Ruminant Nutrition: Dairy: Feed Additives II

T277 Effect of post-ruminal supplementation of phytonutrients on total-tract digestibility, nitrogen losses, and milk production and composition in dairy cows. J. Oh*[1], A. N. Hristov[1], C. Lee[1], K. Heyler[1], T. Cassidy[1], and D. Bravo[2], [1]*The Pennsylvania State University, University Park,* [2]*Pancosma, Geneva, Switzerland.*

The objective of this experiment was to investigate the effects of post-ruminal supplementation of phytonutrients on ruminal fermentation, apparent total-tract digestibility of nutrients, N utilization, and milk production and composition in lactating dairy cows. Eight ruminally cannulated Holstein cows (232 ± 34.1 d in milk) were used in a replicated 4 × 4 Latin square design trial with 23-d periods. Treatments were: (1) control (CON), (2) 2 g/d curcuma oleoresin (CU), (3) 2 g/d garlic extract (GE), and (4) 2 g/d capsicum oleoresin (CA). The phytonutrients were dissolved in ethanol solution and pulse-dosed into the abomasum of the cows once daily, 2 h after feeding for 9 d during each experimental period. Control cows received ethanol solution only. Ruminal pH and ammonia and volatile fatty acid concentrations were not affected (P = 0.40 to 0.97) by treatment. Apparent total tract digestibility of nutrients (dry and organic matter, crude protein, and fiber fractions) was similar (P = 0.34 to 0.91) among treatments. Total urinary-N, urinary urea-N, fecal-N, and total-N excretions were also not affected (P = 0.22 to 0.91) by treatment. Relative to CON, GE decreased (P = 0.04) dry matter intake (DMI, 21.2 vs. 19.9 kg/d, respectively). Milk yield was decreased (P = 0.04) with GE and CA compared with CON (35.8, 35.2 and 37.4 kg/d, respectively). Treatments did not affect milk composition and somatic cell count, 4% fat-corrected milk yield, and milk fat and true protein yields. In conclusion, post-ruminal supplementation of phytonutrients had no effect on ruminal fermentation, nutrient digestibility, N utilization, and milk composition in dairy cows. The decreased DMI with GE resulted in decreased milk yield and CA decreased milk yield without affecting DMI. The production effects from this trial have to be interpreted with caution due to the short duration of the treatment and milk data collection periods (9 and 6 d, respectively).

Key Words: phytonutrients, digestibility, dairy cow

T278 Effects of plant extracts on microbial population, methane emission and ruminal fermentation characteristics in in vitro. E. T. Kim*[1], K.-S. Min[2], C.-H. Kim[2], S. C. Kim[1], and S. S. Lee[1], [1]*Division of Applied Life Science (BK21 Program), Gyeongsang National University, Jinju, Gyeongsangnamdo, Republic of Korea,* [2]*Hankyong National University, Anseong, Gyeonggido, Republic of Korea.*

This study was conducted to evaluate effects of plant extracts on methanogenesis and rumen microbial diversity in in vitro. Plant extracts (*Artemisia princeps* var. *orientalis*; wormwood, *Allium sativum* for. *Pekinense*; garlic, *Allium cepa*; onion, *Zingiber officinale*; ginger, *Citrus unshiu*; mandarin orange, *Lonicera japonica*; honeysuckle) were obtained from Plant Extract Bank at Korea Research Institute of Bioscience and Biotechnology. The rumen fluid was collected before morning feeding from a fistulated Holstein cow fed timothy and commercial concentrate (TDN; 73.5%, crude protein; 19%, crude fat; 3%, crude fiber; 12%, crude ash; 10%, Ca; 0.8%, P; 1.2%) in the ratio of 3 to 2. The 30 mL of mixture, comprising McDougall buffer and rumen liquor in the ratio of 4 to 1, was dispensed anaerobically into serum bottles containing 0.3 g of timothy substrate and plant extracts (1% of total volume, respectively) filled with O_2-free N_2 gas and capped with a rubber stopper. The serum bottles were held in a shaking incubator at 39°C for 24 h. Total gas productions in all added plant extracts were higher (P < 0.05) than that of control, and that of ginger extract was highest (P < 0.05). The methane emission was highest (P < 0.05) at control, but lowest (P < 0.05) at garlic extract which was reduced about 20% of methane (40.2 vs. 32.5 mL/g DM). Other plant extracts were also led to decrease methane emission (wormwood; 8%, onion; 16%, ginger; 16.7%, mandarin orange; 12%, honeysuckle; 12.2%). Total VFAs concentration and pH were not influenced by the addition of plant extracts. Acetate to propionate ratios of garlic and ginger extracts were lower (P < 0.05, 3.36 and 3.38 vs. 3.53) than that of the control. Real-time PCR indicted that all plant extracts affected to the decrease of the ciliated-associated methanogen population, while the fibrolytic bacteria population was increased. In particular, *F. succinogens* community was increased by wormwood, garlic, mandarin orange and honeysuckle extracts, while *R. flavefaciens* population was inhibited by wormwood and garlic extracts, and *R. albus* diversity was influenced by mandarin orange and honeysuckle extracts.

Key Words: methanogenesis, plant extracts, real-time PCR

T279 Adding plant oils to dairy goat diets: Changes in milk fatty acids with sampling time. A. L. Martínez Marín[1], P. Gómez-Cortés[2], G. Gómez Castro[1], M. Juarez[2], L. M. Pérez Alba[1], M. Pérez Hernández[1], and M. A. de la Fuente*[2], [1]*Universidad de Córdoba, Córdoba, Spain,* [2]*Instituto de Investigación en Ciencias de la Alimentación, Madrid, Spain.*

Knowing the time at which the responses to dietary plant oil addition are clear in milk fat could shorten experimental periods and give clues on rumen and mammary metabolism of fatty acids (FA). Our aim was to find out changes in milk fat FA composition from 1 h to 21 d after introducing 3 differently unsaturated plant oils in dairy goat diets. Twelve midlactation multiparous goats were randomly allocated to one of 4 dietary treatments: Control (basal diet, no added oil) or the same basal diet added with 48 g/d of either high oleic sunflower oil, regular sunflower oil (RSO), or linseed oil (LO). Basal diet was made of alfalfa hay (0.33) and pelleted concentrate (0.67). Milk samples were taken at 0 (covariate), 1, 12, 24, 72, 120, 192, 312 and 504 h. Milkings at 0, 1 and 12 h were stripped out by hand after an intravenous dose of oxytocin. Analysis of fatty acid methyl esters (FAME) was performed by gas chromatography. MIXED procedure of SAS was used to analyze milk fat FA contents (g/100 g total FAME). Seventy 2 FA were identified and quantified in milk fat. The same differences between Control and oil treatments found at 504 h were also observed at 312 h in 12 chosen relevant FA, 5 sums of FA (saturated, mono- and polyunsaturated, total trans-18:1 and total conjugated linoleic acid) and linoleic to α-linolenic acid ratio. Both vaccenic (VA) and rumenic acid (RA) contents with LO treatment started showing differences with Control at 12 h (1.65 vs. 0.61; P < 0.001 and 0.60 vs. 0.30; P < 0.001). Between RSO and Control, differences of VA contents started at 12 h (1.87 vs. 0.69; P = 0.016) and that of RA at 24 h (0.79 vs. 0.36; P = 0.048). Content of α-linolenic acid with LO was different of Control at 1 h (0.18 vs. 0.13; P = 0.019); this difference increased at 12 h (0.34 vs. 0.13; P < 0.0001) and kept growing until 504 h (0.73 vs. 0.17; P < 0.0001). Reliable results of milk FA changes can be obtained at sampling times lower than 21 d and these modifications can help in the study of rumen and mammary metabolism of dietary FA.

Key Words: dairy goat, plant oil, fatty acid

T280 Supplementing rumen-protected Met and Lys in low protein diets based on corn distillers grains fed to lactating dairy cows. N. E. Lobos*[1], G. A. Broderick[2], and M. J. de Veth[3], *[1]University of Wisconsin, Madison, WI, [2]U.S. Dairy Forage Research Center, Madison, WI, [3]Balchem Corporation, New Hampton, NY.*

Feeding rumen-protected Met (RPM) and Lys (RPL) may allow feeding lower CP diets to dairy cows, thereby increasing N efficiency and reducing environmental impact. Moreover, RPL supplementation may improve the value of corn distillers dried grains plus solubles (DDGS) because its RUP is limiting in Lys. A trial tested experimental preparations of RPM and RPL that provided 15 g/d of DL-Met and 26 g/d of L-Lys. Forty lactating Holstein cows were blocked by DIM and parity into 8 squares in a replicated 5 × 5 Latin square trial with 5 dietary treatments: 1) low CP control (14.9% CP) without supplement, diet 1) top-dressed with 2) RPM, 3) RPL, 4) RPM + RPL, and 5) high CP control (16.8% CP) without supplement. As fed, all diets contained (DM basis): 31% alfalfa silage, 31% corn silage, 7.7% DDGS, 2.4% mineral-vitamin premix and 31% NDF. Diets 1–4 contained 25% corn grain plus 3.3% solvent soybean meal (SBM); diet 5 contained 21% corn grain, 1.9% solvent SBM, 3.6% expeller SBM, and 1.9% corn gluten meal. Periods were 3-wk (total 15 wk); data from wk-3 were analyzed using Proc Mixed in SAS. Contrasts and LS-means are reported in the table. Relative to the low CP control, feeding higher CP increased DMI and yield of milk and protein, but also increased MUN. No RPM main effect was observed; however, the RPM*RPL interaction for milk yield was significant because RPM increased milk 1.2 kg/d but milk was unaltered on RPM + RPL. Feeding RPL reduced ECM/DMI because numerically greater DMI had no effect on ECM yield. In this trial, feeding RPM, but not RPL, increased milk yield on low CP diets containing SBM plus DDGS.

Table 1.

CP, %:	14.9	14.9	14.9	14.9	16.8				
RPM, g/d:	0	15	0	15	0	Contrasts			
RPL, g/d:	0	0	26	26	0	CP	RPM	RPL	RPM*RPL
Variable									
DMI, kg/d	24.2	24.4	25.0	24.2	25.5	<0.01	0.34	0.29	0.08
Milk, kg/d	38.0	39.2	38.7	38.0	40.2	<0.01	0.58	0.54	0.05
Milk/DMI	1.60	1.65	1.59	1.60	1.61	0.58	0.12	0.16	0.28
ECM, kg/d	35.1	35.6	35.0	34.2	36.9	0.07	0.72	0.25	0.31
ECM/DMI	1.47	1.48	1.42	1.42	1.46	0.87	0.80	0.04	0.86
Fat, kg/d	1.41	1.41	1.39	1.34	1.46	0.22	0.53	0.14	0.42
Protein, kg/d	1.09	1.11	1.10	1.09	1.16	0.02	0.92	0.72	0.37
MUN, mg/dL	9.8	10.1	10.2	10.4	13.6	<0.01	0.29	0.07	0.78

Key Words: corn distillers dried grains, rumen-protected methionine, rumen-protected lysine

T281 Performance and diet digestibility of dairy cows supplemented with *Bacillus subtilis* spores. V. L. Souza[2], V. A. Silveira[1], N. M. Lopes[1], O. F. Zacaroni[1], R. A. M. Pereira[3], J. A. de Freitas*[2], R. Almeida[2], and M. N. Pereira[1], *[1]Universidade Federal de Lavras, Lavras, Brazil, [2]Universidade Federal do Parana, Curitiba, Brazil, [3]Empresa de Pesquisa Agropecuaria de Minas Gerais, Lavras, Brazil.*

This experiment evaluated the supplementation of dairy cows with *B. subtilis*. Trial 1 used 18 Holsteins (246 DIM) in a crossover design, with 39-d periods, and a 10-d washout between periods. Treatments were orally dosed daily: $3x10^9$ viable spores/d of *B. subtilis* C-3102 (Calpis Co. Ltda, Tokyo, Japan) or placebo. Cows were individually fed a TMR: 50% corn silage, 4.1% Tifton hay, 20.2% soybean meal, 11.2% high moisture corn, and 10.2% citrus pulp. Diet digestibility was evaluated by total collection of feces during d 33 to 35. The NDF digestibility was 52.6% of intake for *B. subtilis* and 55.8 for placebo ($P = 0.28$), OM digestibility was 72.1 and 72.6, respectively ($P = 0.69$). No treatment effect on digesta passage rate and chewing activity were detected ($P > 0.21$). Milk yield was 25.2 kg/d for placebo and 25.4 for *B. subtilis* ($P = 0.66$), DMI was 18.3 ($P = 0.91$). There was no treatment effect on milk solids and MUN ($P > 0.17$). Milk SCC was 97,000 cells/mL for *B. subtilis* and 99,000 for placebo ($P = 0.91$). Proportion of cows with positive *B. subtilis* culture in feces was 22% for placebo and 67% for *B. subtilis*. In trial 2, 30 cows (161 DIM) received the treatments for 16 weeks, in a covariate adjusted randomized block design. Milk samples were obtained weekly. Body weight and condition score were evaluated at 4-week intervals. The TMR contained 42.4% corn silage, 21.8% soybean meal, 15.6% ground corn, and 15.8% citrus pulp. Data was analyzed as repeated measures over time with Mixed of SAS. *B. subtilis* increased the daily secretions of milk (25.3 vs. 23.6 kg, $P = 0.02$) and protein (0.816 vs. 0.763 kg, $P = 0.01$), and had no effect on fat and lactose secretions ($P > 0.35$). The milk and protein yield response was consistent along the entire trial ($P > 0.90$ for the interaction of week and treatment). Milk SCC was 952,000 for *B. subtilis* and 747,000 for placebo ($P = 0.56$). *B. subtilis* tended to decrease MUN from 20.8mg/dL to 19.3 ($P = 0.06$). There was no treatment effect on body weight and condition score ($P > 0.33$). The supplementation of *B. subtilis* spores increased milk and protein yield when the trial was performed with high SCC cows, but the mechanism for the response was not elucidated.

Key Words: *Bacillus subtilis*, direct-fed microbial, probiotic

T282 Milk fatty acids composition of dairy ewes fed increasing levels of an unprotected CLA (UnCLA) supplement. D. R. M. Alessio[1], M. Baldin[1], R. Dresch[1], J. Souza[2], M. A. S. Gama[3], M. P. Soares[4], and D. E. Oliveira*[5,1], *[1]Centro de Ciências Agroveterinárias, UDESC, Lages, SC, Brasil, [2]Esalq/USP, Piracicaba, SP, Brasil, [3]Embrapa, CNPGL, Juiz de Fora, MG, Brasil, [4]Instituto Federal Catarinense, Araquari, SC, Brasil, [5]Centro de Educação Superior do Oeste, UDESC, Chapecó, SC, Brasil.*

This study aimed to evaluate the changes in milk fatty acids (FA) composition in ewes fed increasing doses of UnCLA (29.9% of trans-10, cis-12 CLA as methyl esters). Twenty-three primiparous Lacaune ewes with 40 ± 10 DIM, milk yield of 1.73 ± 0.26 kg/d and 52.1 ± 5.0 kg of BW were fed the following dietary treatments during 14d in an 28-d experimental period: Control (C): 30g of Megalac-E, n = 5; T10: 20g of Megalac-E plus 10g of UnCLA, n = 6; T20: 10g of Megalac-E plus 20g of UnCLA, n = 5 and T30: 30g of UnCLA; n = 7. The fat supplements were mixed into the concentrate (1.0 kg/d) and fed individually in 2 equal meals after a.m. and p.m. milkings. Ewes grazed paddocks of a tropical pasture as the only source of forage. Milk samples were collected on the 14th d of experimental period and analyzed for FA profile. Data were analyzed as a completely randomized design using the REG procedure of SAS. The desaturase indexes and the concentration of ≤C16 FA were linearly decreased, whereas the concentration of >C16, trans-10 C18:1 and the sum of trans-C18:1 FA was linearly increased as the CLA dose increased (Table 1). Moreover, treatments T10, T20 and T30 resulted in 191, 445 and 745% increases in milk trans-10, cis-12 CLA content and 131, 320 and 424% increases in trans-10, cis-12 CLA secretion into milk fat, respectively. The transfer efficiencies of trans-10, cis-12 CLA from diet into milk were 2.16, 1.97 and 1.63% (SE = 0.16; $P = 0.39$) for T10, T20 and T30, respectively. Incremental inclusion of UnCLA changed in a linear manner the milk fatty acid profile to a greater proportion of trans monounsaturated, longer chain fatty acids.

Table 1. Milk fatty acid responses to increasing levels of UnCLA

g/100 g FA	C	T10	T20	T30	SE	P^1
Summary						
<C16	32.0	29.3	26.3	27.9	0.69	0.018
C16 + C16:1	27.4	24.5	24.1	23.2	0.47	0.001
>C16	40.5	46.0	49.5	48.7	1.01	0.001
Desaturase Index						
14:1/14:0+14:1	0.010	0.008	0.008	0.006	0.001	0.001
16:1/16:0+16:1	0.025	0.025	0.022	0.020	0.001	0.017
18:1/18:0+18:1	0.559	0.532	0.501	0.473	0.010	0.001
CLA/18:1 t11+CLA	0.319	0.271	0.258	0.256	0.009	0.010

[1]Significance of linear responses. Quadratic responses were not significant ($P > 0.05$).

Key Words: CLA, dairy ewes, milk fatty acid composition

T283 Effect of monensin and tallow on methane estimation and protozoan and bacterial populations in dairy cows rumen. A. R. Castillo-Gonzalez*[1], M. E. Burrola-Barraza[1], J. A. Ortega-Gutierrez[1], M. I. Rivas-Martinez[2], and A. Chavez-Martinez[1], *[1]Facultad de Zootecnia y Ecología, Chihuahua, Chihuaha, México, [2]Colegio de Postgraduados, Texcoco, Edo. de México, México.*

The objective was to evaluate the effect of monensin and/or tallow in the diet of lactating cows on the populations of protozoa and bacteria and the estimation of methane. The ruminants have a significant contribution to the greenhouse gases, due to the production of methane. Recent efforts have been made to explore the effect of different additives (monensin, tallow, etc) on rations and its effect on rumen's microbial populations responsible of methanogenesis. The basal diet was formulated in a ration F:C (Forage:Concentrate) of 40:60. The treatments were: The control diet without additive (T1), control diet plus monensin (3.3g/d, T2), control diet plus tallow (3.0%, T3) and control diet plus monensin and tallow (3.3 g/d, 3.0%, T4). The animals were fed 2 times daily (0800 and 1500 h) and milked twice a day (0400 and 1300 h). An experimental design of 4 × 4 Latin square were used. Samples were collected from 13 to 15 d. Quantification of protozoa, bacteria and VFA′s (to estimate the production of methane with the Wollin equation) were conducted. Data were analyzed using PROC MIXED of SAS; means comparison was made using orthogonal contrasts. Results showed that protozoan population exhibited changes upon the inclusion of different additives to rations ($P \leq 0.06$) among treatment, the combination of monensin and tallow resulted in the lowest protozoan population size ($P \leq 0.0001$) with an average of 5.49 ± 0.07 Log10. Bacteria population size did not change ($P \geq 0.83$) ; however, it was observed a reduce on the population size from 1.3 to 0.68 fluorescence unity (FU). Meanwhile, methane estimate was reduced ($P \leq 0.05$), from 57.3 ± 1.1 mmol/mL in the control diet (T1) to 53.2 ± 1.1, 55.4 ± 1.1 and 54.4 ± 1.1 mmol/mL in T2, T3 and T4, respectively. In conclusion, the most efficient additive to reduce methane production was monensin (3.3%).

Key Words: additives, ionophores, ruminal microorganisms

T284 Hepatic transcriptomics in dairy cows supplemented with SmartamineM or MetaSmart during the peripartal period. J. S. Osorio*[1], P. Ji[1], S. L. Rodriguez-Zas[1], D. Luchini[2], R. E. Everts[1], H. A. Lewin[1], J. K. Drackley[1], and J. J. Loor[1], *[1]University of Illinois, Urbana, [2]Adisseo, Alpharetta, GA.*

We used a newly-developed bioinformatics tool (Dynamic Impact Approach, DIA; M. Bionaz, P. Kathiravan, S. Rodriguez-Zas, W. Hurley, and J. Loor, PLoS One; http://dx.doi.org/10.1371/journal.pone.0032455) that allows visualizing the dynamic adaptations of biological pathways to evaluate the impact of Methionine (M) supplementation on liver transcriptome. Twenty 4 multiparous Holstein cows were fed a control diet (ME, n = 8; 1.47 Mcal/kg DM prepartum and 1.67 Mcal/kg DM postpartum), ME plus MetaSmart (MS, n = 8; Adisseo France S.A.S.), or ME plus Smartamine M (SA, n = 8; Adisseo France S.A.S.). All cows received a common diet (1.24 Mcal/kg DM) during the far-off period [-50 to −21 d in milk (DIM)]. Treatments started at −21 DIM and continued through 30 DIM. MetaSmart (0.19% of DM prepartum and 0.18% of DM postpartum) and SA (0.07% of DM prepartum and postpartum) were top-dressed on the ME diet. Percutaneous liver biopsies for microarrays using a 13,000-gene oligonucleotide microarray were performed at −10, 7, and 21 DIM. Analysis of variance with a false discovery rate (FDR) correction resulted in 2,664 differentially expressed genes (DEG) with a treatment × day (T × D) interaction (FDR <0.10, uncorrected $P = 0.02$). For the DIA analysis the whole data set with Entrez gene IDs, FDR, fold-change, and post-hoc P value between the 3 treatments at each time point were uploaded. A cut-off of FDR = 0.05 and p-value = 0.05 was applied during analysis. The greatest degree of change in terms of up- and downregulated DEG due to T × D occurred with MS vs. ME, e.g., more than half DEG were downregulated by MS at −10 but at 7 DIM ca. 500 DEG were downregulated and > 300 downregulated. The pattern at 21 DIM resembled closely that at −10 DIM. The DIA analysis revealed that MS vs. ME led to an inhibition of cyanoamino acid metabolism at −10, 7, and 21 DIM but it activated taurine/hypotaurine metabolism at 7 DIM. Another novel feature of MS vs. ME was the activation at 7 DIM of riboflavin and nicotinamide metabolism and pantothenate (CoA) biosynthesis. Analysis of DEG due to T × D within treatments revealed that carbohydrate metabolism was among the top-impacted pathways due to ME and SA particularly at 7 vs. −10 DIM when flux of gluconeogenesis and TCA cycle were markedly activated. In contrast to cows fed MS, both ME and SA led to marked activation of glutathione metabolism at 7 vs. −10 DIM. Overall, results from the bioinformatics analysis seem to suggest that the 2 sources of M elicit distinct effects on the liver transcriptome. Such adaptations might be of biological relevance in terms of liver function, dry matter intake, and optimal milk production.

Key Words: systems biology, pathway analysis, genomics

T285 Production response of lactating dairy cows in a confinement operation to a commercial probiotic. J. K. Bernard* and N. A. Mullis, *University of Georgia, Tifton.*

Thirty-six lactating Holstein cows from the Dairy Research Center at the University of Georgia -Tifton Campus were used in a 10 wk randomized design trial to determine the effects of feeding a commercial probiotic (ProDairy, Donaghys Industries Ltd., Christchurch, New Zealand) on dry matter intake, milk yield and milk composition of lactating Holstein cows. The probiotic is a blend of non-viable lactobacillus species and fermentation extracts including amino acids, vitamins, amylase, and cellulose. During the first 2 wk of the trial all cows were fed the control diet and data collected were uses as a covariate in the statistical analysis. At the end of wk 2, cows were assigned randomly to one of 2 treatments (control (CONT) or supplemental probiotic (PRO) for the following 8 wk. A basal diet was fed to cows once daily behind Calan gates as a TMR in amounts to provide at least 5% refusal. PRO was added to the TMR at a rate of 10 mL/cow/d and mixed for 10 min before feeding. There were no differences in DMI between treatments; 25.3 and 25.4 kg/d for CONT and PRO, respectively. Cows fed PRO have increased yield (kg/d) of milk ($P = 0.001$), protein ($P = 0.05$), and SNF ($P = 0.002$) compared with CONT; 32.7, 0.94 and 2.74 and 30.9, 0.91,

and 2.58, respectively. Interactions of treatment and wk were observed for these variables because the difference between CONT and PRO increased throughout the 8 wk experimental period. No differences ($P > 0.10$) were observed among treatments in concentration of milk fat, protein, lactose, or SNF or yield of milk fat and lactose. Concentrations of MUN tended ($P = 0.10$) to be lower for PRO compared with CONT (15.03 and 15.44 mg/dl, respectively). No differences were observed in change of BW or body condition score. Results of this trial indicate that PRO stimulated improvements in nutrient utilization that supported higher yield of milk, protein and SNF.

Key Words: probiotic, milk yield, milk composition

T286 Evaluating in situ procedures for testing lipid encapsulated products — lysine as an example. T. F. Gressley*[1], M. J. de Veth[2], N. K. Diana[1], and E. Mackey[1], *[1]University of Delaware, Newark, [2]Balchem Corporation, New Hampton, NY.*

Standardized in situ methods for evaluating lipid encapsulated products have not been described. This study evaluated the effect of bag size and rinsing technique on in situ DM and Lys disappearance from 2 Lys products encapsulated using different technologies. Product A was a matrix type encapsulate (Italian manufacture) and product B was a core-shell encapsulate (US manufacture). Products were tested in 5 × 10 and 10 × 20 cm in situ bags with 50 µm pores (1 g and 5 g product/bag, respectively). Bags were placed without pre-wetting in the rumen of a lactating cow and incubated for 4 or 24 h, removed, and either hand or machine rinsed. Hand rinsed bags were individually rinsed in running tap water at 13°C for 90 s. Machine rinsed bags were rinsed in a front-loading washing machine using the cold water gentle cycle for 8 rinses of 1 min each. Rinsed bags were immediately dried at 50°C for 48 h. Unground subsamples from each bag were analyzed for N by combustion, and Lys content calculated as N × 5.22. Initial samples analyzed 29 and 52% Lys for products A and B, respectively. DM and Lys retention following in situ incubation were calculated as percent of initial sample. All combinations of product, bag size, time in the rumen, and rinsing technique were assessed in quadruplicate. For Lys retention, coefficients of variation (CV) averaged across all quadruplicates were 6.6% for 5 × 10 cm bags, 2.5% for 10 × 20 cm bags, 2.9% for hand rinsing, and 3.9% for machine rinsing. Both DM and Lys retention were affected by product, time, and product × time ($P < 0.001$). Product A retained 92% of DM and 85% of Lys at 4 h and 79% of DM and 50% of Lys at 24 h. Product B retained 95% of DM and 95% of Lys at 4 h and 89% of DM and 84% of Lys at 24 h. Lys retention was not impacted by bag size but was affected by rinsing technique ($P = 0.01$; 80% for hand rinsing vs. 77% for machine rinsing). This small difference may be due to greater microbial N retention in the hand rinsed bags or greater loss of product during machine rinsing. We recommend that in situ testing of rumen protected lipid encapsulated products use 10 × 20 cm bags and machine rinsing.

Key Words: encapsulate, rumen-protected lysine, in situ

T287 Effects of PEG and water on condensed tannin deactivation and nutrient digestibility of sainfoin in Holstein cows. H. Khalilvandi-Behroozyar*[1,2], M. Dehghan-Banadaky[1], K. Rezayazdi[1], and F. Ghaziani[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

Antinutritional factors such as condensed tannins affect nutritive value of forages for ruminants. Antinutritional factors can decrease nutrient availability for ruminants through limitations in microbial digestion or reduced enzymatic activity in post ruminal sections of gastrointestinal tract. Sainfoin is a legume forage with medium to high tannin content. Sainfoin is high quality hay, but little information is available about effects of condensed tannins on nutrient availability of sainfoin. The objective of this study was to examine tannin deactivation effects on nutrient availability from sainfoin. Second cut forage was shade dried and chopped (3 to 5 cm length), and then exposed to nothing (Control) or 5% (wt/vol) solution of polyethylene glycol (PEG 6000 MW) that was sprayed on the forage (vol/wt ratio of 1:1). Water soaking was applied with tap water (vol/wt ratio of 4:1). Treatments were carried out at an ambient temperature of 25°C for 20 min with hand shaking for water, and overnight for PEG. Water was added to forage just before feeding in an in vivo trial. The extractable CT content was determined (Butanol-HCl reagent). Ruminally fistulated Holstein cows (3 multiparous, 680 ± 20kg of BW) were used in 3 × 3 change over design. Each period consisted of 10 d for adaptation and 7 d for rectal fecal and forage sample collection. Acid insoluble ash was used as internal marker for determining digestibility of nutrients. Forages were fed as sole diet (0800 and 1600) along with mineral/vitamins to meet 110% of maintenance requirements of dairy cows. Digestibility coefficients determined and MIXED PROC of SAS was used for statistical analysis at 0.05 probability level. Water and PEG deactivated 92.06 and 98.57% of CT, respectively. Digestibility coefficients of ether extract, organic matter and acid detergent fiber were not statistically different ($P \leq 0.05$). Differences of means for neutral detergent fiber and CP digestibility among treatments were statistically significant. Tannin deactivation might be responsible for increasing digestibility of crude protein and plant cell wall.

Table 1. Effects of tannin deactivation with PEG and water on nutrient digestibility of sainfoin (g/100 g, CP %)

Treatment	EE	NDF	ADF	CP	OM
Control	72.16	49.25[b]	45.53	63.26[b]	60.41
Water	76.72	58.44[ab]	53.72	73.86[ab]	71.93
PEG	73.81	60.18[a]	51.55	75.42[a]	69.30
SEM	7.273	1.504	4.779	0.619	1.551

[a,b]Means within each column with different superscript letters are statistically different ($P \leq 0.05$).

Key Words: condensed tannin, digestibility, sainfoin

T288 Effect of dietary methionine supplementation in early lactation dairy cows I: dry matter intake, milk yield, milk composition and component yields. A. H. Souza*[1], P. D. Carvalho[1], A. R. Dresch[1], L. M. Vieira[1,2], K. S. Hackbart[1], D. Luchini[3], S. Bertics[1], N. Betzold[4], M. C. Wiltbank[1], and R. D. Shaver[1], *[1]University of Wisconsin-Madison, Madison, [2]University of Sao Paulo-VRA, SP 05508, Brazil, [3]Adisseo, Alpharetta, GA, [4]U.S. Dairy Forage Research Farm, Prairie du Sac, WI.*

Experimental objectives were to measure the effects of supplementing methionine during the postpartum period on lactation performance by dairy cows. Holstein cows (n = 72), were housed in a single pen from day −21 to calving and fed the same basal diet (6.68 Lys % MP, 2.24 Met %MP and 1.23 MCal/kg). From calving to 70 DIM cows were housed in tie-stalls and milked twice daily. Animals were blocked by parity and calving date and randomly assigned to 2 treatments differing in level of dietary methionine supplementation: 1) Methionine (MET); diet composed of (%DM) corn silage (39.7), alfalfa silage (21.8), HMSC (17.2), roasted soybeans (8.6), grass hay (4.6), canola meal (4.0), mineral-vitamin mix (2.7) and ProVAAL Ultra (w/Smartamine, 1.4), formulated

to deliver 2875 g MP with 6.8 Lys %MP and 2.43 Met %MP; 2) Control (CON); cows fed the same basal diet but replacing ProVAAl Ultra by ProVAAL Advantage (no added Smartamine), formulated to deliver 2875 g MP with 6.8 Lys %MP and 1.89 Met %MP. Data were analyzed with repeated measures model (proc Mixed, SAS 9.3) including fixed effects of treatment, week and treatment by week interaction, parity as a covariate, and the random effect of cow. The DMI and milk yield measurements were daily and milk samples were collected from both AM/PM milkings twice weekly for determination of milk composition. Methionine supplementation increased ($P < 0.01$) milk protein% (2.92 vs. 2.75) and solids-not-fat (SNF) % (8.73 vs. 8.54). Intake of DM, milk yield, milk fat% and MUN were unaffected ($P > 0.10$). Interestingly, although milk protein yield was not different ($P = 0.11$) between the treatments, an interaction between methionine supplementation and milk production was detected ($P < 0.01$); with ~9% increase in milk protein yield for cows producing below 34 kg/d compared with ~3% increase in cows producing above this level starting at 2nd week of lactation. Dietary methionine supplementation increased milk protein and SNF% in milk in early lactation cows; however, milk protein yield response to methionine supplementation varied by production level. Supported by Adisseo, USDA Grant 2010-85122-20612.

Key Words: dairy cow, methionine, milk production

T289 Effect of dietary antioxidant and increased rumen unsaturated fatty acid load on milk fat yield and fatty acid composition. J. C. Ploetz,* C. L. Preseault, and A. L. Lock, *Michigan State University, East Lansing.*

This study examined the impact of increasing rumen unsaturated fatty acid load (RUFAL) in the absence or presence of an antioxidant on feed intake, yield of milk and milk components, and milk fatty acids (FA). Twenty-eight Holstein cows (172 ± 59 DIM) were assigned to treatment in a randomized complete block design. Treatments were a control diet (CON) or an antioxidant (AOX; Agrado Ultra [dry blend of ethoxyquin and propyl gallate], Novus International, Inc.) supplemented diet (6.1 g/d). In period 1 (3 wks) no supplemental corn oil was fed; in periods 2, 3, and 4 (2 wks each) corn oil was supplemented at 0.75, 1.5, and 3.0% of the diet (DM basis) to incrementally increase RUFAL. Total dietary fatty acids were 2.7, 3.3, 3.9, and 5.1% of the diet for the 0.0, 0.75, 1.5, and 3.0% corn oil diets, respectively. The final 3 d of each period were used for sample and data collection. Data were analyzed using date as a repeated measure and preliminary 3.5% fat-corrected milk (FCM) as a covariate. There was no effect of AOX on any of the variables measured; thus data from AOX and CON were combined to assess the effects of dietary corn oil concentration (increasing RUFAL). Increased RUFAL decreased milk yield (44.3, 44.2, 43.0, and 38.4 kg/d, $P < 0.001$), fat concentration (3.38, 3.34, 3.10, and 2.43%, $P < 0.0001$), and fat yield (1.49, 1.46, 1.33, and 0.93 kg/d, $P < 0.001$). DMI decreased (30.2, 29.5, 28.2, and 27.8 kg/d, $P < 0.0001$) and there was a quadratic response for feed efficiency (FCM/DMI, 1.44, 1.46, 1.43, and 1.15, $P < 0.001$) with increased RUFAL. Milk FA concentration of C18:1 *t*10 was 0.84, 1.10, 1.92, and 6.29 g/100 g FA ($P < 0.001$) and for C18:2 *t*10, *c*12 was 0.001, 0.001, 0.007 and 0.030 g/100 g FA ($P < 0.001$) with increased RUFAL. On a FA yield basis, increasing RUFAL decreased < C16 FA (394, 376, 309, and 162 g/d, $P < 0.001$) and C16 FA (533, 477, 400, and 258 g/d, $P < 0.001$). There was a quadratic effect on > C16 FA (560, 609, 616, and 511 g/d, $P < 0.001$). Increasing RUFAL through the addition of corn oil to the diet decreased milk fat production and feed efficiency, and altered the FA composition of milk fat. Supplementation with AOX did not overcome the milk fat depression induced by increased RUFAL.

Key Words: milk fat depression, unsaturated fatty acids, antioxidants

T290 Effects of condensed tannins on ruminal VFA profile in fistulated Holstein cows fed sainfoin (*Onobrychis vicifolia*). H. Khalilvandi-Behroozyar*[1,2], M. Dehghan-Banadaky[1], K. Rezayazdi[1], and F. Ghaziani[1], *[1]Department of Animal Science, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, University of Urmia, Urmia, West Azerbaijan, Iran.*

Condensed tannins as antinutritional factors can limit different microbial actions in ruminants digestive tract. Sainfoin is a tanniferous forage with variable content of phenolic compounds. Our previous experiments using sainfoin showed that sainfoin condensed tannins are very active and limit ruminal protein and cell wall degradability, as shown incremental trend in forages treated for tannin deactivation. In previous experiments, treatment of sainfoin hay with water and polyethylene glycol (PEG) have major effects on deactivation of phenolic compounds and increased significantly ruminal cell wall and protein degradability, compared with alkaline chemicals (data not shown). The objective of this study was to examine effects of sainfoin treatment with water and PEG on ruminal VFA production and profile. Second-cut forage was shade dried and chopped (3 to 5 cm length), and then exposed to nothing (Control) or 5% (wt/vol) solution of polyethylene glycol (PEG 6000 MW) that was sprayed on the forage (vol/wt ratio of 1:1). Water soaking was applied with tap water (vol/wt ratio of 4:1). Treatments were carried out at an ambient temperature of 25°C for 20 min with hand shaking for water, and overnight for PEG. Water was added to forage just before feeding in an in vivo trial. The extractable CT content was determined (butanol-HCl reagent). Ruminally fistulated Holstein cows (3 multiparous, 680 ± 20kg of BW) were used in 3 × 3 change over design. Each period consisted of 10 d for adaptation and 7 d for sample collection. Forages were fed as sole diet (0800 and 1600) along with mineral/vitamins to meet 110% of maintenance requirements of dairy cows. Rumen fluid was obtained in 2 consecutive days (d 15 and 16) from ventral rumen via vacuum pump at before feeding ($t = 0$) and 2, 4 and 8 h after morning meal. Rumen fluid instantly squeezed through 4 layer cheesecloth and preserved with 1 mL of 50% sulfuric acid and frozen at −20°C until analysis for VFA. Volatile fatty acids were determined using a Philips PU4410 apparatus. Peaks of individual fatty acids were determined according to pure standards and quantified. Methyl-4-valeric acid was used as internal standard. MIXED PROC of SAS 9.1 was used for statistical analysis at 0.05 probability level. Treatments were able to decrease condensed tannin up to 90 percent compared with control, but there were no statistical differences among dietary treatments. Total concentration of VFA increased until 4 h after feeding and then slightly decreased. Polyethylene glycol treated sainfoin was resulted in higher molar concentration of total VFA, compared with control and water treated forages in 4 and 8 h after feeding.

Table 1. Effects of sainfoin treatment with water and PEG on rumen VFA (percentage of total; total VFA represented as mM)

	PEG	Water	Control	SEM
Before feeding				
Acetate	81.76	79.80	80.18	0.571
Propionate	11.36	11.24	12.33	0.725
Total	64.93	47.40	54.50	7.499
2 h				
Acetate	77.07	75.64	75.01	2.07
Propionate	15.16	17.60	15.95	1.379
Total	88.90	86.63	102.23	8.711
4 h				
Acetate	78.23	77.20	78.64	1.041
Propionate	13.87	13.67	12.62	0.891
Total	103.83	89.73	91.76	11.885
8 h				
Acetate	79.64	79.37	80.80	0.156
Propionate	12.37	11.99	11.68	0.175
Total	94.60	85.25	80.75	4.888

Key Words: sainfoin, volatile fatty acids, condensed tannin

T291 Influence of ionophore source and a proprietary nutrition supplement on the performance and rumen metabolism of Holstein calves previously fed a high plane of milk replacer. K. K. Guatam*[1], C. J. Cobb[1], B. S. Obeidat[1], M. L. Galyean[1], B. L. Miller[2], J. A. Davidson[2], K. L. Perfield[3], T. A. Brooks[1], and M. A. Ballou[1], *[1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, [2]Land O' Lakes Purina Feed, Gray Summit, MO, [3]Elanco, Greenfield, IN.*

Objective was to evaluate the effect of ionophore source with or without a proprietary nutrition package on performance and rumen metabolism in Holstein calves (3 mo old; initial BW = 101 kg) previously fed a high plane of milk replacer. In Exp. 1, 125 calves were randomly assigned to 5 treatments in 25 pens (5 calves/pen). Treatments were administered in a concentrate pellet and included: lasalocid without package (BOV-N), lasalocid with package (BOV-Y), monensin without package (RUM-N), monensin with package (RUM-Y), and High-forage control with monensin without package (HF). The respective pellet was restricted to a DMI of 4.1 kg/calf in BOV-N, BOV-Y, RUM-N, AND RUM-Y and 1.6 kg / calf in HF. Alfalfa hay was offered ad libitum. No differences ($P > 0.05$) in DMI were reported among diets; however, metabolizable energy intake and ADG were less ($P < 0.05$) for calves fed the HF. Gain:feed was greater ($P < 0.05$) in HF diet compared with BOV-Y, RUM-N, RUM-Y; whereas BOV-N did not differ from all groups. In exp. 2, 10 cannulated calves were used to evaluate rumen metabolism. Calves were fed with same diets used in Exp. 1. Alfalfa DM digestibility (DMD) at 24 and 36 h was greatest ($P < 0.05$) in HF. In addition, alfalfa DMD at 36 h was greater ($P < 0.05$) for RUM-Y vs. BOV-Y and RUM-N, and tended to be greater ($P = 0.11$) than BOV-N. Pellet DMD at 24, 36, and 48 h was greatest ($P < 0.05$) for HF. In addition, pellet DMD at 24, 36, and 48 h for RUM-Y was greater ($P < 0.05$) and tended to be greater ($P < 0.07$) for BOV-N and BOV-Y, respectively. Ammonia concentration was greater ($P < 0.05$) for BOV-N than BOV-Y, RUM-N, and HF at 6 h. The use of ionophore and a proprietary nutrition package did not improve the performance of growing calves; however, as expected HF calves consumed less metabolizable energy and had lower ADG than calves fed the higher concentrate diets. Calves fed the high-forage diet had improved DMD of both the alfalfa hay and the concentrate pellet. In addition, among calves fed the higher concentrate diets, monensin plus the nutrition package had improved dry matter digestibility of both the alfalfa hay and pellet.

Key Words: ionophore, performance, rumen metabolism

T292 Effect of feeding *Bacillus subtilis* spores on performance of Holstein dairy calves. V. L. de Souza[1], J. A. de Freitas*[1], S. L. Viechinieski[5], P. H. N. Pinto[2], M. N. Pereira[3], and J. C. Souza[4], *[1]Federal University of Parana, Curitiba, Parana, Brazil, [2]FAG, Cascavel, Parana, Brazil, [3]Federal University of Lavras, Lavras, Minas Gerais, Brazil, [4]Federal University of South of MAto Grosso, Aquidauana, Mato Grosso do Sul, Brazil, [5]Iguacu Farm - Star Milk, Vera Cruz do Oeste, Parana, Brazil.*

The objective was to determine effects of feeding *Bacillus subtilis* spores on performance of dairy calves in the first 60 d of age. Twenty Holstein female calves were allocated to a sequence of 2 treatments (*Bacillus subtilis* or placebo) in a completely randomized design. Treatments were: 0.3 g/calves/d (3×10^9 viable spores/cow/d) of *Bacillus subtilis* C-3102 (Calpis Co. Ltda, Tokyo, Japan) or placebo. The *Bacillus subtilis* spores were mixed with milk and fed directly to the calves. The animals were housed in individual hutches, with free access to water, starter feed (20% CP), and 8L of liquid diet (milk, 3 times a day) until weaning which occurred abruptly at the ninth week of life. The starter feed intake was recorded daily and body weight measurements were taken on d 0 and 60 of the comparison period. The initial weight was 37.8 kg for control and 35.5 for direct-fed microbial. Weaning weight was 77.55 kg for calves control and 81.6 kg for *Bacillus subtilis*, respectively. There was no treatment effect ($P > 0.05$) on DM of liquid or starter intake between the 2 groups. The average daily gain was 0.664 kg/d for placebo and 0.769 kg for *Bacillus subtilis* ($P = 0.25$), respectively. Feed conversion (ADG/DM intake) was 0.50 for placebo and 0.49 kg for *Bacillus subtilis* ($P = 0.99$). The supplementation of *Bacillus subtilis* spores did not induce lower performance of dairy calves.

Key Words: *Bacillus subtilis* spores, dairy calves, performance

T293 Interaction between vitamin E and rumen-protected conjugated linoleic acid on milk composition in grazing dairy cows. M. Ramírez-Mella[1], O. Hernández-Mendo[1], J. E. Ramírez-Bribiesca[1], R. D. Améndola-Massiotti[2], M. M. Crosby-Galván[1], J. A. Burgueño-Ferreira[3], and G. Aranda-Osorio*[2], *[1]Colegio de Postgraduados, Montecillos, Texcoco, México, [2]Universidad Autónoma Chapingo, Chapingo, Texcoco, México, [3]Centro Internacional de Mejoramiento de Maíz y Trigo, Estado de México, México.*

The objective of this study was to evaluate the effect of supplementing protected conjugated linoleic acid (CLA) and vitamin E on the amount of milk fat from lactating grazing dairy cows. Eight milking Holstein cows were used in a rotational grazing system on a mixed pasture of lucerne (*Medicago sativa*) and orchard grass (*Dactylis glomerata*). They got 2 kg of concentrated during each milking (4 kg/d), which contained 5 g of protected CLA. Treatments were: T1) control (basal diet + CLA), T2) 4000 (control + 4000 IU of vitamin E), T3) 8000 (control + 8000 IU of vitamin E), and T4) 12000 (control + 12000 IU). Milk production was measured individually, and determination of milk fat, protein and lactose content were carried out by using an infrared milk analyzer. A crossover experimental design was used and the results were analyzed using the SAS MIXED procedure. There was no difference ($P \geq 0.05$) in milk production or protein and lactose content among treatments, which agrees with those results reported by other authors, who included similar amounts of vitamin E, when adding fat in the diet. Milk fat content was

similar ($P \geq 0.05$) among treatments, however, it remained low (2.6%, on average), which, according with different authors, was due to the inclusion of fat on the diet. Thus, it is concluded that even using high doses of vitamin E in the diet of grazing milking cows, these amounts were insufficient to alleviate the milk fat decline when CLA are added to the diet, so it is indeed necessary more research to reverse this effect, because it is important to have milk with higher levels of CLA.

Key Words: CLA, dairy cattle, milk quality

T294 Assessment of lysine released from rumen-protected lysine products exposed to high and low moisture TMR over 24 hours. P. Ji,* C. S. Ballard, R. E. Clark, B. M. Sweeney, and C. Kokko, *William H. Miner Agricultural Research Institute, Chazy, NY.*

A study was conducted to evaluate the stability of 6 rumen-protected lysine products (RPL) when incorporated into TMR diets with different DM contents. Three loads (~350 kg/load) of each of 2 TMR diets only varying in DM content (40.5% as LD vs. 51.8% as HD) were prepared with Super Data Ranger. Duplicate Ziploc bags containing RPL (2 ± 0.03 g) and no RPL (as control samples) were filled and mixed well with 200 ± 1 g TMR from each load. Bags were stored at room temperature (21°C) for 0, 6, 18, and 24 h to simulate RPL exposure to TMR when mixed and delivered once per day. At the end of each time point, bag contents were transferred to strainer bags and soaked in 1 L Milli-Q water containing 500 mg Arg (as internal standard) for ~1 min to solubilize the Lys released in the TMR. Solution was filtered (0.45 μm) and frozen (−80°C) until filtrate was analyzed for Lys using ultra performance liquid chromatography. The Lys content for samples containing RPL were corrected for background Lys measured in control samples and Lys release (LR, %) was calculated. Data were analyzed as split-plot design with PROC MIXED of SAS. Results show that all RPL exhibited increased LR when exposed to TMR over time with differing magnitudes of LR ($P < 0.01$). The DM of TMR did not affect LR of most RPL; however, the overall LR of AminoShure-L was greater in TMR with LD than HD (5.9 and 3.0%, respectively; $P < 0.01$). A significant interaction of TMR DM and exposure time was observed for AminoShure-L and MetaboLys ($P < 0.01$). Our results indicate that less feeding frequency may result in greater Lys loss from RPL due to longer exposure in TMR before consumption by the cow.

Table 1. Lysine release (%) of RPL at 0, 6, 18, and 24 h in TMR

RPL product	0	6	18	24	SEM	*P*-value
AminoShure-L	0.2^{ax}	1.3^{ay}	6.4^{b}	9.8^{c}	0.47	≤ 0.01
LysiPEARL	6.2^{a}	39.7^{b}	47.0^{c}	48.4^{c}	1.66	≤ 0.01
Megamine-L	3.4^{a}	9.8^{b}	16.5^{c}	19.2^{d}	0.76	≤ 0.01
MetaboLys	0.7^{a}	0.8^{a}	2.2^{b}	3.8^{c}	0.25	≤ 0.01
USA Lysine	0.4^{a}	54.2^{b}	51.4^{b}	53.4^{b}	1.72	≤ 0.01
AjiPro-L	0.5^{a}	0.7^{a}	1.3^{b}	1.3^{b}	0.17	≤ 0.01

$^{a–d}P < 0.05$; $^{x,y}P < 0.10$.

Key Words: rumen-protected Lys, TMR, lysine

T295 Does mechanical mixing of TMR compromise protection efficacy of rumen-protected lysine products? P. Ji,* C. S. Ballard, R. E. Clark, B. M. Sweeney, and C. Kokko, *William H. Miner Agricultural Research Institute, Chazy, NY.*

A study was conducted to determine if mechanical mixing of TMR compromises the ruminal protection efficacy of 6 rumen-protected Lys products (RPL). A Super Data Ranger (SDR) loaded with 350 kg of TMR formulated for high producing cows was used to simulate the routine mixing procedure on a dairy farm. Dacron bags were filled with 1 ± 0.03 g RPL and heat-sealed. Triplicate bags per in situ time point were either mixed with diet for 6 min at full speed in SDR as treatment or indwelled in a bucket of same TMR diet for 6 min as control. Three loads of mixing were performed. After mixing, bags were incubated in the rumen of 3 cannulated cows for 0, 6, 12, and 24 h, one mixing load (control and treatment) per cow. After incubation, bags were gently hand-washed and paper-patted, then air-dried for more than 24 h. Dried RPL residue collected from each bag was acid hydrolyzed with 3 N HCl at 90°C in oven for 60 min and brought to a 100 mL volume with 0.2 mol/L HCl buffer. The solution was filtered and the concentration of Lys was determined by ultra performance liquid chromatography for calculation of ruminal disappearance of Lys (RD, %). Data for each RPL were analyzed separately as a randomized complete block design with MIXED procedure of SAS. The results showed that mechanical mixing increased RD ($P < 0.05$) of LysiPEARL (68.5 vs. 74.7%), MetaboLys (15.0 vs. 17.1%), and USA Lysine (72.2 vs. 75.9%), and tended to increase RD of Megamine-L (51.2 vs. 52.9%; $P = 0.06$), but did not affect that of AminoShure-L (30.6 vs. 33.0%; $P = 0.12$), and AjiPro-L (13.0 vs. 13.1%; $P = 0.92$). Length of ruminal incubation significantly increased RD of all RPL with varying magnitudes ($P < 0.01$). All RPL except AminoShure-L exhibited a significant interaction between mixing treatment and length of ruminal incubation ($P < 0.05$). In conclusion, some RPL are more vulnerable than others to damage caused by mechanically mixing in a TMR that compromises their ruminal protection. However, due to different shape and particle size of RPL, the specific gravity and ruminal passage rate may vary and should be considered when comparing potential ruminal Lys loss of RPL.

Key Words: rumen-protected Lys, mechanical mixing

T296 Ionophore source in a calf starter influences the performance of calves during the immediate post-weaned period. C. J. Cobb*[1], B. S. Obeidat[1], D. L. Hanson[1], M. D. Sellers[1], B. L. Miller[2], J. A. Davidson[2], K. L. Perfield[3], and M. A. Ballou[1], *[1]Department of Animal and Food Sciences, Texas Tech University, Lubbock, [2]Land O' Lakes Purina Feed, Gray Summit, MO, [3]Elanco, Greenfield, IN.*

Objective was to determine if the source of ionophore (monensin vs. lasalocid) in a calf starter influences the performance of calves during the first 3 mo of life. One hundred and 21 Holstein calves (2 ± 1 d old) were randomized to 1 of 2 treatments: Calf starter supplemented with 50 g / ton of monensin (n = 60) or 50 g / ton of lasalocid (n = 58). The ingredient composition of the 2 calf starters were identical and contained 22% crude protein on dry matter (DM) basis. The starter was offered ad libitum after the first week of life. All calves were fed a similar high plane of milk replacer nutrition before weaning. Weaning was initiated during the 7th wk by removing the PM feeding and calves were completely weaned when consuming 800 g DM daily after d 53. Body weights were measured at d 0, 10, 21, 53, 67, and 91. Peripheral blood samples were collected at d 3, 10, 21, 45, 47, 53, and 91 and analyzed for plasma concentrations of glucose, urea nitrogen, and haptoglobin. There was a treatment × time interaction ($P < 0.01$) on starter DM intake; whereas calves fed lasalocid consumed more starter DM during the post-weaning period, from wk 8 to 13 (average of 9.1% increase in starter DM intake among lasalocid). In addition, average daily gains of calves fed lasalocid were greater ($P < 0.05$) during the immediate post-weaned period from d 53 to 67 (0.903 vs. 0.822 ± 0.0259 kg/d). There were no treatment differences in average daily gains ($P > 0.271$) at any other periods. There was no treatment ($P = 0.189$) or treatment × time interaction ($P = 0.617$) on the efficiency of feed utilized for body weight gain. Last,

there were no treatment ($P > 0.346$) or treatment × time interactions ($P > 0.339$) on the plasma concentrations of glucose, urea nitrogen, or haptoglobin. Feeding a calf starter with lasalocid increased starter intake over the entire post-weaning period and increased average daily gains, but only during the immediate post-weaning period.

Key Words: calf starter, ionophore, performance

T297 Effects of microbial additives on nutrient metabolism in continuous culture of rumen contents. W. Braman* and L. C. Solórzano, *Chr. Hansen Inc., Milwaukee, WI.*

Reports indicate that microbial additives based on multiple strains of *E. faecium* show positive responses in ruminal fiber and nitrogen metabolism both, in vitro and in vivo. A study was conducted to determine the effects of microbial additives on nutrient metabolism in continuous culture of rumen content when the diet contained 28% starch. Lactation rations were formulated to support 45.5 kg of milk production. The study was comprised of 5 treatments: Control Diet, Control Diet + 3 strains of *E. faecium* + yeast (T1), Control Diet + 2 strains of *E. faecium* + 1 strain of *Lactococcus lactis* (T2), Control Diet + 2 strains of *E. faecium* + yeast (T3); Control Diet + 3 strains of *E. faecium*, + 1 strain of *Lactococcus lactis* + yeast (T4), Control Diet + 2 strains of encapsulated *E. faecium* + Lactococcus lactis (T5) and Control Diet + *Lactobacillus plantarum* (T6). All treatments were added at 2 g/head/day equivalent. Continuous culture fermentations were conducted using conditions simulating rumen parameters of a lactating dairy cow. These conditions were: liquid dilution rate of 13%/h, solids dilution rate of 4.55%/h, solids retention time of 22 h, feed intake 100 g DM/d, a feeding frequency of 25 g DM, 4 times daily at 6 h intervals, a fermentation temperature of 39°C. Culture pH was recorded at 0.5 h intervals. Each diet was fermented in triplicate 9-d fermentations, with effluent samples composited for analysis during the last 3 d. Parameters analyzed included: DM, OM, CP, ADF, NDF, NSC, VFAs, nitrogen partitioning, microbial growth and microbial efficiency. Data were analyzed using the General Linear Model Procedures of SAS. A Duncan's Multiple Range Test at the 5% level of probability was used to detect differences among treatments. There were no differences from the control in the digestibility of OM ($P > 0.33$), CP ($P > 0.35$), NDF ($P > 0.81$), ADF ($P > 0.33$) and NSC ($P > 0.33$) for any of the treatments. There was a numerical ($P < 0.14$) increase in DM digestion compared with the control (67.7%) for treatments T2 (71.9%), T4 (73.8) and T6 (69.8%). Compared with that of the control (0.064), the production of butyrate (moles/d) was suppressed ($P < 0.01$) by treatments T2 (0.054), T3 (0.053), 4T (0.045) and T6 (0.049). This resulted in lower ($P < 0.01$) molar proportions of butyrate in these treatments as well. There were no differences due to the treatments for the partition of N ($P > 0.31$), microbial growth ($P > 0.19$) and microbial efficiency measurements ($P > 0.31$). The microbial parameters results from this experiment are not in agreement with previously published studies. Microbial additives reduced the production and molar proportions of butyrate and numerically increased the digestibility of DM.

Key Words: digestion, microbial additives, rumen culture

T298 Immunometabolic indices in dairy cows supplemented with Smartamine M or MetaSmart during the peripartal period. J. S. Osorio*[1], E. Trevisi[2], P. Ji[1], D. Luchini[3], J. K. Drackley[1], G. Bertoni[2], and J. J. Loor[1], *[1]University of Illinois, Urbana, [2]Universita Cattolica del Sacro Cuore, Piacenza, Italy, [3]Adisseo, Alpharetta, GA.*

The early postpartal period is characterized by marked changes in hormonal, metabolic, and immune/stress-like conditions all of which may contribute to regulating dry matter intake (DMI) and the supply of nutrients to mammary gland. Peripartal cows are in negative methionine (M) balance due to increased requirements of tissues and cells for methylated compounds and M for milk protein production. Therefore, supplementation of rumen-protected M during the peripartal period may improve yield of milk and protein, and also help coordinate immunometabolic adaptations of the cow. Twenty-four multiparous Holstein cows were fed a control diet (ME, n = 8; 1.47 Mcal/kg DM prepartum and 1.67 Mcal/kg DM postpartum), ME plus MetaSmart (MS, n = 8; Adisseo France S.A.S.), or ME plus Smartamine M (SA, n = 8; Adisseo France S.A.S.). All cows received a common diet (1.24 Mcal/kg DM) during the far-off period [-50 to −21 d in milk (DIM)]. Treatments started at −21 DIM and continued through 30 DIM. MetaSmart (0.19% of DM prepartum and 0.18% of DM postpartum) and SA (0.07% of DM prepartum and postpartum) were top-dressed on the ME diet. Blood samples were collected at −21, −10, 7, 14, and 21 DIM for profiling of 21 markers of liver function, metabolism, oxidative stress, and inflammation. Concentration of cholesterol (CHOL) and the negative acute-phase protein albumin (ALB) decreased (time $P < 0.05$) around calving but increased by 21 DIM regardless of treatment. Paraoxonase concentration followed a similar pattern (time $P < 0.01$) as CHOL and ALB. Whereas glutamic-oxaloacetic transaminase (GOT) concentration increased (time $P < 0.01$) from −10 through 21 DIM regardless of treatment, bilirubin (BIL) increased between −10 and 7 DIM and then decreased through 21 DIM. However, the increase in BIL tended (treatment × time $P = 0.09$) to be lower in cows fed SA. Those responses were indicative of alterations in liver function particularly after calving. The concentration of haptoglobin, inflammation marker, did not change markedly at 7 vs. −21 or −10 DIM and decreased gradually by 21 DIM regardless of treatment. However, cows fed ME tended ($P = 0.09$) to have greater overall ceruloplasmin concentration due to markedly greater (treatment × time $P = 0.03$) concentration at −10 DIM. That response was suggestive of a more pronounced inflammatory-like status precalving. Despite an increase (time $P < 0.01$) in concentration after calving, reactive-oxygen metabolite concentration did not differ between treatments. Overall, preliminary data provide some evidence that M supplementation during the peripartal period could ameliorate inflammatory-like conditions characteristic of this period. As such, M may play a role in stimulating DMI after calving and promoting normal milk production.

Key Words: methionine, oxidative stress

T299 Ruminal biohydrogenation and abomasal fatty acid flow in dairy cows fed with fatty acids unsaturated sources. J. E. Freitas Jr.*[1], R. V. Barletta[1], K. Havartine[2], S. L. D. A. Robassini[1], M. D. S. Oliveira[3], B. C. Venturelli[1], E. F. Jesus[1], F. G. Vilela[1], G. D. Calomeni[1], J. R. Gandra[1], T. S. Canaes[1], and F. P. Rennó[1], *[1]University of São Paulo, Pirassununga, SP, Brazil, [2]Penn State University, University Park, [3]State University Julio de Mesquita, Jaboticabal, SP, Brazil.*

The aim of this study was to evaluate ruminal biohydrogenation and abomasal fatty acids flow in dairy cows supplemented with unsaturated fatty acids sources. Eight Holstein cows in the mid lactation (80 ± 20 d in milk; mean ± SD) cannulated in the rumen and abomasum (580 ± 20 kg of weight; mean ± SD) with milk yield of 25 kg/d were assigned randomly into 2 4 × 4 Latin squares and fed the following diets: 1) control (C); 2) refined soybean oil (inclusion of 3% in the total dry matter); (SO); 3) whole soybean raw (WS) (inclusion of 16% in the total dry matter) and; 4) calcium salts of unsaturated fatty acids (CSFA) (inclusion of 3% in the total dry matter). Milk yield and the dry matter intake were measured daily throughout the experimental period. The marker NDFi was used to determine the abomasal dry matter flow. Ruminal

contents were evacuated manually through the ruminal cannula at 4.5 h after feeding on d 20, and at 2.5 h before feeding on d 21 of each period. Fractional rates of fatty acids biohydrogenation and passage by the rumen were calculated utilizing the model that accounts for transfer of fatty acids among ruminal pools. Data were analyzed using PROC MIXED of SAS 9.1 according with the orthogonal contrasts (C vs SO + WS + CSFA); (SO vs WS + CSFA) and (WS vs CSFA). The diet with SO increased the biohydrogenation rate to the C18:2 in relation the WS and CSFA diets ($P \leq 0.05$) (93.71 vs 86.35; 82.34% respectively). There was tendency to decrease of the biohydrogenation rate of fatty acid C18:3 by contrast 2 (93.18 vs 86.49; 80.36%; $P \leq 0.06$). The cows fed CSFA showed 29.46% more C18:3 in the abomasal fatty acid flow in relation the cows fed with WS diet (5.60 vs 3.95 g/d). The use of whole soybeans raw and calcium salts of unsaturated fatty acids decrease ruminal biohydrogenation rate in dairy cows in the mid lactation.

Key Words: abomasum, linoleic acid, whole soybeans

T300 Evaluation of models ruminal biohydrogenation in dairy cows fed unsaturated fatty acids sources. J. E. Freitas Jr.*[1], R. V. Barletta[1], K. Harvatine[2], V. P. Bettero[1], M. D. S. Oliveira[3], B. C. Venturelli[1], R. Gardinal[1], J. R. Gandra[1], C. E. Araújo[1], F. G. Vilela[1], V. G. C. Lacuna[1], and F. P. Rennó[1], *[1]University of São Paulo, Pirassununga, SP, Brazil, [2]Pennsylvania State University, University Park, [3]State University Julio of Mesquita, Jaboticabal, SP, Brazil.*

The aim of this study was to evaluate 2 models ruminal biohydrogenation in dairy cows fed unsaturated fatty acids sources. Eight Holstein cows in the mid lactation (80 ± 20 d in milk; mean ± SD) cannulated in the rumen and abomasums (580 ± 20 kg of weight; mean ± SD) with milk yield of 25 kg/d were assigned randomly into two 4 × 4 Latin squares, fed following diets: 1) control (C) (inclusion of 2.66% of fatty acids); 2) refined soybean oil (inclusion of 5.14% of fatty acids) (SO); 3) whole soybean raw (WS) (inclusion of 5.00% of fatty acids) and; 4) calcium salts of unsaturated fatty acids (CSFA) inclusion of 5.10% of fatty acids). Corn silage was used as roughage in 50% of the total dry matter). Two models were used to calculate the rate biohydrogenation: Model A): calculated by disappearance rate using abomasal fatty acids flow, and fatty acids intake and; Model B): calculated by ruminal turnover, fractional passage rate and fractional biohydrogenation rate and ruminal pool for each fatty acid. The marker NDFi was used to determine the abomasal dry matter flow. Ruminal contents were evacuated manually through the ruminal cannula at 4.5 h after feeding on d 20, and at 2.5 h before feeding on d 21 of each period. Data were analyzed using PROC MIXED of SAS 9.1 according with the orthogonal contrasts (C vs SO+WS+CSFA); (SO vs WS+CSFA) and (WS vs CSFA). The mean biohydrogenation rate (C18:2) to the model A was 88.58% and to model B 81.95% including all diets. The diets with lipids sources showed greater biohydrogenation rate of the linolenic acid in relation to control diet by model A ($P \leq 0.05$) (76.31 vs 80.01%). Model B caused tendency ($P \leq 0.09$) to decrease biohydrogenation rate of linolenic acid when compared with SO and CSFA diets (88.45 vs 77.5%). Diet WS decreased the biohydrogenation of C18:2 in 16.68% and 5.09% in relation to SO diet. Model B results in higher rates of BHB compared with model A. However, lower rates of ruminal biohydrogenation can occur due to methodological errors.

Key Words: mid lactation, passage rate, whole soybeans