T258 Oocyte and embryo quality of dairy cows fed omega 3 and 6 fatty acids sources in the transition period and early lactation. J. R. Gandra,* R. D. Mingoti, L. C. Verdurico, R. V. Barletta, J. E. Freitas Jr., C. S. Takiya, T. H. A. Vendramine, R. Gardinal, and F. P. Rennó, *1University of Sao Paulo, Sao Paulo, Brazil.*

The objective of this study was to evaluate the supplemental sources of omega 3 and 6 fatty acids and the effects in oocyte and embryo quality of dairy cows fed during the transition period and early lactation. Forty-eight Holstein cows were divided into 4 experimental groups in randomized design. The animals were randomly assigned to receive one of 4 treatments: 1) control (C; n = 12), without fat sources in the pre and postpartum, 2) flaxseed (FS; n = 12), in which cows were fed 60 and 80 g/kg of DM of flaxseed in the pre and postpartum, respectively; 3) whole raw soybeans (WS; n = 12), in which cows were fed 120 and 160 g/kg of DM of whole raw soybeans in the pre and postpartum; 4) calcium salts of unsaturated fatty acid (CSFA; n = 12; Megalac-E), in which cows were fed 24 and 32 g/kg of DM of calcium salts of unsaturated fatty acid in pre and postpartum. The experimental diets were fed from 35 d before the estimate calving, and provided until 84 d of lactation, formulated to meet the nutritional requirements of each period (pre and postpartum). The procedure for follicular aspiration (FA) was performed in 2 periods: 35 ± 7 d of lactation (FA1) and 60 ± 7 d of lactation (FA2). After FA the oocytes were classified grade I, II, III, atretic and degenerate. Only submitted in vitro fertilization (IVF) oocytes with grade I, II and III. Data were analyzed using PROC MIXED of SAS 9.1, with the effect of diet, aspiration (FA) and interaction as fixed effects, and animal has random effect. The data were analyzed by orthogonal contrasts (C vs. WS+CSFA+FS; WS vs. CSFA; and FS vs WS+CSFA. FA effect ($P < 0.05$) was observed for the number of degenerate and viable oocytes (3.00 vs 1.82) and (4.65 vs 8.70), respectively to FA1 and FA2. There was interaction ($P < 0.05$) between the fatty acids sources and FA in the number of viable embryos. Effect ($P < 0.05$) were observed for the contrast FS vs WS+CSFA, when analyzing the number of viable embryos (1.27 vs. 0.73), respectively. The oocyte and embryo quality of dairy cows fed omega 3 and 6 fatty acids sources in the transition period and early lactation influenced the oocyte and embryo quality in post-partum period.

Key Words: fat sources, follicular aspiration, transition period

T259 Effects of different PUFAs supplementation during the postpartum periods of early lactating dairy cows. I: Milk production and composition. E. Dirandeh[1], A. Towhidi*[1], M. Ganjkhanlou[1], S. Zeinoaldini[1], Z. Ansari Pirsaraei[2], and A. R. Zarenezhad[3], *[1]Department of Animal Science, Faculty of Agricultural Science and engineering, University College of Agriculture and Natural Resources, University of Tehran, Karaj, Tehran, Iran, [2]Department of Animal Science, Faculty of Animal Science and Fishery, Sari University of Agricultural and Natural Resources, Sari, Mazandaran, Iran, [3]Mahdasht Dairy Farm, Sari, Mazandaran, Iran.*

The objectives were to determine the effect of diet enriched in α-linolenic acid (n-3), or linolenic acid (n-6) on milk production and composition in lactating dairy cows. Ninety high-yielding multiparous Holstein dairy cows with no over clinical illnesses were blocked according to calving date and parity. Cows were assigned randomly to be fed either 1-soybean whole roast (S, n = 30), or 2-linseed (L, n = 30), or 3-palm oil as a source of saturated fatty acid (C, n = 30) from calving until first heat after d 40 postpartum (dpp) and then half of the cows in each treatment group were switched to receive either linseed (L) or saturated fatty acid (C) from first heat after d 40 to 120 dpp. There was no difference between groups (mean ± SEM) in parity (3.0 ± 1.90) or BCS at calving (3.2 ± 0.07). Milk yield were recorded daily throughout the experiment. Milk samples were taken weekly (Monday and Thursday mornings) and analyzed for fat, protein and lactose contents by infrared analysis at the National Milk Records Laboratory, Sari, Iran, using AOAC reference method No. 972.16 (AOAC 1990). Data were analyzed with PROC MIXED of SAS. Result showed milk yield was not affected by diet ($P = 0.36$). Milk yield increased over time ($P < 0.001$), while no treatment × day interaction was detected ($P = 0.35$). Milk composition was similar among diets, except milk fat percentage that was lower in LL group than other groups ($P = 0.01$). Concentration and yield of milk fat did not change from wk 0 to 4 in cows fed linseed and soybean whole roast, whereas milk fat concentration declined from 3.54 to 3.20% ($P < 0.05$) and from 1.25 to 1.14 kg/d ($P < 0.05$), respectively, from wk 0 to 4 in cows fed palm oil. Milk protein percentage and yield did not differ between the dietary groups in our study. There was no difference in lactose, total solid and SNF in the milks.

Key Words: dairy cow, n-3 fatty acid, n-6 fatty acid

T260 Effects of lipid and propionic acid infusions on feed intake of lactating dairy cows. S. E. Stocks* and M. S. Allen, *Michigan State University, East Lansing.*

Propionic acid is more hypophagic when hepatic acetyl CoA concentration is elevated during early lactation. The objective of this experiment was to evaluate effects of intravenous lipid infusion and intraruminal propionic acid infusion on feed intake of lactating cows. Eight multiparous, ruminally cannulated, Holstein dairy cows (81 – 252 d in milk) were used in a replicated 4x4 Latin square experiment with a 2x2 factorial arrangement of treatments. One cow was removed because of an adverse reaction to the lipid infusion. Treatments were propionic acid (PR) infused intraruminally at 0.5 mol/h for 18 h starting 6 h before feeding or sham control (CO), and intravenous jugular infusion of lipid (TG, Intralipid 20%) or saline (SA, 0.9% NaCl) infused at 0.5 L/h for 24 h starting 12 h before feeding. Changes in plasma concentrations of metabolites and hormones and hepatic concentration of acetyl CoA from before infusion until the end of infusion were evaluated. No interactions of treatments were observed for DMI or changes in concentration for metabolites or hormones. Infusion of PR decreased DMI 15% (16.1 vs. 19.0 kg/12 h, $P = 0.02$) compared with CO but lipid infusion did not affect DMI over the 12 h infusion period. Infusion of PR tended to decrease hepatic acetyl CoA concentration (−5.7 vs. −2.4 nmol/g wet tissue, $P = 0.09$) compared with CO, consistent with PR decreasing DMI by stimulating oxidation of acetyl CoA. Contrary to our expectations, TG did not increase concentrations of NEFA ($P = 0.75$) or BHBA ($P = 0.48$) in plasma, or acetyl CoA in liver ($P = 0.65$), and did not increase milk fat yield ($P = 0.67$), suggesting that the infused TG was stored or oxidized by extrahepatic tissues. As a result, there was no interaction between PR and TG for DMI. While the effect of PR on DMI was consistent with our previous results, this model was not useful to evaluate the interaction between PR and TG related to control of feed intake by hepatic oxidation.

Key Words: hepatic oxidation, control of feed intake, propionate metabolism

T261 Relationships between ruminal volatile fatty acid concentrations, milk production, digestibility, and milk fatty acid composition in dairy cows. A. N. Hristov*[1], K. J. Shingfield[2], P. Huhtanen[3], J. L. Firkins[4], and K. Harvatine[1], *[1]The Pennsylvania State University, University Park, [2]MTT Agrifood Research Finland, Jokioinen, Finland, [3]Swedish University of Agricultural Sciences, Umeå, Sweden, [4]The Ohio State University, Columbus.*

The objective of this meta-analysis was to investigate the relationships between ruminal volatile fatty acids (VFA) concentrations (acetate, Ac; propionate, Pr, valerate, Va), their molar proportions, mol/100 mol (p), Ac:Pr ratio (AcPr), milk production, digestibility, and milk fatty acid (FA) composition in dairy cows as a potential on-farm tool for monitoring rumen function. The data set used in the analysis contained 496 individual cow data from 27 experiments conducted at 4 locations. Data were analyzed using the CORR and MIXED (with study as random effect) procedures of SAS. Average DMI and milk yield of the cows were: 22 (SD = 4.7) and 32 kg/d (SD = 10.3), respectively. The greatest correlation (all $P < 0.001$) for DMI was with Va and pVa (r = 0.38 and 0.34) and Pr (0.22). The greatest correlation for milk yield was with AcPr (−0.44), Pr and pPr (0.43 and 0.42), and Va (0.36). Best fit (based on the Akaike Information Criterion) models for milk yield and DMI included Pr and pVa and total VFA, respectively. Correlations of milk fat and protein contents with rumen VFA were weaker: −0.18 (Pr and pPr), 0.16 (AcPr), and 0.15 (pAc) and −0.11(Pr), respectively. Milk fat yield correlated with Va and pVa (0.19 and 0.16) and milk protein yield with AcPr (−0.40), Ac (−0.35), and Pr and pPr (0.37). Digestibility of NDF correlated with Va and pVa (−0.43 and −0.45), Ac (0.42), and Pr and pPr (−0.33 and −0.38). The greatest correlations between rumen VFA and milk FA were: for Ac with 18:1 (c11–18:1, −0.50; c16–18:1, 0.49; and c13–18:1, −0.46) and 22:4n-3 (−0.40); for pAc with 22:4n-3 (−0.67), t6–8-18:1 (−0.48), c9–10:1 (0.48), and t13–18:1 and 22:6n-3 (−0.47); for Pr with c9–10:1 (−0.42), c9–17:1 (0.42), and 22:6n-3 (0.39); for pPr with c11–18:1 (0.50), 22:4n3 (0.49), and c9–10:1 (−0.47); and for Va with c9-t1218:2 (0.59), total c18:2 (0.52), t9–14:1 (0.50), and c16–18:1 (−0.43). This analysis indicated that milk yield in dairy cows correlated positively with Pr and inversely with AcPr. The greatest correlations of VFA and milk FA were for Ac with c11–18:1 and for Va with c9-t1218:2.

Key Words: dairy cow, milk fatty acid, volatile fatty acid

T262 Occurrence and concentration of mycotoxins, molds and yeasts in total mixed rations from South Dakota and Minnesota dairy farms. F. Diaz-Royon*[1], A. Garcia[1], K. F. Kalscheur[1], K. A. Rosentrater[2], J. S. Jennings[3], and K. Mjoun[3], *[1]Dairy Science Department, South Dakota State University, Brookings, [2]Department of Agricultural and Biosystems Engineering, Iowa State University, Ames, [3]Alltech South Dakota, Brookings.*

Because of mycotoxin degradation in the rumen, dairy cattle can resist better than other livestock the adverse health effects associated with their exposure. At the same time though, dairy cattle are subjected to greater production stress which may increase their susceptibility to mycotoxins. Total mixed rations (TMR) from twenty-seven large dairy farms in eastern SD and western MN were collected during the summer and fall of 2011. Samples were screened for mycotoxins, molds, and yeasts to provide an overview of their prevalence in dairy diets. Ten subsamples of 0.5 kg each were taken from the feed bunk of high-production dairy cow pens. Subsamples were composited and later split in two 0.8-1.0 kg samples. After vacuum sealing, one sample was stored at −20°C for mycotoxin analyses and the second sample was stored at 4°C for mold and yeast analyses. Samples were quantitative analyzed for 18 different mycotoxins through high performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS). Detection limit was 0.5 ppm except for fumonisin B1 and aflatoxin B1, which were 1.0 and 0.05 ppm, respectively. Molds and yeasts were counted by direct plate dilution. In addition, mold isolates were identified using the conventional microscopic tape method in samples with mold growth equal to or higher than 1,000 cfu/g. There were 26 TMR samples (96.3%) positive for vomitoxin, with a maximum concentration of 0.8 ppm. These samples contained fumonisin concentrations lower than U. S. Food and Drug Administration maximum acceptable limits for use in animal feeds. Other mycotoxins were not detected. Mold growth was lower than 1,000 cfu/g in 15 TMR samples (55.5% of the samples) and higher than 10,000 cfu/g in 7 samples (25.9%). *Aspergillus* and *Mucor* were the fungi genera most isolated. On average yeast content in TMR was 1.9×10^6 cfu/g with only one sample (3.7%) showing yeast counts below 1,000 cfu/g. In spite of vomitoxin being detected in 96.3% of the TMR samples analyzed during the summer and fall of 2011, its concentration was always below that considered a health hazard by the FDA.

Key Words: mycotoxins, dairy cows, total mixed ration

T263 Feed restriction, but not L-carnitine infusion, affects the liver transcriptome with an evident induction of gluconeogenesis and inhibition of energy production and sterol synthesis in mid-lactating dairy cows. H. Akbar,* M. Bionaz, D. B. Carlson, S. L. Rodriguez-Zas, R. E. Everts, H. A. Lewin, J. K. Drackley, and J. J. Loor, *University of Illinois, Urbana.*

Abomasal carnitine infusion during acute feed restriction has been shown to increase hepatic fatty acid oxidation and decrease liver lipid accumulation in dairy cows. Using mid-lactating dairy cows we studied the effects of abomasal L-carnitine (20 g/d) in combination with restricted dry matter intake (DMI; 50% of previous 5-d average) on the hepatic transcriptome. Eight lactating Holstein cows were used in a replicated 4 × 4 Latin square design with 14-d periods. A 2 × 2 factorial arrangement was used to determine the effects of water infusion + ad libitum DMI, water infusion + restricted DMI, carnitine infusion + ad libitum DMI, or carnitine infusion + restricted DMI. Liver biopsies were obtained to extract RNA for transcriptome profiling using a bovine microarray consisting of 7,872 cDNA. Analysis of variance (false discovery rate $P \leq 0.15$ for overall treatments effect; equivalent to uncorrected $P \leq 0.05$) identified significant changes in transcriptomics only for feed restricted cows without any carnitine effect, resulting in 312 (155 downregulated, 157 upregulated) differentially expressed genes. Quantitative PCR (qPCR) was performed to confirm array data and measure expression of additional genes not present on the array. The qPCR data confirmed the effect of feed-restriction but not of carnitine treatment. Feed restriction increased expression of GPX3, involved in the detoxification of hydrogen peroxide, and of genes associated with gluconeogenesis (PC, PDK4), inflammation (SAA3), and signaling (ADIPOR2); whereas, it downregulated BBOX, key for L-carnitine biosynthesis, and the transcription factor HNF4A. Functional analysis of microarray data was carried out using Ingenuity Pathway Analysis, DAVID, and the novel dynamic impact approach (DIA). The functional analysis of DEG by feed restriction uncovered as the most relevant and inhibited functions biosynthesis of cholesterol and energy generation by mitochondrial respiration. Associated with the decrease of energy production the data also indicated a decrease in glucose catabolism, particularly through the glycolysis/pyruvate/TCA cycle and pentose phosphate pathways. Data indicated a decrease in fatty acid oxidation and ketone body production by feed restriction. Microarray and qPCR results revealed that in mid-lactating cows feed restriction but not L-carnitine affects the liver transcriptome with an evident inhibition of cholesterol synthesis and energy production. In contrast, gluconeogenesis

increased and glucose catabolism decreased. We interpreted those results as a likely response of liver to spare energy for the lactating mammary gland. This study provides new and unexpected insights on the effect of negative energy balance on liver transcriptome in mid-lactating cows.

Key Words: bioinformatics, microarray, negative energy balance

T264 A comparison of methods to analyze physical effective factor and physically effective NDF in TMR and orts. S. D. Ranathunga,* K. F. Kalscheur, and D. P. Casper, *Dairy Science Department, South Dakota State University, Brookings.*

The objectives were to compare available methods to measure physical effective factor (pef) and physically effective NDF (peNDF) in TMR and orts while using different forage and dried distillers grains with solubles (DG) concentrations. Four Holstein cows were assigned to a 4 × 4 Latin square in a 2 × 2 factorial arrangement of treatments. Diets contained either low forage (LF; 41% of diet DM) or high forage (HF; 60% of diet DM) with DG at 0 or 18% of diet DM. The pef and peNDF of TMR and orts were measured using 3 methods: 1) Dry sieving method using 12 screens (DS), 2) Penn State shaker box using top 3 screens (PS), and 3) Z-box (Z). Average pef and peNDF for all TMRs differed ($P < 0.05$) between the 3 methods (55.5, 79.7, and 52.6% and 16.0, 22.9, and 15.2%, for DS, PS, and Z, respectively). Average pef and peNDF of the orts were greater ($P < 0.05$) for PS (65.4, 82.4, and 63.6% and 22.2, 27.8, and 21.6%) compared with other methods. There was a forage × DG effect on pef of TMR and pef and peNDF of orts irrespective of the method. The peNDF of TMR was affected by forage and DG concentrations irrespective of the method. The results suggest that PS method overestimates pef and peNDF of TMR and orts and interpretation of data are different compared with the DS method. Estimates pef and peNDF values of TMR and orts by the Z method are closer and interpretation of data similar to that of the DS method.

Table 1.

	LF		HF		
Method	0DG	18DG	0DG	18DG	SEM
TMR-pef-DS	52.0^{c}	48.4^{c}	63.8^{a}	58.0^{b}	0.76
PS	77.2^{b}	74.1^{b}	84.9^{a}	82.6^{a}	
Z	45.0^{c}	44.0^{c}	63.2^{a}	58.1^{b}	
TMR-peNDF-DS	13.0^{b}	13.7^{b}	18.5^{a}	18.7^{a}	0.24
PS	19.3^{d}	21.0^{c}	24.6^{b}	26.7^{a}	
Z	11.2^{b}	12.5^{b}	18.3^{a}	18.8^{a}	
Orts-pef-DS	63.3^{b}	50.4^{c}	72.3^{ab}	75.6^{a}	2.36
PS	79.5^{ab}	72.7^{b}	90.1^{a}	87.2^{a}	
Z	59.5^{b}	47.0^{c}	75.8^{a}	71.9^{a}	
Orts-peNDF-DS	18.8^{b}	16.2^{b}	25.8^{a}	28.1^{a}	0.77
PS	23.6^{b}	23.3^{b}	31.9^{a}	32.3^{a}	
Z	17.7^{b}	15.1^{b}	26.9^{a}	26.7^{a}	

$^{a–d}$Means within a row with different superscripts differ ($P < 0.05$).

Key Words: forage, pef, peNDF

T265 Effect of post-ruminal supplementation of phytonutrients on bacterial diversity in feces of dairy cows. J. Oh*[1], A. N. Hristov[1], C. Lee[1], K. Heyler[1], T. Cassidy[1], S. Dowd[2], and D. Bravo[3], *[1]The Pennsylvania State University, University Park, [2]MR DNA Molecular, Shallowater, TX, [3]Pancosma, Geneva, Switzerland.*

The objective of this experiment was to investigate the effect of post-ruminal supplementation of phytonutrients on bacterial diversity in feces of lactating dairy cows. Eight ruminally cannulated Holstein cows (232 ± 34.1 DIM) were used in a replicated 4 × 4 Latin square design trial with 23-d periods. Treatments were control (CON) and 2 g/d of curcuma oleoresin (CU), garlic extract (GE), or capsicum oleoresin (CA). Treatments 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. Fecal samples (8) were collected from the rectum on d 6 and 7 of phytonutrient supplementation and analyzed for bacterial diversity using 16S rDNA bacterial tag-encoded FLX amplicon pyrosequencing (bTEFAP). Predominant genera bacteria were *Clostridium*, *Eubacterium*, *Ruminococcus*, *Bacteroides*, *Oscillospira*, and *Odoribacter* (8 to 10% of the total population) and were not affected ($P > 0.11$) by treatment. Predominant species were *Eubacterium siraeum*, *Oscillospira guilliermondii*, and *Fecalibacterium prausnitzii* (7 to 10%) and were also not affected ($P > 0.11$) by treatment. Compared with CON, GE increased ($P < 0.04$) the proportion of bacteria of the genera *Thiococcus* (0.06 and 0.12%), *Borrelia* (0.01 and 0.06%), and *Lactococcus* (0.01 and 0.08%) and CU, GE, and CA decreased *Flexithrix* (0.05 vs. 0.02, 0.02, and 0.01%). Compared with CON, GE increased ($P < 0.03$) the proportion of *B. garinii* (0.01 and 0.06%) and *L. lactis* (0.01 and 0.07%). Relative to CON, CU and CA decreased ($P < 0.05$) *Prevotella multiformis* (0.06, 0.02, and 0.003%) and CU decreased ($P < 0.05$) *Clostridium stercorarium* (0.06 and 0.02%). The proportion of *F. dorotheae* was lower ($P < 0.04$) for CU, GE, and CA (0.02, 0.02, and 0.01 vs. 0.05% for CON). In conclusion, post-ruminal supplementation of phytonutrients did not alter the major bacterial species in feces of dairy cows, although some bacteria such as *Thiococcus*, *Borrelia*, and *Lactococcus* were enhanced by GE and species such as *Prevotella multiformis* and *F. dorotheae* were inhibited by the phytonutrients.

Key Words: phytonutrients, fecal bacteria, dairy cow

T266 Applicability of the plasma free amino acid dose response approach for determining lysine bioavailability of ruminally protected lysine products. N. L. Whitehouse*[1], E. S. Fletcher[1], A. F. Brito[1], and C. G. Schwab[2], *[1]University of New Hampshire, Durham, [2]Schwab Consulting LLC, Boscobel, WI.*

It has been long recognized that lysine (Lys) is a limiting AA for lactating dairy cows. As a result, several ruminally-protected Lys (RP-Lys) supplements are now available for increasing Lys concentrations in metabolizable protein (MP). However, there is no universally accepted procedure for obtaining estimates of efficacy of these products. To evaluate 4 different RP-Lys supplements, we examined the dose-response relationships between plasma free Lys concentrations and intestinally (Trial 1) or abomasally (Trials 2–4) infused Lys in lactating dairy cows. Four Latin square trials were conducted; 2 with basal diets formulated to have low concentrations of Lys in MP and 2 with high concentrations (according to NRC, 2001). At the start of the trials, DIM of cows were: 63–123 (Trial 1), 60–70 (Trial 2), 175–245 (Trial 3), and 90–130 (Trial 4). Blood samples collected from the tail vein were centrifuged, deproteinized, and composited by cow/d with plasma stored at −80°C until AA analysis. Our data (see table below) showed a linear response between plasma Lys concentrations and infused Lys, corroborating results from previous research. We also observed that expressing plasma Lys concentrations either as μg/mL or as μM, before expression as a percentage of total AA, resulted in relatively similar regression equation parameters as well as similar variation and bias estimations. It is concluded that the plasma free AA response approach can be utilized for determining Lys bioavailability of RP-Lys supplements.

Table 1. Regression parameters for incremental levels of infused lysine

Trial	MPLys, %	Infused Lys, g/d	Slope	Intercept	r^2	SE	RMSE
Lys, % Total AA (μ/mL)							
1	5.27	0, 16.5, 33, 49.5, 66	0.027	2.95	0.97	0.38	0.142
2	5.54	0, 8, 16, 24, 32	0.048	2.81	0.90	0.31	0.238
3	6.81	0, 14, 28, 42, 56, 70, 84	0.024	5.21	0.96	0.45	0.166
4	6.81	0, 30, 60	0.022	4.51	0.98	0.22	0.151
Lys, % Total AA (μM)							
1	5.27	0, 16.5, 33, 49.5, 66	0.024	2.49	0.97	0.32	0.122
2	5.54	0, 8, 16, 24, 32	0.037	2.45	0.84	0.27	0.236
3	6.81	0, 14, 28, 42, 56, 70, 84	0.022	4.81	0.84	0.38	0.315
4	6.81	0, 30, 60	0.018	3.79	0.97	0.19	0.134

Key Words: lysine, bioavailability technique, dose response

T267 Physiological variables associated with reproductive success in dairy cows with different prepartum feeding strategies. F. C. Cardoso,* N. V. L. Serão, and J. K. Drackley, *University of Illinois, Urbana.*

To investigate the association between physiological factors and reproductive performance (days to conception; DTC) in dairy cows fed different prepartal dietary energy regimens, cow-level data from 408 cows from 7 different experiments by our group from 1993 to 2010 were analyzed. Treatments were classified as controlled energy (CE; median NE_L intake = 13.7 Mcal/d) or high energy (HE; median NE_L intake = 22.1 Mcal/d) diets fed during far-off (FO) or close-up (CU) dry periods. Principal component (PC) analysis was conducted on 8 variables: glucose wk 3 (GLU3), glucose wk 4 (GLU4), β-hydroxybutyrate wk 1 (BHBA1), insulin wk 2 (INS2), nonesterified fatty acids wk −1 (NEFA-1), energy-corrected milk wk 4 (ECM4), fat corrected milk wk 4 (FCM4), and milk urea nitrogen wk 4 (MUN4). Prior to analyses, the multinormality of the variables was assessed. The effect of PCs was investigated using linear and logistic (LR) regressions. For LR analysis, animals were classified in 2 groups as high (>131) or low (<121) DTC. All analyses were carried out using SAS 9.2 (SAS Institute, Inc.). PCs with eigenvalues (λ) greater than 0.9 were extracted, and only loadings greater than 0.4 were discussed. PC scores (PCS) were generated for each extracted PC. Four PCs were extracted from the analysis, accounting for 80.1% of total variability. The PC loadings indicated that, for PC1, increased ECM4 and FCM4 were associated with decreased INS2, GLU3, and GLU4. PC2 represented animals with higher NEFA-1 and BHBA1. PC3 had higher values for ECM4, FCM4, GLU3, and GLU4, whereas PC4 had higher values for MUN4 and INS2. Regressing PCS of PC2 on PC1 indicated that the relation between these PCs differed between diets ($P < 0.01$). For increased values of PC1, HE cows had increased values of PC2, whereas those fed CE showed decreased values of PC2. Inclusion of PCs in a logistic model revealed that high values of PC2 result in increased DTC (OR = 1.604, $P = 0.09$). In conclusion, PCs explained and predicted reproductive success in dairy cows. These 8 variables might be used to predict reproductive success.

Key Words: principal component, transition period, reproductive performance

T268 Plasma responses to intra-ruminal or post-ruminal administration of 2-hydroxy-4-methylthio-butanoic acid and its isopropyl ester in dairy cattle to evaluate rumen escape. G. I. Zanton,* S. E. Bettis, and M. Vazquez-Anon, *Novus International, Inc., St. Charles, MO.*

Two experiments were conducted to evaluate the rumen escape of methionine precursors. In experiment 1, 4 rumen cannulated, Holstein steers (718 ± 16 kg) were bolus dosed intra-ruminally (R) or post-ruminally via the omasal canal (O) with 80 mg of DL 2-hydroxy-4-methylthio-butanoic acid (HMTBa as MFP)/kg BW at the time of normal feed distribution. Treatments were administered during 2, 7 d periods according to a crossover design. Plasma samples were prepared from blood taken from the coxygeal vein at 0, 1.5, 3, 6, 9, 12, 18, 24, 30, 36, and 48 h after treatment administration. Plasma was analyzed for concentration of DL HMTBa. In experiment 2, the model developed in experiment 1 was used to compare the rumen escape of HMTBa provided as MFP to the isopropyl ester of HMTBa (HMBi). Eight rumen cannulated Holstein steers (548 ± 27 kg) were blocked by weight and randomly assigned to 4 treatment sequences according to a replicated 4 × 4 Latin square design. Treatment administration, sampling, and analysis were conducted as in experiment 1. Mean responses in area under the curve (AUC) were analyzed in the mixed procedure of SAS with significance declared at $P < 0.05$. In experiment 1, steers consumed 12.5 kg of DM or 1.7% of BW. Plasma HMTBa AUC was greater for O than for R (151 vs 73 ± 6 mg/L). By comparing the plasma HMTBa AUC for R relative to O, it was determined that MFP had a rumen escape of 49%. For experiment 2, DMI was 12.5 kg/d and 2.3% of BW and not affected by treatment or site of administration. Plasma HMTBa AUC was higher for HMBi (O: 169, R: 99 mg/L) than MFP (O: 135, R: 54 mg/L) at both sites; there was no interaction. By comparing the plasma HMTBa AUC for R relative to O for both sources, it was determined that HMBi had a rumen escape of 60% and MFP had a rumen escape of 41%.

Key Words: dairy, HMTBa, methionine

T269 Casein and fatty acid fractions in milk are affected by parity and nutritional regulated body condition score at the beginning of the transition period in dairy cows under grazing conditions. V. Artegoitia*[1,2], A. Meikle[2], L. Olazabal[3], J. P. Damian[2], M. L. Adrien[1], D. A. Mattiauda[1], J. Bermudez[1], A. Torre[3], and M. Carriquiry[1], *[1]Facultad de Agronomía, Universidad de la República Oriental del Uruguay, Montevideo, Uruguay, [2]Facultad de Veterinaria, Universidad de la República Oriental del Uruguay, Montevideo, Uruguay, [3]Laboratorio Tecnológico del Uruguay, Montevideo, Uruguay.*

The study objective was to evaluate the effect of BCS [high (H) vs. low (L)] at 30 d before calving (−30 d) on milk casein and fatty acid (FA) fractions in primiparous (P; PH, n = 13; PL, n = 9) and multiparous (M; MH, n = 9; ML, n = 8) Holstein cows fed under grazing conditions. Cows were offered different planes of nutrition of long-term pastures from −100 to −30 d pre-calving, to generate 0.5 BCS unit differences between groups at the end of the period. Milk samples were collected at wk 2 and 8 of lactation (WOL). Means were considered to differ when $P \leq 0.05$. Milk, protein, and fat yields and somatic cell counts (SCC) were greater in M than P cows, and milk protein and fat yields decreased from WOL 2 to 8 in all cows. Casein content was greater in M than P cows, decreased from WOL 2 to 8, and it was greater for MH cows than the other groups at WOL 2. Milk β-casein was less and κ-casein was greater in M than P cows, which could be due to a greater degradation of β-casein by plasmin activity associated to greater SCC in M cows' milk. The κ-casein was greater in PL than in PH cows, consistent with higher insulin concentrations in the former cows at the end of the treatment.

The de novo (4:0 to 15:1) and mixed origin (16:0 to 16:1) FA in milk fat increased, whereas preformed FA (≥17:0) decreased from WOL 2 to 8, associated with the early (WOL 2) lipid mobilization for milk synthesis. Mixed origin FA tended to be affected by BCS ($P = 0.10$), as they were greater for ML than MH cows. Saturated FA tended to be greater ($P = 0.06$) and MUFA were less in milk fat of M than P cows. Milk PUFA, particularly n-3 FA, were greater in H than L cows at WOL 2 for M cows and WOL 8 for P cows suggesting a greater dry matter intake in these cows. Milk CLA was greater in H than L cows and decreased from WOL 2 to 8 only in PL cows. Results indicate that casein and fatty acid fractions in milk are affected by parity and by BCS at the beginning of the transition period in dairy cows on grazing conditions.

Key Words: body reserves, milk composition

T270 Arterial amino acid concentrations drives milk yield in postpartum transition dairy cows. M. Larsen* and N. B. Kristensen, *Department of Animal Science, Aarhus University, Foulum, Tjele, Denmark.*

Previous studies based on splanchnic amino acid fluxes and milk protein output showed that dairy cows mobilize close to 5 kg of essential amino acids in the first month postpartum. The present study aimed at investigating the effects of alleviating the protein deficiency in postpartum transition dairy cows on mammary uptake of amino acids. Eight Holstein cows (second lactation) were used in a complete randomized design with repeated measurements at 4, 15, and 29 d in milk (DIM). At the calving day, cows were assigned to either continuous abomasal infusion of casein (CAS) or water (CTRL). All cows were fed the same diet. Abomasal casein infusion was profiled as 360 g/d at 1 DIM, 720 g/d at 2 DIM, followed by daily reductions of 19.5 g/d ending at 194 g/d at 29 DIM. Arterial and mammary venous blood sample sets were obtained bihourly at sampling days. Data was analyzed using PROC MIXED in SAS with treatment, DIM, and the interaction as fixed effects. Cow was considered as random effect, and DIM within cow as repeated measurement. Dry matter intake was unaffected ($P = 0.36$) by treatment. Milk yield increased more rapidly after calving with CAS ($P < 0.01$) and averaged 43.8 ± 1.0 kg/d with CAS and 36.6 ± 1.0 kg/d with CTRL. Milk protein yield with CAS was 1,664 ± 39 g/d at 4 DIM compared with 1,212 ± 86 g/d for CTRL, whereas milk protein yield did not differ at 29 DIM (1383 ± 48 g/d; interaction: $P = 0.02$). The calculated utilization of infused casein to milk protein was 64% at 4 DIM and 60% at 29 DIM. Arterial concentrations and mammary venous—arterial concentration differences for essential amino acids (EAA) were higher at 4 DIM with CAS as compared with CTRL, but did not differ at 29 DIM (interactions: $P = 0.02$ and $P = 0.03$, respectively). Mammary extraction rate of EAA was unaffected by CAS ($P = 0.37$) and DIM ($P = 0.58$). Circulating levels of insulin and IGF-1 were unaffected by CAS ($P = 72$ and $P = 36$, respectively). In conclusion, these results suggest that mammary uptake of amino acid is driven by arterial supply during initiation of lactation, thus, extra metabolizable amino acids supplied to postpartum transition cows are efficiently used for milk protein production.

Key Words: transition dairy cows, protein requirement, amino acids

T271 Productive performance of dairy cows fed with omega 3 and 6 fatty acids sources in the transition period and early lactation. J. R. Gandra,* L. C. Verdurico, R. D. Mingoti, R. V. Barletta, J. E. Freitas Jr., C. E. Araújo, K. A. Koyama, G. D. Calomeni, E. Ferreira de Jesus, and F. P. Rennó, *University of Sao Paulo, Sao Paulo, Brazil.*

The objective of this study was to evaluate the supplemental sources of omega 3 and 6 fatty acids and the effects in the milk yield and composition of dairy cows fed during the transition period and early lactation. Forty 8 Holstein cows were divided into 4 experimental groups in randomized design. The animals were randomly assigned to receive one of 4 treatments: 1) Control (C; n = 12), without fat sources in the pre and postpartum, 2) Flaxseed (FS; n = 12), in which cows were fed 60 and 80g/kg of DM of flaxseed in the pre and postpartum, respectively; 3) Whole raw soybeans (WS; n = 12), in which cows were fed 120 and 160g/kg of DM of whole raw soybeans in the pre and postpartum ; 4) Calcium salts of unsaturated fatty acid (CSFA; n = 12, Megalac-E), in which cows were fed 24 and 32g/kg of DM of calcium salts of unsaturated fatty acid in pre and postpartum. The experimental diets were fed since 35 d before the estimate calving, and provided until 84 d of lactation, 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 12th week of lactation. Data were analyzed using PROC MIXED of SAS 9.1, with the effect of diet, time and interaction has fixed effects, and animal has random effect. The data were analyzed by orthogonal contrasts (C vs. WS+CSFA+FS; WS vs. CSFA; and FS vs WS+CSFA). There was no effect of the fatty acids sources on the milk yield, FCM, protein and lactose (kg/d and %). The average milk yield was 32.27 kg/d, where diets C, FA, WS and CSFA were (31.80, 31.43, 31.81 and 33.57 kg / d), respectively. There was effect of lipid sources for milk fat (kg /d and %) and in the contrast WS vs CSFA (3.52% vs. 2.88%); (1.09 kg/d vs 0.92 kg/d) respectively. The productive performance of dairy cows fed with omega 3 and 6 fatty acids sources in the transition period and early lactation was only affected in the milk fat yield and percent.

Key Words: fat sources, productive performance, transition period

T272 Effects of 18-carbon fatty acids on triacylglycerol accumulation in bovine mammary epithelial cells in vitro. R. L. Cui, J. Q. Wang,* H. Y. Wei, D. P. Bu, X. M. Nan, H. Hu, P. Sun, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The objective of this study was to investigate the effects of concentration and ratio change of stearic acid, oleic acid, linoleic acid and linolenic acid on triacylglycerol (TG) accumulation in bovine mammary epithelial cells, and to reveal their contribution to TG accumulation when the four 18-carbon fatty acids (18-C FA) existed in medium simultaneously. Bovine mammary epithelial cells from a 3-year old lactating (ca. 100 DIM) Chinese Holstein dairy cow were incubated at 38°C in DMEM/F12 medium containing 1 g/L fatty acid-free bovine serum albumin (BSA), 5 mg/L prolactin, 5 mg/mL insulin, 5 mg/mL Holo-transferrin, 5 mg/mL progesterone, 10^{-7} mol/L hydrocortisone, 10 ng/mL bovine epithelial growth factor and 5 mg/mL bovine estradiol. An orthogonal L_{16} (4^5) test was performed in this experiment, and the concentrations of 18-C FAs were all 25, 50, 75 and 100 μM. TG contents in cells and medium were tested by Triglyceride Quantitation Kit after different combination performed for 24 h. In addition, every treatment had 3 replicates in this experiment, and ANOVA of SAS was used to analyze the experimental data. The results showed that stearic acid was the most important determinant of TG accumulation when the four 18-C FAs existed in medium simultaneously, and TG accumulation tended to increase as stearic acid concentration increased. The combination of 100 μM stearic acid, 100 μM oleic acid, 50 μM linoleic acid, 75 μM linolenic acid was the the optimal combination for increasing TG accumulation in our experiment. In conclusion, stearic acid was the most important determinant of TG accumulation when the four 18-C FAs were all available.

Key Words: bovine mammary epithelial cells, 18-carbon fatty acids, triacylglycerol

T273 Effects of 18-carbon fatty acids on cell proliferation and triacylglycerol accumulation in bovine mammary epithelial cells in vitro. R. L. Cui, J. Q. Wang,* H. Y. Wei, D. P. Bu, X. M. Nan, H. Hu, P. Sun, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

The objective of this study was to investigate the effects of 18-carbon fatty acids (18-C FAs) on cell proliferation and triacylglycerol (TG) accumulation in bovine mammary epithelial cells in vitro. Bovine mammary epithelial cells from a 3-year old lactating (ca. 100 DIM) Chinese Holstein dairy cow were incubated at 38°C in DMEM/F12 medium containing 1g/L fatty acid-free bovine serum albumin (BSA), 5mg/L prolactin, 5 mg/mL insulin, 5 mg/mL holo-transferrin, 5 mg/mL progesterone, 10^{-7} mol/L hydrocortisone, 10 ng/mL bovine epithelial growth factor and 5 mg/mL bovine estradiol. Epithelial cells were cultured with different concentration of stearic acid, oleic acid, linoleic acid and linolenic acid for 24 h, separately, and the cells cultured without any fatty acids served as control. Then the MTT experiment was performed and the TG contents in medium were detected by Triglyceride Quantitation Kit. In addition, every treatment had 3 replicates in this experiment, and ANOVA of SAS was used to analyze the experimental data. In MTT experiment, the concentrations of 4 18-C FAs were all 0, 0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400 μmol/L, and the results showed that the cell proliferation was inhibited significantly when the cell cultured with 200 or 400 μmol/L stearic acid, oleic acid, linoleic acid or linolenic acid ($P < 0.05$). According to MTT results, the concentrations of 4 18-C FAs were 0, 25, 50, 100 μmol/L for detecting TG contents, and the results showed that TG contents in medium were increased in a concentration-dependent manner from 0 to 100 μmol/L ($P < 0.05$). In conclusion, high concentration (200 or 400 μmol/L) of 18C-FAs can inhibit the cell proliferation, and TG accumulation can be increased as the concentration of 18-C FAs increase.

Key Words: bovine mammary epithelial cells, 18-carbon fatty acids, cell proliferation

T274 Lipopolysaccharide-induced alterations in milk fatty acid composition and mRNA expression of genes related to fatty acid metabolism. Y. D. Zhang, J. Q. Wang,* D. P. Bu, T. Hu, X. M. Nan, H. Hu, R. L. Cui, and L. Y. Zhou, *State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, China.*

Two experiments were conducted to evaluate the effect of lipopolysaccharide (LPS) on milk fat production and fatty acid composition. Eight multiparous Holstein cows (185 ± 30 DIM) received external pudic arterial administration of LPS (*E. coli* O111:B4) at 0.01 μg/kg BW in 10 mL of sterile saline (0.9% NaCl; treatment) or saline only (control) according to a crossover design. The experiment consisted of a 7-d preliminary period and 2 7-d treatment periods. Milk samples were collected at 0, 6, 12, and 24 h after LPS infusion on d 1 and twice daily from d 2 to 7. In the second experiment, the expression of genes for fatty acid metabolism in dairy cows was studied by incubating mammary epithelial cells with LPS at 0, 0.1, or 10 ng/mL for 24 h using RT-qRCR. All data were analyzed using the MIXED procedure of SAS (SAS Institute Inc., 2001, Cary, NC). In the in vivo study, milk yield ($P = 0.19$), milk fat concentration ($P = 0.34$), and milk fat yield ($P = 0.56$) were not affected with LPS infusion. LPS altered the milk fatty acid composition, resulting in an increase in the proportion of unsaturated fatty acids and short-chain fatty acids (<16:0) and a decrease in the proportion of saturated fatty acids and long-chain fatty acids (>16:0). In the in vitro experiment, LPS did not affect fatty acid-binding protein FABP3 or FABP4 mRNA expression ($P > 0.05$), but increased transporter CD36 mRNA expression ($P < 0.01$). Treatment with 0.1 ng/mL LPS increased fatty acid synthesis enzyme FASN, ACACA, ACSS2 and fatty acid metabolism gene PPARG, PPARGC1A mRNA expression ($P > 0.05$) compared with the control. Differential regulations of fatty acid synthesis enzyme FASN, ACACA, ACSS2 and fatty acid metabolism gene PPARG, PPARGC1A mRNA expression were observed with 10 ng/mL LPS. LPS alters fatty acid metabolism, functioning as an important factor affecting milk fat synthesis.

Key Words: fatty acids metabolic-related gene, LPS, milk fat composition

T275 Hepatic expression of GH-IGF axis genes in Holstein cows with different nutritional managements during early lactation. A. L. Astessiano*[1], P. Chilibroste[2], M. Fajardo[2], J. Laporta[1], J. Gil[2], D. A. Mattiauda[1], A. Meikle[3], and M. Carriquiry[1], *[1]1School of Agronomy, UDELAR, Montevideo, Uruguay, [2]2School of Veterinary Medicine, UDELAR, Paysandú (EEMAC), Uruguay, [3]3School of Veterinary Medicine, UDELAR, Montevideo, Uruguay.*

Multiparous cows (n = 25) were used in a randomized block design to study the effects of nutrition during the first 60 d postpartum (DPP) on endocrine profiles and hepatic gene expression. Cows were assigned to 3 treatments (TREAT): TMR) total mixed rations (30 kg DM/d offered; 45% forage, 55% concentrate); G1) 50% pasture in one (am) grazing session (7 h; pasture allowance = 15kg DM/d) + 50% TMR (15kg DM/d offered) and G2) 50% pasture in 2 (am/pm) grazing sessions (11h; pasture allowance = 15 kg DM/d) + 50% TMR (15 kg DM/d offered). Blood and liver biopsies were obtained at −40, −20, 10 and 55 DPP to quantify insulin and IGF-I, and mRNA expression of GH-IGF axis by real time PCR. Means from a repeated measures analysis differed when $P < 0.05$. Cow BCS decreased during the postpartum and at 55 DPP was greater in TMR than G1 and G2 cows. Milk energy output did not differ among TREAT (27.8, 26.4 and 23.4 ± 1.4 Mcal/d for TMR, G1 and G2). Serum IGF-I decreased from pre to postpartum and did not differ among TREAT while serum insulin decreased from pre to postpartum in G1 and G2 remaining stable in TMR cows. Hepatic GHR and IGFBP4 mRNA were not affected by TREAT or DPP. Expression of GHR1A mRNA decreased from −20 to 55 DPP while IGF-I mRNA tended ($P < 0.10$) to decrease from −20 to 10 DPP and to increase at 55 DPP. The IGFBP2 mRNA increased from −20 to 10 DPP but decreased thereafter at 55 DPP, being its abundance at 55 DPP less in TMR than G1 and G2 cows. In contrast, IGFBP3 mRNA tended ($P < 0.10$) to decrease from −20 to 10 DPP and increased at 55 DPP, being the decrease at 10 DPP more evident In G1 and G2 cows. The IGFBP1 mRNA increased from −20 to 55 DPP while IGFBP5 and IGFBP6 mRNA increased from −40 or −20 to 10 DPP and remained elevated at 55 DPP. However, IGFBP6 mRNA tended ($P = 0.06$) to be greater in G2 than TMR and P1 cows. Decreased serum IGF-I in early lactation was associated to decreased IGF-I mRNA and to changes in hepatic IGFBP synthesis which modulate bioavailability and stability of circulating IGF-I. Cows fed TMR showed a better metabolic status (greater BCS, serum insulin, IGBP3/IGBP2 mRNA ratio) during early lactation

Key Words: nutrition, transition cow, somatotropic axis

T276 New discovery on bovine glutathione peroxidase 3. H. R. Khazanehei,* P. Eck, and J. C. Plaizier, *University of Manitoba, Winnipeg, MB, Canada.*

Due to a high-energy demand for milk production, high-yielding dairy cows often experience a negative energy balance during early lactation. As a consequence, lipid is mobilized from adipose tissues and transported to the liver to provide energy through β-oxidation. This process produces reactive oxygen species (ROS), which can cause many disorders and cell damages. An experiment was conducted to assess the liver gene expression pattern before and after parturition and at the initiation of lactation. Liver biopsies were obtained at wk −3, 1 and 4 relative to parturition. Differential gene expression was assessed by affymetrix microarray analysis and FlexArray 1.6.1. An FDR-adjusted P lower than or equal to 0.1 and a fold-change greater than 2 were considered as a cut-off point to indicate significant up- or downregulation of genes. Gene networks were assembled using Ingenuity Pathway Analysis (IPA). The most overexpressed gene (5.08 fold change) at wk 1 was glutathione peroxidase 3 (GPX3). This indicates its non-redundant function in situations of metabolic and oxidative stress, where it detoxifies hydrogen peroxide. A further database query revealed that the bovine glutathione peroxidase 3 gene has not been characterized. Therefore we combined information from genetic databases (NCBI Gene Bank, UniGene, UCSC, ENSMBL) to hand curate and annotate the genetic locus and transcript variants, using assemblies in Sequencher 4.8. We identified 5 exons, and based on EST alignments exon 2 can be skipped in a minority of transcripts. This seems to be a bovine specific splice variant, since this event is not observed in other species alignments (http://genome.ucsc.edu/cgi-bin/hgTracks).

Key Words: dairy cow, gene expression, glutathione peroxidase 3