M167 Toxicological study of gandul forage (Cajanus cajan). M. Duron-Velazquez[1], G. Tirado-Estrada*[1], I. Mejia-Haro[1], F. Jaramillo-Juarez[2], R. Larios-Gonzalez[1], H. Silos-Espino[1], and F. Nieto-Muñoz[1], [1]*ITEL, Ags., El Llano, Ags. Mexico*, [2]*Universidad Autonoma de Aguascalientes*, *Aguascalientes, Ags., Mexico*.

The objective of this study was to evaluate the toxicological content of gandul forage in vitro and in vivo in wethers sheep, two stages were carried out, the first one consisted in the in vitro evaluation of gandul forage, in which tannins (colorimetric method of Folin-Dennis and saponines (level of color intensity, method of thin layer chromatography) were analyzed in four sampling sites (S1, S2, S3 and S4) dividing the plant in three vegetative strata in each site (bottom = EB, medium = EM, top = EA), and three different ages (young plants = PJ, medium = PM and mature = PA). The saponines analysis also was carried out in flowers of the sites 2, 3 and 4 (FS2, FS3 and FS4). The second stage consisted in observe signs of toxicity in 20 sheep (18 kg BW) fed diets with different concentrations of gandul (0, 15, 30, 45 and 60 % of DM for T1, T2, T3, T4 and T5, respectively) and assigned to one of five treatments in a completely randomized design and evaluated by GLM of SAS using ANOVA and tukey tests. Blood samples were collected to determine albumin and transaminases concentrations (aspartate aminotransferase = GOT, and alanine aminotransferase = GTP). Also, liver samples were collected to carry out a histological study, for which, five wethers, one of each treatment were sacrificed. The results indicated no toxicity. In the first stage, the highest value of tannins (gTA equivalents) was for PM (12.52), followed by S1EB (11.86) and the lowest value for PJ (2.71), the average value (7.9) was below 8, which is considered a marginal value of toxicity . For saponines, the highest value was for S1EB and S1EM, otherwise, in FS4 and PJ, saponine presence was not observed. T5 obtained the highest serum albumin concentration (7.084 u/l). For GOT, T3 obtained the highest value (38.72 u/l) and the lowest value for T4 (25.4 u/l), likewise, for GTP, the highest value was obtained by T3 (61.7 units) and the lowest by T2 (21.1 units). In liver, all samples presented a normal histological organization. In the in vivo study, no toxicity was present; ADG, and feed intake were adequate for all treatments. Gandul forage did not present toxicity in in vivo and in vitro studies.

Key Words: Saponines, Tannins

M168 Characterization of a negative halothane gene commercial multibreed swine population for growth and conformation traits in tropical western Thailand. S. Koonawootrittriron[1], M. A. Elzo*[2], and T. Suwanasopee[1], [1]*Kasetsart University*, *Bangkok, Thailand*, [2]*University of Florida*, *Gainesville*.

The Thai market demands lean pork. Producers are attempting to meet this demand by breeding pigs of larger size and lean content. Pietrain is one of the major breeds used to achieve this goal. Unfortunately, Pietrain has a high frequency of halothane (i.e., porcine stress syndrome) genes that can produce low quality meat and death by heat stress under conditions of high temperature and humidity. The objective of this research was to evaluate a large commercial negative halothane gene multibreed swine population in western Thailand for growth and conformation traits. Breeds represented were Pietrain (P), Large White (LW), and Landrace (L). Boars from all breeds were mated to P sows. LW females were only used to produce replacement boars. This mating strategy resulted in 4 breed groups of piglets: P, L, F1 LW×P, and F1 L×P. Pigs were kept in open barns, and received the same nutrition, management, and health care. Data consisted of 37,628 birth weights (BW) and 12,404 weaning weights (WW), and 2,980 body lengths (BL), shoulder widths (SW), hip widths (HW) and ages at first estrus (AE) from pigs born from 2003 to 2006. Genetic parameters and estimated breeding values were computing using multivariate animal models (BW-WW and BL-SW-HW-AE). Fixed effects were contemporary group (year-month), sex, parity of dam, direct heterosis, and animal genetic group. Random effects were animal, dam (BW-WW only), and residual. Computations were performed using ASREML. All fixed effects were important for all traits ($P < 0.001$). Estimates of heritabilities for direct genetic effects were 0.09 ± 0.02 for BW, 0.08 ± 0.02 for WW, 0.13 ± 0.03 for BL, 0.18 ± 0.04 for SW, 0.15 ± 0.04 for HW, and 0.33 ± 0.06 for AE. Maternal heritabilities were 0.22 ± 0.01 for BW and 0.20 ± 0.01 for WW. Monthly phenotypic means tended to increase for BW, BL, and HW, and to decrease for AE. Monthly genetic means tended to increase for HW and to decrease for AE.

Key Words: Halothane, Pig, Tropical

Lactation Biology: Mechanisms Regulating Lactation and Mammary Function

M169 Effects of dietary supplementation with flax during prepuberty on mammary development and circulating prolactin and estradiol concentrations. C. Farmer*[1], H. V. Petit[1], and A. V. Capuco[2], [1]*Agriculture and Agri-Food Canada*, *Sherbrooke, QC, Canada*, [2]*USDA-ARS*, *Beltsville, MD*.

The possible role of dietary flax on mammary development of prepubertal gilts was investigated. Fifty-seven gilts were fed one of four diets from 88 d of age until slaughter (day 212 ± 1). Diets were: standard, CTL (n=14); 10% flaxseed supplementation, FS (n=13); 6.5% flaxseed meal supplementation, FSM (n=15); and 3.5% flaxseed oil supplementation, FSO (n=15). All diets were isonitrogenous, isolipidic and isocaloric. Jugular blood samples were obtained on days 78 and 210 and assayed for prolactin and estradiol. 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 fatty acid profiles in extraparenchymal tissue were evaluated. Dietary flax increased ($P \leq 0.001$) the concentrations of polyunsaturated fatty acids (PUFA) and decreased those of saturated (SFA, $P < 0.01$) and monounsaturated (MUFA, $P < 0.001$) fatty acids in mammary extraparenchymal tissue. This was largely due to the inclusion of FS or FSO ($P < 0.01$), but not FSM. Circulating concentrations of prolactin and estradiol were unaltered by treatments ($P > 0.1$). Dry matter content of parenchymal tissue was the only mammary compositional value affected, showing an increase with flax addition ($P < 0.05$). Diet did not alter ($P \geq 0.1$) BrdU labelling index or estrogen receptor localization. Within mammary parenchyma, estrogen receptors were present in epithelial cells but not adipocytes, a novel demonstration of potential estrogen targets in gilt mammary gland. Dietary supplementation with flax as seed, meal or oil, brought about expected changes in fatty acid profile in mammary extraparenchymal tissue but neither the alteration in fatty acid profile nor the presence of lignans had beneficial effects on hormone concentrations or mammary development.

Key Words: Flax, Mammary Development, Swine

M170 Developmental changes in the milk fat globule membrane proteome during the transition from colostrum to milk. T. A. Reinhardt* and J. D. Lippolis, *National Animal Disease Center, ARS, USDA*, *Ames, IA*.

Shotgun Proteomics, using amine-reactive isobaric tags (iTRAQ) was used to quantify protein changes in milk fat globule membranes (MFGM) that were isolated from day 1 colostrum and compared to MFGM from day 7 milk. Eight Holstein cows were randomly assigned to 2 groups of 4 cow sample pools for a simple replication of this proteomic analysis using iTRAQ. iTRAQ labeled peptides from the experiment sample pools were fractionated by strong cation exchange chromatography followed by further fractionation on a micro-capillary high performance liquid chromatograph connected to a nanospray-tandem mass spectrometer. Data analysis identified 138 bovine proteins in the MFGM with 26 proteins up-regulated and 19 proteins down-regulated in day 7 MFGM compared to colostrum MFGM. Mucin 1 and 15 were up-regulated greater than 7 fold in MFGM from day 7 milk compared to colostrum MFGM. The tripartite complex of proteins of adipophilin, butyrophilin and xanthine dehydrogenase were individually upregulated in day 7 MFGM 3.4, 3.2 and 2.6 fold respectively compared to colostrum MFGM. Additional proteins associated with various aspects of lipid transport, synthesis and secretion such as acyl-CoA synthetase, lanosterol synthase, lysophosphatidic acid acyltransferase, and fatty acid binding protein were up-regulated 2.6 -5.1 fold in day 7 MFGM compared to colostrum MFGM. In contrast, apolipoproteins A1, C-III, E and A-IV were down-regulated 2.6-4.3 fold in day 7 MFGM compared to colostrum MFGM. These data demonstrate that quantitative shotgun proteomics has great potential to provide new insights into mammary development.

Key Words: Proteomics, Milk Fat Globule Membrane, Lactation

M171 Temporal effect of *trans*-10, *cis*-12 conjugated linoleic acid on mammary lipogenic gene expression. J. K. Kay[1,2], C. E. Moore[1], D. E. Bauman[3], R. P. Rhoads[1], S. R. Sanders[1], A. F. Keating[1], and L. H. Baumgard*[1], [1]*University of Arizona*, *Tucson*, [2]*Dexcel*, *Hamilton, New Zealand*, [3]*Cornell University*, *Ithaca*.

Trans-10, *cis*-12 conjugated linoleic acid (CLA) reduces milk fat synthesis and mammary lipogenic gene expression in lactating dairy cows. It is unknown however, if these genes are collectively down regulated by a global nuclear transcription factor or if one key enzyme is directly affected and reduction in other lipogenic genes is due to lack of substrate (i.e. malonyl CoA) availability/requirement, or an alternative indirect mechanism. We investigated the temporal effects (0, 12, 24 and 72 h) of intravenous (IV) *trans*-10, *cis*-12 CLA infusion (13.1 g *trans*-10, *cis*-12 CLA/d) on mammary acetyl CoA carboxylase (ACC), fatty acid synthetase (FAS), Δ*9*-desaturase (SCD), and fatty acid binding protein (FABP) mRNA abundance. Dry matter intake, milk yield and yield and content of milk protein and lactose were not affected by CLA infusion. However, CLA infusion progressively reduced milk fat content and maximum milk fat depression (MFD; 49%; $P<0.05$) occurred at 72 h. Overall mRNA expression of ACC and FAS decreased ($P<0.05$) and SCD tended to decrease ($P<0.09$) but FABP was not altered by CLA infusion. By 24 h post infusion initiation, ACC mRNA abundance was reduced ($P<0.05$) by 44% while FAS and SCD tended to be reduced ($P<0.08$) by 47 and 46%, respectively. At 72 h, magnitude of reduction and level of significance did not alter from the 24 h time point even though MFD continued to progress. The similar temporal effects of *trans*-10, *cis*-12 CLA on ACC, FAS and SCD mRNA abundance suggests these lipogenic genes are down regulated collectively by *trans*-10, *cis*-12 CLA, probably via a global modulator, rather than inhibition of a specific key lipogenic gene.

Key Words: CLA, Milk Fat, Gene Expression

M172 Expression profiling of proteins involved in CLA metabolism in mammary tissue and mammary gland epithelial cells. Y. C. Jin[1], H. G. Lee*[1], J. A. Han[1], J. H. Li[1], K. H. Kim[1], N. K. Lee[1], Y. J. Kim[2], M. K. Song[3], and Y. J. Choi[1], [1]*School of Agricultural Biotechnology, Seoul National University*, *Seoul, Korea*, [2]*Department of Food Science & Biotechnology, Korea University*, *Chochiwon*, [3]*Department of Animal Science, Chungbuk National University*, *Chungbuk, Korea*.

This study was conducted to observe proteins involved in CLA metabolism comparing the difference of protein expression among control (no additional fatty acid), vaccenic acid (trans-11 C18:1, a precursor for CLA biosynthesis) and CLA i.v. infusion in the abdomen of 9 lactating rats. The rats were perfused with 120ml of heparinized DMEM. The mammary tissue samples were biopsied by surgical biopsy instrument in lactating rats. Likewise we sought to compare the difference of protein expression among control (BSA), vaccenic acid and CLA added to the medium of differentiated HC11 cells. The protein samples of the mammary tissue and epithelial cells were analyzed by two-dimensional electrophoresis. We identified the specific spots using ESI-Q-TOF and a protein search engine. In rats, vaccenic acid treatment increased the level of cis-9, trans 11 CLA and vaccenic acid in mammary tissue. In addition, CLA treatment increased the level of trans-10, cis-12 CLA and cis-9, trans-11 CLA in mammary tissue ($P<0.05$). The most striking differences in protein abundance between the control and vaccenic acid treatment were seen in 5 specific spots, and 7 spots were detected between saline and CLA treatment. Likewise in HC11 cells, the most striking differences in protein abundance between the control and vaccenic acid treatment were seen in 3 specific spots and 2 spots were detected between BSA and CLA treatment. So we examined the expression of mRNA in the mammary tissue and epithelial cells by real-time PCR, which substantiated 4 genes differentially expressed overall in mammary tissue and epithelial cells. Our results suggest that the identified proteins may be related to CLA biosynthesis and apoptosis of the mammary tissue induced by CLA.

Key Words: Conjugated Linoleic Acid, Mammary Gland, Proteomics

M173 Effects of heat stress vs. underfeeding on milk fatty acid composition. M. D. O'Brien*, J. B. Wheelock, A. J. La Noce, M. L. Rhoads, R. P. Rhoads, M. J. VanBaale, R. J. Collier, and L. H. Baumgard, *University of Arizona*, *Tucson*.

Determining heat stress (HS) effects on lactation variables is complex as they are confounded with reduced nutrient intake. To separate the differences between HS and decreased feed intake on milk fatty acid composition, we conducted two HS experiments (EXPS) where a thermal-neutral (TN) control group was pair-fed to match nutrient intake with the heat-stressed cows. In both EXPS, the diet was similar and consisted primarily of alfalfa hay and steamed-flaked corn. HS conditions were cyclic to mimic an AZ July day, with temperatures

ranging from 29.7 to 39.2°C. After a TN period of ad libitum feed intake, cows entered HS or underfeeding (UF) periods which lasted for either 9 d (exp 1; n = 12) or 7 d (exp 2; n = 24). In both EXPS rectal temperature increased during HS (averaging 40.5°C at 1400 hr) while DMI decreased by ~30%. By design, UF cows had similar intakes while still producing more milk (> 7 kg/d) indicating UF accounts for only ~50% of HS-induced decreased milk yield. There were little differences in milk fatty acid parameters between experiments, therefore main effects of HS and UF are presented. Milk fat content did not differ (3.73) between treatments. Compared to UF, HS decreased (>10%) the MUFA and PUFA content and increased the saturated fatty acid content of milk fat. Specifically, HS decreased the content of 18:2, 18:3, 18:1 *trans*-6-8, 18:1 *trans*-9 and 18:1 *trans* -11 and increased biohydrogenation end products (18:0 & 16:0), but had no effect on 18:1 *trans*-10 and 18:1 *trans*-12. HS decreased the Δ^9-desaturase index (34.9 vs. 39.4) and reduced the milk fat *cis*-9, *trans*-11 CLA content (2.97 vs. 5.16 mg/g). On a fatty acid origin basis (as a % of total molar yield), HS increased the contribution of 16:0 and 16:1, but *de novo* and preformed derived fatty acids did not contribute differently between treatments. Independent of reduced feed intake, HS markedly effects milk fatty acid composition and this is characterized by a decrease in PUFA and most biohydrogenation intermediates with a corresponding increase in the resulting saturated end products.

Key Words: Heat Stress, Milk Fatty Acids, Biohydrogenation

M174 Stearoyl-CoA desaturase gene expression and fatty acid concentrations in bovine tissues. E. Mosley, B. Hatch, K. Hunt, A. Morrison, C. Roberts, D. Sevier, and M. McGuire*, *University of Idaho*, *Moscow*.

The stearoyl-CoA desaturase (SCD) enzyme is important in maintaining lipid fluidity. However, the relationship between the level of gene expression and activity of the SCD enzyme is not well defined. The objective of this study was to determine if there was an association between the basal gene expression of SCD and occurrence of the products of SCD (14:1c9, 16:1c9, 18:1c9, and 18:2c9t11) in various tissues. Samples were obtained from animals (2 lactating Holstein cows and 2 mixed breed market steers) at slaughter. Heart, skeletal muscle, liver, lung, rumen, large intestine, small intestine, intestinal adipose, and mammary (cows only) samples were frozen in liquid nitrogen and stored at -80°C. RNA was extracted and converted to cDNA for quantitative real time PCR analysis of the SCD gene. Extracted lipid was converted to fatty acid methyl esters and analyzed by gas chromatography. In descending order, intestinal adipose, mammary, lung, heart, and small intestine tissues had the highest expression of SCD (3,037,718 ± 1,989,208; 1,468,529 ± 199,409; 1,319,235 ± 403,596; 1,123,247 ± 345,633; and 931,783 ± 250,632 copies/100 ng RNA, respectively), while skeletal muscle, large intestine, rumen, and liver tissues contained lower levels of expression (519,809 ± 142,239; 385,048 ± 232,297; 359,063 ± 54,836; and 354,244 ± 156,812 copies/100 ng RNA, respectively). Considerable variation was detected among animals independent of gender (cow vs steer). The desaturase indices for each substrate of SCD (product/(substrate+product)) were similar across tissues. There was an inverse correlation ($r = -0.79$, $P < 0.0001$) between total PUFA and total SCD product concentrations across all tissues. No relationship was detected between the level of gene expression and occurrence of SCD fatty acid products in tissues or desaturase indices. A greater understanding of the regulation of tissue fatty acids is necessary.

Key Words: Stearoyl-CoA Desaturase, Real Time PCR, Fatty Acid

M175 Expression of PPAR and LXR nuclear hormone receptor families are not modified during milk fat depression induced by diet or treatment with trans-10, cis-12 conjugated linoleic acid (CLA). K. J. Harvatine* and D. E. Bauman, *Cornell University*, *Ithaca, NY*.

Milk fat synthesis can be inhibited by intermediates of ruminal fatty acid biohydrogenation including trans-10, cis-12 CLA. These biohydrogenation intermediates signal a coordinated down-regulation of genes involved in mammary fatty acid synthesis, transport and esterification. We have previously reported decreased expression of SREBP1, SREBP1 activating proteins and Spot 14 during diet-induced milk fat depression and treatment with trans-10, cis-12 CLA. Regulation of nuclear hormone receptors (NR) during milk fat depression is of interest as NR are key regulators of lipogenic genes and CLA is known to modify expression and activation of some NR. Tissue profiling identified only LXRalpha as responsive to lactation with increased expression in lactating compared to nonlactating mammary tissue. Expression of NR and NR responsive genes during milk fat depression was investigated by Real-Time PCR using mammary tissue of cows during diet-induced milk fat depression (low forage, high oil diet: LF/HO) and 3d intravenous infusion of trans-10, cis-12 CLA (10 g/d). The LF/HO diet and CLA treatment reduced milk fat yield by 38 and 24%, respectively. Expression of PPARalpha, beta and delta was not modified by treatment. Expression of mitochondrial fatty acid oxidation enzymes (CPT1a and ACADVL) was increased approximately 50% by LF/HO treatment but expression of a peroxisomal oxidation enzyme (ACOX) was unaffected. Expression of LXRalpha and beta and the LXR responsive gene ABCA1 was not modified by treatment. Some members of the LXR and PPAR gene families are expressed in the lactating mammary gland; however, their expression is not modified during diet or trans-10, cis-12 CLA induced milk fat depression.

Key Words: Nuclear Receptors, Milk Fat Synthesis, Lipogenesis

M176 Production and physiological indicators to select pasture-based dairy cows suitable for extended lactations. J. K. Kay, P. W. Aspin, C. V. C. Phyn, J. R. Roche, D. A. Clark*, and E. S. Kolver, *Dexcel*, *Hamilton, New Zealand*.

In pasture-based systems cows are traditionally managed to calve seasonally and annually in order to maximize pasture utilization. Increased productivity and declining reproductive performance has led to the incorporation of longer calving intervals and extended lactations, however, individual cows vary in their ability to maintain milk production for an extended lactation. Identifying production and physiological markers to predict suitable cows for extended lactations would allow dairy farmers to make early decisions to withhold mating or to continue milking specific non-pregnant cows through the typical dry months. Fifty-six genetically divergent North American and New Zealand Holstein Friesians were allocated to three pasture-based dietary treatments (0, 3 and 6 kg concentrate DM/cow/d), and breeding was withheld to target a 600 d lactation. Within each treatment (genotype × diet), milk solids (MS; protein + fat) yield during the extended lactation (>296 DIM) was positively correlated with MS yield from the previous season ($P < 0.05$; $r^2 = 0.15$), MS yield from the first 296 DIM ($P < 0.01$; $r^2 = 0.19$) and daily MS yield at a theoretical dry-off date (~296 DIM; $P < 0.01$; $r^2 = 0.43$). In contrast, MS yield during the extended lactation was negatively correlated with BCS at theoretical dry-off ($P < 0.01$; $r^2 = 0.37$). Hormone and metabolite data from wk

1-10 post-partum demonstrated a positive association ($P < 0.01$; $r^2 = 0.24$) between NEFA levels and extended lactation MS production, whereas glucose, insulin, and IGF-I were negatively associated ($P < 0.01$; $r^2 = 0.23, 0.37, 0.26$, respectively) with extended lactation MS production. Overall, milk production, BCS and early lactation hormone and metabolite data may be useful criteria to identify animals that will undergo a successful extended lactation.

Key Words: Extended Lactation, Predictors

M177 Milk from cows at involution reduces MAC-T cell survival. G. Tremblay*[1], P. Bernier-Dodier[1], L. Delbecchi[2], G. F. Wagner[3], B. G. Talbot[1], and P. Lacasse[2], [1]*Université de Sherbrooke*, *Sherbrooke, QC, Canada*, [2]*AAFC-Dairy and Swine R&D Center*, *Sherbrooke, QC, Canada*, [3]*University of Western Ontario*, *London, ON, Canada*.

Several data indicate that mammary gland involution is under a local control. However, the exact nature of this control is still unknown. The objective of this experiment was to verify the presence of factors in milk that can reduce mammary cell survival during milk stasis. Milk samples were obtained from nine Holstein cows in late lactation that received a unilateral milking for 14 days. Left forequarter and right hindquarter were milked twice a day while the two other quarters were dried off (d 0). Milk samples were taken from forequarters on d -7, 1, 2, 7, and 14 for an *in vitro* assay. In the *in vitro* assay, milk samples were added to DMEM/F12 medium at a final concentration of 10% and incubated for 9 h with confluent MAC-T cells. Survival was quantified by the metabolic turnover of tetrazolium salt (XTT). The assay showed a ~98% survival for each milk sample except those from dry quarters on d 7 and 14, which showed a reduction of cell survival to 83.7% ($P < 0.001$) and 74.8% ($P < 0.005$), respectively, in comparison with cells incubated with milk from milked quarters at d 14. These results suggest that milk from dry quarters contains a factor able to reduce cell survival. Among the factors present in milk, stanniocalcin (STC) increased ($P < 0.001$) 2.5- and 3.2-fold after 7 and 14 d of milk stasis, respectively. There was a negative correlation (Pearson coefficient of -0.605) between STC concentration in milk and cell survival. Stanniocalcin is involved in the homeostasis of calcium and has been reported to induce apoptosis in chondrocytes *in vitro*. Even if the exact contribution of this hormone in the involution process is not known, its role in cell death has to be further investigated.

Key Words: Stanniocalcin, Mammary Gland, Involution

M178 Different milking frequencies alter stanniocalcin content in cow's milk. P. Bernier-Dodier*[1], P. Lacasse[2], G. F. Wagner[3], B. G. Talbot[1], and L. Delbecchi[2], [1]*Université de Sherbrooke*, *Sherbrooke, QC, Canada*, [2]*AAFC-Dairy and Swine R&D Center*, *Sherbrooke, QC, Canada*, [3]*University of Western Ontario*, *London, ON, Canada*.

Several lines of evidence suggest that there is a factor in milk that exerts a local control on lactation and mammary gland involution. Previously, we have shown that estradiol reduces milk production and increases the expression and concentration of stanniocalcin (STC) in the mammary gland of cows. We hypothesized that this hormone may be implicated in the involution process. To verify this hypothesis, we modulated the involution rate by manipulation of the milking frequency. Ten Holstein cows in mid-late lactation were differentially milked, two quarters being milked once daily and the two others thrice daily for 8 weeks. Milk samples were taken from forequarters every week to determine the STC content (RIA), the BSA content (colorimetric assay), and the protease activity (zymography). A significant difference in milk yield ($P < 0.0001$) was observed during the treatment between the thrice daily milked glands (increase) and the once daily milked glands (decrease). Milk yields (kg/d) were 12.6, 8.0, 6.5, and 8.8 for once daily milked glands, and 13.5, 16.3, 16.2, and 14.6 for thrice daily milked glands, on weeks 0, 4, 8, and 10 (week 2 post-treatment) respectively. At week 4, a higher rate of apoptosis was observed in the glands milked once daily compared with the glands milked thrice daily (0.83% and 0.38% respectively; $P < 0.05$), as detected by TUNEL analysis. This effect on apoptosis was not detected at week 8. In the majority of the cows (7 out of 10), zymographic analyses showed an increase in protease activity only in the milk from once-daily milked quarters, indicating an active remodelling of these glands. Milk BSA concentration was significantly increased in the once-daily milked quarters ($P < 0.001$), an augmentation possibly caused by an opening of the tight junctions in those quarters. Finally, milk STC concentration increased in all quarters during the treatment ($P < 0.001$); however, this increase was higher in the once-daily milked quarters than in the thrice-daily milked ones ($P < 0.01$). Our data indicate that milking frequency alters milk STC concentration and that this effect may be related to gradual involution of the mammary gland.

Key Words: Mammary Gland, Involution, Stanniocalcin

M179 Reduced nursing frequency decreases milk output and alters SOCS and TPH1 gene expression in the mouse mammary gland. W. Olea*, D. Torres, J. George, and D. L. Hadsell, *Baylor College of Medicine*, *Houston,TX*.

Reduced milking frequency in cows lowers milk production. In rats decreased nursing frequency below 4 times daily causes mammary involution. The mechanism by which this effect occurs is poorly understood. Recent studies in cows have shown that suppressors of cytokine signaling (SOCS) are regulated by milking frequency. The hypothesis for this study was that decreased nursing frequency in lactating mice would decrease milk production and increase mammary expression of SOCS genes and other negative feedback regulators of lactation. To test this hypothesis milk output and mammary gene expression was measured in dams that were allowed to nurse either ad-libitum (AL) or four times daily (4X). On day 1 of lactation, groups of CD-1 dams (10 mice /treatment) were given weight-normalized litters of 10 pups each. On day 8 postpartum the interval nursing was started in the 4x group. For this group the dams were given 4 1-hour nursing periods with their litters over a 24 hour period (5am, 11am, 5pm, and 11pm). Litter weight was measured daily. On day 14 post partum, milk production was measured in both groups by the weigh-suckle-weigh method after a 5 hour period of separation from the litters. Mammary tissue was then collected for analysis. Total RNA prepared from the inguinal mammary gland was analyzed for α-lactalbumin, lactoferrin, SOCS1, SOCS2, SOCS3, Cish, and TPH1 mRNA abundance using RT-qPCR. Milk output was lower ($P<0.05$) in 4X than AL (0.45±0.12g and 1.09±0.15g, respectively) but mammary morphology appeared similar between 4X and AL. Abundance of the mRNAs for α-lactalbumin, lactoferrin, SOCS2, and TPH1 was higher ($P<0.05$) in 4x than AL. Abundance of the mRNAs for SOCS1 was lower ($P<0.05$) in 4X than AL. Abundance of the mRNAs for SOCS3

and Cish were similar among 4X and AL. These results suggest that altered gene expression for SOCS1, SOCS2 and TPH1 in the mammary gland may mediate the loss of milk production that occurs with reduced nursing frequency in mice.

Key Words: Lactation, Milking Frequency, SOCS

M180 Gene expression profiling in bovine mammary gland during onset of lactation. K. A. Finucane[1], T. B. McFadden[1], J. P. Bond[1], J. J. Kennelly[2], and F.-Q. Zhao*[1], [1]*University of Vermont*, *Burlington*, [2]*University of Alberta*, *Edmonton, Alberta, Canada*.

The mammary gland undergoes dramatic functional and metabolic changes during the transition from late pregnancy to lactation. To better understand the molecular events underlying these changes, we analyzed expression profiles of approximately 23,000 gene transcripts in bovine mammary gland at day 5 before parturition and day 10 after parturition. A total of 1531 transcripts were significantly up-regulated while 2910 transcripts were down-regulated ($P < 0.05$). Gene ontology analysis showed that the main up-regulated genes were associated with transport activity (amino acid, nucleotide, glucose and ion transporters), lipid and carbohydrate metabolism (lipoprotein lipase, acetyl-Coenzyme A synthetase and carboxylase, 6-phosphofructo-2-kinase, etc.), and regulation of transcription and translation (transcription factor-like 1, CBP/p300-interacting transactivator, tRNA synthetase, etc.) while the main down-regulated genes were associated with cell cycle and proliferation (cyclins, cyclin-dependent kinase, etc.), protein and RNA degradation (proteasomes, thiolesterase, pinin, RNA binding motif protein, etc.), DNA replication and chromosome organization (histone, histone deacetylases, DNA polymerase accessory subunit, etc.) and microtubule-based processes (microtubule associated protein tau, kinesin, tubulins, etc.). The increased ($P < 0.05$) expression of glucose transporter GLUT1 mRNA during lactation was verified by quantitative reverse transcription/PCR. GLUT1 protein also tended to increase during lactation ($P = 0.13$). Furthermore, GLUT1 protein was primarily localized in mammary ductal epithelia and blood vessel endothelia before parturition, but was predominantly localized in the basolateral and apical membranes of mammary alveolar epithelial cells during lactation. Our microarray data provide insights into the molecular events in the mammary gland at the onset of lactation, indicating the up-regulation of genes involved in milk synthesis concomitant with the inhibition of those related to cell proliferation.

Key Words: Functional Genomics, Microarray, Periparturition

M181 Co-localization of glucose transporter-1 and hexokinase-1 in response to lactogenic hormones and media glucose concentration in bovine mammary epithelial cells. M. Dai* and J. P. Cant, *University of Guelph*, *Ontario, Canada*.

Hexokinase (HK) catalyzes the first step in intracellular glucose metabolism. Prior experiments revealed that glucose transport across the plasma membrane of bovine mammary epithelial cells appears to involve translocation into an intracellular compartment to which HK has access. The glucose transporter GLUT1 is the primary glucose transporter isoform expressed in bovine mammary gland during lactation. However, the isozyme of HK expressed in the bovine mammary gland remains unknown to date. Four isoforms of HK have been identified, and tissue-specific patterns of expression for the isozymes suggest unique roles for each in metabolism. In the present work, we tested the hypothesis that HK1 is expressed in bovine mammary gland, and co-localizes with GLUT1 in the presence of glucose and lactogenic hormones. Western blotting of mammary tissue with anti-HK1 revealed a band in the 75kDa region that is smaller than what has previously been reported for HK1 in other species. Confocal immunofluorescent microscopy of mammary epithelial cells revealed that co-localization of GLUT1 and HK1 is induced by lactogenic hormone complex (insulin, prolactin and hydrocortisone) at varied media glucose concentrations. Without lactogenic hormones, the immunofluorescent density of GLUT1 was low, and HK1 appeared not to co-localize with GLUT1 when media glucose concentration was increased to 25 mM. These results demonstrate that changes in GLUT1 expression and the co-localization of GLUT1 and HK1 in response to lactogenic hormones are consistent with the regulation of mammary glucose uptake and metabolism.

Key Words: Hexokinase, Glucose Transporter, Co-localization

M182 Presence of functional phosphodiesterases in dairy cow's mammary gland. V. Dostaler-Touchette*[1], C. Guillemette[2], F. J. Richard[2], and P. Y. Chouinard[1], [1]*Institut des nutraceutiques et des aliments fonctionnels, Université Laval*, *Québec, Québec, Canada*, [2]*Centre de recherche en biologie de la reproduction, Université Laval*, *Québec, Québec, Canada*.

One possible way to improve the secretion of milk constituents is to modulate cyclic nucleotide (cAMP, cGMP) pathways. Intracellular cyclic nucleotides are degraded by the enzyme family called phosphodiesterases (PDE). Previous studies have shown that using non-specific PDE inhibitors like caffeine improved milk production. The objective of this study was to investigate the critical role of PDEs in the lactation of dairy cows. In order to understand the enzymatic expression pattern in the mammary gland, tissue samples were taken randomly from an udder obtained from a local slaughterhouse. Using 1 μM of cAMP, PDE essays (n=5) reported a total activity of 45.5±5.5 fmol/min per μg of total protein. Rolipram, a specific PDE4 inhibitor, showed a sensible activity of 15.9±5.5 fmol/min/μg of total protein, supporting that PDE4 is responsible for one third of the total enzymatic activity in the mammary gland. Transcript analysis using RT-PCR revealed that PDE4D was amplified from specific primers designed from rat, mouse, human and validated with bovine. Expected size fragment was obtained in a 1% agarose gel. Sequence analysis of the amplicon resulted in 99% homology to PDE4D. It is therefore possible to give this subfamily the credit for the Rolipram-sensitive PDE activity in the cow's udder. Moreover, western blotting using a specific PDE4D antibody has confirmed that the protein of this isoenzyme is present. In conclusion, these results not only demonstrate the presence of PDE4D transcript and protein, but also show an active enzyme, suggesting a functional role of PDE4D in bovine mammary gland.

Key Words: Dairy Cow, Mammary Gland, Phosphodiesterase

M183 Modulation of cellular activity of glutathione peroxidase by L-selenomethionine in primary cultures of bovine mammary gland epithelial cells. S. G. Miranda*[1,2], Y. J. Wang[2], N. G. Purdie[2], V. Osborne[2], B. L. Coomber[2], and J. P. Cant[2], [1]*University of Zulia*,

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Cytosolic glutathione peroxidase (GPx1; E.C. 1.11.1.9) is a selenoenzyme that catalyzes the reduction of hydrogen peroxide, or organic hydrogen peroxides, and protects cells from oxidative damage. We have hypothesized that selenium supply influences mammary epithelial cell (MEC) survival by increasing GPx1 activity. A study was designed to evaluate the effect of L-selenomethionine (SeMet) on the activity of GPx1 in bovine MEC. Six Holstein lactating cows with udders free of infection were slaughtered and parenchymal tissue was harvested to prepare cells for primary culture. MEC were enzymatically monodispersed and collected by filtration. Western Blotting analysis detected GPx1 in the mammary parenchyma and MEC. Secretory cells were plated on collagen gel matrix in 24-well plates and fed with media which was replaced every 24 h. After reaching 80% confluence, MEC were incubated with media containing 0, 10, 20 or 50 nM SeMet for 3 d. GPx1 activity, total MEC and cell viability were recorded daily. Statistical analysis was carried out using one-way ANOVA with significance declared at $P<0.01$. Enzymatic activity of GPx1 in MEC increased linearly ($P<0.01$) as SeMet increased in the medium. Cells cultured in 20 and 50 nM SeMet had significantly higher ($P<0.01$) activity of GPx1 (46.3 ± 4.0, 46.3 ± 4.0 nmole NADPH/min per mg protein, respectively) than those in 0 and 10 nM SeMet (30.5 ± 4.0, 38.9 ± 4.0 nmole NADPH/min per mg protein, respectively). The total number of MEC (4.5×10^5) and their viability (91.7%) were significantly increased ($P<0.01$) in 50 nM SeMet *vs.* 0, 10, and 20 nM SeMet (2.4, 2.4, and 2.5×10^5, respectively, and 77.2, 79.4, and 80.5%, respectively). Results suggest that SeMet can increase the GPx1 activity, number and viability of lactating bovine MEC.

Key Words: Selenomethionine, Glutathione Peroxidase, MEC

M184 Prostaglandins A1 (PGA1) and E1 (PGE1) alter heat shock protein 70 (HSP-70) gene expression in bovine mammary epithelial cells (BMEC). J. L. Collier*[1], M. B. Abdallah[1], L. L. Hernandez[1], J. V. Norgaard[2], and R. J. Collier[1], [1]*University of Arizona*, *Tucson*, [2]*Danish Institute of Agricultural Sciences*, *Tjele, Denmark*.

PGA1 is known to induce heat shock protein (HSP) synthesis in a wide variety of mammalian cells resulting in protection against cellular stresses while PGE1 is associated with alteration of hypothalamic set point during fever. We tested the effects of PGA1 and PGE1 on HSP-70 gene expression in BMEC at thermoneutral (TN, 37°C) and during heat shock (HS, 42°C). Primary BMEC were cast in collagen gels using Dulbecco's Modified Eagle's media (DMEM) containing 10 ng/ml insulin, 25 ng/ml epidermal growth factor, 75 ng/ml insulin-like growth factor-1, 0.1% BSA and antibiotic/antimycotic at 37°C in 5% CO2. Cultures grew into ductal structures for 8 days with media changes at 48 hour intervals. On day 9 cultures were divided into 3 groups with Controls (C) receiving the growth media, another group received growth media containing 8ug/ml PGA1 and the final group received growth media containing 8ug/ml PGE1. Cultures were then equally divided and moved to TN or HS incubators for up to 16 h with samples removed at 0, 1, 2, 4, 8 and 16h. At each time point, 4 collagen cultures were pooled and immediately placed in TRIzol and stored at -80°C until extracted for RNA. Isolated RNA was evaluated by Nanodrop and was reverse transcribed into cDNA. Expression of HSP-70 was measured by real time-PCR using a Bio Rad IQ5 system with analysis by the delta delta CT method. Hypoxanthine phosphoribosyltransferase 1 (HPRT-1) was used as the housekeeping gene. Addition of PGA1 increased HSP-70 expression in both TN and HS at all time points with much greater expression differences detected during HS. Peak fold increases in HSP-70 expression over time zero for C and PGA1 in HS at 8 h were 16.3 vs. 122.3 ($P<0.0001$). Addition of PGE1 significantly reduced HSP-70 gene expression compared to C and PGA1 in both TN and HS at all time periods except 16h ($P<0.001$). At 16h, HSP-70 expression was higher in cultures containing PGA1 or PGE1 compared to C (2.2 and 0.8 vs. 0.5 fold over time zero; $P<0.03$). We conclude that PGA1 and PGE1 alter HSP-70 expression in BMEC in TN and HS environments.

Key Words: Heat Shock Protein 70, Gene Expression, Prostaglandins

M185 Suitability of foremilk somatic cell counts to estimate total quarter somatic cell count. O. Wellnitz[1], M. Woloszyn[2], and R. M. Bruckmaier*[1], [1]*University of Bern*, *Bern, Switzerland*, [2]*DeLaval International AB*, *Tumba, Sweden*.

In dairy practice, testing of quarter foremilk for the detection of mastitis (by California Mastitis Test (CMT) or by determination of somatic cell count (SCC)) is well established. However, the use of foremilk SCC to estimate total quarter SCC as a basis for calculation of whole udder and bulk tank SCC has not been systematically investigated. Therefore, a study was conducted in 19 dairy cows during 10 consecutive days, i.e. 20 consecutive milkings. Foremilk samples were taken from each quarter after a 1 min udder preparation for SCC measurement by a DeLaval cell counter (DCC). During machine milking, the whole milk of each quarter was collected separately and the SCC of each quarter milk was also determined and 1512 pairs of quarter recordings were evaluated by various linear regression analyses. Quarter milk SCC ranged from 2 to 3100 while foremilk SCC ranged from 5 to 5400 × 1000 cells/ml, respectively. The linear regression of foremilk and total quarter milk measurements of all quarters based on log10 SCC had a slope of 0.43 (R2=0.70). To characterize the predictability of total quarter milk SCC by foremilk measurements at different SCC levels, the samples were classified into different foremilk SCC (<20 (n=217), 20-50 (n=419), 50-100 (n=300), 100-300 (n=373), 300-500 (n=99), and > 500 (n=111) × 1000 cells/ml). The percentage of quarters with numerically higher SCC in the total milk than in the foremilk were 70.5, 46.3, 42.0, 23.3, 19.2, and 6.3% in the groups with foremilk levels of <20, 20-50, 50-100, 100-300, 300-500, and >500 × 1000 cells/ml, respectively. The regression coefficients ($p<0.001$) in these groups were 1.54, 1.05, 1.01, 0.75, 0.67, and 0.44, respectively. In conclusion, whole quarter SCC is higher than foremilk SCC at very low SCC levels. At higher foremilk SCC, whole quarter SCC is mostly lower with an increasing difference between foremilk and whole quarter values.

Key Words: Somatic Cell Count, Foremilk

M186 17β-hydroxysteroid dehydrogenase and β-casein transcripts detected in bovine milk somatic cells. D. A. Pape-Zambito*[1], C. A. Gifford[2], T. L. Ott[1], and R. S. Kensinger[1], [1]*The Pennsylvania State University*, *University Park*, [2]*University of Idaho*, *Moscow*.

Bovine milk contains 17β-Estradiol (E2) but the origins of this E2 in milk are undefined. Because E2 is fat-soluble, E2 in milk may be due to passive diffusion of E2 from plasma. However, we showed

that milk E2 concentrations were lower than plasma concentrations in early pregnancy, but were higher than plasma E2 concentrations in late pregnancy. Limited literature is available on enzymes with the ability to convert estrone (E1) to E2 in the mammary gland. The objective of this study was to determine if somatic cells obtained from milk generate 17β-hydroxysteroid dehydrogenase (17β-HSD) mRNA. Production of β-casein (β-CN) mRNA was used to verify presence of mammary epithelial cells (MEC) in somatic cell samples. Primers specific for bovine β-CN, 17β-HSD 7 and 17β-HSD 12 were designed from known and predicted sequences in the NCBI database. Milk was collected from 9 Holstein cows: 3 from each trimester of pregnancy. Milk was centrifuged, fat removed, and the supernantant decanted. The cellular pellet containing somatic cells was washed with PBS and resuspended in Trizol reagent (Invitrogen). RNA was extracted and RT-PCR utilized to determine presence of transcripts for β-CN, 17β-HSD 7, and 17β-HSD 12. The PCR conditions were as follows: 95C for 5 min, 40 cycles of 95C for 30 s, 64C for 30 s, and 68C for 45 s, a final extension step at 68C for 10 min, followed by agarose gel electrophoresis. Milk somatic cells from all 9 cows expressed β-CN, indicating a MEC population. In addition, 17β-HSD 7 and 17β-HSD 12 sequences were detected in cells of cows from all trimesters of pregnancy. The PCR products were cloned, sequenced, and verified against the NCBI database. These data are consistent with the hypothesis that cells within the mammary gland are capable of converting E1 to E2. Additional experiments are needed to determine which cell type(s) express 17β-HSD transcripts from milk-derived somatic cells.

Key Words: Estradiol, Mammary Gland, Steroidogenesis

M187 Estimation of heritability, repeatability and genetic trend for milk yield of Iranian buffalo in Khuzestan province of Iran using a univariate repeatability animal model. H. Farhangfar*[1], B. Zinvand[2], and F. Amirlou Abolfathi[3], [1]*University of Birjand*, *Birjand, Iran*, [2]*Azad University of Shooshtar*, *Shooshtar, Iran*, [3]*Jihade Agriculture of Khuzestan*, *Iran*.

In order to estimate heritability, repeatability and genetic trend for milk yield (adjusted to 305,2x) a total of 1214 records from lactation 1 through 5 of 795 Iranian buffaloes calving from 1993 to 2003 and distributed in 189 herds in Khuzestan province was used. A univariate repeatability animal model was applied to analyze the records. In the model, fixed environmental factors were herd-year-month of calving (contemporary group), lactation order and age linear covariate nested in the lactation. Additive genetic and permanent environmental random effects were also included in the model. Additive genetic relationship among animals was partially complete due to major lack of sire or dam identification. Restricted maximum likelihood estimates of variance components were obtained (via Average Information algorithm) using WOMBAT software. The results obtained in the present study showed that heritability and repeatability of milk yield were 0.071 and 0.075 respectively. This indicates that there was not only low additive genetic but also low permanent environmental variation among animals over the first 5 lactations suggesting that temporary environmental variation made up a large proportion of total phenotypic variance. Based upon regression of average predicted breeding value of animals with records on year of first calving it was also revealed that there was no statistically significant genetic trend over the period of time.

Key Words: Genetic Parameters, Milk, Iranian Buffalo

National ADSA Production Division Graduate Poster Competition

M188 Effect of feeding two forages at two levels with and without Rumensin to high producing Holstein cows on animal performance. C. M. Martinez*, Y. H Chung, T. W. Cassidy, V. Ishler, K. S. Heyler, and G. A. Varga, *The Pennsylvania State University*, *University Park*.

Two studies were conducted to evaluate the effects of feeding corn silage (study I) or grass silage (Study II) based diets at two levels with and without Rumensin on DMI, milk production and composition and blood metabolites. In both studies, 8 multiparous high producing Holstein cows were used (BW=698 kg±16 and DIM=194 d±3 for study I; BW=656 kg±16 and DIM=124 d±3 for study II) in a replicated 4x4 Latin square design with a 2x2 factorial treatment arrangement to evaluate the effects of forage level with and without Rumensin supplementation (300 mg/cow/d, top dressed). In study I, diets were formulated to contain 50 or 60% forage (DM basis) in which corn silage comprised 70% and western hay comprised 30% of the total forage in the diet. In study II, diets were formulated to contain 50 or 55% forage (DM basis) in which grass silage comprised 55% and corn silage comprised 45% of the total forage in the diet. The length of each period was 4 wks and samples were collected during the last wk. Results from study I (corn silage based diet) showed that DMI was higher ($P < 0.01$) for the 50% forage diets (29.6 kg/d) compared to the 60% forage diet (28.3 kg/d). In study II, DMI was lower ($P < 0.05$) for cows that were supplemented with Rumensin when compared to cows with no supplementation (25.9 vs. 24.7 kg/d, respectively). Milk total solids% was reduced ($P < 0.05$) with Rumensin supplementation (11.8%) versus no Rumensin supplementation (12.3%). Milk urea nitrogen was higher ($P < 0.059$) for cows consuming the 55% forage diet (10.1 mg/dL) than for cows provided the 50% forage diet (8.81 mg/dL). Within each forage source, milk yield and fat corrected milk were not affected by forage level source or Rumensin in the diet (40.9 and 42.4 kg/d, for study I; 41.6 and 41.7 kg/d for study II, respectively). Data from these studies showed that higher forage inclusion in dairy rations can be attained without affecting milk production or major components such as milk fat or protein % for either corn silage or grass silage based diets. Rumensin supplementation may affect milk components and DMI depending on the level and type of forage fed.

Key Words: Rumensin, Forage Level, Silage

M189 Conjugated linoleic acids attenuate lymphocyte proliferation and interleukin-4 production in bovine peripheral blood mononuclear cells challenged with concanavalin-A. C. Caldari-Torres*, W. R. Collante, and L. Badinga, *University of Florida*, *Gainesville*.

During the perinatal period, immune functions such as lymphocyte response to mitogens and production of antibodies are depressed in dairy cows. The objective of this study was to examine the short-term effects of *cis*-9,*trans*-11 and *trans*-10,*cis*-12 isomers of conjugated linoleic acid (CLA) on lymphocyte proliferation, tumor necrosis