TH103 Identification of internal controls for quantitative PCR in mammary tissue from lactating cows receiving various lipid supplements. A. K. G. Kadegowda*[1], M. Bionaz[2], B. Thering[2], L. S. Piperova[1], R. A. Erdman[1], and J. J. Loor[2], [1]*University of Maryland*, *College Park*, [2]*University of Illinois*, *Urbana*.

Dietary lipid supplements affect mammary lipid metabolism through changes in lipogenic gene expression. Quantitative PCR (qPCR) is one of the most sensitive, reliable, and accurate techniques available to date for gene expression analysis. However, variation introduced in qPCR data by analytical/technical errors needs to be accounted for via normalization using appropriate internal control genes (ICG). Objectives were to mine individual bovine mammary microarray data on >13,000 genes across 66 cows from two independent studies to identify the most suitable ICG for normalization of qPCR. In addition to unsupplemented control diets, cows were fed saturated or unsaturated lipids (fish oil, Energy Booster) for 21 d, or were infused with supplements (Butterfat, CLA mixture, long-chain fatty acids) into the abomasum to modify milk fat synthesis and fatty acid profiles. GeneSpring® GX identified 49 genes that did not vary in expression across the 66 samples. Subsequent gene network analysis revealed that 22 of those genes were not co-regulated. Among those, COPS7A, CORO1B, DNAJC19, EIF3K, EMD, GOLGA5, MTG1, UXT, MRPL39, GPR175, and MARVELD1 (sample/reference expression ratio = 1 ± 0.1) were selected for qPCR analysis upon verification of goodness of BLAT/BLAST sequence and primer design. Relative expression of B2M, GAPDH, and ACTB, previously used as ICG in bovine mammary, was highly unstable (0.9 ± 0.6) across studies. Gene stability analysis, via geNORM software, uncovered EMD, MARVELD1, MRPL39, GPR175, UXT, and EIF3K as the most stable genes and, thus, suitable as ICG. geNorm also indicated that use of 3 to 5 ICG was most appropriate for calculating a normalization factor. Overall, results showed that the geometrical average of at least three among EMD, MARVELD1, MRPL39, GPR175, UXT, and EIF3K is ideal for normalization of mammary qPCR data in studies involving lipid supplementation of dairy cows.

Key Words: House Keeping Genes, Quantitative PCR, Lipid Supplements

TH104 Gene network analysis in mammary tissue of lactating cows receiving abomasal infusions of butterfat, long-chain fatty acids, or CLA. A. K. G. Kadegowda*[1], L. S. Piperova[1], S. L. Rodriguez-Zas[2], R. E. Everts[2], H. A. Lewin[2], R. A. Erdman[1], and J. J. Loor[2], [1]*University of Maryland*, *College Park*, [2]*University of Illinois*, *Urbana*.

Abomasal infusion of butterfat and long-chain fatty acids (LCFA) induced the most contrasting responses in the concentrations of milk FA with ≤ 16-carbons (greater with butterfat) and polyunsaturated FA (greater with both) relative to control. To better characterize gene networks associated with lipid synthesis and other cellular processes that might be responsive to dietary lipid, mammary tissue from lactating cows receiving no lipid (control; n = 6) or abomasal infusions of 400 g/d butterfat (n = 4), 245 g/d LCFA (59% cocoa butter + 36% olive oil + 5% palm oil; n = 4), or 100 g/d conjugated linoleic acid (CLA, 10 g of t10c12 CLA; n = 6) was used for gene expression analysis. Transcript profiling was conducted with a 13,257 bovine oligonucleotide (70-mers) array. Annotation was based on similarity searches using BLASTN against human, mouse and bovine RefSeq, human, mouse, and bovine UniGene, and bovine TIGR. Cy3- and Cy5-labelled cDNA from mammary and a reference standard were used for hybridizations. ANOVA ($P \leq 0.01$) identified >300 differentially expressed genes in mammary due to treatments. Relative to control, LCFA infusion resulted in 295 genes and butterfat in 101 genes with changes in expression of 1.5-fold or greater. Gene network analysis identified inflammatory response (54 genes), cell growth/proliferation (104 genes), and cell death (90 genes) as modified families of related genes due to LCFA vs. control. Similar analysis in cows receiving butterfat identified lipid metabolism (14 genes), molecular transport (15 genes), and cell morphology (16 genes) as gene families most affected. Interestingly, signaling pathways most affected by butterfat included acute-phase response and complement system. Overall, results indicate that supply of short and medium chain FA (butterfat) vs. LCFA affect expression of previously-unrecognized cellular gene networks.

Key Words: Lactation, Genomics, Fatty Acids

TH105 Differential expression of lipid transporters and their regulators during the lactation cycle in the bovine mammary gland. O. Mani[1], M. T. Sorensen[2], K. Sejrsen[2], R. M. Bruckmaier*[1], and C. Albrecht[1], [1]*University of Bern*, *Switzerland*, [2]*University of Aarhus*, *Denmark*.

The transport of lipophilic compounds across mammary gland (MG) epithelial cells determines milk lipid content and composition. ATP-binding cassette (ABC) transporters are known to play a pivotal role in cellular lipid efflux. As only scarce information is available about the transfer of lipids and cholesterol in the MG, we investigated the expression of lipid transporters and their regulators during lactation and dry period (DP) in dairy cows.

Repeated MG biopsies were taken from 10 cows at the end of lactation (d 347), during DP (d 48, 16 ante partum - ap) and subsequent lactation (d 14, 42, 88, 172 post partum - pp). mRNA levels of ABCG2, ABCA1, ABCA7, ABCG1 and NPC1, their regulatory genes LXRa and PPARg, and regulators of sterol synthesis SREBF1 and SREBF2 were determined by real-time RT-PCR.

mRNA levels of ABCG2 were significantly increased pp and throughout lactation as compared to the DP ($P<0.0001$). ABCA1 and ABCA7 were elevated during the DP ($P=0.0197$ and <0.0001, resp.) as compared to mid and late lactation and downregulated pp ($P=0.0443$ and 0.0003, resp.). ABCG1 showed no significant changes between the different functional stages of the MG. The intracellular cholesterol transporter NPC1 as well as LXRa and PPARg were elevated pp ($P=0.0003$, 0.0271 and 0.0375, resp.). SREBF1 was increased throughout lactation ($P<0.0001$) as compared to the DP; a similar but not significant expression pattern was observed for SREBF2.

These results indicate that lipid transporters show differential expression between lactation and DP. This may be due to physiological changes in the MG like immigration of macrophages or accumulation of fat due to loss of liquid in the involuting MG. To test these hypotheses and to elucidate underlying molecular mechanisms cellular localization of candidate transporters in the MG will be investigated.

Key Words: Mammary Gland, Lipid Transporter, Dairy Cow

TH106 Unprotected conjugated linoleic acid (CLA) negatively affects milk production and secretion of milk components in dairy ewes. D. E. de Oliveira*[1], M. P. Soares[1], F. J. Bianchett[1], R. Fornazier[1], M. R. Fachinello[1], M. Girardi[1], D. Fernandes[1], D. Soster[1], M. A. S. da Gama[2], M. G. C. D. Peixoto[2], S. de O. Juchem[3], and L. O. Tedeschi[4], [1]*Universidade do Estado de Santa Catarina*, *Chapecó 89801-070, SC, Brasil*, [2]*Embrapa Gado de Leite*, *Juiz de Fora, MG, Brasil*, [3]*University of California*, *Davis*, [4]*Texas A&M University*, *College Station*.

Feeding conjugated linoleic acid (CLA) in a rumen-inert form to dairy ewes has been shown to increase milk production, alter milk composition, and increase the CLA content in milk fat. However, few studies utilized unprotected CLA sources. The objective of this study was to evaluate the effects of an unprotected CLA supplement (28% of c-9, t-11 and 28% of t-10, c-12 isomers) on milk yield and composition of dairy ewes. Twenty four lactating Lacaune ewes (40 to 70 DIM) were used in a cross-over design and received two treatments: 1) Control (C) and 2) CLA (30 g/d). The CLA supplement was mixed into the concentrate (1.2 kg/d, as-fed) and fed in two equal meals after morning and afternoon milkings. Ewes were individually fed 2.5 kg/d of corn silage and grazed a pasture of annual ryegrass and white clover. Each experimental period consisted of 21 d: 7 d for adaptation and 14 d for data collection. All ewes were fed the C diet for 7 d before the second collection period to prevent any carry-over effect. Milk production was daily recorded and milk samples were collected at each milking and a daily composite was obtained. Milk samples were analyzed for contents of fat, protein, lactose, and somatic cell count (SCC). Data were analyzed as repeated measurement design using PROC MIXED procedure of SAS, assuming period and ewe within treatment sequence as random effects. The CLA treatment decreased milk fat content (CLA = 3.86, C = 5.62%; P<0.01, SD= 0.15), milk yield (CLA = 1.73, C = 1.88 kg/d; P<0.01, SE= 0.08) and milk lactose content (CLA = 4.48, C = 4.55%; P<0.01, SD= 0.06). Ewes fed CLA had greater milk protein content (CLA = 5.00, C = 4.91%; P<0.01, SE= 0.06) and linear SCC score (CLA = 3.93, C = 3.34; P<0.01, SE= 0.25). Secretion of all milk components was reduced by CLA. As previously shown in dairy cows (Bell and Kennelly, 2003, JDS 86:1321-1324 and Keating AF et al., Domest. Anim. Endocrinol (2007)), it is possible that relatively large amounts of c-9, t-11 and t-10, c-12 CLA isomers might have reached the abomasum of these ewes and resulting in precocious involution of mammary gland.

Key Words: Conjugated Linoleic Acid, Mammary Gland, Milk Components

TH107 Effects of rumen-protected choline administration on mRNA expressions of selected enzymes involved in mammary lipid metabolism. L. Pinotti*[1], F. D'Ambrosio[1], R. Bruckmaier[2], C. Albrecht[2], V. Dell'Orto[1], and A. Baldi[1], [1]*University of Milan*, *Milan, Italy*, [2]*University of Bern*, *Bern, Switzerland*.

Aim of this study was to investigate the effects of rumen-protected choline administration on mRNA expressions of selected enzymes involved in mammary lipid metabolism. Eight pregnant multiparous Saanen goats were assigned to one of two experimental groups: CTR, control group, no choline supplementation; RPC, supplemented with 4 g/day choline chloride in rumen-protected form (Sta-Chol 50%, Ascor Chimici, Forlì, Italy). Treatment was administered individually from day -30 to day 30 relative to kidding. Through the first month of lactation milk yield and composition were measured weekly. On day 28 in milk, from the same animals mammary tissue samples were collected under sterile conditions. Samples were stored at –80°C until RNA extraction. Synthesis of cDNA was performed by reverse transcription-polymerase chain reaction. Glyceraldehyde 3-phosphate dehydrogenase and beta-actin were chosen to confirm a constant gene expression level in the investigated total RNA extractions. Quantitative analysis of PCR products was carried out from cDNA fragments of FAS (Fatty acid synthase), LPL (Lipoprotein Lipase), SREBP-1 (Sterol regulatory binding protein-1), SREBP-2 (Sterol regulatory binding protein-2), and PPARγ (Peroxisome proliferator-activated receptor gamma). mRNA expression levels have been normalized to GAPDH relative to total RNA and presented as logarithm dualis. Data were analyzed by the general linear model procedure of SAS. Milk yield and milk composition did not differ between groups. Moreover, choline supplementation did not affect the mRNA expressions of the enzymes involved in mammary lipid metabolism. However, present results are based on a limited number of goats, thus possible choline effects at mammary gland levels in lactating dairy goats deserve further investigation.

Key Words: Choline, Mammary Gland, Lipid Enzymes

TH108 Hormonal influence on mammary tissue composition in pre-pubertal Holstein heifers. B. P. Huderson*[1], B. T. Velayudhan[1], S. E. Ellis[2], and R. M. Akers[1], [1]*Virginia Polytechnic Institute and State University*, *Blacksburg*, [2]*Clemson University*, *Clemson, SC*.

It is known that mammary gland growth and development are hormonally regulated. However, hormonal regulation is poorly understood in pre-pubertal heifers. The purpose of these two studies was to evaluate the effects of exogenous bovine somatotropin (bST) or ovariectomy on mammary tissue composition in pre-pubertal Holstein heifers. In a first experiment, 19 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 treatments every three weeks beginning on day 23. All animals were fed milk replacer and calf starter for eight wk and thereafter starter and hay. Mammary parenchyma (PAR) and fat pad (MFP) were harvested for analysis of protein, lipid and DNA at two times (after the second or after the fourth injection). There was a significant increase in total DNA, total protein, DNA/100 kg BW and protein/100 kg BW due to day and a significant treatment by day interaction on DNA and lipid concentration in PAR. In MFP there was a treatment by day interaction for DNA and protein/100 kg BW. In the second study, 36 heifers were randomly assigned to one of two treatments, ovariectomy (OVX; n=18) or sham (INT; n=18). All animals were fed traditional milk replacer and calf starter. Treatments were applied on 40 d of age and mammary samples were harvested either at 55, 70, 85, 100, 130, or 160 d age. Composition of PAR was not affected by ovariectomy (p>0.05), while MFP protein concentration was decreased in OVX (26.6 vs 22.6 ± 1.2 μg/mg MFP). Total protein as well as DNA and lipid per 100 kg BW in MFP were significantly affected by age. There was an age by treatment interaction effect on total DNA and lipid in MFP. In conclusion, bST and ovariectomy impacted the composition of MFP but not PAR.

Key Words: Mammary, bST, Ovariectomy

TH109 Feeding genistein to prepubertal gilts stimulates their mammary development. C. Farmer*[1], S. Gilani[2], M.-F. Palin[1], H. Weiler[3], M. Vignola[4], R. K. Choudhary[5], and A. V. Capuco[5], [1]*Agriculture and Agri-Food Canada, Dairy and Swine R & D Centre*, *Sherbrooke, QC, Canada*, [2]*Nutrition Research Division, Health Canada*, *Ottawa, ON, Canada*, [3]*McGill University*, *Ste-Anne-de-Bellevue, QC, Canada*, [4]*Nutreco Canada Agresearch*, *St-Romuald, QC, Canada*, [5]*USDA-ARS, Bovine Functional Genomics Lab*, *Beltsville, MD, USA*.

The possible role of dietary genistein on mammary development of prepubertal gilts was investigated. Forty-five gilts were fed one of three diets from 90 d of age until slaughter (day 183 ± 1). Diets were: without soya (CTL0, n=15); soya-based commercial (CTLS, n=15); and soya-based commercial with 2.3 g/d of genistein (GEN, n=15). All diets were isonitrogenous and isocaloric. Jugular blood samples were obtained on days 89 and 183 to determine concentrations of genistein, prolactin, estradiol, IGF-I and cross-linked N-telopeptide of type I collagen (NTx, day 183 only). At slaughter, mammary glands were excised, parenchymal and extraparenchymal tissues were dissected and composition of parenchymal tissue was determined. Histochemical analyses of mammary parenchyma were performed and mRNA level of specific genes determined. Dietary genistein increased parenchymal protein ($P < 0.05$) while decreasing dry matter ($P < 0.05$) and tending to lower fat content compared to the CTLS, but not the CTL0, diet. There was more parenchymal DNA (1.26 vs. 0.92 mg/g, $P < 0.05$) in GEN than CTLS gilts. Circulating concentrations of hormones or NTx were not affected by GEN ($P > 0.1$) but concentrations of genistein were greater ($P < 0.0001$) in GEN than CTLS gilts. Percent ERα-positive epithelial cells was lower ($P < 0.05$) in GEN than CTLS gilts whereas BrdU labelling index was unaltered ($P > 0.1$). Transcript levels for ERα, ERβ, IGF-I, EGF, EGFR and TGFα were not altered by treatments ($P > 0.1$). Feeding dietary genistein to prepubertal gilts led to hyperplasia of mammary parenchymal tissue.

Key Words: Genistein, Gilts, Mammary Development

TH110 Evidence that prolactin does not drive the milk yield response to frequent milking in early lactation. J. G. Titus*[1], H. M. Crawford[2], E. H. Wall[1], G. E. Dahl[2], and T. B. McFadden[1], [1]*University of Vermont*, *Burlington*, [2]*University of Illinois*, *Urbana*.

Frequent milking of dairy cows in early lactation causes an increase in milk yield that persists throughout lactation, however the mechanisms regulating this response are unknown. We hypothesized that prolactin (**PRL**) released during additional milkings stimulates the increase in milk yield; therefore blockade of PRL secretion in frequently-milked cows should eliminate the milk yield response. Our objectives were to determine effects on milk yield and to gain insight into potential mechanisms of action by measuring mammary cell proliferation and apoptosis as well as expression of genes associated with angiogenesis. Eighteen multiparous Holstein cows were assigned to one of three treatments for the first 21 d of lactation: twice-daily milking (**2×**), four-times daily milking (**4×**), or four times daily milking plus administration of the prolactin inhibitor, bromocriptine (**Bromo**) at two of the four milkings. Milk weights were recorded daily and blood samples were obtained on d 1, 4, 7, 14 and 21 of lactation for quantifying plasma PRL. Mammary biopsies were taken at 8 ± 3 DIM for determination of [3H]-thymidine incorporation into DNA in vitro, mammary cell apoptosis, and isolation of total RNA. During treatment, milk yield was lower in the 2× group than in the 4× or Bromo groups (33.4, 37.3 or 38.0kg/d, P<0.001), but was not different between 4X and Bromo treatments (P>0.4). Mean PRL concentrations were greater in 4× cows than in 2× or Bromo cows (12.4, 9.2 and 7.0ng/mL, P<0.01), but did not differ between 2X and Bromo cows (P>0.1). Incorporation of [3H]-thymidine was greater in 2X cows relative to both of the 4X groups (P<0.01) whereas mammary cell apoptosis was not affected by treatment (P≥0.9). Mammary expression of PRL receptor, insulin-like growth factor 2, insulin-like growth factor binding protein 5, and the vascular endothelial growth factor receptors, flt-1 and flk-1 did not differ among treatments (P>0.9), suggesting that angiogenesis was not affected. We conclude that increases in plasma PRL do not drive the milk yield response to frequent milking in early lactation.

Key Words: Frequent Milking, Prolactin, Angiogenesis

TH111 Reduced nursing frequency during prolonged lactation in the mouse decreases milk production and increases mammary expression of tryptophan hydroxylase 1 (TPH1), but does not accelerate mammary gland remodeling. D. L. Hadsell*[1], W. Olea[1], D. Torres[1], J. George[1], and R. J. Collier[2], [1]*Baylor College of Medicine*, *Houston, TX*, [2]*The University of Arizona*, *Tucson*.

We have observed that lactating mouse dams nursed 4 times per day (4X) maintained lactation, but had lower milk yields by the weigh-suckle-weigh method, than dams nursed ad libitum (AL). Therefore, we hypothesized that decreased nursing frequency would also decrease lactation persistence, increase mammary gland remodeling, and alter the expression of genes linked to milk production (α-lactalbumin), mammary involution (lactoferrin) and mammary secretory cell feedback inhibition (TPH1). To test this hypothesis, milk output, mammary epithelial and adipocyte content, and mammary gland gene expression was measured on days 8, 14, or 28 postpartum in dams (n=5-16/treatment group) that nursed either AL or 4X for up to 3 weeks. Milk yield (1.3±0.1 g) was lower on day 14 in 4X than AL, which had similar yield to day 8 of the pretreatment period (2.4±0.3 g). On days 14 and 28, milk production in both groups was similar, and both were lower than that observed on day 8. Mammary epithelial content in 4X dams, as determined in cytokeratin 8 stained tissue sections, was higher than AL on day 28 (70±2 and 56±2 %). Alveolar luminal area was greater in 4X than AL on both days 14 (4030±201 and 3307±207 μm^2) and 28 (3293±246 and 2010±275 μm^2). Mammary adipocytes in perilipin-stained tissue sections, were larger in AL than 4X on day 28 (374±53 and 134±5 μm^2). Expression of α-lactalbumin and lactoferrin genes was greater on day 28 than day 8 postpartum, but not affected by nursing frequency. Expression of the TPH1 gene was higher in 4X than AL on day 14 (4.2±0.9 and 1.7±.3 fold over day 8), but similar to AL on day 28. These data suggest that reduced nursing frequency decreases milk synthesis through a mechanism involving serotonin biosynthesis, but may also delay the normal mammary gland remodeling that occurs with prolonged lactation. 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: Milking Frequency, Mammary Involution, Serotonin

TH112 Evaluation and classification of milking disorders in Swiss dairy cattle. C. J. Belo[1], S. Schlegel[2], J. Moll[3], and R. M. Bruckmaier*[1], [1]*University of Bern*, *Bern, Switzerland*, [2]*Swiss Federal Institute of Technology Zurich*, *Zurich, Switzerland*, [3]*Swiss Brown Cattle Breeders Federation, Zug and ASR*, *Bern, Switzerland*.

Morphological dysfunction of the udder and the teat as well as pathophysiological reduction of the release of oxytocin perturb or inhibit milk removal. To evaluate the incidence of these disorders and to classify them as anatomical or pathophysiological, questionnaires were sent to 2099 Swiss dairy farms with at least 25 dairy cows representing the major breeds in Switzerland (Brown Swiss: BS, Holstein: HO, Red Holstein x Simmental: SI). A high percentage of the questionnaires were returned (BS: 78%, HO: 73%, SI: 72%). Thus we have obtained information on a total of 67548 cows. Of these cows, 2642 (BS: 5%, HO: 3%, SI: 3%) were reported with poor milkability and 1198 problem cows (BS: 2.5%, HO: 1%; SI 1%) were regularly treated with oxytocin. In addition, 242 of the dairy farms that had reported animals with poor milkability were contacted by phone and asked for more details about the reported disorders and the specific activities performed in case of problem. The farmers reported milk ejection disorders (Si: 76%, HO: 63%, BS: 48%), poor milkability (BS: 34%, HO: 21%, SI: 7%) and externals factors such as stray voltage, engine disturbances and other animal diseases (BS: 28%, HO: 16%, SI: 15%). Apart from using oxytocin, the farmers reported careful teat stimulation, massage of vulva and udder, air blown into the vagina or homeopathy. About half the dairy farms have culled the animals because of the problem.
51 cows were selected in 18 farms which had reported a supposed disturbed milk ejection, i.e. an incomplete delivery of the milk based on a lack of oxytocin release. The presence of milk ejection disorder was investigated by recording the milk flow with a mobile unit (LactoCorder). After cessation of spontaneous milk flow, oxytocin was injected iv. and the amount of residual milk was measured. In 18% of the tested cows disturbed milk ejection was not detected, contrary to the assumption of the farmers.

Key Words: Milk Ejection, Inhibition, Cattle

TH113 Prestimulation combined with a short waiting time before cluster attachment affects milk removal in dairy cows. S. Kaskous*[1] and R. M. Bruckmaier[2], [1]*Damascus University*, *Damascus, Syria*, [2]*University of Bern*, *Bern, Switzerland*.

The suitability of combining manual prestimulation with a short waiting time before cluster attachment and machine milking was investigated. 21 dairy cows in their second to sixth lactation were divided in three lactational stages with seven animals each, early (<100 d), mid (100 to 200 d) and late (>200 d) lactation. In addition, cows were classified based on their estimated degree of udder filling as a ratio between the actual milk yield and the maximum storage capacity. During the experiment, cows were machine milked twice daily at 5 a.m. and 4 p.m. and milk flow curves were recorded. The animals were manually prestimulated for 15, 30 or 45 s, followed by a waiting time of 0, 30, 45 or 60 s, i.e. 12 different treatments were applied for each animal. The treatments were performed in a random sequence for each animal, but remained unchanged for two subsequent milkings (morning and evening). Total milk yield did not significantly differ between treatments in all stages of lactation and at all degrees of udder filling. Average milk flow (AMF) and peak flow rate (PFR) in early lactation, and similarly in mid lactation (udder filling 60-100%) were highest after a prestimulation of 30 s followed by a waiting time of 30 or 45 s, or after a prestimulation for 45 s without additional waiting time ($p<0.05$). In late lactation and at low degrees of udder filling (20-40 %), AMF and PFR were highest after a prestimulation of 45 s combined with a waiting time of 30, 45 or 60 s ($p<0.05$). In conclusion, total milk yield does not differ between prestimulation treatments and additional waiting time as applied in the present study. An optimal course of milk removal is reached after a prestimulation of 30 s, combined with a waiting time of 30 to 45 s in early and mid lactation. In late lactation a prestimulation for 45 s is required, combined with an additional waiting time of 30 to 60 s.

Key Words: Prestimulation, Waiting Time, Milking

TH114 Identification of internal controls for quantitative PCR in swine mammary gland during pregnancy and lactation. S. Tramontana[1,2], M. Bionaz*[2], A. Sharma[2], D. E. Graugnard[2], E. A. Cutler[2], P. Ajmone-Marsan[1], W. L. Hurley[2], and J. J. Loor[2], [1]*Università Cattolica del Sacro Cuore*, *Piacenza, Italy*, [2]*University of Illinois*, *Urbana*.

High-throughput microarray analysis is an efficient means of obtaining a genome-wide view of transcript profiles across physiological states. However, quantitative PCR (qPCR) remains the chosen method for high-precision mRNA abundance analysis. Essential for reliability of qPCR data is normalization using internal control genes (ICG). Identification of reliable ICG is now, more than ever before, a fundamental step for accurate gene expression profiling. To identify swine ICG, we mined mammary microarray data on >13,000 genes at -34, -14, 0, 7, 14, 21, and 28 d relative to parturition in 27 cross-bred primiparous gilts. GeneSpring GX analysis revealed *TBK1*, *PCSK2*, *PTBP1*, *API5*, *VAPB*, *QTRT1*, *TRIM41*, *TMEM24*, *PPP2R5B*, and *AP1S1* as the most stable genes (sample/reference = 1 ± 0.2). We also included 9 genes previously identified as ICG in bovine mammary. Co-regulation on these 19 genes was assessed using gene network analysis, which identified *AP1S1*, *API5*, *MTG1*, *VAPB*, *TRIM41*, *MRPL39*, and *RPS15* as having no known co-regulation. *UXT* and *ACTB* were added to this list and mRNA abundance of the 9 genes measured by qPCR. Expression of all ICG chosen decreased ($P < 0.05$) markedly during lactation. Gene stability analysis, via geNorm software, identified *API5*, *VABP*, and *MRPL39* as the most stable ICG and indicated that the use of 3 ICG was most appropriate for calculating a normalization factor. In a previous study with bovine mammary tissue, decreased mRNA of stably-expressed genes during lactation was apparent, and due to a dilution effect brought about by large increases in expression of highly abundant, metabolism-related genes. To verify this effect, highly-abundant mammary genes such as *CSN1S2*, *SCD*, and *LTF* were evaluated by qPCR. The tested ICG had a negative correlation with these genes, demonstrating the presence of a dilution effect as in bovine mammary tissue. Overall, results underscore the importance of proper validation of internal controls for qPCR and highlight the limitations of using time effects as the criteria for selection of appropriate ICG.

Key Words: Genomics, Involution, Milk Synthesis

TH115 Serial mammary biopsies in cows do not alter overall milk production. H. Dover*, M. VandeHaar, J. Liesman, O. Patel, L. De Vries, and K. Plaut, *Michigan State University*, *East Lansing*.

Serial biopsies of the mammary gland are useful in studies of mammary biology; however, many researchers assume the cow's long term milk production might be impaired. The objective of this study was to determine the effect of mammary biopsies during late lactation through the dry period on overall milk production. Six multigravid cows were biopsied approximately 275-290 days in milk (biopsy 1), 7 days after dry off (biopsy 2), 3 weeks (biopsy 3) and 1 week (biopsy 4) before expected calving date. Animals were sedated with xylazine hydrochloride, and the biopsy site was numbed with lidocaine gel before lidocaine injection. A biopsy tool (AgResearch, Hamilton, NZ) powered by a cordless drill was used to obtain approximately 1 g of mammary tissue per biopsy. Immediately after biopsies 1 and 4, a teat cannula was inserted into the biopsied quarter to drain any accumulated blood. For approximately 3 days, cows were hand stripped at each milking until blood clots were no longer observed in the milk. Cows joined the milking herd after biopsy 1 and 4. Analysis of milk yields during the lactation prior to and following the biopsies indicate that milk production in biopsied cows was not different from the herd ($P>0.1$). At 30-60 days in milk, primiparous cows averaged 35 kg/day of milk prior to biopsy and 48 kg/day in the lactation following biopsy; for multiparous cows, milk yield averaged 50 kg/day prior to biopsy and 54 kg/day in the lactation following biopsy. The use of teat cannulae after biopsy appears to improve cow comfort. Results show that serial mammary biopsies do not impair milk production in dairy cows.
Project support: National Research Initiative Competitive Grant no. 2006-35206-16719 from the USDA CSREES.

Key Words: Mammary Gland, Biopsy, Milk Production

TH116 Effect of dry period length on calving related disorders. M. S. Gulay*[1], M. J. Hayen[2], K. C. Bachman[2], and H. H. Head[2], [1]*Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Physiology*, *Burdur, Turkey*, [2]*University of Florida*, *Gainesville*.

Objectives were to evaluate effects of dry period (60 vs. 30 d), with or without estradiol cypionate (ECP) injections, on incidence rates of digestive problems and displaced abomasum (DP), retained fetal membranes (RFM), metritis (MET), clinical mastitis (MAS), ketosis (KET), or foot problems (FOOT) during the first 60 d postpartum. One hundred eighty-nine multiparous Holstein cows from two separate trials were combined for analyses. Dry period treatments included 30 d dry+ECP (30DE; n=48), 30 d dry (30D; n=44) and 60 d dry (60D; n=97). Single injections of ECP (7.5 ml; 15 mg; 30DE) or 7.5 ml of cottonseed oil (30D) were supplemented i.m. at final milk removal; 60D group was the control group and received no ECP or cottonseed oil. Across all treatment groups the incidence rates (number of diseased cows divided by the total number of cows) for DP, RFM, MET, MAS, KET, and FOOT were 8.5, 6.4, 12.7, 19.6, 4.2, and 9.5%, respectively. No significant Chi-Square values ($P<0.1$) were detected among the treatment groups. Incidence rates of DP, RFM, MET, MAS, KET, and FOOT for 30DE, 30D and 60D groups were 2.8, 6.5, and 12.4%; 6.8, 4.2, and 7.2%; 9.1, 10.4, and 15.5%; 22.7, 18.8 and 18.6%; 4.6, 4.2 and 4.1%; and 9.1, 6.3, and 11.3%, respectively. The proportions of sick cows in the 3 groups (number of cows having one or more cases of diseases divided by the total number of cows) were 36.4, 35.4, and 48.5%, respectively. When 30DE and30D groups were combined and compared to the 60D group, a significant difference was detected between short and longer dry period, but only for DP ($P<0.05$). Moreover, proportions of sick cows in the combined 30D dry group also tended to be lower (38.9 vs. 48.5%; $P=0.08$). Odds ratio values suggested that cows given longer dry periods were 1.68 times more likely to get sick during first 60 d after calving. Thus, results indicated that shortening dry period length (30D) with or without supplemental ECP did not negatively affect postpartum calving disorders compared to cows given 60D and improved health status slightly.

Key Words: Dry Period Length, Transition Period, Diseases

TH117 Dietary energy management during pregnancy and its effects on transition health in dairy heifers. M. S. Laubach[1], D. B. Carlson[2], L. Mabasa[2], K. S. Cho[2], A. W. Fowler[2], and C. S. Park*[2], [1]*South Dakota State University*, *Brookings*, [2]*North Dakota State University*, *Fargo*.

The objective of this study was to determine if a gestational one stair-step compensatory nutrition regimen (SSCN) affects metabolic and immune status of dairy heifers during the transition period. The experiment consisted of two sequential trials (20 heifers for trial 1; 10 heifers for trial 2). Holstein heifers (95 d in gestation) averaging 511 kg of body weight were assigned into two groups: control and treatment (SSCN). The control diet [14% crude protein (CP); 2.35 Mcal/kg metabolizable energy (ME)] was intended for a constant gain of 0.45 kg/d throughout the last two trimesters of gestation. The SSCN heifers were fed a restriction diet (18.5% CP; 2.35 Mcal/kg ME) during the second trimester at 70% of the ME intake of control heifers, and a realimentation diet (14% CP; 3.05 Mcal/kg ME) during the third trimester. Blood was collected around parturition on d -14, -11, -9, -7, -5, -4, -3, -2, -1, 0 (within 3 h of calving), 1, 2, 3, 4, 5, 7, 9, 11, and 14 to monitor various metabolites, total white blood cell counts (WBC), and lymphocyte populations [cluster of differentiation 3 (CD3), CD4 and CD8]. In both trials, serum glucose, insulin, triglycerides, and nonesterified fatty acids were not different between groups before or after parturition. SSCN heifers before parturition tended to have higher WBC ($P = 0.06$); however, after parturition there was no difference between groups. The CD8 cells tended to be higher ($P = 0.10$) in the SSCN group compared with the control before parturition and were higher ($P = 0.02$) after parturition; the increase of CD8 cells in SSCN heifers may be related to the enhancement of immune cell production due to increased compensatory energy metabolism from the realimentation diet during the last trimester. SSCN heifers yielded 4% more milk than control heifers (34.8 vs. 36.1 kg/d; $P = 0.18$). These results indicate that SSCN did not affect general metabolic parameters, but moderately improved immune status of transition heifers.

Key Words: Transition Heifer, Compensatory Growth

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