 ± 0.04 Mcal/kg for DG, CO, and CON, respectively). Tissue energy was greater ($P = 0.05$) in DG and CO compared with CON (5.51, 6.48, and 2.71 ± 0.98 for DG, CO, and CON, respectively). Nitrogen balance was greater ($P = 0.03$) in DG than CO (91.1 vs 56.6 g/d for DG and CO, respectively). Added oil and calcium sulfate to diets containing RFDDGS may be a viable option to reduce methane emissions without affecting energy balance in lactating dairy cows.

Key Words: dried distillers grains and solubles, energy, methane

M293 Calves fed with oregano and green tea extracts alter slightly their blood redox status. V. Fischer¹, M. de Paris¹, S. C. B. Stivanin¹, E. F. Vizzotto¹, M. B. Zanela², C. Klein¹, V. Stone¹, and C. Matte¹, ¹Universidade Federal do Rio Grande do Sul, Porto Alegre, RS, Brazil, ²Empresa Brasileira de Pesquisa Agropecuaria, Pelotas, RS, Brazil.

This work aimed to investigate the effect on antioxidant status in dairy calves fed milk with *Origanum vulgare* or *Camellia sinensis* extracts. Twenty-four calves were randomly assigned to one of the following treatments: control (CO, no plant extract), green tea extract (GTE, 30 mg/kg BW/day) and oregano extract (OE 60mg/kg BW/day) from birth to weaning. Calves received 4 L/d of milk from birth to 60 d of life when they were weaned. Plant extracts were added into the milk immediately before feeding. Blood samples were collected by jugular vein puncture on d 1, 30 and 60 of life and further analyzed for redox status parameters. Data were submitted to variance analysis, using the Mixed procedure of SAS according to a completely randomized design, considering the effect of diet (n = 3), period (n = 3), diet by period interaction, calves as random effect and days as repeated measures. Overall, calves of CO group showed higher values for glutathione peroxidase (GPx) activity than calves of GTE and OE groups. Calves of the OE group tended to show higher values for superoxide dismutase (SOD) activity than calves of GTE group. There were no differences for the remaining parameters evaluated between groups. On d 30 calves from groups CO tended to show higher values for dichlorofluorescein oxidation in the plasma (DCFP) than GTE and OE groups (2678, 2201, 2067 nmol/mg of protein, $P < 0.10$), but on d 60, these results were reversed as calves of CO group showed lower values for DCFP than calves of GTE and OE groups (2111, 3242, 3468 nmol/mg, $P < 0.05$). Calves

fed milk with green tea or oregano extracts show little changes in the blood redox status.

Table 1 (abstract M293).

Item	Means (SE)		
	Control	Green Tea	Oregano
SOD ¹	26.2 ± 1.63 ^{cd}	23.3 ± 1.68 ^d	28.8 ± 1.86 ^c
CAT ¹	3.45 ± 0.28 ^a	3.35 ± 0.29 ^a	3.31 ± 0.32 ^a
GPX ²	9.84 ± 0.7 ^a	7.38 ± 0.7 ^b	7.03 ± 0.8 ^b
GSH ²	0.07 ± 0.02 ^a	0.095 ± 0.02 ^a	0.05 ± 0.02 ^a
Carbonyl ²	1.93 ± 0.16 ^a	1.74 ± 0.16 ^a	2.02 ± 0.18 ^a
Thiols ²	0.63 ± 0.03 ^a	0.66 ± 0.03 ^a	0.70 ± 0.04 ^a

^{a,b} or ^{c,d}Means in the same row followed by different letters differ (Lsmeans, $P < 0.05$) or tend to differ ($P < 0.10$).

¹In U/mg.

²In nmol/mg.

Key Words: plant extract, pre-weaning calf, redox

M294 Effects of a pulse dose of propionate on metabolic response in lactating dairy cows during the postpartum period. K. M. Kennedy* and M. S. Allen, Michigan State University, East Lansing, MI.

Our long-term hypothesis is that hepatic oxidation of acetyl CoA is stimulated by anaplerosis of the tricarboxylic acid (TCA) cycle by propionate, causing an increase in hepatic energy charge. The objective of this study was to determine potential bottlenecks associated with propionate metabolism. Six cows (4 to 18 d postpartum) were used in a crossover design (3 d with 1 d rest). Cows were blocked from feed 1 h before treatment and received a pulse dose to the rumen of either 500 mL of water (control) or 2.0 moles of propionic acid (PA) in a 500 mL solution. Plasma and liver samples were collected immediately before dosing (T0) and at 10 (T10) and 20 (T20) min post-dosing. Liver samples were analyzed for acetyl CoA (A-CoA), propionyl CoA (P-CoA), methylmalonyl CoA (M-CoA), succinyl CoA (S-CoA), succinate, fumarate, and malate and plasma was analyzed for propionate. Samples were standardized relative to T0 (T0 = 0). Data were analyzed with the Proc Mixed procedure in SAS (v.9.4). The PA treatment increased plasma propionate at T10 compared with control but propionate declined rapidly (interaction $P = 0.04$). PA tended to decrease A-CoA, did not affect P-CoA, tended to increase M-CoA and S-CoA, and increased succinate, fumarate and malate. Although interactions of treatment and time were not detected for liver metabolites, M-CoA and S-CoA were greater at T20 than T0 ($P = 0.01$ for both) and numerically greater than T10 indicating possible bottlenecks for metabolism of M-CoA and S-CoA.

Table 1 (abstract M294).

Metabolite	Control		2 mol of PA		SE	P-value		
	T10	T20	T10	T20		Trt	Time	Trt × Time
Plasma propionate	-0.001	0.170	5.690	1.545	1.111	<0.01	0.05	0.04
A-CoA	0.006	0.214	-0.475	0.063	0.202	0.08	0.04	0.341
P-CoA	1.232	0.206	1.228	0.271	0.779	0.96	0.14	0.96
M-CoA	-0.163	-0.191	0.265	1.655	0.588	0.07	0.27	0.25
S-CoA	-0.223	-0.319	0.132	1.406	0.502	0.06	0.26	0.19
Succinate	-0.048	0.004	0.247	0.488	0.152	<0.01	0.14	0.32
Fumarate	-0.021	-0.162	0.419	0.349	0.149	<0.01	0.49	0.81
Malate	0.013	-0.130	0.479	0.410	0.188	0.01	0.54	0.83

M-CoA requires vitamin B₁₂ and its supplementation may improve efficiency of propionate metabolism by alleviating that bottleneck. Our research identified M-CoA and S-CoA as possible metabolic bottlenecks to examine in future work.

Key Words: propionate, metabolism, hepatic oxidation

M295 Milk and methane production in lactating dairy cattle consuming distillers dried grains and solubles or canola meal.

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The use of co-products as an alternative feed source is a common practice when formulating dairy rations. A study using 12 multiparous (79 ± 16 DIM; mean ± SD) lactating Jersey cows, was conducted to evaluate the effects of dried distillers grains with solubles (DDGS) or canola meal (CM) on milk and methane production. A replicated 4 × 4 Latin square design was used to compare 4 different dietary treatments over 4 periods. Each of the 4 periods utilized 23 d for adaptation with 5 d of collection. Treatments were composed of a control (CON) containing corn and soybean meal and no co-products, a treatment diet containing 10% (DM basis) DDGS (DDGS), 10% DDGS treatment with an alternative distillers grains source (ADDGS), and a 10% canola meal (CAN) treatment. Co-products were included in partial replacement for corn and soybean meal. Indirect calorimeters were used to sample methane. Dry matter intake and milk yield were similar ($P > 0.55$) between all treatments averaging 17.5 ± 0.78 kg/d and 24.1 ± 0.80 kg. Milk protein and fat percentage was similar across treatments ($P \geq 0.43$) averaging 3.64 ± 0.04% and 6.18 ± 0.17%, respectively. Compared with CON, the addition of DDGS, CAN, or ADDGS did not affect total methane produced ($P = 0.54$) averaging 340.2 ± 19.59 L/d. When expressing methane per unit of DMI, all treatments were similar ($P = 0.75$) averaging 19.6 ± 1.26 L/kg/d. Heat production per day of metabolic body weight tended to be lowest in CON ($P = 0.06$) followed by DDGS, ADDGS and CAN (203.83, 210.73, 215.23, 227.70 ± 6.27 heat production/d/MBW, respectively). Milk urea nitrogen (MUN) was also affected by treatment ($P < 0.01$), CON and CAN were not different (20.7 and 19.9 ± 0.62 mg/dl, respectively) and DDGS and ADDGS were not different (18.1 and 18.1 ± 0.62 mg/dl, respectively). Results of this study indicate that milk production; milk components and methane production are not adversely affected when feeding the current inclusion rate of common co-products replacing both corn and soybean meal.

Key Words: dried distillers grains and solubles, methane, canola meal

M296 Increasing the diet concentrations of fat and hemicellulose on energy utilization and methane production in lactating Jersey cattle.

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Feeding fat to lactating dairy cows may decrease methane production additionally relative to cellulose fermentation of hemicellulose is believed to result in less methane. However, these factors have not been studied simultaneously. Eight multiparous, lactating Jersey cows averaging 98 ± 30.8 DIM and BW of 439.3 ± 56.7 kg were used in a twice replicated 4 × 4 Latin square to determine the effects of fat

and hemicellulose individually and together on energy utilization and methane production using a headbox type indirect calorimetry method. To manipulate the concentration of fat, porcine tallow was included at either 0 or 2% of the diet DM. The concentration of hemicellulose was manipulated by changing the inclusion rate of corn silage, alfalfa hay, and soybean hulls resulting in either 11.3% or 12.7% hemicellulose (DM basis). The factorial arrangement of treatments were low fat low hemicellulose (LFLH), low fat high hemicellulose (LFHH), high fat low hemicellulose (HFLH), and high fat high Hemicellulose (HFHH). Neither fat nor hemicellulose affected DMI ($P \geq 0.25$) averaging 16.2 ± 1.18 kg/d across treatments. Likewise, treatments did not affect ($P \geq 0.51$) milk production averaging 23.0 ± 1.72 kg/d. The inclusion of fat tended ($P = 0.10$) to decrease methane produced per kg of DMI from 24.8 to 22.7 ± 1.61 L/kg while hemicellulose had no effect ($P = 0.37$). Increasing hemicellulose increased ($P = 0.01$) NDF digestibility from 40.6 to 50.3 ± 2.91%. Methane per unit of digested NDF tended to decrease ($P = 0.11$) from 64.4 to 46.9 ± 0.70 L/kg with increasing hemicellulose while fat had no effect ($P = 0.70$). An interaction between hemicellulose and fat content on net energy intake was observed. Specifically, increasing hemicellulose in low fat diets tended ($P = 0.08$) to increase net energy intake but this was not observed in high fat diets. Results confirm methane production may be decreased with the inclusion of fat while energy intake of lactating dairy cows is improved by increasing hemicellulose in low fat diets.

Key Words: fat, hemicellulose, indirect calorimetry

M297 Increasing the concentration of linolenic acid in diets fed to Jersey cows in late lactation does not affect methane production.

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Although the inclusion of fat has shown to reduce methane production in ruminants, relatively little research has been conducted on comparing the source and profile of fatty acids on methane production in lactating dairy cows. A study using 8 multiparous (325 ± 17 DIM) (mean ± SD) lactating Jersey cows was conducted to determine effects of feeding canola/tallow vs. extruded byproduct containing flaxseed as a fat source on methane emissions and diet digestibility in late lactation dairy cows. A crossover design with 35 d periods (28 d adaption and 7 d collections) was used to compare 2 different fat sources. Diets contained approximately 50% forage mixture of corn silage, alfalfa hay, and brome hay with only the concentrate mixture changing between diets to include either 1) a conventional corn/soybean meal/canola meal with tallow, or 2) a conventional corn/soybean meal diet with an extruded byproduct containing flaxseed (EXF) as the fat source. Diets were balanced to decrease corn and canola meal and replace them with EXF to increase linolenic acid supply (31.2 vs 201.6 g/d) to the rumen. Methane production was measured using headbox-style indirect calorimeters. Milk production was similar ($P = 0.38$; 17.4 ± 1.04 kg/d) as well as DMI ($P = 0.26$; 15.4 ± 0.71 kg/d) across treatments. Milk fat was similar ($P = 0.87$; 5.88 ± 0.25%) and milk protein ($P = 0.69$; 4.08 ± 0.14%) across treatments. For methane, production was similar ($P = 0.90$) for total production (352.0 vs. 349.8 ± 16.43 L/d for CM vs. EXF, respectively). Methane production per unit of DMI was similar ($P = 0.34$) and averaged 10.5 ± 0.57 L/kg. Similarly, methane production per unit of energy corrected milk was similar ($P = 0.30$) for fat source and averaged 7.01 ± 0.68 L/kg. Heat production was similar ($P = 0.98$) averaging 21.1 ± 1.02 Mcal/d. Digestibility of NDF, CP, DM, OM, and starch were similar ($P \geq 0.22$) by diet and averaged 53.6, 73.3, 67.5, 69.9 and 96.1 for NDF,

CP, DM, OM, and starch, respectively. Results indicate that increasing C18:3 may not affect methane emissions or digestibility of the diet.

Key Words: linolenic acid, methane, milk

M298 The effects of feeding a high- or low-plane of milk pre-weaning on IGF-1 and IGFBP in dairy heifers. J. Haisan^{*1}, M. Oba¹, D. J. Ambrose², and M. A. Steele¹, ¹University of Alberta, Edmonton, AB, Canada, ²Livestock Research Section, Agriculture and Forestry, Edmonton, AB, Canada.

The objective of this experiment was to determine the effects of a high-plane of milk before weaning on plasma insulin-like growth factor 1(IGF-1) and insulin-like growth factor binding proteins (IGFBP) concentrations and their relationship in group-housed calves. Twenty-six female Holstein calves were fed 4 2-L feedings of colostrum for the first 2 d after birth and randomly assigned to either a HIGH (10 L/d; n = 13) or LOW (5 L/d; n = 13) plane of milk. All calves were allowed 2.5L of pasteurized whole milk per meal until 7 weeks of life before a 10-d weaning transition began, where milk was reduced by 10% per day, resulting in all calves being weaned at 9 weeks. Calf starter and water were provided ad libitum throughout. Pre-weaning average daily gain was 0.90 kg/d for HIGH and 0.65 kg/d for LOW treatments, however, no difference was seen post-weaning. Blood samples were taken starting at 0830 h at 1, 3, 5, 7 and 9 weeks of life and analyzed for IGF-1 and IGFBP-2, -3, -4 and -5. Pre-weaning, IGF-1 concentrations were greater for HIGH than LOW (198.96 vs. 120.39 ng/mL, $P < 0.01$), but did not differ post-weaning. Plasma levels of IGFBP-2 were lower in HIGH calves pre-weaning than in LOW (483.64 vs. 714.86 ng/mL, $P < 0.05$), with no difference post-weaning. There was no effect of treatment on concentrations of IGFBP-3, -4 or -5 pre- or post-weaning. However, a treatment by week interaction existed during the pre-weaning period for IGFBP-3. Overall, from wk 1 to 9, a treatment by week interaction existed for IGFBP-2 and IGFBP-3, and the ratio of IGFBP-3 to IGFBP-2. These results suggest that a high plane of nutrition pre-weaning increased IGF-1 concentration before the weaning transition, and exerts effects on plasma concentrations of IGFBP.

Key Words: IGF, IGFBP, dairy calf

M299 Interactions between levels of flaxseed oil and corn grain particle size on milk fatty acid profile in Jersey cows. V. Brossillon¹, A. F. Brito^{*2}, S. F. Reis², D. C. Moura³, J. G. B. Galvão Jr.⁴, C. Côrtes¹, and A. S. Oliveira⁵, ¹Ecole Supérieure d'Agricultures, Angers, France, ²University of New Hampshire; Department of Biological Sciences, Durham, NH, ³Programa de Pós Graduação em Ciência Animal; Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil, ⁴Instituto Federal de Educação do Rio Grande do Norte,

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Flaxseed oil (FO) is the richest source of the essential n-3 fatty acid (FA) α -linolenic acid (ALA). We hypothesize that corn of 2 particle sizes [cracked corn (CC) vs. ground corn (GC)] and different degradation rates could interact with FO leading to shifts in ruminal biohydrogenation (BH) pathways that ultimately affect milk fatty acids (FA). Sixteen mid-lactation organically certified Jersey cows were randomly assigned to treatments in a replicated, factorial 4 \times 4 Latin square design with 24-d periods. Treatments were fed as TMR (55:45 forage-to-concentrate ratio) consisting (DM basis) of (1) 0% FO + 28.3% CC, (2) 0% FO + 27.1% GC, (3) 3% FO + 28.3% CC, and (4) 3% FO + 27.1% GC. Diets averaged 19.5% starch, and 4.9 and 7.6% ether extract for 0 and 3% FO, respectively. Corn grain mean particle size averaged 2,047 μ m (CC) and 580 μ m (GC). Contrasts were used to compare: FO level, corn particle size, and oil \times corn interaction. ALA was the only FA in milk that showed an oil \times corn interaction ($P = 0.02$); cows fed 3% FO + GC showed the greatest proportion of ALA in milk (0.82 g/100 FA), and those fed 0% FO + CC the least (0.55 g/100 g FA). Except for c-9, c-12 18:2, all remaining milk FA increased with feeding 3% vs. 0% FO (Table 1). Increased milk 18-C FA with 3% FO may be explained by increased 18-C FA intake. Compared with cows fed CC, those fed GC had decreased 18:0 and c-9 18:1, and increased c-9, c-12 18:2, which may be linked to differences in FA profile and ruminal BH between corn sources. Overall, changes in milk FA profile appear to be largely influenced by level of FO supplementation or corn grain source, but not by the FO \times oil interaction.

Key Words: corn grain, flax oil, milk fatty acid

M300 Replacing conventional or brown midrib corn silage with brown midrib sudangrass silage in the diets of lactating dairy cows. K. F. Kalscheur^{*} and G. E. Brink, U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.

Forages that use less water, but are high in digestibility, are sought as alternatives to traditional forages such as corn silage. Brown midrib (BMR) sudangrass is an example of alternative forage for corn silage. The objective of this study was to evaluate whether BMR sudangrass silage (SS) can replace 2 types of corn silage with differing fiber digestibilities [conventional (CONV) or BMR corn silage (CS)] in the diets of lactating dairy cows. Forty-eight Holstein cows in mid- to late-lactation were assigned to 1 of 4 treatments in a randomized complete block design. Cows were fed a common covariate diet for 2 weeks followed by 8 weeks of experimental diets. Diets were formulated to contain 40% CS, 20% alfalfa silage, and 40% concentrate on DM basis. Sudangrass silage was included in experimental diets at either 0 or 10% of the diet

Table 1 (abstract M299). Milk FA profile

FA, g/100 g	Flax oil		Corn grain		SEM	Contrasts ($P > F$)	
	0%	3%	Cracked	Ground		Oil	Corn
18:0	12.7	16.4	14.9	14.2	0.45	<0.001	<0.01
c-9 18:1	15.3	18.7	17.4	16.6	0.33	<0.001	<0.001
c-9, c-12 18:2	2.02	1.83	1.87	1.98	0.05	<0.001	<0.01
ALA	0.57	0.76	0.63	0.70	0.02	<0.001	<0.001
t-10 18:1	0.19	0.32	0.26	0.26	0.01	<0.001	0.90
t-11 18:1	1.03	1.82	1.43	1.42	0.09	<0.001	0.99
c-9, t-11 18:2	0.36	0.57	0.47	0.46	0.02	<0.001	0.72

DM replacing either 10% CONV or BMR CS. All other ingredients (high moisture corn, canola meal, roasted soybeans, soyhulls, and minerals and vitamins) were included equally for all diets. Cow was the experimental unit. Dry matter intake (DMI) averaged 25.2 kg/d and was not affected by the type of CS used nor by the inclusion of SS in the diets ($P > 0.05$). Similarly, milk production (averaged 40.0 kg/d) and was not affected by type of CS nor SS inclusion. Milk fat percentage increased 0.15% for cows fed the addition of 10% SS compared with cows fed 0% SS. Milk protein, lactose, and total solids percentage was not affected by dietary treatments. Milk protein yield was greater (0.054 kg/d; $P = 0.03$) for cows fed 0% SS compared with cows fed 10% SS. Because the dietary CP% was slightly greater for diets containing 10% SS compared with 0% SS (17.2 vs 16.2%), MUN responded similarly (11.1 vs. 9.6 mg/dL; $P = 0.001$). Energy-corrected milk (ECM) and feed efficiency (defined as ECM/DMI) was not affected by changes in diet because of similar intake and performance. Overall, the inclusion of 10% SS as a replacement for either CONV or BMR CS did not negatively affect lactation performance. BMR SS can be a successful replacement for CS where CS availability is limited.

Key Words: BMR sudangrass, corn silage, milk production

M301 The role of fat in distillers grains and solubles on the rumen bacterial community. E. Castillo-Lopez^{*1}, C. Jenkins², N. Aluthge², T. Wesley², S. Fernando², and P. Kononoff², ¹Universidad Nacional Autonoma de Mexico-FESC, Cuautitlan, Estado de Mexico, Mexico, ²University of Nebraska-Lincoln, Lincoln, NE.

This study evaluated the effects of feeding dried distillers grains with solubles (DDGS) or reduced-fat DDGS (RFDG) on the rumen bacterial community composition. Four ruminally cannulated Holstein dairy cows were used in a 4 × 4 Latin square over 28-d experimental periods. Treatments (DM basis) were (1) CONT, a diet with no distillers grains; (2) DG, inclusion of 20% DDGS; (3) rFDG, inclusion of 20% RFDG; and (4) MIX, inclusion of 10% DDGS and 10% RFDG. The DDGS and RFDG were included by replacing corn grain and soybean meal. At the end of each period, the rumen bacterial community was evaluated by sequencing the 16S rRNA gene. The phylum *Bacteroidetes* decreased and *Firmicutes* tended to increase when distillers grains and solubles were fed. No treatment effect was observed on the predominant bacterial families *Prevotellaceae* ($P = 0.51$), *Lachnospiraceae* ($P = 0.67$), *Veillonellaceae* ($P = 0.69$), *Spirochaetaceae* ($P = 0.45$) and *Paraprevotellaceae* ($P = 0.87$) with averages across treatments of 29.37 ± 1.430%, 7.23 ± 0.367%, 5.89 ± 0.963%, 3.04 ± 0.218%, and 2.21 ± 0.151%, respectively. However, the abundance of the genus *Ruminococcus* tended ($P = 0.06$) to increase with estimates of 1.72 and 2.22 ± 0.230% for CONT and treatments containing distillers grains and solubles, respectively. In addition, the abundance of the genus *Bulleidia* increased ($P < 0.01$) with estimates of 0.35 and 0.46 ± 0.027% for CONT and treatments containing distillers grains and solubles, respectively. The archaeal genera *Methanobrevibacter* ($P = 0.86$) and *Methanosphaera* ($P = 0.85$) were not affected by treatment and averaged 1.64 ± 0.234% and 0.11 ± 0.023%, respectively, across treatments. Overall, this study demonstrates that DDGS or RFDG can be used to replace corn and soybean meal in dairy rations; however, in doing so, the abundance of certain bacterial taxa within the rumen are shifted.

Key Words: distillers grains, Illumina DNA sequencing, ruminal microbiome

M302 Effects of selected feed additives to improve growth and health of dairy calves. L. Salazar¹, C. Cortinhas^{*2}, T. Acedo²,

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We aimed to evaluate the effect of supplementation of monensin (MON), probiotics or essential oils on performance and health of suckling dairy calves from 6 to 60 d of age, and its residual effect 15 d after weaning. Fifty Holstein calves were fed 5 L/d of raw milk and starter feed. The products were provided by DSM Brasil SA, and the treatments were control, MON (30 mg/kg of starter), probiotic *E. faecium* (PROB, 70 mg/kg of starter, cfu/kg 7.0E+09), essential oils (EO, 300 mg/kg of starter; composed by thymol, guaiacol, eugenol, vanillin, salicylaldehyde and limonene), and EOPROB (treatments PROB + EO). The DMI and fecal score (scored from 1 to 4) were daily measured, and animals were weighed every 15 d. Two 48 h digestibility trials were performed at d 20–28 (period 1) and 50–56 (period 2), with total feces collection. The experiment was designed in completely randomized blocks with 10 replications, and date of entrance was used as block. Treatment effects were tested using ANOVA and means were compared by Student's *t*-test at $P < 0.10$. In digestibility trials, periods were included as repeated measures. The DMI before weaning was greater for EO (903.03 g/d) compared with MON (794.34 g/d) and EOPROB (783.12 g/d) ($P < 0.05$). The EO ($P < 0.026$) and MON ($P < 0.010$) improved health and decreased the incidence of diarrhea demonstrated by the lower fecal score. Average daily gain (ADG) did not differ among treatments ($P > 0.10$) before weaning. After weaning, EO had greater ADG (917.50 g/d) compared with control (615.80 g/d) and PROB (592.60 g/d). Feed efficiency of EO (0.72 g/g) improved over control (0.36 g/d), MON (0.49 g/d) and PROB (0.36 g/g). During the digestibility trials, greater intakes of DM (1097.80 g/d), CP (237.91 g/d), and NDF (268.59 g/d) were observed during period 2 compared with period 1 ($P < 0.001$). Animals fed PROB had greater intakes of DM ($P < 0.022$; 1184.56 g/d), CP ($P < 0.022$; 254.63 g/d) and NDF ($P < 0.030$; 320.36 g/d) than animals fed EOPROB. Digestibility of NDF was greater in animals fed MON ($P < 0.012$). In summary, EO can replace MON to improve the health of young dairy calves and can be used as an alternative to prevent diarrhea.

Key Words: essential oils, probiotic, dairy calf

M303 The effects of varying undigested NDF and physically effective NDF content of fresh cow rations on metabolism in multiparous Holstein cows. S. E. LaCount^{*}, B. M. Leno, C. M. Ryan, and T. R. Overton, Department of Animal Science, Cornell University, Ithaca, NY.

The objective of this study was to determine the effect of varying undigested NDF at 240 h (uNDF₂₄₀) and physically effective NDF (peNDF) content of fresh cow rations on metabolism. Multiparous Holstein cows ($n = 56$) were fed a common prepartum ration beginning 28 d before expected parturition and assigned randomly at calving to 1 of 2 postpartum diets differing in content of uNDF₂₄₀ and peNDF. High fiber (HF; 35.3% NDF, 12.1% uNDF₂₄₀, 25.0% peNDF; $n = 27$) and low fiber (LF; 32.8% NDF, 9.5% uNDF₂₄₀, 21.4% peNDF; $n = 29$) treatment diets were formulated for equivalent metabolizable protein (110 g/kg DM) and starch (24.8% DM), with higher fiber levels achieved through the addition of straw. At 29 DIM, cows fed HF were switched to the LF diet and all cows were fed the LF diet through 42 DIM. Blood samples were collected 2×/wk prepartum, daily from d 0 through 7 DIM, 3×/wk through 21 DIM and 2×/wk to 42 DIM. Liver biopsies were obtained from a subset of 40 cows on d 7 ± 1.1 (mean ± SD) and 14 ± 1.0 postpartum and incubated in an *in vitro* system to determine liver capacity to convert [¹⁻¹⁴C] propionate and [¹⁻¹⁴C] palmitic acid to end products. Data were analyzed by repeated measures ANOVA with the

random effect of cow within treatment and fixed effects of treatment, time, and treatment \times time. A treatment \times time effect was observed for plasma NEFA and was higher for cows fed HF particularly from 21 to 31 DIM ($P = 0.01$), plasma β -hydroxybutyrate was higher for cows fed HF from 12 to 31 DIM ($P < 0.01$), and plasma glucose was lower for cows fed HF from 9 to 27 DIM ($P < 0.01$) compared with cows fed LF. Cows fed LF tended to have greater liver oxidation of palmitate to CO_2 (12.23 nmol/(g·h) vs. 10.94 nmol/(g·h); $P = 0.15$) and lower conversion to esterified products (226.9 nmol/(g·h) vs. 248.2 nmol/(g·h); $P = 0.10$) than cows fed HF. Conversion of palmitate to acid soluble products was not different between treatments and no effects on in vitro liver propionate metabolism were observed. Changes in plasma metabolites and liver fatty acid metabolism were consistent with lower dry matter intake of cows fed the HF diet.

Key Words: transition cow, fiber, metabolism

M304 Pre- and post-weaning performance and health of dairy calves fed complete pelleted calf starters formulated for three different starch levels. D. Ziegler^{*1}, H. Chester-Jones¹, B. Ziegler², and S. Schuling², ¹University of Minnesota, Waseca, MN, ²Hubbard Feeds Inc, Mankato, MN.

One-hundred twelve (2 to 5 d old) individually fed Holstein heifer calves (38.6 \pm 0.56 kg) from 3 commercial dairies were randomly assigned to 1 of 4 calf starter (CS) treatments formulated for varying starch levels to evaluate pre- (d 1–42) and post weaning (d 43–56) calf performance and health. The study was conducted between August and October, 2016. All treatment diets included a non-medicated 20% CP:20% fat milk replacer (all milk protein) fed at 0.28 kg in 2 L of water 2 \times daily from d 1 to d 35 and 1 \times daily from d 36 to weaning at d 42; supplemented daily with neomycin sulfate and oxytetracycline at 22mg/kg BW for 14 d. Calf starter treatments were as follows 1) texturized starter (TS) formulated for 30% starch DM basis, (TS30); 2) complete pelleted starter (CPS) formulated for 18% starch DM basis, (CPS18); 3) CPS formulated for 24% starch DM basis, (CPS24); 4) CPS formulated for 30% starch DM basis, (CPS30); Water and CS were offered free choice from d 1 to 56. Pre-weaning (d 1–42), 0.55 vs. 0.48 kg/d and post weaning gains (d 43–56) 1.03 vs. 0.83 kg/d were greater for calves fed TS30 ($P < 0.05$) vs. CPS18, CPS24 and CPS30. Overall 56 d gain was greater for TS30 vs. CPS18, CPS24 and CPS30, 0.67 vs. 0.57 kg/d. Gain/feed (d 1–42) was greater for TS30 vs. CPS18, CPS24 and CPS30, 0.62 vs. 0.56 kg/d ($P < 0.05$). Overall gain/feed (d 1–56) was greater for TS30 vs CPS18, CPS24 and CPS30, 0.57 vs. 0.52 kg/d. There were no differences in daily fecal scores or health costs. Under conditions of this study, calf performance was reduced with a CPS regardless of starch level compared with TS30. Cost savings with a CPS may still provide economical gains over TS30.

Key Words: calf performance, calf starter, starch.

M305 Total fatty acid and rumen unsaturated fatty acid load variation in commercial TMR, forages, and corn grain. J. P. Goeser^{*1,2}, J. Karlen¹, D. Meyer¹, and A. L. Lock³, ¹Rock River Laboratory Inc., Watertown, WI, ²University of Wisconsin-Madison, Madison, WI, ³Michigan State University, Lansing, MI.

Risk of dietary-induced milk fat depression and troubleshooting low milk fat tests may be partially alleviated when the fatty acid (FA) content and profiles for individual feeds are known. Our first objective was to expand on feed library FA data by describing population statistics for total FA content (TFA, % DM) and rumen unsaturated FA load (RUFAL) for commercial dairy farm feeds. Our second objective was to determine if knowing TFA and feed type was sufficient to predict RUFAL (% DM and % TFA). Commercial farm legume and/or grass hay (n = 69) and silage (n = 129), corn silage (n = 115), corn grain (n = 35), and small grain silages (n = 46) were selected for FA analyses from samples submitted from across the US for routine nutritional analysis based upon near-infrared spectral diversity. Total FA concentration and profile was determined by gas-liquid chromatography. TFA (% DM) was calculated by summing individual FA concentrations and RUFAL determined by summing unsaturated 18-carbon FA. RUFAL was expressed as both % DM and % TFA to explore the variation across feeds and within FA content. Data were analyzed by SAS JMPPro v11. RUFAL (% TFA and % DM) was related to TFA and feed type using backward elimination. Population statistics for TFA and RUFAL are presented in Table 1. RUFAL (% DM) was related to TFA (linear and quadratic effects), feed, a TFA interaction with feed (each with $P < 0.001$; model adj. $R^2 = 0.99$). RUFAL (% TFA) was related to TFA (linear and quadratic effects) and feed (each with $P < 0.001$; model adj. $R^2 = 0.77$). The coefficient of variation (percentage; Table 1) suggest that TFA varies greater than FA profile. Results suggest that feed TFA (% DM) can predict RUFAL (% DM), however relationships may not be linear and are feed dependent.

Key Words: milk fat, fatty acid, nutrition

M306 Feeding rumen-protected methionine pre- and postpartum increases milk protein content and yield in early-lactation. M. Z. Toledo^{*1}, R. S. Gennari¹, R. V. Barletta¹, P. L. J. Monteiro Jr.¹, C. A. Gamarra¹, A. B. Prata¹, J. R. R. Dorea¹, A. Jones¹, D. Luchini², G. I. Zanton³, M. E. Van Amburgh⁴, J. O. Giordano⁴, R. D. Shaver¹, and M. C. Wiltbank¹, ¹University of Wisconsin-Madison, Madison, WI, ²Adisseo, Alpharetta, GA, ³USDA, US Dairy Forage Research Center, Madison, WI, ⁴Cornell University, Ithaca, NY.

Objectives were to evaluate the effects of feeding rumen-protected methionine (MET) from 23 d (\pm 12) before calving until 98 DIM on lactation performance and DMI of dairy cows. Multiparous Holstein cows (n = 223) were enrolled before calving and housed in replicated close-up (n = 4; 10 cows each) and lactation pens (n = 6; 16 cows each) in a free-stall barn, milked 2 \times daily, pen-fed a basal diet formulated to contain 14.5% and 16.2% CP in close-up and lactation periods, respectively and

Table 1 (abstract M305). Commercial farm forage and grain population descriptive statistics

Feed	TFA mean			RUFAL mean			RUFAL mean	
	(%DM)	SD	CV	(%TFA)	SD	CV	(%DM)	SD
Legume/grass hay	0.92	0.26	27.9	52.3	7.03	13.5	0.49	0.17
Legume/grass silage	1.26	0.51	40.3	63.8	10.0	15.7	0.84	0.42
Corn silage	1.54	0.32	20.6	75.2	4.62	6.10	1.18	0.29
Corn grain	2.89	0.67	23.1	84.4	1.76	2.09	2.45	0.59
Small grain silage	1.11	0.40	35.6	64.7	9.17	14.2	0.74	0.32

randomly assigned to either (1) MET, fed 12 g of Smartamine M mixed with 215 g of distillers grains (DDGS) with 1441 g of metabolizable protein (MP), 6.86% Lys and 2.67% Met and 26 g of Smartamine M mixed with 201 g DDGS, 3300 g of MP, 6.72% Lys and 2.56% Met as % of MP, close-up and lactation periods, respectively; or (2) Control (CON), fed 227 g of DDGS either close-up and lactation, with 1432 g of MP, 6.89% Lys and 2.15% Met and 3292 g MP, 6.75% Lys and 2.08% Met as % of MP, close-up and lactation periods, respectively, with mixes that were incorporated into TMR. Milk yields were recorded daily and milk composition and milk components yields determined weekly. For lactation pens, DMI was recorded daily and the average of the period when pens were full was used for the analysis. The statistical models contained pen as the experimental unit and DMI and production variables were analyzed by a linear mixed model and by a linear mixed model with repeated measures, respectively, using PROC MIXED of SAS. Feeding MET increased milk protein percentage (2.82% vs. 2.95%, $P < 0.001$) and yield (1.39 kg/d vs. 1.44 kg/d, $P = 0.04$), with no difference in ECM, 3.5% FCM, fat, lactose, somatic cells count and milk urea nitrogen. The MET treatment had no effect on DMI (27.0 vs 27.2 kg/d, $P = 0.67$) or milk yield (50.0 kg/d vs. 49.3 kg/d, $P = 0.33$). Feeding MET pre- and postpartum improved lactation performance by increasing milk protein percentage and yield, however, DMI, milk yield and other milk components were unaffected by treatment.

Key Words: methionine, transition cow, milk protein

M307 Effects of temporal supply of propionic acid on feeding behavior of cows in the postpartum period. G. Maldini^{*1,2} and M. S. Allen¹, ¹Michigan State University, East Lansing, MI, ²CAPES, Brasilia, DF, Brazil.

Appetite suppression during the postpartum (PP) period is likely caused by a signal related to hepatic oxidation of fuels. Faster absorption and hepatic uptake of propionate is expected to accelerate hepatic oxidation, stimulating satiety sooner, reducing meal size. However, if excessive propionate absorption from the rumen results in less efficient hepatic extraction of propionate, effects on meal size might diminish and hepatic oxidation following meals might be extended decreasing meal frequency. The objective of this study was to determine the temporal effects of propionic acid (PA) infused intraruminally at initiation of meals on feeding behavior. Eight ruminally-cannulated, multiparous cows in the PP period were utilized in a 4x4 Latin square design experiment. Cows were blocked by parturition and randomly assigned to treatment sequence within square. Treatments were infusion of 1.25 (HIGH) or 0.5 mol (LOW) of PA at initiation of meals over 5 min (FAST) or 15 min (SLOW). Infusions were triggered at each meal for 12 h. A 24-h recovery period was allowed between infusion days to reduce carry-over effects of treatment. HIGH decreased DMI compared with LOW (7.2 vs 11.2 kg/12 h, $P = 0.001$) by decreasing meal frequency (5.8 vs 7.5 meals/12 h; $P = 0.02$). HIGH decreased eating time (103 vs 127 min/12 h, $P = 0.002$) and reduced eating rate (52 vs 66 g/min; $P < 0.01$) compared with LOW but did not affect meal size ($P = 0.38$). FAST increased meal length compared with SLOW (28.2 vs 22.7 min; $P = 0.05$) but tended to decrease eating time (109 vs 122 min/12 h; $P = 0.06$) and did not affect meal size ($P = 0.68$) or meal frequency ($P = 0.16$). No interactions of treatments were detected for DMI or feeding behavior parameters ($P > 0.32$). Treatment effects on milk yield were not detected. The lack of effect of infusion rate on meal size, along with the reduction in DMI by HIGH compared with LOW by decreasing meal frequency rather than meal size, suggests that propionate flux to the liver might have exceeded the liver's capacity for first-pass extraction of propionate from

the blood, likely extending hepatic oxidation longer after meals for the higher propionate dose.

Key Words: liver metabolism, feed intake, oxidation of fuels

M308 Comparing choline bioavailability of two rumen-protected choline products using milk betaine as a biomarker in the lactating dairy cow. M. J. de Veth^{*1}, M. Cooney², and P. French³, ¹BioNarus LLC, Cary, NC, ²phdR&D, Fort Atkinson, WI, ³Feed Components LLC, East Troy, WI.

During the periparturient period, supplementation of rumen-protected choline (RPC) has reduced liver triacylglycerol accumulation and improved animal performance in the dairy cow. Recently milk betaine (Bet) was identified as biomarker for choline bioavailability, making it possible to compare the effectiveness of RPC products at delivering choline for absorption. Our objective was to compare the choline bioavailability of 2 commercial RPC products, ReaShure (RES; Balchem Corporation, New Hampton, NY) and Excential Rumenpass (ERP; Orffa, Werkendam, the Netherlands), using milk betaine as a biomarker for choline absorption. Nine lactating Holstein cows (173 \pm 8 DIM) were used in a replicated 5 \times 5 Latin square design (one square incomplete), with 7-d treatment periods and a 3-d interval between periods. Treatments were (1) control (0 g/d choline), (2) 12.5 g/d choline fed as RES, (3) 25 g/d choline fed as RES, (4) 12.5 g/d choline fed as ERP, (5) 25 g/d choline fed as ERP. Choline chloride was the choline form for both products; RES and ERP contained 22.2% and 19.8% choline ion, respectively. Cows were fed twice daily and RPC products mixed with 25% of TMR to ensure treatment consumption. Milk samples from d7 were analyzed for Bet using liquid chromatography-stable isotope dilution-multiple reaction monitoring mass spectrometry. No changes in DMI or milk yield were observed with choline treatment ($P > 0.40$). The concentration and yield of milk Bet increased with ERP relative to RES ($P = 0.02$). The concentration of milk Bet (control – 83.5 μ M) increased linearly ($P = 0.05$) with ERP (84.8 and 99.6 μ M for 12.5 and 25 g/d ERP, respectively). Similarly, the yield of milk Bet (control – 0.42 g/d) increased linearly ($P = 0.02$) with ERP (0.38 and 0.51 g/d for 12.5 and 25 g/d ERP, respectively). No relationship was found between RES dose and milk Bet concentration ($P = 0.44$; 78.1 and 74.8 μ M for 12.5 and 25 g/d RES, respectively) or RES dose and milk Bet yield ($P = 0.76$; 0.38 and 0.38 g/d for 12.5 and 25 g/d RES, respectively). Overall, in this experiment the choline bioavailability of ERP was greater than RES when using milk betaine as a biomarker for choline absorption.

Key Words: choline, bioavailability, dairy cow

M309 Effects of supplementation with a combination of palmitic and stearic acids on dry matter intake, milk yield, and component production: a meta-analysis. M. D. Sellers^{*}, T. L. Harris, and J. R. Lofton, *Milk Specialties Global Animal Nutrition, Eden Prairie, MN.*

To date, a cumulative meta-analysis of the effects of supplementation with a combination of prilled C16:0 and C18:0 fatty acids (PFA) that includes all study designs has not been completed. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and milk component production responses when lactating cows were supplemented with PFA. Data were extracted from 25 peer-reviewed publications and the final data set included 73 treatments, with 39 treatments supplementing PFA and 34 non-supplemented control treatments (CON). Dietary nutrient concentrations (DM%; range) were 17.1% CP [12.0 – 20.1%], 26.1% starch [17.3 – 37.4%], and 33.7% NDF

[27.2 – 48%]. A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between PFA and CON treatments as well as standard errors of the differences between means are reported. The average amount of C16:0 and C18:0 supplementation across studies was 632 ± 222.4 g/d. DMI was not affected by PFA supplementation (22.01 vs 22.07 ± 0.18 kg/d; $P = 0.75$), while net energy intake increased with PFA supplementation (37.59 vs 35.46 ± 0.62 Mcal/d; $P < 0.01$). PFA increased MY (33.78 vs 32.55 ± 0.26 kg/d; $P < 0.01$) and milk fat percentage (3.53 vs 3.45 ± 0.03%; $P < 0.01$). There was no change in milk protein concentration (3.12 vs 3.14 ± 0.02%; $P = 0.34$) and a tendency for decreased milk lactose concentration (4.85 vs 4.89 ± 0.02%; $P = 0.06$) with PFA. Yields of milk fat and milk protein were increased (1.17 vs 1.11 ± 0.01 kg/d and 1.04 vs. 1.01 ± 0.01 kg/d, respectively; $P < 0.01$) with PFA supplementation, while milk lactose yield was unaffected (1.83 vs 1.78 ± 0.03 kg/d; $P = 0.16$). Supplementation with a combination of C16:0 and C18:0 fatty acids resulted in a significant increase in MY, milk fat and milk protein yield and net energy intake, while causing no appreciable decrease in DMI.

Key Words: palmitic acid, stearic acid, supplemental fat

M310 Withdrawn

M311 Effects of supplementation with calcium salts of palm fatty acid distillate on dry matter intake, milk yield, and component production: A meta-analysis. T. L. Harris*, M. D. Sellers, and J. R. Loften, *Milk Specialties Global Animal Nutrition, Eden Prairie, MN.*

A cumulative meta-analysis examining the effects of all calcium salts of palm fatty acid distillate (CS) on production responses that includes all study designs is not available. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and component production in lactating dairy cows supplemented with CS. Data included in the study were extracted from 31 peer-reviewed publications which included 74 treatment means that were supplemented with CS and 65 non-supplemented control (CON) diets. Dietary nutrient concentrations (DM%; range) were 17.4% CP [14 – 23.3%], 32.0% starch [23.4 – 42.2%], and 34.0% NDF [24.8 – 51.7%]. The average feeding amount of CS was 661.7 ± 275.5 g/d. A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between CS and

CON treatments as well as standard errors of the differences between means are reported. Calcium salts decreased DMI (20.04 vs 20.97 ± 0.181 kg/d; $P < 0.01$), however, NE_L intake did not differ among treatments (36.85 vs 35.98 ± 0.550 Mcal/d; $P = 0.11$). Milk yield increased (32.61 vs 31.82 ± 0.260 kg/d; $P < 0.01$) with CS supplementation. Milk fat percentage increased (3.48 vs 3.42 ± 0.023%; $P < 0.01$), milk protein percentage decreased (3.03 vs 3.12 ± 0.011%; $P < 0.01$), and milk lactose percentage did not differ (4.99 vs 4.97 ± 0.021%; $P = 0.40$) with CS supplementation. Yields of milk fat and lactose increased (1.13 vs 1.08 ± 0.012 kg/d; $P < 0.01$; 1.48 vs 1.38 ± 0.014 kg/d; $P < 0.01$; respectively), while protein yield was unchanged (1.00 vs 1.01 ± 0.011 kg/d; $P = 0.45$). Supplementation with CS in lactating cows has mixed effects on production, as evidenced by decreased DMI, milk protein percentage, and milk protein yield and increased MY, milk fat percentage and yield, and milk lactose yield.

Key Words: calcium salt, palm fatty acid distillate, supplemental fat

M312 Replacing ground corn with liquid molasses decreases production performance in dairy cows offered low-starch diets.

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Production performance data regarding the use of liquid molasses (LM) or ground corn (GC) as the sole supplemental NSC source or in different combinations to dairy cows offered low-starch diets are lacking. The objective of this study was to evaluate the effects of feeding incremental amounts of LM on milk yield and composition, and apparent total-tract nutrient digestibility in Jersey cows. Sixteen multiparous Jersey cows (99 ± 41 DIM and 462 ± 38.2 kg of BW) were randomly assigned to treatment sequences in a replicated 4 × 4 Latin square design with 14 d for diet adaptation and 7 d for data and sample collection. Diets were fed as TMR and consisted (DM basis) of 52% grass-legume baleage, 8% grass hay, 8.5% soyhulls, 2.5% roasted soybean, and 15% flaxseed meal. Ground corn was replaced by incremental amounts of LM at 0, 4, 8, or 12% of diet DM. Diets averaged 9.7 and 0.0%, 7.0 and 1.7%, 4.3 and 3.3%, and 1.64 and 5.0% of starch and LM-added sugars, respectively; CP and NDF concentrations averaged 19.0 and 43.5% across diets. Results are presented in Table 1. Intake of DM, as well as MUN and yields of milk and milk fat and protein decreased linearly

Table 1 (abstract M312). Production performance and nutrient digestibility

Item	Molasses:Corn ratio (% diet DM)				SEM	$P > F$	
	0:12	4:8	8:4	12:0		L	Q
DMI, kg/d	19.1	18.6	18.0	17.6	0.68	0.05	0.96
Milk yield, kg/d	18.9	18.0	17.8	16.8	1.44	<0.01	0.94
Milk fat, %	5.28	5.37	5.28	5.25	0.16	0.51	0.42
Milk fat, kg/d	1.01	0.93	0.96	0.84	0.05	<0.01	0.32
Milk protein, %	4.03	4.04	4.02	3.99	0.13	0.31	0.42
Milk protein, kg/d	0.76	0.70	0.72	0.63	0.04	<0.01	0.70
MUN, mg/dL	16.8	16.4	15.8	16.0	0.54	0.04	0.25
OM digestibility, %	72.7	71.2	72.1	72.1	1.06	0.83	0.44
NDF digestibility, %	64.7	64.2	65.0	65.2	1.28	0.67	0.72

with replacing GC with LM. Decreased milk yield is likely explained by the drop in DMI. Conversely, milk fat and protein concentrations, and OM and NDF total-tract digestibilities did not differ across treatments. Overall, it appears that the amount of LM-added sugars, particularly at the greatest level of supplementation depressed ruminal fermentation processes negatively affecting DMI and milk yield.

Key Words: dairy cow, ground corn, molasses

M313 Effects of supplementation with palmitic acid-enriched fat products on dry matter intake, milk yield, and component production: A meta-analysis. M. D. Sellers, T. L. Harris, and J. R. Loften*, *Milk Specialties Global Animal Nutrition, Eden Prairie, MN.*

Recent research has demonstrated unique production responses to various supplemental fat products. Data on effects of supplementation with palmitic acid-enriched fat products (PA) on milk and component responses are limited. The objective of the current analysis was to examine dry matter intake (DMI), milk yield (MY), and milk component production responses in lactating dairy cows supplemented with PA. Data included in the study were extracted from 7 peer-reviewed publications, which included 12 treatment means that were supplemented with PA and 8 non-supplemented control (CON) diets. Dietary nutrient concentrations (DM%, range) were 16.3% CP [15 - 18.7%], 26.9% starch [16.1 - 29.3%], and 31.3% NDF [25.1 - 34.3%]. A random-effects model with the random effect of study was chosen to estimate the mean of the sampling distribution of possible effect sizes, and studies were weighted by the inverse of their variance. Weighted mean differences between PA and CON treatments as well as standard errors of the differences between means are reported. The average feeding amount of PA was 560 ± 92.5 g/d. Dry matter intake was decreased an average of 0.54 kg/d in cows supplemented with PA versus CON (26.07 vs 26.61 ± 0.169 kg/d; $P < 0.01$). Milk yield was not different between PA and CON (37.99 vs 37.76 ± 0.305 kg/d; $P = 0.45$). There were varying milk component responses to feeding PA. Milk fat percentage increased with PA supplementation (3.98 vs $3.74 \pm 0.03\%$; $P < 0.01$), but milk protein percentage (3.15 vs $3.20 \pm 0.015\%$; $P < 0.01$) and milk lactose percentage (4.76 vs $4.81 \pm 0.009\%$; $P < 0.01$) decreased with PA supplementation. Milk fat yield increased with PA supplementation (1.44 vs 1.35 ± 0.01 kg/d; $P < 0.01$), while milk protein yield did not differ (1.20 vs 1.21 ± 0.013 kg/d; $P = 0.28$), and milk lactose yield tended to decrease (1.80 vs 1.82 ± 0.013 kg/d; $P = 0.07$) with PA supplementation. These results indicate that supplementation with highly enriched PA products decreases DMI and does not affect MY. Moreover, PA supplementation increases milk fat concentration and yield, but decreases concentrations of milk protein and lactose.

Key Words: meta-analysis, palmitic acid, supplemental fat

M314 Effect of crude glycerin combined with virginiamycin on rumen metabolism of Nellore bulls fed with finishing diets. P. de Souza Castagnino*, E. E. Dallantonia, E. San Vito, J. D. Messina, G. Fiorentini, G. Penasso, L. O. Lima, and T. A. Simioni, *Universidade Estadual Paulista (Unesp), Faculdade de Ciências Agrárias e Veterinárias, Jaboticabal, São Paulo, Brazil.*

The objective of this study was to evaluate the effect of virginiamycin (VM) combined with crude glycerin (CG) on pH value, N-NH₃ (mg/100 mL) and VFA (mM). Rumen fistulated bulls (BW = 600 ± 34 kg) were used in a replicated 4×4 Latin square (21-d periods) with 2×2 factorial arrangement of treatments: diets without virginiamycin (VM-)

or with 25 mg/kg of VM in DM (VM+) combined with diets without crude glycerin (CG-) or with 100 g/kg of CG (80% glycerol) in DM (CG+). The sugar cane bagasse was used as the exclusive roughage in the proportion of 20% in DM of diet and crude glycerin replace corn in the diet formulation. Diets were offered on an ad libitum basis at 0700 and 1600 h. Ruminal samples were taken immediately before feeding and at 3, 6, 12, 18 h post feeding on d20 and d21 of the sampling week. Data were analyzed in a replicated 4×4 Latin square with a 2×2 factorial using the MIXED procedure of SAS (SAS Inst., Inc., Cary, NC). Statistical model included the fixed effect of virginiamycin (1 degree of freedom, DF), crude glycerin (1DF) and all interactions. Random effects were period (3DF), bulls (3DF), and residual error. There were no CG \times VM interactions for any variable measured ($P > 0.05$). Diets with CG or VM had similar values of pH (mean = 6.15; $P > 0.05$). CG+ had lower N-NH₃ concentration compared with CG- diets, independent of VM inclusion (24.26 vs. 28.69 mg/100mL; $P > 0.05$). Data showed that CG or VM did not affect the concentration of total VFA (116.92 mM; $P > 0.05$). The molar proportion of acetate was lower in CG+ compared with CG- diets (53.58 vs. 62.70% of total VFA; $P < 0.01$) and it was greater in VM- compared with VM+ diets (59.60 vs. 56.68% ; $P < 0.01$). The molar proportion of propionate was greater in CG+ than CG- diets, independent of VM inclusion (24.47 vs. 18.54% ; $P = 0.0091$). The molar proportion of isobutyrate and isovalerate were not affected by CG or VM ($P > 0.05$). Acetate:propionate ratio was lower in CG+ compared with CG- diets (3.57 vs. 2.36 ; $P > 0.05$). Valerate and butyrate proportion was greater in CG+ than CG- ($P < 0.05$). CG can replace corn at 100 g/kg of DM and VM in Nellore finishing diets without impairing fermentation.

Key Words: crude glycerin, finishing diet, virginiamycin

M315 Comparative analysis of bacterial community composition from the different ruminal ecological niche of Alxa Bactrian camel. J. Zhao*^{1,2}, Z. Yu², and H. Wu¹, ¹Inner Mongolia University for Nationalities, Tongliao, Inner Mongolia, China, ²The Ohio State University, Columbus, OH.

Similar to ruminants, camels, as pseudoruminants, depend on the microbiota in their pseudorumen (a 3-chambered forestomach) to digest fibrous feed. Compared with the ruminal microbiome of cattle and sheep, the microbiome in the pseudorumen of camels is poorly understood. The objective of this research was to characterize the bacteria community compositions partitioned into different niches: liquid phase (LP), solid phase (SP), and epimural phase (EP), of the pseudorumen of Alxa Bactrian camel. Samples of the 3 phases were collected from 6 slaughtered Alxa Bactrian camels. Community composition of bacteria were determined through sequencing 16S rRNA gene amplicons of the V3-V5 hypervariable regions on the Illumina MiSeq platform. Weighted UniFrac analysis revealed that the bacterial community of LP was clearly different from that of SP and EP. From 619,517 quality-checked sequences, 774 operational taxonomic units (OTUs) were identified at a 97% sequence identity. As in the rumen, *Bacteroidetes* (46.6% of the total sequences) and *Firmicutes* (32.8%) were the 2 most predominant phyla, with other minor phyla also being represented: *Verrucomicrobia* (4.4%), *Spirochaetes* (3.3%), *Proteobacteria* (2.8%), *Fibrobacteres* (2.6%), *Tenericutes* (2.4%), and *Lentisphaerae* (1.2%). *Bacteroidetes* was more predominant in LP than in SP and EP, while *Fibrobacteres* was more predominant in SP than in LP. At genus level, a total of 117 taxa were observed across all the samples, but 48 taxa of them were belong to unknown genera. Only 0.1% of the total sequences were assigned to archaea. The results showed that the camel pseudorumenal microbiome was structurally similar but compositionally distinct from that of true

rumen, reflecting the different dietary and genetic impact of camels on their pseudorumenal microbiome. These findings also demonstrated the compositional differences among LP, SP, and EP, indicating that those bacterial communities are specific and adapted to their niches.

Key Words: Alxa Bactrian camel, bacteria, microbiome

M316 Screening of chemically and physically treated corn stover and soybean meal pellet formulations for in situ digestibility in dairy cows. B. C. Dooley^{*1}, C. S. Shouse¹, M. A. Torres-Crespo¹, R. Zecek², and H. A. Ramirez-Ramirez¹, ¹*Iowa State University, Ames, IA*, ²*Pellet Technology USA, Gretna, NE*.

The objective of this screening study was to determine the in situ ruminal digestibility of dry matter (DMD), neutral detergent (NDFD), and acid detergent fiber (ADFD) of 4 corn stover-based experimental formulations of blended material as potential feedstuffs for dairy cows. Each prototype formulation included approximately 42.5% ground corn stover (CS), 15% distillers solubles and varied proportions of soybean meal (SBM) ranging from 33 to 40% to allow inclusion of CaO as a chemical treatment or a custom mixture of fatty acids. Treatments were 1) untreated corn stover (U-CS; 75%NDF, 44% ADF); 2) untreated pelleted blend (U-BLN; 46% NDF, 25% ADF); 3) CaO-treated blend (T-BLN; 44% NDF, 25% ADF); 4) blend with no fatty acid supplementation (NFA; 33% NDF, 23% ADF); and 5) fatty acid-supplemented blend (FA; 29% NDF, 18% ADF). Ten 5-g samples of each prototype and untreated CS were evenly assigned and incubated in the rumen of 2 ruminally-cannulated lactating Holstein cows for 48 h. Data were analyzed using the GLIMMIX procedure of SAS with prototype formulation and cow as fixed effects. By design, U-CS resulted in the lowest ($P < 0.01$) DMD, $37.3 \pm 1.64\%$ followed by T-BLN, which was $65.5 \pm 1.64\%$. Prototypes NFA and FA were similar ($P = 0.27$) and averaged $78.5 \pm 1.64\%$ and U-BLN had the greatest ($P < 0.01$) DMD at $82.0 \pm 1.64\%$. Untreated CS had the least ($P < 0.01$) NDFD at $29.8 \pm 2.28\%$ followed by T-BLN at $44.4 \pm 2.28\%$, NFA and FA were similar ($P = 0.63$) and averaged $50.4 \pm 2.28\%$ whereas NDFD of U-BLN was $67.2 \pm 2.28\%$. Digestibility of ADF was different ($P < 0.01$) across treatments, 25.2 and $38.2 \pm 2.47\%$ for U-CS and T-BLN, respectively; NFA and FA were similar ($P = 0.10$) averaging $45.6 \pm 2.47\%$ and U-BLN had the greatest ($P < 0.01$) ADFD at $59.6 \pm 2.47\%$. Similarities between NFA and FA indicate that additional fatty acids did not alter digestibility. Although additional research is warranted to isolate the effects of chemical composition and mechanical processing, these results indicate that corn stover and SBM-based pellets are highly digestible and may be a suitable feedstuff for dairy cows.

Key Words: corn residue, ruminal digestibility

M317 The development of methodology for ruminal and colon tissue biopsying of Holstein dairy bull calves during weaning. J. K. van Niekerk^{*}, M. Middeldorp, Z. He, and M. A. Steele, *Department of Agricultural, Food and Nutritional Science, University of Alberta, Edmonton, AB, Canada*.

The objective of this study was to develop methodology for biopsying the rumen and colon from dairy calves during weaning. Six Holstein dairy bull calves (45 ± 1.5 kg birth weight) were ruminally cannulated during the second week of life and were fully weaned on d 42. Colon and rumen tissue samples were collected on d 28, 35, 42, 49, 56 and 84. Calves were not sedated but restrained in a chute for sampling. The lubricated distal tip of the endoscope (100 cm length, 9.8 mm diameter) was gradually inserted into the calf's anus. Six (± 12.6 mg)

colon tissue samples were collected (30–40 cm from the calf's anus) every sampling with endoscopic biopsy forceps (single use capture hot biopsy forceps), which were inserted through the instrument channel. The instrument channel was washed with double distilled water and 70% ethanol between calves as well as outside of the endoscope. Furthermore, the endoscope was introduced through the ruminal cannula to harvest ruminal papillae. Endoscopic biopsy of the rumen with endoscopic biopsy forceps (capture hot biopsy forceps and alligator jaws with a needle biopsy forceps) was unsuccessful (85% of the time) because the endoscopic biopsy forceps were unable to excise rumen papillae due to connective tissue. Thereafter, an Allis clamp was used to retrieve the blind sac through the ruminal cannula to perform direct tissue biopsying. Surgical scissors were used to perform tissue biopsying after exteriorization of the blind sac through the ruminal cannula. Colon and rumen samples were washed in PBS and 2 samples submerged in formalin solution and stored at room temperature for light histology analysis and 4 samples were stored in RNA stabilization fluid for molecular biology analysis. The mean RNA integrity number for the rumen papillae and colon samples were 8.9 ± 0.13 and 8.7 ± 0.09 , respectively. In conclusion, endoscopic biopsying can be used for tissue harvest in the colon in young calves. However, it was found that collecting rumen tissue through retracting the rumen and taking samples with surgical scissors was more successful than using an endoscopic biopsy.

Key Words: endoscopic biopsy, colon, rumen biopsy

M318 Functional oils or monensin on milk production and feed efficiency of Holstein cows during the summer. F. P. Rennó^{*1}, C. S. Takiya², G. G. Silva¹, T. A. Del Valle¹, E. M. C. Zilio¹, L. G. Ghizzi¹, and J. Torrent³, ¹*University of Sao Paulo, Pirassununga, SP, Brazil*, ²*Kansas State University, Manhattan, KS*, ³*Oligo Basics, Cascavel, Parana, Brazil*.

Essential (Oligo Basics, Cascavel, Brazil) is a feed additive that contains cashew nut shell liquid (CNSL) and castor oil (CO) as active ingredients. CNSL and CO are considered functional oils (FO) due to their antioxidative and gastro-protective properties. This study aimed to compare the milk production and feed efficiency of cows fed FO or monensin (MON) during the summer season in Pirassununga, Brazil. Thirty-six cows (201 ± 63 DIM, 599 ± 78 kg BW, and 28.7 ± 3.92 kg/d milk yield) were used in a randomized complete block design experiment. Cows were allocated in a barn containing forced ventilation with $25.0 \pm 0.83^\circ\text{C}$ temperature, $81.5 \pm 4.67\%$ of relative humidity, and 75 ± 0.8 ITU during the 5 wk of experiment. Cows within block were assigned to one of treatments: control (CON); FO, 500 mg/kg DM of Essential; or MON, 22 mg/kg DM of MON (Rumensin, Elanco Animal Health, Greenfield, IN). Additives were provided mixed into the concentrate of a TMR fed twice daily. Samples of ingredients and orts were collected weekly to determine DM intake. Cows were milked twice daily, being the milk samples collected weekly during 6 consecutive milkings to determine the contents of fat, protein and lactose by infrared method. Data were analyzed as repeated measures using the PROC MIXED of SAS, and differences were calculated by PDIFF test. Data from one week before the start of experiment were used for covariate adjustments. FO-fed cows had greater milk fat concentration and DM intake than MON (Table 1). In addition, FO numerically increase milk fat concentration and had similar DM intake in relation to CON. FO may improve milk fat concentration of cows in summer.

Key Words: ionophore, milk fat

Table 1 (abstract M318).

Item	Treatment			SEM	P-value ¹	
	CON	MON	FO		Diet	Time
Production, kg/d						
Milk	29.2	29.5	29.1	0.41	0.786	0.003
3.5% FCM	28.7	28.3	29.9	0.43	0.153	<0.001
Milk concentration, g/kg						
Fat	34.6 ^{ab}	32.5 ^b	36.5 ^a	0.51	0.021	0.021
Protein	30.7	30.9	30.7	0.09	0.412	<0.001
Lactose	45.9	46.2	46.3	0.06	0.323	<0.001
DMI, kg/d	24.6 ^a	23.0 ^b	24.5 ^a	0.46	0.021	0.158
Milk yield/DMI	1.21	1.30	1.20	0.03	0.092	0.386

¹Diet by time (wk) interaction effect >0.10 for all variables.

M319 Extracellular amino acids and lysine to methionine ratio affect cell signaling in mammary epithelial cells. P. S. Yoder^{*1,2}, T. Ruiz-Cortes³, and M. D. Hanigan¹, ¹Virginia Tech, Blacksburg, VA, ²Perdue AgriBusiness, Salisbury, MD, ³Universidad de Antioquia, Medellin, Colombia.

Extracellular amino acid (AA) profile may affect intracellular AA concentrations and profile as well as signaling proteins that regulate translation rate. The objective of this work was to assess the effects of various extracellular AA profiles and Lys to Met ratio to determine signaling protein sensitivity. Six AA profiles of DMEM, blood meal (BM), corn gluten meal (CM), casein (CS), blood plasma of cows milking 45 kg/d (CW), and a negative control (NC) represented the profile factor (AAPROF) and Lys/Met ratio unchanged or set to 3:1 was the Lys/Met factor (ML) for a total of 12 treatments with 4 replications. The concentrations of total AA for all treatments except NC (0 mg/L) were set to 659 mg/L (63% of DMEM) which previously was shown to result in maximal stimulation of casein synthesis. Confluent mammary epithelial cells were exposed to treatments for 75 min. Intracellular concentrations of Met, Lys, Leu, Ile, and Thr were affected by AAPROF ($P < 0.02$) whereas only Met and Lys were affected by ML, increasing by 13.6 $\mu\text{mol/L}$ and 11.5 $\mu\text{mol/L}$ ($P < 0.01$). Intracellular Met and Lys concentrations were 145 and 274% ($P < 0.01$) greater for the NC and ML at 3:1 ratio treatment versus other ML 3:1 ratio treatments despite similar extracellular concentrations indicating greater uptake. Within mTOR pathway, mTOR, ribosomal protein S6 kinase 1 (S6K1), and eukaryotic initiation factor 4 E binding protein 1 were induced by AAPROF ($P < 0.01$) while only S6K1 was affected by ML ($P = 0.11$). mTOR pathway proteins had greater phosphorylation for DMEM, BM, CM, CS, and CW versus NC ($P < 0.01$). Blood meal ($P = 0.15$) and CM ($P = 0.06$) had higher phosphorylation than CS and CM tended to

be higher than CW ($P = 0.14$) for S6K1 respectively. For mTOR, BM, CS, and CM tended to have higher phosphorylation than CW ($P = 0.07$). Eukaryotic initiation factor 2 α subunit was unaffected by PROF and ML factors ($P = 0.23$). Setting Lys/Met at a 3:1 ratio had a small positive effect on S6K1 regardless of AA profile. Changes in extracellular AA profiles largely translated to intracellular AA and these varying profiles in general stimulated mTOR pathway related proteins.

Key Words: amino acid, mTOR, translation regulation

M320 Milk enterolactone and fatty acid profile in dairy cows offered flaxseed meal and incremental amounts of liquid molasses. C. P. Ghedini¹, A. F. Brito^{*1}, D. C. Moura², A. S. Oliveira³, and R. A. V. Santana⁴, ¹Department of Biological Sciences, University of New Hampshire, Durham, NH, ²Programa de Pós Graduação em Ciência Animal, Universidade Federal de Mato Grosso, Cuiabá, MT, Brazil, ³Instituto de Ciências Agrárias e Ambientais, Universidade Federal de Mato Grosso—Campus Sinop, Sinop, MT, Brazil, ⁴Instituto Federal de Educação, Ciência e Tecnologia do Norte de Minas Gerais—Campus Arinos, Arinos, MG, Brazil.

Enterolactone (EL) is a mammalian lignan originated from the microbial fermentation of flaxseed in the rumen, and it has been linked to potential human health benefits. However, there is limited information about the impact of liquid molasses (LM) or ground corn (GC) on the microbial output of EL in the rumen and EL transfer to milk. There is also scarce information about the effect of LM or GC or LM:GC mixtures on milk fatty acid (FA). Our objective was to investigate the impact of replacing GC with incremental amounts of LM on milk concentrations of EL and FA. Sixteen multiparous Jersey cows were assigned to treatment in a

Table 1 (abstract M320). Milk enterolactone and FA (g/100 g)

Item	Molasses:corn ratio (% diet DM)				SEM	P > F	
	0:12	4:8	8:4	12:0		L	Q
Enterolactone, nM	246	324	251	324	62.1	0.57	0.58
18:0	11.0	10.7	10.3	9.94	0.32	<0.01	0.78
c-9 18:1	13.6	13.1	13.1	12.9	0.42	<0.01	0.59
c-9, c-12 18:2	1.35	1.36	1.30	1.25	0.03	<0.01	0.10
c-9, t-11 18:2	0.54	0.55	0.56	0.59	0.02	0.03	0.34
c-9, c-12, c-15 18:3	0.62	0.62	0.65	0.66	0.05	0.04	0.60
Σ odd-chain	2.04	2.06	2.09	2.20	0.07	<0.01	0.03
Σ branched-chain	1.93	1.94	1.91	1.94	0.04	0.98	0.47

replicated 4 × 4 Latin square design with 21-d periods. Diets were fed as TMR and consisted (DM basis) of 52% grass-legume baleage, 8% grass hay, 8.5% soyhulls, 2.5% roasted soybean, and 15% flaxseed meal. Ground corn was replaced by LM at 0, 4, 8, or 12% of diet DM. Diets averaged 9.7 and 0.0%, 7.0 and 1.7%, 4.3 and 3.3%, and 1.64 and 5.0% of starch and LM-added sugars, respectively; CP and NDF concentrations averaged 19.0 and 43.5% across diets. Results are presented in Table 1. Concentration of milk EL and Σ branched-chain FA was not affected by treatments. Milk 18:0, c-9 18:1, and c-9, c-12 18:2 decreased linearly, whereas c-9, t-11 18:2 and c-9, c-12, c-15 18:3 increased linearly with replacing GC with LM. These results may be associated with decreased intake of 18-C or shifts in ruminal biohydrogenation. Σ odd-chain FA responded quadratically to LM supplementation, suggesting changes in ruminal microbiota. Overall, changing the dietary NSC profile in cows fed flaxseed meal did not alter milk EL, but affected milk FA.

Key Words: dairy cow, enterolactone, flaxseed meal

M321 Pre-ensiling addition of bacterial inoculant, amylase or both to rehydrated cracked corn. L. C. Solórzano¹, L. L. Solórzano², and A. A. Rodríguez¹, ¹University of Puerto Rico, Mayagüez, PR, ²Independent Researcher, Fitchburg, WI.

Increasing starch digestibility benefits lactational and economic performance of dairy cows. Ensiling increases starch digestibility, but it is affected by the length of ensiling and intensity of fermentation. Increasing the intensity of fermentation by adding a homo-fermentative bacterial inoculant (HBI, 1 g/kg supplying $>9.1 \times 10^{10}$ cfu/g containing *Lactobacillus plantarum*, *Enterococcus faecium*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *P. acidilactici*), a source of α -amylase (AMY, 1.1 g amylase/kg), both (HBI+AMY) or no additives (CTL) to rehydrated cracked corn (RCC; 66% DM) before ensiling was evaluated. Sixteen 1-L glass mini-silos (4/treatment) were vacuumed packed and stored for 90 d at 25°C. Nutritional and fermentation characteristics, in vitro starch digestibility (IVSD) and aerobic stability were determined. Data were analyzed as a completely randomized design. Means were separated using Tukey's test. HBI tended to increase ($P < 0.10$) DM recovery (95.4%) compared with CTL (95.0%) while the addition of AMY (94.2%) or HBI+AMY (94.5%) reduced it. HBI decreased ($P < 0.05$) pH and butyric acid (3.90, 0.01%) compared with CTL (4.03, 0.11%), AMY (3.95, 0.06%) or HBI+AMY (4.00, 0.04%). HBI lowered ($P < 0.05$) butyric acid compared with AMY or HBI+AMY. Ethanol was increased ($P < 0.05$) with AMY (0.38%) compared with CTL (0.30%). Adding HBI (0.23%) or HBI+AMY (0.29%) decreased ($P < 0.05$) ethanol compared with CTL (0.30%). HBI and HBI+AMY decreased $\text{NH}_3\text{-N}$ (0.13 and 0.14% of CP) compared with CTL (0.22% of CP) or AMY (0.17% of CP). AMY and CTL differed between them in $\text{NH}_3\text{-N}$. HBI+AMY tended ($P < 0.13$) to increase IVSD (69.1% of starch) compared with CTL (67.5% of starch) while HBI or AMY (67.5 or 66.6% of starch) tended to decrease it. HBI+AMY decreased the stability (h) of RCC upon aerobic exposure vs. HBI (78 vs 138 h), AMY (>168 h) or CTL (>168 h). HBI or AMY positively influenced the nutritional and fermentation characteristics of RCC. Neither HBI nor AMY increased IVSD. The combination of HBI+AMY tended to increase IVSD but decreased aerobic stability of RCC.

Key Words: ensiling, inoculant, amylase

M322 Relationship between mineral composition of milk and lactation performance. A. R. Alfonso-Avila¹, E. Charbonneau¹, P. Y. Chouinard¹, G.F. Tremblay², D. E. Rico¹, and R. Gervais¹,

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Minerals could be implicated in cellular transport of milk constituents or their precursors in the mammary epithelial cell. Our objective was to examine associations between mineral composition of milk and lactation performance in dairy cows. A total of 120 observations from 2 separate randomized complete block design experiments comprising 60 early-lactation Holstein cows (38 ± 11 DIM; Mean \pm SD) were used. In both experiments, treatment periods lasted 28 d and were preceded by a 1-wk pretreatment collection period. In both trials, diets had a forage-to-concentrate ratio of 40:60 (DM basis) and were supplemented, on a DM basis, with (1) 1.5% K_2CO_3 , (2) 1.8% K_2CO_3 , (3) 2.6% KHCO_3 , (4) 2% KCl, (5) 1.4% Na_2CO_3 , (6) 1.5% K_2CO_3 + 2% soybean oil, (7) 2% soybean oil, or (8) unsupplemented. Milk yield was recorded and samples collected on the last 3 or 5 d of each period, pooled by cow and period, and analyzed for fat, protein, lactose, and mineral concentrations (Na, K, Cl, S, Mg, P, Ca). Associations between minerals and milk yield and component concentrations were assessed using mixed model regressions, considering cow and experiment as random effects, and parity and individual mineral concentrations as fixed effects. Parity was removed from the model when not significant ($P > 0.10$). Milk concentrations of Ca, Mg, P, and S were positively associated with both fat ($P < 0.01$; $R^2 > 0.28$) and protein ($P < 0.01$; $R^2 > 0.30$) contents. Negative relationships were observed between Cl and fat ($P = 0.02$; $R^2 = 0.48$), protein ($P < 0.01$; $R^2 = 0.40$) as well as lactose ($P < 0.01$; $R^2 = 0.71$) contents, whereas K was negatively associated with lactose concentration ($P < 0.01$; $R^2 = 0.62$). Finally, Ca, Mg, and P were negatively related with milk yield ($P < 0.01$; $R^2 > 0.86$), whereas a positive association was observed with Cl ($P < 0.01$; $R^2 = 0.79$). The strong relationship of milk Cl concentration with milk yield suggests that this mineral is involved in milk synthesis. Mineral concentrations in milk are recognized to reflect their cellular levels, it is then possible to explore the biological role of minerals in the secretory mechanisms of milk constituents.

Key Words: chlorine, milk constituent, milk synthesis

M323 Effects of pre-ensiling additions of a bacterial inoculant with or without molasses on rehydrated cracked corn fermentation parameters. L. C. Solórzano¹, L. L. Solórzano², and A. A. Rodríguez¹, ¹University of Puerto Rico, Mayagüez, PR, ²Independent Researcher, Fitchburg, WI.

Ensiling increases starch digestibility of corn, which is affected by the intensity of fermentation. Increasing the intensity of fermentation by adding a homo-fermentative bacterial inoculant (BI, 1 g/kg supplying $>9.1 \times 10^{10}$ cfu/g containing *Lactobacillus plantarum*, *Enterococcus faecium*, *Lactococcus lactis*, *Pediococcus pentosaceus*, *P. acidilactici*), or BI plus molasses at 1.1 g/kg (BI+MOL) or no additives (CTL) to rehydrated cracked corn (RCC; 66% DM) before ensiling was evaluated. Twelve 1-L glass mini-silos (4/treatment) were vacuumed packed and stored for 90 d at 25°C. Data were analyzed as a completely randomized design. Means were separated using Tukey's test. DM recovery (95, 95.4, 95.2%) nor the temperature (19.2, 19.4, 19.3°C) at the opening of the silo were affected by treatment (CTL, BI and BI+MOL, respectively). BI decreased ($P < 0.05$) $\text{NH}_3\text{-N}$ compared with CTL (0.13 vs. 0.22% of CP) or BI+MOL (0.14 (% of CP), which was lower ($P < 0.05$) than CTL. BI (2.06%) increased ($P < 0.05$) water-soluble carbohydrates compared with CTL (1.62%) or BI+MOL (1.71%). BI (3.9) decreased ($P < 0.05$) pH compared with CTL (4.03) or BI+MOL (4.0). BI and BI+MOL decreased ($P < 0.05$) butyric acid compared with CTL (0.01 and 0.02 vs. 0.11%). BI (0.23%) decreased ($P < 0.05$) ethanol compared with CTL (0.30%) but did not differ from BI+MOL (0.28%). Neither BI

nor BI+MOL affected in vitro starch digestibility compared with CTL (66.2, 67.1, 67.5% of starch, respectively). BI+MOL tended ($P < 0.10$) to decrease DM recovery compared with CTL (83.8 vs. 89.9%) or BI (86.1%) after 168 h of aerobic exposure (AE). The average temperature during 168 h of AE was higher ($P < 0.05$) for BI+MOL (22.1°C) compared with CTL (19.7°C) or BI (21.3°C). BI+MOL decreased the aerobic stability (h) of RCC compared with BI (60 vs 138 h) or CTL (>168 h). Additives had some positive effects on fermentation characteristics, however, the use of a homo-fermentative BI, or BI+MOL is not recommended for ensiling of RCC due to their negative effects on DM recovery and aerobic stability upon opening of silos. Additives did not affect starch digestibility.

Key Words: inoculant, molasses, ensiling

M324 Supplementation of blackberry pomace during the transition phase may improve health and reproductive performance of dairy cows. K. Swanson*, S. Akers, K. Estenson, R. Wilson, M. Keller, and G. Bobe, *Oregon State University, Corvallis, OR.*

Dairy cows are during the transition period most susceptible to metabolic and infectious diseases, which adversely affect reproductive performance. Blackberry pomace, the waste product from blackberry processing, is rich in polyphenolic compounds that have anti-inflammatory and anti-oxidative properties. To evaluate the effect of supplementation of blackberry pomace during the transition period on health and reproductive performance of dairy cows, 24 multiparous dairy cows were fed either 0 (Control), 57, or 114 g/d of dried blackberry pomace as top dressing to the TMR from 28 d before to 28 d after calving. Blood samples were collected on approximately d 28, 21, 14, 7, 3, and 1 prepartum, while both blood and milk samples were collected on d 0, 1, 3, 7, 14, 21, and 28 postpartum. Upon completion of the study, serum samples were analyzed for BHB, glucose, FFA, BUN, calcium, and phosphorus concentrations. Reproductive data, including days open and number of times bred before confirmed pregnant, was collected. All data were analyzed using PROC MIXED and PROC GLIMMIX in SAS version 9.4. Fixed effects were blackberry pomace supplementation rate and breed and for repeated data within cows, time and the interaction of time with treatment. Compared with Control, supplementation with 114 g/d decreased the number of days until first heat (33 ± 5 d vs. 48 ± 7 d; $P = 0.02$), first breeding (58 ± 3 d vs. 68 ± 8 d; $P = 0.04$), and days open (73 ± 9 d vs. 122 ± 22 d; $P = 0.002$) and tended to improve pregnancy rate from first breeding ($78 \pm 17\%$ vs. $16 \pm 15\%$; $P = 0.06$). No significant group differences were observed for disease incidence ($P = 0.34$), feed intake ($P = 0.58$), milk yield ($P = 0.36$), or concentrations of serum for BHB ($P = 0.31$), glucose ($P = 0.0525$), FFA ($P = 0.74$), BUN ($P = 0.65$), calcium ($P = 0.46$), and phosphorus ($P = 0.102$). In conclusion,

supplementation of blackberry pomace at 114 g/d during the transition phase may improve health and reproductive performance of dairy cows.

Key Words: blackberry pomace, dairy health, reproductive performance

M325 Evaluation of *Saccharomyces cerevisiae* fermentation products on production, metabolism, oxidative stress, and health of transition dairy cows. K. M. Glosson*¹, I. Yoon², and J. K. Drackley¹, ¹*University of Illinois, Department of Animal Science, Urbana, IL,* ²*Diamond V, Cedar Rapids, IA.*

Yeast culture products have been used in the dairy industry to modify the rumen environment of lactating cows to improve production and reduce metabolic stresses in the transition period. The objective of this study was to determine the effects of feeding *Saccharomyces cerevisiae* fermentation products (Diamond V Original XPC and NutriTek) on the production and health of cows from -26 through 28 DIM. Multiparous Holstein cows ($n = 100$) were randomly assigned one of 4 top-dress treatments that were combinations of the products and ground corn to equal 50 g/d: 1) control, ground corn (CON); 2) 14 g XPC mix (XPC); 3) lower level, 19 g, NutriTek mix (NTH); or 4) higher level, 38 g, NutriTek mix (NTH). Cows were milked 3x/d and milk production and components were summarized by week. Blood samples were collected prepartum based on expected calving date at -26, -17, -14, -10, -7, -4 and -1 d, and postpartum at calving and 1, 3, 5, 7, 10, 14, 17, and 21 d for analysis of blood metabolites. Phagocytosis (PN/PM) and oxidative burst (OBN/OBM) activity of neutrophils (N) and monocytes (M) were used to evaluate immune status at -17, -7, 5, 14, and 28 d. Intake did not differ significantly among treatments through d 28, but cows supplemented with XPC or NTH tended to have greater ECM yield in this period (trt \times time, $P = 0.06$) when compared with the other treatments (CON: 44.3 kg/d; XPC: 45.0 kg/d; NTH: 44.1 kg/d; NTH: 46.9 kg/d). Most blood metabolites and minerals were not different among treatments. Activities of PN (Trt, $P < 0.01$) and OBN (Trt, $P < 0.01$) were increased at d 5 for cows supplemented with NTH when compared with cows given CON or NTH (CON: 53%, 44%; XPC: 58%, 49%; NTH: 53%, 48%; NTH: 64%, 58%). While cows supplemented with NTH appeared to partition more energy toward other metabolic uses than milk production, the difference of innate immune system activity during the first week of lactation and the differences in ECM production shows a possible benefit of supplementing XPC or NTH to transition dairy cows.*

Key Words: *Saccharomyces cerevisiae* fermentation product, transition dairy cow

*Corrected abstract