($P \leq 0.1$) P3 activity (1.87 vs. 0.34 arbitrary units for stearic vs. control, respectively). Activity of P2 was not altered ($P \geq 0.05$) by any treatments and there was no response to glucocorticoids or unsaturated fatty acids for any of the promoters tested. Stearic acid suppresses the activity of bovine PC promoter 1 but enhances the activity of promoter 3 creating a net increase in promoter activity. These data demonstrate the role of fatty acids in regulating PC expression. The specificity of response for PC promoter function to stearic acid in the current data suggests a physiological role for elevated stearic acid concentrations previously observed in transition cows at calving.

Key Words: pyruvate carboxylase, promoter, fatty acids

Lactation Biology

M139 Effects of restricted feeding of prepubertal ewe lamb on growth performance, mammary gland development and first lactation. L. Villeneuve*[1], D. Cinq-Mars[2], and P. Lacasse[3], [1]*Centre d'expertise en production ovine du Québec*, *LaPocatière, QC, Canada*, [2]*Laval University*, *Québec, QC, Canada*, [3]*AAFC, Dairy and Swine Research and Development Center*, *Sherbrooke, QC, Canada*.

The aim of this study was to determine the effects of restricted feeding before puberty on growth performances, mammary gland development and milk production in replacement ewe lambs. At weaning, 72 Dorset ewe lambs were assigned to either an ad libitum diet (A), a restricted diet with good quality forage (14.8% CP, 2.15 Mcal ME/kg DM, 34.7% ADF) (F), or a restricted diet with medium quality forage (13.3% CP, 1.81 Mcal ME/kg DM, 42.8% ADF) (R). The quantity of feeds offered to ewe lambs of group R and F was adjusted in order to get an ADG representing 70% of that of ewe lambs of A group. These diets were offered during 75 d following weaning to cover the allometric phase of mammary gland development. During this period, ADG were respectively 223 and 229 g/d for R and F group compared to 305 g/d for A group ($P<0.0001$). At the end of this period, 28 ewe lambs were slaughtered and their mammary gland was collected. Parenchymal tissue weight tended to be higher for R and F groups with 27,86g and 24,37g respectively compared to 19,34 g for A ($P<0.10$). Stroma weight was greater ($P<0.05$) for A lambs (91.2, 61.9, and 64.4g for A, R and F, respectively). Total DNA and total protein in parenchymal tissue tend to be greater ($P=0.09$ and $P=0.07$ respectively) for R and F. Dry fat free tissue were greater for R and F averaging 1.81mg and 1.50mg respectively against 1.19mg for A ($P<0.05$). The remaining ewe lambs were fed a diet composed of silage and barley until their first lambing. During this period, compensatory growth occurred and ADG were greater ($P<0.01$) for R and F (179 and 175 g/d) than for A ewe lambs (147 g/d). Feed conversion was better ($P<0.01$) for R and F while the DMI was similar for all three groups. Milk fat and protein content were comparable for all animals but standardize milk yield tend to be better for R and F group ($P=0.07$). The results of this study suggest that restricted feeding before puberty improve mammary gland development and milk production without compromising growth performances in ewe lambs.

Key Words: ewe lamb, mammary development, restricted feeding

M140 Effects of intravenous infusion of *trans*-10, *cis*-12 18:2 on mammary lipid metabolism in lactating dairy cows. R. Gervais*[1], J. W. McFadden[2], A. J. Lengi[2], B. A. Corl[2], and P. Y. Chouinard[1], [1]*Université Laval*, *Québec, QC, Canada*, [2]*Virginia Tech*, *Blacksburg*.

It has been previously established that supplementation of *t*10*c*12 18:2 reduces milk fat content and fat deposition in a number of species. The objectives of the study were 1) to examine whether potential mechanisms by which *t*10*c*12 18:2 is reported to affect lipid metabolism in adipose tissue of different species could be partly responsible for the inhibition in milk fat synthesis observed in dairy cows; 2) to investigate the effects of *t*10*c*12 18:2 on the expression of a newly identified isoform of stearoyl-CoA desaturase in bovine mammary tissue. Four primiparous lactating Holstein cows fitted with a jugular catheter were used in a 2×2 crossover design. For the first 5 d of each period, cows were infused intravenously with a 15% lipid emulsion providing 10 g/d of either *c*9*c*12 18:2 (CTL) or *t*10*c*12 18:2 (CLA). On d 5 of infusion, mammary gland biopsies were performed and tissues were analyzed for mRNA expression of acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS), lipoprotein lipase (LPL), stearoyl CoA desaturase-1 (SCD1) and -5 (SCD5), sterol regulatory element-binding protein-1 (SREBP1), interleukin-6 (IL6) and -8 (IL8), and tumor necrosis factor-α (TNFα) by real-time RT-PCR. Compared to CTL, CLA reduced milk fat content and yield by 46 and 38%, respectively ($P<0.05$), and increased the *t*10*c*12 18:2 content in milk fat from 0.05 to 3.53 mg/g ($P<0.05$). Milk yield, milk protein, and DMI were unaffected by treatments ($P>0.15$). Infusion of the CLA treatment reduced the mRNA expression of ACC and FAS by 46 and 57%, respectively and tended to reduce the expression of SCD1 ($P=0.06$) and LPL ($P=0.14$). Abundance of SREBP1 mRNA was reduced by 59% in the CLA treatment ($P<0.05$). However, infusing *t*10*c*12 18:2 did not affect the expression of transcripts for SCD5, TNFα, IL6, and IL8 ($P>0.15$). Results from the current study corroborate the idea that effects of *t*10*c*12 18:2 observed on adipose tissue in animal models and humans are not part of the response in the inhibition of milk fat synthesis in dairy cows. They also support the hypothesis that SCD1 and SCD5 present important differences in their regulation and physiological roles.

Key Words: CLA, lipid metabolism, desaturase

M141 Selection of reference genes for quantitative real-time PCR in mouse mammary gland during different lactation days. X. L. Dong[1,2], J. Q. Wang*[1], D. P. Bu[1], K. L. Liu[1], H. Y. Wei[1], and L. Y. Zhou[1], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*Yangzhou University*, *Yangzhou, China*.

Real-time quantitative PCR (qPCR) is a method for rapid and reliable quantification of mRNA transcription. Selection of high quality reference genes is crucial to interpret the data generated by this method. The aim of this study was to investigate the expression stability of reference genes in the mammary gland of mouse during lactation circle. Six reference genes (*B2M*, *ACTB*, *GAPDH*, *SDHA*, *HPRT1* and *ARBP*) were chosen. The mammary gland samples were obtained from thirty lactating mice that were at 0, 1, 4, 8 and 12 days relative to parturition. Gene expression levels were measured by qPCR. During the different lactation days, the expression of the gene was analyzed by the ANOVA procedure of SAS (Version 6.12, SAS). The expression of *ACTB*,*GAPDH*, *SDHA*,

HPRT1 and *ARBP* was relatively stable (P>0.05), but the *B2M* shows a significant difference (P<0.05). The stability of the selected genes was investigated using geNorm Visual Basic application for Microsoft Excel. Based on geNorm algorithm, the range of expression stability in the genes analysed was (from the most stable to the least stable): *GAPDH/ HPRT1*, *ARBP*, *ACTB*, *SDHA* and *B2M*. We propose the use of two genes *GAPDH* and *HPRT1* as references for normalization of real-time qPCR in mice mammary gland for different lactation days.

Key Words: lactation, reference gene, qPCR

M142 Responses of milk protein and mammary amino acids metabolism to duodenal soybean small peptides and free amino acids infusion in lactating goat. H. Liu, Z.-J. Cao, L. Wang, S.-L. Li*, and L.-B. Wang, *College of Animal Science and Technology, China Agricultural University*, *Beijing, China*.

The effects of infusing soybean small peptides (SSP) or their free amino acids (FAA) into milk protein and the mammary amino acids (AA) metabolism of lactating goats were investigated using duodenal perfusion technique. Six Saanen goats with silicon catheters implanted into their carotid artery and mammary veins were used in crossover design. Blood samples were collected from carotid artery and mammary vein. The AA concentrations in the plasma, as well as in mammary uptakes of AA were monitored. The results showed that all AA concentrations in arterial plasma tended to be increased by SSP and FAA infusion. Differences in the concentrations of most essential amino acids (EAA) were significant between SSP and FAA infusion treatment, except for Val, Ile and Met ($p<0.05$). The concentration of all nonessential amino acids (NEAA), measured after FAA infusion treatment, was the higher ($p<0.05$), and the concentration of Asp, Glu, Ala and Tyr were significantly different ($p<0.05$). The concentration of most EAA in the mammary vein was the higher after FAA treatment, and the concentration of Val, Lys, Leu and Arg were significantly so ($p<0.05$). The mammary uptake of EAA was increased by SSP and FAA infusion. The mammary uptakes of other NEAA were increased by SSP and FAA infusion, except for Asp, Glu and Gly. Most EAA were taken up in excess in the SSP and FAA treatment. The rations of the EAA (except for Met) were low in the SSP treatment, compared to the FAA treatment. Ratios of all NEAA varied among treatments. Compared to the control treatment, significant increases in milk protein yield and milk protein content after the FAA infusion were observed ($p<0.05$); with the SSP infusion treatment, milk yield and milk protein were numerically increased, the difference was not significant. The results suggested FAA could improve milk protein synthesis.

Key Words: soybean small peptides, free amino acid, mammary metabolism

M143 Characterization of the bovine mammary FcRn receptor in mammary cells *in vitro*. C. R. Baumrucker*, Y. Wang, W. Liu, and C.D. Dechow, *The Pennsylvania State University*, *University Park*.

Bovine IgG1 is specifically transported by a process of transcytosis across the mammary epithelial cells during colostrogenesis and the neonate intestinal epithelia by the bovine FcRn receptor [bFcRn]. The FcRn receptor is composed of α heavy chain [FCGRT] and it is non-covalently associated with β2 microglobulin [β2M] in the plasma membrane. Mammary IgG1 appearance in cow colostrum is extremely variable in mass and concentration. We show concentration variance is inversly correlated to lactose mass while total mass of IgG1 remains variable suggesting differences in FcRn capacity. Data with bovine mammary cells in vitro (BME-UV1 & primary cells [MeBo]) shows strong expression of both bFCGRT and bβ2M mRNA when compared with bovine mammary and liver mRNA. In whole mammary tissue, but not the cells, we detected the expression of a bFCGRT variant. With the cellular expression, we set the objectives to characterize the 1) hormonal regulation of the bFCGRT mRNA and 2) to characterize IgG1 binding to the bFCGRT component *in vitro*. We show that the two messages (bFCGRT & bβ2M) were regulated (real time PCR) by hormone combinations that regulate mammary proliferation and differentiation *in vivo*. While insulin (I), cortisol (F), and prolactin (Prl) was not different from control (2% FBS), the absence of Prl showed significant reduction in bFCGRT expression. The application of 17β-estradiol accounted for >2 fold increase in both FcRn components in MeBo cells while the BME-UV1 cells did not respond. Biotinylated bIgG1 binding assays with the mammary cells in vitro indicated specific binding and receptor characteristics. Our findings suggest that bovine mammary cells may be utilized to understand the regulation of the FcRn to bind, internalize, and transcytoses IgG1 into colostrum. Furthermore, it is likely that the use of cell culture will provide a better understanding of the phenotype of FcRn variants and how they may contribute to the difference in IgG1 mass during colostrogenesis.

Key Words: mammary, FcRn, IgG1

M144 *In vitro* culture and characterization of a mammary epithelial cell line from Chinese Holstein dairy cows. H. Hu[1], D. P. Bu[1], J. Q. Wang*[1], Q. Chen[1], X. Y. Li[1], H. Y. Wei[1], L. Y. Zhou[1], and J. J. Loor[2], [1]*State Key Laboratory of Animal Nutrition, Institute of Animal Science, Chinese Academy of Agricultural Sciences*, *Beijing, China*, [2]*University of Illinois*, *Urbana*.

The objective of this study was to establish an in vitro bovine mammary epithelial cell line as a system that could be used to study mammary epithelial cell biology. Mammary tissue from a 3-y old lactating (ca. 100 DIM) Chinese Holstein dairy cow was used as a source of epithelial cells. Tissue was plated on collagen-coated (35 mm) plastic culture dishes and incubated in DMEM/F12 media containing 10% fetal bovine serum (FBS). Fibroblasts and epithelial cells successively grew and extended from mammary tissue during a 3-d culture period after which epithelial cells were obtained by continuous culture and digestion with 0.25% tripsin and 0.02% EDTA. Immunocytochemistry revealed a strong positive response of cytokeratin-18 suggesting that cells harvested exhibited the specific character of epithelial cells. Epithelial cells cultured in growth medium supplemented with supraphysiological concentrations of bovine insulin (5 μg/mL) and hydrocortisone (10^{-7} mol/L) maintained a normal diploid chromosome modal number ($2n = 60$) and were capable of synthesizing and secreting β-casein. Frozen preservation in 90% FBS and 10% DMSO did not influence the growth character, chromosome number, or protein secretion ability of the epithelial cell line. Results indicated that the obtained mammary epithelial cell line is normal in morphology, growth, and cytogenetic characteristics. Thus, this resource might aid in the controlled study of bovine mammary cell biology.

Key Words: mammary epithelial cells, Chinese Holstein dairy cow, tissue culture

M145 Transcriptomics comparison of MacT cells and mammary tissue during pregnancy and lactation. R. Sharma, M. Bionaz, A. K. G. Kadegowda, R. E. Everts, H. A. Lewin, and J. J. Loor*, *University of Illinois*, *Urbana*.

Use of immortalized bovine mammary epithelial cell lines (e.g., MacT) has enriched knowledge on the molecular aspects of bovine mammary physiology. The degree of similarity between these cell lines and mammary tissue at the mRNA level remains unknown. A 13K bovine oligonucleotide microarray was utilized to compare gene expression in bovine mammary tissue prepartum (-30 d) and postpartum (60 d) with MacT cells cultured in lactogenic medium. We identified transcripts which were similar as well as those highly-expressed in mammary tissue (MG) or MacT cells through direct hybridization. Overall, 30.4% (3,073) of transcripts had similar abundance between MG and MacT (FDR ≤ 0.2). Similarity was higher at 60 d (55%) compared with -30 d (43%). Results at 60 d were mainly related to insulin sensitivity, metabolism (e.g., hormones, anion), oligomerization of proteins, and arrest in proliferation. In contrast, at -30 d similarities were related to production of ATP and lipids (e.g., eicosanoids), activation of transcription (e.g. androgen and E2F). Overall, there were comparable numbers of highly-expressed genes (HEG; FDR ≤ 0.05) in MG (822) or MacT (852). The same trend was observed at 60 d (1,380 for MG; 1,350 for MacT). Interestingly, at -30 d the proportion of HEG in MG vs. MacT was nearly doubled (2,092) than that of MacT vs. MG (1,269). Overall HEG in MacT vs. MG were involved in cellular movement, apoptosis, cellular growth and proliferation, morphogenesis and development. In contrast, HEG in MG vs. MacT were related to increases in carbohydrate and lipid metabolism, transport of lipid and Ca2+, morphology of leukocytes, and decreased cell death. Most of HEG in MacT vs. MG at 60 d code for elements of the protein synthesis machinery even though milk protein transcripts were expressed at >12-fold in MG vs. MacT. Results suggest that MacT cells could be useful to study transcriptional regulation of nonlactating mammary tissue. Furthermore, they appear suitable for studies of proliferation, differentiation, and survival and death during lactation.

Key Words: microarray, genomics, epithelial cell

M146 Chinese women dietary behavior in different lactating stages and breast milk levels of fatty acids and iron. L. Xu*[1], Q.-H. Sheng[2], Z.-G. Zhang[3], Q. Gen[3], and L.-W. Zhang[1], [1]*School of Food and Science and Engineering , Harbin Industry University*, *Harbin city, China*, [2]*National Dairy Engineering & Technical Research Center, Northeast Agriculture*, *Harbin city, China*, [3]*Hebei Dairy Engineering & Technical Research Center*, *Shingjiazhuang city, China*.

Doing-the-month is a popular traditional behavior for Chinese mothers after delivery. During this period, lactating women prefer to meat and egg, and have a sedentary traditional habit. These unusual behaviors of mothers make a possible of change in fatty acids and iron compositions in human milk during the early stage of lactating, compared with follow-up months. Therefore, forty volunteers were recruited and assigned into two groups, the postpartum first month group (n=13) and postpartum 2-6 months group (n=27), respectively. The data were analyzed using SPSS version 10.0. This protocol and procedure were approved by Peking University Ethics Committee. The special changes in diet were assessed with indictors of protein, fat, and carbohydrate. Higher protein and lower carbohydrate intakes were indicated in the postpartum first month group, compared with that in postpartum 2-6months group. The fat intakes are similar between the two groups. Compared with "Chinese Dietary Reference Intakes", over 60% of women exceeded the standard in the intake of protein and fat in the postpartum first month group. However, there were over 20% women who had under standard intakes in energy, protein, and carbohydrate in the postpartum first month. For the postpartum 2-6months group, more than 50% of lactating women had lower intakes in protein and carbohydrate than the standard. Furthermore, the individual average content of 13 fatty acids and iron from human milk in different lactating stages were identified. There are no significant differences in the concentration of reported breast milk fatty acids in two groups, except for LA. There is significantly higher concentration of LA in the postpartum first month group than that in postpartum 2-4months group. The decrease in the content of iron was indicated in postpartum 2-6 months group, comparing with the postpartum first month group, from 126.82 μg/100ml to 80.85 μg/100ml. However, there is no statistical significant difference from two groups.

Key Words: lactating women, human milk, fatty acids

M147 Effect of staged ovariectomy on mammary histology and transcript abundance in prepubertal heifers. B. T. Velayudhan*[1], R. M. Akers[1], B. P. Huderson[1], A. Rowson-Baldwin[2], R. C. Hovey[2], and S. E. Ellis[3], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg*, [2]*University of California*, *Davis*, [3]*Clemson University*, *Clemson, SC*.

Previous studies suggest that the magnitude of reduction in mammary growth in response to ovariectomy depends on the age at which surgery was performed. We hypothesized that ovariectomy will reduce mammary epithelial cell proliferation and the magnitude of response will be greater in early ovariectomy. The objectives were to determine the effect of staged ovariectomy on epithelial cell proliferation as well as gene expression in bovine mammary gland. Prepubertal dairy heifers either at 2, 3 or 4 m of age were randomly assigned to one of two treatments, ovariectomized n = 8) or sham operated (n = 12), and mammary parenchyma were harvested 30 d after surgery. Epithelial area measurements and localization of progesterone receptors (PR) and Ki67 positive epithelial cells were determined by histological and immunohistochemical analyses. Gene expression of estrogen receptor-α (ERα), estrogen responsive genes such as stc1 and tfpi, PR and proliferating cell nuclear antigen (PCNA) were determined by quantitative real time PCR using comparative Ct method. Data were analyzed using Mixed model procedure of SAS. Expression of PR was reduced ($P < 0.05$) in ovariectomy at both protein and message level, but there was no difference in the proliferation of epithelial cells ($P = 0.652$) or the percent area of epithelium present ($P = 0.845$). Expressions of stc1, tfpi, and PCNA mRNA were down regulated by ovariectomy ($P < 0.05$) but ERα mRNA was not affected ($P = 0.196$). We did not find an interaction between ovariectomy and age at surgery for epithelial proliferation or transcript abundance. Our data suggest that a 30 d period after ovariectomy in prepubertal dairy heifers is insufficient to produce a marked reduction in proliferation rates in mammary epithelial cells despite the changes in estrogen related gene expression.

Key Words: epithelial proliferation, gene expression, ovariectomy

M148 Variation in expression of genes involved in glucose production and transport in mammary gland, liver and muscle of lactating cows. R. Weikard*, K. Krappmann, B. Brand, T. Goldammer, R. Brun-

ner, and Ch. Kühn, *Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany*.

To identify regulatory mechanisms necessary to accommodate changes associated with lactation, analysis of mRNA expression levels of metabolic key enzymes of gluconeogenesis and glucose transport in mammary gland, liver and muscle of lactating cows was performed using quantitative RT-PCR. Three groups of animals (10 per group) differing in their metabolic status due to high differences in milk production (high, medium, low) were analyzed. In mammary gland of high-lactating (HL) dairy cows, the mRNA expression level of PCK2 known to catalyze the rate-controlling step of gluconeogenesis was significantly upregulated ($p<0.001$). Because in mammary gland mRNA expression levels of other genes included in gluconeogenesis did not vary significantly between groups, an additional metabolic function for PCK2 could be assumed in this tissue. Differences in glucose transport in mammary gland were reflected by divergent in transcript levels of glucose transporters. Whereas GLUT1 was higher expressed in mammary gland of HL cows ($p<0.05$), insulin-responsive GLUT4 and GLUT12 were downregulated in mammary tissue of this group ($p<0.005$ and $p<0.001$). In liver, significant differences of mRNA expression levels were detected for PCCA, which revealed a higher level in the HL group ($p<0.001$). This would indicate on an accelerated propionate metabolism. Furthermore, GLUT8 and GLUT4 showed a marked decrease in mRNA expression ($p<0.001$ and $p<0.001$) in the liver of HL cows. A considerably lower level of gene expression of PCK2 ($p<0.001$) and a substantial increase in the transcript level of FBP2 ($p<0.001$) were observed in muscle of HL cows pointing to affected gluconeogenesis or suggesting specific, yet unrecognized functions of these genes in muscle metabolism. The results presented could provide a basis for the elucidation of the physiological role of the enzymes of gluconeogenesis and glucose transport in different tissues in response to lactation-associated metabolic challenges in dairy cows.

Key Words: lactation, gene expression

M149 Effects of increased milking frequency on milk fatty acid composition in early lactation dairy cows. S. L. Shields*, D. Sevier, J. E. Williams, S. Zaman, P. Rezamand, and M. A. McGuire, *University of Idaho, Moscow*.

Milk production can be increased by more frequent milking; however, it may alter milk fatty acid (FA) composition. Our objective was to determine the effects of increased milking frequency on milk FA profile in early lactation cows, using a unilateral frequent milking (UFM) model. After parturition, sixteen Holstein cows were assigned to UFM in which the left udder half of each cow was milked four-times daily (4×; at 0500, 0900, 1600, and 2000 h) and the right udder half was milked twice daily (2×; at 0500 and 1600 h) with milk yields recorded from 1 through 21 days in milk (DIM) and samples were collected on d 3, 7, 10, 14, and 21. Milk FA composition was determined using gas chromatography (GC). Data were statistically analyzed by paired t-test using SAS (v. 9.2). Milk fat yield (milk yield × milk fat percent) was greater for the udder half milked 4× compared with the udder half milked 2X (0.60 vs 0.39 ± 0.01 kg/day; $P < 0.001$). Milk protein yield was also greater for the udder half milked 4× compared with the udder half milked 2× (0.42 vs 0.31 ± 0.006 kg/day; $P <0.001$). No significant difference was, however, detected in FA (wt% of total FA) synthesized via de novo synthesis for the udder milked 4× compared with the udder half milked 2× including 4:0 (3.91 vs 3.84 ± 0.1), 6:0 (1.75 vs 1.74 ± 0.06), 8:0 (0.91 vs 0.91 ± 0.04), 10:0 (1.86 vs 1.90 ± 0.12), 12:0 (2.10 vs 2.13 ± 0.14), and 14:0 (0.14 vs 0.13 ± 0.01; $P \geq 0.15$ for all). In addition, no significant difference was detected in 18:0 (11.83 vs 11.86 ± 0.28; $P = 0.76$) or two major products of desaturase enzyme, 18:1 c9 (27.0 vs 27.1 ± 0.68) and 18:2 c9 t11 (0.37 vs 0.37 ± 0.01; $P \geq 0.75$ for both), between the udder half milked 4X and the udder half milked 2×. We conclude that using a UFM model, increased milking frequency resulted in greater milk fat yield; however, increased milking frequency did not affect the FA synthesized via *de novo* synthesis, through the action of the desaturase enzyme or from preformed FA. Increased milk fat content, due to increased milking frequency, may have been related to increased FA uptake by the mammary gland.

Key Words: milking frequency, milk composition, milk fatty acid

M150 Energy deprivation inhibits protein synthesis in mammary epithelial cells through an AMPK- and mTOR-dependent pathway. S. A. Burgos* and J. P. Cant, *University of Guelph, Guelph, Ontario, Canada*.

Restriction of energy availability results in decreased milk yield and activity of enzymes involved in milk synthesis. To determine the involvement of the AMP depedent protein kinase (AMPK) and mammalian target of rapamycin (mTOR) signaling pathways in regulation of protein synthesis by energy deprivation, we examined the effects of 2-deoxy-D-glucose (2DG), a glucose analog that inhibits glycolysis, in the activation state of AMPK and mTOR signaling on effector mechanisms of mRNA translation in Mac-T cells. Subconfluent Mac-T cells were incubated in DMEM containing 2.5 mM D-glucose for 16 h and then treated with 10 mM 2DG for 15 min. Cell were harvested by scraping in lysis buffer. For immunoprecipitation, cell lysates were incubated with antibody for 2 h at 4°C with rotation and then protein A sepharose was added for 1 h. Beads were washed in lysis buffer and the precipitates were eluted in SDS sample buffer. For determination of protein phosphorylation state, cell lysates were boiled in SDS sample buffer for 5 min. Equal amounts of immunoprecipitated samples or cell lysate protein were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked, incubated with primary antibody overnight at 4°C, washed, and incubated with secondary antibody. Blots were visualized by enhanced chemiluminescence. We observed that energy depletion by 2DG stimulates AMPK activity, as measured by its phosphorylation at Thr172 and by the phosphorylation of the AMPK target acetyl-CoA carboxylase at Ser-79. The activation of AMPK was associated with downregulation of mTOR signaling, as determined by phosphorylation state of the mTOR translation effectors ribosomal protein S6 kinase and eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1). In addition, 2DG treatment resulted in the enhanced association of eIF4E with the translational repressor 4E-BP1 and reduced formation of the active eIF4E-eIF4G complex. The results suggest that energy deprivation inhibits protein synthesis in mammary epithelial cells through an AMPK- and mTOR-dependent pathway.

Key Words: energy deprivation, mammalian target of rapamycin, protein synthesis

M151 Effect of milking frequency (1 vs. 4x) on milk yield, composition and numbers of gene transcripts for alpha-lactalbumin and beta casein in milk. A. P. Alex*[1], J. L. Collier[1], D. L. Hadsell[2], and R. J. Collier[1], [1]*University of Arizona, Tucson*, [2]*Baylor College of Medicine, Houston, TX*.

Recently published information indicates cytoplasm associated with milk fat globule membranes contains messenger RNA for the milk

proteins casein and alpha-lactalbumin. Furthermore, differences in the concentrations of these transcripts in mammary epithelial cells are reflected in differences in the concentration of these transcript in milk. Objective was to evaluate the effect of 1 vs 4x milking frequency on milk yield & composition as well concentration of messenger RNA transcripts for beta casein and alpha lactalbumin. Mean milk yield (kg/d) on d15, d60, d120 and d230 was 19.0, 18.2, 15.9 and 9.1 from the 4× half and 6.8, 7.2, 5 and 2.7 from the 1x half. Milk fat percent was on average slightly lower (3.34) in milk from 4× halves versus 1× halves (3.7) while protein per cent was slightly higher (2.83 vs. 2.68). Lactose and solids not fat (SNF) percentages were higher in milk from 4× halves than 1x halves. Beta-casein expression in 4× milk at d15, 60, 120 and 230 was 3.2, 2.2, 4.8 and 2.4 fold higher than 1× milk. Alpha-lactalbumin gene expression in milk from 4× halves was 2.9, 3.6, 8.8 and 2.1 fold higher than 1× at d15, 60,120 and 230. Thus transcript numbers were higher in milk from udder halves milked 4x despite slightly lower fat concentrations in milk from these halves versus milk from 1× milked halves. We conclude that transcript concentration for the milk proteins alpha-lactalbumin and beta-casein reflected differences in milk yield associated with differences in milking frequency. *This project was supported by National Research Initiative Competitive Grant no. 2007-35206-17831 from the USDA Cooperative State Research, Education, and Extension Service.*

Key Words: beta casein, alpha lactalbumin, milking frequency

M152 Lactational effects of once- versus twice-daily milkings throughout lactation in two breeds of dairy ewes. A. Santibañez, X. Such*, G. Caja, V. Castillo, and E. Albanell, *G2R, Universitat Autònoma de Barcelona*, *Bellaterra, Spain*.

The effects of once- (1×) vs. twice-daily (2×) milkings throughout lactation on milk yield, milk composition, SCC, lactose, cisternal size and milk fractioning were studied in 2 breeds of dairy ewes differing in milk yield, udder compartments and milkability (Manchega, MN, n = 29; Lacaune LC, n = 37). After the weaning of the lambs (wk 5), ewes were machine milked at 2× and blocked into 2 groups to which milking treatments were applied: 1× (MN, n = 15; LC, n = 18) and 2× (MN, n = 14; LC, n = 19). Individual milk recording was conducted weekly (wk 5 to 25) for milk yield, biweekly for milk composition, monthly for SCC, and 2 times (wk 5 and 14) for lactose. Cisternal area and udder compartments were measured using an oxytocin receptors blocking agent (wk 5 and 14). Reducing milk frequency throughout lactation impaired milk yield differently in each breed (MN, -46%; LC, -25%; $P < 0.01$). A dramatic drop in milk yield was observed between wk 5 and 6 of lactation in the ewes passing from 2× to 1×. Milking frequency did not affect ($P > 0.05$) percentages of major milk components (fat, protein, total solids and casein), the milk of MN ewes being richer in components than LC. During lactation, lactose in blood was not affected by milking frequency, but LC ewes had greater levels of lactose than MN (33.9 vs. 19.5 µml/L, respectively; $P < 0.05$). The SCC was not affected ($P > 0.05$) by milking frequency, but LC ewes had greater values than MN ($P < 0.01$) and, for both breeds, a linear increase ($P < 0.01$) in SCC from 1st to 3rd parity was observed (5.01, 5.02, 5.62 log10 cells/mL). Cisternal and alveolar milk decreased ($P < 0.05$) throughout lactation (wk 4 to 14; 503 to 270 mL; 450 to 164 mL, respectively). Area of the cistern decreased (44.5 to 34.6 cm^2) as lactation progressed, affecting ($P < 0.01$) the fractioning of cisternal milk (S, 282 mL; L, 476 mL). Ewes LC showed greater cisternal area and cisternal milk ($P < 0.05$) than MN (483 vs 267 mL; 46.9 vs. 30.3 cm^2), respectively) which were related to the ability of each breed for adapting to 1× milking frequency.

Key Words: milking frequency, milk fractioning, dairy ewes

M153 Activation of mTOR signaling by insulin-like growth factor-I stimulates translation initiation in mammary epithelial cells. S. A. Burgos* and J. P. Cant, *University of Guelph*, *Guelph, Ontario, Canada*.

In several mammalian systems, the insulin-like growth factor I (IGF-I) mediates its effects through the phosphatidylinositol-3-kinase (PI3K) and mammalian target of rapamycin (mTOR) signaling pathways to regulate protein translation. The objective of this study was to determine the involvement of PI3K and mTOR signaling pathways in regulation of translation initiation by IGF in bovine mammary epithelial cells. Subconfluent Mac-T cells were serum starved for 16 h and then treated with 50 ng/ml IGF-I for 0, 5, 10 or 15 min. For inhibition of PI3K, cells were pretreated with 1 mM wortmaninn for 30 min. Cell were harvested by scraping in lysis buffer. For immunoprecipitation, cell lysates were incubated with antibody for 2 h at 4°C with rotation and then protein A sepharose was added for 1 h. Beads were washed in lysis buffer and the precipitates were eluted in SDS sample buffer. For determination of protein phosphorylation state, cell lysates were boiled in SDS sample buffer for 5 min. Equal amounts of immunoprecipitated samples or cell lysate protein were separated by SDS-PAGE and transferred to PVDF membranes. Membranes were blocked, incubated with primary antibody overnight at 4°C, washed, and incubated with secondary antibody. Blots were visualized by enhanced chemiluminescence. IGF treatment led to increased phosphorylation of protein kinase B at Ser 473was maximal within 5 min of IGF treatment. The effect of IGF on PKB phosphorylation was blocked by wortmannin. IGF treatment resulted in phosphorylation of the tuberous sclerosis complex 2 protein at Thr 1462 and the proline-rich Akt substrate of 40 kDa at Thr 246 by 5 min and was maximal by 10 min. Phosphorylation of ribosomal protein S6 kinase phosphorylation and eukaryotic initiation factor (eIF) 4E-binding protein-1 (4E-BP1) increased progressively up to 15 min. Hyperphosphorylation of 4E-BP1 led to the release of eIF4E and enhanced formation of the active eIF4E-eIF4G complex. IGF-I stimulates translation initiation through mTOR signaling in mammary epithelial cells.

Key Words: insulin-like growth factor, mammalian target of rapamycin, protein synthesis

M154 An intact SREBP pathway is essential for the *trans*-10, *cis*-12 CLA-induced inhibition of de novo fatty acid synthesis in the murine lactating mammary gland. M. R. Foote*[1], K. J. Harvatine[1], J. Monks[2], M. C. Neville[2], Y. R. Boisclair[1], and D. E. Bauman[1], [1]*Cornell University*, *Ithaca, NY*, [2]*University of Colorado*, *Aurora*.

Trans-10, *cis*-12 conjugated linoleic acid (CLA) is a potent inhibitor of de novo fatty acid synthesis in the lactating mammary glands of many species. In dairy cows and mice, this inhibition of milk fat synthesis is accompanied by a coordinated decrease in the expression of key lipogenic genes within the mammary gland, including the sterol response element binding protein 1 (SREBP1), a transcription factor involved in the regulation of hepatic lipid synthesis in rodents. To evaluate the functional role of SREBP in the regulation of milk fat production, we examined CLA-induced inhibition of milk fat synthesis using mice expressing a floxed version of the escort protein SCAP (SREBP cleavage-activating protein), which is necessary to activate the SREBP pathway. Control mice and mice with disrupted SCAP expression were orally dosed with water (n = 8) or with 20 mg of *trans*-10, *cis*-12 CLA (n = 8) for four days during lactation. CLA decreased ($P<0.05$) growth of litters from control dams but had no effect on growth in dams with disrupted SCAP expression. CLA decreased ($P<0.01$) the proportion of de novo fatty acids (C<16:0) in milk of control mice but had no effect

on milk fatty acid composition of mice with disrupted SCAP expression. In addition, CLA decreased (P<0.05) the mammary expression of key lipogenic genes (e.g., SCD1, SREBP1c) in control dams but not in SCAP-disrupted dams. These results demonstrate a functional role for the SREBP signaling pathway in the regulation of mammary synthesis of fatty acids and indicate that an intact SREBP pathway is essential for the *trans*-10, *cis*-12 CLA-induced inhibition of de novo fatty acid synthesis in the mammary gland.

Key Words: CLA, mouse

M155 Low dosage oxytocin treatment induces milk ejection in dairy cows. C. J. Belo and R. M. Bruckmaier*, *University of Bern, Vetsuisse Faculty, Veterinary Physiology*, *Bern, Switzerland*.

Oxytocin (OT) treatment is frequently used in dairy practice to overcome disturbed milk ejection. However, the chronic use of high OT dosages can reduce the response to endogenous OT. We have investigated the use of OT in practical farms and we have tested the suitability of low dosage OT for milk removal. Based on the information from 82 dairy farms the used OT dosages were high, especially when injected i.m. (23 ± 2 IU and 7 ± 1 IU for i.m. and i.v. injections, respectively). We have studied low dosage OT treatment in cows with disturbed milk ejection, twenty six cows routinely treated with OT during milking (group T) and 17 cows without previous OT treatment (group C). After cessation of spontaneous milk flow, OT (0.2, 0.5 or 1 IU in group T; 0.2 or 0.5 IU in group C) was i.v. injected. The time from the injection until cessation of the OT induced milk flow was recorded (response phase). The response phase and the amounts of removed milk in response to the OT injection increased with increasing OT dosage and were 198 ± 27 s and 302 ± 18 s and 3.4 ± 0.7 kg and 6.5 ± 1.3 kg for 0.2 and 0.5 IU in group C, respectively, and 157 ± 15 s, 221 ± 16 s and 288 ± 22 s and 3.2 ± 0.5 kg, 5.5 ± 1.0 kg and 9.7 ± 1.3 kg for 0.2, 0.5 and 1 IU in group T, respectively. Within 20 min from injection OT, plasma concentrations returned to basal levels except for 1 IU where the OT plasma concentrations were still slightly elevated as compared to basal concentrations (P<0.05). The threshold OT concentration at cessation of milk flow after injection of 0.2, 0.5 or 1 IU of OT was calculated based on the OT plasma half-life. The threshold increased with increasing dosages of OT and was higher in group T (88 ± 11 pg/ml, 14 ± 1pg/ml, and 23 ± 2 pg/ml for 0.2, 0.5 and 1 IU, respectively) than in group C (7 ± 1 pg/ml and 11 ± 1 pg/ml for 0.2 and 0.5 IU, respectively). Obviously there is a desensitization of the udder towards OT when the udder is exposed to elevated OT plasma concentration, both short-term during the actual milking and long-term due to chronic high-dosage OT treatment. However, low dosage OT treatments to induce normal milk removal are possible.

Key Words: oxytocin, milk ejection, dairy cow

M156 Effect of exogenous growth hormone and ovariectomy on protein expression of aromatase in prepubertal bovine mammary gland. B. P. Huderson*[1], S. E. Ellis[2], and R. M. Akers[1], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg*, [2]*Clemson University*, *Clemson, SC*.

Recent studies have shown an important role for locally produced estrogen in both humans and transgenic mice. Currently there is limited literature examining local estrogen production in the bovine mammary gland with most concentrating on periparturient animals. The purpose of these studies was to evaluate the effects of administering exogenous bovine somatotrophin (bST) and ovariectomy (OVX) on local aromatase production in mammary gland of prepubertal heifers. In the first experiment, 19 Holstein heifers (7 ± 4 d of age) were randomly assigned to one of two treatments bST (500 mg; n=10) or placebo (0.9% saline; n=9). Animals were administered subcutaneous treatments every three weeks beginning on day 23 of life. All animals were fed milk replacer and calf starter for 8 wk and starter and hay thereafter. Mammary parenchyma (PAR) and fat pad (MFP) were harvested for Western blot analysis. In both PAR and STR, local aromatase production was not affected by bST. In the second study, 22 Holstein heifers were randomly assigned to one of two treatments, ovariectomy (OVX; n=10) or sham (INT; n=12). Treatments were applied at 60, 90, or 120 d of age. Heifers were fed milk replacer and calf starter for the first 8 wk of life, and hay and calf starter thereafter. In PAR, animals operated on at 120 d had more aromatase detected compared to animals operated on at 60 and 90 d. Aromatase was detected in MFP but expression was not affected by treatment. In conclusion, local mammary production of aromatase was not effected by either bST or OVX and was predominantly expressed in MFP as opposed to PAR. Previously our lab reported no effect of OVX on the expression of genes involved in mammary gland proliferation and morphogenesis. Based on our current findings it is plausible that gene expression was not affected because there was no actual change in local aromatase production and therefore no change in local estrogen concentration.

Key Words: aromatase, bST, ovariectomy

M157 Removal of histidine from an intravenous amino acid infusion depresses milk protein and stimulates milk fat production by dairy cows. N. G. Purdie*, C. E. A. Borsy, C. Chui, J. Imada, P. Stahel, C. Longo, A. K. Shoveller, V. R. Osborne, and J. P. Cant, *University of Guelph*, *Guelph, Ontario, Canada*.

A selective decrease in histidine supply to the duodenum of dairy cows has been shown to stimulate milk fat production. To further characterize this response, four Holstein cows in their second lactation (122 ± 9 DIM) were fed a 50% forage TMR (12.1% CP, 1.53 Mcal/kg NELp). They were continuously infused i.v. with 0.9% saline as a negative control (NC), 300 g/d of a mix of essential amino acids in the profile found in milk protein as a positive control (PC), PC minus histidine (PC-His), and PC minus His plus 100 g/d Glu (PC-H+G). Treatments were given in a 4 x 4 Latin square, balanced for carryover effects, of 5-d infusion periods followed by 2 d of rest without infusion. Treatment differences were detected after analysis of variance with PROC MIXED of SAS. Pre-planned orthogonal contrasts were used to detect effects of PC vs. saline, imbalance (PC-His and PC-H+G vs. saline) and His deficiency (PC-His and PC-H+G vs. PC). There were no effects of treatment on feed intake or milk production of cows during the last 2 d of infusion ($P < 0.20$). Infusion of PC increased ($P < 0.05$) plasma concentrations of the essential amino acids Met, Phe, Thr, Ile, Leu, and Val and had no effect on non-essentials ($P > 0.17$) compared to saline. Infusion of the mixes without His caused a similar increase compared to saline in plasma concentrations of Met, Phe, Thr, Ile, Leu, and Val but also increased Arg, Tyr, Ser and Gln ($P < 0.05$). Compared to PC, the removal of His caused plasma His concentration to fall ($P = 0.033$) and branched-chain amino acids to increase ($P < 0.05$). Except for Arg, non-branched chain essential amino acids were not affected by His deficiency ($P = 0.318$). A milk fat stimulation of 95 g/d was observed with His deficiency ($P = 0.077$) but was not significant with imbalance ($P = 0.126$). Milk protein yield decreased 100 g/d with His deficiency ($P = 0.014$) and was not

affected by imbalance (P = 0.478). The protein:fat ratio of milk fell from 0.91 to 0.75 due to His deficiency (P = 0.012).

Key Words: milk composition, milk yield, histidine

M158 Effects of a shortened dry period on milk production and composition in early lactating Holstein cows. S. Safa[1], A. Heravi Moussavi*[1], M. Danesh Mesgaran[1], A. Golian[1], and A. Soleimani[1,2], [1]*Department of Animal Science, Ferdowsi University of Mashhad, Mashhad, Khorasan Razavi, Iran*, [2]*Islamic Azad University-Kashmar Branch, Kashmar, Khorasan Razavi, Iran*.

The study was designed to test the effects of a shortened dry period on milk production and composition in early lactating Holstein cows. Cows were randomly assigned in 1 of 2 treatments: 1) traditional 60 d dry period (n=12) and 2) a shortened 20 d dry period (n= 12). Holstein cows were blocked in pairs based on their previous 305 d milk, parity and expected calving dates. All cows were fed by routine ration of farm (total mixed diet) twice a day and had at all time free access to water. Milk samples were collected from each milking on 1 d per week until 50 d after parturition and composited for analysis of milk composition. The data were analyzed using the MIXED procedure of SAS for a completely randomized design with repeated measures. The reduction in dry period reduced milk production dramatically ($p<0.01$; 38.11 and 28.57 ± 1.1kg/d, respectively). The effect of time was significant and the milk yields increased over the time ($p<0.01$). The cows in different dry periods behaved similarly over the time. Milk protein content tended to increased in the shortened dry period group ($p=0.066$; 3.07 and 3.17 ± 0.04%, respectively). The lactose content was numerically higher in the shortened dry period group ($p=0.07$; 4.59 and 4.73 ± 0.05%, respectively). Milk fat content was similar among the groups. The yields of milk fat ($p<0.01$; 1.45 and 1.09 ± 0.09 kg/d, respectively), protein ($p<0.01$; 1.17 and 0.92 ± 0.06 kg/d, respectively) and lactose ($p<0.01$; 1.76 and 1.36 ± 0.09 kg/d, respectively) were all decreased due to the reduction in dry period. The milk fat content was decreased and yields of protein and lactose increased over the time ($p<0.01$). The results of this study demonstrated that reduction of dry period to 21 d significantly reduced milk production and milk components yields compare with the convention 60 d dry period.

Key Words: dairy cows, dry period, milk production

M159 Udder morphology of the Holstein cows, primiparous and multiparous. M. Porcionato*, J. Negrão, F. Paiva, and T. Delgado, *University of São Paulo*, *Pirassununga, São Paulo, Brazil*.

This trial aimed to evaluate the morphological characteristics of udder and teats with ultrasound, estimate the cisternal areas of mammary gland and teats, as well as the relation between these images and the productive parameters. One hundred lactating primiparous and multiparous Holstein cows at the beginning and at the end of 1st, 2nd, and 3rd, lactations were milked twice a day with mechanical milker. Data were analyzed using SAS (version 8.2). Differences were considered significant at $P<0.05$. The ultrasonography showed higher ($P<0.05$) teat channel in 3rd (12.74 mm) than 1st (14.64 mm) lactation. The distance for the teat to floor was lower ($P<0.05$) in 3rd (48.82 cm) than 1st (57.07 cm) lactation. It was observed high and positive correlation ($r = 0.84$) between cisternal area and cisternal milk. The number of lactation had influence on the morphology of their mammary gland and teats, as well as in the milking duration and milk yield of the Holstein cows. The ultrasound technique used in this trial, carried before early milkings at minimum intervals of eight hours between milkings, presented satisfactory results of cisternal areas estimation of the mammary gland and teats in the primiparous and multiparous cows.

Key Words: adaptation, cisternal milk, morphology

M160 Effects of increased milking frequency on milk yield and selected measures of mammary gland health in lactating cows. S. L. Shields*, D. Sevier, J. Peak, K. S. Seo, P. Rezamand, and M. A. McGuire, *University of Idaho*, *Moscow*.

Increased milking frequency during early lactation increases milk yield; however, it may alter milk composition and measures of mammary gland health. The objective of this study was to determine the effects of increased milking frequency on milk yield and composition, and measures of mammary gland health in a unilateral frequent milking (UFM) model. Sixteen Holstein cows at parturition were assigned to UFM, in which, the left udder half of each cow was milked four-times daily (4×; at 0500, 0900, 1600, and 2000 h) and the right udder half was milked twice daily (2×; at 0500 and 1600 h). Milk yields were measured from 1 through 21 days in milk (DIM) and samples were collected on d 3, 7, 10, 14, and 21. Milk composition and somatic cell count (SCC) was determined. In addition, flow cytometric analysis with bovine monoclonal antibodies was used to identify the composition of leukocytes from the milk samples collected. Data were statistically analyzed in a paired t-test setting by SAS (v. 9.2). No clinical case of mastitis was observed. Milk yield was greater during the first 21 DIM for the udder halves milked 4× as compared with those milked 2× (13.3 vs 9.91 kg/d; $P < 0.001$). Milk fat percent was greater for the udder half milked 4× as compared with the udder half milked 2× (4.96 vs 4.57%; $P = 0.009$). No significant difference was observed in SCC ($P = 0.20$) or the SCC linear score (3.54 vs 2.77 for 4× vs 2×, respectively; $P > 0.9$) between milking frequencies. In addition, no significant difference was detected for cell population of total leukocytes (45.5 vs 40.5% for 4× vs 2×, respectively), granulocytes (12.7 vs 8.75% for 4× vs 2×), mononuclear cells (30 vs 29.2% for 4× vs 2×), or CD4 to CD8 ratio (2.90 vs 2.75 for 4× vs 2×) for the udder halves milked 4× as compared with the udder halves milked 2× ($P \geq 0.46$ for all). We conclude that using a UFM model, increased milking frequency resulted in greater milk yield and milk fat percent; however, milking frequency did not affect selected measures of mammary gland health.

Key Words: milking frequency, somatic cell count, milk leukocyte