LACTATION BIOLOGY I

0404 Temporary alterations to milking frequency, immediately postpartum, modifies expression of milk synthesis and apoptosis genes in the mammary glands of grazing dairy cows.

T. M. Grala[1], J. K. Kay[2], J. R. Roche[2], A. G. Rius[3], and C. V. Phyn*[2], *[1]DairyNZ, Auckland, NZ, [2]DairyNZ, Hamilton, NZ, [3]University of Tennessee, Knoxville.*

Temporary changes to postpartum milking frequency can elicit lactation-long changes in milk production. The hypothesis tested in this experiment was that the immediate and long-term milk yield response to altered milking frequency would correspond to gene expression changes associated with mammary cell secretory activity and/or number. Multiparous, grazing Holstein-Friesian cows (n = 150) were randomly assigned to 1 of 5 groups at calving: milked once daily (1×) for 3 or 6 wk and twice daily (2×) thereafter; milked 2× for the entire lactation (control); or milked thrice daily (3×) for 3 or 6 wk and 2× thereafter. Milk yields were recorded daily and milk composition weekly. Mammary tissue was collected at 3, 6, and 9 wk postpartum (n = 12 cows/treatment), and gene expression measured using quantitative reverse transcription PCR. Data were analyzed using mixed models fitted with REML (Restricted Maximum Likelihood) in GenStat, including: treatment and contrasts to test milking frequency (1×, 2×, 3×), duration (3 wk, 6 wk), and their interaction as fixed effects, and cow as a random effect. Immediate ($P < 0.001$) and lactation-long ($P < 0.01$) decreases in milk and energy-corrected (ECM) production were recorded in cows milked 1× postpartum relative to 2×; however, cows milked 3× only produced greater ($P < 0.05$) milk volumes during the treatment period and did not differ ($P > 0.05$) in ECM production. Transcript levels from genes involved in milk fat (*ACACA*, *FASN*), protein (*CSN1S1*, *CSN2*), and lactose (*LALBA*, *B4GALT1*) synthesis were not altered in cows milked 3×, but were downregulated ($P < 0.05$) at 3 and 6 wk postpartum in cows milked 1×. Decreased ($P < 0.05$) expression of these genes was maintained after 1× cows were switched to 2× milking. Furthermore, at 9 wk postpartum, cows milked 1× for 3 wk had lower ($P < 0.05$) expression of genes involved in fat and lactose synthesis than cows milked 1× for 6 wk. In contrast, apoptotic genes (*PYCARD*, *FAS*) were up-regulated ($P < 0.05$) in cows milked 1×. This effect was still apparent at 9 wk ($P < 0.01$), indicating that greater mammary cell death was maintained post-treatment. In conclusion, greater milk volumes during 3× milking were not associated with altered expression of genes involved in milk synthesis or mammary cell death. However, changes in expression of genes involved in these processes may underpin the long-term reduction in milk and ECM yields in cows milked 1× postpartum.

Key Words: milking frequency, mammary apoptosis, gene expression

0405 Dietary anion-cation difference and daylength differently affect milk calcium secretion pathways.

M. Boutinaud*[1], A. Bondon[1], A. Narcy[2], C. Hurtaud[1], M. Johan[1], J. Couedon[1], and P. Lamberton[3], *[1]INRA, Saint Gilles, France, [2]INRA, Nouzilly, France, [3]INRA, Le Rheu, France.*

Milk is an important source of Ca for growth and development of children. However, it has been recently shown that dairy milk Ca content decreases with long-day photoperiod and varies according to the type of diet. Many proteins are involved in the secretion of Ca into milk by the mammary epithelial cell (MEC). The aim of this study was to identify the role of gene expression of these proteins in the regulation of milk Ca content. A trial was performed according to a Latin square design using 8 dairy cows averaging 103 ± 44 d in milk, with 2 treatments in a factorial arrangement with 4 periods of 14 d. The cows received 2 levels of dietary anion-cation differences (DCAD; 0 mEq/kg DM for d 0 and 400 mEq/kg for d 400) and 2 daylengths (8 h of light/d for short days and 16 h/d for long days). The DCAD treatments were conceived to mimic diets based either on corn silage or on herbage. The cows were exposed to solarium lights providing UVA and UVB. Once per period, MEC were prepared after milk centrifugation by purification using an anti-cytoketatin antibody bound to magnetic beads to study by real-time RT-PCR the mRNA level of genes involved in Ca secretion expressed related to RPLP0 housekeeping gene. Data were analyzed using Mixed procedure. There was no significant interaction between daylength and DCAD level. Milk and Ca yields did not vary with any treatments, averaging 32.7 kg/d and 41.1 g/d, respectively. With d 400 compared with d 0, milk Ca content increased ($P < 0.01$) with no link with casein content. No significant variation was observed on gene expression with DCAD treatment and on kappa casein and a-lactalbumin mRNA levels with any treatments. Milk Ca and casein content were lower with long days compared with short days ($P < 0.05$). The lower Ca secretion was associated with lower mRNA levels for SPCA1, ITPR1, and PMCA1, 3 Ca transporters in milk purified MEC ($P < 0.05$). This work suggests that Ca secretion pathways may be downregulated with long photoperiod, and that could explain a part of the seasonal decrease of milk Ca content during summer. In contrast, no significant variation of gene expression could explain the increase in milk Ca content with d 400.

Key Words: milk calcium, mammary epithelial cell, photoperid, feeding

0406 Infusion of a 5-hydroxy-L-tryptophan (5-HTP) to late-lactation cows impacts circulating calcium and glucose concentrations. J. Laporta*[1], S. A. E. Moore[1], A. P. Prichard[1], M. Olsen[1], B. P. Schnell[1], S. R. Weaver[1], C. R. Cronick[1], R. M. Bruckmaier[2], and L. L. Hernandez[1], [1]*University of Wisconsin-Madison, Madison,* [2]*Veterinary Physiology, Vetsuisse Faculty, University of Bern, Bern, Switzerland.*

Supplementation of 5-hydroxy-L-tryptophan (5-HTP) improved Ca and glucose status in lactating rodents, and serotonin was shown to be an indicator of positive Ca and glucose status in lactating dairy cows. Here, we examined the effect of intravenous infusion of 5-HTP on circulating Ca and glucose concentrations. Using a 4 × 4 latin square design, multiparous Holstein cows (avg. lactation = 3; avg. d in milk = 333 d) were infused with a sterile saline control (CON) or 1 of 3 doses of 5-HTP (TRT; 0.5, 1.0 and 1.5 mg/kg). Infusion periods were 4 d, with a 5-d washout between periods. Cows were infused at a constant rate for 1 h, and blood samples were collected at 0 min (preinfusion), and 5, 10, 30, 60, 90, and 120 min postinfusion. Heart rate (HR), respiration rate (RR), and rectal temperature (TEMP) were recorded every 15 min during infusion and 15 min postinfusion; milk yield (MY) was recorded daily, and manure score (0 to 4, MS) and frequency were recorded during infusions. Data were analyzed using PROC MIXED in SAS (SAS Inst. Inc., Cary, NC). Heart rate, RR, TEMP, and MY were not different between CON and TRT at any dose ($P > 0.05$) and MS was affected by TRT ($P = 0.013$). The MS was similar between CON and 0.5 mg/kg TRT, but different between CON and 1.0 and 1.5 mg/kg TRT (0.44 vs. 1.69 and 2.06 ± 0.33, respectively). Serum Ca and plasma glucose concentrations were measured and area under the curve (AUC) was calculated using the trapezoidal model. For Ca, all 5-HTP doses significantly decreased AUC compared with CON ($P < 0.001$), decreasing the first 30 min postinfusion, increasing and reaching initial Ca concentrations 120 m postinfusion. Mean Ca was greater for CON compared with all TRT doses (1.70 vs. 1.56, 1.60 and 1.59 ± 0.05 m*M*, respectively) and the same was observed for minimum Ca. Glucose AUC was greater for 1.0 and 1.5 mg/kg TRT compared with CON and 0.5 mg/kg TRT ($P = 0.02$). Mean glucose was greater for 1.0 mg/kg TRT compared with CON and 0.5 mg/kg TRT only at 90 m postinfusion ($P < 0.04$). These results demonstrate that 5-HTP stimulates a decrease in circulating Ca, and only the 2 higher doses of 5-HTP increased circulating glucose. In conclusion, 5-HTP differentially affects circulating Ca and glucose concentrations in dairy cattle, suggesting significant impacts on Ca and glucose metabolism during lactation.

Key Words: 5-hydroxytryptophan, calcium, glucose

0407 The dopamine antagonist domperidone increases prolactin concentration and milk production in dairy cows. P. Lacasse* and S. Ollier, *Dairy and Swine R&D Centre, Sherbrooke, QC, Canada.*

In previous studies, we have shown that inhibition of prolactin (PRL) secretion by the dopamine agonist quinagolide reduces milk production of dairy cows (Domest. Anim. Endocrinol. 43:154). The objective of this study was to determine the effects of the administration of a dopamine antagonist and feed restriction on basal and milking-induced PRL concentrations in blood and milk production in dairy cows. Twelve mid-lactation Holstein cows received daily subcutaneous injections of either domperidone (300 mg, DOMP, $n = 6$) or canola oil (CTL, $n = 6$) for 5 wk. During the fifth wk, all cows were fed at 65% of their DMI of the previous wk. Blood samples were collected before morning milking 3 d a wk from d 8 to d 42 (7 d after the last injections). In addition, blood samples were collected during morning milking at d 1 (before the first injection), 2, 29, and 35. Basal PRL concentration was similar among both groups before the start of treatments. Domperidone injection caused a gradual increase ($P < 0.001$) in basal PRL concentration which averaged, on the week prior the feed restriction, 32.2 and 13.9 ± 2.1 ng/mL for DOMP and CTL, respectively. Feed restriction did not affect basal PRL concentration in DOMP cows but reduced it in CTL cows ($P < 0.05$), averaging 28.0 and 7.9 ± 2.4 ng/mL, respectively. Concentration of PRL was still elevated ($P < 0.05$) in DOMP cows 7 d after the last injection, averaging 19.4 and 10.8 ± 2.7 ng/mL for DOMP and CTL, respectively. In CTL cows, the milking-induced PRL above premilking concentration (AUC) was similar at d 1, 2, and 29, but was reduced ($P < 0.05$) during feed restriction (d 35). In DOMP cows, AUC was similar at d 1 and 2, but was reduced ($P < 0.05$) at d 29 and 35. Milk production was similar for both groups before the start of treatments. There was time × TRT interaction ($P < 0.001$) for milk production during the treatment period. Milk production was similar during the first 2 wk of treatments, but was greater ($P < 0.02$) in DOMP cows during the 2 following weeks, averaging 38.0 and 35.0 ± 0.6 kg/d at wk 3 and 38.0 and 35.3 ± 0.7 kg/d at wk 4 for DOMP and CTL, respectively. Milk production declined in both groups during feed restriction but remained greater ($P < 0.05$) in DOMP cows. Milk production of both groups became similar again 5 d after the last injection. Milk composition and DMI were not affected by DOMP. These results support the hypothesis that PRL is galactopoietic in dairy cattle.

Key Words: feed restriction, prolactin

0408 Compensatory feeding of gestating gilts does not affect mammary development of their offspring at puberty. C. Farmer*[1], M. F. Palin[1], and Y. Martel-Kennes[2], [1]*Agriculture and Agri-Food Canada, Dairy and Swine R&D Centre, Sherbrooke, QC,* [2]*La COOP Fédérée, Animal Nutrition Division, St-Romuald, QC, Canada.*

The goal of this project was to determine if feed restriction followed by compensatory feeding of gestating gilts affects mammary development and mammary gene expression of their female offspring at puberty. Gilts were fed a conventional (CTL; $n = 5$) or an experimental (TRT; $n = 3$) dietary regimen. The experimental regimen provided 70% (restriction) and 115% (compensatory) of the protein and DE contents provided by the CTL diet. The restriction diet was given during the first 10 wk of gestation, followed by the compensatory diet until farrowing. Gilts were allowed to farrow, and female offspring from these (11 CTL, 12 TRT) were weighed at birth, weaning (d 20), and puberty (d 211), at which time they were slaughtered and had their mammary glands collected and dissected. Parenchymal tissue samples were collected for molecular biology work and blood samples were obtained the day before slaughter to measure IGF-1 concentrations. The MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with treatment as main effect and sow as a randomized effect was used for statistical analyses. Piglets from TRT sows tended to weigh less at birth (1.31 vs. 1.53 kg, SEM = 0.05, $P = 0.10$) than piglets from CTL sows, but had similar BW at weaning and puberty ($P > 0.10$). Concentrations of IGF-1 at slaughter tended to be greater in gilts from TRT than in gilts from CTL sows (167 vs. 142 ng/mL, SEM = 9, $P = 0.06$). Amounts of parenchymal (534.9 vs. 542.4 g for TRT and CTL gilts, respectively, SEM = 45.0) and extra-parenchymal tissue as well as composition of parenchymal tissue (DM, protein, fat, and DNA contents) were similar across treatments ($P > 0.10$). There were no differences in mRNA abundance for *IGF1*, *IGF2*, ornithine decarboxylase 1 (*ODC1*), prolactin receptor (*PRLR*), signal transducer and activator of transcription 5A (*STAT5A*) or 5B (*STAT5A*) in mammary parenchyma ($P > 0.10$). In conclusion, feed restriction and subsequent compensatory feeding of gestating gilts had no effects on mammary development or mammary gene expression of their female offspring at puberty.

Key Words: diet deprivation, diet over allowance, gestation, mammary development, offspring, sows

0409 Comparative 2D-DIGE proteomic analysis of mammary epithelial cells during lactation reveals protein signatures for lactation persistency and milk yield. J. Janjanam*, S. Singh, M. K. Jena, J. K. Kaushik, A. K. Dang, and A. K. Mohanty, *National Dairy Research Institute, Karnal, India.*

The mammary gland is made up of a branching network of ducts that end with alveoli which surrounds the lumen. These alveolar mammary epithelial cells (MEC) reflect the milk producing ability of farm animals. In our previous study, we reported the proteome profile of functionally differentiated mammary epithelial cells isolated from milk (Janjanam et al., 2013). In this study, we have used 2-D DIGE and mass spectrometry to identify and relatively quantify protein expression changes in MEC during early, peak, and late stages of lactation and also compared differentially expressed proteins in MEC isolated from high and low milk yielding animals. All the animals selected for these studies were in their third or fourth parity. For the comparative proteomic analysis at different stages of lactations, we selected 4 animals (Sahiwal cows) in each group of immediate early (E, d 15 to 30 postparturition), peak (P, d 75 to 100 postparturition), and late stage (L, d 210 to 250 postparturition) of lactation. For the comparative proteomic analysis of high and low milk yield samples, we selected 4 animals each of indigenous Sahiwal cows with high yielding (Hy, ~15 L/day) and low-yielding (Ly, ~5 L/day) breeds and 4 high-yielding cross bred cows (Karan Fries: KF, ~22 L/d) were selected which were at peak stage of their lactation. We have identified 44 differentially expressed proteins during lactation stages, and 28 proteins in high and low milk yielding animals. Bioinformatics analysis showed, a majority of the differentially expressed proteins are associated in metabolic process, catalytic, and binding activity. The differentially expressed proteins were mapped to the available biological pathways and networks involved in lactation. The proteins up-regulated during late stage of lactation are associated with NF-kB stress induced signaling pathways and whereas Akt, PI3K, and p38/MAPK signaling pathways are associated with high milk production mediated through insulin hormone signaling. The differentially expressed proteins reported in our present study could be potential biomarkers associated with lactation persistency and secretory diminution. The findings reported in the present study could benefit to the field of lactation biology. Reference: Janjanam et al. (2013). Proteome analysis of functionally differentiated bovine (*Bos indicus*) mammary epithelial cells isolated from milk. Proteomics 13:3189–3204.

Key Words: lactation, mammary epithelial cells, proteomics

0410 Milk protein synthesis is regulated by lysine and branched chain amino acid deficiencies in lactating bovine mammary glands. J. Doelman*[1], R. V. Curtis[2], M. Carson[1], J. J. M. Kim[2], J. P. Cant[2], and J. A. Metcalf[1], *[1]Nutreco Canada Agresearch, Guelph, ON, Canada, [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.*

The supply of specific essential AA is tightly regulated by the lactating dairy cow to maintain milk protein production. To determine the effect of essential amino acid (EAA) deficiencies and imbalances on milk protein synthesis and metabolic parameters, early lactation fistulated dairy cows (105 ± 12 d in milk) were abomasally infused with either saline, EAA, EAA less lysine, EAA less leucine, or EAA less the branched-chain amino acids (BCAA; isoleucine, leucine, and valine) in a 5 × 5 Latin square design. Cows were fed a diet to provide an NE_L of 6.9MJ/kg DM and 11.7% crude protein. Compared with EAA, a BCAA deficiency significantly decreased plasma concentration of Leu, Val and total BCAA by 72, 67, and 66%, respectively ($P < 0.001$). In response to a leucine deficiency, plasma concentration of Ile and Val increased 71 and 62%, respectively, while plasma leucine decreased 72% ($P < 0.001$). Omission of lysine from the abomasal infusate resulted in a 72 and 77% decline in plasma lysine and asparagine, respectively ($P < 0.04$). Plasma concentrations of β-hydroxybutrytrate and NEFA were not significantly different between treatments. While no significant treatment differences were observed for daily milk production (30.1 kg/d), milk protein yield increased 18% by the EAA infusion over saline ($P = 0.001$), while the omissions of lysine, leucine, and the BCAA decreased milk yield by 10.2, 21.1, and 12.2%, respectively, compared with EAA ($P < 0.03$). In comparison with EAA, milk protein concentration was 0.23 ($P = 0.057$), 0.3 ($P = 0.01$), and 0.29 ($P = 0.01$), percentage points lower for lysine, leucine, and BCAA deficiencies, respectively. The increase in plasma concentration of Ile and Val in response to Leu deficiency suggests that compensatory measures were initiated to maintain substrate supply for milk protein synthesis. These results indicate that protein synthesis in the mammary gland is sensitive to the supply of Lys, Leu, and the BCAA.

Key Words: mammary gland, milk protein synthesis, essential amino acid

0411 Lysine and branched-chain amino acid deficiencies decrease abundances of S6K and eIF2Bε in the mammary glands of lactating dairy cows. J. Doelman[1], R. V. Curtis*[2], M. Carson[1], J. J. M. Kim[2], J. A. Metcalf[1], and J. P. Cant[2], *[1]Nutreco Canada Agresearch, Guelph, ON, Canada, [2]Department of Animal and Poultry Science, University of Guelph, Guelph, ON, Canada.*

There have been recent investigations into the potential role of mRNA translational control in the regulation of synthesis of milk protein in the lactating bovine mammary gland. An infusion subtraction protocol was used to explore effects on abundance and activity state of regulators of mRNA translation in response to specific essential amino acid (EAA) deficiencies and imbalances. Five lactating cows on a diet of 11.7% protein were infused abomasally for 5 d with saline, 563 g/d of a complete EAA mix (equivalent to EAA in 1 kg casein), or EAA without Lys, Leu, or the branched-chain amino acids (BCAA; Ile, Leu, and Val) in a 5 × 5 Latin square design. Data was analyzed using the MIXED procedure in SAS (SAS Inst. Inc., Cary, NC) where period and treatment were considered classification effects and cow considered a random effect. The translation factors examined in mammary biopsies collected on d 5 of each period were ribosomal protein S6 kinase (S6K) to indicate mTOR (mammalian target of rapamycin) activity, eukaryotic initiation factor 2 α (eIF2α) to indicate uncharged tRNA signalling, and eukaryotic initiation factor 2B epsilon (eIF2Bε) to indicate insulin effects. Milk protein yield increased in response to EAA compared with saline ($P = 0.001$), while Lys, Leu, and BCAA deficiencies depressed milk protein yield compared with EAA ($P < 0.03$). Infusion of the complete EAA mix did not affect mTOR activity ($P = 0.65$), but subtraction of BCAA from the mix decreased phosphorylated S6K abundance ($P = 0.05$) and subtraction of Leu tended to decrease phosphorylated S6K ($P = 0.10$). Similarly, abundance of total eIF2Bε was not affected by infusion of EAA ($P = 0.39$) but decreased when BCAA ($P = 0.04$) or Leu ($P = 0.06$) were subtracted. There was a correlation of 0.58 between abundances of phosphorylated S6K and total eIF2Bε. Lys subtraction had no effect on mammary mTOR/eIF2Bε signalling, but the abundance of total S6K tended to be lower during Lys deficiency compared with saline ($P = 0.06$) and EAA ($P = 0.09$). Phosphorylation state of eIF2α was not increased by any of the imbalances or deficiencies. It was concluded that Lys deficiency may impair milk protein yield through a decline in translational activation capacity, indicated by S6K abundance. The BCAA deficiencies may impair milk protein yields through deactivation of mTOR-mediated up-regulation of eIF2Bε abundance.

Key Words: milk protein synthesis; mRNA translation regulation, essential amino acid