

## Ruminant Nutrition: Dairy III

**W318 Milk yield and composition of Holstein cows fed increasing levels of amylolytic enzyme.** Caio Seiti Takiya\*<sup>1</sup>, Gustavo Delfino Calomeni<sup>1</sup>, Thiago Henrique Annibale Vendramini<sup>1</sup>, Thiago Henrique Silva<sup>1</sup>, Guilherme Gomes Silva<sup>1</sup>, Jessica Cristiane Bertoni<sup>1</sup>, Carlos Eduardo Cardoso Consentini<sup>1</sup>, Rodrigo Gardinal<sup>1</sup>, Jefferson Rodrigues Gandra<sup>2</sup>, José Esler Freitas Jr.<sup>3</sup>, and Francisco Palma Rennó<sup>1</sup>, <sup>1</sup>Departamento de Nutrição e Produção Animal da Universidade de São Paulo, Pirassununga, São Paulo, Brazil, <sup>2</sup>Faculdade de Ciências Agrárias da Universidade de Grande Dourados, Itahum, Mato Grosso do Sul, Brazil, <sup>3</sup>Departamento de Zootecnia da Universidade Federal da Bahia, Ondina, Bahia, Brazil.

The objective of this study was to evaluate the effects of increasing doses of amylolytic enzyme in diet of dairy cows on milk yield and composition. The enzyme used comes from *Aspergillus oryzae* extract enriched with  $\alpha$  amylase activity (Amaize, Alltech Inc., Nicholasville, KY). The extract is in powdered form and was offered hand mixed into concentrate. Twenty-four multiparous Holstein cows (average 120 DIM, 32 kg/d of milk yield and 580kg of live weight) were distributed in 4 × 4 Latin square design with 21-d periods and received the following treatments: (1) Control, without Amaize; (2) A150, diet containing 150 FAU/kg of Amaize (diet DM basis); (3) A300, diet containing 300 FAU/kg DM of Amaize (diet DM basis) diet; (4) A450, diet containing 450 FAU/kg of Amaize (diet DM basis). One FAU (fungal  $\alpha$ -amylase unit) is the amount of enzyme which breaks down 1g starch in 1 h based on 11–25 min at pH 4.7 and 30°C. Experimental diet was formulated to achieve 30% of starch content. Corn silage was used as forage source and diet contained 48:52 ratio of forage and grain mixture. Cows were mechanically milked twice daily, and samples were collected proportionally from each milking on d 15, 16 and 17 of each experimental period. Milk samples were analyzed for fat protein and lactose (Milkoscan; Foss Electric, Hillerod, Denmark). Data were subjected to ANOVA and regression using PROC MIXED of SAS (2001). No differences were observed in milk yield and composition (Table 1). Inclusion of exogenous amylolytic enzyme did not improve milk yield and composition of mid lactating cows.

**Table 1 (Abstr. W318)**

Item	Treatment <sup>1</sup>				SEM	P-value <sup>2</sup>	
	0	A150	A300	A450		L	Q
Milk yield, kg/d	32.81	32.72	32.34	32.40	1.37	0.275	0.829
3.5% FCM, kg/d	34.78	34.87	34.19	34.60	1.28	0.543	0.714
Fat, kg/d	1.26	1.28	1.24	1.26	0.05	0.594	0.917
Protein, kg/d	1.02	1.01	1.00	1.01	0.02	0.348	0.212
Fat, %	3.85	3.93	3.86	3.89	0.10	0.793	0.549
Protein, %	3.09	3.09	3.09	3.09	0.02	0.924	0.631

<sup>1</sup>0, 150, 300 or 450 FAU/kg (DM basis) of Amaize (Alltech Inc., Nicholasville, KY).

<sup>2</sup>Linear and quadratic effect.

**Key Words:** a-amylase, *Aspergillus oryzae*, starch

**W319 Biofortified milk: Selenium and vitamin E in cow's diet to improve nutritional components in milk.** Marcia S. V. Salles\*<sup>1</sup>, Arlindo Saran Netto<sup>2</sup>, Luiz C. Roma Junior<sup>1</sup>, Marcus A. Zanetti<sup>2</sup>, Karina Pfrimer<sup>3</sup>, and Fernando A. Salles<sup>1</sup>, <sup>1</sup>APTA, Ribeirão

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Healthy nutrition is a preoccupation of most of the world's population, thus the importance of animal science studies to improve milk nutrient composition. The aim was to study the vitamin E and selenium with sunflower oil (SFO) added to the diet of lactating cows to improve the nutrient profile of milk. Twenty-eight cows were allocated in 4 treatments, as follows: C (control diet); O (4% of SFO in dry matter (DM) diet); A (3,5 mg/ kg DM of organic selenium + 2000 IU of vitamin E/ cow per day); OA (4% of SFO in DM diet + 3,5 mg/ kg DM of organic selenium + 2000 IU of vitamin E/cow per day). Cows were fed with 0.50 of concentrate, 0.42 of corn silage and 0.08 of coast-cross hay (DM). Blood and milk were taken in the last week of trial and analyzed for selenium and  $\alpha$ -tocopherol. Data were analyzed as a RCBD with a factorial treatment structure (GLM/SAS). The addition of selenium and vitamin E in the cow's diet increased selenium and  $\alpha$ -tocopherol serum (0.083, 0.085, 0.337 and 0.389  $\mu$ g/L,  $P < 0.0001$ , 0.022 SEM of selenium and 4.51, 6.26; 6.92 and 8.97 mg/dl,  $P = 0.0009$ , 0.66 SEM of  $\alpha$ -tocopherol for C, O, A and OA, respectively) and milk (0.011, 0.027, 0.235 and 0.358  $\mu$ g/L,  $P < 0.0001$ , 0.033 SEM of selenium and 2.27, 1.56; 3.08 and 2.89 mg/L,  $P = 0.0088$ , 0.36 SEM of  $\alpha$ -tocopherol for C, O, A and OA, respectively). SFO supplementation increased  $\alpha$ -tocopherol in cow's blood ( $P = 0.0097$ ). The antioxidants added to the diet of lactating cows improved the nutrient profile of milk. Financial support: FAPESP.

**Key Words:** nutrient, mineral, vitamin

**W320 Effect of fermented corn silage density and gas filled porosity on corn silage pH and fermentation end-products.** William L. Braman\*, John E. Kurtz, and Keith A. Bryan, *Chr. Hansen Inc., Milwaukee, WI.*

Increasing density (D) of ensiled corn forage by proper harvest dry matter, chop length and silo packing results in decreased presence of oxygen which enhances bacterial fermentation of corn silage (CS). This research measured the effect of CS D and calculated gas filled porosity (P) (Holmes and Muck, 2006) on selected fermentation characteristics. From December, 2012 to July, 2013, 46 dairy operations in the Midwest United States ranging in size from approximately 300 to 4,500 cows (62,500 total) participated in a study to evaluate the effect of silo density (kg/meter<sup>3</sup>) of ensiled CS on the pH, lactic acid (LA), total volatile fatty acids (VFA), ammonia (NH<sub>3</sub>), and ethanol (ETOH), all % of CS DM, of the corresponding corn silage. A minimum of at least 6 core samples from the face of CS bunkers or drive-over piles was consolidated and a composite sample sent to a commercial lab (Rock River Laboratories, Watertown, Wisconsin) for analysis using wet chemistry methods. Some dairies were sampled more than once as they changed sources of CS. The average D was 292.6 kg/meter<sup>3</sup> (range 174.6–350.8). The REG procedure of SAS was used to analyze the relationship between D/P and the dependent variables. There were negative relationships between D and pH ( $R^2 = 0.31$ ,  $P < 0.001$ ), NH<sub>3</sub> ( $R^2 = 0.25$ ,  $P < 0.001$ ), and ETOH ( $R^2 = 0.14$ ,  $P = 0.003$ ). There were positive relationships between D and LA ( $R^2 = 0.26$ ,  $P < 0.001$ ), VFA ( $R^2 = 0.17$ ,  $P < 0.05$ ). Gas filled P was calculated as an alternative measurement of CS D with 0.4 or lower P recommended for proper fermentation. The CS calculated P average was 0.32 (range 0.06–0.69). Regression analysis indicated a negative relationship between P and LA ( $R^2 = 0.23$ ,  $P < 0.001$ ) and VFA ( $R^2 =$

0.18,  $P = 0.001$ ). A positive relationship between P and pH ( $R^2 = 0.30$ ,  $P < 0.001$ ) was observed. These results suggest that increased CS bunker/pile D measured by silage face core sampling is strongly correlated with improved fermentation characteristics of low pH,  $\text{NH}_3$ , ETOH and high VFA and LA concentrations. Gas filled P is an alternative measurement that was correlated with the increased fermentation acids measured and a decreased pH. This work demonstrates the importance of high D on improving the fermentation quality of CS.

**Key Words:** silage, ensiling, density

### **W321 Productive performance of dairy cows fed saturated and unsaturated fatty acids sources in the transition period and early lactation.**

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The objective of the present study was to evaluate milk yield and composition of dairy cows receiving saturated or unsaturated fatty acids (FA) sources during transition period and early lactation. Thirty Holstein cows were divided into 3 experimental groups in randomized complete design. The animals were randomly assigned to receive one of the following treatments: 1) control (C; without fat addition,  $n = 10$ ), basal diet containing (DM basis) 2.5 g/100g of ether extract (EE) in pre and postpartum period; 2) calcium salts of saturated FA (SAT; Magnapac - Tectron Ltd.,  $n = 10$ ), basal diet containing (DM basis) 4.5 g/100g of EE in pre-partum and 5.5 g/100g in postpartum; 3) whole raw soybeans (UNSAT;  $n = 10$ ), basal diet containing (DM basis) 4.5g/100g of EE in pre-partum and 5.5g/100g of EE in postpartum period. The animals were fed individually, and the experimental diets were fed since 35 d before the expected calving date, and provided until 90 d of lactation and were formulated to meet the nutritional requirements of each period (pre and postpartum). Milk yield was measured daily and samples for milk composition were collected weekly from the first to the 13th week of lactation. Data were analyzed using PROC MIXED of SAS 9.1, with the effect of diet, time and interaction as fixed effects, and animal as random effect. The data were analyzed by orthogonal contrasts (C vs. UNS + SAT and UNS vs. SAT). Fatty acids sources did not influence ( $P > 0.05$ ) milk yield, protein and lactose (kg/d and %). The average milk yields were 30.07, 28.86, and 30.85 kg for C, UNS and SAT, respectively. Unsaturated fatty acids source reduced the FCM (28.12 Kg/d vs 33.63 kg/d) ( $P = 0.029$ ), and milk fat (4.16% vs. 3.37%; 1.23 kg/d vs 0.95 kg/d) ( $P = 0.006$ ) ( $P = 0.004$ ) for UNS vs SAT contrast. The supplementation of dairy cows with fatty acids sources in the transition period and early lactation affected the FCM and milk fat yield and percent.

**Key Words:** milk yield, milk composition, fat source

### **W322 Embryo production and oocyte quality of dairy cows fed saturated and unsaturated fatty acids in transition period and early lactation.**

Gustavo D. Calomeni<sup>\*1</sup>, Rodrigo Gardinal<sup>1</sup>, Filipe Zanferari<sup>1</sup>, Caio S. Takiya<sup>1</sup>, Thiago H. A. Vendramini<sup>1</sup>, Jose Esler Freitas Junior<sup>2</sup>, Cybele E. Araújo<sup>1</sup>, Victor C. Galvão<sup>1</sup>, and Francisco P. Renno<sup>1</sup>, <sup>1</sup>Department of Nutrition and Animal Production, School of Veterinary Medicine and Animal Science, University of São Paulo, Pirassununga, São Paulo, Brazil, <sup>2</sup>School of Veterinary Medicine, Federal University of Bahia, Ondina, Bahia, Brazil.

The objective of the present study was to evaluate embryo production and oocyte quality of dairy cows receiving saturated or unsaturated fatty acids (FA) sources during transition period and early lactation. Thirty Holstein cows were divided into 3 experimental groups in randomized complete design. The animals were randomly assigned to receive one of the following treatments: 1) control (C; without fat addition,  $n = 10$ ), basal diet containing (DM basis) 2.5 g/100g of ether extract (EE) in pre and postpartum period; 2) whole raw soybeans (UNSAT;  $n = 10$ ), basal diet containing (DM basis) 4.5g/100g of EE in pre-partum and 5.5g/100g of EE in postpartum period; 3) calcium salts of saturated FA (SAT; MAGNAPAC - Tectron Ltd.,  $n = 10$ ), basal diet containing (DM basis) 4.5 g/100g of EE in pre-partum and 5.5 g/100g in postpartum. The experimental diets were fed from 35 d before the expected calving date, and provided until 90 DIM, formulated to meet the nutritional requirements of each period (pre- and postpartum). The procedure for ovum pick-up (OPU) was performed in 3 periods:  $30 \pm 7$  DIM (OPU1),  $60 \pm 7$  DIM (OPU2) and  $90 \pm 7$  DIM (OPU3). After OPU, the viable oocytes were classified in grade I, II and III. Only oocytes with grade I, II and III were submitted to in vitro fertilization (IVF). Data were analyzed using PROC MIXED of SAS 9.1, with the effect of diet, OPU and interaction as fixed effects, and animal as random effect. The data were analyzed by orthogonal contrasts (C vs. UNS + SAT and UNS vs. SAT). No effects ( $P > 0.05$ ) were observed for OPU, treatment and interaction (treatment\*OPU). No differences were observed among contrasts for total oocytes, viable oocytes, oocytes grade and total embryo produced. The saturated and unsaturated FA supplementation did not influence embryo production and oocyte quality in postpartum period.

**Key Words:** fat source, ovum pick-up, transition period

### **W323 Effect of supplemental OmniGen AF and either a negative or positive DCAD prepartum on intake and blood and urine metabolites from dry off through 10 week postpartum.**

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Multiparous Holsteins (80) were enrolled 60 d before expected parturition through 70 DIM to evaluate the effects of feeding OmniGen AF (OG) and Animate (AN, Phibro Animal Health Corp., Quincy, IL) on DMI, blood and urine metabolites, and milk yield and composition. Cows were blocked by expected calving date and assigned randomly within block to one of 8 treatment combinations arranged as a  $2 \times 2 \times 2$  factorial to provide: 0 (OG-) or 57 g OM (OG+) beginning at dry off through parturition; 0 (POS) or 0.59 kg/d AN (NEG) beginning 28 d before predicted calving; and OG- or OG+ postpartum. Three cows were dropped from the trial due to reasons unrelated to treatments. During the far-off period, no differences were observed in DMI: 13.7 and 14.8 kg/d for OG- and OG+, respectively. Cows fed OG+ had higher ( $P < 0.05$ ) BUN and urinary pH and K concentrations compared with OG-. During the close up period, cows fed NEG had lower ( $P < 0.05$ ) DMI compared with POS; 13.9 and 15.7 kg/d for NEG and POS, respectively. Concentrations of K, Cl, ionized Ca, and ionized Mg were higher ( $P < 0.05$ ) for NEG (4.59, 104.05, 1.28, and 0.68) compared with POS (4.46 mEq/mL, 102.06 mEq/mL, 1.26 mmol/mL, and 0.66 mmol/mL, respectively). Immediately postcalving (0 - 2 DIM), cows fed NEG had lower concentrations of globulin, and urinary pH and Na concentrations but higher Cl ( $P < 0.05$ ): 2.93, 7.59, 31.51, and 55.99 compared with 3.17 g/dl, 7.95, 53.69 mEq/L, and 37.7 mEq/L for NEG and POS, respectively.

Aspartate aminotransferase was greater ( $P < 0.05$ ) for OG- compared with OG+: 127.16 and 106.21 U/L, respectively. No differences were observed in DMI or milk yield postpartum but cows fed OG+ had lower ( $P < 0.05$ ) SCC compared with OG-. Milk fat percentage was higher for cows fed OG+ postpartum compared with those fed OG+ prepartum only. No interactions were observed among OG and AN. Carryover effects of NEG fed prepartum on blood mineral concentrations were observed 0–2 DIM. Supplemental OG did not affect postpartum milk yield but decreased SCC consistent with previous research.

**Key Words:** Animate, blood metabolite, DCAD

**W324 Characterization of ingredient loading accuracy on commercial dairy farms in North America and Europe feeding total mixed rations.** Michael C. Barry\*, *AgModels LLC, Tully, NY.*

Successful implementation of precision feeding is predicated on the assumption that farms can accurately deliver the correct amounts and proportions of ingredients during the feed mixing process. Thirty-three commercial dairy herds of varying management styles across North America and Europe feeding total mixed rations (TMR) were surveyed with respect to ingredient loading accuracy. Across farms, 386,011 individual feed loading records were electronically recorded and compiled during the calendar year of 2014. Deviations from target load amounts were calculated as the difference between actual and the targeted amount of ingredient, both in absolute (kg) and relative (percent of target) terms. Absolute bullseyes were defined as those records with a deviation less than 5 kg from the target and averaged 24.1% across all observations, ranging from 4.3% to 73.9% between farms (median 19.8%). Relative bullseyes were defined as those records with a deviation less than 2% from the target and averaged 46.7% across all observations, ranging from 10.8% to 80.6% between farms (median 42.7%). Absolute deviation values that exceeded an absolute value greater than 62.5 kg from the target were designated as absolute outliers, and represented 11.85% of all values, ranging from 1.3 to 35.3% between farms (median 13.1%). Relative deviation values that differed more than 20% of target values were designated as relative outliers and represented 14.3% of all values and ranged from 2.7 to 40.3% between farms (median value of 15.1%). Several nonsensical values (those with actual values <0 kg) were identified and stratified by farm. 3.8% of all values were deemed as nonsensical and ranged from 0.7% to 10.2% between farms. Deviations were skewed toward positive values, indicating a tendency to overload ingredients. These results indicate a wide range of proficiency of feed loading across farms studied, and would suggest that there exists opportunities to assess and improve quality control procedures and the feed loading protocols on dairy farms feeding total mixed rations.

**Key Words:** total mixed ration, loading accuracy

**W325 Effects of hydroxy versus sulfate forms of trace minerals in milk replacer or starter on dairy calves through weaning.**

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Young dairy calves fed at a higher plane of nutrition may benefit from trace mineral (TM) sources with greater bioavailability, but few data are available for hydroxy forms of Zn, Cu, and Mn. The goal of this study was to evaluate differences in intake, growth, and health status of calves fed milk replacer and starter grain with different sources of TM. Male Holstein calves ( $n = 64$ ) < 1 wk old were transported from a commercial farm to the research facility. Calves were assigned to treat-

ments in a  $2 \times 2$  factorial arrangement of TM source in milk replacer and TM source in starter grain in a randomized complete block design. All calves were fed milk replacer (28% crude protein, 20% fat) at a fixed feeding rate [700 g/d of powder for wk 1, 950 g/d of powder for wk 2–6, and 450 g/d of powder for wk 7] and had ad libitum access to starter [22% CP] and water. Milk replacers were formulated to contain either sulfate (S) or hydroxy (H) TM (70, 10, and 50 mg/kg of Zn, Cu, and Mn, respectively). Starters formulated either with STM or HTM contained 150, 30, and 100 mg/kg of Zn, Cu, and Mn, respectively. All calves were weaned on d 49 and continued to have ad libitum access to water and starter until the end of the experiment at d 63. Body weights and measurements were taken on all calves on a weekly basis. Fecal and respiratory scores were monitored daily and any use of medications was recorded. Final body weight and average daily gain (overall mean of ADG = 0.91 kg/d) did not differ among treatments. Calves fed the HTM milk replacer plus STM starter had greater hip height ( $P = 0.03$ ) and hip height average daily gain ( $P = 0.007$ ). Starter intake was greater in calves fed HTM milk replacer than those fed STM milk replacer ( $P < 0.0001$ ). Calves fed the STM milk replacer had a greater frequency of elevated fecal scores in the first 3 wk of life (odds ratio STM to HTM = 1.80), although use of medication was not different among treatments. Results indicated that calves fed HTM milk replacer and starter had similar overall growth as calves fed STM but maintained a better health status throughout the pre-weaning phase.

**Key Words:** dairy calf, mineral, hydroxy

**W326 Effects of different forage supplement patterns on the growth of Holstein calves.** Zhaohai Wu, Shengli Li, and Zhijun Cao\*, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, Beijing, China.*

This study was conducted to investigate the effects of 2 forage sources supplemented from different time points on the growth performance of Holstein bull calves. Forty Holstein bull calves with similar body weights and birthdate were selected and randomly assigned to 5 treatments with 8 replicates each: control (CON) calves were fed starter feed only, and the other 4 treatments consisted of the same starter feed plus 2 forage sources started from different time points: chopped alfalfa hay started at the 15th day (AW) and the 3rd day (AD), chopped oat hay started at the 15th day (OW) and the 3rd day (OD). All calves were weaned at 56 d, and the study finished one week later. In the pre-weaning period, the starter feed intake was not significantly affected by forage supplement. Moreover, starter feed and total dry matter intake were increased in the first week of post-weaning by feeding forage from the 15th day and providing oat hay. In the pre-weaning period, the average daily gain of calves supplemented forage from the 15th day tended to be higher compared with that from the 3rd day ( $P = 0.079$ ). In the first week of post-weaning, the average daily gain of calves fed forage from the 15th day was significantly higher compared with that from the 3rd day ( $P = 0.045$ ) and CON calves ( $P = 0.032$ ). Meanwhile, the body length of calves pre-weaning improved by feeding oat hay ( $P = 0.071$ ), and the heart girth of calves fed forage from the 15th day tended to be greater than CON calves ( $P = 0.089$ ) in the first week post-weaning. The contents of LDH, AKP, GOT and GPT in the serum of calves were improved by supplementing oat hay and providing forage from the 15th day ( $P < 0.05$ ). The incidence of diarrhea in CON calves were numerically higher than the calves provided forage. In conclusion, forage supplementing did not adversely affect the growth and intake of calves in the pre-weaning period, and the growth performance of calves was improved in the first

week of post-weaning by providing forage from the milk-feeding period, especially offering oat hay from the 15th day.

**Key Words:** calves, oat hay, alfalfa hay

### **W327 Effects of acidified and pasteurized waste milk on calf**

**diarrhea occurrence.** J. Y. Ma<sup>\*1</sup>, X. X. Ren<sup>1</sup>, H. T. Shi<sup>1</sup>, G. Guo<sup>2</sup>, X. Z. Li<sup>2</sup>, and Z. J. Cao<sup>1</sup>, <sup>1</sup>State Key Laboratory of Animal Nutrition, China Agricultural University, Beijing, China, <sup>2</sup>Beijing Capital Agribusiness Group Co., Ltd., Beijing, China.

**Abstract:** Waste milk is unfit for human consumption because it might be harmful for human health. However, it may be acceptable for calf feeding if properly processed. This study was designed to investigate the effect of 2 different sterilization methods of waste milk on diarrhea occurrence of calves. Forty female Holstein calves were randomly assigned into one of 2 groups and fed either acidified (by formic acid) or pasteurized waste milk and defined as treatment group (TT) and control group (CT). The total number of bacteria in milk was detected and found higher in pasteurized milk than in acidified milk ( $3.6 \times 10^4$  vs.  $3.8 \times 10^3$  cfu/mL) on average. The birth weights of calves in 2 groups were similar (TT:  $43.38 \pm 4.100$ ; CT:  $43.83 \pm 4.854$ ;  $P > 0.05$ ). All calves were fed 4 L of colostrum within 1 h after birth and were subsequently fed milk 3 times per day at 0730, 1430, and 1930. Fecal samples were collected from all calves daily for 8 weeks from the third day after birth. Feces were scored by UW-Madison calf health scoring. Calf diarrhea occurrence was described by 3 indexes including diarrhea rate (DA), diarrhea frequency (DF) and fecal indexes (FI). The calculation equations are as bellow. Data were statistically analyzed using the Two-Sample *t*-test for means procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). Diarrhea rate = number of diarrhea calves/total number of calves  $\times$  100%; diarrhea frequency = ( $\sum$  number of diarrhea calves  $\times$  days in diarrhea)/total number of calves  $\times$  number of days on trial  $\times$  100%; fecal index = the sum of fecal score/(total number of calves). Results indicate that the overall calf diarrhea in TT group was lower compared with CT group. DF and FI of TT group were lower than those of CT group (DF:  $10.00 \pm 0.02$  vs.  $15.9 \pm 0.02\%$ ; FI:  $1.41 \pm 0.28$  vs.  $1.59 \pm 0.25$ ;  $P < 0.05$ ). DR values were not significantly different between two groups during the whole trial period of 8 weeks ( $P > 0.05$ ); however, DA, DF and FI within the first 6 weeks (d3 to d42) was lower in TT group than those in CT group (DR: 60 vs. 90%; DF:  $3.75 \pm 1.07$  vs.  $7.50 \pm 1.10\%$ ; FI:  $1.34 \pm 0.029$  vs.  $1.50 \pm 0.03$ ;  $P < 0.05$ ). We conclude that feeding waste milk acidified with formic acid can effectively reduce calf diarrhea occurrence compared with feeding pasteurized waste milk.

**Key Words:** acidification, pasteurization, calf diarrhea

### **W328 Investigating the impact of dietary changes on rumen microbial community during the transition period in Holstein dairy cows using high-throughput sequencing.**

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The transition period is a critical time in the life of a dairy cow and is associated with a dietary shift from a high-forage-based diet (HF) to a high-grain based (HG) diet. This study investigated the impact of such a dietary shift on the structure of the rumen bacterial population. Fourteen Holstein dairy cows were used in this experiment. Rumen digesta samples were obtained using an oral stomach tube 3 weeks before calving (HF) and 9 weeks postcalving (HG). Twenty-eight

samples were collected, and bacterial 16S rRNA genes were sequenced using Roche 454 pyrosequencing with titanium chemistry. Processing of sequences, performing  $\alpha$  and  $\beta$  diversities, and classifying sequences were conducted using MOTHUR. A total of 198,276 non-chimeric sequences were generated. Those sequences were assigned to 16,833 operational taxonomic units based on a 95% genetic similarity for each sample. Shannon and Inverse Simpson indices were calculated for each sample, and revealed an overall reduction ( $P < 0.05$ ) in the diversity of the rumen bacterial population when cows were shifted from the HF to the HG (from  $5.4 \pm 0.2$  to  $4.3 \pm 0.2$  and from  $153 \pm 26$  to  $47 \pm 26$ , respectively). UniFrac was used to test whether HF samples were different from HG samples. The normalized-weighted *P* test was  $< 0.001$ , indicating a significant difference between the 2 bacterial communities; and the calculated UniFrac metric value was 0.968, indicating that most of the branch length belonged to one community or the other. Sequences from HF were allocated mainly into the following phyla: Bacteroidetes ( $38 \pm 4\%$ ), an unclassified group ( $33 \pm 2\%$ ), Firmicutes ( $23 \pm 3\%$ ), and Proteobacteria ( $5 \pm 4\%$ ), whereas samples from HG were allocated mainly into the Proteobacteria ( $36 \pm 4\%$ ), Bacteroidetes ( $34 \pm 4\%$ ), Firmicutes ( $15 \pm 3\%$ ), and an unclassified group ( $14 \pm 2\%$ ). ANOVA with time as the fixed effect showed that Proteobacteria and the unclassified group were significantly different ( $P < 0.001$ ) between HF and HG, and Firmicutes had a tendency ( $P = 0.07$ ) to be different.

**Key Words:** dairy cow, transition period, 16S rRNA

### **W329 Metabolic profile and onset of puberty in dairy heifers fed reduced-fat distillers grains in replacement of forage.**

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Our objective was to determine the effect of increasing the inclusion rate of reduced-fat distillers dried grains (RFDDGS) in replacement of forage in limit-fed diets on the metabolic profile and onset of puberty in dairy heifers. A 16-wk randomized complete block design feeding study was conducted using 48 Holstein heifers ( $199 \pm 2$  d of age) with 3 treatments. Treatments were 30% RFDDGS with 68.5% grass hay (30DG), 40% RFDDGS with 58.5% grass hay (40DG), and 50% RFDDGS with 48.5% grass hay (50DG) on a DM basis. All diets also contained 1.5% mineral mix. Rations were limit-fed at 2.65, 2.50, and 2.35% of BW on a DM basis for 30DG, 40DG, and 50DG, respectively to have similar intakes of CP and energy among treatments. Jugular blood samples were collected on 2 d during wk 0, 4, 8, 12, and 16 for metabolite and metabolic hormone analysis. When heifers weighed 200 kg, coccygeal vein blood samples were taken twice per wk for progesterone analysis to estimate onset of puberty. Blood samples continued until cycling was confirmed via ultrasound for the presence of a corpus luteum. There were no interactions of treatment by wk for any of the metabolites and metabolic hormones measured. Glucose (76.3, 77.7, and 77.3 mg/dL; SEM = 1.60 for 30DG, 40DG, and 50DG, respectively) and leptin (4.42, 4.35, 4.59 ng/mL; SEM = 0.088) were similar ( $P > 0.05$ ) among treatments. There was a quadratic effect ( $P < 0.05$ ) for cholesterol (93.5, 89.2, and 97.1 mg/dL; SEM = 2.16), plasma urea nitrogen (17.8, 17.8, and 19.9 mg/dL; SEM = 0.35), and a quadratic tendency ( $P = 0.05$ ) for IGF-1 (102.7, 100.0, and 109.4 ng/mL; SEM = 3.59). Age at puberty (234.6, 244.3, and 235.5 d; SEM = 13.7) and BW at puberty (246.4, 261.3, and 254.0 kg; SEM = 24.9) were similar ( $P > 0.05$ ) among treatments. These results demonstrate that heifers can be limit-fed diets with greater inclusion rates of RFDDGS and maintain energy status without

accumulating excess adipose tissue as indicated by leptin. Treatments had no detrimental effects on age or BW at puberty; however, at this time no measures were made on reproductive performance post-trial.

**Key Words:** distillers grains, metabolic profile, dairy heifer

**W330 Gastrointestinal tract of healthy 1-week-old Jersey calves is well suited to digest, absorb, and incorporate nutrients into lean tissue even when fed a high plane of milk replacer.** Yu Liang\*<sup>1</sup>, Tyler L. Harris<sup>1</sup>, Jeff A. Carroll<sup>2</sup>, and Michael A. Ballou<sup>1</sup>,

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This study investigated the digestibilities of nutrients as well as nitrogen (N) retention of Jersey calves fed different planes of milk replacer nutrition. Twelve calves were blocked by BW at birth and randomly assigned to either a low (LPN) or high (HPN) plane of milk replacer nutrition. The LPN calves were fed 14.5 g DM/kg BW of a 20% protein and 20% fat milk replacer/d, and HPN calves 20 g DM/kg BW of a 28% protein and 20% fat milk replacer/d. Calves were fed twice daily at 0700 and 1900. All calves were fed 3 L of pooled colostrum within 6 h of birth and then were assigned treatments. Calves were given 1 d to adapt to treatments. The study was divided into two for the last 24 h of each period. Blood samples were collected at the beginning and end of each period and analyzed for plasma glucose and urea N concentrations. All data reported as HPN vs LPN, respectively. Fecal scores were greater (2.01 vs 1.52 ± 0.13;  $P = 0.004$ ) for HPN calves during both periods; however, there was no difference (30.9 vs 31.9 ± 0.6%;  $P \geq 0.253$ ) in the DM percentage of feces. The HPN calves had greater ADG over the entire study (0.211 vs -0.106 ± 0.6 kg/d;  $P < 0.001$ ). There were no differences ( $P \geq 0.239$ ) between treatments in either digestible or metabolizable energy efficiencies, which averaged 92.8 and 83.1%, respectively. There was a treatment × period interaction ( $P = 0.038$ ) on the percentage of intake N retained, whereas calves fed the HPN had a greater N retention during period 1 (88.0 vs. 78.7 ± 1.79%;  $P = 0.004$ ), but was not different from calves fed the LPN during period 2 (85.3 vs. 85.0 ± 1.79%;  $P = 0.904$ ). Therefore, these data indicate that healthy Jersey calves have the capability to digest and absorb the additional nutrients when fed a higher plane of nutrition during the first week of postnatal life. Further, the additional energy and amino N absorbed by calves fed the HPN were incorporated into lean tissue growth at a high efficiency.

**Key Words:** calf, digestibility, nutrition

**W331 Effects of glucose and propionate infusions on milk fat yield: A meta-analysis.** Sarah E. Schmidt\* and Adam L. Lock, Michigan State University, East Lansing, MI.

Milk fat depression (MFD) research has primarily focused on inhibitory effects of rumen biohydrogenation intermediates, particularly *trans*-10,*cis*-12 18:2 (CLA), on fat synthesis in the mammary gland. However, several studies have shown that directly increasing insulin-secretagogues through infusion also results in MFD. The objective of this meta-analysis was to evaluate the effects of glucose and propionate infusions on milk fat yield and energy balance. The analysis included 22 publications representing 27 glucose infusion treatments and 15 propionate infusion treatments. Trial-adjusted values were calculated in a linear mixed model with study included as a random variable and weighted by the inverse of the variance of daily milk yield. Trial-adjusted values for percent change in milk fat yield were regressed across levels of glucose (0.33 to 2.40 kg/d) and propionate (0.25 to 1.52 kg/d) infusion and lines

of best fit were applied ( $r^2 = 0.76$ ;  $r^2 = 0.65$ ). Change in milk fat yield ranged from 1.69% to -20.3% for glucose infusions and from 6.78% to -23.8% for propionate infusions. Increasing glucose and propionate infusion amount increased the magnitude of MFD ( $P < 0.01$ ;  $P < 0.01$ ). Cows in each glucose infusion study were categorized as having high (HF; milk fat >4.09%), moderate (MF; milk fat ≥3.58% and ≤4.09%), or low (LF; milk fat <3.58%) milk fat concentrations. Increasing glucose infusion amount decreased milk fat yield of HF cows at a greater rate compared with LF cows ( $P = 0.03$ ). MF cows also exhibited a greater response to glucose infusion compared with LF cows ( $P < 0.01$ ). Trial-adjusted values for change in energy balance were regressed on percent change in milk fat yield following glucose infusion and a line of best fit was applied ( $r^2 = 0.74$ ). Reducing milk fat yield from 1.69 to -20.3% through glucose infusion increased energy balance (-1.3 to 4.8 Mcal/d;  $P < 0.01$ ). While CLA has been shown to cause MFD, the effects of insulin-secretagogues on energy partitioning should also be considered in the development of a comprehensive model.

**Key Words:** energy balance, meta-analysis, milk fat depression

**W332 Effect of the starch level in diets with soybean or canola meal on the performance of lactating dairy cows.** Juan I. Sanchez-Durte\*<sup>1</sup>, Kenneth F. Kalscheur<sup>2</sup>, and David P. Casper<sup>1</sup>,

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This study was designed to test the impact of reducing corn grain starch with nonforage fiber sources in diets using either soybean meal or canola meal as the primary protein source. Sixteen Holstein cows were assigned to a 4 × 4 Latin square design with 4 periods of 28 d. Treatments were arranged as a 2 × 2 factorial with 2 protein sources [soybean meal (SBM) and canola meal (CM)] and 2 starch levels (21 and 27% of DM). Diets were formulated to contain 16.5% CP and the starch levels were achieved by replacing corn grain with soybean hulls and beet pulp. Protein source × starch interactions ( $P < 0.05$ ) were observed for DMI, feed efficiency (ECM/DMI), fat %, protein %, protein yield, and MUN. Cows fed the 27% starch diet consumed more DMI than cows fed the CM-21% diet. However, there was no interaction for milk yield, as cows fed 27% starch produced 2.5 kg/d more than cows fed 21% starch. Milk fat percentage was the least for cows fed CM-27% and greatest for cows fed SBM-27% and CM-21%. Milk protein percentage and yield was least for CM-21% compared with the other 3 diets. Milk urea nitrogen was least for cows fed CM-27% compared with the other 3 diets. Cows fed 27% starch produced 1.9 kg/d more energy-corrected milk (ECM) than cows fed 21% starch. Feed efficiency was the greatest for cows fed CM-21% and least for cows fed CM-27%. Overall, lower starch % in SBM or CM diets negatively affected DMI, milk yield, ECM, and milk protein percentage and yield, but not feed efficiency and milk fat percentage and yield. Increasing starch level in canola meal diets decreased milk fat percentage, but potentially improved protein balance because less MUN was observed.

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**Table 1 (Abstr. W332).**

Item	SBM		CM		SEM	<i>P</i> > <i>F</i> <sup>1</sup>
	21%	27%	21%	27%		
DMI, kg/d	26.2 <sup>ab</sup>	26.8 <sup>a</sup>	24.7 <sup>b</sup>	27.7 <sup>a</sup>	0.90	I
Milk, kg/d	36.5	38.5	36.6	39.7	1.30	S
ECM, kg/d	39.8	41.8	40.0	41.8	1.23	S
ECM/DMI	1.53 <sup>ab</sup>	1.57 <sup>ab</sup>	1.66 <sup>a</sup>	1.53 <sup>b</sup>	0.06	I
Fat, %	4.08 <sup>ab</sup>	4.14 <sup>a</sup>	4.21 <sup>a</sup>	3.86 <sup>b</sup>	0.16	I
Fat, kg/d	1.49	1.57	1.53	1.51	0.06	NS
Protein, %	3.28 <sup>a</sup>	3.26 <sup>a</sup>	3.15 <sup>b</sup>	3.26 <sup>a</sup>	0.06	I
Protein, kg/d	1.20 <sup>ab</sup>	1.24 <sup>a</sup>	1.15 <sup>b</sup>	1.28 <sup>a</sup>	0.04	I
MUN, mg/dL	12.8 <sup>a</sup>	12.4 <sup>a</sup>	12.6 <sup>a</sup>	11.2 <sup>b</sup>	0.53	I

<sup>ab</sup>Means with different letters differ (*P* < 0.05).

<sup>1</sup>S = starch effect; I = protein source by starch effect; NS = no significant.

**Key Words:** protein, starch, milk production

**W333 The effect of the supplementation of virginiamycin plus monensin on milk performance under grazing conditions in dairy cattle.** Ramiro Desantadina<sup>1</sup>, Luis Casares<sup>2</sup>, Matias Bailleres<sup>3</sup>, Milton Gorocica<sup>2</sup>, and Alejandro Relling<sup>\*1,4</sup>, <sup>1</sup>Fac Cs Veterinarias, UNLP, Argentina, <sup>2</sup>Phibro Animal Health, Argentina, <sup>3</sup>Ministerio de asuntos Agrarios, Buenos Aires, Argentina, <sup>4</sup>IGEVET, CCT La Plata, CONICET, Argentina.

The aim of the present study was to evaluate the effect of virginiamycin (Vm) plus monensin (Mn) on milk production and composition of grazing dairy cows. Eighty dairy cows, with an average of 2.5 lactations, 129 d in milk and a milk yield (MY) of 27.7 kg per day were randomly distributed into 2 groups; Group 1 (n = 40): 300 mg/cow/d Mn (M), and Group 2 (n = 40): 300 mg/cow/d Vm and 300 mg/cow/d Mn (V). Cows were grazed in a rotational grazing system of 2 grass paddocks per day. The cows were milked twice a day and received 4 kg of a pelleted concentrate and mineral supplement in the parlor at each milking. Both groups were also fed once a d 7 kg corn silage, 6 kg grass haylage, 2 kg corn grain, and 2 kg wheat middlings (all quantities as fed). Milk yield and composition were measured on d 0, 17, 34, 52, 66, 78, 90, 101, 111, 120 and 129, and body condition score (BCS) was measured on d 0, 52 and 129. Four-percent fat corrected milk (FCM) was estimated using the following formula  $MY \times (0.383 \times \% \text{ fat} + 0.242 \times \% \text{ protein} + 0.7832) / 3.1138$ . Data were analyzed using a mixed model with repeated measures, using the cow as random effect, and treatment, time and their interaction as fixed effects. The treatment  $\times$  time interaction was not significant in all evaluated variables (Table 1). However, on FCM a trend for time and treatment interaction (*P* = 0.14) was approached: V cows had greater FCM on d 52, 78 (*P* < 0.1) and 101 (*P* < 0.05) than M cows. The V treatment group had numerically greater MY, milk fat and protein content, and BCS at the end of the trial, but differences were not statistically significant (Table 1; all *P* > 0.15). In conclusion, the addition of Vm in diets containing Mn increases FCM during peak- to mid-lactation.

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**Table 1 (Abstr. W333).** Milk yield, composition, and FCM in grazing cows supplemented with Mon (M) and Mon+Vm (V)

Item	Treatment			<i>P</i> -value	
	M	V	Trt	Time	Trt $\times$ Time
MY, kg/d	27.6	28.3	0.46	<0.01	0.64
Milk fat, %	3.34	3.46	0.15	<0.01	0.47
Milk protein, %	3.21	3.24	0.26	<0.01	0.72
FCM, kg/d	25.0	26.1	0.14	<0.01	0.14
d 0 FCM	27.1	26.8	0.80		
d 34 FCM	25.8	26.8	0.33		
d 52 FCM	27.4	25.7	0.06		
d 78 FCM	28.3	26.5	0.07		
d 101 FCM	26.7	24.6	0.04		

**Key Words:** dairy cow, virginiamycin, monensin

**W334 Undegradable aNDFom in non-forage feeds.** Alessandro Maria Zontini\*, Andreas Foskolos, Deborah Ross, and Michael Van Amburgh, Cornell University, Ithaca, NY.

Non-forage fiber feeds are generally highly digestible, however, most calculations of undegradability are made from static calculations based on Chandler et al. (1980) or Weiss et al. (1992). The objective of this study was to analyze the extent of aNDFom degradation in non-forage fiber feeds, to obtain the undegradable aNDFom (uNDF). Samples of 12 feeds (citrus pulp, beet pulp, wheat middlings, soy hulls, corn gluten meal, corn gluten feed, wheat distillers, corn ethanol distillers, flaked corn, rice hulls, soybean meal, and canola meal) were collected, each from 2 providers, and analyzed in duplicate in 3 separate batches for the extent of NDF digestion using the in vitro technique. Samples, 0.5 g, were weighed into Erlenmeyer flasks and 40 ml of Goering and Van Soest (1970) buffer was added to each flask under continuous CO<sub>2</sub>, and incubated in a water bath at 39°C. After 2 h of incubation, 10 mL of mixed rumen fluid from 2 lactating cattle were added to each flask and continuous CO<sub>2</sub> was maintained throughout the fermentation. Fermentations were conducted for 96, 120, and 240 h consistent with previous data from Raffrenato (2011). Residues were filtered on a glass microfiber filter (934-AH, Whataman) with a 1.5 μm pore size to enhance residue recovery. To analyze changes in uNDF the residues were compared with a *t*-test in JMP. For non-forage aNDFom the uNDF was obtained at 120h with the exception of citrus pulp where residues continued to digest out to 240 h compared with 120 h residues (*P*-value = 0.002).

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**Table 1 (Abstr. W334).** The aNDFom (%NDF) residues of feeds after 96, 120, and 240 h of fermentation

Item	Time (h)			SEM	P-value
	96	120	240		
Beet Pulp	22 <sup>a</sup>	19 <sup>b</sup>	17 <sup>b</sup>	0.01	0.004
Canola Meal	40	41	41	0.01	0.79
Citrus Pulp	21 <sup>a</sup>	20 <sup>a</sup>	16 <sup>b</sup>	0.01	0.002
Corn Gluten Feed	16 <sup>a</sup>	14 <sup>ab</sup>	13 <sup>b</sup>	0.01	0.028
Corn Distiller	16	16	14	0.01	0.50
Corn Germ	34	29	27	0.03	0.74
Flaked Corn	14	14	12	0.02	0.73
Rice Hulls	94	93	93	0.01	0.61
Soybean Meal	11	9	9	0.01	0.95
Soy Hulls	10 <sup>a</sup>	9 <sup>ab</sup>	8 <sup>b</sup>	0.01	0.022
Wheat Distiller	28	26	25	0.01	0.20
Wheat Middling	36 <sup>a</sup>	31 <sup>b</sup>	30 <sup>b</sup>	0.01	0.001

<sup>a,b</sup>Values with different letters are statistically different.

**Key Words:** aNDFom

**W335 Effect of two fat supplements differing in saturation on milk production and energy partitioning.** Enhong Liu\*, Courtney L. Preseault, Michael J. VandeHaar, and Adam L. Lock, *Michigan State University, East Lansing, MI.*

Effects of feeding diets containing fat supplements differing in saturation on milk production and energy partitioning were evaluated. Holstein cows ( $n = 32$ ;  $93 \pm 35$  DIM) were randomly assigned to treatment sequence in a crossover design experiment. Treatments were diets containing a saturated fat supplement (2.5% DM palmitic acid-enriched triglyceride [BergaFat T-300], SF) or an unsaturated fat supplement (2.5% DM soybean oil, UF). Diets utilized corn silage and alfalfa silage as forage sources and contained 25% NDF, 18% forage NDF, 32% starch, 18% CP, and 4.6% FA. Treatment periods were 28 d in length with the final 5 d used for sample and data collection. The statistical model included the random effect of cow and fixed effects of treatment and period. Compared with UF, SF increased milk fat concentration (3.07% vs. 2.42%;  $P < 0.01$ ) and yield (1.35 vs. 1.11 kg/d;  $P < 0.01$ ), but reduced milk protein concentration (3.05% vs. 3.12%;  $P < 0.01$ ) and yield (1.40 vs. 1.44 kg/d;  $P < 0.05$ ). Treatment did not alter milk yield (46 kg/d;  $P = 0.6$ ), but SF did increase FCM (41.9 vs. 38.1 kg/d;  $P < 0.01$ ) and ECM (42.6 vs. 39.8 kg/d;  $P < 0.01$ ) compared with UF. DMI and energy intake did not differ between treatments and averaged 25 kg/d and 41.2 Mcal/d, respectively (both  $P > 0.6$ ). However, SF increased the milk to feed ratio (ECM/DMI) compared with UF (1.67 vs. 1.53;  $P < 0.01$ ). Compared with UF, SF reduced BW gain (5.2 vs. 12.8 kg/28 d;  $P < 0.05$ ) but did not alter BCS ( $P = 0.8$ ) or fat thickness over the rump ( $P = 0.7$ ) and rib ( $P = 0.5$ ). SF decreased plasma concentration of insulin (1.18 vs. 1.34  $\mu\text{g/L}$ ,  $P < 0.05$ ), NEFA (122 vs. 137  $\mu\text{Eq/L}$ ,  $P < 0.01$ ), and triglycerides (7.9 vs. 8.5 mg/dL,  $P = 0.05$ ) compared with UF. There was no effect of treatment on plasma concentration of glucose ( $P = 0.3$ ). Compared with UF, SF increased plasma milk energy as a fraction of  $\text{NE}_L$  use (71 vs. 66%,  $P < 0.01$ ) and reduced calculated body energy gain as a fraction of  $\text{NE}_L$  use (3 vs. 8%,  $P < 0.01$ ). In conclusion, the 2 diets resulted in similar  $\text{NE}_L$  intake but the SF diet containing the palmitic acid-enriched triglyceride increased milk fat yield and partitioned more energy toward milk, while the UF diet containing soybean oil reduced milk fat yield and partitioned more energy toward body gain.

**Key Words:** fat supplementation, milk fat, palmitic acid

**W336 Nutrient intake and blood parameters of dairy cows fed sugarcane in different ways of storage.** Viviane B. Ferrari<sup>\*1</sup>, Mauro D. S. Oliveira<sup>2</sup>, and Francisco P. Rennó<sup>1</sup>, <sup>1</sup>University of São Paulo, Pirassununga, São Paulo, Brazil, <sup>2</sup>São Paulo State University, Jaboticabal, São Paulo, Brazil.

This study aimed to evaluate nutrient intake and blood parameters of Holstein cows fed sugarcane based diets. Treatments consisted of fresh chopped sugarcane (FS), hydrolyzed sugarcane (HS) and HS + 6 g live yeast (*Saccharomyces cerevisiae*) (HSY). All treatments contained concentrate with ground corn, soybean meal, cottonseed meal, urea, mineral salt. Roughage:concentrate ratio offered was 60:40. Twelve Holstein cows with body weight of 590 kg were assigned into 4 contemporary  $3 \times 3$  Latin Squares, with 3 periods of 21 d each and 3 treatments. The sugarcane used was IAC 862480 fifth cut and 12 mo of growth, chopped in particle size of 8 mm. The sugarcane was hydrolyzed with 0.5% of hydrated lime (95% of calcium hydroxide) using 0.5 kg of hydrated lime with 2 L of water to each 100 kg of sugarcane. Intake was regulated for animal by daily weighting of diet and orts, allowing for 5 to 10% of orts. Samples of feedstuffs and orts were weekly collected for chemical analyses and determination of dry matter (DMI), neutral detergent fiber (NDFI), crude protein (CPI) and ether extract (EEI) intakes. Blood samples were collected by jugular vein before the first meal for blood parameters determination: glucose (GLU), total protein (TP), total cholesterol (TC), albumin (AL), gamma-glutamyl transferase (GGT), aspartate aminotransferase (AST). Two contrasts were used to test the effect of FS vs. HS and HSY (C1); and HS vs. HSY (C2). Differences between treatments were considered significant at  $P < 0.05$ . FS promoted differences in all nutrient intakes. FS decreased DMI (11.43, 14.78 and 14.38 kg/d;  $P = 0.03$ ); NDFI (5.78, 7.95 and 7.09 kg/d,  $P = 0.02$ ); CPI (1.06, 1.32 and 1.39 kg/d;  $P = 0.03$ ), EEI (0.48, 0.60 and 0.62 kg/d;  $P = 0.03$ ), compared with HS and HSY, respectively. There were no differences for HS vs. HSY on nutrient intakes. There were no differences for none of the studied blood parameters ( $P > 0.05$ ). Fresh chopped sugarcane decreased nutrient intake of Holstein cows compared with hydrolyzed sugarcane.

**Key Words:** crude protein, hydrolysis, neutral detergent fiber

**W337 The effect of increasing concentrations of DL-methionine and HMB on hepatic genes controlling methionine and glucose metabolism.** Dean A. Bowen<sup>\*1</sup>, Nestor D. Luchini<sup>2</sup>, and Heather M. White<sup>1</sup>, <sup>1</sup>University of Wisconsin-Madison, Madison, WI, <sup>2</sup>Adisseo, Alpharetta, GA.

Metabolizable methionine (met) concentrations can be increased by feeding rumen-protected DL-Met (DL) or the isopropyl ester of 2-hydroxy-4-(methylthio)butanoic acid (HMB); however, hepatic response to these compounds have not been comparatively examined. The objective of this experiment was to examine the regulation of key genes in the Met and gluconeogenic pathways in response to doses of DL or HMB that mimic Met deficiency, adequacy, or supplementation in lactating dairy cows. Hepatocytes were isolated from 4 Holstein calves and were maintained as monolayer cultures in FBS for 24h before treatment. Treatments of DL or HMB (0, 10, 20, 40, 60 mM) were added to Met-free media in triplicate and after 24h, cells were collected for RNA isolation and quantification of gene expression by quantitative PCR. Data were analyzed in Proc Mixed of SAS 9.3. Analysis of covariance confirmed equivalent slopes of Met source and final model included source and dose, and random effect of calf within source. There was no main effect of Met source ( $P > 0.1$ ) for any genes examined. Expression of BHMT and MTR, genes that catalyze generation of Met from betaine and homocysteine, decreased ( $P \leq 0.05$ ) with

increasing Met concentration (BHMT: 0.7551, 0.7205, 0.6512, 0.582 ± 0.2563; MTR: 0.8897, 0.8481, 0.7648, 0.6815 ± 0.1763 arbitrary units). When concentrations of Met are high, Met can generate SAM, a methyl donor; however, expression of the gene that catalyzes this reaction, MAT1, was decreased ( $P \leq 0.05$ ) as Met concentration increased (0.7828, 0.7108, 0.5667, 0.4226 ± 0.1554 arbitrary units). Expression of CPT1a, G6Pase, and PEPCKm was not altered ( $P > 0.1$ ) suggesting that increased Met was not used as a gluconeogenic precursor at these concentrations. The decrease in hepatocyte production of Met at higher treatment doses suggest that cellular Met requirements were being met. The lack of influence on gluconeogenic enzymes and the decrease in the enzyme responsible for SAM generation may reflect metabolic priority for Met use at these concentrations, an adequate glucose concentration, or the lack of a Met deficiency before treatment.

**Key Words:** methionine, 2-hydroxy-4-(methylthio)butanoic acid (HMB), hepatocyte

### **W338 aNDFom degradation behavior in nonforage feeds.**

Alessandro Maria Zontini\*, Andreas Foskolos, Deborah A. Ross, and Michael E. Van Amburgh, *Cornell University, Ithaca, NY.*

Previous work conducted in our lab demonstrated that the extent of NDF degradation in nonforage fiber feeds is achieved by 120 h and results in undigested NDF (uNDF). Further, Raffrenato (2011) demonstrated that aNDFom degradation kinetic can be characterized with a dynamic model utilizing 4 data points (0, 30, 120 and 240 h). The objectives of this study were to characterize the degradation behavior of aNDFom, and to determine the time points required to model and calculate the degradation parameters, in nonforage fiber feeds. Samples of 12 feeds (citrus pulp, beet pulp, wheat middlings, soy hulls, corn germ, corn gluten feed, wheat distillers, corn ethanol distillers, flaked corn, rice hulls, soybean meal, and canola meal) were collected, each from 2 providers, and analyzed in duplicates and in 3 separate batches for NDF digestion kinetics using the in vitro technique. Fermentations were conducted for up to 3, 6, 9, 12, 15, 18, 21, 24, 30, 48, 72, 96, 120 h following the methods of Goering and Van Soest (1970). Residues were filtered on a glass microfiber filter (934-AH, Whatman). To study the digestion behavior of non-forage fiber NDF, the degradation curves were plotted on semi-log scale, and analyzed to determine the inflection point of the aNDFom residues. Overall, the natural log-transformation, demonstrated that NDF digestion in nonforage fiber feeds can be described by one degradable pool (no inflection points). To determine the time points, 7 combinations of 4 data points corresponding to: 0 h, 2 intermediate time points, and uNDF were used as inputs. The corresponding slope and intercept of each regression (observed on predicted) were then compared. The accuracy of the model did not allow for detecting statistically significant differences in slope and intercept among time point combinations, however 0, 12, 72, and 120 h were the time points that provided model predictions with an average slope and intercept closest to unity and zero respectively (intercept = 0.04, slope = 0.95). Thus, for routine determination of non-forage fiber digestibility and the estimation of rates of degradation for use in nutrition models, these time points provided adequate information among the feeds analyzed.

**Key Words:** aNDFom

**W339 Can potential digestible fiber affect dietary crude protein level in lactating dairy cows? Milk production and feeding behavior.** H. R. Mirzaei Alamouti\* and B. Mohtashami, *Department of Animal Science, University of Zanjan, Zanjan, Iran.*

Nitrogen (N) excretion from dairy farming systems is a major environmental challenge. An experiment was conducted to determine the effects of dietary potential digestible fiber (pdF) and crude protein (CP) levels in mid lactating Holstein cows on milk production and content, feeding behavior and nitrogen efficiency. Sixteen primiparous ( $n = 8$ ) and multiparous ( $n = 8$ ) cows (BW = kg and DIM = 118 ± 50 d) were allocated to 1 of 4 diets in balanced randomized complete block design in 2 periods (28 d). The diet containing: 1) 19% CP and low pdF, (HPLDF), 2) 17% CP and low pdF, (MPLDF), 3) 15% CP and high pdF, (LPHDF), and 4) 17% CP and high pdF, (MPHDF). The diets were balance for pdF by using different levels of dried alfalfa hay and corn silage in forage proportion of diets. Cows were fed individually and daily dry matter intake was recorded. Daily milk production and bi-weekly milk content were determined. Feeding behavior was recorded in 24 h with exception in milking times. Data were analyzed as repeated measurement with proc MIXED by using SAS software. The diets, periods, times and 2 or 3 ways interactions were constant effects and cows nested in diet and residual errors were random effects. Cows fed the diets with high pdF had greater DMI (17.87, 17.46, 18.44, and 18.60 kg/d, for HPLDF, MPLDF, LPHDF, and MPHDF respectively) and lower (21.8, 17.56, 12.86, and 15.78 for HPLDF, MPLDF, LPHDF, and MPHDF respectively) milk urea nitrogen ( $P < 0.01$ ) than cows fed low pdF diets. Milk Production and content and blood metabolites were not significantly different among the experimental diets. There was no significant difference among diets in parities and periods. There was significant difference ( $P < 0.05$ ) among diets in eating activity and cows fed to high pdF diets had greater eating activity (246.85 vs 261.2 min/d for high pdF vs low pdF). Results from this study indicate that increasing cp content in diets with low pdF and also increasing pdF content of diets with mid or low CP content can improve mid lactating Holstein cows' milk production and efficiency.

**Key Words:** potential digestible fiber, nitrogen efficiency, milk production

**W340 Effects of prill size of a palmitic acid-enriched fat supplement on yield of milk and milk components and nutrient digestibility of dairy cows.** Jonas De Souza\*, Joshua L. Garver, Courtney L. Preseault, and Adam L. Lock, *Michigan State University, East Lansing, MI.*

Effects of prill size of a palmitic acid-enriched fat supplement (PA; 85% C16:0) on feed intake, nutrient digestibility, and production responses of dairy cows were evaluated. Twenty-four Holstein cows (120 ± 44 DIM) were randomly assigned to treatment sequence in a 4 × 4 Latin square design. Treatments were a control diet (CON; no added PA), or 2.0% PA added either as a small prill size (SM; 284 ± 12.4 μm), medium prill size (MD; 325 ± 14.7 μm), or large prill size (LG; 600 ± 17.8 μm) supplement. PA replaced soyhulls in the ration and diets contained 21% forage NDF, 16% CP and 26% starch. Periods were 21 d in length with the final 5 d used for sample and data collection. Indigestible NDF was used as an internal marker to estimate fecal output and nutrient digestibility. The statistical model included the random effect of cow and period and the fixed effect of treatment. Overall, PA increased milk fat content (4.25 vs. 3.99%;  $P < 0.01$ ), milk fat yield (1.48 vs. 1.39 kg/d;  $P < 0.01$ ), tended to increase 3.5% FCM (39.2 vs. 37.7 kg/d;  $P = 0.10$ ), and improved feed efficiency (FCM/DMI; 1.51 vs. 1.42;  $P < 0.01$ ). However, compared with CON, PA treatments did not affect DMI, body condition score, or yields of milk, protein, and lactose. Overall, PA increased NDF digestibility (45 vs. 42%;  $P < 0.01$ ) and reduced the digestibility of 16-carbon FA (72 vs. 79%;  $P < 0.01$ ) and total FA (77 vs. 80%;  $P < 0.01$ ). Compared with CON, PA reduced the yields of de novo-synthesized (<16-carbon) milk FA (251 vs. 297 g/d;  $P < 0.01$ )



and preformed (>16-carbon) milk FA (383 vs. 430 g/d;  $P < 0.01$ ), and increased the yield of 16-carbon milk FA (457 vs. 378 g/d;  $P < 0.01$ ). There was no effect of prill size of PA on DMI, yield of milk and milk components, or feed efficiency. However, LG increased 16-carbon FA digestibility compared with MD (74 vs. 71%;  $P < 0.05$ ) and SM (74 vs. 72%;  $P < 0.05$ ). Additionally, LG increased total FA digestibility compared with MD and SM (78, 76 and 76%, respectively;  $P < 0.05$ ). These results demonstrate that PA increased milk fat concentration and yield and feed efficiency. Although prill size affected FA digestibility, this had no effect on animal performance.

**Key Words:** fatty acid digestibility, feed efficiency, milk fat

**W341 Changes in dairy cattle performance due to addition of a live yeast product.** Heidi A. Rossow<sup>\*1</sup>, Tim Riordan<sup>2</sup>, Andy Riordan<sup>2</sup>, Dennis Ervin<sup>3</sup>, and Dari Brown<sup>3</sup>, <sup>1</sup>University of California, Davis, Davis, CA, <sup>2</sup>Nutri-Systems Inc., Clovis, CA, <sup>3</sup>Phileo Lesaffre Animal Care, Milwaukee, WI.

Live yeast products have been proposed to increase nutrient utilization and milk production. Therefore the purpose of this trial was to determine if a live yeast product (LYP; BioCell 20, Phileo Lesaffre Animal Care, Milwaukee, WI) administered at 3g/cow/d to cows at a commercial dairy could increase milk yield and milk component production. All milking cows were milked 2 times a day and given LYP in the TMR in an OFF, ON, OFF, ON, OFF design where each ON or OFF period lasted 45 d and LYP was added to TMR during the ON periods only. Only data from cows that had at least one Dairy Herd Improvement Association milk test during each period remained in the data analyses (1903). Pens were fed the same respective diet (Fresh cow, milk cow, milk heifer or pregnant cow TMR) twice per day. Statistics were performed using Proc GLM (SAS Institute, 2013). To compensate for differences in days in milk (DIM) and milk production due to a period effect, treatment was nested within period, repeated measures for cow and covariates pen, DIM, diet, relative value, milk yield during period 1 (control period) and fresh date were included in the statistical model. There were no differences in least squares means of parity and small differences in least squares means DIM among periods. Average DIM in periods 2–5 were not different ( $P < 0.05$ ; range 143–298 d). Overall, daily milk yield (31.8 and 32.9.0 kg,  $P < 0.0001$ ) and milk protein (1.02 and 1.08 kg,  $P < 0.0001$ ; 3.25 and 3.31%,  $P < 0.0001$ ) for control and LYP supplemented treatments, respectively, were higher for cows fed LYP. Therefore LYP did increase milk yield and milk protein kg and % but decreased milk fat % (3.71 and 3.60%,  $P < 0.0001$ ), respectively.

**Key Words:** live yeast, dairy cow performance

**W342 Effects of feeding frequency and adding plant oil to diet on performance and feeding behavior of lactating Holstein dairy cows.** H. R. Mirzaei Alamouti<sup>\*</sup> and K. Akbari, *Department of Animal Science, University of Zanjan, Zanjan, Iran.*

The objectives of this experiment were to investigate the effect of feeding frequency and oil supplementation to the diet on performance and feeding behavior of lactating dairy cows. Twenty 4 lactating Holstein cows, 12 primiparous, (BW = 626 ± 58 kg and DIM = 195 ± 44 d) and 12 multiparous, (BW = 617 ± 25 kg and DIM = 207 ± 39 d) were randomly assigned to the 4 diets contained 2.5 percent supplemental oil or no oil and feeding frequency of 3 or 1 time per day in a randomized complete block design with a 2 × 2 factorial arrangement. Frequency of feed delivery and oil supplementation had no effect on dry matter intake. Oil supplementation reduced slug feeding especially with feeding once per

day ( $P < 0.05$ ). Feeding frequency and oil supplementation interaction tended to significant for milk yield ( $P = 0.09$ ), as there was a numerical increase in milk yield in 3 time feeding with oil diet. The cows fed oil diet in 3 time feed delivery had lower milk protein ( $P < 0.05$ ), but milk fat increased with 1 time feeding and oil supplementation ( $P < 0.05$ ). The results showed that oil supplementation to mid lactating cows diet can decrease compromised effects of decreasing feeding frequency.

**Key Words:** feeding frequency, oil supplementation, chewing behavior

**W343 Effect of feeding hay during the nursery phase of calf rearing.** F. Xavier Suarez-Mena<sup>\*</sup>, James D. Quigley, T. Mark Hill, and Rick L. Schlotterbeck, *Nurture Research Center, Provimi North America, Brookville, OH.*

Recommendations on the value of feeding forage to calves before weaning are inconsistent. This study aimed to evaluate the effects of feeding long hay during the nursery phase on calf performance. Treatments were to not feed hay or feed hay to calves throughout the 56-d trial. Forty-eight calves (45.4 ± 1.18 kg BW, 2 to 3 d of age) were fed a milk replacer (MR; 22% CP, 20% fat as-fed basis) powder at 568 g as-fed daily diluted at 12.5% solids. Calves were fed MR daily in 2 equal meals from 0 to 39 d, then at 313 g of MR powder daily (AM meal only) for d 40 to 42. All MR offered was consumed. Starter, hay, and water were fed ad libitum. Hay and starter were fed in separate pails. Calves were housed in individual pens bedded with straw in a nursery with natural ventilation and no added heat through d 56. Feces were scored daily using a 1 to 5 scale with 1 being normal and 5 being watery. Medical treatments were recorded daily. Calves were weighed initially and weekly thereafter. Body condition score (1 being thin and 5 being obese) and hip widths were measured initially and every 2 weeks thereafter. The average temperature in the nursery was 23°C with a range from 12 to 36°C. The average relative humidity was 78% with a range from 40 to 97%. Data were analyzed as a completely randomized design. Repeated measures analysis was performed on pre-weaning (0 to 42 d), post-weaning (42 to 56 d), and overall (0 to 56 d) data. Initial measurements differed for hip widths ( $P < 0.05$ ) among treatments. Pre-weaning hip width change tended ( $P < 0.09$ ) to be less for calves fed hay. Other measurements did not differ ( $P > 0.05$ ). Hay intake was highly variable and averaged 6.1% of total intake overall. Hay intake as a percentage of overall intake ranged from less than 1 to 20% among the 24 calves fed hay. Hay intake was not related to starter intake. Feeding hay did not improve calf performance as measured in this study.

**Key Words:** starter intake, hay, performance

**W344 Effects of non-fiber carbohydrate level in low energy diets on production and health responses in peripartum Holstein cows.** H. R. Mirzaei Alamouti<sup>\*</sup> and P. Panahiha, *Department of Animal Science, University of Zanjan, Zanjan, Iran.*

Effects of rumen fermentability of carbohydrate and nonfiber carbohydrate during late gestation and early lactation on production and metabolic status of periparturient cows were studied. Sixteen multiparous and 40 primiparous cows were used in a randomized complete block design and assigned at random to 1 of 2 treatments in pre- and postpartum. The cows were fed diets as total mixed ration (TMR) with similar crude protein and energy (low energy diets) content, but different source of starch (ground barley vs. corn grain) from 20 ± 2 d relative to expected calving until calving. At parturition, half of the animals from each prepartum treatment were assigned to a lactation diet with 2 level of nonfiber carbohydrate until d 28 and, all cows received the same

high energy lactation diet until d 90. The cows fed prepartum diet had a positive energy balance during the prepartum period. Blood samples were taken at -20, +1 and +21 d relative to calving. Data were separately analyzed for multi- and primiparous cows by proc Mixed of SAS with diet and time as fixed effect and animal nested in diet as random effect. There was no significant difference in milk content except milk lactose in primiparous cows fed prepartum diets. Milk production was higher for primiparous cows fed barley diet in prepartum and high NFC diet in postpartum, and also corn diet in prepartum and low NFC in postpartum. Milk fat content for multiparous cows was different. There was significant difference between  $\beta$ -hydroxybutyrate, albumin and nonesterified fatty acid for primiparous cows and total protein, alanine amino acid transferase and BHBA for multiparous cows in postpartum. There were differences in some reproductive parameters. Generally, the responses of fresh cow to nonfiber carbohydrate content in lactating diet is depends on fermentability of starch in low energy close-up diet.

**Key Words:** transition period, nonfiber carbohydrate

#### W345 Increase in total solids of whole milk and its effects on development of dairy calves.

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Objectives were determine the best total solids (TS) concentration in liquid diet consisting of whole milk (WM) and milk replacer (MR) and its effects on preweaning calves feed intake and performance. Sixty crossbred Holstein-Gyr calves were distributed in 4 treatments, maintaining the balance for initial body weight and total protein concentration in blood serum and genetic composition. The treatments consisted of WM with the increasing addition of MR (Sprayfo Violet SSP), to adjust the TS to 12.50; 15.00; 17.50 and 20.00%, after the measurement the TS in WM using Brix refractometer (DD-3 MISCO Palm Abbe Digital), which had the degree brix value converted to TS (Moore et al., 2009). TS contents verified in the treatments after the addition of MR were 13.50; 16.10; 18.20 and 20.40%. The calves were fed 6 L/d, divided in 2 equal meals (8 and 16h) provided in buckets, from 5 to 55 d of age. From 56 to 59 d, the volume was reduced to 3 L/d (8h). At 60 d the animals were weaned. Starter (Soylac Rumen 20% CP) and water were provided ad libitum throughout the experiment. Feed intake, feed efficiency, average daily gain (ADG), structural body measures and days of diarrhea were measured. Data were analyzed by ANOVA using the PROC GLM procedure of SAS. Increasing TS resulted in a linear increase in water intake, total DMI, ADG, withers height and hearth girth. Starter intake, days of diarrhea, hip width and feed efficiency did not change as the TS increased up to 20.4%.

**Key Words:** milk system, milk replacer

#### W346 Increase in total solids of whole milk and its effects on development in postweaning calves.

Rafael Alves de Azevedo\*<sup>1</sup>, Pâmela Michéli Furini<sup>1</sup>, Sâmara Raiany de Almeida Rufino<sup>1</sup>, Fernanda Samarini Machado<sup>2</sup>, Mariana Magalhães Campos<sup>2</sup>, Aloma Eitere Leão<sup>1</sup>, Paulo Campos Martins<sup>1</sup>, Ângela Maria Quintão Lana<sup>1</sup>, and Sandra Gesteira Coelho<sup>1</sup>, <sup>1</sup>Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil, <sup>2</sup>EMBRAPA Dairy Cattle, Coronel Pacheco, Minas Gerais, Brazil.

This study aimed to evaluate the effects of intensive whole milk feeding in calves on postweaning feed intake and performance. Sixty crossbred Holstein-Gyr calves were distributed in 4 treatments until the weaning. The treatments consisted of WM with the increasing addition of MR (Sprayfo Violet SSP), to adjust the TS to 12.5; 15.0; 17.5 and 20.0%, after the measurement the TS in WM using Brix refractometer (DD-3 MISCO Palm Abbe Digital), which had the degree brix value converted to TS (Moore et al., 2009). The calves were fed 6 L/d, divided in 2 equal meals (8 and 16h) provided in buckets, from 5 to 55 d of age. From 56 to 59 d, the volume was reduced to 3 L/d (8h). At 60 d the animals were weaned. Starter (Soylac Rumen 20% CP) and water were provided ad libitum throughout the experiment. Corn silage was provided ad libitum to calves from 70 d of age. Feed intake, average daily gain (ADG) and structural body measures were measured in postweaning calves. Data were analyzed by ANOVA using the PROC GLM procedure in SAS. Results are presented in Table 1. Starter, corn silage and water intakes and ADG in postweaning calves did not change as the TS increased up to 20.4%. Increasing TS resulted in a tendency of linear increase in withers height and final body weight.

**Table 1 (Abstr. W346).** Feed intake and performance of postweaning calves fed liquid diet with increasing total solid content during preweaning period

Item	Treatment, % TS				SEM	P-value
	13.5	16.1	18.2	20.4		
Starter intake, g DM/d	2088.91	2073.93	2140.40	2230.93	53.13	0.69
Corn silage intake, g DM/d	201.1	170.4	188.7	201.2	12.33	0.79
Water intake, kg/d	8.4	8.1	9.1	8.9	0.20	0.27
ADG, g/d	999.57	976.77	971.75	1010.23	25.44	0.94
Withers height, cm	91.9	92.5	93.5	94.0	0.40	0.08
Final body weight, kg	101.5	104.9	107.0	110.7	1.60	0.07

**Key Words:** milk system, milk replacer

#### W347 Effect of dietary starch content on the occurrence of subacute ruminal acidosis (SARA) and inflammation in fresh dairy cows.

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Multiparous ruminally cannulated Holstein cows (n = 16) were fed either a lower starch [21% starch, 37% neutral detergent fiber (NDF)] or higher starch (27% starch, 32% NDF) diet from 1 to 21 d in milk (DIM) to assess the effect of dietary starch on rumen fermentation, rumination, energy metabolites, and inflammatory markers. Diets contained 28% corn silage, 22% haycrop silage, 2% straw, and 48% concentrate. Corn meal was replaced partially with soyhulls and wheat middlings in the lower starch diet. Cows were fed a close-up diet (16% starch, 44% NDF) for 21 d. Cows were housed in a bedded pack, fed individually, and milked 3x/d. Rumen pH was measured at 1-min intervals and rumination was measured continuously from -14 to 21 DIM. Rumen and blood samples were collected on -14, 1, 2, 3, 4, 5, 6, 7, 9, 13, 17, and 21 DIM at 0 and 6 h post feeding. Data were analyzed as a completely randomized design by ANOVA with the MIXED procedure of SAS with model effects of treatment, time, treatment x time, and covariate (data from -14 to -8 DIM). Cows averaged 20.4 ± 0.7 kg dry matter intake/d and 42.1 ± 1.9 kg milk/d with 4.6 ± 0.2% fat and 3.5 ± 0.1% true protein. Daily mean rumen pH was higher (6.19 vs. 6.04, SE = 0.05, P = 0.03), time with pH < 5.8 was lower (1.6 vs. 4.5 h/d, SE = 0.7, P < 0.01), and rumen ammonia was higher (10.1 vs. 7.1 mg/dL, SE = 0.6, P < 0.01) for cows fed the lower starch diet. Diet did not affect (P >

0.10) rumination ( $481 \pm 18$  min/d), rumen volatile fatty acids ( $115 \pm 3$  mM), or acetate to propionate ratio ( $3.23 \pm 0.13$ ). Cows fed the lower starch diet had lower ( $P < 0.10$ ) 6-h serum haptoglobin [ $0.10$  (95% CI  $0.06$ – $0.14$ ) vs.  $0.17$  (95% CI  $0.11$ – $0.27$ ) mg/mL] and serum amyloid A [ $22$  (95% CI  $12$ – $38$ ) vs.  $53$  (95% CI  $28$ – $101$ ) ug/mL]. Diet did not affect ( $P > 0.10$ ) 0-h nonesterified fatty acids ( $680 \pm 58$  uEq/L), 0 and 6-h  $\beta$ -hydroxybutyrate ( $1.0 \pm 0.2$  mM), 6-h interleukin (IL)  $-1\beta$  ( $57.3 \pm 5.1$  pg/mL), 6-h IL-6 ( $457 \pm 42$  pg/mL), or 6-h tumor necrosis factor- $\alpha$  ( $40.7 \pm 2.7$  pg/mL). The nutritional strategy that is used during the transition period is critical for minimizing the risk of SARA and controlling inflammation.

**Key Words:** transition cow, starch, acidosis

**W348 Physical and enzymatic hydrolysis characteristics of ruminal protozoal glycogen.** Mary Beth Hall\*, *US Dairy Forage Research Center, USDA-ARS, Madison, WI.*

The characteristics of microbial glycogen have not been well described, but have implications for its analysis and digestion characteristics. A series of analyses, and comparisons carried out as randomized complete block designs were performed on glycogen isolated from protozoa from ruminal inoculum incubated in vitro with glucose. The isolated protozoal glycogen (IPG) was in the form of water and 0.9% NaCl insoluble granules 1.3 to 1.9 mm in length. IPG was not obviously birefringent under polarized light.  $\alpha$ -Glucan was measured by AOAC Official Method 2014.10 as detected glucose  $\times 0.9$ .  $\alpha$ -Glucan contents of beef glycogen, wheat starch (WS), corn starch (CS), and IPG on a dry matter (DM) basis were 98.1, 99.5, 100.0, and 98.3%, respectively. Duplicate samples (each 50 mg in 5 mL water) were incubated at successively increasing temperatures (22, 37, 50, 57, 65, 70, 75, 80, and 85°C) for 30 min with vortexing every 5 min for 5 s. Gelatinization temperature was determined as the point at which transmittance % at 650 nm departed from baseline measurements of unheated samples. WS, CS, and IPG had gelatinization temperatures of 57, 65, and 65°C, respectively. Subsequent enzymatic hydrolysis of 0.2 mL of the sample suspensions was performed with addition of 1 mL 0.1 M Na acetate buffer (pH 5.0), and 4 U amyloglucosidase incubated for 2 h at 39°C. Digested  $\alpha$ -glucan as a percentage of initial CS, WS, and IPG were 8.3, 9.0, and 24.7% for ungelatinized vs. 100.0, 88.9, and 95.6% for gelatinized (85°C) samples, respectively. Effects of sample, gelatinization, and sample  $\times$  gelatinization all were  $P < 0.01$  (standard error of the difference; SED = 0.4). Incubation of ungelatinized suspensions of IPG for 24 h at 57°C with 0, 1.8, or 4 U amyloglucosidase with Na acetate buffer gave  $\alpha$ -glucan values of 0.03, 14.3, and 15.0% of DM, respectively ( $P < 0.01$ , SED = 0.06). Similar to native starch, gelatinization was required to achieve more extensive enzymatic hydrolysis of IPG. This suggests that protozoal glycogen digestion characteristics may be similar to those of native starch, and that IPG requires gelatinization for analysis involving enzymatic hydrolysis.

**Key Words:** rumen microbe, protozoa, glycogen

**W349 The determination of the concentrations of isoforms of vitamin E in tissues, milk and blood via HPLC after short-term feeding in dairy cows.** Y. Qu\*<sup>1</sup>, T. H. Elsasser<sup>2</sup>, J. R. Newbold<sup>3</sup>, E. E. Conner<sup>2</sup>, and K. M. Moyes<sup>1</sup>, <sup>1</sup>University of Maryland, College Park, MD, <sup>2</sup>Agricultural Research Service, US Department of Agriculture, Beltsville, MD, <sup>3</sup>Cargill Innovation Center, Velddriel, the Netherlands.

The objective of this study was to determine the pattern of change in the concentrations of the 4 isoforms of vitamin E ( $\alpha$ -,  $\beta$ -,  $\gamma$ - and

$\delta$ -tocopherol) in bovine tissues (liver, mammary and muscle), blood and milk after short-term feeding of a vegetable-derived oil (Tmix) particularly enriched with  $\gamma$ - and  $\delta$ -isoforms (9%  $\alpha$ -, 1%  $\beta$ -, 24%  $\delta$ - and 62%  $\gamma$ -tocopherol). Healthy multiparous Holstein cows (>90 DIM) were assigned to dietary control (Notoc,  $n = 4$ ) or tocopherol-fed treatment groups ( $n = 5$ : TOC; ~260g Tmix/cow/d, top-dressed) for 9 consecutive days. Milk and blood samples were collected in the mornings on d 0 and d 10 from the 5 TOC cows; tissues were harvested from those same cows on d 10 of treatment and the cohort of physiologically similar Notoc cows. Isoform concentrations were determined in samples by HPLC. Data were analyzed by Student's  $t$ -test and ANOVA. Delta-TOC was not detected in tissues, blood or milk. Concentrations of  $\gamma$ -TOC were increased ( $P < 0.001$ ) in tissues, blood and milk with feeding ( $P < 0.001$ ). In all tissues,  $\alpha$ -TOC concentrations were higher ( $P < 0.001$ ) than  $\gamma$ -TOC before and after feeding. Concentrations of both  $\alpha$ - and  $\gamma$ -TOC were higher in blood and milk after feeding Tmix ( $P < 0.05$ ). The content of  $\alpha$ -TOC was higher than  $\gamma$ -TOC ( $P < 0.001$ ) in milk and blood before and after feeding. In conclusion, short-term feeding of Tmix oil resulted in increases in the  $\gamma$ -isoform, largely no measurable content or significant change in the  $\beta$ - or  $\delta$ -isoforms, and a variable change in the  $\alpha$ -isoform of TOC across various tissues and biological fluids of the cow.

**Key Words:** concentration, cow, vitamin E

**W350 Total replacement with organic minerals regulates endometrial gene expression patterns that improve reproductive performance status in dairy heifers.** Daniel E. Graugnard\*, Allison C. Smith, Sylvie Andrieu, and Kristen M. Brennan, *Alltech Center for Animal Nutrigenomics and Applied Animal Nutrition, Nicholasville, KY.*

Reproductive performance in dairy cows is a major factor determining herd efficiency and profitability. The objective of this study was to evaluate the effect of dietary organic minerals on gene expression profiles of endometrial tissue. A total of 18 Holstein heifers (10–15 mo) were randomly assigned to 2 groups ( $n = 9$ /treatment) and supplemented for 90d with an organic premix (consisting of Bioplex Zn, Bioplex Cu, Bioplex Mn and EconomasE) or a standard inorganic premix (control). Endometrium was biopsied at d90 of supplementation and RNA was hybridized to the Affymetrix Bovine Genome Array. Hierarchical clustering analysis resulted in 2 different groups as a function of the pairwise distances corresponding to the treatments. Relative expression analysis between treatments resulted in a total of 580 differentially expressed genes ( $P < 0.05$ ; 398 upregulated; 182 downregulated), which enriched ( $P < 0.05$ ) different pathways related to reproductive performance: (1) Dendritic cell maturation, which regulates endometrium cell differentiation and the vascular responses associated with implantation; (2) Relaxin hormone signaling, which confers beneficial effects on the endometrium for implantation; (3) G $\alpha$ q signaling function of smooth muscle tissue like endometrium; and (4) Thrombin signaling, which promotes coagulation and the binding and aggregation of platelets and facilitating the rapid adherence of neutrophils, monocytes and lymphocytes to the endothelial cell layer to overcome exogenous challenge or play an essential role in early pregnancy. In conclusion, supplementation with organic minerals potentially benefits reproductive performance, including improved regulation of the estrous cycle, shorter calving intervals, and improved implantation rates.

**Key Words:** organic mineral, endometrium, cattle

**W351 Comparative bioavailability of lysine in three commercial rumen-protected lysine products using the in vivo plasma lysine response method.** Heather A. Tucker<sup>1</sup>, Makoto Miura<sup>2</sup>, Izuru Shinzato<sup>3</sup>, and Catherine S. Ballard<sup>\*1</sup>, <sup>1</sup>William H. Miner Agricultural Research Institute, Chazy, NY, <sup>2</sup>Ajinomoto Co., Inc., Kawasaki-ku, Kawasaki-shi, Japan, <sup>3</sup>Ajinomoto Heartland Inc., Chicago, IL.

Lysine bioavailability of second generation AjiPro-L (A2G; Ajinomoto Heartland, Inc.) has been determined using the plasma free amino acid dose response technique. The objective of this study was to compare bioavailability of MetaboLys (MBL; H. J. Baker & Bro., Inc.) and USA Lysine (USA; Kemin Industries Inc.) relative to A2G following the same technique. Fourteen multiparous lactating Holstein cows (113 ± 28 d in milk) were housed in a tie stall facility and used in a replicated 7 × 7 Latin square design with 7-d periods. Cows were blocked by days in milk and milk production, and assigned randomly to treatment sequence. A common basal diet adequate in Lys was fed proportionately at 3 intervals (33.4% at 0500 h, 33.3% at 1300 h, and 33.3% at 2100 h). Treatments included Lys supplemented at 0, 30, or 60 g/d from A2G, MBL, or USA and were administered 1 h before each feeding on d 2 through 7 of each period in amounts proportional to feed offered to simulate diet inclusion. Blood samples were obtained from each cow on d 6 and 7 of each period from the tail vein at 2-h intervals starting at 0600 h. Plasma, pooled by day, was analyzed for amino acid (AA) concentrations. Data were reduced to a period mean for each cow and analyzed using the MIXED procedure of SAS. REG procedure was used to generate linear regression models for each rumen-protected Lys product using the values of Lys (μmol) and Lys as a percentage of total AA (μmol-basis) to determine the degree of elevation of plasma Lys. Relative to A2G, estimated bioavailability of MBL and USA were determined using the slope ratio assay technique. Dry matter intake and milk yield ( $P > 0.10$ ) did not differ among treatments. The slope for A2G was numerically greater (0.183;  $r^2 = 0.72$ ) when compared with the slope for MBL (0.03;  $r^2 = 0.58$ ) or USA (0.07;  $r^2 = 0.99$ ) when expressing the concentration of plasma Lys as absolute value (μmol). Calculated bioavailability of MBL and USA was 18.3 and 38.2% of the bioavailability of A2G, respectively. Characterizing relative bioavailability of rumen-protected Lys in dairy cattle provides a means for comparing economic value of commercially available products.

**Key Words:** lysine, bioavailability, rumen-protected

**W352 Plane of milk replacer nutrition influences the resistance to an oral *Citrobacter freundii* opportunistic infection in Jersey calves at 10 days of age.** Yu Liang<sup>\*1</sup>, Jeff A. Carroll<sup>2</sup>, and Michael A. Ballou<sup>1</sup>, <sup>1</sup>Texas Tech University, Department of Animal and Food Sciences, Lubbock, TX, <sup>2</sup>USDA-ARS, Lubbock, TX.

This study investigated how early life plane of milk replacer nutrition influences the resistance to an opportunistic enteric challenge with *Citrobacter freundii*. Twenty colostrum-fed Jersey calves were blocked by BW at birth and randomly assigned to either a low (LPN) or high plane of nutrition (HPN) treatment. The LPN calves were fed 400 and 450 g DM/d of a 20% CP and 20% fat milk replacer during the 1st wk and thereafter, respectively. The HPN calves were fed 600 and 700 g DM/d of a 28% CP and 20% fat milk replacer during the 1st wk and thereafter, respectively. All calves were challenged orally with  $1 \times 10^8$  cfu of a stationary phase culture of *Citrobacter freundii* at d 10. Fecal scores were recorded throughout the study and rectal temperatures were recorded daily beginning on d 10. Fecal and plasma samples were collected at d 10, 12, 14, 16, 18, 20, 22, and 24. Fecal samples were analyzed for DM percentage and plasma for urea nitrogen, glucose, and haptoglobin concentrations. All calves were harvested at d 24 and

ileal tissues were analyzed for morphology. All data reported as LPN vs HPN, respectively. Water intakes increased to a greater extent among HPN calves after the challenge ( $P \leq 0.021$ ). There was no difference in DM percentage of feces between treatments (21.9 vs  $19.9 \pm 1.34\%$ ;  $P = 0.291$ ) despite HPN having greater fecal scores ( $2.0$  vs  $2.5 \pm 0.07$ ;  $P = 0.001$ ). Rectal temperatures were greater among the HPN calves ( $38.4$  vs  $38.6 \pm 0.05^\circ\text{C}$ ;  $P = 0.024$ ). Additionally, the HPN calves had numerically greater peak plasma haptoglobin concentrations after the challenge ( $266$  vs  $511 \pm 108.0$  μg/mL;  $P = 0.118$ ) and a tendency to have a greater total mucosal height of the distal ileum ( $752$  vs  $921 \pm 59.1$  μm;  $P = 0.059$ ). Therefore, these data indicate that calves fed a HPN during the neonatal period have a slightly greater acute phase response to an opportunistic bacterial enteric infection. Furthermore, these data reiterate that fecal scores should not be the only measure of enteric health, especially when evaluating the health effects associated with plane of nutrition.

**Key Words:** calf, disease, nutrition

**W353 Pre-weaning calf responses to lysine: I. Development and evaluation of functions explaining nitrogen retention responses to dietary lysine and body weight.** Juan J. Castro Marquez<sup>\*</sup>, Robin R. White, and Mark D. Hanigan, Department of Dairy Science, Virginia Tech, Blacksburg, VA.

The objective of this study was to define the nitrogen retention (NR) response of growing dairy calves as a function of dietary lysine intake (DLI) and body weight (BW). Raw, individual calf data from 3 published papers were collected. Lysine intake and NR were expressed as g per kg BW. Twelve models were derived relating NR, DLI and BW. Three equation forms (logistic, Michaelis-Menten or exponential), 2 effect types (fixed effects or mixed-effects with random study effect) and 2 error assumptions (equal error variance or heterogeneous error variance) were considered. The models were evaluated and compared based on their root mean squared prediction error (RMSPE), concordance correlation coefficient (CCC) and any mean and slope bias revealed by residual analysis. The asymptotic and rate-determining parameters of each function were assumed to vary as a function of BW and the primary explanatory variable within each equation form was DLI. Models varied notably in their predictive capacity and RMSPE ranged from 14.4 to 58.9%. A fixed-effects, logistic model with equal error variance returned the lowest RMSPE (14.4%), highest CCC (0.94) and had no appreciable mean or slope bias. All parameters were statistically significant ( $P < 0.01$ ). This equation,  $\text{NR} = (0.89 - 0.0067 \times \text{BW} + 0.000015 \times \text{BW}^2) \div (1 + e^{-((\text{Lys} - (0.0024 \times \text{BW} * e^{-0.013 \times \text{BW}})) / 0.032))})$ , estimated that a young calf (50 kg BW) could potentially achieve a maximal NR efficiency of 2.72 g/g DLI/kg BW and at 250 kg BW a maximal NR efficiency of 1.25 g/g DLI/kg BW could be reached. Nitrogen retention efficiency reduced substantially as BW increased. Increasing DLI promoted NR at low BW more effectively than at high BW. Although DLI affects short-term protein accretion responses, NR rate was primarily dependent on calf maturity.

**Key Words:** calf, nitrogen retention, amino acid

**W354 Saliva sodium, potassium, and phosphorus concentrations of post-peak lactating Holstein cows are not affected by dietary fiber or protein content.** J. A. D. R. N. Appuhamy<sup>\*1</sup>, M. Niu<sup>1</sup>, T. Teweldebrhan<sup>1</sup>, A. Leytem<sup>2</sup>, R. Dungan<sup>2</sup>, and E. Kebreab<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of California, Davis, CA, <sup>2</sup>USDA-ARS, Northwest Irrigation Research Lab, Kimberly, ID.

Knowledge on saliva sodium (Na), potassium (K), and phosphorus (P) concentrations are important in modeling electrolyte, water, and P partitioning in dairy cows. Saliva mineral concentrations in ruminants are strongly associated with saliva secretion rates, which can be affected by diet composition. The objective of this study was to examine saliva Na, K, and P concentrations in lactating cows fed 2 levels of forage and CP contents. Saliva samples were collected from 12 lactating Holstein cows randomly assigned to a 2 × 2 factorial arrangement of 2 forage (alfalfa hay) levels [38 (LF) vs. 53% (HF)] and 2 CP levels [15.2 (LP) vs. 18.5% (HP)] over 3 periods. Saliva samples were acidified (pH = 5–6) and diluted by a factor of 50 using deionized water (pH = 4.0). The filtered samples were then analyzed for Na, K, P, and calcium (Ca) concentrations by inductively coupled plasma atomic emission spectroscopy. Treatment effects on saliva mineral concentrations were analyzed with mixed-effect models including random cow effect. Mean dietary concentrations and total intake (via feed and water) of Na, K, and P were 0.36 ± 0.01, 1.04 ± 0.10, and 0.40 ± 0.01% of DM and 81 ± 10, 236 ± 40, and 93 ± 9 g/cow/d, respectively. Only total K intake was negatively affected by dietary fiber content ( $P < 0.01$ ). Saliva concentrations of Na and K were on average 70.3 ± 6.7 and 21.9 ± 1.3 mmol/L and were negatively correlated ( $r = -0.40$ ,  $P < 0.01$ ). A unit increase in average daily temperature (T, °C) increased and decreased saliva K ( $P = 0.08$ ) and Na ( $P = 0.05$ ) concentrations by 0.9 ± 0.5 and 4.9 ± 2.4 mmol/L, respectively. Saliva K concentration decreased by 0.010 ± 0.005 mmol/L ( $P = 0.07$ ) for unit increase in BW. Saliva P concentration was on average 1.9 ± 0.4 mmol/L, and increased and decreased by 0.004 ± 0.002 ( $P = 0.10$ ) and 0.61 ± 0.18 mmol/L ( $P < 0.01$ ) for unit increase in BW and T. Saliva P concentration was negatively related to saliva Ca concentration ( $r = -0.37$ ,  $P < 0.01$ ). Dietary fiber or CP contents did not have significant effect on any of the saliva mineral concentrations. Information on BW, T, and relationship with other minerals are important in determining Na, K, and P partitioning in lactating dairy cows.

**W355 Do the viability and dose of *Saccharomyces cerevisiae* affect the digestibility, ruminal fermentation, and performance of lactating dairy cattle?** Y. Jiang<sup>\*1</sup>, R. M. Martins<sup>2</sup>, I. M. Ogunade<sup>1</sup>, M. A. Bamikole<sup>3</sup>, F. Amaro<sup>2</sup>, W. Rutherford<sup>4</sup>, S. Qi<sup>4</sup>, F. Owens<sup>4</sup>, B. Smiley<sup>4</sup>, K. G. Arriola<sup>1</sup>, C. Staples<sup>1</sup>, and A. T. Adesogan<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of Florida, Gainesville, <sup>2</sup>Animal Science Department, Federal University of Vicosa, Vicosa, Minas Gerais, Brazil, <sup>3</sup>Department of Animal Science, University of Benin, Benin City, Nigeria, <sup>4</sup>DuPont Pioneer, Johnston, IA.

This study was conducted to examine the effect of the dose and viability of supplemental *Saccharomyces cerevisiae* on ruminal fermentation and performance of lactating dairy cows. Four ruminally cannulated lactating cows (284 ± 18 DIM) were assigned to 4 treatments arranged in a 4 × 4 Latin square design with four 21-d periods. Cows were fed a non-acidotic total mixed ration (46.8% corn silage, 8.5% wet brewers grain and 44.7% concentrate, dry matter basis). The diet was supplemented with no yeast (Control, CON) or with a low dose of live yeast ( $5.7 \times 10^7$  cfu/day; LLY), a high dose of live yeast ( $6.0 \times 10^8$  cfu/day; HLY), or a high dose of killed yeast ( $6.0 \times 10^8$  cfu/day before heating at 80°C; HDY). Ruminal pH and temperature were measured with indwelling probes. Ruminal fluid was collected 0, 2, 4, 6, 8 and 10 h after the morning feeding on d 21 for analysis of volatile fatty acids (VFA) and ammonia-N. All data were analyzed using the GLIMMIX procedure of SAS (SAS Institute, Cary, NC). In vivo digestibility was measured using chromic oxide as a marker. Supplemental LLY increased yields of milk (29.9 vs 31.8 kg/d;  $P = 0.04$ ), milk fat (1.09 vs 1.17 kg/d;  $P = 0.08$ ) and milk protein (0.95 vs 1.02 kg/d;  $P = 0.05$ ), feed efficiency (0.85 vs 0.90;  $P = 0.03$ ), acetate to propionate ratio ( $P = 0.07$ ), and in vivo apparent

digestibility of dry matter and neutral and acid-detergent fiber ( $P = 0.02$ , 0.02 and 0.02, respectively). Feeding HLY instead of LLY decreased milk yield (31.8 vs 30.1 kg/d;  $P = 0.07$ ) and feed efficiency (0.90 vs 0.86 kg/d;  $P = 0.05$ ). Supplementing with killed instead of live yeast decreased concentrations of acetate ( $P = 0.06$ ), propionate ( $P = 0.05$ ) and total VFA ( $P = 0.05$ ). Supplementing with HDY instead of CON increased lactate ( $P = 0.14$ ) concentration and acetate to propionate ratio ( $P = 0.08$ ) and decreased the minimum ruminal pH ( $P = 0.10$ ). The low dose of live yeast increased dry matter and neutral-detergent fiber digestibility, milk production and feed efficiency but the high dose of killed or live yeast did not.

**Key Words:** yeast, dairy cow, milk yield

**W356 Pre-weaning calf responses to lysine: II. Sensitivity and optimization of nitrogen retention responses to dietary lysine and body weight.** Robin R. White\*, Juan J. Castro Marquez, and Mark D. Hanigan, Department of Dairy Science, Virginia Tech, Blacksburg, VA.

The objective of this study was to use a model relating lysine (Lys) intake and weight gain in calves to identify optimal alternative protein sources for use in calf diets to minimize cost of gain (COG). A nonlinear optimization was used to minimize COG by adjusting milk replacer formulation subject to biological and practical constraints. The optimizer balanced milk replacer formulas for calves on a weekly basis from wk 1 through 8 of age. Body weight was diet dependent and updated weekly. Energy and mineral requirements of calves were calculated weekly following NRC (2001). Biological, practical and economic constraints were used to define the feasible set of ingredient combinations. Daily Lys intake was used to estimate nitrogen retention for each age group and change in body protein content was tracked. A literature search of calf slaughter balance studies was used to derive allometric equations relating body protein content to body fat, water and ash content. These functions were differentiated and change in body weight was calculated as the sum of the changes in body protein, fat, water and ash. Diet cost was estimated from current ingredient prices and dietary inclusion rates. Sensitivity of the model outputs to feedstuff Lys, protein content and digestibility was performed by taking 10,000 random draws of these parameters from normal distributions, running the growth model and calculating final weight and COG. Standard deviations in feedstuff Lys, protein content and digestibility were sourced from the National Animal Nutrition Program website. The optimal diet relied on a milk protein base with steadily increased inclusion of wheat gluten as animals aged. Blood meal and meat meal were also identified as key alternative protein sources. Model solutions were very robust. After 8 weeks calves weighed 87.8 ± 0.1 kg and COG was \$0.358/kg ± \$0.001/kg. Protein content and digestibility and Lys significantly ( $P < 0.001$ ) affected COG and BW and protein digestibility was quantitatively the most important parameter to estimate.

**Key Words:** calf, nitrogen retention, optimization

**W357 Effects of corn treated with foliar fungicide at various times of application on in situ ruminal digestibility of corn silage in Holstein cows.** Katie J. Haerr<sup>\*1</sup>, Naina M. Lopes<sup>2</sup>, Japheth D. Weems<sup>1</sup>, Carl A. Bradley<sup>1</sup>, Marcos N. Pereira<sup>2</sup>, Michael R. Murphy<sup>1</sup>, Gary M. Fellows<sup>3</sup>, and Felipe C. Cardoso<sup>1</sup>, <sup>1</sup>University of Illinois, Urbana, IL, <sup>2</sup>Universidade Federal De Lavras, Lavras, MG, Brazil, <sup>3</sup>B.A.S.F. Corporation, Research Triangle Park, NC.

The main objective was to explore the associations of corn plant foliar fungicide application on harvested whole-plant corn silage digestibility. Treatments were control (CON), corn received no foliar fungicide application; treatment 1 (1×), corn received one application of pyraclostrobin foliar fungicide (PYR; Headline; BASF Corp. Florham Park, New Jersey) at corn stage V5; treatment 2 (2×), corn received 2 applications of foliar fungicides, PYR at corn stage V5, and a mixture of pyraclostrobin and metconazole (PYR+MET; Headline AMP; BASF Corp. Florham Park, New Jersey) at corn stage R1; and treatment 3 (3×), corn received 3 applications of foliar fungicides, PYR at corn stage V5, PYR+MET at corn stage R1, and PYR+MET at corn stage R3. Corn was harvested at the same time and ensiled for 7 mo. Three cannulated lactating multiparous Holstein cows ( $376 \pm 28$  DIM) were used to measure corn silage in situ digestibility. Dried unground corn silage was put into 288 (3 time points/treatment/cow)  $10 \times 20$  cm bags and incubated for 0, 2, 4, 8, 12, 48, 72 or 96 h. A sample of unground undried corn silage was also placed into a  $20 \times 40$  cm bag and incubated for 48 h. The degradable portion of DM was greater ( $P = 0.01$ ) for corn silages treated with fungicide when compared with CON (0.36, 0.42, 0.40 and 0.47 for CON, 1×, 2×, and 3×, respectively). There was a linear ( $P < 0.001$ ) effect of treatment frequency on the proportion of DM digestibility. The 2 different sizes of bags used ( $10 \times 20$  vs.  $20 \times 40$  cm) for the in situ digestibility technique were different ( $P < 0.02$ ) for DM, NDF, ADF, CP, and starch for 48h digestibility. Larger bags had greater ( $P < 0.03$ ) digestibility for DM (33, 35%), and lower digestibility for NDF (42, 35%), and ADF (47, 39%), than smaller bags. In conclusion, corn silage with fungicide application had higher DM digestibility and a trend for a lower rate of digestion as well as linear effects for decreasing DM solubility, increasing DM degradability, and decreasing rate of DM digestion.

**Key Words:** in situ digestibility, corn silage, fungicide

**W358 A multivariate mixed effects model to estimate the energetic efficiencies of synthesizing milk components.** Luis E. Moraes<sup>\*1</sup>, James G. Fadel<sup>1</sup>, David P. Casper<sup>2</sup>, and Ermias Kebreab<sup>1</sup>, <sup>1</sup>University of California-Davis, Davis, CA, <sup>2</sup>South Dakota State University, Brookings, SD.

The current Northern American feeding system for dairy cows suggests that new feeding systems calculate milk energy requirements as individual requirements for milk fat, protein and lactose. Extant models from the literature provide efficiencies that are unrealistically larger than the partial efficiency of utilizing dietary metabolizable energy (ME) for milk production ( $k_L$ ) or require inputs that are often not available to nutritionists when formulating diets. The objective of this study was to develop a model to estimate partial efficiencies of simultaneously synthesizing milk fat, protein and lactose from dietary ME and body stores. The model was developed as a multivariate mixed effects model and it was structured such that the partial efficiencies of synthesizing individual milk components additively determined  $k_L$  and the partial efficiency of utilizing body stores for milk production ( $k_T$ ). The new energy balance function described the trivariate response vector comprising milk fat, protein and lactose energy outputs as functions of the animal's ME intake and tissue energy balance. The model was fitted with 930 energy balance records from 244 Holstein lactating cows. Measurements were conducted through indirect calorimetry at the former USDA Energy Metabolism Unit at Beltsville, Maryland. The partial efficiencies of synthesizing milk fat, protein and lactose additively determined a  $k_L$  of 0.62 (0.009) and a  $k_T$  of 0.80 (0.020) which are in good agreement with the partial efficiencies adopted by the NRC (2001). The net energy requirement for maintenance ( $NE_M$ ) estimated with this

model was 0.34 (0.015) MJ/kg BW<sup>0.75</sup> which is virtually identical to the  $NE_M$  adopted by the NRC (2001). The determination of the ME required to synthesize each milk component may be further evaluated with the estimation of the fraction of the ME intake directed to each milk component and through the estimation of the partial efficiencies of individually synthesizing each milk component. Therefore, the estimated partial efficiencies can be directly implemented in the current Northern American net energy system.

**Key Words:** energy, efficiency, milk component

**W359 Effects of feeding slow-release NPN and microbial fermentation extracts on ruminal pH, ammonia and volatile fatty acids.** Fernando Diaz-Royón<sup>\*1</sup>, Alvaro D. Garcia<sup>1</sup>, Kenneth F. Kalscheur<sup>2</sup>, and Kamal Mjoun<sup>3</sup>, <sup>1</sup>Dairy Science Department, South Dakota State University, Brookings, SD, <sup>2</sup>U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI, <sup>3</sup>Alltech, Brookings, SD.

The objective of this study was to examine the effect of partial substitution of soybean meal with a product containing slow-release NPN and microbial fermentation extracts [(OPT); Optimase Alltech Inc., Nicholasville, KY] on rumen fermentation. This is a companion study to a performance experiment (Díaz-Royón et al., 2014, J. Dairy Sci. 97 (E-Suppl. 1):332). Four cannulated, lactating Holstein cows were randomly assigned to a  $4 \times 4$  Latin square in a  $2 \times 2$  factorial arrangement of treatments. High (HF) and low forage (LF) diets contained, respectively, 61% and 46% forage, with and without OPT. Forages consisted of 75% corn silage and 25% alfalfa hay (DM basis). Experimental diets containing OPT (125 g/cow/day) were designed to partially replace soybean meal 48% CP (0.625 kg) with forage fiber [corn silage (0.225 kg) and alfalfa hay (0.075 kg)] and non-forage fiber [soybean hulls (0.200 kg)]. Increasing forage produced the expected pH increase (6.15 vs. 6.05 for HF and LF, respectively;  $P < 0.01$ ) whereas ruminal pH was not affected by OPT inclusion. Ruminal ammonia concentration tended to be greater in cows fed HF than LF diets (8.07 vs. 7.28 mg/dL;  $P = 0.08$ ), whereas OPT had no effect. Cows fed HF diets had a lower total VFA concentration compared with those fed LF (86.5 vs. 91.6 mM;  $P = 0.02$ ). For cows fed HF diets, molar proportions of acetate (66.0 vs. 64.4%;  $P < 0.01$ ), isobutyrate (0.89 vs. 0.82%;  $P < 0.01$ ), and isovalerate (1.80 vs. 1.58%;  $P < 0.01$ ) were greater, whereas those for propionate were lesser (19.4 vs. 21.1%;  $P < 0.01$ ). Total VFA proportions increased by 6.4% in HF diets supplemented with OPT; however, it decreased by 4.4% on LF diets (forage  $\times$  OPT,  $P$  value = 0.03). Feeding OPT increased molar proportions of acetate ( $P < 0.01$ ), decreased propionate ( $P < 0.01$ ), isobutyrate ( $P < 0.01$ ), and isovalerate ( $P < 0.01$ ), but did not change molar proportions for butyrate and valerate. Partial replacement of soybean meal with OPT in dairy cow diets altered ruminal fermentation in a manner that suggests improved fiber digestion.

**Key Words:** slow-release NPN, microbial fermentation extract, dairy cows

**W360 Relationship between total-tract starch digestibility and fecal starch content in dairy cows.** Marcos N. Pereira<sup>\*1,2</sup>, Eugenio F. Barbosa<sup>1</sup>, and Renata A. N. Pereira<sup>3,2</sup>, <sup>1</sup>Universidade Federal de Lavras, Lavras, MG, Brazil, <sup>2</sup>Better Nature Research Center, Ijaci, MG, Brazil, <sup>3</sup>Empresa de Pesquisa Agropecuária de Minas Gerais, Lavras, MG, Brazil.

Fecal starch content has utility to estimate starch digestibility in dairy herds. We evaluated the relationship of total-tract starch digestibility and

fecal starch content in individually fed lactating Holsteins. The data set from 5 experiments conducted at the Better Nature Research Center had 190 cow/observations fed at ad libitum intake. Starch content of feces, orts, and feeds were analyzed enzymatically at the Federal University of Lavras laboratory (Hall, 2009). Fecal production was measured by total collection of feces in buckets by trained personal. Feces were collected concurrent to defecation during 3 8 h sampling periods and weighed. The second and third sampling periods begun 8 h later than the previous sampling, to avoid a major disturbance to the animals while still representing a 24-h collection period. Fecal aliquots (equal fresh weight basis) were immediately frozen during the collection period and a composite sample was formed. Diets contained 37 to 42% whole plant corn silage in DM and mature finely ground corn grain and/or high moisture corn at 17 to 26% of DM. Cows BW, DMI, and milk yield (MY) during the period of fecal sampling were obtained. Mean  $\pm$  SD (Min-Max) were: BW 636  $\pm$  73 kg (500–860), DMI 20.74  $\pm$  2.93 kg/d (11.88–27.83), MY 31.16  $\pm$  6.22 kg/d (13.70–47.20), DMI/BW 3.28  $\pm$  0.45% (2.07–4.57), MY/DMI 1.51  $\pm$  0.25 (0.70–2.41), fecal starch content 5.09  $\pm$  2.42% of DM (1.05–13.08), starch intake 6.021  $\pm$  0.985 kg/d (3.374–8.356), consumed diet starch content 29.06  $\pm$  2.84 (22.74–37.72), starch digestibility 94.96  $\pm$  2.60% of intake (86.24–99.18). The correlation coefficient (*r*) of starch digestibility and fecal starch content was  $-0.924$ , higher than with starch intake ( $-0.219$ ), diet starch content ( $0.136$ ), DMI ( $-0.362$ ), DMI/BW ( $-0.376$ ), MY ( $-0.363$ ), and MY/DMI ( $-0.121$ ). Based on stepwise procedure, Starch digestibility (% of intake) =  $100.02 - 0.9945 \times \text{fecal starch content (\% of DM)}$  ( $r^2 = 0.854$ ,  $P < 0.01$ ). Model  $R^2$  was 0.878 when diet starch content was included and 0.882 when MY was further added (0.15 significance required for entry into the model). Fecal starch content was a good predictor of starch digestibility for this data set of cows sampled on similar experimental condition and fed corn based diets.

**Key Words:** starch in feces, starch digestibility, corn digestion

**W361 Relationship between milk urea nitrogen and milk protein ratio with dietary and non-dietary variables in commercial dairy herds.** Liliana Fadul-Pacheco<sup>\*1</sup>, Doris Pellerin<sup>1</sup>, P. Yvan Chouinard<sup>1</sup>, Michel A. Wattiaux<sup>2</sup>, and Edith Charbonneau<sup>1</sup>, <sup>1</sup>Université Laval, Quebec, Quebec, Canada, <sup>2</sup>University of Wisconsin-Madison, Madison, WI.

Milk urea nitrogen (MUN) can be used as a tool for the nutritional and environmental management of dairy cows as an estimate of the efficiency of protein metabolism. Using MUN to milk protein ratio (MUN/PROT) was suggested to improve the prediction. Thus, the aim of this study was to better understand the relationships between MUN and MUN/PROT with non-nutritional and nutritional variables in commercial dairy herds. Data recorded by Valacta (Dairy Production Center of Expertise Quebec-Atlantic) for the years 2009 to 2011 was used and originally comprised 3,481,705 test-day records (275,758 cows in 3,140 herds). The analysis was restricted to data from Holstein cows, between 1 and 305 DIM, reducing the number of admissible records for the analysis to 1,339,156 (189,913 cows in 2,522 herds). Average ( $\pm$ SD) milk production, fat content and protein content were 30.8  $\pm$  8.5 kg/d, 3.89  $\pm$  0.60% and 3.25  $\pm$  0.33%, respectively. MUN was statistically different between milkings (10.1 and 10.7 mg/dL for AM and PM, respectively;  $P < 0.001$ ). MUN/PROT was also statistically different between milkings (31.1 and 32.1 mg urea-N/kg milk CP for AM and PM, respectively;  $P < 0.001$ ). Average MUN was statistically different between parities (10.2 and 10.5 mg/dL for first and second or more lactation, respectively;  $P < 0.001$ ). MUN/PROT was also affected by parity (30.7 and 32.0 mg urea-N/kg milk CP for first and second or more lactation, respectively;

$P < 0.001$ ). Pearson correlations between MUN or MUN/PROT and diet variables were low, the highest correlation for both parameters being  $r = 0.16$  ( $P < 0.001$ ) with crude protein content. Both parameters were negatively correlated with milk fat percentage ( $r = -0.19$  and  $r = -0.29$  for MUN and MUN/PROT, respectively;  $P < 0.001$ ) and with the estimated breeding value for protein content ( $r = -0.10$  and  $r = -0.22$  for MUN and MUN/PROT, respectively;  $P < 0.001$ ). MUN/PROT was also correlated with daily milk production ( $r = 0.20$ ;  $P < 0.001$ ). These results show that in commercial conditions MUN and MUN/PROT are affected by factors other than diet composition.

**Key Words:** lactating dairy cow, MUN, MUN/protein ratio.

**W362 Effects of corn treated with foliar fungicide at various times of applications on milk production of Holstein cows.** Katie J. Haerr<sup>\*1</sup>, Naina M. Lopes<sup>2</sup>, Marcos N. Pereira<sup>2</sup>, Gary M. Fellows<sup>3</sup>, and Felipe C. Cardoso<sup>1</sup>, <sup>1</sup>University of Illinois, Urbana, IL, <sup>2</sup>Universidade Federal De Lavras, Lavras, MG, Brazil, <sup>3</sup>B.A.S.F Corporation, Research Triangle Park, NC.

The objective of this study was to determine if corn treated with foliar fungicide and ensiled as corn silage would increase milk production and efficiency in dairy cattle. This study utilized 64 Holstein cows with parity 2.53  $\pm$  1.5, BW 653  $\pm$  80 kg, and 161  $\pm$  51 d in milk (DIM). Cows were blocked and randomly assigned to 1 of 4 treatments to be included in the total diet (35% of the DM as corn silage). Treatments were as follows: Control (CON), corn silage with no applications of a foliar fungicide, Treatment 1 (1 $\times$ ), corn silage received application of pyraclostrobin (PYR) foliar fungicide (Headline; BASF Corp.) at corn stage V5; treatment 2 (2 $\times$ ), corn received the same application as 1 $\times$  and another application of a mixture of PYR and metconazole (MET; Headline AMP; BASF Corp.) at corn stage R1; and treatment 3 (3 $\times$ ), corn the same applications as 2 $\times$  as well as third application of PYR and MET at R3. Corn was harvested at the 3/4 milk line stage of growth and ensiled for 200 d. The trial was conducted in 2 consecutive periods each consisting of 1 wk for adaptation (covariate) followed by 5 wk of measurements where cows received assigned treatments. Body weight, BCS, and lame scores were assessed weekly. Milk production, and DMI were measured daily. Milk samples for milk composition analysis were collected during wk 5. Blood samples were taken on d1 (covariate) and d 29 to assess blood metabolites. Data were analyzed using a MIXED procedure in SAS (v9.4). Dry matter intake was 23.78, 22.95, 19.54, and 21.33 kg for CON, 1 $\times$ , 2 $\times$  and 3 $\times$ , respectively. There was a linear ( $P = 0.08$ ) tendency for DMI. Milk yield (34.5, 34.5, 34.2, 34.4 kg/d) and milk components did not differ ( $P > 0.05$ ) among treatments. However, there were trends for increased FCM/DMI (1.65 vs. 1.47.  $P = 0.08$ ) and ECM/DMI (1.60 vs. 1.43.  $P = 0.09$ ) for cows fed corn silage with fungicide compared with CON ( $P < 0.09$ ). Serum glucose was lower for cows receiving treated silage with fungicide when compared with CON (51.1 vs. 63.4 mg/dL,  $P < 0.01$ ). In conclusion, cows receiving corn silage treated with foliar fungicide had increased feed conversion when compared with CON.

**Key Words:** milk yield, corn silage, fungicide

**W363 Increased plasma NEFA lowers the ratio of sphingomyelin to ceramide in Holstein cows.** J. Eduardo Rico<sup>1</sup>, Luciano S. Caixeta<sup>2</sup>, Yves R. Boisclair<sup>2</sup>, and Joseph W. McFadden<sup>\*1</sup>, <sup>1</sup>West Virginia University, Morgantown, WV, <sup>2</sup>Cornell University, Ithaca, NY.

Saturated fatty acids can antagonize insulin sensitivity in rodents by increasing ceramide (Cer) levels in liver and plasma. Cer can be

generated via de novo synthesis, sphingomyelin (SM) hydrolysis, or sphingolipid recycling. In turn, Cer can be glycosylated to form mono-hexosylceramide (GlcCer) and lactosylceramide (LacCer). Analyzing the ratio between sphingolipids, within identical acyl chain length and saturation, can be a means to understand Cer metabolism. Our objective was to evaluate whether an increase in plasma NEFA can modify the sphingolipid ratio in cows. Six nonpregnant, nonlactating Holstein dairy cows (682 kg ± 22), were used in a crossover design with treatments consisting of i.v. infusion (100 ml/h) of either saline (control) or triacylglycerol (TG) emulsion (Intralipid 20%; Fransenius Kabi) for 16 consecutive hours. The feeding level was set at 120% of estimated energy requirement. Blood was collected at routine intervals. LC/MS was used to profile sphingolipids in plasma. Log-transformed data were analyzed using a mixed model with repeated measures. Nonparametric correlations were analyzed. TG infusion increased plasma NEFA by 454% at 3 h relative to control ( $P < 0.01$ ) with no further increase at 16 h. Ratio of C16:0-SM to C16:0-Cer (C16:0 SM: Cer) decreased 9% by 3 h of TG infusion, relative to control ( $P < 0.05$ ). C22:0, C22:1, and C24:0 SM: Cer decreased 27, 52, and 17% by 16 h of TG infusion, relative to control ( $P < 0.01$ ). TG infusion did not modify C16:0-dihydro SM: Cer; however, TG-infusion lowered the ratio of C18:0-SM to C18:0-dihydro-SM ( $P < 0.01$ ). C16:0 and C18:0 Cer:GlcCer decreased 24 and 13% by 16 h of TG infusion, relative to control ( $P < 0.01$ ). In contrast, C24:0 Cer:GlcCer and C26:0 Cer:GlcCer increased with TG infusion, relative to control ( $P < 0.05$ ). C18:0, C22:0, and C24:1 GlcCer: LacCer increased in TG-infused cows, relative to control ( $P < 0.01$ ). C16:0 Cer:GlcCer and C24:0 SM: Cer were negatively correlated with NEFA, and C24:0 Cer:GlcCer was positively correlated with NEFA ( $P < 0.01$ ). Increasing plasma NEFA lowers the ratio of SM to Cer in dairy cows, consistent with a shift in metabolism toward Cer accumulation.

**Key Words:** ceramide, dairy cow, insulin resistance

**W364 Effects of clay (EcoMix) after an aflatoxin challenge on milk production and blood metabolism of Holstein cows.** Saige A. Sulzberger<sup>\*1</sup>, Sergey Melnichenko<sup>2</sup>, and Felipe C. Cardoso<sup>1</sup>, <sup>1</sup>University of Illinois, Urbana, IL, <sup>2</sup>United Minerals Group, Kyiv, Ukraine.

Oral supplementation of clay to dairy cattle has been reported to reduce toxicity of aflatoxin in contaminated feed. The objective of this study was to determine the effects of 3 levels of dietary clay (EcoMix) supplementation after an aflatoxin challenge. Ten multiparous rumen-cannulated Holstein cows (BW = 669 ± 20kg, 146 ± 69 DIM), were assigned to 1 of 5 treatments in a completely randomized replicated 5 × 5 Latin square design. Periods consisted of a 14-d (d 1–14) adaptation period followed by a 3-d aflatoxin challenge (d 14–16; 100 µg of AFB1/kg of diet DM administered intra-ruminally). Cows were sampled daily during the last week of the period (d 14–21). Treatments were: POS, no EcoMix plus aflatoxin challenge; 0.5%, 1%, 2%, percentage of dietary DMI as EcoMix; and CON, no EcoMix and no aflatoxin challenge. Statistical analysis was performed using the MIXED procedure of SAS. Two contrasts CONT1 (POS vs. CON), CONT2 (POS vs. average of 0.5%, 1%, and 2%) were compared along with the linear and quadratic treatment effects. Milk samples were collected daily and tested with AFM<sub>1</sub> snap test (SNP; IDEXX, Inc.). Cows in CON had no positive SNP tests, while cows in POS had 14.2 ± 0.44 ( $P < 0.001$ , CONT2) positive SNP tests. Cows supplemented with EcoMix had lower number of positive SNP tests (0.5% = 13.9, 1% = 13.1, 2% = 12.4, SEM = 0.4,  $P = 0.04$ ) than cows in POS (14.2, CONT2). Cows supplemented with EcoMix through the challenge had lower 3.5% FCM (0.5% = 38.2 kg, 1% = 39.3 kg, 2% = 38.4kg, SEM = 1.8,  $P = 0.06$ ), ECM (0.5% = 37.1 kg, 1% = 37.1

kg, 2% = 37.1 kg, SEM = 1.6,  $P = 0.05$ , and Fat kg/d (0.5% = 1.36 kg, 1% = 1.43 kg, 2% = 1.40 kg, SEM = 0.09,  $P = 0.09$ ) than cows in POS (41.3 kg, 39.6 kg, 1.5 kg, respectively, CONT2). Serum cholesterol and creatine phosphokinase concentrations were higher for cows fed EcoMix (232.6 mg/dL ± 8.3,  $P = 0.08$ , 140.1 U/L ± 9.3,  $P = 0.04$ , respectively) than cows in POS (227.4 mg/dL ± 8.4, 126.3 U/L ± 9.3, respectively). In conclusion, oral supplementation of EcoMix decreased the total number of positive SNP tests after an aflatoxin challenge and seems to work as a flow agent for aflatoxin contamination in dairy cattle.

**Key Words:** clay, aflatoxin, milk yield

**W365 Discrepancies in milk urea nitrogen analysis among milk processing laboratories in Pennsylvania.** Holley L. Weeks<sup>\*</sup> and Alexander N. Hristov, Department of Animal Science, The Pennsylvania State University, University Park, PA.

Milk urea-N is used by dairy nutritionists and producers to monitor dietary protein intake and is indicative of N utilization in lactating dairy cows. Two experiments were conducted to explore discrepancies in MUN results among 3 laboratories and one experiment to evaluate the effect of 2-bromo-2-nitropropane-1,3-diol (bronopol) on MUN. In Experiment 1, 10 replicates of bulk tank milk samples, collected from the Pennsylvania State University's Dairy Center over 5 consecutive days, were sent to 3 milk processing laboratories located in Pennsylvania. Average MUN differed ( $P \leq 0.05$ ) between Laboratory A (Foss 4000; 14.9 ± 0.40 mg/dL), Laboratory B (Foss FT + 600; 6.5 ± 0.17 mg/dL), and Laboratory C (Foss 6000; 7.4 ± 0.36 mg/dL). In Experiment 2, milk samples were spiked with urea at 0, 17.2, 34.2, and 51.5 mg/dL of milk. Two 35-mL samples from each urea level were sent to the 3 laboratories used in Exp. 1. Average analyzed MUN was higher than expected (calculated for each laboratory based on the control; 0 mg added urea): for Laboratory A (23.2 vs. 21.0 mg/dL;  $P = 0.001$ ), Laboratory B (18.0 vs. 13.3 mg/dL;  $P < 0.001$ ), and Laboratory C (20.6 vs. 15.2 mg/dL;  $P < 0.001$ ). In Experiment 3, replicated milk samples were preserved with 0 to 0.30 g bronopol/35 mL milk (at 0.02 g increments) and submitted to one milk processing laboratory that analyzed MUN using 2 methods. Samples with increasing amounts of bronopol ranged in MUN concentration from 7.7 to 11.9 ± 0.27 mg/dL (linear increase due to bronopol;  $P < 0.001$ ) and from 9.0 to 9.3 ± 0.05 mg/dL ( $P = 0.06$ ) when analyzed on Foss 4000 or CL10, respectively. In this experiment, bronopol was calculated to contribute 7.9% of the total N in milk for the average milk vial (0.21 ± 0.003 g bronopol/ 35 mL milk) when milk protein was 3.01% and MUN was 8.5 mg/dL. In summary, MUN concentrations vary due to analytical procedures used by the milk processing laboratories. Amount of bronopol used to preserve the milk sample may also have an effect on MUN. Thus, it is important to maintain consistency in milk sample preservation and analysis to ensure precision and accuracy of MUN results.

**Key Words:** milk analysis, milk urea-N, bronopol

**W366 Plasma long-chain acylcarnitines are elevated in overweight dairy cows experiencing greater lipolysis and insulin resistance during late pregnancy.** J. Eduardo Rico<sup>\*</sup>, Rachel E. Cokeley, and Joseph W. McFadden, West Virginia University, Morgantown, WV.

Excess saturated fatty acids can impair mitochondrial β-oxidation in overweight monogastrics. In turn, long-chain acylcarnitines (LCAC) can accumulate in plasma, biomarkers for insulin resistance in humans. Our objective was to determine whether plasma LCAC are associated



with changes in estimated insulin sensitivity in dairy cows. Our data included multiparous Holstein cows grouped according to BCS at d -30 prepartum: lean (BCS < 3.0; n = 10) or overweight (BCS > 4.0, n = 11; OVER), with blood collected at d -45, -30, -15, -7, and 4, relative to calving. Profiling of LCAC was achieved using LC/MS. Data were analyzed using a mixed model with repeated measures including the random effect of cow and the fixed effects of BCS and time. Nonparametric correlations were analyzed. We previously demonstrated that OVER had greater NEFA mobilization and lower insulin sensitivity at d -30, -15 and -7 prepartum ( $P < 0.05$ ), relative to lean. LC/MS detected C14:0-, C16:0-, C18:0-, and C20:0-LCAC, representing 12, 31, 48, and 9% of total plasma LCAC, respectively. Concomitant with lipolysis, plasma C14:0-, C16:0-, C18:0-, and C20:0-LCAC, and total LCAC increased ( $P < 0.01$ ). Plasma C16:0- and C20:0-LCAC, and total LCAC levels were greater in OVER at d -7 ( $P < 0.05$ ). Plasma C14:0- and C18:0-LCAC levels were higher in OVER ( $P = 0.10$ ). A BCS  $\times$  time interaction was observed, where OVER had greater plasma C18:0- and C20:0-LCAC levels at d -7 ( $P = 0.06$  and  $P < 0.05$ , respectively). Plasma C14:0-, C16:0-, C18:0-, and C20:0-LCAC, and total LCAC were positively correlated with NEFA ( $P < 0.01$ ). Utilizing previously acquired GC/MS data, circulating C16:0- and C18:0-LCAC were positively correlated with mobilized palmitic acid, stearic acid, and lipolysis marker glycerol ( $P < 0.01$ ). Plasma C14:0-, C16:0-, C18:0-, and C20:0-LCAC, and total LCAC were negatively correlated with insulin sensitivity ( $P < 0.01$ ). Our data demonstrate greater plasma LCAC in insulin resistant, overweight periparturient dairy cows; however, the origin and involvement of LCAC in the progression of insulin resistance is unclear.

**Key Words:** acylcarnitine, insulin resistance, periparturient dairy cow

**W367 Effects of the dose and viability of *Saccharomyces cerevisiae* yeast on the diversity of ruminal microbes as analyzed by Illumina MiSeq sequencing and qPCR.** Y. Jiang<sup>\*1</sup>, I. M. Ogunade<sup>1</sup>, S. Qi<sup>2</sup>, F. Owens<sup>2</sup>, B. Smiley<sup>2</sup>, W. Rutherford<sup>2</sup>, C. Staples<sup>1</sup>, and A. T. Adesogan<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of Florida, Gainesville, FL, <sup>2</sup>DuPont Pioneer, Johnston, IA.

The objective was to examine the effect of the dose and viability of *Saccharomyces cerevisiae* (SC) on the ruminal microbial population of lactating dairy cows. Four ruminally cannulated lactating cows (284  $\pm$  18 DIM) were used in a study with a 4  $\times$  4 Latin square design with four 21-d periods. Cows were fed a TMR supplemented with no SC (Control) or a low dose of live SC (5.7  $\times$  10<sup>7</sup> cfu/day; LLY), a high dose of live SC (6.0  $\times$  10<sup>8</sup> cfu/day), or a high dose of killed SC (6.0  $\times$  10<sup>8</sup> cfu/day before heating at 80°C). Ruminal fluid collected 0, 2, 4, 6, 8 and 10 h after the morning feeding on d 21 was strained through cheesecloth to obtain liquid (LF) and solid (SF) fractions. Microbial diversity was examined by high throughput Illumina MiSeq sequencing of the V4 region of the 16S rRNA gene. Populations of select ruminal bacteria and protozoa were also quantified by qPCR. Data were analyzed using the GLIMMIX procedure of SAS. In the SF and LF, *Prevotella* was the most abundant genus (23.8 and 49.1%) followed by *Fibrobacter* (10.7 and 1.5%) and *Succinivibrionaceae* (7.1 and 4.6%), respectively. Supplemental LLY increased the prevalence of *Butyrivibrio* ( $P = 0.11$ ) and *Ruminococcus* ( $P = 0.04$ ) in the LF. Supplementing with live instead of dead SC increased the prevalence of *Ruminococcus* in the LF and SF ( $P \leq 0.05$ ). Supplemental killed SC decreased the prevalence of *Lachnospiraceae* ( $P = 0.06$ ) and *Coproccoccus* ( $P = 0.13$ ) in the SF and increased those of *Ruminobacter* and *Porphyromonadaeae* in the SF and LF ( $P \leq 0.12$ , respectively). The qPCR results showed that in the SF, supplementing with LLY or with live instead of killed SC increased the prevalence of *F. succinogenes* ( $P = 0.14$  and 0.04, respectively). Supplemental killed

SC decreased the prevalence of protozoa ( $P = 0.05$ ) and *B. fibrosolvans* ( $P = 0.14$ ). In the LF, supplemental LLY decreased the prevalence of *R. albus* ( $P = 0.12$ ) and increased that of *S. ruminantium* ( $P = 0.14$ ). Unlike the high dose of live or killed SC, feeding the low SC dose or live instead of killed SC increased the prevalence of fiber-degrading bacteria.

**Key Words:** yeast, rumen microbes, MiSeq

**W368 Effects of direct-fed *Bacillus pumilus* 8G-134 during the transition period on health of Holstein cows.** Shaoyu Luan<sup>1</sup>, Elizabeth Galbraith<sup>2</sup>, Megan Duersteler<sup>2</sup>, and Felipe C. Cardoso<sup>\*1</sup>, <sup>1</sup>University of Illinois, Urbana, IL, <sup>2</sup>Dupont Nutrition and Health, Waukesha, WI.

The objective of this study was to evaluate the effects of a direct-fed microbial (DFM; *Bacillus pumilus* 8G-134) fed during the transition period on health of Holstein cows. Forty-three multiparous cows were fed a total mixed ration and assigned to 2 treatments in a randomized completely block design. Cows in the direct-fed microbial treatment (DFMt, n = 21) received 5.0  $\times$  10<sup>9</sup> cfu of *B. pumilus* direct-fed microbial in 28g of maltodextrin carrier, whereas cows in the control treatment (CON, n = 22) received 28g of maltodextrin carrier alone. Treatments were top-dressed daily. Treatments were applied from 21 d before calving to 154 d after calving. Blood samples were collected on d 5 and 14 after calving. Cows that had nonesterified fatty acids (NEFA) serum concentrations higher than 0.7 mEq/L were classified as high (HNEFA). Cows that had blood  $\beta$ -hydroxybutyrate (BHBA) concentrations higher than 1.2 mmol/L were classified as experiencing sub-clinical ketosis (SCK). Cows that had serum haptoglobin concentrations higher than 20  $\mu$ g/mL were classified as positive (POS). The immunoglobulins, IgA, IgG, and IgM were quantified in milk collected during the first week after calving and serum. Statistical analysis was performed using the MIXED, GLIMMIX and FREQ procedures of SAS. Cows on CON tended to have greater odds (OR = 3.55;  $P = 0.09$ ) of being classified as POS than CON cows on d 14. Treatment DFMt had higher (584  $\pm$  34  $\mu$ g/mL;  $P = 0.03$ ) IgA concentrations in milk than CON (478  $\pm$  35  $\mu$ g/mL) cows on the first week after calving. Cows on CON tended to have greater odds of being classified as HNEFA than DFMt cows on d 14 (OR = 3.21;  $P = 0.07$ ). Cows on CON tended to have greater odds of being classified SCK than DFMt cows on d 5 (OR = 3.85;  $P = 0.06$ ). Cows on CON tended to have higher odds for lower (<2) FS than DFMt cows (OR = 1.03;  $P = 0.08$ ). In conclusion, cows receiving DFMt tended to have lower incidence of SCK and to have higher immunity than cows receiving CON. Therefore, supplementation with DFMt seems to contribute for a sound transition period for dairy cows.

**Key Words:** transition period, direct-fed microbial, immunity

**W369 Bacterial communities in rumen fluid from lactating Holstein cows from Washington dairies.** Elizabeth D. Benda<sup>\*1</sup>, Nicola F. Beatty<sup>1</sup>, Janet E. Williams<sup>1</sup>, Matthew L. Settles<sup>1</sup>, John P. McNamara<sup>2</sup>, and Mark A. McGuire<sup>1</sup>, <sup>1</sup>University of Idaho, Moscow, ID, <sup>2</sup>Washington State University, Pullman, WA.

The rumen serves as a fermentation tank for bacteria to break down nutrients from feed for the benefit of the cow. Culture-dependent methods have identified the most prevalent genera as *Ruminococcus*, *Fibrobacter*, *Butyrivibrio*, and *Prevotella*. The objective of the study was to determine if the bacterial community in rumen fluid differed within a herd or across dairies within a given geographic region as assessed by a culture-independent method. In this study, rumen fluid samples from 82 cows across 4 different dairies in Washington were

collected via stomach tube and filtered through cheesecloth. Cows within a herd were in the same pen and fed the same ration. DNA was extracted using the Qiagen QIAamp DNA Stool Mini Kit, amplified via PCR with primers targeting the V1-V3 hypervariable region of the 16S rRNA, and amplicons sequenced using an Illumina Miseq v3 paired-end 300-bp protocol for 600 cycles. Sequence reads were processed using the custom python application dbcAmplicons. Sequencing data at different taxonomic levels were categorized by percent relative abundance for each cow. The least squares means of the top 15 genera were computed and compared among dairies to look at bacterial population differences using Generalized Linearized Mixed Models (SAS v9.3). Significance was declared at  $P \leq 0.05$ . Across all cows, *Prevotella* was the bacterial genus with the greatest relative abundance ( $44.8 \pm 1.06$ ), followed by *Hallella* ( $3.29 \pm 0.11$ ), *Treponema* ( $2.94 \pm 0.15$ ) and *Paraprevotella* ( $1.98 \pm 0.06$ ). Within a herd, the bacterial community of rumen fluid was similar among cows. However, each of the top 15 genera differed in abundance across dairies. These results suggest that cattle within a specific dairy have similar bacterial communities, but bacterial populations can differ across different dairies even within the same geographical region. Further work is necessary to determine the cause of the different levels of abundance of the top genera among dairies.

**Key Words:** rumen, microbial communities, dairy

**W370 Effects cobalt source on rate and extent of DM and NDF degradation *in vitro*.** Claudio F. Vargas-Rodriguez<sup>\*1</sup>, Abigail J. Carpenter<sup>1</sup>, Jeffrey DeFrain<sup>2</sup>, and Barry Bradford<sup>1</sup>, <sup>1</sup>Kansas State University, Manhattan, KS, <sup>2</sup>Zinpro Corp., Eden Prairie, MN.

Positive effects on fiber degradation have been observed when supplemental cobalt (Co) was added to diets for ruminants, but dose-dependent effects of different Co sources on ruminal fermentation have not been tested. Our objective was to determine the effects of different sources and concentrations of Co on *in vitro* fermentation rate, fermentation end products, and DM and NDF disappearance. Ruminant fluid was collected from heifers fed a wheat straw based diet (49%) with no supplemental Co, and fermentation substrate (46% NFC, 25% NDF, 22% CP) contained no measurable Co. Different inclusion levels (0.0, 0.1, 0.5, 1.0, 2.0, 5.0, 10.0, and 15.0 ppm) of Co glucoheptonate (S1) and Co carbonate (S2) were tested *in vitro* during study 1. Gas production was recorded every 15 min, and after 24 h, pH was measured and contents of each flask were used to determine NDF and DM disappearance (NDFD and DMD). Experiment 2 evaluated the effects of Co (S1 and S2 at 0, 0.33, 1, 3, and 9 ppm) on gas production, ruminal VFA and NH<sub>3</sub> concentration. In both studies, each treatment combination had 4 replicates and samples were incubated for 24 h; asymptotic gas production curves were modeled with NLIN of SAS using the Gauss-Newton fit method. Gas production kinetic values and all other data were modeled to assess the effects of Co concentration, source, and their interaction. Differences were declared at  $P < 0.05$  and tendencies at  $P < 0.10$ . NDFD was increased by S1 at concentrations between 0.1 and 1.0 ppm ( $55.0 \pm 1.8\%$ ), but decreased at 15 ppm (30.9%). S2 had no effect on NDFD across the concentrations tested. Moderate levels of Co tended to increase DMD, and S1 significantly increased this parameter compared with S2 ( $63.5$  vs.  $61.1\% \pm 1.6\%$ ), but interactions were not detected. Effects of Co source on gas production kinetics and pH change were inconsistent between experiments, largely because of dramatic negative effects at 15 ppm. Propionate concentration increased by S1 at 1.0 ppm, but not by S2 (dose by source interaction). Doses of 9 ppm Co decreased branched-chain VFA and NH<sub>3</sub> concentrations after 24-h incubation. In summary, Co does affect ruminal fermentation; however,

concentrations of 9 ppm and greater may be inhibitory, particularly with a highly soluble source of Co.

**Key Words:** mineral, fermentation, ruminant

**W371 Effects of long-term omega 6 fatty acid supplementation on blood metabolites of Holstein cows during transition period and early lactation.** Rodrigo Gardinal<sup>\*1,3</sup>, Gustavo Delfino Calomeni<sup>1</sup>, Filipe Zanferari<sup>1</sup>, Caio Seiti Takiya<sup>1</sup>, Thiago Henrique Aniballi Vendramini<sup>1</sup>, Jose Esler Freitas Junior<sup>2</sup>, Jose Eduardo Portela Santos<sup>3</sup>, and Francisco Palma Renno<sup>1</sup>, <sup>1</sup>University of Sao Paulo, Sao Paulo, SP, Brazil, <sup>2</sup>Federal University of Bahia, Salvador, BA, Brazil, <sup>3</sup>University of Florida, Gainesville, FL.

The objective of this study was determine the effects of feeding omega 6 ( $\omega 6$ ) fatty acids (FA) during an extended period of dry period until early lactation on blood metabolites of dairy cows. Thirty Holstein cows were used in 4 experimental groups in randomized design. The animals were randomly distributed to the following diets: Groups -90, -60, -30 and 0, supplemented with  $\omega 6$  FA source (12% of whole raw soybeans - WRS, on DM basis) 90,60 and 30 d before expected calving date and from the calving date (0) until 90 d in milk. Diets were formulated according to NRC (2001) recommendations. Ether extract (EE) averaged 4.8 and 2.8% for diets containing WRS and without WRS respectively. The blood samples were taken on days -49, -35, -21, -14 and -7 of expected calving date, at calving and on d 7, 14, 21, 35 and 70 of lactation. Blood concentrations of glucose (GLU), total cholesterol (CHOL), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), triglycerides (TRI), total protein (TP), urea, gamma-glutamyl transferase (GGT) and aspartate aminotransferase (AST) were measured. Data were subject to PROC MIXED of SAS 9.3, diet were considered as fixed effect and time, interaction between diet and time, animal and residual error as random effect. The data were analyzed using polynomial regression using PROC REG of SAS 9.3. Linear decrease ( $P < 0.05$ ) in GGT concentration and linear increase of TP and CHOL concentration were observed as the length of  $\omega 6$  FA supplementation was longer. Quadratic effect ( $P < 0.05$ ) was observed in AST blood concentration and the highest value was presented by cows of group -30 (86.4 U/L). Interaction effect ( $P < 0.05$ ) was detected in GLU, TRI and VLDL blood concentration. The higher length of  $\omega 6$  FA supplementation during pre-partum may improve hepatic metabolism of cows during transition period and early lactation.

**Key Words:** dry period, linoleic acid, whole raw soybean

**W372 Organic trace minerals during the transition period: 1. Supplementing Zn, Mn and Cu from AvailaMins and Co from CoPro improves postpartal performance of dairy cows.** J. S. Osorio<sup>\*1</sup>, E. Trevisi<sup>2</sup>, J. K. Drackley<sup>1</sup>, M. T. Socha<sup>3</sup>, and J. J. Loor<sup>1</sup>, <sup>1</sup>University of Illinois at Urbana-Champaign, Urbana, IL, <sup>2</sup>Università Cattolica del Sacro Cuore, Piacenza, Italy, <sup>3</sup>Zinpro Corporation, Eden Prairie, MN.

Beneficial effects of supplementing organic trace minerals (AAC) to lactating dairy cows have been reported. However, there are few studies evaluating AAC supplementation during the transition period. Thirty-seven Holstein dairy cows were enrolled at 60 d prior dry-off (~110 d before calving) and remained on experiment until 30 d in milk (DIM). Cows were offered a common diet supplemented entirely with inorganic trace minerals (INO) from -110 to -30 d before calving. Cows received a common prepartal (1.5 Mcal/kg DM, 15% CP) and postpartal (1.76 Mcal/kg DM, 18% CP) diet. Both diets were partially supplemented with

an INO mix of Zn, Mn, and Cu to supply 35, 45, and 6 ppm, respectively, of the total diet DM. Cows were assigned to treatments in a randomized complete block design, receiving an oral bolus with a mix of INO (n = 21) or AAC (n = 16) containing Zn, Mn, Cu, and Co to achieve 75, 65, 11, and 1 ppm, respectively, in total diet DM. Inorganic trace minerals were provided in sulfate form and AAC were supplied via AvailaZn, AvailaMn, AvailaCu, and CoPro (Zinpro Corp., Eden Prairie, MN). Blood glucose and NEFA were measured at -30, -14, 3, 15, and 30 DIM. Liver biopsies were harvested at -30, -15, 10, and 30 DIM. BHBA was measured via Precision Xtra every other day from 1 to 15 d postpartum. Data were analyzed using the MIXED procedure of SAS. Prepartal DMI was lower ( $P = 0.06$ ) in AAC cows. In contrast, a tendency ( $P = 0.11$ ) was detected for diet by time ( $D \times T$ ) interaction that resulted in ca. 2 kg/d greater postpartal DMI in AAC. Milk and milk protein yield had a  $D \times T$  ( $P < 0.05$ ), because AAC cows produced ca. 3.3 kg/d more milk and 0.14 kg/d more protein during the first 30 DIM. Although blood glucose, NEFA, and liver triacylglycerol were not affected ( $P > 0.56$ ) by diet, the Precision Xtra BHBA was lower ( $P = 0.02$ ) in AAC than INO (1.44 vs 2.18 mmol/L). The positive response in milk yield and milk protein observed in AAC cows might be partly explained by the beneficial effect of AAC on postpartal DMI. Greater BHBA in cows fed INO suggests a mild-to-severe state of ketosis.

**Key Words:** metabolite, trace mineral, transition cow

**W373 Evolution of milk freezing point depression during the year in Holstein and Normande dairy cows.** Catherine Hurtaud<sup>1</sup>, Elise Vanbergue<sup>1,2</sup>, Sophie Lemosquet<sup>1</sup>, Ségolène Colette<sup>3</sup>, Yves Gallard<sup>3</sup>, and Luc Delaby<sup>1</sup>, <sup>1</sup>INRA-Agrocampus Ouest UMR1348 Pegase, Saint-Gilles, France, <sup>2</sup>Institut de l'Élevage, F-35650 Le Rheu, France, <sup>3</sup>INRA, Domaine du Pin-au-Haras, Exmes, France.

Milk freezing point depression (FPD) content is regarded as being relatively stable in cows' milk during lactation. Since the 30s, its measurement is a means to determine if water has been added to the milk. However, results from commercial herds have suggested that changes in milk FPD occurred during the year. The reason of those variations is unclear. However, dairy cow feeding might be an explanation. The objective of this experiment was to compare the characteristics of milk (especially milk FPD measured by MIR spectrometry) based on low input grass-based system compared with corn silage-based system, across 2 breeds of dairy cows (Holstein vs. Normande). Sixty-four dairy cows were observed from calving to drying off. Two feeding systems were compared. The Intensive system was designed to maximize individual performance, with a high energy diet (in winter, corn silage with 30% concentrate; in spring, summer and autumn periods, pasture with 4 kg/d of concentrate supplemented with corn silage from July). The Grass system was designed to decrease inputs (in winter, conserved grass with no concentrate; in spring, summer and autumn, pasture with no concentrate). The experimental design was a continuous design. Data were analyzed by using SAS mixed procedure. There was no significant effect of breed and feeding system on milk FPD. There was a significant effect of month of sampling and month of lactation. Milk FPD did not change from calving to 7th month of lactation. After that date, it largely decreased. These effects could be related to the high temperature in summer, specific feeding (grazing), or restricted access conditions for watering. There was also a significant effect of rank of lactation. FPD was higher for multiparous cows. This difference could be due to the opening of mammary tight junctions and a different repartition of ions and lactose on both sides of the epithelial barrier. Surprisingly FPD did not correlate with lactose content the most important osmotic agent in

mammary epithelial cells. Some investigations have to be done to explain the mechanisms of FPD and its physiological variations.

**Key Words:** milk, dairy cow, freezing point depression

**W374 Influence of calcified seaweed supplementation on rumen pH, digestive efficiency, and health in lactating dairy cows fed an acidosis inducing diet.** B. P. Molloy<sup>\*1</sup>, E. W. Neville<sup>2</sup>, S. J. Taylor<sup>1</sup>, A. W. Fahey<sup>2</sup>, and F. J. Mulligan<sup>2</sup>, <sup>1</sup>Celtic Sea Minerals Ltd., Carrigaline, Cork, Ireland, <sup>2</sup>College of Food Science, Veterinary Medicine and Agriculture, University College Dublin, Dublin, Ireland.

Subacute ruminal acidosis (SARA) is a significant problem on intensively managed dairy farms throughout the world. High producing dairy cows are unable to acquire sufficient nutrients from forage-based diets to meet their needs during early lactation. Therefore, forage based diets are often supplemented with high-energy starch-rich ingredients to meet their caloric demand. High consumption of rapidly fermentable ingredients can cause excessive acidification of the rumen decreasing fiber digestion and milk fat %, inducing rumenitis, laminitis, reduced reproductive performance and liver abscesses. The objective of this experiment was to evaluate the potential for 3 different treatments to prevent SARA and the associated decrease in digestive efficiency. A highly fermentable diet containing 380 g of starch and 300g of total dietary NDF was fed to 4 ruminally fistulated lactating dairy cows. The diet was composed from grass silage, corn silage and concentrates and fed at a 45:55 forage to concentrate ratio. The diets were either not supplemented (control) or supplemented with calcified seaweed (CS), calcified seaweed and marine magnesium oxide (CS + MMgO) or sodium bicarbonate (SB) 4 treatments. A range of parameters investigating the effect of supplementation on rumen physiology were analyzed including pH, volatile fatty acid production, fiber digestion, rate of passage, total-tract digestibility, milk yield and milk quality. Rumen pH was measured every 10 min over 3 d during each experimental period, 25.4% of control, 3.2% of CS, 2.8% of CS + MgO and 13.2% of SB readings were <5.5. All treatments maintained rumen pH above 5.5 for significantly longer ( $P < 0.0001$ , respectively) than the control. The CS and CS + MMgO were significantly more effective ( $P < 0.0001$ ,  $P < 0.000$ , respectively) than the SB in maintaining rumen pH above 5.5 (min). Supplementing with CS or CS + MMgO has the potential to maintain rumen pH above 5.5 for a longer period of the day.

**Key Words:** acidosis, calcified seaweed, rumen

**W375 Evaluation of an on-farm tool to estimate physically effective neutral detergent fiber of forages and total mixed rations.** Sarah E. Schuling<sup>\*1</sup>, Eric J. Staudinger<sup>1</sup>, Jeff A. Rortved<sup>1</sup>, Paul M. Windschitl<sup>1</sup>, Greg L. Golombeski<sup>1</sup>, and Kurt W. Cotanch<sup>2</sup>, <sup>1</sup>Hubbard Feeds Inc., Mankato, MN, <sup>2</sup>William H. Miner Agricultural Research Institute, Chazy, NY.

The objective of this experiment was to evaluate the use of a 3 screen Penn State Particle Separator box (19.0-, 8.0-, and 4.0-mm screens; PSPS4mm) to estimate physically effective factor (pef) and physically effective neutral detergent fiber (peNDF) of forages and total mixed rations (TMR) using the Ro-Tap method as the gold standard. Samples of corn silage (CS; n = 21), alfalfa silage (AS; n = 21), and TMR (n = 20) were collected from 20 Midwest dairy farms in summer/fall 2014. Four gallons of each sample were collected and mixed thoroughly. Samples were split and analyzed for pef using PPS4mm and Ro-Tap with standard shaking methods. Sample nutrient composition was

determined at Dairyland labs (Arcadia, WI). The pef was calculated as percentage of sample retained above 4.0-mm and 1.18-mm screens for PSPS4mm and Ro-Tap, respectively. The peNDF (%DM) of each sample was calculated as pef x NDF. Dry matter intake, milk yield, and milk protein and fat content were recorded for each herd. The REG procedure of SAS was used to determine the relationship between pef and peNDF estimated using PSPS4mm and Ro-Tap. The Stepwise Selection procedure was used to determine variables that affect herd milk components. The PSPS4mm was a good predictor of pef and peNDF in TMR and forages ( $R^2 = 0.93$  and  $0.98$ ; slope =  $0.86$  and  $0.91$ , respectively). For the CS, AS, and TMR samples, average pef estimated with Ro-Tap was (mean  $\pm$  SD)  $87.4 \pm 3.99$ ,  $83.5 \pm 4.34$ , and  $64.3 \pm 6.24$ , respectively, and average pef estimated with PSPS4mm was (mean  $\pm$  SD)  $88.4 \pm 4.58$ ,  $87.6 \pm 2.92$ , and  $64.2 \pm 6.35$ , respectively. Significant variables for predicting herd milk fat content were pef, concentrate intake, and milk yield (model  $R^2 = 0.55$ ). Significant variables for predicting milk protein content were pef and forage intake (model  $R^2 = 0.42$ ). The PSPS4mm is a useful tool to estimate pef and peNDF of forages and TMR. The pef within and across sample type varied, so estimating pef of individual samples on-farm will allow for more precise formulation of ration peNDF, which affects herd milk components.

**Key Words:** physically effective NDF, Penn State Particle Separator, milk component

**W376 The effects of choice feeding during preweaning period on preweaning and postweaning growth performance of dairy calves.** Mohammad Wakil Hassani and Murat Gorgulu\*, *Cukurova University Agriculture Faculty Department of Animal Science, Adana, Turkey.*

The aim of the study was to investigate the effects of choice feeding in preweaning period on growing performance of calf performance pre and postweaning period. Twenty-eight male and 28 female Holstein calves were used to test 2 feeding systems (TMR, total mixed ration, containing 10% alfalfa hay and choice feeding) and 2 sex (male and female) in a factorial arrangement. Before weaning TMR calves were fed with TMR containing 90% calf starter and 10% alfalfa hay and after weaning all calves were fed with the same TMR containing 50% calf grower and 50% alfalfa hay. Choice fed calves were fed with feed ingredients in TMR ad libitum and simultaneously. The choice fed calves before weaning preferred the diet containing lower alfalfa (10% vs. 5.78%,  $P < 0.05$ ) and barley (52.29% vs. 15.87%,  $P < 0.05$ ), and higher wheat bran (17.28% vs. 30.07%,  $P < 0.05$ ) and SBM (17.73 vs. 45.39%,  $P < 0.05$ ). Sex had no significant effects on diet preferences ( $P > 0.05$ ). Choice feeding increased feed and nutrient intake (protein and fiber) and daily gain significantly ( $P < 0.05$ ). After weaning, sex and feeding system during preweaning period had no effects on any parameters investigated ( $P > 0.05$ ). But sex and feeding system interaction had significant effects on daily gain, feed and nutrient intakes ( $P < 0.05$ ). The male calves fed TMR before weaning consumed more feed and nutrients and had higher daily gain than females but, choice fed calves in both sex had similar daily gain, feed and nutrient intake after weaning. When overall performance were evaluated, male calves had higher daily gain than females ( $P < 0.05$ ). Sex  $\times$  feeding system interaction had significant effects on feed and nutrient intake ( $P < 0.05$ ). The male calves fed with TMR consumed more feed and nutrients than the females ones but this differences disappeared in choice feeding group. In conclusion, the results revealed that choice feeding may improve growth performance of calves by increasing protein intake before weaning and this effect may disappear after weaning. The female calf gave better response to

choice feeding in respect to feed intake. This work was supported by Research Fund of the Cukurova University.

**Key Words:** choice feeding, calf, feeding system

**W377 Effects of day of gestation and feeding regimen in Holstein  $\times$  Gyr cows on apparent total-tract digestibility, nitrogen balance, and fat deposition.** Polyana P. Rotta\*<sup>1,2</sup>, Sebastiao C. Valadares Filho<sup>1</sup>, Terry E. Engle<sup>2</sup>, Luiz Fernando Costa e Silva<sup>1,2</sup>, Marcos I. Marcondes<sup>1</sup>, Fernanda S. Machado<sup>3</sup>, Tathyane R. S. Gionbelli<sup>1</sup>, Breno C. Silva<sup>1</sup>, and Marcos V. C. Pacheco<sup>1</sup>, <sup>1</sup>Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, <sup>2</sup>Colorado State University, Fort Collins, CO, <sup>3</sup>Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil.

This study investigated how feeding regimen (FR) alters apparent total-tract digestibility, performance, N balance, excretion of purine derivatives, and fat deposition in Holstein  $\times$  Gyr cows at different days of gestation (DG). Forty-four pregnant multiparous Holstein  $\times$  Gyr cows with an average initial body weight of  $480 \pm 10.1$  kg and an initial age of  $5 \pm 0.5$  yr old were allocated to 1 of 2 FR: ad libitum (AL;  $n = 20$ ) and maintenance level (ML;  $n = 24$ ). Maintenance level was considered to be 1.15% of body weight on a dry matter (DM) basis and met 100% of the energy requirements, whereas AL provided 190% of total net energy requirements. Data for hot and cold carcass dressing, fat deposition, average daily gain, empty body gain, and average daily gain without the gravid uterus were analyzed as a  $4 \times 2$  factorial design. Intake, apparent total-tract digestibility, N balance, urinary concentration of urea, and purine derivatives data were analyzed as repeated measurements taken over the 28-d period. Pregnant cows were slaughtered on 4 different DG: 139, 199, 241, and 268 d. Overall, DM intake decreased as DG increased. This decrease observed in DM intake may be associated with the reduction in ruminal volume caused by the rapid increase in fetal size during late gestation. We observed an interaction for DM and organic matter apparent total-tract digestibility between FR and DG; at 150, 178, and 206 d of gestation, ML-fed cows had greater DM and organic matter apparent total-tract digestibility values than AL-fed cows. Rib fat thickness, mesentery, and kidney, pelvic, and heart fat were greater in AL-fed than in ML-fed cows at all DG, with the exception of rib fat thickness on d 139. Ad libitum-fed cows excreted more N in their feces and urine compared with ML-fed cows. Pregnant cows that were fed at maintenance had greater digestibility during some DG, excreted less N in feces and less N and urea in urine, and deposited less fat in the body. We therefore recommend ML (1.15% of body weight with 93% of roughage) as a FR for pregnant dry cows; however, during the last month of gestation, AL seems to be the most appropriate FR to avoid loss of body weight.

**Key Words:** ad libitum, maintenance, performance

**W378 Effects of day of gestation and feeding regimen in Holstein  $\times$  Gyr cows on maternal and fetal visceral organ mass.** Polyana P. Rotta\*<sup>1,2</sup>, Sebastiao C. Valadares Filho<sup>1</sup>, Terry E. Engle<sup>2</sup>, Luiz Fernando Costa e Silva<sup>1,2</sup>, Marcos I. Marcondes<sup>1</sup>, Mariana M. Campos<sup>3</sup>, Tathyane R. S. Gionbelli<sup>1</sup>, Luis H. R. Silva<sup>1</sup>, Edilane C. Martins<sup>1</sup>, Flavia A. S. Silva<sup>1</sup>, and Faider A. C. Villadiego<sup>1</sup>, <sup>1</sup>Universidade Federal de Viçosa, Viçosa, Minas Gerais, Brazil, <sup>2</sup>Colorado State University, Fort Collins, Colorado, <sup>3</sup>Embrapa Gado de Leite, Juiz de Fora, Minas Gerais, Brazil.

This study investigated the influence of day of gestation (DG) and feeding regimens (FR) on maternal and fetal visceral organ mass in Holstein

× Gyr cows. Forty-four pregnant multiparous Holstein × Gyr cows with an average initial body weight of  $480 \pm 10.1$  kg and an average initial age of  $5 \pm 0.5$  yr were allocated to 1 of 2 FR: ad libitum (AL;  $n = 20$ ) or maintenance level (ML;  $n = 24$ ). Maintenance level was considered to be 1.15% of body weight (dry matter basis) and met 100% of the energy requirements; AL provided 190% of the total net energy requirements. Cows were individually fed a corn silage and concentrate-based diet composed of 93% roughage and 7% concentrate (dry matter basis) as a total mixed ration twice daily. Pregnant cows were slaughtered at 4 DG: 139 ( $n = 11$ ), 199 ( $n = 11$ ), 241 ( $n = 11$ ), and 268 ( $n = 11$ ) d, which was followed by necropsy. Mass of heart, liver, and gastrointestinal tract was greater in AL- than in ML-fed cows. Mammary gland mass was greater in AL- than in ML-fed cows, and the greatest mass was observed at 268 d of gestation. Feeding regimen did not influence fetal body weight in this study. The majority of the visceral organ masses were similar in fetuses from cows fed AL or ML. These data indicate that maternal feed restriction does not affect the development of most fetal organs or fetal development; however, some maternal organs are affected by the FR provided. Moreover, the negative effect on mammary gland mass caused by ML feeding will probably not affect the subsequent lactation because the crude protein concentration in the mammary gland increased with ML feeding. However, we suggest that the AL diet in pregnant dry cows should be provided with caution because the amount of fat in the mammary gland increased at 268 d of gestation.

**Key Words:** fetal development, gastrointestinal tract, mammary gland

**W379 Dietary supplementation of palm- versus high-linoleic safflower oil to mid-lactating Holstein cows: Intake and milk fat yield.** Shahryar Kargar<sup>1</sup>, Clayton M. Stoffel<sup>2</sup>, Lou E. Armentano<sup>3</sup>, and Francisco E. Contreras-Govea<sup>\*3</sup>, <sup>1</sup>Department of Animal Sciences, College of Agriculture, Shiraz University, Shiraz, Iran, <sup>2</sup>Papillon Agricultural Co., Easton, MD, <sup>3</sup>Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.

Cows were fed diets supplemented with either palm oil (PO, rich in C16:0 and C18:1) or high-linoleic safflower oil (SO, rich in C18:2) at 1.5% of dietary DM. Sixty-four primiparous and multiparous, cows with an average of  $100 \pm 21.7$  DIM,  $48.6 \pm 10.3$  kg milk yield per d, and  $657 \pm 70.3$  kg body weight at trial initiation were fed the 2 diets for 56 d, after a 2-week covariance period. Thirty-two primiparous and multiparous cows were assigned to one diet, and the other 32 cows to the other diet. The experimental design was a randomized complete block, blocking by parity. Cows were housed in a free-stall barn equipped with roughage intake control system gates (Insentec BV, Marknesse, the Netherlands), which recorded individual cow feed intake continuously. Milk yield was measured daily, and milk composition and cow's body weight were measured weekly. Data were analyzed as a randomized complete block design with diet, parity, week, and interactions as fixed effects, cow as random effect, and week as repeated measurement (SAS Institute, 2003). There was not difference in DMI (26.6 kg/d) and milk yield (46.5 kg/d) between the 2 diets ( $P > 0.05$ ) but feeding PO instead of SO raised milk fat concentration (3.88 vs. 3.55%) and yield (1.79 vs. 1.65 kg/d) ( $P < 0.05$ ), but milk protein yield tended ( $P = 0.10$ ) to increase for PO (1.39 kg/d) than SO (1.36 kg/d). Feeding SO increased *trans*-C18:1 including *trans*-6/8, *trans*-9, *trans*-10, and *trans*-12. For cows fed PO vs. SO, yields of de novo (<16 carbons; 447 vs. 412 g/d) and preformed (>16 carbons; 701 vs. 662 g/d) fatty acids were no difference between the 2 diets. Yield of mixed origin fatty acids (C16:0 + C16:1) increased for cows fed PO (515 vs. 379 g/d), possibly due to less inhibition of endogenous synthesis of C16 when feeding less dietary C18:2; as well as providing more exogenous dietary C16 from palm

oil. These results confirm the greater milk fat depressing effects of oils containing higher concentration of C18:2, as safflower has, relative to a combination of C18:1 and C16:0 as palm oil.

**Key Words:** palm oil, high-linoleic safflower oil, milk fat

**W380 Short- and medium-term changes in glucose metabolism and insulin sensitivity of dairy calves offered different amounts of milk replacer early in life.** Cristina Yunta<sup>1</sup>, Marta Terré<sup>1</sup>, and Alex Bach<sup>\*2,1</sup>, <sup>1</sup>Department of Ruminant Production, IRTA (Institut de Recerca i Tecnologia Agroalimentàries), Caldes de Montbui, Spain, <sup>2</sup>ICREA (Institució Catalana de Recerca i Estudis Avançats), Barcelona, Spain.

The objective of the present study was to evaluate the consequences of 3 allowances of milk replacer (MR) during the first 2 mo of life on short- and medium-term glucose metabolism and insulin sensitivity (IS) of dairy replacement heifers. Forty-five newborn female Holstein calves ( $40.7 \pm 4.94$  kg) after receiving colostrum were randomly allocated to 4, 6, or 8 L/d of MR until 63 d of life ( $n = 15$ ). A glucose tolerance test (GTT) was performed at 42, 86 and 300 d of life. Area under the curve for plasma insulin (IAUC), clearance rates of glucose (CRG) and insulin (CRI), insulin to glucose rate (ItoG), and IS were calculated. Data were analyzed using a mixed-effects model with repeated measures. There were no differences ( $P = 0.67$ ) in CRG among treatments, although they decreased from  $10.1 \pm 0.55\%/min$  at 42 d of age to  $6.7 \pm 0.56\%/min$  at 300 d of age. Interestingly, CRI was greatest ( $P < 0.05$ ) at 42 d of age, and calves fed 8 L/d had the greatest ( $P < 0.05$ ) ICR throughout the study. Insulin release (measured as IAUC) after a GTT increased ( $P < 0.005$ ) with age, and the increase observed between 42 and 300 d of life was more ( $P < 0.05$ ) marked in calves that received 4 (from  $1,000 \pm 234.0$  to  $3,319 \pm 242.7$   $\mu U/mL \times 60$  min) or 6 L/d (from  $1,538 \pm 226.1$  to  $3,887 \pm 242.6$   $\mu U/mL \times 60$  min) than in those receiving 8 L/d (from  $1,735 \pm 226.1$  to  $2,940 \pm 242.6$   $\mu U/mL \times 60$  min). The amount of MR offered had short- and medium-term effects on ItoG, with calves fed 4 and 6 L/d having lower ( $P < 0.05$ ) values ( $145.2 \pm 16.60$   $\mu U/mg$ ) than calves fed 8 L/d ( $215.3 \pm 16.02$   $\mu U/mg$ ) independently of age. Insulin sensitivity tended ( $P = 0.07$ ) to be lesser in calves fed 8 than in calves fed 4 or 6 L/d ( $1.39 \pm 0.04$  vs  $1.82 \pm 0.05$  mL/min  $\times$   $\mu U/mL$  per kg of BW, respectively). This difference was mainly due to a low IS of calves fed 8 L/d at 42 d, but as age increased, IS tended ( $P = 0.06$ ) to become progressively similar among treatment groups. It is concluded that offering 4 L of MR twice daily elicits a decrease in IS and an increase in ItoG while animals are consuming MR, and the IS returns to normal values over time, but the increase in ItoG is maintained with age.

**Key Words:** calves, enhanced feeding, metabolism

**W381 Energy expenditure in crossbred (Holstein x Gyr) calves differing in phenotypic residual feed intake.** Juliana Mergh Leão<sup>\*1</sup>, Fernanda Samarini Machado<sup>2</sup>, Alexandre Lima Ferreira<sup>2</sup>, Mariana Magalhães Campos<sup>2</sup>, Juliana Campos Carneiro<sup>3</sup>, Paulo Campos Martins<sup>1</sup>, Juliana Aparecida Mello Lima<sup>2</sup>, Thierry Ribeiro Tomich<sup>2</sup>, Luiz Gustavo Ribeiro Pereira<sup>2</sup>, Rayanne Soalheiro de Souza<sup>1</sup>, and Sandra Gesteira Coelho<sup>1</sup>, <sup>1</sup>Universidade Federal de Minas Gerais-UFMG, Belo Horizonte, Minas Gerais, Brazil, <sup>2</sup>Embrapa Dairy Cattle, Juiz de Fora, Minas Gerais, Brazil, <sup>3</sup>Instituto de Ciências Agrárias da UFMG, Montes Claros, Minas Gerais, Brazil.

The aim of this study was to evaluate the energy expenditure in crossbred Holstein-Gyr (F<sub>1</sub>) calves at 50 d of age with different phenotypes for residual feed intake (RFI) by measuring the respiratory gas exchanges

with a face-mask. Eighteen calves were housed in individual sand bed stalls in the experimental farm of Embrapa Dairy Cattle (Coronel Pacheco, Brazil). All animals were fed 6 L/d of whole milk (TS; 11.75%), divided in 2 equal meals (7 and 15h). Solid diet consisting of 95% of pelleted calf starter (88% DM; 20% CP and 3% Fat) and 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. Phenotypic RFI was calculated for each animal as the difference between actual dry matter intake (DMI) and expected DMI. Expected DMI was computed for each animal by regressing average daily DMI on conceptus-adjusted mean BW<sup>0.75</sup> and conceptus-adjusted ADG over a 60 d period. Twelve animals were ranked by RFI into low (efficient) and high (inefficient) groups. Oxygen consumption, carbon dioxide and methane production data were recorded in calves with 50 d age using Sable System (Sable Systems, Henderson, NV) attached to a facemask over a 2 d period, 3 h after morning milk supply, during 20 min. Heart rate (HR) was recorded during 20 min with Polar equine transmitter and monitor (RS800CX G3, Polar Electro Inc., Finland). Daily energy expenditure was calculated as heat production estimated from Brouwer equation (Brouwer, 1965). Methane production was not detected and urinary nitrogen was neglected in calculations. Data were analyzed as a completely randomized design by ANOVA using the GLM procedure of SAS software (version 9.4; SAS Institute Inc., Cary, NC). High RFI calves had DMI 12.39% higher ( $P < 0.05$ ) than the low RFI group (1.07 and 0.941 Kg/d, respectively), but body weight was higher by 0.6% ( $P < 0.05$ ) for the low RFI group. Heart rate did not differ between the groups. High RFI animals presented higher energy expenditure (177.64 kcal/kg of metabolic weight – MBW) than low RFI animals (144.40 kcal / kg of MBW).

**Key Words:** facemask, heat production, performance

**W382 The effect of different energy and nitrogen sources on in vitro fiber digestion of high and low quality roughages.** Christian W. Cruywagen\* and Mari Strauss, *Stellenbosch University, Stellenbosch, South Africa.*

Three energy sources (starch, sucrose and pectin) were used alone (without any N addition), or in combination with either soybean meal or urea as N source to investigate their effect on the in vitro digestion of NDF from alfalfa hay and wheat straw. The ratio of energy source to forage substrate was 50:50 on a hexose equivalent:NDF basis. Forage control treatments, without any energy or N sources added, were also included. The Goering/Van Soest incubation medium was used with slight modification; ammonium bicarbonate and tryptose were omitted (for the treatments with energy sources alone), or replaced by a similar amount of N from either soybean meal or urea. In the forage control treatments, the medium was used without modification. The trial was repeated in 6 runs. Samples were incubated at 39°C for 6 or 30 h. The substrate control data were analyzed per time according to a main effects ANOVA. For the factorial experiment, the data were also analyzed per time, but according to a 3 way factorial ANOVA with substrate, energy source and nitrogen source as factors. The 3 way interactions were interpreted with LSD multiple comparisons and Bonferroni tests. Significance was declared at  $P < 0.05$ . After 6 h of fermentation, NDF digestion was higher ( $P < 0.01$ ) in alfalfa hay (13.6%) than in wheat straw (8.1%), but energy and nitrogen source had no effect in either forage. After 30 h of fermentation, sucrose with soybean meal (35.8%) did not differ from starch and soybean meal (29.0%), but compared with no supplementation (26.4%) it was the only combination that increased NDF digestion of alfalfa hay significantly ( $P < 0.01$ ). In wheat straw, pectin with soybean meal (16.5%) did not differ from sucrose with soybean meal (12.4%), sucrose with urea (12.6%), pectin alone

(15.0%) or starch alone (13.5%), but compared with no supplementation (11.5%) it was the only combination that increased wheat straw NDF digestion significantly ( $P < 0.02$ ). In the current study, it appeared that sucrose and soybean meal was the best energy-nitrogen combination for alfalfa hay NDF digestion, whereas pectin and soybean meal was the best combination for wheat straw NDF digestion.

**Key Words:** NDF digestion, energy source, nitrogen source

**W383 Milk fatty acid profile of dairy cows fed omega 3 and 6 fatty acid sources during transition period and early lactation.**

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The objective of this study was to evaluate the effects of omega 3(n-3) and 6 (n-6) fatty acids (FA) supplementation on milk FA profile of dairy cows in early lactation. Forty 8 Holstein cows were divided randomly distributed into 4 groups to receive one of the diets: 1) Control (C, n = 12), without FA supplementation; 2) Flaxseed (WF, n = 12), addition of 60 and 80 g/kg diet DM of whole flaxseed (n-3 FA source) during pre and postpartum, respectively; 3) Soybean (WRS, n = 12), addition of 120 and 160 g/kg diet DM of whole raw soybean (n-6 FA source) during pre and postpartum, respectively; 4) Calcium salts of FA (CSFA, n = 12), addition of 24 and 32 g/kg diet DM of Megalac E (n-6 FA source) during pre and postpartum. The diets were offered from 35 d of expected calving date until 84 DIM and were formulated according to NRC (2001). Milk samples were collected weekly, proportionally of each milking (6h00 and 15h00) and proportionally of each cow yield. The extraction of lipids was made according to Feng et al. (2004) and methylated according to Kramer et al. (1997). Fatty acids were quantified by gas chromatography. Data were subjected to PROC MIXED of SAS (2004) using as fixed effect diet and weeks, and using as random effect interaction between week and diet and residual error. Orthogonal contrasts were performed (C vs WRS+CSFA+CSFA; WRS vs CSFA and WF vs WRS+CSFA). Fat supplemented diets increased total saturated FA compared with control ( $P < 0.01$ ) and tended to increase C18 unsaturated FA ( $P = 0.08$ ). Fatty acids with less than 16C were decreased with and FA with higher than 16C were increased FA supplementation. Whole raw soybeans increased C18:3, C18:2 and decreased C18:1 trans-11 FA compared with CSFA. However, SCFA presented higher unsaturated / saturated ratio than cows fed WRS. Increased cis-9, trans-11 FA was observed in cows fed WRS and CSFA when compared with WF. Whole flaxseed did not increase unsaturated FA in milk and SCFA increased the total unsaturated FA acids in milk. Supplementation of n-3 and n-6 FA altered milk fatty acid profile of early lactating cows.

**Key Words:** calcium salts of fatty acid, flaxseed, soybean

**W384 The effect of feeding nucleotides on milk production, reproductive performance and immunity in lactating Holstein dairy cows.**

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Preliminary evidence suggests that nucleotide feeding improves fertility in dairy cows, most likely mediated by an improvement in general and (or) local immunity of the reproductive tract. The objective of this research was to determine the effect of supplementing nucleotides on dairy cow milk production, reproductive performance and immunity. Two hundred and 60 adult ( $\geq 2$  lactations) Holstein dairy cows (87 d in milk) were divided in 2 groups and fed a standard diet (40% forage, 60% concentrate) without (CONTROL) or with nucleotides supplement (NUCLEOFORCE COWS, Biobierica S.A.) included at 4 g/cow/day during a period of 7 mo (May through December, 2014). Milk production (daily) and composition (monthly) were measured throughout the experimental period, and days from calving to first insemination and calving to pregnancy recorded. All cows were re-vaccinated against clostridia after 6 weeks in treatment. Serum IgG ( $n = 160$ ) were determine immediately before and 8 weeks after vaccination, and mucus from the vagina ( $n = 34$ ) was collected on the day of insemination at least 6 weeks after vaccination to measure IgA concentrations. Data were analyzed with PROC GLM of SAS and differences declared at  $P < 0.05$ . There were no differences ( $P > 0.10$ ) in milk production (42.8 kg/d), milk fat content (2.85%) and yield (1.23 kg/d), milk protein content (3.13%) and yield (1.34 kg/d) or somatic cell counts (387,000 counts/mL) between treatments. However, the ratio pre-vaccination vs. post-vaccination serum IgG tended to be higher ( $P < 0.08$ ) in the nucleotides supplementation (1.04) compared with CONTROL (0.93) treatment. Concentrations of IgA in vaginal mucus were higher ( $P < 0.05$ ) in nucleotides supplementation compared with CONTROL (2274 vs. 2078 ng/mL) treatment. Results indicate that the supplementation with nucleotides improved the indicators of general and reproductive tract immunity in dairy.

**Key Words:** immunity, nucleotide, dairy cow

**W385 Evaluation of a proprietary blend of essential oil and cobalt on a commercial dairy.** Olivia Kuester\* and David Casper, *South Dakota State University, Brookings, SD.*

An 18 week field trial was conducted on a commercial dairy in southwest Minnesota equipped with 2 Lely robotic milking units. This study was designed to evaluate the response of feeding a proprietary essential oil and cobalt product (EOC) on the lactational performance and nutrient digestibility of lactating Holstein dairy cows. Cows were divided between 2 pens ( $57 \pm 2$  cows and  $59 \pm 3$  cows for treatment [EOC] and control [C] pens, respectively), based on cow parity ( $2.65 \pm 1.52$  and  $2.33 \pm 1.20$ ), days in milk (DIM) ( $184 \pm 103$  and  $154 \pm 94.2$ ), and milk production ( $35.4 \pm 11.3$  kg/d and  $36.9 \pm 11.3$  kg/d) before study initiation. Cows were fed either an EOC or C total mixed ration (TMR) 2x/d. Cows fed the EOC averaged  $2.72 \pm 0.11$  milkings/d and cows fed C averaged  $2.80 \pm 0.13$  milkings/d. Production data were collected daily from Lely Time for Cows (T4C) robotic milking software and was reduced to weekly observations for each cow. Fecal samples were collected weekly from each pen based on a composite from 15 cows. Weekly TMR samples were collected from EOC and C pens and were composited monthly. Milk samples were collected once every 2 weeks from all cows and were analyzed for milk components and somatic cell counts (SCC) by the Dairy Herd Improvement Association (DHIA). Daily milk production was not different ( $P = 0.26$ ) between EOC and C fed cows, but was numerically greater for cows fed EOC ( $41.9 \pm 1.91$  kg/d) than cows fed C ( $38.8 \pm 2.05$  kg/d). The deviation in milk yield was greater ( $P = 0.01$ ) for cows fed EOC (0.42 kg/d) than cows fed C ( $-0.75$  kg/d). Total-tract NDF digestibility was numerically greater ( $P = 0.36$ ) for cows fed EOC (54.4% NDFD) than cows fed C (50.6% NDFD). Cows fed EOC had lower ( $P = 0.0002$ ) milk urea nitrogen

(MUN) ( $15.2 \pm 0.14$  mg/dL) than cows fed C ( $15.9 \pm 0.14$  mg/dL). Feeding the proprietary EOC product on a commercial dairy operation, which used robotic milking units numerically increased milk production and total-tract NDF digestibility.

**Key Words:** essential oil, cobalt, commercial dairy

**W386 Evaluation of rumen undegradable protein sources on lactational performance of Holstein dairy cows.** Heather A. Tucker<sup>1</sup>, Shane M. Fredin\*<sup>1</sup>, Heather M. Dann<sup>1</sup>, Kurt W. Cotanch<sup>1</sup>, Catherine S. Ballard<sup>1</sup>, Les W. Berghorn<sup>2</sup>, and Rick J. Grant<sup>1</sup>, <sup>1</sup>William H. Miner Agricultural Research Institute, Chazy, NY, <sup>2</sup>Afgritech LLC, Watertown, NY.

This study compared the effects of feeding a proprietary blend of heat-treated canola and soybean meal (AminoMax; Afgritech LLC; Watertown, NY) or heat-treated soybean meal (AminoPlus; Ag Processing Inc.; Omaha, NE) on lactational performance of dairy cows. Forty-two lactating Holstein dairy cows (21 per treatment) averaging  $866 \pm 98$  kg of BW and  $146 \pm 30$  DIM at study initiation were used in a randomized complete block design with a 2-wk covariate period followed by an 8-wk treatment period. Treatments were diets containing (DM basis) 7.7% AminoMax or AminoPlus. Diets also contained 19.3% conventional corn silage, 19.3% brown midrib corn silage, 14.0% hay crop silage, and 39.7% concentrate mix (excluding AminoMax or AminoPlus). Both diets contained 15.7% CP, 30.9% NDF, and 25.4% starch. Dry matter intake and milk yield were collected daily and milk composition was determined over 3 milkings once per week. Cow served as the experimental unit. Data from the last 7 d of the covariate period were used as a covariate adjustment. Data were analyzed by ANOVA using the MIXED procedure of SAS containing model effects of covariate, treatment, time, and treatment  $\times$  time. Dry matter intake ( $27.4 \pm 0.2$  kg/d), solids-corrected milk (SCM) yield ( $43.8 \pm 0.8$  kg/d), milk fat yield ( $1.76 \pm 0.04$  kg/d), and milk true protein yield ( $1.39 \pm 0.02$  kg/d) were unaffected by diet ( $P > 0.10$ ). Milk lactose yield tended to increase ( $2.14$  versus  $2.06 \pm 0.03$  kg/d;  $P = 0.09$ ) for cows fed AminoPlus. Milk urea N concentration was decreased for cows fed AminoMax ( $11.4$  vs.  $9.6 \pm 0.2$  mg/dL;  $P < 0.01$ ). Efficiency of SCM production (SCM/DMI;  $1.59 \pm 0.02$  kg/kg) and N efficiency ( $0.322 \pm 0.005$  kg/kg) were unaffected ( $P > 0.10$ ) by diet. In conclusion, lactational performance did not differ between the products, but N utilization appeared to be improved for cows fed AminoMax.

**Key Words:** lactation, rumen undegradable protein

**W387 Evaluation of industry growth chart equations from birth until first calving of Holstein heifers fed a high plane of nutrition.** Marie E. Iwaniuk\*<sup>1</sup>, Jill A. Davidson<sup>2</sup>, Catherine M. Bradley<sup>2</sup>, and Tom J. Earleywine<sup>3</sup>, <sup>1</sup>University of Maryland, College Park, MD, <sup>2</sup>Purina Animal Nutrition, Gray Summit, MO, <sup>3</sup>Land O'Lakes Animal Milk Products, Shoreview, MN.

The 2013 Penn State Customized Heifer Growth Chart (PS) was created as a tool to enable dairy producers to create herd-based heifer performance goals and track the performance of individual heifers until first calving. The prediction equations used in the program were derived from body weight (BW) and hip height (HH) benchmarks established by the 2001 *Nutrient Requirements of Dairy Cattle* and as previously reported BW and HH prediction equations (Heinrichs et al., 1992; Kertz et al., 1998). These prediction equations were derived from data sets in which heifers were fed standard diets; however, these prediction equations have yet to be evaluated for heifers fed a higher

plane of nutrition. The objective of this study was to evaluate the PS prediction equations for BW and HH growth using data of heifers fed a high plane of nutrition at the Purina Animal Nutrition Center. PS growth curves for BW and HH were predicted using mature cow herd data as target parameters. To evaluate the PS equations, residuals were regressed on centered predicted values to detect the presence of mean and/or linear biases using methods published by St-Pierre et al. (2006). For BW, the linear bias was not significant ( $P = 0.2131$ ); however, a significant mean bias ( $P < 0.05$ ) indicated that the PS equations overestimate BW by an average of 1.63kg for heifers fed a high plane of nutrition. In regards to HH growth, a significant linear bias (0.53cm;  $P < 0.05$ ) demonstrated that the PS equations overestimate HH from wk 0 to wk 5 and underestimate HH from wk 20 to wk 100 for heifers fed a high plane of nutrition. In addition, a significant mean bias indicated that the PS equations underestimate HH by an average of 5.41cm ( $P < 0.05$ ). Moreover, the presence of mean biases for both BW and HH further indicates that collectively the PS equations do not accurately describe overall growth patterns of heifers fed a high plane of nutrition. Therefore, new BW and HH equations should be developed that adjust for level of nutrition.

**Key Words:** Penn State Customized Heifer Growth Chart, growth curve

**W388 Milk urea nitrogen as a predictor of urinary nitrogen excretion in late lactation dairy cows fed four levels of dietary crude protein.** Tiago Barros\* and Michel A. Wattiaux, *Department of Dairy Science, University of Wisconsin-Madison, Madison, WI.*

Our objective was to assess the relationship between milk urea nitrogen (MUN, mg/dL) and blood urea nitrogen (BUN, mg/dL), and the excretion of urinary nitrogen (UN, g/d), and urinary urea nitrogen (UUN, g/d) in late lactation dairy cows. Holstein cows (means  $\pm$  SD; 736  $\pm$  18 kg BW; 224  $\pm$  54 DIM at the start of the trial) were used in a 16-pen study with 8 cows per pen, and fed one of 4 TMR containing either 11.9, 13.4, 14.4, or 16.3% CP (DM basis) for 12 weeks. Cows ( $n = 4$  per pen) were sampled for BUN on wk 4, 8 and 12. Daily MUN (infrared analysis) was derived from sampling 4 cows per pen in 2 consecutive milkings in wk 3, 7 and 11. In the same 3 weeks, urine volume was estimated using creatinine concentration in spot urine samples collected 6 h before and 6 h after feeding in groups of 4 randomly selected cows in each pen. Linear models were built using PROC REG and differences in slopes were tested using PROC GLM in SAS. Week of sampling did not affect the slope between BUN and MUN ( $P = 0.11$ , Table 1), but affected the slope of the relationship between UN and MUN ( $P = 0.042$ ) and between UUN and MUN ( $P = 0.029$ ). Increased dietary CP resulted in increases in UUN/UN ratio (0.54, 0.68, 0.74, and 0.81). However, there was no difference in slope when UN or UUN were regressed against MUN. Excretion of non-urea-N was constant (40.8 g/d). Four published models showed a slope bias and over-predicted UN when applied to our data set (227, 214, 176 and 125 g/d compared with 116 g/d observed UN). The week effect observed here, and the inadequacy of previous models suggested that best equations to predict UN from MUN should consider stage of lactation.

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**Table 1 (Abstr. W388).**

Y variable	X variable	Week	Intercept	SE	Slope	SE	R <sup>2</sup>	RMSE
BUN	MUN	3	0.19 <sup>1</sup>	0.79	0.96	0.076	0.94	0.94
		7	1.70 <sup>1</sup>	1.23	0.75	0.116	0.75	1.72
		11	-1.99 <sup>1</sup>	2.20	1.18	0.214	0.68	1.81
UN	MUN	3	47.4	7.3	6.95 <sup>b</sup>	0.704	0.87	8.65
		7	29.3	9.1	8.15 <sup>ab</sup>	0.084	0.90	12.50
		11	3.70 <sup>1</sup>	11.4	10.90 <sup>a</sup>	1.106	0.87	9.32
UUN	MUN	3	5.80 <sup>1</sup>	5.98	7.93 <sup>b</sup>	0.576	0.93	7.07
		7	-12.2	9.46	9.62 <sup>ab</sup>	9.46	0.90	13.01
		11	-35.5	10.6	11.54 <sup>a</sup>	1.032	0.90	8.70

<sup>ab</sup>Slopes among weeks for each set of regressions with a different superscript differ at  $P < 0.05$ .

<sup>1</sup>Intercept is not different from zero at  $P < 0.05$ .

**Key Words:** nitrogen excretion, BUN, MUN

**W389 Phosphorous excretion and digestibility in Jersey and Holstein consuming corn milling co-products.** Gabriel Garcia Gomez\*, Alison Foth, and Paulk 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  $\pm$  12.9 kg) and 8 Jersey (BW = 429.1  $\pm$  13.0kg) multiparous, lactating cows (93  $\pm$  20 d DIM) in a repeated switchback design. The concentration of P in the test treatments were 0.44% and 0.59%  $\pm$  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 versus 1.27  $\pm$  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 versus 89.70  $\pm$  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  $\pm$  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  $\pm$  0.07%, respectively;  $P = 0.36$ ), digestibility (32.3 versus 29.0  $\pm$  2.83%, respectively;  $P = 0.40$ ) and P efficiency (19.4 vs 18.0  $\pm$  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.

**W390 Relationship between protein molecular structure and protein metabolic characteristics of co-products from bio-oil processing (rapeseed meal, canola meal and soybean meal) in dairy cattle.** Xinxin Li\*<sup>1,2</sup>, Yonggen Zhang<sup>2</sup>, and Peiqiang Yu<sup>1,2</sup>, <sup>1</sup>*Department of Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada,* <sup>2</sup>*College of Animal Science and Technology, Northeast Agricultural University, Harbin, China.*



This study was aimed to detect the relationship between protein inherent molecular structure features and protein rumen degradation kinetics and intestinal digestibility of 3 types of co-products from bio-oil processing including rapeseed meal, canola meal and soybean meal for dairy cattle. Three sources of rapeseed meal, 3 sources of soybean meal and 3 sources of canola meal were collected in 2014. The protein molecular structure in terms of chemical functional groups in these feed samples was analyzed using attenuated total reflectance-Fourier transform infrared spectroscopy (ATR-FT/IR) instrument. Rumen protein degradation kinetics was determined using in situ nylon bag method. Intestinal digestibility of rumen undegraded feed protein were determined according to the 3-step in vitro procedure with 12h pre-rumen incubated feed samples. The PROC MIXED procedure of SAS 9.3 was used to analyze the univariate protein spectral data, protein degradation kinetics and intestinal digestibility data. After normality test of the data, PROC CORR of SAS with a SPEARMAN option was used to investigate the relationship between protein molecular structure and metabolic characteristics in dairy cattle. Significances were declared at  $P < 0.05$ . The results showed that the ratio of protein amide I to amide II spectral peak height had a highly negative correlation with rumen undegraded protein (RUP<sup>NRC</sup>;  $r = -0.67$ ,  $P = 0.05$ ), intestinal absorbable rumen degraded protein (IADP;  $r = -0.67$ ,  $P = 0.05$ ), intestinal digestible protein (IDP;  $r = -0.77$ ,  $P < 0.05$ ) and total digestible protein (TDP;  $r = -0.67$ ,  $P = 0.05$ ). Additionally, the ratio of protein secondary structure  $\alpha$ -helix to  $\beta$ -sheet height was negatively correlated with rumen undegradable protein (RUP<sup>NRC</sup>;  $r = -0.67$ ,  $P < 0.05$ ), rumen degradable protein (EDCP;  $r = -0.83$ ,  $P < 0.05$ ), and total digestible protein (TDP;  $r = -0.93$ ,  $P < 0.05$ ). The results indicated that protein molecular structure was highly related with its nutrient supply to dairy cattle.

**Key Words:** protein molecular structure, protein metabolic characteristics, correlation study

**W391 The effect of decreasing dietary cation-anion difference in the prepartum diet on dry matter intake, milk production and milk composition in multiparous Holstein cows.** B. M. Sweeney<sup>\*1</sup>, C. M. Ryan<sup>1</sup>, K. Zanzalari<sup>2</sup>, D. Kirk<sup>2</sup>, and T. R. Overton<sup>1</sup>, <sup>1</sup>Department of Animal Science, Cornell University, Ithaca, NY, <sup>2</sup>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 dry matter intake (DMI), milk production and milk composition. 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 DCAD 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. Repeated measures analyses were conducted using the MIXED procedure of SAS with linear and quadratic effects of decreasing prepartum DCAD as contrasts. A quadratic effect of decreasing DCAD on prepartum DMI was observed (CON = 14.6 kg/d, MED = 15.1 kg/d, LOW = 14.1 kg/d;  $P < 0.01$ ). Postpartum DMI as a percent of body weight through wk 3 increased linearly with decreasing DCAD (CON = 2.94%, MED = 3.04%, LOW = 3.15%;  $P = 0.03$ ) and tended to be increased through wk 9 ( $P = 0.07$ ). During wk 1 to 3, milk yield (CON = 40.5 kg/d, MED = 42.1 kg/d, LOW = 43.8 kg/d;  $P = 0.03$ ) and fat yield were increased linearly ( $P = 0.13$ ) by decreasing prepartum DCAD resulting in a trend for greater 3.5% fat-corrected milk ( $P = 0.07$ ). Protein percent decreased linearly ( $P < 0.01$ ) with no effect on yield. Lactose yield ( $P = 0.02$ ), total solids content ( $P = 0.01$ ) and total solids yield ( $P = 0.06$ ) were increased

linearly, with a trend for greater energy-corrected milk for cows fed a lower DCAD ( $P = 0.08$ ). Milk urea nitrogen increased linearly ( $P = 0.04$ ) and there tended to be a quadratic effect on somatic cell score ( $P = 0.06$ ). Effects of prepartum DCAD on milk yield and composition were smaller when assessed from wk 1 to 9. Cows fed lower prepartum DCAD had linearly decreased protein percent ( $P = 0.02$ ) through wk 9 with a trend for a quadratic effect on yield ( $P = 0.11$ ). Ultimately, there was no difference in fat or energy-corrected milk through wk 9. Feeding decreasing prepartum DCAD linearly increased DMI and milk yield during early lactation.

**Key Words:** dietary cation-anion difference, transition cow

**W392 Effect of *Bacillus pumilus* on performance of primiparous dairy cows fed low or high starch diets.** D. N. Lobão da Silva<sup>\*1</sup>, Z. Sawall<sup>1</sup>, J. Guillen<sup>1</sup>, E. Galbraith<sup>2</sup>, T. Parrott<sup>3</sup>, M. Endres<sup>1</sup>, and N. B. Litherland<sup>1</sup>, <sup>1</sup>University of Minnesota, Saint Paul, MN, <sup>2</sup>DuPont Nutrition and Health, Waukesha, WI, <sup>3</sup>DuPont Industrial Biosciences, Waukesha, WI.

*Bacillus* species offer advantages as direct-fed microbials (DFMs) because their spores tolerate the harsh environment of the gastrointestinal tract and some strains can produce enzymes such as xylanase which breaks down xylan and the bonds that link lignin to cellulose, potentially increasing dietary fiber digestibility. This study aimed to investigate the effects of *Bacillus pumilus* 8G-134 (BP) supplementation on performance of primiparous dairy cows fed low (20%, LS) or high (27%, HS) starch diets from calving to 112 DIM. We hypothesized that BP would improve total-tract nutrient digestibility and performance of cows fed LS or HS diets postpartum. Forty-eight ( $n = 12$ /treatment) primiparous cows were assigned to a common prepartum diet 42d before expected calving date. At calving, cows were fed a LS or HS lactation diet and BP carrier or BP was top dressed on the TMR once daily to provide  $5 \times 10^9$  cfu/cow/day until 112 DIM. Factors combined produced 4 treatments: LS+carrier (LSCO); LS+BP (LSBP); HS+carrier (HSCO); HS+BP (HSBP). Fecal samples collected on d 7, 14 were analyzed for NDFd. Data were analyzed using the MIXED procedure of SAS. Apparent total-tract NDFd was greater ( $P = 0.05$ ) for diets supplemented with BP, by 4.4% (LS) and 8.5% (HS) which tended to increase milk yield, 3.5% FCM and 3.5% ECM by 13.9%, 14.9% and 15.2% for HS diets, but increases in LS supplemented diets were less apparent. The 112 d of BP supplementation increased DMI and milk fat yield, and tended to improve yields of 3.5% FCM and milk protein. Results indicated that BP supplementation to primiparous cows can increase fiber digestibility and milk yield resulting in potential increases in profitability.

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**Table 1 (Abstr. W392).** Intake and lactation performance

Item (kg/d)	Treatments				SEM	<i>P</i> -value		
	LS		HS			S	BP	S × BP
	CO	BP	CO	BP				
<b>28 DIM</b>								
DMI	15.27	16.15	14.23	15.08	0.90	0.16	0.25	0.98
Milk	27.96	28.30	25.92	29.52	1.21	0.73	0.10	0.18
3.5% FCM	28.07	28.57	25.75	29.60	1.24	0.60	0.08	0.17
3.5% ECM	28.33	28.80	26.03	29.99	1.24	0.66	0.08	0.16
<b>112 DIM</b>								
DMI	18.97	20.58	18.85	20.30	0.94	0.73	0.01	0.89
3.5% FCM	31.63	33.00	30.87	33.62	1.71	0.95	0.06	0.51
Milk fat	1.10	1.15	1.06	1.18	0.07	0.87	0.04	0.42
Milk protein	0.97	1.03	1.00	1.07	0.04	0.30	0.06	0.92

**Key Words:** *Bacillus pumilus*, dietary starch, primiparous

**W393 Balancing dairy cattle diets for methionine or all essential amino acids relative to energy at negative and adequate levels of rumen nitrogen.** Ryan J. Higgs<sup>1</sup>, Brian K. Sloan<sup>2</sup>, Larry E. Chase<sup>1</sup>, Charles G. Schwab<sup>3</sup>, and Michael E. Van Amburgh<sup>\*1</sup>,

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Updates have been made to the Cornell Net Carbohydrate and Protein System which includes estimations of recycled urea nitrogen, rumen protozoa production and yield, endogenous N secretions and a new system for calculating post-ruminal N digestion. The objective was

to evaluate the ability of the updated model to balance diets of high producing dairy cattle below or close to requirements for both rumen N and EAA. Sixty-four high producing dairy cows (100 ± 31 DIM) were randomly assigned to one of 4 treatments: 1) limited in Met, MP and rumen N (Base); 2) adequate in Met but limited MP and rumen N (Base+M); 3) adequate in Met and rumen N, but limited MP (Base+MU); 4) adequate in MP, rumen N and balanced for all EAA (Positive). Dietary CP was 13.5, 13.6, 14.6 and 15.6% DM and model predicted dietary MP balance was -231, -310, -142 and 33 g/d for the Base, Base+M, Base+MU and Positive treatments, respectively. No differences were observed in DMI or milk yield (24.1 to 24.8 and 40.0 to 41.8 kg/d, respectively). Energy corrected milk, fat and true protein yield were greater (3.3, 0.09 and 0.11 kg/d, respectively; *P* < 0.001) in cows fed the Positive compared with the Base treatment. True protein concentration in milk was higher (*P* < 0.001) and milk fat tended to be higher (*P* < 0.10) in cows fed the Positive and Base+MU treatments than cows fed the Base and Base+M treatments. Bacterial growth was predicted to be depressed by 16% and 17% for the Base and Base+M treatments, respectively, due to the predicted rumen N balance and this prediction corresponded with lower (*P* < 0.05) apparent total-tract NDF digestion. The study demonstrated high levels of milk yield can be achieved when diets are formulated on rumen N balance and EAA, even when CP is less than 14% DM provided adequate AA are supplied to the small intestine. The highest milk yield was achieved when considering all EAA in the ration formulation process.

**Key Words:** CNCPS, nitrogen, amino acid