M339 The effect of decreasing dietary cation-anion difference in the prepartum diet on urine pH and plasma minerals in multiparous Holstein cows. B. M. Sweeney*[1], C. M. Ryan[1], T. Stokol[2], K. Zanzalari[3], D. Kirk[3], and T. R. Overton[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Department of Population Medicine and Diagnostic Sciences, Cornell University, Ithaca, NY, [3]Prince Agri Products Inc., Quincy, IL.*

The objective of this study was to determine the effect of decreasing dietary cation-anion difference (DCAD) in the prepartum period on prepartum urine pH and peripartum plasma mineral concentrations. Multiparous Holstein cows (n = 89) were allocated randomly to one of 3 prepartum diets formulated with decreasing DCAD: CON (+17.5 mEq/100 g DM), MED (+3.6 mEq/100 g DM), or LOW (−10.9 mEq/100 g DM), beginning 24 d before expected parturition. Analyzed DCADs were +18.3, +5.9, and −7.4 mEq/100 g DM. Cows were fed a common postpartum diet from parturition until 63 d in milk (DIM). Blood was collected 1 × /wk prepartum, 2 × in 24 h postpartum, 1 × /d until 5 DIM, and 3 × /wk until 14 DIM. Repeated measures analyses were conducted using the MIXED procedure of SAS with linear and quadratic effects of decreasing prepartum DCAD as contrasts. There was a quadratic effect on urine pH (CON = 8.20, MED = 7.84, LOW = 5.98; $P < 0.01$). There tended to be an interaction between DCAD and parity (2nd lactation vs. 3rd and greater) for prepartum Mg ($P = 0.08$) such that Mg decreased with decreasing DCAD for older cows ($P < 0.05$) but not for 2nd lactation cows. Postpartum Ca increased linearly with decreasing DCAD (CON = 8.84, MED = 8.89, LOW = 9.19; $P < 0.01$) with greater increases through 5 DIM (DCAD × Day; $P = 0.06$). There tended to be an interaction between DCAD and parity ($P = 0.06$) such that Ca was increased more for older cows fed lower DCAD levels (CON = 8.68, MED = 8.63, LOW = 9.16; $P < 0.01$) than for 2nd lactation cows. Postpartum P tended to decrease linearly ($P = 0.08$). For 2 d postpartum cows fed LOW had lower Mg (DCAD × Day; $P < 0.01$). Incidence of hypocalcemia (HC, plasma Ca < 8.5 mg/dL) was significantly different at 1 DIM ($P = 0.02$). When parity groups were analyzed separately, incidence of HC in older cows was decreased by decreasing prepartum DCAD at 0.6 ($P = 0.07$), 1 and 2 DIM ($P < 0.01$) but did not differ for 2nd lactation cows. Decreasing prepartum DCAD improved Ca status, decreased incidence of HC, and had varied effects on other minerals over the periparturient period.

Key Words: hypocalcemia, dietary cation anion difference, transition cow

M340 Canola meal in dairy cow diets with varying concentration of starch sources. Nadeesha K. Jayasinghe[1], Kenneth F. Kalscheur*[2], Jill L. Anderson[1], and David P. Casper[1], *[1]Dairy Science Department, South Dakota State University, Brookings, SD, [2]US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

Synchronization of the degradability of non-structural carbohydrate and rumen degradable protein has been identified as an effective method of increasing intestinal AA flow through increased microbial protein synthesis and more efficient ruminal fermentation, thereby increasing performance of dairy cows. Therefore, the objective was to determine the performance of lactating cows fed either corn and barley starches at varying proportions in diets containing canola meal as the major source of supplemental protein. Twelve multiparous and 4 primiparous Holstein cows (94 ± 25 DIM) were used in a 4 × 4 Latin square design with 28-d periods. The ratio of starch from ground corn and rolled barley within each treatment was 100:0, 67:33, 33:67, and 0:100. Diets contained 36% corn silage, 20% alfalfa haylage, and 44% concentrate (DM basis). Varying proportions of corn and barley had no effect ($P > 0.10$) on dry matter intake (26.5 kg/d) or milk production (41.2 kg/d). Milk fat percentage (3.52%) and yield (1.42 kg/d) and milk protein percentage (2.95%) and yield (1.21 kg/d) were not affected by starch. Lactose percentage (4.86, 4.83, 4.90, and 4.88%, for 100:0, 67:33, 33:67, and 0:100, respectively) and MUN (14.8, 14.5, 15.4, and 15.1 mg/dL) responded cubically ($P < 0.05$) to the changes in dietary starch proportions. Treatments did not affect energy-corrected milk (40.6 kg/d) nor feed efficiency (1.53). Increasing the proportion of barley to corn had no effect on the molar proportion of ruminal acetate and butyrate, however, propionate increased quadratically as barley increased in the diets ($P < 0.01$). Ruminal ammonia concentration averaged 11.5 mg/dl and was not affected by starch source. Apparent total-tract digestibilities of DM, OM, and NDF decreased linearly ($P < 0.05$) and, CP and ADF tended to decrease linearly ($P < 0.10$) when the proportion of barley starch increased in the diet. Total-tract digestibility of starch was not affected by starch source and averaged 95.5%. Overall, lactation performance was not affected by feeding varying proportions of corn and barley when the diets were formulated with canola meal as the primary protein supplement.

Key Words: canola meal, starch source, lactating dairy cow

M341 Dietary grape marc supplementation alters the milk protein and fatty acid profile produced by pasture-based dairy cattle. Reuben Harland[1], Aysha Morrow[1], Roland Harrison[1], Jana Kraft[2], and Sabrina L. Greenwood*[1,2], *[1]Lincoln University, Lincoln, New Zealand, [2]The University of Vermont, Burlington, Vermont.*

Grape marc (GM) is a byproduct of the wine-making industry and is a rich source of polyphenols. This experiment evaluated the efficacy of feeding supplementary GM to late-lactation dairy cows fed pasture-based diets as a means to manipulate the milk fatty acid (FA) and protein content produced. The effects of condensed tannins (CT) within GM were determined through the feeding of polyethylene glycol (PEG), a CT inhibitor. Forty late-lactation Friesian x Jersey cows were used in a 4 × 2 factorial design, receiving either (1) pasture only (control), (2) pasture + 350 g PEG/cow/d, (3) pasture + 2 kg DM GM/cow/d, (4) pasture + 2 kg DM GM/cow/d + 350 g PEG/cow/d, (5) pasture + 4 kg DM GM/cow/d, (6) pasture + 4 kg DM GM/cow/d+ 350 g PEG/cow/d, (7) pasture + 6 kg DM GM/cow/d, or (8) pasture + 6 kg DM GM/cow/d + 350 g PEG/cow/d. Cows were offered increasing amounts of GM for 10 d and maintained on the full amount of supplement for a further 6 d. Milk yield was determined at each milking. Milk samples were collected at a.m. and p.m. milking on d 0 and 16 and analyzed for milk components (milk fat, protein, and lactose), protein profile (α-, κ- and β- casein, α-lactalbumin, and β-lactoglobulin variants A and B) and FA profile by ANOVA using GenStat. Total milk and component yields were not affected by GM or PEG ($P > 0.05$). Of the short-chain FA analyzed (n = 13; C4 to C15), 63% were negatively affected ($P < 0.05$) by GM inclusion and 15% increased with PEG intake. PEG positively affected 25% of long-chain FA (n = 24; C17 to C26), while 29% were negatively affected by GM and 8% were positively affected by GM. Inclusion of GM did not affect the content (mg/mL) of any milk protein analyzed. Cows that received dietary PEG had lower α- ($P = 0.06$) and β- ($P = 0.02$) casein content than those not receiving PEG. In conclusion, GM

negatively affected the profile of some short-chain (de novo synthesized) and long-chain (diet-derived) FA, the effects of which could not be fully eradicated by dietary PEG inclusion (CT inhibition). Dietary PEG inclusion decreased the content of α- and β- casein, suggesting that diet CT can increase the output of these proteins in milk.

Key Words: casein, fatty acid, byproduct

M342 Integrating nutrient and hormonal effects on mTOR phosphorylation in the mammary cell. Juan J. Castro Marquez* and Mark D. Hanigan, *Virginia Tech, Blacksburg, VA.*

The objective of this work was to integrate experimental data on the effect of insulin, EAA and acetate into a model of transcription control in the mammary cell. Current representations of milk protein synthesis consider energy and dietary protein effects separately with no regulation other than by limiting production when supply of either is short. In reality, protein synthesis is simultaneously affected by EAA, energy substrates and hormones, by upregulating the initiation and elongation phases of mRNA translation. A central element to this process is the mammalian target of rapamycin (mTOR) which transfers nutritional and hormonal signals onto translation initiation and elongation proteins that, through phosphorylation, modulate protein synthesis. Phosphorylation data for protein kinase B (Akt), AMP activated protein kinase (AMPK), mTOR, eukaryotic elongation factor binding protein (4EBP1) and eukaryotic elongation factor 2 (eEF2) from a series of experiments in MACT cells and lactogenic mammary tissue slices conducted over the past 5 years was used to build a dynamic model representing each one of the above signaling proteins in their phosphorylated and dephosphorylated states. Akt and mTOR phosphorylation reactions were defined as Michaelis-Menten, whereas AMPK, 4EBP1 and eEF2 representations used mass action kinetics. Inference was based on resampling with replacement and resulting nonparametric confidence intervals (CI). The affinity constants for insulin effects on Akt (CI = 6.6, 32.8 ug) and isoleucine on mTOR (CI = 90, 443 uM) were within physiological concentration ranges, but the rate constant for acetate effects on AMPK was null (CI = 2.71×10^{-9}, 9.8×10^{-8}). Sensitivity analysis coefficients indicate isoleucine was the strongest driver (0.71) of mTOR phosphorylation compared with insulin (0.01) and acetate (0.01). Phosphorylated Akt, AMPK, mTOR, 4EBP1 and eEF2 were predicted without bias and had errors of 16, 33, 29, 28 and 33%, respectively. Insulin and isoleucine effects seem to be well represented but more work is needed to improve our understanding of the role of acetate and other AA on mTOR regulation.

Key Words: mTOR, casein, modeling

M343 Prediction of daily energy status in early and mid lactation using milk and body traits. Päivi Mäntysaari*[1], Tuomo Kokkonen[2], Martin Lidauer[1], and Esa A. Mäntysaari[1], *[1]Natural Resources Institute Finland, Green technology, Jokioinen, Finland, [2]Department of Agricultural Sciences, University of Helsinki, Helsinki, Finland.*

Monitoring cow's energy status at the individual level in early lactation is important for management, but also for breeding purposes. Energy status of a cow can be estimated by calculating the energy balance (EB) from cow's energy intake and output. Alternatively, indicator traits such as body weight (BW) and body condition score (BCS) changes and milk fat-protein ratio (FP) have been proposed. However, precision of these predictions has been low. This may be related to the lack of precision in estimated EB itself, because standard estimates for energy requirements are used in its calculation. We used the plasma nonesterified fatty acids (NEFA) concentration as a biomarker of energy mobilization and energy status, and addressed associations between NEFA concentration and energy status indicators. The data included 10032 daily BW, intake and milk, 279 BCS, and 261 NEFA measurements of 56 Nordic Red primiparous dairy cows. Plasma samples for NEFA were collected twice on lactation wk 2 and 3 and once on wk 20. The milk samples were taken on the same days as NEFA samples and on monthly test days. Daily BWs were smoothened by a regression model with fixed effect of days in milk and random animal part. The NEFA concentration on wk 20 was considered as base level and deviations from base level (dNEFA) were used in calculations. The mean (±sd) ECM (kg/d), milk fat and protein (%), BW (kg) and BCS (1–5) were 30.3 (±4.60), 4.43 (±0.51), 3.59 (±0.32), 574 (±53), and 3.19 (±0.38), respectively. On lactation wk 2 and 3 the average NEFA concentrations were 0.704 (±0.363) and 0.526 (±0.275), and 0.123 (±0.035) mmol/l on lactation wk 20. From all indicators daily BW change had the highest correlation (0.57) with NEFA, followed by daily FP (0.53) and BCS change (−0.20). To predict dNEFA by available indicators, a multiple linear regression model was developed. The best fit was achieved with a model including BW change, FP, BCS and its change, BCS*BCS change, and days in milk. The correlation between predicted and observed dNEFA was 0.77, which was higher than the correlation (0.69) between dNEFA and EB.

Key Words: dairy cow, energy balance

M344 Performance of dairy calves receiving probiotic containing *Bacillus subtilis* and *Bacillus licheniformis*. Thais M. Torrezan[1], Jackeline T. Silva[1], Nathalia B. Rocha[1], Evangelina Miqueo[1], Fernanda L. M. Silva[1], Samyra Baldassin[1], and Carla M. M. Bittar*[1,2], *[1]University of Sao Paulo, ESALQ, Piracicaba, Sao Paulo, Brazil, [2]CNPq, Brasilia, DF, Brazil.*

Methods to improve calf growth and health, with reduced antibiotics use are essential for a successful and profitable dairy. This study evaluated the performance and fecal pH of dairy calves receiving milk replacer supplemented or not with probiotic. Twenty-four newborn Holstein male calves were utilized in a randomized blocks experimental design, and distributed into 2 treatments: Control: no probiotic supplementation; Supplementation of 2g/d of a *Bacillus subtilis* (1.6×10^9 cfu) and *Bacillus licheniformis* (1.6×10^9 cfu) containing probiotic (Bioplus, Chr. Hansen), via milk replacer. Calves were individually housed, with free access to water and starter (20% CP and 80% TDN), and received 4L/d of milk replacer (FeedTech, DeLaval, 21.6:15.5, 12.5% solids) until the eighth week of life, when weaned. Feed intake was monitored daily; while body weight, height withers, heart girth, hip width and fecal pH were weekly measured. Data were analyzed as repeated measures, considering treatment, age, and their interaction effects. There were no probiotic supplementation effects on growth and starter intake ($P > 0.05$). As animals were growing, all parameters were significantly affected by age ($P > 0.05$); however, there were no treatment and age interaction effect ($P > 0.05$). On the other hand, probiotic supplementation tended to reduce fecal pH, suggesting a change in intestine microorganisms and probably a reduction on pathogens growth.

Contd.

Table 1 (Abstr. M344). Performance of calves receiving milk replacer supplemented with probiotics

	Treatment			$P <$[1]		
Iten	Control	Probiotic	SEM	T	A	T × A
Initial BW, kg	36.54	35.47	0.82	0.48	—	—
Weaning BW, kg	57.22	56.64	1.99	0.89	—	—
ADG, kg	0.39	0.44	0.03	0.49	0.0001	0.81
Average starter intake, g/d	372.8	362.0	28.6	0.89	0.0001	0.72
Withers height, cm	78.8	79.2	0.2	0.68	0.0001	0.98
Heart girth, cm	81.1	80.7	0.4	0.71	0.0001	0.39
Hip width, cm	22.0	22.1	0.09	0.68	0.0001	0.86
Fecal pH	6.75	6.79	0.04	0.07	0.001	0.02

[1]T = treatment effect; A = age effect; T × A = treatment and age interaction effect.

Key Words: additive, health, supplement

Table 1 (Abstr. M345). Main effect means and SE to intake and growth performance parameters in crossbred (Holstein x Gyr) calves differing in phenotypic residual feed intake (RFI)

Parameters	Low RFI	High RFI	SE	*P*-value
RFI, kg DM/d	−0.13	0.07	—	—
ADG, kg/d	0.730[a]	0.746[a]	0.02	>0.05
DMI, kg/d	0.941[b]	1.074[a]	0.03	<0.05
Water intake, L/d	1.00[a]	1.38[a]	0.20	>0.05
Milk intake, kg/d	0.803[a]	0.795[a]	0.04	>0.05
Hip height, cm	92.25[a]	91.87[b]	0.96	<0.05
Chest circumference, cm	94.00[a]	93.50[b]	1.33	<0.05
Rump width, cm	25.67[a]	25.50[a]	0.40	>0.05
Withers height, cm	88.20[a]	87.25[b]	0.89	<0.05

Means within rows followed by the same letter are not significantly different ($P \geq 0.05$).

Key Words: measurement, body weight, efficiency

M345 Growth performance in Crossbred (Holstein x Gyr) calves differing in phenotypic residual feed intake on pre-weaned period. Juliana Mergh Leão*[1], Fernanda Samarini Machado[2], Mariana Magalhães Campos[2], Juliana Campos Carneiro[3], Paulo Campos Martins[1], Isabela Carvalho Costa[4], Paulo Sérgio Dornelas Silva[4], Brenda Karoline Alcântara Faria[3], Juliana Aparecida Mello Lima[2], Rayanne Soalheiro de Souza[1], and Sandra Gesteira Coelho[1], *[1]Universidade Federal de Minas Gerais-UFMG, Belo Horizonte, Minas Gerais, Brazil, [2]Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, [3]Instituto de Ciências Agrárias da UFMG, Montes Claros, Minas Gerais, Brazil, [4]Instituto Federal de Educação, Ciência e Tecnologia do Sudeste de Minas Gerais-IFSEMG, Rio Pomba, Minas Gerais, Brazil.*

The aims of this study were to quantify the variation in residual feed intake (RFI) of calves F_1 (Holstein × Gyr) until 60 d of age and evaluate their productive performance. Eighteen calves received colostrum after birth (10% of body weight) and were housed in individual sand bed stalls in the experimental farm of Embrapa Dairy Cattle (Coronel Pacheco, Brazil). All animals were subjected to the same nutrition management which consisted of 6 L of whole milk (TS; 11.75%) in equal amounts twice a day. Solid diet consisting of 95% of concentrated (88% DM; 20% CP and 3% Fat), 5% Tifton 85 hay (81% DM; 13.4% CP; 72.8% NDF; 32.3% ADF) and water were provided ad libitum from the first day of life. Feed solid diet, milk and water intakes were measured daily and body weight and morphometric measurements (withers height, hip height, chest circumference and rump width) were done at birth and at 60 d of age. RFI was calculated for each animal as the difference between actual DMI and expected DMI. Expected DMI was computed for each animal by regressing average daily DMI on mean $BW^{0.75}$and ADG over a 60 d period. Twelve animals were ranked according to the RFI into 2 groups: low (efficient) and high (inefficient). The data were analyzed in a completely randomized design by ANOVA using GLM procedure of SAS. High RFI calves had DMI 12.39% higher than the low group ($P < 0.05$). There was no difference in ADG, water intake and rump width between RFI groups ($P > 0.05$).Withers height, hip height, chest circumference were higher ($P < 0.05$) for the low RFI group.

Contd.

M346 Dietary grape marc supplementation lowers urinary nitrogen excretion from pasture-based dairy cattle. Aysha Morrow[1], Reuben Harland[1], Roland Harrison[1], Jana Kraft[2], and Sabrina L. Greenwood*[1,2], *[1]Lincoln University, Lincoln, New Zealand, [2]The University of Vermont, Burlington, Vermont.*

On-farm nitrogen (N) losses in pasture-based dairy systems are an environmental issue. Grape marc (GM) is a byproduct that contains condensed tannins (CT) and low crude protein concentrations, constituents that alter N partitioning in ruminants. The objective of this experiment was to determine if GM could decrease the urine N concentration and the urine N output (g N/d) from cows grazing a typical perennial ryegrass/white clover pasture. A second objective of the current experiment was to determine if the CT in GM are a component causing any changes in fecal and urine N concentrations through the use of polyethylene glycol (PEG), a CT inhibitor. The experiment was arranged in a 4 × 2 factorial design, including 4 levels of GM supplementation (0, 2, 4, and 6 kg DM/cow/d) and 2 levels of PEG (0, and 350 g/cow/d) in addition to their daily pasture allowance. Forty Friesian x Jersey crossbred multiparous cows in late lactation were evenly divided into one of 8 treatment groups. Cows were fed increasing amounts of GM for 10 d and fed the full treatment amounts of GM for a further 6 d. The pasture DMI were estimated for each treatment group daily, and intakes of GM and PEG were determined for each cow daily. Urine, feces, plasma and milk samples were collected on d 0, 10, and 16 of the trial. Fecal N % and DM % were analyzed, as well as urine N %, and concentrations of urine ammonia, urine urea-N, plasma urea-N, and milk urea-N. Estimated urine N excretion (g N/d) was calculated. Results were analyzed using GenStat. The dietary inclusion of PEG decreased fecal N % ($P < 0.001$), while dietary supplementation with GM lowered urine N % ($P = 0.005$) and urine urea ($P = 0.003$) concentrations. Cows fed GM also had lower plasma urea-N ($P = 0.024$) and milk urea-N ($P = 0.026$) concentrations. There was a tendency toward lower ammonia (mmol/L; $P = 0.09$) and N excretion in urine (g N/d; $P = 0.08$) due to the supplementation of dietary GM. These results indicate that the dietary inclusion of grape marc is effective at increasing fecal N and reducing urine N concentrations and that the CT within GM aids in increasing fecal N loss.

Key Words: nitrogen loss, environment, byproduct

M347 Energy expenditure and methane emission in dairy heifers using the face-mask method. Carlos Alberto Alves Oliveira Filho[1], Fernanda Samarini Machado[2], Alexandre Lima Ferreira[2], Luiz Gustavo Ribeiro Pereira*[2], Thierry Ribeiro Tomich[2], Mariana Magalhães Campos[2], José Augusto Gomes Azevêdo[3], Rogério Martins Maurício[4], Alexandre Vieira Chaves[5], and Camilla Flávia Portela Gomes Silva[6], *[1]Universidade Estadual do Sudoeste da Bahia, Itapetinga, Bahia, Brazil, [2]Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, [3]Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil, [4]Universidade Federal de São João Del Rei, São João Del Rei, Minas Gerais, Brazil, [5]Faculty of Veterinary Science, Sydney, New South Wales, Australia, [6]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Santa Inês, Bahia, Brazil.*

The aim of this study was to evaluate the effect of feeding levels (FL) and breed (B) on energy expenditure as heat production (HP) and methane emission in dairy heifers using the face-mask method. Twenty-four heifers, 8 Holstein, 8 Gyr and 8 Holstein-Gyr crossbreed (F_1) with average live weight (LW) of 440 ± 88 kg and average age of 27.5 ± 0.8 mo, were housed in tie stall and randomly distributed to the treatments in a 2 × 3 factorial design (feeding level of 1.17% LW or 1.46% LW, on dry matter (DM) basis and breed). The diet was offered as a total mixed ration (700g/kg of corn silage and 300g/kg of concentrate, on DM basis). O_2 consumption, CO_2 production and CH4 emission data were measured using Sable System (Sable Systems, Henderson, NV) coupled with a face-mask for 30 min per day, for 3 d. Heart rate (HR) (beat/min) was registered over 72 h using Polar Equine transmitter (model RS800CX G3, Polar Electro Enc.). Three measurements of oxygen pulses (O2P) (mL O_2/beat) were registered. Total daily O2 consumption (L/d) was calculated as O2P times daily mean HR. Daily HP was calculated as total daily O_2 consumption times the constant 20.47 KJ/L of O_2. Data were subjected to ANOVA and means were compared by Student-Newman-Keuls test ($P < 0.05$). No effect of the interaction between B and FL was observed for any of the variables analyzed. HR, O2P and HP (kcal/kg $LW^{0.75}$) did not differ among B and FL. F_1 heifers presented higher DM intake (DMI) and higher daily mean gain (DMG) in comparison to Holstein and Gyr breeds (6.18, 5.36 and 4.14 kg DM/d; 674.3, 480.8 and 435.4 g/d, respectively). Animals fed at 1.46% LW level presented higher DMI, DMG, BW and methane emission (g/d). Methane emission was higher for F_1 animals (161.3 g/d), but did not differ from Holstein breed (141.1 g/d). Animals from Gyr breed fed at 1.17% LW presented lower DMI, DMG and BW as well as inferior CH4 production (98.3 g/d). This research project was funded by FAPEMIG, CAPES, CNPq and Embrapa.

Key Words: greenhouse gas, heat production, zebu

M348 Methane production in dairy cows consuming corn milling co-products. K. G. Saathoff*[1], C. J. R. Jenkins[1], S. C. Fernando[1], D. Hostetler[2], and P. J. Kononoff[1], *[1]Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE, [2]The School of Veterinary Medicine and Biomedical Sciences, University of Nebraska-Lincoln, Lincoln, NE.*

A study using 4 multiparous Holstein dairy cows, which were 93.5 ± 22.2 DIM, was conducted to determine the effect of conventional and corn milling co-products, specifically dried distillers grains and solubles (DDGS), on milk production, composition, and methane production. A 4 × 4 Latin square was utilized and included 4 treatments, namely a zero control (C) and diets that contained 30% of the diet DM as either conventional DDGS (ConDG), or 15% reduced fat DDGS (RFDG) or a mixture (Mix) of 15% conventional DDGS and 15% reduced fat DDGS. In all 3 treatment diets, DDGS were included in replace of corn and soybean meal. Cows were housed and fed in individual stalls and fed once per day and milked twice per day for 4–28 d periods. During the last 2 d methane production was measuring using indirect calorimeters. Cows consuming DDGS consumed more ($P = 0.05$) feed (22.7, 24.8, 26.4 and 27.3 ± 1.57 for the C, RFDG, Mix and ConDG respectively). Likely in response feed intake, milk yield was also increased ($P < 0.01$) by feeding DDGS (29.4, 39.4, 38.2, and 38.0 ± 3.22 for the C, RFDG, Mix and ConDG respectively). The concentration of fat in the milk was not affected ($P = 0.47$) by treatment and averaged 3.41 ± 0.29%. In comparison, protein in milk was higher ($P = 0.05$) in consuming DDGS (2.74, 2.85, 2.95 and 2.91 ± 0.12 for the C, RFDG, Mix and ConDG respectively). Although total methane was not different ($P = 0.69$) across treatments averaging 443.8 ± 20.6 L/d, cows consuming DDGS produced less ($P = 0.01$) methane per unit of milk produced (14.6, 11.3, 12.1 and 12.1 ± 0.84 kg milk/kg feed for the C, RFDG, Mix and ConDG respectively). Results of this study further support the notion that corn milling co-products may be used to replace both corn and soybean meal in dairy rations and also suggest that doing may also result in less methane per unit of milk produced.

Key Words: methane, dried distillers grains and solubles

M349 Implementing multi-variate statistical process control to detect variability on a commercial dairy farm. Robb W. Bender*[1,2], James A. Barmore[2], David E. Cook[1], and David K. Combs[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]GPS Dairy Consulting LLC, Calmar, IA.*

The objective of this study was to characterize variability in both individual animal and pen data in data streams on a well-managed commercial dairy farm. Additional objectives were to assess the effect of outside events on the variability of data streams and utilize multivariate statistical process control (SPC) to improve detection time of out-of-control data streams. A 1,400-cow dairy in Eden, Wisconsin, was equipped with milk meters and rumination/activity collars to record individual cow milk production, rumination, physical activity, and pen-based feed intake. Data were collected over a 3 mo period. Milk production was analyzed for out-of-control data points via the Shewhart procedure, and rumination, physical activity and feed intake were analyzed multivariately via the MVP procedures of SAS. On this dairy, milk production averaged 45.1 kg, with a standard deviation of 1.3 kg among days within a pen, 11.5 kg among individual cows within a pen, and 20.1 kg among days within individual cows. Rumination (min per day) averaged 441.6 min, with a standard deviation of 14.0 among days within a pen and 120.7 min among individual cows within a pen. Physical activity (measured in arbitrary units) averaged 489.2, with a standard deviation of 17.1 among days within a pen, 103.4 among individual cows within a pen. Feed intake (DMI kg/cow/d) averaged 26.6 kg, with a standard deviation of 1.8 kg among days within pens. Multi-variate SPC increased sensitivity when compared with individual single-variate SPC analyses. Out-of-control milk production values were preceded by a deviation from normal variance in the multi-variate analysis of rumination, physical activity, and feed intake. Thus, multivariate SPC could be used as an early determinant of extreme variability in data streams on a commercial dairy.

Key Words: statistical process control, variation, dairy

M350 Evaluation of apparent starch digestibility in commercial dairy herds. R. A. Silva[1], J. H. Carneiro[1], I. Q. Carvalho[2], J. F. Santos[3], R. B. Navarro[4], P. F. Menegucci[5], M. Caetano[6], D. P. D. Lanna[7], and R. Almeida*[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Fundação ABC, Castro, PR, Brazil, [3]Castrolanda Cooperativa Agroindustrial, Castro, PR, Brazil, [4]Capal Cooperativa Agroindustrial, Arapoti, PR, Brazil, [5]Chr. Hansen, Valinhos, SP, Brazil, [6]University of Adelaide, Roseworthy, SA, Australia, [7]ESALQ/USP, Piracicaba, SP, Brazil.*

The objective of this study was estimate the apparent total-tract starch digestibility (ATTSD) in dairy herds, as well as its correlation with corn ensiling time. There were 20 commercial herds in the Paraná State, South Brazil, TMR-fed with predominantly Holstein cows. In all herds, only the high-producing groups were included in the study, with an average milk yield of 41 ± 6 L/cow/d, representing 33 ± 13% of lactating cows, and a total of 1655 cows. To check the influence of silage fermentation time on the starch digestibility, each farm was sampled in 2 distinct periods; fall season when most producers were using corn silage (CS) with short ensiling time (132 ± 132 d), and spring season with CS of longer ensiling time (260 ± 46 d). In each period 3 samples were collected per herd; TMR, CS and feces. Starch contents were determined in quadruplicate by enzymatic colorimetric method. To estimate ATTSD 2 equations were used: only with fecal starch as input (Eq1 = 100 × (1–0.0125 × %fecal starch)); and using lignin as marker (Eq2 = 1 − ((%TMR lignin/%fecal lignin) × (%fecal starch/%TMR starch))) × 100). The average values of 40 samples of corn silage and TMR were 31.15 ± 3.44% DM, 42.36 ± 3.86% NDF, 21.19 ± 2.11% ADF, 2.49 ± 0.68% lignin, 30.45 ± 4.29% starch, and 45.38 ± 3.75% DM, 34.99 ± 3.21% NDF, 16.72 ± 2.16% ADF, 1.92 ± 0.66% lignin, 27.02 ± 4.37% starch, respectively. The average fecal starch content was 3.40 ± 3.1% and 3.93 ± 2.50% for spring and fall samples, respectively. Apparent TTSD estimates were 95.42 ± 2.38% and 95.43 ± 5.15% to Eq1 and Eq2, respectively, with a high correlation between them (r = 0.83; $P < 0.01$). Positive correlation between ensiling time and ATTSD calculated by Eq2 was also detected (r = 0.31; $P < 0.05$), showing that CS with longer fermentation contributes positively to the increased starch utilization. We also observed moderate negative correlation between CS dry matter and ATTSD (r = −0.28; $P < 0.05$), indicating that dried CS reduces TTSD. In general, dietary starch was efficiently utilized, but some factors such as ensiling time and plant maturity influence starch digestibility of the dairy cow diets.

Key Words: corn silage, fecal starch

M351 Effects of partial replacement of corn and alfalfa silage with tall fescue hay on total-tract digestibility and lactation performance in lactating dairy cows. Robb W. Bender*, Fernanda Lopes, David E. Cook, and David K. Combs, *University of Wisconsin-Madison, Madison, WI.*

Our objective was to evaluate the effects of partial replacement of corn and alfalfa silage with tall fescue hay on total-tract NDF digestibility and lactation performance in dairy cows. Twenty-four primi- (75 ± 35 DIM) and 40 multi-parous (68 ± 19 DIM) Holstein cows were blocked by parity and randomly assigned to 1 of 4 treatment groups in a pen equipped with 32 feeding gates to record intake by cow. Each gate was randomly assigned to 1 treatment group, thus each cow had access to all 8 gates within the respective treatment and cow was the experimental unit. Treatments were formulated to partially replace corn silage (CS) and alfalfa silage (AS) with tall fescue hay (TF) as follows (DM basis): 67% CS and 33%AS (control), 60%TF and 40%AS (60TF40AS), 60%TF and 40%CS (60TF40CS), and 33%TF and 67%CS (33TF67CS). The experiment was a 7-week continuous lactation trial with a 2-week covariate period. Data were analyzed using the MIXED procedure of SAS. The model included parity, treatment, week, and relevant interactions as fixed effects and cow within treatment as a random effect. Dry matter intake and milk production did not differ, and averaged 23.5 and 41.4 kg/d, respectively. Fat concentration, protein yield and concentration, and SCC did not differ and averaged 4.00%, 1.28 kg/d, 3.15%, and 92.3, respectively among all treatments. Fat yield was greater ($P < 0.01$) for the control (1.65 kg/d), 60TF40AS (1.66 kg/d), and 33TF67CS (1.66kg/d) treatments compared with the 60TF40CS treatment (1.50 kg/d). Total-tract dry matter digestibility and total-tract organic matter digestibility did not differ, and averaged 67.9% and 70.0%, respectively. Total-tract NDF digestibility was lowest ($P < 0.01$) for control (40.8%), and greater ($P < 0.01$) for 60TF40CS (51.2%) than 60TF40AS (45.9%). 33TF67CS (46.7%) had similar total-tract NDF digestibility to the latter 2 treatments. Inclusion of highly digestible tall fescue grass hay has the potential to replace corn silage and alfalfa silage without influencing DMI and production.

Key Words: NDF, fiber digestion, dairy cow

M352 Methyl-donors choline and methionine differentially alter hepatic methyl carbon metabolism. Tawny L. Chandler*[1], Courtney L. McCourt[1], and Sandra J. Bertics[1], Barbara A. Barton[2], and Heather M. White[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]Balchem Corporation, New Hampton, NY.*

Overlap in hepatic methyl pathways highlights a role for potential competition and compensation of methyl donors. The objective of this experiment was to examine the regulation of genes controlling methyl group transfer in response to increasing concentrations of choline chloride (CC), DL-Met (dLM), and added fatty acids (FA). Primary hepatocytes isolated from 4 Holstein calves were maintained as monolayer cultures for 24 h before treatment with CC (33, 100, 200, 450 μM) and dLM (16, 30, 100, 300 μ*M*), with or without a 1 m*M* FA cocktail in a factorial design. Concentrations mimicked expected physiological concentrations. After 24 h, media was collected for quantification of reactive oxygen species (ROS) by fluorometric assay and cells were collected for quantification of gene expression. Data were analyzed using PROC MIXED of SAS 9.4 with linear and quadratic contrasts in a model with fixed effect of treatment and random effects of calf. Interactions were not significant and therefore only main effects are discussed. Met can be generated from betaine via BHMT, or from homocysteine via MTR which also serves as the final step in regeneration of met after methyl-group donation. Increasing dLM concentration did not alter BHMT expression but did decrease ($P = 0.003$) MTR expression. Increasing concentration of CC did not alter BHMT but did increase ($P = 0.02$) MTR expression, suggesting that CC plays a key role in regeneration of met after methyl group donation. FA increased ($P = 0.05$) BHMT expression but decreased ($P = 0.0001$) MTR expression, which favors regeneration of met that is coupled with downstream glutathione production, which may aid the cell with oxidative stress associated with FA metabolism. Both CC and dLM decreased ($P = 0.02$) expression of MAT1A, the enzyme that generates SAM from met. PEMT expression was not affected by CC or dLM suggesting that dLM was not used to generate phosphatidylcholine. ROS tended to decrease ($P = 0.08$) with increasing CC treatment but was not changed with dLM treatment. These data suggest that CC may play a critical role in donating methyl groups and in decreasing ROS within the hepatocyte.

Key Words: choline, methionine, methyl-donor

M353 The effect of nitrate or live yeast culture on methane mitigation in a continuous culture system. Caitlyn M. Massie*[1], Benjamin A. Wenner[1], Amanda M. Gehman[2], Zhongtang Yu[1], Kelly C. Wrighton[1], and Jeffrey L. Firkins[1], *[1]The Ohio State University, Columbus, OH, [2]Alltech, Nicholasville, KY.*

Nitrates have been successfully fed to dairy cows to decrease methane emissions in several experiments. In the nitrate assimilatory pathway, bacteria reduce nitrate to nitrite to ammonia. Because the second step can be rate-limiting, nitrite accumulation poses health risks such as methemoglobinemia, which would hinder adoption of nitrate feeding to compete with methanogens for H_2 while assimilating the N from nitrate into microbial protein. The yeast *Saccharomyces cerevisiae* has the potential to anaerobically respire nitrite through its cytochrome c oxidase; it also can stimulate populations of bacteria that express nitrate and nitrite reductases. For this project, 4 dual flow continuous culture fermenters were used in 5 periods with 7 d of adaptation and 3 d of sampling. Fermenters were fed 40 g DM (50:50 ratio of concentrate:alfalfa pellet). Treatments were arranged in a 2 × 2 factorial with NO_3 (1.5% of DM) or urea as an isonitrogenous control and without or with Yea-Sacc (Alltech Inc., Nicholasville, KY) fed at a recommended 0.010 g/d. Gas production was measured over 3 d by closed circuit respirometry; 1 fermenter's gas production was omitted for all periods (unrelated to treatment). Objectives were to test the hypothesis that the combination of live yeast culture and nitrate would mitigate methane production in continuous culture compared with the control (a statistical interaction). However, there were no interactions ($P > 0.10$). The main effect of nitrate decreased ($P < 0.05$) CH_4 emission compared with urea control (29.6 vs 21.0 mmol/d). There was no difference ($P > 0.10$) for H_2 emission for nitrate or yeast (averaging 0.149 mmol/d; SEM = 0.051), but the main effect of nitrate was decreased ($P < 0.01$) for aqueous H_2 concentration compared with urea (1.23 vs 1.88 μM). Total VFA production (averaging 148 mmol/d; SEM = 15) and acetate:propionate (averaging 3.37, SEM = 0.12) did not differ ($P > 0.10$) among treatments. Nitrate decreased methanogenesis without affecting H_2 variables. No interactions were detected, but live yeast might offer a useful protection against incompletely adapted rumen microbial populations.

Key Words: dairy, methane, yeast

M354 Performance, heat production and methane emission in dairy heifers under different nutritional plans. Carlos Alberto Alves Oliveira Filho[1], Fernanda Samarini Machado[2], Alexandre Lima Ferreira[2], Luiz Gustavo Ribeiro Pereira*[2], Thierry Ribeiro Tomich[2], Mariana Magalhães Campos[2], José Augusto Gomes Azevêdo[3], Rogério Martins Maurício[4], Alexandre Vieira Chaves[5], and Camilla Flávia Portela Gomes Silva[6], *[1]Universidade Estadual do Sudoeste da Bahia, Itapetinga, Bahia, Brazil, [2]Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, [3]Universidade Estadual de Santa Cruz, Ilhéus, Bahia, Brazil, [4]Universidade Federal de São João Del Rei, São João Del Rei, Minas Gerais, Brazil, [5]University of Sydney, Sydney, New South Wales, Australia, [6]Instituto Federal de Educação, Ciência e Tecnologia Baiano, Santa Inês, Bahia, Brazil.*

This study aimed to evaluate the effect of feeding levels (FL) and breed (B) on performance, heat production (HP) and enteric CH_4 emission in dairy heifers. Thirty 6 heifers, 12 Holstein, 12 Gyr and 12 crossbreed Holstein-Gyr (F1) with average live weight (LW) of 445.8 ± 98 kg and average age of 27.5 ± 0.8 mo, were housed in tie stall and randomly distributed to the treatments in a 3x3 factorial design (feeding levels of 1.95% LW, 1.46% LW and 1.17% LW, on dry matter (DM) basis and breeds). The diet was offered as a total mixed ration (700g/kg of corn silage and 300g/kg of concentrate, on DM basis; 140g of crude protein (CP) per kg of DM). Respiratory exchanges (oxygen consumption and CO_2 and CH_4 production) were measured over 2 periods of 24h using 4 open-circuit respiration chambers and a Sable System (Sable Systems, Henderson, NV) of Embrapa's Bioenergetic Laboratory (Coronel Pacheco, Minas Gerais, Brazil). The equation from Brouwer was used to estimate HP. Data were subjected to ANOVA and means were compared through the Student-Newman-Keuls test ($P < 0.05$). Significant effects were found for the interaction between B and FL for DM, organic matter (OM) and neutral detergent fiber (NDF) intakes (kg/d, %LW and g/kg $LW^{0.75}$) and enteric methane emissions (L/day, g/kg DMI, g/kg OMI and g/kg NDFI). Animals fed at 1.17% LW level presented lower HP (kcal/kg $LW^{0.75}$), feeding efficiency (FE) (kg LW/kg DM_{ing}) and CH_4 emission (L/kg $LW^{0.75}$). Gyr breed presented lower heat production (132.5 kcal/kg $LW^{0.75}$) and CH_4 emission (2.03 L/kg $LW^{0.75}$). For Gyr heifers, FE was higher than Holstein heifers but did not differ ($P > 0.05$) from F1 animals (0.11 and 0.10 Kg LW/kg DMI, respectively). F1 heifers presented higher ($P < 0.05$) daily mean gain (DMG), but did not differ ($P > 0.05$) from Holstein breed (0.81 and 0.67 kg/day, respectively). Animals fed at the level of 1.17% of LW presented lower DMG (0.40 kg/day). This research project was funded by Fapemig, CAPES, CNPq and Embrapa.

Key Words: greenhouse gas, respirometric chamber, zebu

M355 Effects of rumen-protected choline and B vitamins during the transition period on serum metabolites and milk composition in periparturient dairy cattle. C. M. Melo[1], L. C. Copetti[1], O. F. Stuani[2], R. Locatelli-Dittrich[1], and R. Almeida*[1], *[1]Universidade Federal do Paraná, Curitiba, PR, Brazil, [2]Safeeds Nutrição Animal, Toledo, PR, Brazil.*

The effects of rumen-protected choline and B vitamins (RPB) on β-hydroxybutyrate (BHBA), nonesterified fatty acids (NEFA), total cholesterol, triglycerides, HDL, LDL and VLDL and milk fat to protein ratio (FPR) were evaluated in periparturient dairy cows. In a commercial farm in Southern Brazil, 132 Holstein cows (104 multiparous and 28 primiparous) were blocked by parity, expected day of calving and body condition score (BCS). The supplementation period was 18 d pre and 21 d postpartum. Cows in the treatment group were individually top-dressed with 100 g/d Vicomb (Jefo, Quebec, Canada), to provide 20.9 g of rumen-protected choline plus protected riboflavin and folic acid, while the control cows (CON) were supplemented with corn meal. Five blood samples were collected from each animal (14 d and 7 d before calving, at calving, 7 d and 14 d after calving). Additionally 4 samples were collected (3, 5, 7 and 10 d after calving) for BHBA analysis using a Precision Xtra meter. Milk samples to estimate FPR were collected on d 7, 14 and 21 after parturition. Data were analyzed using MIXED procedure of SAS with a model containing the effects of block, treatment, time, and treatment × time interaction as fixed effects and cow within treatment as a random effect. The incidence of hyperketonemia (BHBA concentration ≥1.2 mmol/L) was 25.0% (16/64) for RPB and 30.0% (19/63) for CON. Body weight and BCS did not differ ($P > 0.05$) between RPB and CON cows in all 3 observations (21 d before calving, at calving, and 21 d after calving). No differences on serum metabolites between RPB and CON cows were detected ($P > 0.05$). NEFA concentrations peaked ($P < 0.01$) at calving; 0.82 ± 0.04 mmol/L, whereas BHBA peaked ($P < 0.01$) at 7 d after calving; 0.78 ± 0.05 mmol/L. The lowest cholesterol concentration was at calving; 73.78 ± 1.53 mg/dL. Finally no milk FPR differences ($P > 0.05$) were observed between RPB and CON cows in the first 3 weeks after calving; 1.29 *vs*. 1.31. In the particular

conditions of this on-farm trial with low incidence of hyperketonemia no benefits on RPB supplementation were detected.

Key Words: cholesterol, ketosis, lipoproteins

M356 Microbial protein synthesis of Jersey heifers supplemented with chitosan or omega-6 fatty acids source. Murilo Vendramini[1], Helder Amaral[1], Hayne Araki[1], Marcia Vaz[2], Dargon Salvia[1], Euclides Oliveira[1], Rafael Goes[1], Marcelo Barros[2], Bruno Secundino[1], and Jefferson Gandra*[1], *[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, MS, Brazil, [2]Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil.*

The objective of this study was to evaluate the microbial protein synthesis of Jersey heifers supplemented with chitosan or omega-6 fatty acids (FA) source. Eight animals (average body weight of 158.62 ± 1.75 kg, mean ± SD) were used in replicated 4 × 4 Latin square experimental design, balanced and contemporary, in 2 × 2 factorial arrangements. The experimental period consisted of 18 d (12 d for adaptation and 6 d for data collection) and 5 d were used for wash out. The experimental diets were (1) control (CO), without omega-6 FA and chitosan supplementation; (2) whole raw soybean (WRS, source of omega-6 FA) with 200 g/kg of DM of WRS); (3) chitosan (CHI), with 2 g/kg of DM of chitosan; (4) chitosan and WRS (CHWS), with chitosan and WRS in the same level used in previously cited treatments. The diets were formulated according to the NRC (2001) to achieve weight gain of 700 g/day. Spot urine samples were collected on d 17 of each period 4 h after feeding. The analyses were performed according to Chen and Gomes (1992). Data were analyzed using PROC MIXED of SAS 9.3.Interaction effect ($P < 0.05$) was observed between CHI and WRS for uric acid (mmol/L), which CHI reduced (1.74 mmol/L) and CHIWS increased uric acid (3.34 mmol/L). Animals fed CHI presented decrease ($P < 0.05$) in total purine and absorbable purine derivatives. The output of microbial nitrogen and protein (g/d) was reduced ($P < 0.05$) when heifers were supplemented only with CHI. Animals fed CHI presented decrease in the output of microbial nitrogen and protein in 31.55, 35.53 and 15.07% when compared with CO, WRS and CHWS, respectively. The chitosan supplementation reduced the microbial protein synthesis of dairy Jersey heifers.

Key Words: nitrogen metabolism, purine derivative, rumen manipulation

M357 Nutritional value of hemp byproducts as ruminant feeds. George N. Gozho* and Jan C. Plaizier, *University of Manitoba, Winnipeg, MB, Canada.*

Hemp byproducts have potential as ruminant feeds, especially now that very low tetrahydrocannabinol (THC) hemp varieties contents are available in Canada. Three hemp by-products, i.e., hemp meal, hemp bran, and hemp meal fines, were, therefore, analyzed for their value as ruminant feeds. These values were compared with ruminant feeds that these byproducts may replace, including canola meal, soybean meal, and grain screenings. Analyses included proximate analysis, in vitro dry matter digestibility (IVTDMD) analysis using the Dairy II system, and in situ rumen degradability using Dacron bags. The rumen in situ dry matter digestibilities were fitted to an exponential equation using the SAS nonlinear regression procedure. Calculated parameters included washing loss (A), potential digestibility after washing (B), potential total digestibility (A + B) and digestibility rate (c). Results showed that hemp meal contained less CP, and more fiber and fat, and had lower in situ and in vitro dry matter digestibilities than soybean meal. Hemp meal had a similar CP content, contained more fiber and fat, and had lower in situ and in vitro dry matter digestibilities than canola meal. Hemp bran had a very high fiber content and was less digestible than grain screenings. Hemp seed fines had a similar chemical composition than hemp meal, but it was more digestible than hemp meal. Hemp meal could be an alternative for other high protein feeds, but its relatively low digestibility must be taken into account.

Table 1 (Abstr. M357). Evaluation of hemp byproducts for ruminant feeds

	Hemp meal	Hemp bran	Hemp seed fines	Canola meal	Soybean meal	Grain screenings
CP, % DM	36.2	15.9	29.1	37.1	52.9	10.3
NDF, % DM	56.5	76.2	52.1	30.1	10.5	49.7
ADF, % DM	34.0	56.0	29.6	18.0	5.5	27.5
Crude fat, %	9.5	12.1	ND	2.4	0.7	2.4
Ash, % DM	6.3	3.2	4.5	6.6	6.7	8.2
IVTDMD, %	49.4	46.7	70.8	67.2	86.5	54.1
Rumen digestibility						
A,%	37.3	23.0	61.0	47.4	37.3	35.3
B, %	23.3	6.33	16.6	40.5	63.2	48.0
A+ B, %	61.6	31.4	76.6	87.5	99.9	83.3
c, %/h	0.08	0.01	0.25	0.06	0.08	0.12

Key Words: hemp byproduct, ruminant, feed

M358 Effect of corn type, particle size, enzymes, and time ensiled on chemical composition of rehydrated corn silage. Naina M. Lopes*[1,2], Marcos N. Pereira[2], and Felipe C. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Universidade Federal de Lavras, Lavras, MG, Brazil.*

Utilization of enzymes have been reported to improve feedstuff quality for dairy cows. The objective of this study was to evaluate 2 corns, Flint (F) and Floury (D); 2 particle sizes, grinded Fine (Fi) or Coarse (Co); application of 3 enzyme combination, amylase (A), protease (P) and both (AP); during 3 times of ensiling (TP) at 1, 3, or 5 mo in a complete randomized design resulting in 36 treatments. Each of the 5 silos for each treatment (replicates) consisted of 1kg of corn at 35% moisture and were stored in vacuum sealed bags at environmental temperature. Enzymes used were 650 g/t of amylase and 200 g/t of protease or the combination of the 2. Corn weight, pH and temperature were measured at time of bag opening. Samples were analyzed for starch, crude protein (CP) and prolamin concentration. Statistical analysis was performed using the MIXED procedure in SAS. Corn F had higher ($P < 0.001$) CP (9.52 vs. 7.61%DM) and prolamin (9.70 vs. 8.3 2%DM) before ensiling than D. On TP5, F had increased prolamin concentration (7.95%DM, $P = 0.003$) than D (4.91%DM). Corn F had lower weigh variation (11.34 vs. 12.12 g, $P < 0.0001$), lower starch concentration (65.03 vs. 66.63%DM, $P = 0.01$) and lower NFC ($P < 0.0001$) than D. Particle size Co had lower DM (29.28 vs. 29.41%; $P = 0.04$), higher weigh variation (12.12 vs. 11.34 g, $P = < 0.0001$) and higher pH (4.07 vs. 3.98, $P < 0.0001$) than Fi. Enzymes P and AP had lower prolamin concentration for A (6.09%DM), P (4.62%DM) and AP (4.92%DM, $P < 0.0001$); for starch, A had lower concentration for A (64.40%DM), P (65.30%DM) and AP (67.80%DM, $P = 0.0002$). Enzyme A had lower NFC, for A (8.75%DM), P (8.86%DM) and AP (8.89%DM, $P < 0.0001$). Corn weight increased with time 1.73g in TP1, 11.4g inTP3 and 22.02g in TP5 ($P < 0.0001$). Corn CP decreased ($P = 0.04$) with time ensiled from TP1 to TP 3 by 0.35%DM. Prolamin concentration changed ($P =$

0.01) during time of ensiling, 5.19, 4.0 and 6.43%DM for TP1, TP3 and TP5, respectively. In conclusion, P seemed to have reduced the matrix starch protein. Small particle size, enzyme and time ensiled, made F better feed but not as good as D.

Key Words: matrix starch protein, amylase, protease

M359 Nitrogen balance of Jersey heifers supplemented with chitosan or omega-6 fatty acids source. Murilo Vendramini[1], Helder Amaral[1], Maria Gabriela Lobo[1], Marcia Vaz[2], Natalia Silva[1], Euclides Oliveira[1], Rafael Goes[1], Marcelo Barros[2], Caio Takiya[3], and Jefferson Gandra*[1], *[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, MS, Brazil, [2]Faculdade de Ciências da Saúde, Universidade Federal da Grande Dourados, Dourados, MS, Brazil, [3]Departamento de Nutrição e Produção Animal, Universidade de São Paulo, Pirassununga, Brazil.*

The objective of this study was to evaluate the nitrogen balance of Jersey heifers supplemented with chitosan or omega-6 fatty acids (FA) source. Eight animals (average body weight of 158.62 ± 1.75 kg, mean ± SD) were used in replicated 4 × 4 Latin square experimental designs, balanced and contemporary, in 2× 2 factorial arrangements. The experimental period consisted of 18 d (12 d for adaptation and 6 d for data collection) and 5 d were used for wash out. The experimental diets were (1) control (CO), without omega-6 FA and chitosan supplementation; (2) whole raw soybean (WRS, source of omega-6 FA) with 200 g/kg of DM of WRS); (3) chitosan (CHI), with 2g/kg of DM of chitosan; (4) chitosan and WRS (CHWS), with chitosan and WRS in the same level used in previously cited treatments. The diets were formulated according to the NRC (2001) to achieve weight gain of 700 g/day. Blood samples were collected on d 15 of each period before feeding, by puncture of coccygeal vein and immediately centrifuged at 2000 g × 15 min, supernatant serum was transferred do tubes and submitted to analyses. Spot urine samples were collected on d 17 of each period 4 h after feeding. For the evaluation of nitrogen compounds were dosed concentrations of urea and creatinine (mg/dL) in blood and urine by enzymatic colorimetric method. Data were analyzed using PROC MIXED of SAS 9.3.Animals fed CHI presented lower ($P < 0.05$) concentration of urea and higher ($P < 0.05$) concentrations of creatinine (0.80 mg/dL) in blood than other diets. Higher concentration of urea (43.12 mg/dL) was observed for heifers fed WRS and lower concentration of creatinine than others experimental diets. The renal clearance of urea (24 h period) presented highest value ($P < 0.05$) for animals fed WRS and lowest ($P < 0.05$) for animals fed CHI. Fractional urea excretion (%) was highest for animals fed CHI and lowest for animals fed WRS. Chitosan and omega-6 FA source supplementation altered the nitrogen balance of dairy Jersey heifers.

Key Words: rumen manipulation, renal clearance, soybean

M360 Effects of amylase and protease on degradability and gas production of rehydrated corn grain silage. Naina M. Lopes*[1,2], Marcos N. Pereira[2], and Felipe C. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Universidade Federal de Lavras, Lavras, MG, Brazil.*

Protein starch matrix is a physical barrier to starch digestion in corn grain. The objective of this study was to evaluate 2 corns, Flint (F) and Floury (D); 2 particle sizes, grinded Fine (Fi) or Coarse (Co); application of 3 enzyme combinations, amylase (A), protease (P) and both (AP); during 3 times of ensiling (TP) at 1, 3, or 5 mo in a complete randomized design resulting in 36 treatments. Each of the 5 silos for each treatment (replicates) consisted of 1kg of corn at 35% moisture and were stored in vacuum sealed bags at environmental temperature. Enzymes used were 650 g/t of amylase and 200 g/t of protease or the combination of the 2. Samples were analyzed for in vitro degradability for 18 h and fermentation of gas production for 24 h (Ankom RFS, Ankom Tech.). Statistical analysis was performed using the MIXED procedure in SAS. Corn F had lower soluble fraction (Fs) degradability (6% of incubated, $P < 0.0001$) but higher degradable fraction (Fd, $P = 0.02$) than D (42 and 43% of incubated, respectively). Corn Co had higher indigestible fraction (Fi, 53% of incubated, $P = 0.002$) then Fi (51% of incubated). Enzyme P resulted in higher ($P < 0.0001$) Fd (43% of incubated) and AP (44% of incubated) than A (39% of incubated). Addition of AP reduced Fi portion and increased the rate of degradation ($P < 0.0001$) over time. Time points 3 and 5 had higher Fd (30% on TP1 vs. 50% on TP3 and 5; $P = 0.002$) than TP1, thus, the rate of degradation increased in the last month of ensiling (from 0.02 to 0.04; $P = 0.004$). Timepoint 3 had a higher ($P < 0.0001$) absolute pressure 37.42 psi for TP1, 42.81 psi for TP3 and 38.08 psi for TP5 and cumulative pressure 9.77 psi for TP1, 16.5 psi for TP3 and 8.92 psi for TP5 ($P < 0.0001$). Data from gas production were divided in 2 periods: 0–12 h and 12–24 h. Period 1 (6.17 psi) had lower ($P < 0.001$) gas production than period 2 (17.30 psi). In conclusion, corn ensiled and treated with enzymes for 3 mo had improved in vitro degradability than non-ensiled corn. Treated corn has great potential to improve farmer's profitability when fed to cattle.

Key Words: enzyme, corn fermentation, gas production

M361 Stereo microscopy and scanning electron microscopy of manure samples from late lactation dairy cows when fed cobalt-lactate in a high-forage total mixed ration. Jon P. Pretz*[1], Jianping Wu[2], Madam Jao[2], Bill Holloway[3], Del Davis[3], and David P. Casper[1], *[1]South Dakota State University, Brookings, SD, [2]Gansau Agricultural University, Lanzhou, Gansu, China, [3]Ralco Inc., Marshall, MN.*

Feeding higher Co amounts showed increased ruminal fiber digestion. Cobalt-lactate is a highly rumen soluble source of Co and has demonstrated increased ruminal molar acetate concentrations and decreased ruminal ammonia concentrations when lactating dairy cows were fed a high forage ration (Pretz et al., 2014). These results indicated increased fiber digestion and synthesis of microbial protein. Twenty-four late-lactation (μ = 238 DIM and 36.5 kg milk) Holstein dairy cows (10 primiparous and 14 multiparous), were block by milk yield, DIM, and parity and randomly assigned to 1 of 2 treatments being: 1) control (C): ration containing cobalt carbonate fed at 58 mg/cow/d and 2) (T): ration containing 5 g/cow/d of a 1% Co-lactate product (Co-Max). Total mixed rations (TMR) were 70% forage (60% alfalfa baleage and 40% corn silage) and 30% of the respective experimental grain mix on a DM basis. Lactational responses were previously reported. This study further evaluated the total-tract nutrient digestion by collecting TMR and manure samples for measuring nutrient digestibility via internal markers (acid insoluble ash and iron). In addition, manure samples were submitted for stereo microscopy and scanning electron microscopy evaluation. Manure samples were collected at 4 h intervals for 3 consecutive d from each cow during wk 3 and 4. Total-tract DM (53.7 and 56.8% for C and T, respectively), CP (63.8 and 64.0%), NDF (46.3 and 48.9%), and ADF (39.8 and 42.9%) digestibility coefficients were numerically greater, but not significantly ($P > 0.10$) different for cows fed T compared with cows fed C. Stereo microscopy demonstrated that manure samples from cows fed T were more transparent than manure samples from cows fed C. Scanning electron microscopy indicated small visible improvements in digestibility of fiber components observed as appearance of hollow pits in the fiber particles. Feeding additional Co as cobalt-lactate visu-

ally appeared to increase digestibility of fiber particles when evaluated by stereo and scanning electron microscopy.

Key Words: scanning electron microscope, cobalt-lactate, stereo microscopy

M362 Plasma metabolites of Jersey heifers supplemented with chitosan or omega-6 fatty acid source. Helder Amaral[1], Murilo Vendramini[1], Leticia Parangaba[1], Grazilele Rosa[1], Caio Takiya[2], Euclides Oliveira[1], Rafael Goes[1], Antonio Machado[1], André Santos[1], and Jefferson Gandra*[1], *[1]Faculdade de Ciências Agrárias, Universidade Federal da Grande Dourados, Dourados, MS, Brazil, [2]Departamento de Nutrição e Produção Animal, Universidade de São Paulo, Pirassununga, Brazil.*

The objective of the present study was to evaluate plasma metabolites of blood of Jersey heifers supplemented with chitosan or omega-6 fatty acids (FA). Eight Jersey heifers (average body weight of 158.62 ± 1.75 kg, mean ± SD) were used in replicated 4 × 4 Latin square experimental design, balanced and contemporary, in 2 × 2 factorial arrangement. The experimental period consisted of 18 d (12 d for adaptation and 6 d for data collection) and 5 d were used for wash out. The experimental diets were (1) control (CO), without omega-6 FA and chitosan supplementation; (2) whole raw soybean (WRS, source of omega-6 FA) with 200g/kg of DM of WRS); (3) chitosan (CHI), with 2 g/kg of DM of chitosan; (4) chitosan and WRS (CHWS), with chitosan and WRS in the same level used in previously cited diets .The diets were formulated according to the NRC (2001) to achieve weight gain of 700 g/day. Blood samples were collected on d 15 of each period before feeding, by puncture of coccygeal vein and immediately centrifuged at 2000 g × 15 min, supernatant serum was transferred do tubes and submitted to analyses of cholesterol (CHO), low density cholesterol (LDL), high density cholesterol (HDL) urea and blood urea nitrogen (UN) by enzymatic colorimetric method. Data were submitted to PROC MIXED of SAS 9.3. The CHI diet decreased ($P < 0.05$) and WRS increased ($P < 0.05$) blood concentrations of TC, LDL, urea and UN. Heifers presented blood concentration (mg/dL) of TC (78.12 and 131.25), LDL (44.15 and 92.35), urea (35.50, 43.12) and UN (16.54 and 20.09) for fed CHI and WRS, respectively. Interaction effect for HDL blood concentration was observed ($P < 0.05$) between CHI and WRS. The animals fed CHWS presented higher concentration (46.37 mg/dL), than WS (32.87 mg/dL), CO diet (28.00 mg/dL), and CH (23.75 mg/dL).Chitosan and omega 6 fatty acid influenced plasma metabolites of Jersey dairy heifers.

Key Words: alternative rumen modulator, metabolic profile, rumen manipulation

M363 Effect of top-dressing rumen-protected methionine in lactating Holstein cows: I. Profile of plasma amino acids, milk yield, and milk composition. Mateus Z. Toledo*[1], Giovanni M. Baez[1], Eduardo Trevisol[1], Nelson E. Lobos[1], Alvaro Garcia-Guerra[1], Jerry N. Guenther[1], Daniel Luchini[2], Randy D. Shaver[1], and Milo C. Wiltbank[1], *[1]University of Wisconsin-Madison, Madison, WI, [2]Adisseo, Alpharetta, GA.*

Experimental objectives were to evaluate the effects of supplementation with rumen-protected methionine (RPM) from 31 ± 2 until 127 ± 2 DIM on circulating amino acid concentrations and lactation performance of dairy cows. Holstein cows (n = 309) were housed in a freestall barn, milked twice daily, fed a basal diet formulated to 16.7% CP to deliver 2521 g of metabolizable protein (MP) with 6.93 lysine as % of MP and randomly assigned to once daily top-dressing with either: 1) RPM, 21.2 g of Smartamine M mixed with 38.8 g of dry distillers grains (2.34 methionine as % of MP) or 2) Control (CON), 60 g of dry distillers grain (1.87 methionine as % of MP). Plasma was assayed for free amino acids by gas chromatography using a commercial kit (EZ:faast, Phenomenex). Amino acid data were analyzed using a linear mixed model with repeated measures. Milk yield and composition were determined monthly and analyzed using a linear mixed model with treatment as a fixed effect and enrollment week as a random effect. Cows treated with RPM had increased milk protein content at all 3 milk tests (mean 58.7, 86.0, 113.5 DIM) (2.96 vs. 3.03%, $P = 0.003$; 3.02 vs. 3.07%, $P = 0.02$; 3.05 vs. 3.12%, $P = 0.02$) with no difference in milk yield. Blood samples were collected from a subset of cows (n = 8 CON; n = 12 RPM) at 0, 3, 6, 9, 12, 18, and 24 h after feeding RPM. Plasma methionine did not differ between treatments at 0 ($P = 0.37$) and 3 h ($P = 0.50$) but was greater in RPM cows at 6 ($P = 0.03$) and 9 h ($P < 0.0001$), peaking at 12 h (52.4 vs 26.0 nmol/mL, $P < 0.0001$), decreasing by 18 h ($P = 0.12$), and back to basal by 24 h ($P = 0.44$). Plasma lysine ($P = 0.47$) and histidine ($P = 0.42$) were unaffected by treatment. Another subset of cows (n = 16 CON; n = 24 RPM) was evaluated at 12 h after top-dressing. Cows fed RPM had increased methionine in both primiparous (23.9 vs. 46.4; $P = 0.007$) and multiparous (23.5 vs. 38.7; $P = 0.02$) cows. Thus, top-dressing RPM resulted in a surprisingly large but acute change in circulating methionine and a small but consistent change in milk protein content.

Key Words: methionine, dairy cattle, milk protein

M364 Milk protein and fat production are regulated by histidine and glucose supply in the lactating dairy cow. John Doelman*[1,2], Michelle Carson[1], John P. Cant[2], and John A. Metcalf[1], *[1]Nutreco Canada Agresearch, Guelph, ON, Canada, [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.*

The lactational response to essential amino acid (EAA), histidine and glucose supply was determined in 6 early lactation fistulated dairy cows (92 ± 17 DIM) fed a diet to provide an NE_L of 6.9 MJ/kg DM and 11.7% crude protein. Treatments consisted of abomasal infusions of saline (Sal), 1 kg/d glucose (Glc), 563 g/d of an EAA mix (equivalent to EAA in 1kg casein) with (EAA + Glc) and without (EAA) glucose, or EAA less histidine with (–His + Glc) and without glucose (–His) in a 6 × 6 Latin square design. Data were analyzed using the MIXED procedure in SAS where period and treatment were fixed effects and cow a random effect. Milk yield was significantly increased in response to EAA + Glc compared with Sal ($P < 0.001$), while the removal of histidine from EAA + Glc significantly decreased milk yield ($P < 0.001$). Compared with Sal, milk protein yield increased 146 g/d ($P < 0.001$) in response to EAA. The –His treatment decreased milk protein yield 266 g/d ($P < 0.001$) compared with EAA, and EAA + Glc generated an increase of 346 g/d over –His + Glc ($P < 0.001$). Milk protein concentration increased 0.21 and 0.23 percentage points during EAA and EAA + Glc infusion compared with Sal ($P \leq 0.003$), while the imbalance created with the omission of histidine, with and without glucose, significantly reduced milk protein concentrations to below those observed for Sal ($P \leq 0.002$). With the exception of the complete EAA treatment, milk fat yield was significantly greater for –His compared with all other treatments ($P \leq 0.03$). Milk fat concentration was 0.99 and 1.08 percentage points greater for –His compared with Glc ($P < 0.001$) and EAA + Glc ($P < 0.001$). Supplemental glucose did not stimulate a milk protein response and amplified the negative effects of a histidine deficiency. Additionally, histidine deficiency generated a positive milk fat response while glucose

Table 1 (Abstr. M366).

Item	Strategy					
	L-H	S-H	H-H	L-S	S-S	H-S
Farms, no.	12	14	12	6	12	8
Cows sampled	178	192	187	90	194	131
% HYK	9.0	15.0	12.0	7.8	33.3	23.7
BHBA mmol/L	0.46[a]	0.51[ac]	0.53[ac]	0.52[ac]	0.69[b]	0.58[bc]
[95% CI]	[0.41–0.53]	[0.46–0.57]	[0.48–0.59]	[0.45–0.60]	[0.62–0.77]	[0.52–0.65]
Farms/risk group (%)						
Low	8 (66.7)	7 (50.0)	8 (66.7)	5 (83.3)	2 (16.7)	3 (37.5)
Moderate	4 (33.3)	6 (42.9)	4 (33.3)	1 (16.7)	4 (33.3)	3 (37.5)
High	0	1 (7.1)	0	0	6 (50.0)	2 (12.5)

[a,b,c]Row means with different superscript letters differ ($P < 0.05$) Tukey's HSD.

supplementation inhibited milk fat production. These results demonstrate the effect of histidine and glucose supply on lactation performance.

Key Words: amino acid, dairy cow, milk protein

M365 Development of an in vitro subacute ruminal acidosis (SARA) model. Allan B. Chestnut*[1], Jim M. Aldrich[1], Tammy K. Miller Webster[2], Wenping Hu[1], Wibe B. Fokkink[1], and Howard G. Bateman[1], *[1]Provimi North America, Brookville, OH, [2]Rumen Fermentation Profiling Laboratory, West Virginia University, Morgantown, WV.*

To test efficacy of additives to reduce SARA, a protocol was developed to model SARA conditions using an in vitro continuous culture fermentor system at the Rumen Fermentation Profiling Laboratory, West Virginia University. A BASE diet was formulated with a 60:40 forage:concentrate (F:C) ratio (DM basis). Inclusion of corn silage, alfalfa balage, grass hay, ground corn (GC), SBM, corn gluten meal, corn gluten feed, soybean hulls, dried molasses, sodium bicarbonate and a mineral-vitamin supplement were, respectively, 36.1, 12.1, 12.1, 11.00, 13.7, 1.0, 1.0, 8.3, 2.0, 2.0 and 1.8% of DM. Mean particle size of GC was 870 µm (SD = 2.7). In Trial 1, diet SARA1 was formulated by reducing the BASE diet F:C ratio to 52:48. Corn was increased to 20.9% with 50% (DM basis) of the GC replaced with steam flaked corn (SFC). Treatments were: BASE diet fed as 25-g meals 4 times daily for 10 d (B) and BASE diet fed as 25-g meals 4 times daily for 7 d followed by the SARA1 diet fed as 50-g meals 2 times daily for 3 d (S1). Treatments were replicated in triplicate. Data collected the last 3 d included pH at 30-min intervals and NDF digestibility. Daily average pH were calculated for each fermentor. All data were analyzed using PROC MIXED of SAS. Average and minimum pH were analyzed with a repeated-measures model. Fermentor was treated as a random variable and AR(1) was selected as the appropriate covariance structure. Average pH of S1 (6.25) vs B (6.28) was similar ($P = 0.15$). Minimum pH of S1 (6.06) vs B (6.15) tended to be less ($P = 0.08$). NDF digestion was less ($P < 0.01$) for S1 (41.5%) vs B (46.6%). In Trial 2, treatment S1 was replaced with S2 by altering the SARA1 diet to create SARA2 as follows: corn replaced sodium bicarbonate and the GC:SFC ratio changed to 1:2. Trial 2 protocol was similar to Trial 1. Average pH of S2 (6.17) was less ($P < 0.05$) than B (6.30). Minimum pH of S2 (5.88) was less ($P < 0.01$) than B (6.18). NDF digestion was less ($P < 0.01$) for S2 (30.5%) vs B (40.4%). It was concluded that using the tested protocol with diets BASE and SARA2 provided an appropriate SARA model.

Key Words: subacute ruminal acidosis, in vitro, model

M366 Association of peripartal nutritional strategy with concentration of postpartum β-hydroxybutyrate in dairy cows. Allison B. Lawton*[1], Sabine Mann[2], Winfield S. Burhans[3], Daryl V. Nydam[2], Christine A. Rossiter-Burhans[4], Michael Tetreault[4], and Thomas R. Overton[1], *[1]Department of Animal Science, Cornell University, Ithaca, NY, [2]Department of Population Medicine and Diagnostic Sciences, College of Veterinary Medicine, Cornell University, Ithaca, NY, [3]Dairy-Tech Group, South Albany, VT, [4]Poulin Grain, Newport, VT.*

The objective was to determine the effect of transition cow nutritional management on β-hydroxybutyrate (BHBA) concentration of postpartum dairy cows. Commercial Holstein herds (n = 64) based in New York and Vermont were enrolled in a prospective cohort study in one of 6 herd nutritional strategy groups: 1) low energy dry cow (<16% starch), high energy lactating (>25% starch) (L-H); 2) Step-up dry (far-off < 16% starch, close-up > 16% starch), high energy lactating (S-H); 3) high energy dry (>16% starch), high energy lactating (H-H); 4) low energy dry, step-up fresh (fresh < 25% starch, high > 25% starch) (L-S); 5) step-up dry, step-up fresh (S-S); 6) high energy dry, step-up fresh (H-S). Blood samples were collected from 972 cows, 3–14 DIM and BHBA concentration measured using a Precision Xtra meter. Concentrations of BHBA were log-transformed and a multivariable linear model was used to assess the fixed effects nutritional strategy, season, and parity on BHBA concentration with farm as a random effect. The proportion of cows with hyperketonemia (HYK; BHBA ≥1.2 mmol/L) in each nutritional strategy as well as risk group (low: <15%, moderate: ≥15%, <40%, high: ≥40% of sampled cows HYK) was evaluated. Multiparous cows had higher BHBA concentrations than primiparous (0.61 [0.57–0.67 CI] vs. 0.48 [0.43–0.53 CI] mmol/L, $P < 0.0001$) and concentrations were higher in summer compared with winter (0.61 [0.56–0.67 CI] vs. 0.48 [0.44–0.53 CI] mmol/L, $P < 0.0001$; Table 1). Overall, a step-up approach to both dry and fresh diets led to the highest BHBA concentration among all strategies in this study.

Key Words: transition period, β-hydroxybutyrate, nutritional strategy

M367 Evaluation of rumen outflow in dairy cows by use of reticular and omasal sampling as an alternative to sampling from abomasal cannula. José Esler Freitas Jr.*[1], Tiago Dell Vale[2], Vitor Pereira Bettero[2], Marjorye Kametani[2], Pablo Gomes Paiva[2], Rodrigo Gardinal[2], Caio Seiti Takiya[2], Filipe Zanferari[2], Thiago T. H. A. Vendramini[2], Elmerson Ferreira Jesus[2], Gustavo Delfino Calomeni[2], and Francisco Palma Renno[2], *[1]Department of Animal Science, Federal University of Bahia, Salvador, Bahia, Brazil, [2]Department of Nutrition and Animal Production, Faculty of Veterinary Medicine, University of São Paulo, Pirassununga, São Paulo, Brazil.*

The aim of this study was to evaluate the effects rumen outflow in dairy cows fed unsaturated fatty acid by use of reticular and omasal sampling as an alternative to sampling from the abomasal canal. Four Holstein dry cows cannulated in the rumen and abomasum (602 ± 21 kg of body weight; mean ± SD) were assigned randomly into a 4 × 4 Latin square design experiment, fed the following diets: (1) control (C), without unsaturated fatty acid supplementation; (2) soybean oil (SO), addition of 3% of refined soybean oil in DM basis of total diet; (3) whole raw soybean (WS), addition of 16% of whole raw soybeans in DM basis of total diet; and (4) calcium salts of unsaturated fatty acids (CSFA), addition of 3% of CSFA in DM basis of total diet. Nutrient flow was calculated using the reconstitution system based on 3 markers (cobalt-EDTA, ytterbium chloride and indigestible NDF). Large and small particles and the fluid phase were recovered from digesta collected of different sites. Samples were collected 8 times during 20 4 h and were combined into one pool sample for further reconstitution. Data were analyzed using PROC MIXED of SAS 9.1 using the Tukey test to test pair wise comparisons. The DM flow was higher ($P < 0.01$) to abomasum compared with reticulum and omasum (4.0; 4.0 and 4.8 kg to sites reticulum, omasum and abomasum respectively). However, the NDF flow was higher ($P < 0.01$) (1.5; 1.2 and 1.4 kg to reticulum, omaso and abomasums respectively), to reticulum compared with omaso and abomasums. There was no difference for the 18:3 flow. The 18:2 flow was higher (50.9; 10.3 and 24.3 g to sites reticulum, omasum and abomasum respectively), to reticulum compared with omaso and abomasums, and 18:1 flow was less ($P < 0.01$) for to omasum compared with reticulum and abomasum. The reticular sampling technique provided reliable estimates for DM flow and fatty unsaturated fatty acids in dairy cows

Key Words: dairy cow, omasal sampling, nutrient flow

M368 Feed milk value and protein supply to dairy cows of new co-products (carinata meal) from bio-fuel processing in comparison with canola meal. Yajing Ban*, David A. Christensen, John J. McKinnon, and Peiqiang Yu, *Department of Animal and Poultry Science, College of Agricultural and Bioresources, University of Saskatchewan, Saskatoon, Saskatchewan, Canada.*

Brassica carinata is a newly developed oilseed for bio-fuel production in Canada. The bio-fuel processing results in a large amount of co-products (carinata meal), which could potentially be utilized as a protein source for animal feed. However, there is little research on metabolizable protein characteristics of carinata meal for dairy cattle. The objectives of this study were to determine differences among carinata meal, extruded carinata meal and canola meal for dairy cattle in terms of (1) rumen protein degradation, (2) intestinal digestion of protein, (3) total truly digestible protein supply, and (4) feed milk value. The animal trial was carried out in University of Saskatchewan dairy research facility. Statistical analyses were performed using PROC MIXED procedure of SAS 9.3 with significance declared at $P \leq 0.05$. The results indicated that extruded carinata meal had higher rumen degraded protein than canola meal ($P \leq 0.05$). Intestinal digestible protein was the highest in canola meal but the lowest in extruded carinata meal ($P < 0.0001$), while total digestible protein was the highest in extruded carinata meal but the lowest in canola meal ($P < 0.0001$). Extruded carinata meal had the most truly absorbed rumen-synthesized microbial protein in the intestine and degraded protein balance, but had the least truly absorbed rumen undegraded feed protein and truly digested protein in the small intestine ($P \leq 0.05$). The canola meal was the highest in truly absorbed rumen undegraded feed protein in the small intestine but the lowest in truly absorbed rumen-synthesized microbial protein in the intestine and degraded protein balance ($P \leq 0.05$). Feed milk values had no significant difference in carinata meal and canola meal using the Dutch DVE/OEB model. In NRC model, canola meal was the highest in FMV. The results indicated that carinata meal could be used as a potential protein supplement. Extrusion processing seems to have a negative effect on protein utilization and production in dairy cattle.

Key Words: carinata meal, metabolizable protein characteristics, feed milk value

M369 Dairy calves changes in serum total protein and albumin concentration according to time after colostrum intake. Nathalia B. Rocha[1], Fernanda L. M. Silva[1], Jackeline T. Silva[1], Carolina C. F. Monteiro[2], Marília R. Paula[1], and Carla M. M. Bittar*[1,3], *[1]University of Sao Paulo, ESALQ, Piracicaba, SP, Brazil, [2]Universidade Federal de Pernambuco, Recife, PE, Brazil, [3]CNPq, Brasilia, DF, Brazil.*

Determination of serum total protein (TP) is being used as a tool to evaluate failure of passive immune transfer in dairy calves. However, time of evaluation recommendation is not well established. The objective of this study was to evaluate these changes, aiming a better recommendation of the better time to assess TP in neonatal calves. Blood form 47 cows was drawn just after calving, as well as from the calves at 0 (before colostrum feeding), 1, 2, 4, 6, 12, 24, 48, 72, 96 and 120h after colostrum intake. Crossbred calves (Holstein × Gir and Jersey × Gir) were separated from their mothers and 3L of high quality colostrum were fed. Hematocrit was determined and samples were centrifuged for determination of TP using a refractometer and albumin and TP using a commercial enzymatic kit. Regression curves were constructed for each parameter according to time, using SAS PROC REG. Correlations between mother TP at calving and calf TP at different times were also calculated using SAS PROC CORR. Quadratic regressions showed higher R^2 for all parameters. Total protein concentration estimated by the protein refractometer was higher than that determined by enzymatic method, as shown by the higher intercept in both linear and quadratic prediction equation (Table 1). Correlation between cow's TP at calving were positive and significant only at time 0 ($r = 0.2989$; $P < 0.05$) and 1h ($r = 0.24591$; $P < 0.1$). The quadratic behavior of the regression analysis of TP, by both methods, suggest that the maximum time after colostrum feeding for a better evaluation of passive immune transfer is around 12–24h after colostrum feeding.

Contd.

Table 1 (Abstr. M369).

Prediction equation[1]	R^2	$P <$
Albumin = 2.32 − 0.00038 T	0.0009	0.497
Albumin = 2.39783 − 0.00951T + 0.00008507T^2	0.0418	0.0001
TPe = 5.304999 + 0.01931 T	0.2876	0.0001
TPe = 4.79507 + 0.08244 T – 0.00058577 T^2	0.5176	0.0001
TPr = 6.13537 + 0.02468 T	0.2486	0.0001
TPr = 5.53535 + 0.10027 T – 0.00070451 T^2	0.4233	0.0001

[1]TPe = TP determined by enzymatic method; TPr = TP estimated by refractometer.

Key Words: refractometer, passive immune transfer

M370 Relationship between rumen methanogens and methane production in crossbred Holstein-Gyr steers. Shirley Motta de Souza[1], Daniela Batista Oss[2], Luiz Gustavo Ribeiro Pereira*[1], Cláudia Braga Pereira Bento[2], Hilário Cuquetto Mantovani[2], Marcos Inácio Marcondes[2], Fernanda Samarini Machado[1], Thierry Ribeiro Tomich[1], Mariana Magalhães Campos[1], Adriana Santana Carmo[1], Ellen de Almeida Moreira[1], Sávio Augusto Toledo Moreira[1], and Pedro Braga Arcuri[4], *[1]Brazilian Agricultural Research Corporation-Embrapa (Dairy Cattle), Juiz de Fora, MG, Brazil, [2]Federal University of Viçosa, Viçosa, MG, Brazil, [3]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [4]EMBRAPA Liaison Officer for Multilateral, Regional & National Entities in Europe, Rome, Italy.*

The aim of this study was to evaluate the bacterial community composition by denaturing gradient gel eletrophoresis (PCR-DGGE). Eighteen steers Holstein × Gyr with average body 8 155 ± 5 kg d^{-1} were randomly distributed in a completely randomized design with 3 treatments and 6 repetitions. The diet was calculated for average daily gain of 1.2 kg/d and an average weight of 240 kg, using the requirements for crossbred steers estimated by BR-Corte. Forage:concentrate ratio (based on DM) used was 60:40 and the animals received dietary treatment 1.2% DM of BW; 1.9% DM of BW and ad libitum intake, as maintenance, intermediate and high gain treatment, respectively. The daily feed intake was recorded and animals were weighed weekly each 28 d. The production of methane enteric by the animals was measured by open-circuit respiration chambers for 2 consecutive 24-h days. To assess the genetic diversity of the ruminal microbial community, 50 mL of rumen fluid samples were collected at the slaughter. DNA was extracted and processed by phenol-chloroform and bead beating method. PCR reaction used universal primers to amplify the V3 region to amplify the 16S rRNA of archaea Nested-PCR was performed to amplify a shorter region of the archaea 16S rRNA, using the primers ARC344f-GC/517r for archaea. PCR-DGGE patterns were analyzed using BioNumerics software 5.1 with which hierarchical cluster comparisons were carried out to group similar profiles and to generate a binary matrix of band classes. All the images were normalized using the internal control samples and the comparison among whole profiles was performed using the Dice similarity coefficient. The total number of the detected bands represented the species richness. Shannon-Wiener index was calculated based on relative band intensity and the total of number bands of each DGGE profile. The statistical analyses were done using the software R. The methane emission was affected by the treatments ($P < 0.05$) but there was no effect of the treatments on the richness index also by Shannon-Wiener ($P > 0.05$). No differences in archaeal population were detected between treatments.

Key Words: archaea, greenhouse gas, global warning

M371 Evaluation of different oral rehydration protocols for dairy calves affected by diarrhea. Evangelina Miqueo[1], Thais M. Torrezan[1], Nathalia B. Rocha[1], Jackeline T. Silva[1], Marília R. Paula[1], Samyra Baldassin[1], and Carla M. M. Bittar*[1,2], *[1]University of Sao Paulo, ESALQ, Piracicaba, SP, Brazil, [2]CNPq, Brasilia, DF, Brazil.*

Different oral rehydration therapies are used to restore the electrolyte level in diarrheic dairy calves. Most of them demand great amount of labor and time, however the use of commercial supplements administered with the liquid diet may make it easier. The objective of this study was to evaluate 3 rehydration protocols to determine the most effective in restoring electrolytes and water while maintaining animal performance. Thirty male Holstein bull calves were blocked by birth weight and serum total protein 24 h after birth, and distributed to one of 3 protocols of oral rehydration when presenting fecal score higher than 3 in a scale of 1 to 5: (1) Common electrolytes oral solution: 25 g dextrose, 10 g of sodium bicarbonate and diluted 5 g of sodium chloride in 1 L of water supplied at 10:00 and at 14:00); (2) commercial electrolyte solution vial for calves added to the milk replacer; and (3) Common electrolytes oral solution containing 2.2 g of glutamine and glutamate diluted in 1 L of water supplied at 10:00 and 1L at 14:00 h. All animals received 4 L/d milk replacer divided into 2 meals (7 h and 17 h) and were weaned with 56 d. Starter concentrate and water intake were measured daily and calves were weighed weekly. Although there were no significant differences among treatments for consumption, weight gain and days in diarrhea, voluntary water intake was significantly higher for diarrheic calves that received Glutellac, which together with the simplicity of use, represent the major advantages of this method of rehydration.

Table 1 (Abstr. M371). Performance of dairy calves receiving different rehydration treatments when presenting diarrhea

Item	Dextrose solution	Commercial electrolytes	Dextrose + AA solution	SEM	$P <$
Total DMI, g/d	713	747	670	41.3	0.41
Starter intake, g DM/d	267	199	225	37.0	0.35
Milk replacer intake, g DM/d	447	448	445	2.2	0.6
Total water intake, L/d	3.50	2.96	3.31	0.20	0.201
Voluntary water intake, L/d	1.50[b]	2.96[a]	1.30[b]	0.20	0.0001
Average weight, kg	40.7	40.6	39.1	2.5	0.63
ADG, g	309	340	292	32.4	0.53
Feed efficiency	0.36	0.34	0.37	0.03	0.35

Key Words: amino acid, dextrose solution, commercial electrolyte

M372 Pretreatment with saturated and unsaturated fatty acids regulates [1-^{14}C] C16:0 metabolism in Madin-Darby bovine kidney cells. Katherine E. Boesche*, Stephanie L. Koser, and Shawn S. Donkin, *Purdue University, West Lafayette, IN.*

Metabolic fates of fatty acids (FA) may be influenced by circulating FA concentration. Previous work in our lab demonstrated an ability of C18:3n-3 *cis* to ameliorate gene expression of pyruvate carboxylase (PC) after depression by either C16:0 or C18:0. PC catalyzes oxaloacetate (OAA) synthesis and ostensibly links gluconeogenesis and FA metabolism. Our objective was to determine effects of copresence of saturated and unsaturated FA pretreatments on cellular partitioning of [1-^{14}C] C16:0 metabolism to CO_2 or acid-soluble products (ASP) in Madin-Darby bovine kidney (MDBK) cells. Cells at 80% confluence were exposed for 21h to either individual FA bound to BSA (C16:0,

C18:0, C18:1n-9 *cis* or C18:3n-3 cis) or FA cocktails in 10:90, 25:75, 50:50, 75:25 or 90:10 ratios for combinations of C16:0: C18:3n-3 *cis* or C18:0: C18:3n-3 *cis* or C18:1n-9 *cis*: C18:3n-3 *cis*. Total pretreatment FA concentration was 1.0 m*M*. Following pretreatment, cells were then incubated in the presence of 1.0 m*M* [1-^{14}C] C16:0 for 3h. Pretreatments with either C16:0 alone or C18:0 alone significantly ($P < 0.01$) depressed subsequent oxidation of [1-^{14}C] C16:0 to ASP by 62.7% and 41.2%, respectively, compared with C18:3n-3 *cis* pretreatments. Pretreatments with C18:1n-9 *cis* either alone or in any combination with C18:3n-3 *cis* did not significantly ($P > 0.10$) depress subsequent oxidation of [1-^{14}C] C16:0 to ASP. Similar patterns were seen with [1-^{14}C] C16:0 oxidation to CO_2. ASP production from [1-^{14}C] C16:0 was positively correlated ($r = 0.68$, $P < 0.01$) with PC gene expression levels while CO_2 production from [1-^{14}C] C16:0 did not show a correlation ($r = 0.30$, $P > 0.10$) with PC expression. Activation of PC gene expression by unsaturated FA may play a critical role in setting the capacity for OAA synthesis and determining metabolic fates of FA. Results show the regulation of ketone production by bovine kidney cells in response to saturated and unsaturated FA pretreatments.

Key Words: fatty acid oxidation, ketogenesis, pyruvate carboxylase

M373 Ca(OH)$_2$-treated corn stover as an alternative for haycrop forage or corn silage in diets for lactating dairy cows. Brittany A. Casperson*[1], Aimee E. Wert-Lutz[2], and Shawn S. Donkin[1], *[1]Purdue University, West Lafayette, IN, [2]ADM Alliance Nutrition, Quincy, IL.*

Nutritive value of crop residues may be improved through prestorage treatment with Ca(OH)$_2$. The objective of this experiment was to determine the effect of maximal substitution of either haylage or corn silage with Ca(OH)$_2$-treated corn stover on feed intake, milk production and milk composition in lactating dairy cows. Corn stover was processed by chopping, rehydrating, and treating with 6.6% Ca(OH)$_2$ (DM basis), and stored in bag silos. Six mid-lactation multiparous Holstein cows were assigned to one of 2 groups and randomized within group to a Latin Square design to receive a TMR without any added Ca(OH)$_2$-treated stover (CON), a TMR where Ca(OH)$_2$-stover replaced alfalfa haylage at 15% of the diet (HYLGsub), or a TMR where Ca(OH)$_2$-stover replaced corn silage at 19% of the diet DM (CSsub). Diets were evaluated in a 3 × 3 replicated Latin square consisting of 3 21-d periods. Cows were individually fed in tie stalls. The first 14 d of each period were used for diet adaptation followed by 7 d of data collection. Milk production was not different ($P = 0.77$) among treatments. DMI was reduced ($P < 0.05$) for HYLGsub and CSsub diets when compared with CON (24.5, 21.8, 20.6 ± 1.0 kg/d for CON, HYLGsub, and CSsub, respectively). Milk fat percent was decreased ($P < 0.05$) with inclusion of Ca(OH)$_2$-treated corn stover (3.92, 3.66, 3.69 ± 0.15%, CON, HYLGsub, and CSsub, respectively) and milk fat yield tended to decrease ($P = 0.09$). Milk protein percentage was reduced ($P < 0.05$) by Ca(OH)$_2$-treated corn stover inclusion (3.27, 3.16, and 3.11 ± 0.06% for CON, HYLGsub, and CSsub, respectively) but milk protein yield was unaffected ($P = 0.32$). Energy-corrected milk production per unit of DMI (kg/kg) was greater ($P < 0.05$) for cows fed diets containing Ca(OH)$_2$-treated corn stover (1.12, 1.26, and 1.30 ± 0.05 CON, HYLGsub, and CSsub, respectively). Results show that Ca(OH)$_2$-treated corn stover can replace haycrop forages or up to 31% of the corn silage in diets for dairy cows without negatively impacting milk production while simultaneously improving the efficiency of conversion of feed to milk.

Key Words: corn stover, alternative forage, feed conversion efficiency

M374 Composition of rumen microbiota alters following diet-induced milk fat depression in dairy cows. Elnaz Azad[1], Daniel E. Rico[2], Hooman Derakhshani*[1], Kevin J. Havartine[2], and Ehsan Khafipour[1], *[1]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, [2]Department of Animal Science, Penn State University, University Park, PA.*

An experiment was conducted to explore the effect of diet-induced milk fat depression (MFD) on the global profile of rumen microbiota. Eight ruminally cannulated cows were subjected to time-course induction of and recovery from diet-induced MFD in a replicated design with 3 periods of 21 d. Briefly, MFD induction followed control and recovery followed MFD. A high-fiber, low-oil diet was fed during the control and recovery periods, and a low-fiber, high-oil (LFHO) diet was fed during the induction period. Whole ruminal digesta samples were collected and subjected to high-throughput Illumina sequencing of the V3-V4 hypervariable regions of bacterial 16S rRNA gene. On average, 40,562 high-quality sequences were generated per sample. Taxonomic classification of sequences unraveled the presence of 15 different bacterial phyla across all samples, among which Bacteroidetes (46.96%), Firmicutes (35.95%), Proteobacteria (7.5%), Spirochaetes (2.64%), and Fibrobacteres (2.10%) were identified as predominant members of rumen microbiota. When compared with control and recovery-associated communities, the diversity of rumen microbiota was significantly decreased following consumption of LFHO diet ($P < 0.001$). Moreover, PERMANOVA analysis of weighted UniFrac distances of microbial communities also revealed distinct clustering pattern for LFHO-associated microbiota compared with control and recovery diet ($P = 0.002$ and $P = 0.007$, respectively). Based on linear discriminant analysis effect size (LEfSe), the proportions of several members of rumen microbiota were also found to be significantly ($P < 0.05$) altered in response to dietary-induced MFD; the proportion of family Succinivibrionaceae (Proteobacteria) and genera *Shuttleworthia* and *Catonella* (Firmicutes) were found to be significantly increased in response to LFHO diet, while fibrolytic genera, such as *Fibrobacter* (Fibrobacteres) and *Ruminococcus* (Firmicutes) were found to be relatively more abundant during the control/recovery periods. Here, we demonstrated the complex dynamics of rumen microbiota that underlie diet-induced MFD.

Key Words: milk fat depression, 16S rRNA sequencing, rumen microbiota

M375 Effects of *Saccharomyces cerevisiae* fermentation product on rumen fermentation during heat stress. Kristy L. Dorton*, Tracy Werner, Jason Lin, Abigail Souder, Adam M. Brainard, Joan Butler, and Ilkyu Yoon, *Diamond V, Cedar Rapids, IA.*

Effects of *Saccharomyces cerevisiae* fermentation product (SCFP; Original XPC) on rumen volatile fatty acid (VFA) and lipopolysaccharide (LPS) concentrations during heat stress were measured in a crossover experimental design consisting of 2 28 d-periods. Eight cannulated Jersey cows (nonlactating, non-pregnant) were housed in tie stalls containing individual feed bins and an automatic watering system. Cows were fed a mixture of chopped grass hay and a grain mix twice daily and 1 of 2 treatments. Treatments consisted of 14 g Control (grain mix) or 14 g SCFP and were fed before the morning feeding. The THI of the barn was maintained at 80 to 83 during the trial. Rumen fluid samples were collected from 5 locations within the rumen (cranial dorsal, cranial ventral, central rumen, caudal dorsal, and caudal ventral) every 3 h from 0900 h to 2100 h on d 26, 27 and 28, composited within cow, and strained. Samples were analyzed for VFA and LPS concentrations. Data were analyzed using the fit model procedure of JMP. Cows supplemented with SCFP had greater ($P < 0.0001$) concentrations of

total VFA, acetate, propionate, and butyrate than control cows (103.46 vs. 95.19; 69.14 vs. 63.78; 18.75 vs. 17.21; 12.16 vs. 10.80 mM, respectively). Molar proportions of acetate and propionate were not affected by treatment. Molar proportion of butyrate was higher ($P < 0.002$) for cows supplemented with SCFP than control cows (11.72 vs. 11.32%, respectively). Molar proportions of valerate, isobutyrate and isovalerate were lower ($P \leq 0.007$) for cows supplemented with SCFP (1.01 vs. 1.08; 1.18 vs. 1.27; 1.11 vs. 1.20%, respectively). Although not significantly different, cows supplemented with SCFP tended to have lower trend ($P = 0.16$) LPS concentrations than control cows (17,327 vs. 20,156 endotoxin units). Results show that SCFP can maintain better rumen function in cows subjected to heat stress as indicated by higher rumen VFA concentrations. This effect could be the result of stabilized rumen microbial populations, as indicated by the reduced tendency of LPS concentrations in the rumen.

Key Words: *Saccharomyces cerevisiae* fermentation product, VFA, LPS

M376 Peroxisome proliferator-activated receptor β/δ regulates glucose uptake in bovine mammary epithelial cells. Jayant Lohakare*[1,2], Johan Osorio[2], and Massimo Bionaz[2], *[1]College of Animal Life Sciences, Kangwon National University, Chuncheon, South Korea, [2]Oregon State University, Corvallis, OR.*

A previous study showed that Peroxisome Proliferator-Activated Receptor β/δ (PPARβ/δ) activation inhibits glucose uptake in bovine aortic endothelial cells. We hypothesize that inhibition of PPARβ/δ can increase glucose uptake in immortalized bovine mammary alveolar cells (MACT) and increases lactose synthesis. To test our hypothesis, we treated MACT cells with PPARβ/δ synthetic agonist (GW501516) and antagonist (GSK3787) and assessed PPARβ/δ activation, live and dead cell count, and glucose uptake. MACT cells plated at 10,000 cells/well in 96 well plates were transfected with a PPAR Response Element (PPRE X3-TK-luc) plasmid using 0.3 μL/well of TransIT-X2 Dynamic Delivery System (Mirus) in Opti-MEM media without fetal bovine serum (FBS). Cells were treated 24h after transfection in triplicates with 10 and 1,000 n*M* of GW501516, GSK3787, or a 1:1 combination of them plus ethanol as control in high-glucose DMEM medium with 10% FBS. After 24 h of treatment, a nuclear staining (NucBlue Live) was added and 2 images/well were obtained using an inverted fluorescent microscope (DMI6000B, Leica Microsystems, Germany). Luciferase activity was measured via a luminometer and normalized by the number of viable cells measured using CellProfiler software. Glucose concentration in the medium was measured using a Blood Glucose Meter kit (Safeway) and glucose uptake/viable cell was estimated. Data were analyzed using GLIMMIX of SAS. Significance was declared with $P < 0.05$. More than 2-fold increase of luciferase compared with control ($P < 0.001$) was observed with both doses of GW501516 and a dose-dependent decrease of luciferase was observed with GSK3787. The number of viable cells was negatively affected by 10 n*M* of GSK3787 ($P < 0.05$) but was positively affected with 1,000 n*M* of GSK3787 (16,990 vs. 20,966 ± 521 cells/well). No effect on cell viability was observed with GW501516. There was a tendency ($P = 0.06$) for an overall effect of treatments on glucose uptake. Cells treated with GW501516 had a lower glucose uptake (7.2 vs. 10.4 ± 1.6 ng/cell) compared with cells treated with GSK3787. In conclusion, the use of 1,000 n*M* of GSK3787 successfully inhibited PPARβ/δ activity and increased glucose uptake in MACT cells. It remains to be determined if the increase in glucose uptake observed results in higher lactose synthesis.

Key Words: PPARβ/δ, mammary alveolar cells (MACT), glucose uptake

M377 Estimation of biohydrogenation in dairy cows fed unsaturated fatty acids by use of reticular and omasal sampling as an alternative to sampling from abomasal cannula. José Esler Freitas Jr.*[1], Tiago Dell Vale[2], Vitor Pereira Bettero[2], Marjorye Kametani[2], Pablo Gomes Paiva[2], Rodrigo Gardinal[2], Caio Seiti Takiya[2], Filipe Zanferari[2], Thiago T. H. A. Vendramini[2], Elmerson Ferreira Jesus[2], Gustavo Delfino Calomeni[2], and Francisco Palma Renno[2], *[1]Department of Animal Science, Federal University of Bahia, Salvador, Bahia, Brazil, [2]Department of Nutrition and Animal Production, Faculty of Veterinary Medicine, University of São Paulo, Pirassununga, São Paulo, Brazil.*

The aim of this study was to evaluate the biohydrogenation in dairy cows fed unsaturated fatty acid by use of reticular and omasal sampling as an alternative to sampling from the abomasal canal. Four Holstein dry cows cannulated in the rumen and abomasum (602 ± 21 kg of body weight; mean ± SD) were assigned randomly into a 4 × 4 Latin square design experiment, fed the following diets: (1) control (C), without unsaturated fatty acid supplementation; (2) soybean oil (SO), addition of 3% of refined soybean oil in DM basis of total diet; (3) whole raw soybean (WS), addition of 16% of whole raw soybeans in DM basis of total diet; and (4) calcium salts of unsaturated fatty acids (CSFA), addition of 3% of CSFA in DM basis of total diet. Nutrient flow was calculated using the reconstitution system based on 3 markers (cobalt-EDTA, ytterbium chloride and indigestible NDF). Large and small particles and the fluid phase were recovered from digesta collected of different sites. Samples were collected 8 times during 20 4 h and were combined into one pool sample for further reconstitution. Data were analyzed using PROC MIXED of SAS 9.1 using the Tukey test to test pair wise comparisons. There were not effects of sampling sites, on biohydrogenation of 18:3 (85.6; 97.9 and 93.2% of intake for to digesta reticular, omasal and abomasal respectively). However, the biohydrogenation of C18:2 (84.2; 93.4 and 85.1% of intake for to digesta reticular, omasal and abomasal respectively) ($P < 0.01$) and C18:1 (63.2; 83.7 and 62.9% of intake for to digesta reticular, omasal and abomasal respectively) were overestimated by use sampling omasal in relation to use reticular sampling and abomasal ($P < 0.01$).The reticular sampling technique provided reliable estimates for biohydrogenation of 18:2 and 18:1. The omasal sampling technique can overestimate to biohydrogenation.

Key Words: dairy cow, fatty acid, linoleic acid

M378 Milk odd- and branched-chain fatty acid profile is affected by lactation stage in dairy cows. Eric Baumann*, P. Yvan Chouinard, Yolaine Lebeuf, and Rachel Gervais, *Université Laval, Quebec, QC, Canada.*

The odd- and branched-chain fatty acid (OBCFA) profile of milk fat has emerged as an interesting, non-invasive tool for evaluating rumen fermentation. These fatty acids are synthesized by different rumen microbial populations, absorbed in the intestine, and taken up by the mammary gland to be incorporated in milk fat. It is well known that milk fat composition is influenced by stage of lactation; proportion of short chains (de novo synthesis) being low initially and increasing with days in milk. However, literature is scarce about the specific effects of stage of lactation on milk OBCFA concentrations. Seven Holstein dairy cows were followed during the experiment. Five data collection and sampling periods were conducted on d 60 to 70, d 120 to 130, d 210 to 220, and d 300 to 310 of a lactation, and on d 5 to 15 of the subsequent lactation. During that period, cows were fed total mixed rations based on grass/legume and corn silages, cracked corn, soybean meal, and corn gluten meal, and formulated to meet their energy and nutrient requirements according to NRC (2001). The results indicate that stage of lactation do

affect expression of various milk OBCFA; these effects would need to be considered in the development of models aiming to predict rumen parameters based on milk OBCFA.

Table 1 (Abstr. 378). Milk fat concentrations (mg/g) of individual OBCFA

	5-15 DIM	60-70 DIM	120-130 DIM	210-220 DIM	300-310 DIM	*P*-value
Fatty acid						
11:0	0.20^{c}	0.46^{bc}	0.49^{b}	0.86^{a}	0.69^{ab}	<0.01
13:0	0.41^{d}	0.93^{c}	0.99^{bc}	1.38^{a}	1.20^{ab}	<0.01
15:0	5.47^{d}	9.86^{c}	11.17^{b}	12.50^{a}	12.32^{ab}	<0.01
17:0	3.79^{a}	2.41^{b}	2.31^{bc}	2.07^{c}	2.22^{bc}	<0.01
c9 17:1	1.85^{a}	0.85^{b}	0.76^{b}	0.77^{b}	0.79^{b}	<0.01
Odd	12.04^{d}	14.77^{c}	16.05^{b}	17.90^{a}	17.52^{a}	<0.01
ai 13:0	0.15	0.21	0.22	0.16	0.24	0.60
ai 15:0	2.20^{b}	4.74^{a}	5.22^{a}	4.88^{a}	4.85^{a}	<0.01
ai 17:0	4.26^{a}	3.99^{ab}	4.10^{a}	3.60^{b}	3.67^{ab}	<0.01
Anteiso	6.60^{b}	8.94^{a}	9.54^{a}	8.65^{a}	8.79^{a}	<0.01
i 14:0	0.62^{b}	1.54^{a}	1.82^{a}	1.45^{a}	1.66^{a}	<0.01
i 16:0	2.06^{c}	3.42^{ab}	3.92^{a}	2.98^{b}	3.28^{ab}	<0.01
i 18:0	3.29^{c}	5.25^{ab}	5.99^{a}	4.64^{b}	5.17^{ab}	<0.01
Even iso	3.29^{c}	5.25^{ab}	5.99^{a}	4.64^{b}	5.17^{ab}	<0.01
i 13:0	0.24^{b}	0.33^{a}	0.39^{a}	0.34^{a}	0.35^{a}	<0.01
i 15:0	5.01^{c}	10.03^{b}	12.12^{a}	10.60^{a}	10.76^{a}	<0.01
i 17:0	3.32^{a}	2.88^{b}	3.03^{ab}	2.74^{b}	2.70^{b}	<0.01
Odd iso	8.57^{c}	13.24^{b}	15.54^{a}	13.68^{ab}	13.80^{ab}	<0.01

Key Words: milk fat synthesis, odd- and branched-chain fatty acids, stage of lactation

M379 Ruminal and production effects of supplementing high and low forage dairy rations with a live yeast culture. Maegan E. Weatherly*[1], Amanda M. Gehman[2], Amanda M. Lisembee[2], Joey D. Clark[1], Laurel L. Ball[2], and Jeffrey M. Bewley[1], *[1]University of Kentucky, Lexington, KY, [2]Alltech Inc., Nicholasville, KY.*

The objective of this study was to assess the effect of yeast supplementation in high and low forage diets on rumen and production parameters. Four, ruminally fistulated, multiparous, mid-lactation, Holstein cows were housed in a tie-stall barn at the University of Kentucky Coldstream Dairy from October 29, 2013 to February 7, 2014. A 4 × 4 Latin square design with a 2 × 2 factorial arrangement of treatments was used. Cows were assigned to 1 of 4 treatments each period including (1) low forage (LF), (2) low forage with 10 g/d yeast (Yea-Sacc; Alltech Inc., Nicholasville, KY; LFY), (3) high forage (HF), or (4) high forage with 10 g/d yeast (HFY). Periods 1 to 3 consisted of 21 d and period 4 was 18 d. Treatment periods were followed by a 7-d washout period where cows were gradually adjusted to the next ration. Dry matter intake was recorded daily. Daily rumination was recorded using HR Tags (SCR Engineers Ltd., Netanya, Israel). Rumen papillae were biopsied from each cow once per feeding period and analyzed for expression of enzymatic genes and transcriptional regulators. The GLM procedure of SAS (Version 9.3 SAS Institute, Inc., Cary, NC) was used to evaluate the fixed effects of cow, period, forage, yeast, and the interaction of forage and yeast on each parameter. Rumen papillae gene expression data were analyzed using a MIXED model in SAS. Rumination time and DMI were the only production parameters significantly influenced by treatment ($P < 0.01$). Dry matter intake was 17.05, 13.41, 19.44, and 20.29 ± 1.40 kg/d for cows on the LF, LFY, HF, and HFY treatments, respectively. Rumination time was 442.88, 323.09, 433.34, and 475.50 ± 21.93 min/d for cows on the LF, LFY, HF, and HFY treatments, respectively. Expression of peroxisome proliferator-activated receptor γ, sterol regulatory element-binding transcription factor 1, and oxoglutarate dehydrogenate ornithine carbamoyl transferase were significantly upregulated by yeast supplementation ($P \leq 0.05$). The upregulation of genes that affect metabolism of VFA during yeast supplementation may suggest the importance of this product on rumen stabilization.

Key Words: yeast, rumen papillae gene expression, rumination

M380 Effects of milk replacer and multivitamin-mineral supplementation on metabolism and rumen development in heat-stressed dairy calves. Steven J. Blair*[1], Cathleen C. Williams[1], Bruce F. Jenny[1], Ashley H. Dolejsiova[1], and Thomas J. Earleywine[2], *[1]Louisiana State University, Baton Rouge, LA, [2]Land O'Lakes Animal Milk Products, Shoreview, MN.*

Seventy-one neonatal Holstein calves (40 female; 31 male) were used in a randomized block design with a 2 × 2 factorial arrangement of treatments to evaluate the effects of milk replacer (MR) feeding management alone or in combination with a multivitamin and electrolyte supplement on growth performance and mitigation of heat stress in southeast Louisiana. Milk replacer treatments consisted of Land O'Lakes Herdmaker Supreme (20% CP, 20% fat; CON) and Land O'Lakes Warm Front (27% CP, 10% fat; WF). Supplemented calves received either 0 or 20 mL of Palamountains Calf Boost (CB) in MR once daily. Calves were offered MR treatments and water and calf starter (20% CP) ad libitum beginning on d 2. All milk replacer was mixed at 15% solids. Calves consuming CON were fed 2.28kg MR twice daily. Calves on WF were fed 2.72kg MR twice daily for the first 3 weeks of life, and 3.86kg twice daily until weaning. Beginning on d 42, MR feeding was reduced to 1 time per day for all treatment groups to decrease MR intake by 50%. On d 49 calves were weaned. Calves remained in their hutches until d 56 to determine immediate post weaning performance. Blood was collected on d 14, 28, 42, and 56 for analysis of plasma urea nitrogen (PUN), glucose, and β-hydroxybutyrate (BHBA), as well as rumen fluid for analysis of volatile fatty acids (VFA) and pH. Data were analyzed using the PROC MIXED procedure in SAS. A main effect of milk replacer composition on PUN was observed, with calves fed WF having greater concentrations ($P < 0.05$) than CON. Glucose concentrations decreased ($P < 0.05$) as calves aged. There was no treatment effect ($P > 0.05$) on plasma BHBA, but concentrations increased ($P < 0.05$) as calves aged. Likewise, there was no treatment effect ($P > 0.1$) on rumen acetate, propionate, butyrate, and total VFA concentrations; however, concentrations increased ($P < 0.05$) as calves aged. No effects of treatment or time were observed ($P > 0.05$) for rumen pH. These data indicate that milk replacer composition and feeding management and multivitamin mineral supplements do not affect negatively metabolism or rumen development in young dairy calves.

Key Words: calf milk replacer, multivitamin-mineral supplement, heat stress

M381 Validation of a radio frequency system for monitoring feeding behavior and intake of feed and water in young cattle. Baltazar Ruas de Oliveira Júnior[1], Marcelo Neves Ribas[2], Fernanda Samarini Machado[3], Juliana Aparecida Mello Lima[1], Luigi Francis Lima Cavalcanti[2], Mario Luiz Chizzotti[4], Rafael Alves de Azevedo*[1], and Sandra Gesteira Coelho[1], *[1]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [2]CNPq, RHAE–SEVA Engenharia, Projeto Intergado, Contagem, MG, Brazil, [3]EMBRAPA Dairy Cattle,*

Juiz de Fora, MG, Brazil, [4]Federal University of Viçosa, Viçosa, MG, Brazil.

The objective was to validate a radio frequency system for monitoring individual feeding behavior, water and feed consumption in young cattle housed in group. Thirty 5 Holstein-Gyr crossbred heifers, fitted with an ear tag containing a unique passive transponder, were distributed in 3 groups of 12, 12 and 11 animals per period and had free access to 12 electronic feed bins and 2 electronic water bins (Intergado, Contagem, Brazil). The system documented the visit duration and feed and water intake by recording animal's identification tag, bin number, initial and final times of visits, and the difference of feed/water weight at start and end for each bin visit. Feed bins were monitored by time-lapse video recording over 4 d and the water bins over 6 d. Video data on animal behavior were compared with those generated by the system. Feed and water consumption were measured using an external scale. For each feed bin, 2 feeding events were monitored using manual weighing's immediately before and after the animal's visit and the difference between them was assumed as feed intake (n = 24 observations). For water bins there were made 60 manual weighing's. These data were compared with those recorded by the system. Video and manual weighing data were regressed on the electronic feeding behavior and feed and water intakes data to evaluate system's precision and accuracy. The system showed a high specificity (98.98 and 98.56% for the feed and water bins, respectively) and sensitivity (99.25 and 98.74%, respectively) for identifying animal's presence or absence. Duration of feed and water bins visits, and feed and water consumption per visit estimated by the system were highly correlated and precise (R^2 = 0.917, 0.963, 0.973 and 0.986, respectively) when compared with observed video and manual weighing data. Feed daily intake per visit registered electronically and manual weighing differed by less than 150 g. It was concluded that Intergado system is a useful tool for monitoring feeding behavior, water and feed intakes in young cattle housed in group.

Key Words: electronic monitoring, heifer, precision farming

M382 Evaluation of two techniques used to dislodge bacteria from particles contained in rumen digesta. Jared V. Judy*, Chad J. R. Jenkins, Samodha C. Fernando, and Paul J. Kononoff, *University of Nebraska-Lincoln, Lincoln, NE.*

The objective of this study was to estimate the concentration of bacterial crude protein (BCP) in pellets isolated from ruminal digesta using a preparatory step of either blending or shaking to dislodge bacteria from rumen particles. Using a completely randomized design, 2 multiparous, lactating Holstein cows (DIM 229 ± 7d, DMI 36.1 ± 2.5 kg/d, milk yield 37.7 ± 5.6 kg/d) (mean ± SD), fitted with ruminal cannulas were fed the same diet once daily at 0930 h. Two hours post feeding, approximately 2.5 kg of rumen contents were collected from each cow, then thoroughly mixed and separated into 2 aliquots (blend or shake) then samples were strained through 4 layers of cheesecloth. Particle associated bacteria were separated from the solid portion of rumen contents by adding and equal amount of McDougal's buffer as was collected in the filtrate and physically shook or blended in a commercial blender for 1 min., followed by straining through 4 layers of cheesecloth. Fluid collected after shaking or blending, as well as fluid retained from the initial straining where combined together. Each sample underwent differential centrifugation which yielded bacterial pellets consisting of fluid associated bacteria and particle associated bacteria. DNA was then extracted from bacterial pellets and from the non-centrifuged samples of rumen content particles. The DNA from the bacterial pellets and samples of rumen content were subjected to real-time PCR using the TaqMan assay. Primers and a probe were designed from the DNA encoding part of the 16S rRNA for bacteria. The concentration of BCP using these 2 methods to dislodge bacteria did not differ (P = 0.42) (13.9 ± 5.0 and 7.5 ± 1.9 mg BCP/g DM for shake vs. blend, respectively). Results suggest that BCP concentration is not different between shaking or blending to dislodge bacteria, however, further research should examine and attempt to identify the large amount of analytical variation observed in both techniques.

Key Words: bacteria, bacterial crude protein, rumen

M383 Effect of an exogenous fibrolytic enzyme on the performance of dairy cows consuming a diet with a high proportion of bermudagrass silage. Andres A. Pech Cervantes*, Kathy G. Arriola, Jorge E. Zuniga, Ibukun M. Ogunade, Yun Jiang, Thiago F. Bernardes, Charles R. Staples, and Adegbola T. Adesogan, *Department of Animal Sciences, University of Florida, Gainesville, FL.*

We previously reported that milk production by dairy cows was increased by adding specific xylanase-rich (XYL) and xylanase-cellulase enzymes to corn silage-based diets containing 0 or 10% bermudagrass silage. This study examined effects of adding XYL on the intake and performance of lactating dairy cows consuming a TMR formulated with a greater proportion of bermudagrass silage. Endoglucanase and xylanase activities of XYL were 3,283 and 46,281 μmol/min/mL, respectively. Forty lactating Holstein cows (16 multiparous and 24 primiparous; 21 ± 3 DIM; BW 589 ± 73 kg) were stratified by milk production and parity and assigned randomly to Control and XYL diets. The TMR (CP of 16.2% of DM, NDF of 36.4% of DM, and NE_L of 1.65 Mcal/kg of DM) contained 20% bermudagrass silage, 25% corn silage, and 55% concentrate (DM basis). Immediately before the a.m. (0700 h) and p.m. (1300 h) feedings, the enzyme was sprayed on the XYL diet at the rate of 1 mL/kg of TMR DM in a Calan data ranger and mixed. A second data ranger was used to feed control cows. Cows were fed experimental diets for 70 d after they were fed a common diet for a 9-d covariate period. The experiment had a randomized complete block design. The statistical model included effects of enzyme, parity, week, and their interactions as well as covariate milk production or DMI. The random effect was cow nested within treatment. Application of XYL did not (P > 0.10) affect milk yield (35.1 vs. 36.2 kg/d), DM intake (24.0 vs 23.7 kg/d for XYL and Control), fat-corrected milk (FCM) (36.1 vs. 36.9 kg/d), yields of milk fat (1.29 vs. 1.31 kg/d) and protein (1.07 vs. 1.08 kg/d), milk fat concentration (3.65 vs. 3.61%), and body weight change (0.26 vs. 0.33 kg/d) compared with control cows. However, cows fed the diet treated with XYL had greater milk protein concentration (P = 0.01; 3.02 vs. 2.95%) and tended to have less feed efficiency (P = 0.06; 1.52 vs. 1.57 kg of FCM/kg of DMI) compared with cows fed the control diet. Adding XYL to a diet containing 20% bermudagrass silage and 25% corn silage did not improve DM intake or milk production.

Key Words: bermudagrass silage, milk, enzyme

M384 Effects of intensive whole-milk feeding in calves on subsequent feeding behavior of dairy heifers. Camila Flávia de Assis Lage[1], Mariana Magalhães Campos[2], Fernanda Samarini Machado[2], Paulo Campos Martins[1], Luigi Francis Lima Cavalcanti[3], Marcelo Neves Ribas[3], Luiz Gustavo Ribeiro Pereira[2], Thierry Ribeiro Tomich[2], Rafael Alves de Azevedo*[1], and Sandra Gesteira Coelho[1], *[1]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [2]EMBRAPA Dairy Cattle, Coronel Pacheco, Minas GG, Brazil, [3]CNPq, RHAE–SEVA Engenharia, Projeto Intergado, Contagem, MG, Brazil.*

This study aimed to evaluate the effects of intensive whole milk feeding in calves on subsequent feeding behavior of 58 Holstein-Gyr females. Up to 56 d of age, calves received 6 L/d of 4 different liquid diets consisting of whole milk with the increasing addition of milk replacer (Sprayfo Violet SSP) to adjust the concentration of total solids (TS) to 13.5 (n = 15), 16.1 (n = 15), 18.2 (n = 13), 20.4% (n = 15). After weaning, animals were randomly housed in 4 paddocks, each one equipped with 3 electronic feed bins and one electronic water bin (INTERGADO, Brazil) in Embrapa Dairy Cattle facilities, Brazil. The diet (70% corn silage and 30% concentrate, 195 g of CP/kg, DM basis) was fed ad libitum, twice a day, until 210 d of age. Only events with registered intake were used, and the following results were calculated: Ingestion rate (IR, g/s), average bunk visit duration (AVD, min), daily visit duration (DVD, h) and daily visit frequency (VF, events). Due to the natural right skewness of IR and AVD distributions, their daily median, instead of mean, were used to represent their trends across study period. All variables were analyzed as a completely randomized design with repeated measures using linear mixed model approach. Age and TS were evaluated as fixed effects, while animals as random. The necessity to model error dependence and heteroscedasticity was evaluated by monitoring Schwarz criterion. The effect of TS was decomposed into orthogonal polynomials of linear and quadratic degrees. Significance was declared at $P < 0.05$. All variables were consistently influenced by animal's age, where IR was increased as animals become older (0.837 ± 0.075 g/s at 100 d old versus 1.617 ± 0.072 g/s at 210 d old). An interaction effect between TS and age was detected for IR, where TS linear increased IR for animals older than 190 d of age. None of visit variables were affected by TS levels (AVD = 3.70 ± 0.37 min, DVD = 2.50 ± 0.12 h, VF = 35.16 ± 3.21 events). Intensive whole-milk feeding in calves caused minor effects on subsequent feeding behavior of dairy heifers.

Key Words: milk replacer, intake, precision farming

M385 Comparison of the RQUICKI estimate of insulin sensitivity with glucose and insulin tolerance in periparturient dairy cows. Sina Saed Samii*, J. Eduardo Rico, Alice T. Mathews, Cassandra L. Orndorff, Amanda N. Davis, and Joseph W. McFadden, *West Virginia University, Morgantown, WV.*

The revised quantitative insulin sensitivity check index (RQUICKI) has been utilized to evaluate insulin resistance in dairy cows; however, discrepancies between RQUICKI and direct measurements of insulin sensitivity are documented. Our objective was to compare RQUICKI with glucose and insulin tolerance in non-fasted peripartal dairy cows. Multiparous Holstein cows were grouped by BCS at d −28 prepartum: lean (BCS 2.91 ± 0.13; n = 7) or overweight (OVER; BCS 4.03 ± 0.21; n = 7). Diets were formulated to meet nutrient requirements. An intravenous insulin challenge (0.1 IU/kg BW; ITT) was performed on d −26 and −13, relative to expected calving, and 5 DIM. An intravenous glucose challenge (0.3 g/kg BW; GTT) was performed 24 h post- ITT . Blood and milk were collected routinely. Data were analyzed using a mixed model with repeated measures (fixed effects of BCS and day). Effects are presented as changes relative to lean cows, unless described otherwise. OVER had lower DMI, and lost more BCS and BW postpartum ($P < 0.05$). Adiposity had no effect on milk yield, milk protein yield, and SCC; however, milk fat yield was greater in OVER ($P < 0.05$). OVER had increased plasma NEFA and BHBA ($P < 0.05$). Prepartum plasma insulin levels were higher in OVER ($P < 0.05$). Although plasma glucose levels declined with time ($P < 0.01$), BCS did not modify plasma glucose. RQUICKI values were lower for OVER pre- and postpartum ($P < 0.05$). Postpartum cows had lower insulin-stimulated glucose disposal, relative to prepartum cows ($P < 0.01$). Following insulin-stimulated glucose disappearance, return to basal glucose in OVER was delayed by 60 min ($P < 0.05$). BCS had no effects on GTT; however, post-glucose challenge area under the curve (AUC) for 180 min and clearance rate (%/min) for the first 30 min were lower for postpartum cows, relative to prepartum cows ($P < 0.05$). Before and after calving, OVER experienced greater glucose-stimulated reductions in NEFA and AUC for 180 min following glucose challenge ($P < 0.05$). Observed inconsistencies between RQUICKI and tolerance testing may be due to direct measurements in the fed state.

Key Words: glucose tolerance, insulin resistance, transition cow

M386 Evolving the plasma free AA dose-response technique to determine bioavailability of Met in RP-Met supplements. Devan L. Chirgwin*[1], Nancy L. Whitehouse[1], Andre F. Brito[1], Charles G. Schwab[2], and Brian K. Sloan[3], *[1]University of New Hampshire, Durham, NH, [2]Schwab Consulting, LLC, Boscobel, WI, [3]Adisseo, Alpharetta, GA.*

The plasma free AA dose-response technique has been proposed as the standard approach for arriving at estimates of efficacy for rumen-protected Lys supplements. Results of the first replicate of a 5 × 5 Latin square study, reported last year [J. Dairy. Sci. (97(E-Suppl. 1):763], confirmed that a positive relationship also exists between increasing amounts of absorbed Met and plasma Met and total sulfur AA (TSAA) concentrations. The objective of adding a second replicate was to complete the study, and using the combined data set, to determine if using plasma Met or TSAA concentrations (μM) is the more precise response parameter and whether expressing either as a % of total AA (TAA) or total essential AA (TEAA) would reduce the error of calculated estimates of Met-bioavailability. Experimental protocol was as previously described: namely, 5 rumen-cannulated Holstein cows (74–222 DIM) were fed a Met-deficient basal diet with identical treatments. Combined data were analyzed using PROC MIXED and PROC REG of SAS. Outlier analysis, using ± 2.0 SD away from the mean for plasma Met and TSAA concentrations, resulted in removal of all data for one cow. Plasma Met and TSAA concentrations (μ*M*), and both expressed as %TAA and %EAA, were regressed on 0, 12, and 24 g of infused Met and 0, 15, and 30 g of fed Met. Slopes (and associated CV, %) for infused and fed Met were 1.40 and 1.04 (3.68 and 1.85) for Met, 0.067 and 0.048 (4.92 and 3.18) for Met as %TAA, and 0.152 and 0.112 (4.48 and 3.22) for Met as %TEAA. Corresponding values for total TSAA were 2.00 and 1.64 (1.58 and 2.57) for μ*M* concentrations, 0.108 and 0.079 (2.79 and 2.48) for %TAA, and 0.234 and 0.184 (2.67 and 3.48) for %TEAA, respectively. Estimates of bioavailability (and 95% CI) of the RP-Met supplement for the 6 respective methods of expression were 74.4 (2.1), 71.6 (1.9), 73.7 (1.3), 81.8 (3.3), 72.7 (1.3) and 78.6 (2.3). We conclude the plasma free AA dose-response technique is precise, and because Met is a precursor to other sulfur AA, TSAA (μ*M*) is the most appropriate response parameter for estimating Met bioavailability of RP-Met supplements.

Key Words: methodology, methionine, bioavailability

M387 Effects of intensive whole-milk feeding in calves on subsequent performance and feed efficiency of crossbred dairy heifers. Camila Flávia de Assis Lage[1], Mariana Magalhães Campos[2], Fernanda Samarini Machado[2], Paulo Campos Martins[1], Luigi Francis Lima Cavalcanti[3], Marcelo Neves Ribas[3], Luiz Gustavo Ribeiro Pereira[2], Thierry Ribeiro Tomich[2], Rafael Alves de Azevedo*[1], and Sandra Gesteira Coelho[1], *[1]Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, [2]EMBRAPA Dairy Cattle, Coronel Pacheco,*

MG, Brazil, [3]CNPq, RHAE – SEVA Engenharia, Projeto Intergado, Contagem, MG, Brazil.

This study aimed to evaluate the effects of intensive whole-milk feeding in calves on subsequent performance and feed efficiency in growing heifers. Up to 56 d of age, Holstein-Gyr calves received 6 L/d of 4 different liquid diets consisting of whole milk with the increasing addition of milk replacer (Sprayfo Violet SSP) to adjust the concentration of total solids (TS) to 13.5 (n = 15), 16.1 (n = 15), 18.2 (n = 13), 20.4% (n = 15). After weaning, animals were housed in 4 paddocks, each one equipped with 3 electronic feed bins and one electronic water bin (Intergado, Brazil) in the experimental farm of Embrapa Dairy Cattle, Brazil. The same diet (70% corn silage and 30% concentrate, dry matter basis; 195 g of CP/kg of DM) was fed in ad libitum, twice a day, until 210 d of age. Daily feed intake (DFI) and water intake (WI) were registered by the electronic system. Average daily gain (ADG) was determined from regression of weekly BW measurements so feed conversion (FC) could be calculated. Longitudinal data was analyzed as a completely randomized design with repeated measures using linear mixed models, where animal's age and TS were fixed effects while animal was considered a random effect. The necessity to add random components to model error dependence and heteroscedasticity was evaluated based on Akaike's Information Criterion. Initial body weight, air temperature and humidity were added as co-variables. Average and cumulative results were analyzed by linear regression, where only the fixed effect of TS was evaluated ($\alpha = 0.05$). DFI and WI were only influenced by age, however in both cases an interaction effect was observed, although none supplementation level caused a steady superiority across period for any variable. This result was corroborated by cumulative DFI and WI that were not influenced by TS. ADG was decreased by increasing nutritional management (e.g., 916 ± 30 and 833 ± 29 g/d for animals with 13.5 and 20.4%, respectively), what also reflected in a higher FC to animals in higher TS (13.19 ± 0.45 vs 14.55 ± 0.44 in animals that received 13.5 and 20.4% of FD, respectively).

Key Words: milk replacer, feeding, performance

M388 Immediate and long-term effects of niacin feeding to fresh dairy cows: 1. Ketosis and fertility. J. M. Havlin*[1], P. H. Robsinson[1], and J. E. Garrett[2], *[1]University of California, Davis, Davis, CA, [2]Qualitech, Chaska, MN.*

During the fresh period after calving through ~21 d postpartum, dairy cows are often in negative energy balance (NEB) due to high energy demands to support rapidly rising milk output at a time of relatively low dry matter intake (DMI). This NEB makes cows susceptible to ketosis, fatty liver, metritis, and displaced abomasum, which can lead to decreased performance and eventual culling. A possibility to reduce the extent of NEB is to feed niacin (Ni) as nicotinic acid (NA) to reduce milk fat production, thereby minimizing body weight loss to reduce ketogenesis, all to reduce the extent of NEB thereby creating a more successful lactation. Multiparity Holstein cows (672) on a California dairy farm were used from 14 d pre-calving through 150 d in milk (DIM). While in the close-up dry pen (−14 to 1 DIM), cows were comingled and fed the same total mixed ration (TMR), in the fresh pens (1 to ~22 DIM) cows were fed the same TMR, except for inclusion of ruminally protected (RP) Ni (rumen escape estimate = 66%), in separate pens at 0, 3.5, 7 or 14 g NA/cow/d. Cows were comingled in the high pen (~23 to 150 DIM) and fed the same TMR. DMI was tabulated by treatment in the fresh pens, blood samples were collected for NEFA and BHBA analysis during the dry and fresh periods, and fertility data was tabulated through 150 DIM. Feeding 3.5 g/d RPNi increased DMI from 19.3 to 21.5 kg/d in the fresh period, but RPNi at 14 g/d reduced it to below Control cows (Quadratic $P = 0.07$). Ketosis prevalence (% cows with BHBA ≥ 1.44 mg/dl) decreased from 36 to 20% in the fresh period with 3.5 g/d, but RPNi at 14 g/d did not differ from levels of Control cows (Quadratic $P = 0.06$). Niacin feeding had no effect on any fertility measure, with averages for 1st service conception (%) being 44.6 ± 4.38, pregnancy (%) being 76.2 ± 3.70 and services/conception being 1.58 ± 0.082. Short-term fresh period RPNi feeding at the 3.5 g/d level reduced the incidence of ketosis caused by NEB, but feeding higher levels removed those benefits. No RPNi feeding level during the fresh period affected fertility parameters through 150 DIM.

Key Words: niacin, ketosis, fertility

M389 Effects of supplementation with a rumen-protected lysine product on production in high-producing dairy cows. Meagan Cooney*[1] and Izuru Shinzato[2], *[1]phdR&D, East Troy, WI, [2]Ajinomoto Heartland, Chicago, IL.*

The objective of this study was to determine the effects of supplementation and withdrawal of a rumen-protected lysine product, AjiPro-L 2nd generation (2G), on production performance of high-producing dairy cows. Ninety-six multiparous Holstein cows averaging 2.9 ± 1.3 lactations and 83.6 ± 36.7 DIM at the commencement of the trial were assigned at random to one of 12 pens that received either a negative control ration (n = 4), positive control ration with added metabolizable lysine from an animal protein (n = 4), or AjiPro-L 2G added metabolizable lysine from a rumen-protected amino acid (n = 4). The 8wk experimental period consisted of 2 periods; a 4-wk treatment period followed by a 4-wk carryover period. During the treatment period, animals received negative, positive or AjiPro-L 2G treatments, whereas during the carryover period all animals received the positive control treatment. Milk yield, energy-corrected milk yield, water consumption and DMI were recorded daily and milk composition was measured weekly. Data from only cows that completed the entire duration of the study were used in the analysis. Days in milk categories of either high (114 ± 17.7) or low (53 ± 21.3) were also analyzed. Results showed that water consumption and DMI was significantly increased compared with baseline *(*$P < 0.05$) and MUN levels were significantly decreased compared with baseline ($P < 0.01$) for animals receiving AjiPro-L 2G during the treatment period. When comparing DIM categories, results revealed a significant difference ($P < 0.05$) in change of milk production, ECM, pounds of fat and pounds of lactose in comparison to baseline across treatments. For these parameters, high DIM animals receiving AjiPro-L 2G had greater milk yield, and the least reduction of ECM, pounds of fat, and pounds of lactose from baseline compared with the positive and negative treatments. During the carryover period all parameters measured, except for MUN ($P < 0.05$), were not different between treatments compared with baseline. Under the conditions of this experiment, supplementation of AjiPro-L 2G had the greatest effect on cows averaging 114 DIM in which milk production and milk components were maintained in post-peak cows.

Key Words: rumen-protected lysine, dairy cow, production performance

M390 Immediate and long-term effects of niacin feeding to fresh dairy cows. 2. Body condition and milk production. J. M. Havlin*[1], P. H. Robsinson[1], and J. E. Garrett[2], *[1]University of California, Davis, Davis, CA, [2]Qualitech, Chaska, MN.*

During the fresh period after calving through ~21 d postpartum, dairy cows are often in negative energy balance (NEB) due to high energy

demands to support rapidly rising milk output at a time of relatively low dry matter intake (DMI). Multiparity Holstein cows (672) on a California dairy farm were used from 14 d pre-calving through 150 d in milk (DIM). While in the close-up dry pen (−14 to 1 DIM), cows were comingled and fed the same total mixed ration (TMR), in the fresh pens (1 to ~22 DIM) cows were fed the same TMR, except for inclusion of ruminally protected (RP) Ni (rumen escape estimate = 66%) in separate pens at 0, 3.5, 7 or 14 g nicotinic acid/cow/d. Cows were comingled in the high pen (~23 to 150 DIM) and fed the same TMR. Milk production and body condition (BCS) was measured every 2 wk in the fresh pen and every 4 wk in the high pens. At 7 ± 3.9 DIM, there was no effect of RPNi on milk (39.3 ± 0.89 kg) or component yields. However at 21 ± 3.9 DIM there were quadratic trends ($P < 0.20$), with highest outputs at 3.5 g/d feeding and lowest at 14 g/d. However at the 1st high pen milk sampling after cessation of RPNi feeding (48 ± 8.0 DIM), milk and component yields slumped for cows previously fed 3.5 g/d resulting in lower yields vs Control (53.2 vs 51.8 kg milk/d; 1.75 vs 1.67 kg fat/d), but over the next 3 mo they converged with Control. In contrast, milk and component yields of cows previously fed 14 g/d rebounded on the 1st high pen milk sampling after cessation of feeding RPNi resulting in higher yields vs Control (53.2 vs 55.3 kg milk/d; 1.75 vs 1.82 kg fat/d). There was no effect of RPNi feeding on BCS during the fresh period, but BCS of cows previously fed 3.5 g/d decreased more than Control cows in high pens, maintaining a lower BCS through ~80 DIM, but by 150 DIM there was no difference with Control. In contrast the BCS of cows previously fed 14 g/d decreased from the fresh period through 150 DIM. RPNi had small effects on animal performance while supplemented, but cessation of feeding caused short-term rebound carry-over effects. However, performance of cows on all RPNi levels had largely converged by 150 DIM.

Key Words: niacin, milk, BCS

M391 Lactational performance of cows fed extruded linseed on commercial dairy herds. Amélie Beauregard*[1,2], Marie-Pierre Dallaire[1], Rachel Gervais[1], and P. Yvan Chouinard[1,2], *[1]Université Laval, Quebec, QC, Canada, [2]Insitute of Nutrition and Functional Foods, Quebec, QC, Canada.*

The objective of this study was to determine the effect of feeding extruded linseed (EL) on milk yield and milk composition, including fatty acid profile, and enteric methane output on commercial settings. Thirty dairy herds averaging 55 cows and 30.1 kg of milk/d with 4.0% fat and 3.3% protein were recruited for this study. Twenty-eight herds had Holstein cows, while 2 herds had Jersey and Holstein cows, and all of them were located in the province of Quebec, Canada. A first group of 15 herds was randomly selected to be used as control (CTL). Cows from the remaining 15 herds were fed between 200 and 900 g of EL (mixture of linseed:wheat bran, 70:30; Valorex, Combourtillé, France) per cow per day (average 700 g/d) according to their lactation stage and their level of production. Diet compositions were then adjusted to cover the animals' nutrient requirements. The feeding trial was 6 mo in length, and data collected during a 2-mo interval before the experimental period were used as covariates. Actual milk yield was higher in herds fed EL as compared with CTL (28.5 vs. 27.4 L/d; $P = 0.01$). Milk fat content was similar between treatments (4.08 kg/hL; $P = 0.20$), but milk fat yield tended to be higher in herds fed EL as compared with CTL (1.15 vs. 1.12 kg/d; $P = 0.09$). Feeding EL decreased milk protein content (3.30 vs 3.37 kg/hL; $P < 0.01$), but had no effect on milk protein yield (0.94 kg/d; $P = 0.17$) when compared with CTL. Milk fat contents of *cis*-9,*cis*-12,*cis*-15 18:3 (6.72 vs 4.79 mg/g; $P < 0.01$), and other fatty acids of the n-3 family (18:4, 20:4, 20:5, 22:5, 22:6; $P \leq 0.05$) were higher in herds fed EL as compared with CTL. Methane output, as estimated using a proprietary equation (WO 2009 156453 A1) based on milk yield and fatty acid profile, was 9.4% lower in dairy herds fed EL as compared with CTL (12.9 vs. 14.2 g/L milk; $P < 0.01$). In conclusion, feeding moderate amount of EL (700 g/d, providing 200 g of oil) was efficient to mitigate methane emission while maintaining animal performance and increasing the n-3 FA content in milk fat.

Key Words: cow, extruded linseed, methane

M392 Bacterial communities in the gastrointestinal tract of preruminant dairy calves. Janet E. Williams*, William I. Loucks, Elizabeth D. Benda, Nicola F. Beatty, Katelyn M. Steinkamp, Matthew E. Doumit, and Mark A. McGuire, *University of Idaho, Moscow, ID.*

Nutritional factors are known to influence the development of the gastrointestinal (GI) tract in preruminants. However, the effect of different lipid sources on the bacterial communities in the GI tract of young calves has not been well described. Therefore, the aim of this study was to utilize high-throughput sequencing to investigate the GI microbiota of preruminant calves fed supplemental polyunsaturated fatty acids. Calves (n = 8) were fed milk (4 L per d) plus 3% supplemental oil containing either palm oil or a combination of conjugated linoleic acid and flaxseed oil in a ratio of 1:2 (CLA/FLAX) for 50 d beginning 3–6 d after birth. After euthanasia, digesta samples from the rumen, omasum, abomasum, duodenum, cecum, and large intestine (LI) were collected. DNA was extracted using the Qiagen DNA Stool Mini kit and bacterial DNA amplified using primers targeting the V1-V3 hypervariable region of the 16S rRNA gene. Amplicons were sequenced using an Illumina MiSeq v3 paired-end 300-bp protocol for 600 cycles. Sequences were processed and classified using the custom python application dbcAmplicons. Principal coordinate analysis at the genus level revealed 3 clusters: (1) rumen, omasum, abomasum; (2) duodenum; (3) cecum and LI. All 3 clusters had large proportions of reads that could not be classified at the genus level: 1) 39.9% ± 1.9; 2) 76.2% ± 5.4; 3) 56.4% ± 2.6. Clusters 1 and 2 were enriched in *Prevotella* (24.4% ± 1.6 and 7.2% ± 2.7, respectively) and *Succinivibrio* (8.7% ± 1.4 and 2.8% ± 2.1, respectively) while Cluster 3 had similar proportions of *Prevotella* (6.2% ± 2.2), *Bacteroides* (6.2% ± 1.9), and *Oscillibacter* (4.3% ± 0.6). Using generalized linear mixed models, rumen, omasum, and abomasum bacterial communities from calves fed palm oil had greater ($P < 0.05$) abundances of *Succinivibrio* (13.10% ± 1.9 vs 4.1% ± 1.0), *Oscillibacter* (2.7% ± 0.4 vs 1.1% ± 0.3), and *Paraprevotella* (2.5% ± 0.4 vs 1.4% ± 0.3), and lower ($P < 0.05$) abundances of *Prevotella* (20.4% ± 1.8 vs 28.4% ± 2.1) as compared with those from calves fed CLA/FLAX. The implications of different ruminal bacterial composition on growth and tissue development in preruminant calves warrant further research.

Key Words: preruminant, microbiome, gastrointestinal

M393 Application of tri-axial accelerometers to determine the grazing behavior of dairy cows in a commercial dairy herd. Pieter J. M. Raedts*, Rajneet S. Sohi, Indunil Kulatilleke, and Markandeya Jois, *La Trobe University, Melbourne, Victoria, Australia.*

In grazing dairy farming systems measurement of feed intake is problematic, especially due to lack of practical methods to determine the quantity of pasture that cows graze. Our aim was to measure relative DM intake by grazing behavior using tri-axial accelerometers. Tri-axial accelerometers (ActiGraph) were set to continuous recording at 60 Hz and attached to collars around the neck of 10 lactating dairy HF and

HF-Jersey cross cows (HF 82.5% to 100%) on a commercial dairy herd in Northern Victoria, Australia. After 16 full days of recording, the sensors were removed from the collars and recorded RAW data downloaded from the sensors. The 16 d RAW data were used to determine grazing time, bites and intensity. Occasional observational data of cow behavior was used to validate behavior of cows as determined from the activity counts. All cows in the herd were offered the same ration (flat-feeding), consisting of approximately 1/3rd of DM as concentrate and 2/3rd of DM as forage. Forage was predominantly grazed pasture. Herd test results during the trial provided data regarding milk production and somatic cell count (SCC) of individual cows. The data set (n = 10) showed AVG per day grazing time of 340 min (265 to 397), 21,390 grazing bites (16,409 to 25,810) and 541,957 counts for grazing intensity (345,094 to 641,808). Two cows had a high SCC (>2,500,000/mL), indicating subclinical mastitis, and were excluded from the data set used for correlations. The cows (n = 8) had an AVG daily milk yield of 30.6 L (17.7 to 39.1) and 108 d in lactation (62 to 134). The correlation between number of daily bites and daily milk yield was $R^2 = 0.80$, while grazing time (minutes per day) had a correlation to milk yield of $R^2 = 0.69$. These results suggest that tri-axial accelerometer sensors are useful in determining grazing behavior of lactating dairy cows.

Key Words: grazing, bites, lactating

M394 Effect of acetate and *trans*-10,*cis*-12 CLA on milk production in lactating dairy cows. Natalie L. Urrutia*, Michel Baldin, Jackie Y. Ying, and Kevin J. Harvatine, *The Pennsylvania State University, University Park, PA.*

During CLA-induced milk fat depression (MFD) acetate and glucose are spared from milk fat synthesis and are available for other metabolic uses. Acetate is the major carbon source spared and although acetate deficiency does not cause milk fat depression the effect of acetate supply on lactation and the effect of spared acetate during MFD is not clear. The objective of this study was to compare the effect of CLA and acetate equivalent to that spared during MFD on milk production. Nine multiparous, lactating, ruminally cannulated Holstein cows (244 ± 107 DIM; mean ± SD) were randomly assigned to treatments in a 3 × 3 Latin square design. Experimental periods were 14 d in length and included 4 d for treatment and 10 d for washout period. Cows received the following treatments: control (CON), acetate [ACE; continuous infusion of 7 *M*/d acetate pH 6.1 (rumen)], or CLA [10 g/d *trans*-10,*cis*-12 CLA (abomasal)]. Milk samples were collected on the last 2 d of treatment for determination of fat, lactose and protein concentration, and milk fatty acid profile. Data were analyzed using the fit model procedure of JMP Pro. The model included the random effects of cow nested in sequence, sequence and period and the fixed effect of treatment. Dry matter intake, protein and lactose yield and percentage were not affected by treatments. Milk yield tended to be increased 11% in ACE ($P = 0.09$) compared with CON (22.8, 23.4 and 25.4 kg/d for CON, CLA and ACE, respectively). Milk fat yield increased 20% in ACE ($P = 0.04$) and decreased 23% in CLA ($P < 0.02$) and milk fat percent was decreased 30% by CLA ($P < 0.001$), but was not affected by ACE compared with CON. Concentration and yield of de novo FA ($P < 0.001$), while concentration of preformed FA (>C16) was increased by CLA ($P < 0.001$), compared with CON. Yield of de novo FA, palmitic acid and total C16 FA was increased by ACE ($P < 0.05$ for all) and concentration of palmitic acid was higher ($P < 0.05$) in ACE, compared with CON. In conclusion, acetate supply has an effect on milk production and milk fat synthesis, and spared acetate during MFD may improve energy status.

Key Words: acetate, CLA, milk fat synthesis

M395 Exogenous fibrolytic enzyme in dairy cows diets: Milk yield and composition. Thiago Henrique da Silva*[1], Caio Seiti Takyia[1], Thiago Henrique Anibale Vendramini[1], Filipe Zanferari[1], Elmeson Ferreira de Jesus[2], and Francisco Palma Rennó[1], *[1]University of São Paulo, Pirassununga, São Paulo, Brazil, [2]São Paulo State University, Jaboticabal, São Paulo, Brazil.*

Exogenous fibrolytic enzymes can be a feature to improve fiber digestion and performance of dairy cows. This study was aimed to evaluate the effect of exogenous fibrolytic enzymes on milk yield and composition. Twenty-four Holstein cows (180.2 ± 54.3 DIM; 662.6 ± 88.2 kg BW) were assigned in a replicated 4 × 4 Latin square design, with 21-d periods. Dietary treatments were: 0 (control), 8 (low), 16 (middle) and 24 (high) g of enzyme/cow/day (Fibrozyme, Alltech Inc., Nicholasville, KY) of total mixed ration based on corn silage. Milk yield was recorded by a computer twice daily at 0600 and 1600 h. Milk sample was collected proportionately to a.m. and p.m. milking, and analyzed for fat, protein and lactose by near infrared reflectance spectroscopy. Data were subjected to ANOVA and simple polynomial regression using the SAS software, version 9.0. There was no effect of fibrolytic enzyme supplementation on milk yield (30.24 vs. 30.22 vs. 30.52 vs. 29.89 kg/d) and 3.5% fat-corrected milk (FCM) yield (30.96 vs. 31.70 vs. 31.33 vs. 30.71 kg of 3.5% FCM/d) for control, low, middle and high supplementation, respectively. Also, milk composition was not affected ($P > 0.05$) by enzyme supplementation just like yield fat, protein and lactose. A possible explanation for these results is that animals were not with energy challenge. Results of present study indicated that the use of exogenous fibrolytic enzymes did not improve performance of mid-lactation dairy cows.

Key Words: digestion, fiber, energy

M396 Milk urea:allantoin ratio is a useful marker of efficiency of protein utilization in dairy cows. Pieter J. M. Raedts*, Devin A. Benheim, Ashlee J. Hammond, Jargal Menghe, and Markandeya Jois, *La Trobe University, Melbourne, Australia.*

Dietary protein is extensively degraded in the rumen into ammonia some of which which is in turn used by rumen bacteria to synthesize microbial protein. Excess ammonia is converted to urea in the liver and excreted in urine and milk. Microbial protein (MCP) is digested and absorbed as amino acids and nucleic acids. Nucleic acids are degraded in the liver into allantoin and excreted in the urine and milk. The ratio of urea to allantoin therefore is an indicator of efficiency of utilization of protein in the feed. Our aim was to investigate the efficiency of protein utilization by determining the urea:allantoin ratio in the milk of cows in the 1, 2, 3 and 6+ months of lactation. Herd test milk was collected from 44 Holstein Friesian (HF) and HF-Jersey cross cows (HF 75% to 100%) on a commercial dairy herd in Northern Victoria, Australia. The herd consisted of 2 cohorts, one in early lactation and one in the second half of lactation. All cows in the herd were offered the same ration (flat-feeding), consisting of approximately one-third of DM as concentrate and two-thirds of DM as grass based forage. Part of the forage was grazed pasture consisting predominantly of perennial English ryegrass (*Lolium perenne*) with a scattering of clover and some weeds. The milk was analyzed for urea concentration by an enzymatic method. Allantoin concentration was determined using high performance liquid chromatography. The MUN:MAC ratio was significantly different ($P < 0.05$) between cows in 1st month of lactation (27.92 ± 2.28) and cows in 2nd, 3rd or 6+ months in lactation (34.84 ± 1.68, 43.89 ± 5.35, 45.60 ± 3.42, respectively). This ratio was also significantly different ($P < 0.05$) between cows in 2nd month of lactation and 6+ months of lactation. Cows in the first month of lactation had a significantly higher MAC

and lower MUN ($P < 0.05$) than cows 3 or more months in lactation. These results indicate that cows in early lactation are more efficient in the utilization of dietary protein

Key Words: milk, urea, MUN

M397 Use of chloride concentration to identify ration sorting by dairy cattle. Heidi Rossow*, *University of California, Davis, Davis, CA.*

Currently differences in proportions of particle sizes between the ration fed and the residual ration indicates if dairy cattle are sorting their feed. However, results using the Penn State Particle Sorter (PSPS) can be variable depending on the dry matter of the ration and how vigorously and consistently PSPS is shaken for each sample. Examining differences in chloride concentration (CC) of the ration between the ration fed and residual ration may be an easier and more accurate method to assess ration sorting by dairy cattle. Therefore, the objective of this study is to examine if CC in the ration fed compared with the residual ration could also be used to assess ration sorting by dairy cattle. Ten samples each of the ration fed and the residual ration were collected from 2 pens, including cows close to calving and cows 30–200 d in milk, from 5 dairies in Tulare County CA. Total CC, and CC from each tray of the PSPS was measured by soaking 30 g of sample in 200 mL of de-ionized water for 2 h and then measuring CC using an Oakton waterproof SaltTestr meter (Oakton Instruments, Vernon Hills IL) with a range of 0 to 1% chloride. Overall CC increased with decreasing particle size with means (standard deviations) of CC of 0.195% (0.048), 0.254% (0.041), 0.264% (0.055) and 0.277% (0.080) for top, middle, screen and bottom trays of the PSPS, respectively. Statistics were performed using Proc GLM (SAS Institute, 2013) with CC and PSPS % from each tray regressed on sample type (ration fed or residual ration) for each dairy and pen and if different ($P < 0.05$), then the ration was sorted. For 8 pens representing 4 dairies, results for sorting (fed vs residual) were consistent between PSPS and CC. However for 1 dairy (2 pens), CC indicated sorting and PSPS did not. Therefore, results from CC and PSPS indicate that CC maybe an equivalent method to identify ration sorting but more research needs to be done to determine why results were not consistent on 1 dairy.

Key Words: chloride concentration, ration sorting, Penn State particle sorter

M398 Pre- and postweaning performance of nursery calves offered texturized calf starters with varying protein levels for 56 days. Bruce Ziegler*[1], David Ziegler[2], Hugh Chester-Jones[2], Daniel Schimek[1], and Sarah Schuling[1], *[1]Hubbard Feeds, Inc., Mankato, MN, [2]University of Minnesota Southern Research and Outreach Center, Waseca, MN.*

One hundred and four (2 to 5 d old) individually fed Holstein heifer calves (39.5 ± 0.69 kg BW) were randomly assigned to 1 of 4 treatments to evaluate pre- (d 1–42) and postweaning (d 43–56) calf performance when fed texturized calf starters varying in crude protein (CP) level. Texturized calf starter (CS) treatments were 1) 15% CP; 2) 18% CP; 3) 21% CP, and 4) 24% CP, as-fed. All calves were fed 0.28 kg of milk replacer (MR; 20% protein:20%fat) in 1.99 L water twice daily for the first 35 d and once daily from d 36 to 42. From d 1 to 14, 1:1 neomycin:oxytetracycline was added to the MR solution to provide 22 mg/kg BW/d. Calf starter and water were fed free choice throughout the trial. Linear (L) and quadratic (Q) contrasts were used to differentiate effects of CS CP level on growth performance. There were no differences among treatments in average daily gain pre- or postweaning. There was a Q effect of treatment on daily gain for d 1 to 56 ($P < 0.01$). Daily gains were 0.71, 0.76, 0.76, and 0.70 kg for the 15%, 18%, 21% and 24% CP CS treatment, respectively. There was a Q effect for hip height gain ($P = 0.02$). Hip height gains were 10.2, 11.1, 11.0 and 9.9 cm for the 15%, 18%, 21% and 24% treatments, respectively. Preweaning CS intake was similar across treatments. Postweaning CS intake linearly decreased with increasing CP levels, d 43 to 56 ($P < 0.01$) and overall ($P < 0.03$). Preweaning gain/feed increased with CS CP level up to 21% CP then decreased (Q; $P = 0.04$). A similar overall 56 d response in gain/feed occurred (Q; $P = 0.05$). Gain/feed d 1 to 56 was 0.53, 0.55, 0.56, and 0.55 kg for 15%, 18%, 21% and 24% CP treatments, respectively. Fecal scores, scouring days and treatment costs were similar among treatments. b-Hydroxybutyrate levels at 42 d were the lowest in calves fed the 15% CP CS. Calf starter intake decreased with increasing CP levels but did not directly relate to calf performance. Under the conditions of this stud, there was no benefit of feeding CS CP levels above 21%.

Key Words: calf performance, calf starter, crude protein

M399 Pre- and postweaning performance and health of dairy calves fed milk replacers vs. pasteurized waste milk. David Ziegler*[1], Hugh Chester-Jones[1], David Cook[2], and Julian Olson[2], *[1]University of Minnesota Southern Research and Outreach Center, Waseca, MN, [2]Milk Products, Chilton, WI.*

The objectives of this study were to compare pre- (d 1 to 49) and post weaning (d 50 to 56) performance of calves fed a milk replacer (MR) formulated with similar crude protein (CP) and fat (F) concentrations to pasteurized waste milk (PWM) and a combination of PWM and a low (F) and high CP MR. One hundred and five (2 to 5 d old) individually fed Holstein heifer calves (38.8 ± 0.73 kg) were randomly assigned to 1 of 4 milk treatments. Milk treatments included 1) all-milk, non-medicated MR 20% CP: 20% F fed at 0.34 kg in 2.38 L of water 2x daily from d 1 to 42 and 1x daily from d 43 to weaning at d 49 (CON); 2) all-milk, non-medicated MR 26% CP: 31% F supplemented with additional fatty acids fed as in CON (MRS); 3) pasteurized waste milk 28.4% CP: 30.1% F fed as in CON, feeding rate was adjusted daily based on measured solids (PWM); 4) PWM fed 2× daily with 0.22 kg solids supplemented with 0.12 kg of an all milk non-medicated 24% CP: 7% F MR as in CON, adjusted for solids as in PWM (WMS). Calf starter (CS;18%CP) and water were fed free choice d 1 to 56. Waste milk was collected twice a week from one farm then sampled, cooled, and pasteurized before each feeding. Calves fed PWM and WMS avg. 0.85 kg/d gain vs. 0.72 kg/d for calves fed CON and MRS ($P < 0.05$) for the 56 d study. Hip height gain avg.13 cm for PWM and WMS vs.11 cm for CON and MRS ($P < 0.05$). There were no differences in intake of milk solids, avg. 29.7 kg for 49 d. Intake of CS, d 1 to 56, was highest for WMS (48.2 kg) with CON and PWM being intermediate (avg. 38.2 kg) and MRS the lowest (31.9 kg; $P < 0.05$). Gain/feed was highest ($P < 0.05$) for PWM (0.69 kg) with MRS (0.65 kg) being intermediate and CON and WMS the lowest ($P < 0.05$; avg. 0.64 kg).There were no differences in daily fecal scores across treatments. Days with fecal scores = 4 and health costs were higher ($P < 0.05$) for MRS vs. CON, PWM and WMS. From d 57 to d 84 there were no differences in ADG across treatments. Under conditions of this study calves fed WMS had greater CS intake than CON, MRS and PWM. Calves fed MRS did not enhance performance over CON.

Key Words: calf performance, milk replacer, pasteurized waste milk.

M400 Evaluation of the effects of direct-fed microbials, microbial fermentation products, and digestive enzymes on milk yield and milk components in dairy cattle in the tropics. Karen Espino-Mercado, Coral Castillo-Caballero, Jaime Curbelo-Rodríguez, and Guillermo Ortiz-Colón*, *University of Puerto Rico at Mayaguez, Mayaguez, PR, Puerto Rico.*

The effect of direct-fed microbials, fermentation products (FP) and digestive enzymes on milk yield and milk composition was evaluated in dairy cattle. Lactating Holstein dairy cows (n = 40) from a commercial dairy herd were divided in 4 groups (n = 10 each) and blocked by days in milk (DIM; < 100 DIM or ≥100 ≤ 171 DIM) and balanced by parity number. Animals had a 2-week adaptation period. The balanced groups were randomly assigned to one of the following treatments: Control (C): 21g wheat middling; Treatment 1 (T1): 21g of a commercial mixture of *A. oryzae* FP, *B. subtilis* FP, *L. acidophilus* FP, yeast and amylase. Treatment 2 (T2): 18g Wheat middlings + 3g of a commercial mixture of *A. niger* FP, *A. oryzae* FP, α amylase, pectinase, endo- glucanase, β- glucanase, xylanase, and mannanase. Treatment 3 (T3): 11g Wheat middlings + 10g of a commercial mixture of *B. subtilis*, amylase and α amylase. Each treatment was top-dressed on the pelleted feed offered at the parlor during every morning milking. Milk production by cow was collected twice a day for 14 weeks using the AfiLab AfiMilk system. Milk urea nitrogen (MUN) and ketone body concentrations in milk by cow was collected once a week for 14 weeks using the Porta BHB milk ketone test and MUN was determined by the Teco Diagnostic Vet- MUN Reagent strips. There was no interaction between treatment, parity and/or period ($P > 0.10$). Average milk production (kg/cow/per day) during the experiment was C 21.4 ± 2.12; T1 19.7 ± 2.11; T2 19.8 ± 2.23; T3 21 ± 2.11 ($P > 0.05$). Average milk BHB concentration (μmol/L) per treatment was C 90.29 ± 13.08; T1 100.39 ± 12.04; T2 89.71 ± 13.12; T3 99.17 ± 12.57 ($P = 0.2121$). Treatment neither had an effect on MUN. Mean MUN (mg/dL) per treatment was C 8.42 ± 1.3; T1 8.38 ± 1.20; T2 8.58 ± 1.44; T3 8.63 ± 1.26 ($P = 0.8533$). In conclusion, in this experiment under tropical conditions, DFM, FP and digestive enzymes, resulted in no change in milk production, MUN and BHB concentrations in milk.

Key Words: direct-fed microbial, digestive enzyme, fermentation product

M401 Phosphorous excretion and digestibility in Jersey and Holstein consuming corn milling co-products. Gabriel Garcia Gomez*, Alison Foth, and Paul Kononoff, *University of Nebraska-Lincoln, Lincoln, NE.*

Excess dietary phosphorous (P) in dairy cows diet may result in increased excretion of this mineral. Additionally, P accumulation in the soil may be a result of high concentrations of P when manure is applied to cropland. The objective of this study was to evaluate P intake, digestibility and excretion when dairy cows consumed rations containing reduced fat distillers grains (RFDDGS). Data from this study originated from an energy balance study in which RFDDGS was included at 28.8% of the ration DM. In this study, corn was reduced from 22.9 to 8.95% and soybean meal was reduced from 14.8 to 0% of the ration DM in the control and co-product (Co-P) diet, respectively. The study included 8 Holstein (BW = 693.8 ± 12.9 kg) and 8 Jersey (BW = 429.1 ± 13.0kg) multiparous, lactating cows (93 ± 20 DIM) in a repeated switchback design. The concentration of P in the test treatments were 0.44% and 0.59% ± 0.01% DM for the control and Co-P diet, respectively. The intake and excretion of P was estimated through feed sampling and total collection of feces. All feed and fecal samples were analyzed for P. Concentration of P in feces was lower in control diet compared with Co-P (0.97 vs. 1.27 ± 0.05%, respectively; $P < 0.01$). Excretion of P was less for cows fed the control diet compared with the Co-P diet (62.34 vs. 89.70 ± 3.82 g/d, respectively; $P < 0.01$). The excretion of P per kg of milk yield was higher in cows fed Co-P diet compared with control diet (21.7 and 15.8 ± 1.29 g/kg, respectively; $P < 0.01$). There was no difference between Holstein and Jersey in concentration of P in the feces (1.16 vs. 1.08 ± 0.07%, respectively; $P = 0.36$), digestibility (32.3 versus 29.0 ± 2.83%, respectively; $P = 0.40$) and P efficiency (19.4 vs. 18.0 ± 1.63g/kg, respectively; $P = 0.55$) across treatments. Results of this study suggest that rations formulated containing RFDDGS should be adjusted for P to reduce P excretion by dairy cows.

Key Words: phosphorus, excretion, digestibility

M402 Variability in diets of lactating dairy herds . Maria P. Turiello*[1], Marco Sambataro[1], Agustin Turiello[1], Claudina Vissio[1], and Alejandro Relling[2,3], *[1]Universidad Nacional de Rio Cuarto, Facultad de Agronomia y Veterinaria, Cordoba, Argentina, [2]Universidad Nacional de La Plata, Facultad de Ciencias Veterinarias, Buenos Aires, Argentina, [3]IGEVET CCT CONICET, Buenos Aires, Argentina.*

The objective of this study was to determine daily ration and feed bunk variability in physical composition of TMR offered to lactating cows in dairy herds. Four commercial herds (12 pens) in the south of Cordoba province, Argentina, were visited during 3 consecutive days on February. Fresh TMR offered in the morning was sampled to assess particle size distribution with a Penn State Particle Separator. Two samples were taken at the beginning and 2 at the end of the feed bunk to determine TMR variability for each wagon. Daily ration variability was expressed as CV, and calculated by dividing the SD of each particle length % over the 3 d period by the average of them over the same period. The effect of place at the feed bunk on particle size distribution was analyzed with linearized mixed models using the mixed procedure of InfoStat. Place at the feed bunk (beginning vs end) was included as a fix effect. Herd and mixer wagon within herd were included in the model as random effects. The greatest daily CV was found with long and fine particles (17.4 and 17.6% respectively) but all of them where greater than 8%. We observed a great variability and a significant difference between places of TMR samples in the short particle size sieve (Table 1). These results may be indicating heterogeneity on the feed delivered due to procedures of loading and mixing the TMR. It is very important to determine factors of management and infrastructure associated with variability of TMR to improve consistency and then to increase profitability of dairy farms.

Table 1 (Abstr. M402). Distribution (mean ± SD) of particles

	Mixer wagon		
Particles (%)	Beginning	End	*P*-value
Long	17.0 ± 2.84	17.6 ± 2.85	0.52
Medium	34.8 ± 3.80	34.1 ± 3.80	0.34
Short	35.7 ± 2.01	34.4 ± 2.01	0.05
Fine	12.5 ± 2.16	13.9 ± 2.16	0.11

Key Words: TMR variability, particle size distribution

M403 Mineral blood serum status of Holstein cows during the warm and cold seasons. Pedro Meda-Alducin*, Maximino Huerta-Bravo, Gustavo De la Torre-López, Baldomero Alarcón-Zúñiga, and Raymundo Rangel-Santos, *Posgrado en Producción Animal, Departamento de Zootecnia, Universidad Autónoma Chapingo, Texcoco, México.*

Heat stress decreases productive and reproductive performance of dairy cows, and it may be related to mineral status. The aim was to quantify serum concentrations of Cu, Zn, Fe, Ca, Mg and K in Holstein cows during warm (WS, ITH = 72) and cold seasons (CS, ITH = 56). Cows (n = 240) with 2 to 6 lactations and 30–60 DIM, milked 3 times per day were selected from 3 commercial dairy farms (F1-F3) in an arid zone of México. Cows were fed similar diets during both seasons, within farm, with a total mixed ration (corn silage, flaked corn, soybean meal, wheat bran, concentrated, alfalfa and wheat hay, molasses and minerals, according to NRC (2001) requirements. Blood samples were obtained from coccygeal vein with Vacutainer tubes, serum was separated by centrifugation at 3000 rpm during 15 min, and stored at −20°C until their analysis. Mineral content was quantified using an atomic absorption spectrophotometer. Data were analyzed using a lineal model with a 2x3 season x farm arrangement. The interaction season x farm effected ($P < 0.001$) all minerals studied. Concentrations of Cu, Zn, K and Mg in blood serum of cows were not dependent on season. However, Fe was higher ($P < 0.05$) during CS compared with WS in the cows from the 3 farms, while the reverse was true for Ca. Overall, Cu, Zn, Fe, Ca, Mg and K concentrations in blood serum were below the normal range in 65, 35, 26, 0, 35 and 38% of cows during the WS, while corresponding values during the CS were 42, 45, 4, 40, 52 and 38%.It is concluded that the dairy cows studied have several mineral problems that depend more of the interaction season × farm than season. It is recommended to study water quality.

Table 1 (Abstr. M403). Means of minerals (mg/L) in blood serum of Holstein cows in warm and cold seasons

		Mineral (mg/L)					
Season	Farm	Cu	Zn	Fe	Ca	Mg	K
Warm	1	0.92[a]	0.94[a]	1.60[c]	105.53[a]	18.23[b]	173.58[c]
Cold	1	0.84[a]	0.69[a]	1.97[b]	94.95[b]	20.30[a]	234.81[a]
Warm	2	0.77[b]	0.80[a]	1.68[c]	108.38[a]	19.02[b]	194.11[b]
Cold	2	0.78[b]	0.92[a]	2.01[b]	60.27[c]	10.96[c]	128.62[d]
Warm	3	0.68[c]	0.87[a]	1.55[c]	112.64[a]	20.49[a]	126.81[d]
Cold	3	0.88[a]	0.89[a]	2.64[a]	89.16[b]	18.36[b]	222.67[a]
P > F		<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
SEM[1]		0.03	0.08	0.08	1.83	0.33	3.88
Normal range		0.8–1.5	0.8–1.4	1.3–2.5	80–110	18–30	160–215

[a–d]Means without a common letter in the same column are different.

Key Words: mineral diagnosis, heat stress, Holstein cows

M404 Effects of rumen-protected methionine or choline supplementation on vaginal discharge and uterine cytology of Holstein cows. Cassandra S. Skenandore*[1], Diego A. Velasco Acosta1,2, Zheng Zhou[1], Maria I. Rivelli[1], Marcio N. Corrêa[2], Daniel Luchini[3], and Felipe C. Cardoso[1], *[1]University of Illinois, Urbana, IL, [2]Federal University of Pelotas, Pelotas, Brazil, [3]Adisseo S.A.S., Alpharetta, GA.*

Fertility in dairy cows has been declining in recent years. Supplementation with methionine has been shown to improve reproductive health. Seventy-one pregnant Holstein cows entering their 2nd or greater lactation were fed the same basal diet and randomly assigned to 4 treatments from 21 d before calving to 30 DIM. From −21 d to calving cows were fed a close-up diet. From calving to 30 DIM cows were fed a fresh cow diet, and from 30 to 72 DIM a high cow diet. Treatments were: CON (n = 16, fed the basal diets with a Lys:Met = 3.5:1), MET (n = 20, fed the basal diets + Smartamine M to a Lys:Met = 2.9:1), CHO (n = 16, fed the basal diets + 60 g/d Reashure), and MIX (n = 19; fed the basal diets plus Smartamine M to a Lys:Met = 2.9:1 and 60 g/d Reashure). Starting at d 31 cows were randomly re-assigned to 2 treatments: (CON; n = 36, fed the basal diet with a Lys:Met = 3.4:1) or (SM; n = 36, fed the basal diet + Smartamine M to a Lys:Met = 2.9:1). Cows were evaluated at 4, 7, 10, 13, 15, 17, and 30 d after calving for the presence of secretion by inserting the Metricheck device into the cow's vagina. Sample appearance was scored from 0 to 3 and smell was scored 0 or 3 according to Sheldon et al. (2006), and combined in a final score (S). On 15, 30, and 72 d after calving, the uterine endometrium of all cows was sampled using an endocervical brush (cyto-brush) and streaked onto slides. Each slide was examined and counted by the same person for the presence of endometrial polymorphonuclear (PMN) cells. Statistical analysis was performed using the MIXED procedure of SAS. There was no treatment effect ($P = 0.16$) for S up to 17 DIM. Cows receiving MIX had a lower (0.38 ± 0.3, $P = 0.03$) S at 30 DIM than CON (1.15 ± 0.3), MET (1.08 ± 0.4), or CHO (2.11 ± 0.4). There were no treatment differences ($P = 0.93$) for the percentage of PMN cells at 15 or 30 DIM. At 72 DIM, cows in SM had lower (5.33 ± 3.6%, $P = 0.01$) PMN cells than CON (10.17 ± 3.6%). In conclusion, supplementing cows with Smartamine M after 30 DIM seems to have beneficial effect on cows' uterine health.

Key Words: methionine, endometritis, PMN

M405 Milk yield and composition in cows fed calcium salts of polyunsaturated fatty acids of different particle sizes. Maxime Leduc*[1,2], Rachel Gervais[1], Yolaine Lebeuf[1,2], and P. Yvan Chouinard[1,2], *[1]Université Laval, Québec, QC, Canada, [2]Institue of Nutrition and Functional Foods, Québec, QC, Canada.*

Feeding unsaturated fatty acids (FA) as Ca salts (CS) has been proposed as a way to protect ruminal microbes from the adverse effects of dietary oils. However, ruminal dissociation of CS may limit the efficiency of this protection. Industrial processes used to obtain CS of FA lead to the production of commercial feeds with a range of different particle sizes. We hypothesized that feeding CS as large particles will improve their inertness in the rumen and improve animal performance. CS of unsaturated FA were obtained from Virtus Nutrition LLC (Corcoran, CA). On a FA basis, the preparation contained 22.4% 18:1 n-9, 14.7% 18:2 n-6, 31.9% 18:3 n-3. The product was sieved through a 1.9-mm screen, and the retained particles were identified as coarse CS (CCS). The small particles were ground through a 0.864-mm sieve, and identified as fine CS (FCS). A mixture of unprotected FA, as triglycerides, with a composition similar to that of the CS served as control. Eight Holstein cows were used in a 4 × 4 Latin square design. Treatments were: 1) ruminal dosing of unprotected FA (negative control; N-CTL); 2) ruminal dosing of FCS; 3) ruminal dosing of CCS; and 4) abomasal dosing of unprotected FA (positive control; P-CTL). Treatments were adjusted to provide 600 g FA per day, and were offered daily in 2 equal boluses for 14 d, followed by 14-d washout intervals. Pre-planned contrasts were used to compare CCS with i) N-CTL; ii) FCS; and iii) P-CTL. Milk yield was 30.0 kg/d for CCS, and was similar in cows fed N-CTL (29.4 kg/d; $P = 0.70$) or FCS (29.6 kg/d; $P = 0.82$), and lower in cows fed P-CTL (25.8 kg/d; $P = 0.01$). Milk fat content was 3.48% for CCS, and was lower in milk from cows fed N-CTL (3.01%; $P < 0.01$) or FCS

(3.15%; $P = 0.03$), and higher in milk from cows fed P-CTL (3.84%; $P < 0.01$). Concentrations of trans-10, cis-12 18:2 in milk was 0.41 mg/g of fat for CCS, and was higher for N-CTL (0.69 mg/g; $P < 0.01$) or FCS (0.69 mg/g; $P < 0.01$), and was similar for P-CTL (0.35 mg/g; $P = 0.29$). In conclusion, feeding CCS prevented ruminal production of trans-10, cis-12 18:2 and maintained a higher milk fat content as compared with unprotected FA or FCS.

Key Words: dairy cow, Ca salt, fatty acid

M406 Using the NRC (2001) model to examine the relationships between predicted supplies of metabolizable Met and Lys and actual yields of milk and milk protein: A subject revisited. Nancy L. Whitehouse*[1], Andre F. Brito[1], Charles G. Schwab[2], and Brian K. Sloan[3], *[1]University of New Hampshire, Durham, NH, [2]Schwab Consulting, LLC, Boscobel, WI, [3]Adisseo, Alpharetta, GA.*

We previously reported relationships between predicted supplies of MP, MP-Met, and MP-Lys (NRC, 2001) and yields of milk and milk protein. The original database consisted of model evaluations of 464 diet treatments from experiments published in the *Journal of Dairy Science* (JDS) from 1976 through 2003. Results indicated yields of milk and milk protein were more accurately predicted by supplies of the first limiting AA rather than by supplies of MP. To establish a more robust database, an additional 550 diet treatments from experiments published in JDS in 2004–2014 were evaluated. For the current analysis, the following criteria were imposed: NE_L allowable milk ≥ MP allowable milk, MP allowable milk within ± 6 kg of actual milk yield, MP balance between −250 to 100 g/d, Lys ≤7.2% of MP, and Met ≤2.4 of MP. To develop the regression equations for MP-Met, and to help ensure that Met was more limiting than Lys, the Lys/Met ratio in MP had to be ≥3.20/1. To generate the regression equations for MP-Lys, the Lys/Met ratio in MP had to be ≤3.15/1. It was preferred this ratio be ≤3.00/1, but that was too restrictive for the available data set. The PROC REG procedure of SAS was used to generate the regression equations. The resulting regression equations describe the relationship between measured milk yields and MP, MP-Met and MP-Lys supplies: MP (n = 455): $y = 0.01397x + 1.76875$, $R^2 = 0.74$; MP-Met (n = 260): $y = 0.84054x - 1.18403$, $R^2 = 0.78$; and MP-Lys (n = 29): $y = 0.25722x - 1.48198$, $R^2 = 0.70$. The equations describing the relationship between milk protein yields and MP, MP-Met and MP-Lys were MP (n = 455): $y = 0.47131x - 67.02527$, $R^2 = 0.82$; MP-Met (n = 260): $y = 28.47206x - 178.08057$, $R^2 = 0.86$; and MP-Lys (n = 29): $y = 9.07155x - 217.19663$, $R^2 = 0.85$. In contrast to the previous equations, these are linear in nature and biologically more correct. We conclude these updated Met and Lys regression equations more accurately predict relationships between MP-Met and Lys supplies and milk and milk protein yields, and can be used by NRC users to determine if milk and milk protein yields are potentially being limited by these 2 AA.

Key Words: methionine, lysine

M407 Milk fatty acid profile in cows fed calcium salts of polyunsaturated fatty acids of different particle sizes. Maxime Leduc[1,2], Rachel Gervais*[1], Yolaine Lebeuf[1,2], and P. Yvan Chouinard[1,2], *[1]Université Laval, Québec, QC, Canada, [2]Institue of Nutrition and Functional Foods, Québec, QC, Canada.*

Feeding unsaturated fatty acids (FA) as Ca salts has been proposed as a way to protect them against ruminal biohydrogenation. However, dissociation of Ca salts in the rumen limits the efficiency of this protection. Industrial processes used to obtain Ca salts of FA lead to the production of commercial feeds with a range of different particle sizes. We hypothesized that unsaturated FA in large particles are physically protected against ruminal biohydrogenation. Calcium salts of polyunsaturated FA were obtained from Virtus Nutrition LLC (Corcoran, CA). On a FA basis, the preparation contained 22.4% 18:1n-9, 14.7% 18:2n-6, 31.9% 18:3n-3. The product was sieved through a 1.9-mm screen. The retained particles were saved, and identified as coarse Ca salts. The particles less than 1.9-mm were ground through a 0.864-mm sieve, and identified as fine Ca salts. A mixture of unprotected FA, as triglycerides, with a composition similar to that of the Ca-salts served as control. Eight Holstein cows were used in a 4 × 4 Latin square design. Treatments were N-CTL) ruminal dosing of unprotected FA, used as negative control; FCS) ruminal dosing of fine Ca salts; CCS) ruminal dosing of coarse Ca salts; and P-CTL) abomasal dosing of unprotected FA, used as positive control. Treatments were adjusted to provide 600 g FA per day, and were offered in 2 equal boluses at 1000 and 1600h for 14 d, followed by 14-d washout intervals. Pre-planned contrasts were used to compare CCS with i) N-CTL; ii) FCS; and iii) P-CTL. Milk fat content of 18:2n-6 was 25.4 mg/g for CCS, and was lower in cows fed N-CTL (16.5 mg/g; $P = 0.01$) or FCS (15.0 mg/g; $P < 0.01$); and higher in cows fed P-CTL (35.2 mg/g; $P < 0.01$). The concentrations of 18:3n-3 in milk fat was 28.9 mg/g for CCS, and was lower in cows fed N-CTL (7.0 mg/g; $P = 0.01$) or FCS (14.6 mg/g; $P = 0.08$, tendency); and higher in cows fed P-CTL (92.2 mg/g; $P < 0.01$). In conclusion, feeding CCS appeared to have partially prevented ruminal biohydrogenation, and increased milk fat content of polyunsaturated FA as compared with dietary unprotected oil or FCS.

Key Words: dairy cow, Ca salt, unsaturated fatty acids

M408 The effect of linseed oil supplementation on rumen microbiota composition in lactating dairy cows. H. M. Tun*[1], E. Khafipour[1], and C. Benchaar[2], *[1]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, [2]Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada.*

The effect of linseed oil (LO) supplementation to red clover silage (RCS)- or corn silage (CS)-based diets on rumen microbiota composition was studied in 12 rumen-cannulated lactating dairy cows in a 4 × 4 Latin square design (35-d periods) with a 2 × 2 factorial arrangement of treatments. Rumen liquid and solid samples were collected on d 18 of each period. DNA was extracted and V4 region of 16S rRNA was amplified and subjected to illumina paired-end sequencing. In both rumen liquid and solids, the LO supplementation reduced bacterial α-diversity in CS-based diet but not in RCS ($P < 0.05$). The LO supplementation altered the β-diversity of rumen microbiota in both CS- and RCS-based diets though the magnitude of shift was greater in CS. The LO supplementation reduced the abundances of several bacterial phyla including Actinobacteria, Chloroflexi, Fibrobacteres, Firmicutes, Plantomycetes, SR1, Spirochetes and Tenericutes, and increased the abundances of Bacterioidetes, Elusimicrobia, Lentisphaerae, Proteobacteria and Synergistetes in the CS-based diet ($P < 0.05$) but did not change the proportion of the abovementioned phyla in the RCS-based diet. The LO supplementation reduced methanogenic *Methanobrevibacters* in both diets, but the magnitude of depression found to be greater in CS compared with RCS. The LO supplementation increased *Methanosphaera* population with a greater magnitude in the CS-based diet compared with RCS ($P < 0.05$). The LO supplementation also negatively affected the proportion of several beneficial rumen bacteria including *Bifidobacteria*, *Fibrobacter* and *Mollicutes* with greater magnitude in the CS-based diet compared with RCS ($P < 0.05$). Data mechanistically explains how

LO supplementation differentially affected CH_4 emission and animal production in CS- vs. RCS-based diets observed in the parallel study to this. The LO supplementation more effectively reduced the CH_4 emission (26%) in CS vs. RCS (9%), which might be due to greater reduction in *Methanobrevibacters*. Also, LO supplementation reduced DMI, fiber digestion, and yields of milk fat and protein only in the CS-based diet, which might be due to its negative effect on fibrolytic populations.

Key Words: linseed oil, rumen microbiota, methane emission

M409 The effects of linseed oil supplementation on fecal microbiota in lactating dairy cows. H. M. Tun*[1], E. Khafipour[1], and C. Benchaar[2], *[1]Department of Animal Science, University of Manitoba, Winnipeg, MB, Canada, [2]Agriculture and Agri-Food Canada, Dairy and Swine Research and Development Centre, Sherbrooke, QC, Canada.*.

The effect of linseed oil (LO) supplementation to red clover silage (RCS)- or corn silage (CS)-based diets on rumen microbiota composition was studied in 12 rumen-cannulated lactating dairy cows in a 4 × 4 Latin square design (35-d periods) with a 2 × 2 factorial arrangement of treatments. Fecal samples were collected on d 24–29 of each period and pooled. DNA was extracted and V4 region of 16S rRNA was amplified and subjected to Illumina paired-end sequencing. DNA was extracted and V4 region of 16S rRNA was amplified and subjected to illumina paired-end sequencing. The feces from cows fed RCS-based diets had greater microbial α-diversity compared with CS, but LO supplementation did not affect α-diversity of microbial community. In contrast, the LO supplementation altered the β-diversity of fecal microbota only in the CS-based diet ($P = 0.001$). Among 17 bacteria phyla found in fecal samples, the LO supplementation reduced both Actinobacteria and Bacteroidetes populations and increased Fimicutes under the CS-based diet ($P < 0.05$). However, under the RCS-based diet, the LO supplementation showed no significant effect on any of bacterial phyla. In CS-based fed cows, LO supplementation suppressed abundances of 2 genera from Bacteriodales family, unclassified RF16 and unclassified Rikenellaceae, as well as the genus SHD231 in Anaerolinaceae family ($P < 0.05$). The 16S rRNA sequencing generated taxa belonged to Euryarchaeota in which no archaeal genera were found to be associated with LO supplementation. Data suggest that LO supplementation only affected fecal microbiota in the CS-based diet and that the source of the basal forage diet influences the effectiveness of LO supplementation and composition of microbiota even in the hindgut of dairy cows.

Key Words: linseed oil, fecal microbiota, methane emission

M410 Effects of acetate, and propionate infusion and pH on VFA production. Sandip Ghimire*[1], Benjamin A. Wenner[2], Richard A. Kohn[3], Jeffrey L. Firkins[2], and Mark D. Hanigan[1], *[1]Virginia Polytechnic Institute and State University, Blacksburg, VA, [2]The Ohio State University, Columbus, OH, [3]The University of Maryland, College Park, MD.*

Four continuous culture fermenters were used to determine the effect of varying VFA concentrations and pH on VFA production. The treatments were applied in the fermenters over 4 periods in a 4 × 4 Latin square design. The 4 treatments were: control, 20 mmol/d acetate infusion (INFAC), 7 mmol/d propionate infusion (INFPR), and low pH (LOWPH). For the LOWPH, buffer composition was adjusted to lower pH by 0.5 units compared with control (ranging from 6.62 to 6.97). The fermenters were fed 40 g of a pelleted 50:50 alfalfa: concentrate diet once daily. After 7 d of adjustment, filtered liquid effluent (4 mL) was sampled at 0, 2, 4, 6, 8, 12, 16, and 22 h after feeding for VFA concentration analysis. Acetate and butyrate production were not affected by treatments. Production of propionate was higher in LOWPH ($P < 0.05$) compared with control and INFPR (Table 1). The hourly production of propionate was not different between treatments ($P > 0.1$) except at 2 h when it was higher in LOWPH compared with INFPR ($P > 0.05$). The acetate to propionate ratio (AP ratio) in INFPR was higher than other treatments ($P < 0.05$). The AP ratio of LOWPH was lower than control. These results reveal that propionate production was increased by low pH, and higher concentration of propionate increased the AP ratio in continuous culture, thus affecting VFA recycling.

Table 1 (Abstr. M410). Effect of acetate infusion, propionate infusion, and low pH on VFA production[1]

VFA (mmol/d)	INFAC	INFPR	LOWPH	Control	SE	*P*-value
Acetate	88	93	89	91	5.2	0.56
Propionate	34.7^{ab}	30.8^{b}	38.4^{a}	33.4^{b}	1.8	0.01
Butyrate	15.3	15.4	14.1	14.7	0.9	0.12
Acetate: Propionate	2.55^{ac}	3.03^{a}	2.32^{c}	2.73^{b}	0.1	<0.01

[1]Production was calculated after subtracting infused VFA from entry rate.

Key Words: fermenter, VFA, pH

M411 Effect of linseed meal on animal performance and oxidative stability of omega 3 enriched milk in Holstein dairy cows. Daniel E. Rico*, Rachel Gervais, Lauriane Schwebel, Yolaine Lebeuf, and Yvan Chouinard, *Département de Sciences Animales, Université Laval, Quebec, QC, Canada.*

Linseed meal antioxidants could help prevent oxidative degradation of omega-3 enriched milk. Six Holstein dairy cows (120 ± 30 DIM, 36.3 ± 6.5 kg milk/d; Mean ± SD) were used in a replicated 3 × 3 Latin Square design (20-d periods; 14 d of adaptation) investigating the effect of linseed meal on animal performance and oxidative stability of omega-3 enriched milk. Linseed oil was abomasally infused continuously to all cows at 243 ± 23 g/d, and dietary treatments were: 1) Linseed meal (16.5% of DM; LS), Canola meal (16.5% of DM) + 7000 units of vitamin E/kg DM (VE), Canola meal (16.5% of DM; CON). Milk yield was recorded and sampled for composition analyses on the last 3 d of each period. Oxidation measurements were done in fresh milk collected on d 17. Data were analyzed using the MIXED procedure of SAS (SAS 9.3, The SAS institute, Cary, NY) including the random effects of period and cow, and the fixed effects of square and treatment. Preplanned contrasts were CON vs. LS and VE vs. LS. Milk yield and fat corrected milk were not affected by treatment and averaged 34.2 ± 2.3 and 32.8 ± 2.3 (mean ± SE), respectively. Milk fat concentration tended to be lower in LS (3.76%) relative to CON (4.00%; $P = 0.06$), but was no different from VE (3.73%). Milk protein concentration was higher in LS (3.38%) compared with CON (3.30% $P = 0.01$), and was not different from VE (3.38%). The concentration of lactose and the yields of fat, protei, and lactose were not different among treatments. The concentration of C18:3 n-3 was not affected by treatment and averaged 5.1 ± 0.79% of total milk fatty acids. Treatments had no effect on the concentration of dissolved oxygen, redox potential or conjugated diene hydroperoxides of fresh milk, which averaged 5.5 ± 0.17 mg/L of milk, 148.5 ± 24.1 mV and 0.92 ± 0.06 mmol/L, respectively. However, VE reduced milk concentrations of the volatile lipid oxidation products propanal, hexanal, and 1-octen-3-one by >73% ($P < 0.05$) and tended to reduce hept-*cis*-4-enal concentrations by 90% ($P = 0.06$) relative to

LS, whereas there were no differences between LS and CON. Vitamin E may help prevent oxidative degradation of milk moderately enriched in omega-3, whereas linseed meal has no effect.

Key Words: dairy cow, omega-3, linseed meal.

M412 Effect of potassium carbonate and soybean oil supplementation on lactational performance in early-lactating dairy cows fed a high-concentrate diet. A. Rene Alfonso Avila*[1], Edith Charbonneau[1], P. Yvan Chouinard[1], Gaëtan F. Tremblay[2], and Rachel Gervais[1], *[1]Université Laval, Quebec, QC, Canada, [2]Agriculture and Agri-Food Canada, Quebec, QC, Canada.*

Research suggests that the decrease in milk fat synthesis observed in dairy cows fed rations high in concentrates or supplemented with vegetable oils could be prevented by increasing dietary cation-anion difference (DCAD) and/or potassium supply. The objective of this study was to evaluate the effect of potassium carbonate (K_2CO_3) on lactational performance of early-lactating dairy cows fed diet supplemented with soybean oil (SBO). Eight primiparous and 20 multiparous Holstein cows averaging 39 ± 9 DIM (Mean ± SD) were used in a randomized complete block design (7 blocks) based on DIM and number of calving with a 2 × 2 factorial arrangement of treatments. Within each block, cows were fed a basal diet formulated to achieve 40% forage (58% corn silage) and 60% concentrate (47% non-fibrous carbohydrates), with 0 (DCAD: +95 mEq/kg) or 1.5% K_2CO_3 (DM basis; DCAD: +316 mEq/kg), and 0 or 2% SBO. Effects of K_2CO_3, SBO and their interaction were evaluated. Treatment period was 28 d in length, plus 1 wk pretreatment collection period, used as covariate and the last 5 d used for data and sample collection. Dry matter intake was not affected by treatments (24.8 ± 1.2 kg/d; $P = 0.56$), but milk yield was increased when SBO was added to the diet (41.8. vs 38.6 kg/d; $P = 0.01$). Milk protein content was decreased by SBO (3.02% vs. 3.23%; $P = 0.01$), but a similar milk protein yield was observed among treatments (1.26 ± 0.01 kg/d; $P = 0.19$). Milk fat percentage (3.30 ± 0.07%; $P = 0.21$) was not affected by treatments. Feeding SBO tended to decrease milk fat yield, exclusively when cows were fed a diet without K_2CO_3 (0% K_2CO_3: 1.26 vs. 1.33 kg/d, 1.5% K_2CO_3: 1.31 vs. 1.34 kg/d; interaction: $P = 0.09$). 4% fat-corrected milk was increased with K_2CO_3 supplementation in cows fed SBO diets (37.4 vs. 35.0 kg/d), whereas the opposite effect was observed for cows receiving diets without SBO (35.4 vs. 36.9 kg/d; interaction: $P = 0.06$). Milk urea nitrogen was decreased by SBO (14.2 vs. 16.2 mg/dL; $P = 0.03$) and K_2CO_3 (13.9 vs. 16.5 mg/dL; $P = 0.01$). In conclusion, the effect of K_2CO_3 on milk production and composition is affected by dietary unsaturated fatty acid supplementation.

Key Words: DCAD, milk fat synthesis, potassium carbonate

M413 Ratio between plasma sphingolipids reveals acyl-chain specific changes during the transition from pregnancy to lactation in Holstein cows. Sina Saed Samii*, J. Eduardo Rico, Alice T. Mathews, and Joseph W. McFadden, *West Virginia University, Morgantown, WV.*

The ratio between sphingolipids is a means to understand sphingolipid biology, an analysis utilized in biomedicine to study ceramide-mediated insulin resistance. Our objective was to evaluate whether the ratio between plasma sphingolipids in dairy cows is modified during the peripartum. Multiparous Holstein cows were grouped by BCS at d −28 prepartum: lean (BCS 2.91 ± 0.13; n = 7) or overweight (OVER; BCS 4.03 ± 0.21; n = 7). Diets were formulated to meet nutrient requirements. Blood was collected routinely from d −21 to 21. LC/MS was used to profile 37 Cer, monohexosylceramides (GlcCer), lactosylceramides (LacCer), and SM in plasma. Log-transformed data were analyzed using a mixed model with repeated measures (fixed effects of BCS and day). Nonparametric correlations were analyzed. NEFA mobilization increased during transition, more so in OVER ($P < 0.01$). Ratio of C16:0-SM to C16:0-Cer (C16:0 SM:Cer) increased during transition ($P < 0.01$) and tended to be lower in OVER ($P < 0.1$). C18:0 SM:Cer reached a nadir at calving ($P < 0.01$). In contrast, C24:0 and C22:1 SM:Cer progressively decreased with time ($P < 0.01$). C16:0, C22:0, and C26:0 Cer:GlcCer decreased during transition ($P < 0.01$). C18:0 Cer:GlcCer tended to display a biphasic response (increased then declined; $P = 0.08$). Neither BCS nor day modified C24:0 Cer:GlcCer. C16:0 and C18:0 GlcCer:LacCer increased and decreased, respectively, as calving approached ($P < 0.01$). After calving, C24:0 GlcCer:LacCer declined until d 21 ($P < 0.05$). C18:0 and C24:0 SM:Cer, C16:0 and C20:0 Cer:GlcCer, and C18:0 and C24:1 GlcCer:LacCer were negatively correlated with NEFA (r = −48 to −0.22; $P < 0.01$). In contrast, C16:0 GlcCer:LacCer was positively correlated with NEFA (r = 0.57; $P < 0.01$). C18:0 and C26:0 Cer:GlcCer were negatively correlated with estimated insulin sensitivity (RQUICKI; r = −0.35 to −0.29; $P < 0.01$), whereas, C20:0 Cer:GlcCer and C18:0 GlcCer:LacCer were positively correlated with RQUICKI (r = 0.19 to 0.29; $P < 0.05$). Research will need to determine whether changes in plasma fatty acids are related to the ratio between acyl-chain specific sphingolipids.

Key Words: ceramide, dairy cow, sphingomyelin

M414 Effects of feeding protected unsaturated fatty acids (Persia Fat) on Insulin resistance parameters of fresh Iranian Holstein dairy cows. Hamed Khalilvandi-Behroozyar[1], Mehdi Dehghan-Banadaky*[2], Mohammad Ghaffarzadeh[3], Kamran Rezayazdi[2], and Essa Dirandeh[4], *[1]Department of Animal Science, Urmia University, Urmia, West Azerbaijan, Iran, [2]Department of Animal Science, University of Tehran, Karaj, Alborz, Iran, [3]Chemistry and Chemical Engeenering Research center of Iran, Tehran, Iran, [4]Department of Animal Science, Sari University of Agriculture and Natural resources, Sari, Mazandaran, Iran.*

The onset of insulin resistance (IR) in fresh dairy cows, will promote sparing of glucose, but the ability of insulin to inhibit HSL and suppress NEFA release from adipose may be impaired. The aim of this study was to evaluate the effects of feeding different FA in dairy cows on IR parameters. Twenty-four multiparous Iranian Holstein cows were used −30 d to 50 DIM. Dietary treatments consisted of (1) Prilled Palm (PO) [Energizer RP10, 2 and 2.25% DM in pre- and postpartum]; (2) Ca-salts of sunflower oil [Persia Fat-SO]; (3) Ca-salts of fish oil [Persia Fat- FO] and (4) equal amounts of Persia Fat-FO and Persia Fat-SO (2.2 and 2.5% of dietary DM in pre- and postpartum). Cows were weighed on 2 consecutive days to determine the doses of glucose for IVGTT(20 DIM, by administering 0.25 g/kg BW glucose i.v.) and insulin for insulin challenge (by 0.1 IU/kg BW insulin i.v. followed by saline). Blood samples were collected at −30 to180 min relative to administration of glucose and 120 min to insulin. The areas under the curve (AUC) of glucose and insulin during IVGTT and IC were calculated using the trapezoidal method. The parameters obtained from IVGTT and IC, were analyzed using PROC MIXED of SAS. The model included the fixed effects of treatment and sequence, and the random effects of period and cow within sequence. A covariate was used to adjust for differences in glucose concentration before the challenges. Statistically significant lower glucose and higher Insulin concentration in PO fed cows, in line with higher TNF-α and glycerol levels can be a hint to lower insulin sensitivity compared with Persia Fat fed animals. Accordingly, PO

impaired glucose and NEFA CR (%/min) during IVGTT and glucose, insulin and NEFA during IC, reflecting lower responsiveness to insulin. The highest and lowest AUC (mg/dL) for glucose, NEFA and insulin during IVGTT, determined for PO and Persia Fat- Fish fed animals, respectively. After IC data support lower response to insulin in the case of glucose, NEFA and Insulin according to lower clearance rate for PO fed animals than Persia Fat. NEFA AUC30 (mEq/L) values were −13817 for PO vs. −16338, −18275 and −19462 for SO, Mix and FO. Overall, data support the idea that feeding rumen protected PUFA can modulate insulin resistance in fresh dairy cows.

Key Words: inflammation, palm oil, fish oil

M415 A novel method to determine rumen biohydrogenation kinetics of alpha-linolenic acid (18:3 n-3). Michel Baldin*[1], Natalie L. Urrutia[1], Daniel E. Rico[2], Kelsie Baxter[1], Yun Ying[1], and Kevin J. Harvatine[1], *[1]Penn State University, University Park, PA, [2]Université Laval, Québec, QC, Canada.*

Biohydrogenation (BH) of unsaturated fatty acids (FA) has been extensively studied in vitro allowing inferences on BH pathways and kinetics. However, BH rates and intermediates formed in vitro may not parallel BH pathways in vivo. The objective was to develop an in vivo method to determine the rate of α-linolenic acid (18:3 n-3) BH and identify intermediates formed. Eleven rumen cannulated high-producing Holstein cows [40 ± 6 kg milk/d (Mean ± SD)] were fed at a rate of 6%/h of expected total DMI a diet balanced to 29% NDF and 5.9% EE (1.5% soybean oil). A single bolus consisting of 200 g of flaxseed oil (53% 18:3) and 15 g of tridecanoic acid (13:0) was mixed with rumen contents and rumen digesta was collected at −1, 0.1, 0.5, 1, 2, 3, 4, 6 and 8 h relative to the bolus. Samples were immediately placed in dry ice, stored at −20°C, freeze-dried, methylated and analyzed by gas chromatography using 17:1 and 19:0 as internal standards. Data were first analyzed using PROC Mixed with repeated measures for time point comparison. Second, the disappearance of 13:0 and 18:3 was fit to a single exponential decay model using the nonlinear procedure of JMP Pro. The bolus increased total fat in the rumen from 4.3 to 6.0% and enriched 13:0 concentration from 0.04 to 2.2% of FA and 18:3 concentration from 2.0 to 11.3% of FA. The fractional rate of disappearance of 13:0 was 0.4%/min (r^2 = 0.98) and of 18:3 was 2.5%/min (r^2 = 0.99), with 18:3 reaching pre-bolus concentration within 4 h. Assuming that 13:0 disappeared only by passage, 18:3 disappeared by passage and biohydrogenation, and the rate of passage of 13:0 and 18:3 are the same, the extent of bolused 18:3 BH was 85%. The concentration of *cis*-9,*trans*-11,*cis*-15 18:3 peaked at 1.2% of FA at 1 h (8-fold increase), *trans*-11,*cis*-15 18:2 peaked at 3.9% of FA at 2 h (13-fold increase), and *trans*-11 18:1 peaked at 6.6% FA at 3 h (43% increase). The in vivo method resulted in the expected extent of biohydrogenation and biohydrogenation intermediates, but the rate of ruminal biohydrogenation of 18:3 was much higher than that commonly observed in vitro.

Key Words: biohydrogenation, fatty acid