(22.2 ± 0.3%). Hay NSC was 8.9 ± 0.05%. Insulin concentrations were higher in grazing horses than in horses restricted to hay (P = 0.012). Group mean insulin concentrations of grazing horses and NSC levels in the pasture were related ($r^2 = 0.032$, P < 0.001). Individual mean insulin response was proportional to the increase in insulin per unit of NSC ($r^2 = 0.601$, P = 0.008). Sinusoidal circadian patterns in NSC ($r^2 = 0.507$, P < 0.001) and insulin in grazing horses ($r^2 = 0.121$, P < 0.001) had similar frequency (P = 0.36), with changes in insulin delayed by 30 min. The percent change in insulin was 2.5 times that of NSC. The circadian patterns in NSC were attributable to WSC which comprised 93%, and there was no evident pattern in starch (7%). This study indicates that circulating insulin in grazing horses is associated with circadian variation in pasture NSC.

Key Words: Horse, NSC, Insulin

W98 Seasonal variation in cool season grasses. L. Lawrence^{*1}, S. Hayes¹, R. Allman², and G. Rich³, ¹University of Kentucky, Lexington, ²The Farm Clinic, Lexington, KY, ³Rich Equine Nutrition Consulting, Memphis, TN.

Pasture is an important nutritional resource on Central Kentucky horse farms, however both pasture availability and pasture composition will vary during the year. The purpose of this study was to identify variations in the nutritional composition of cool season pasture grasses commonly found in horse pastures. Samples of cool season grasses (tall fescue, orchard grass and blue grass) were collected from Central Kentucky horse pastures every month for 5 y (2000-2004). Pastures were maintained on fall fertilization schedules based on soil sampling and were regularly clipped to maintain a forage height below 25 cm. Samples were analyzed for crude protein (CP), acid detergent fiber (ADF), calcium (Ca), and phosphorus (P). Digestible energy (DE) content was estimated from CP and ADF concentration (NRC, 1989). Mean values were calculated across all months, plant types and years and also by month across years and plant types. Across all years and months the cool season pastures sampled in this study contained 20.1 $\pm\,4.4\%$ CP, 28.6 $\pm\,4.9\%$ ADF, 0.46 $\pm\,0.08\%$ Ca and 0.41 $\pm\,0.08\%$ P, on a dry matter basis. Concentrations of CP, ADF, Ca and P were affected by month (P < 0.05). Pasture quality, based on CP and estimated DE, increased in the spring, decreased in the summer and then increased again in the fall. Mean CP concentration (DM basis) ranged from a high of $24.9 \pm 1.7\%$ in April to a low of $14.6 \pm 2.4\%$ in July. Mean CP content exceeded 18% from September through May. Mean ADF concentration ranged from $34.5 \pm 3.6\%$ in July to $21.5 \pm 2.7\%$ in April. Digestible energy was highest in April $(3.21 \pm 0.17 \text{ Mcal/kg})$ and lowest in July $(2.26 \pm 0.19 \text{ Mcal/kg})$. Calcium concentration was lowest in the late spring and highest in the fall. For lactating mares and growing horses, calcium intakes of 1.6 to 2 g/Mcal of DE have been suggested (NRC, 1989). During April and May, the Ca:DE ratio in the cool season grasses was below 1.6. Based on these observations, including legumes in Central Kentucky horse pastures may be warranted.

may have compensated for their lower milk solid content. We conclude

that milk solid content in the previous lactation is not an effective way

W100 Effects of milking interval on milk constituents from various

fractions of ewe milk. A. Dzidic*1, M. Kaps1, and R. Bruckmaier2,

¹University of Zagreb, Zagreb, Croatia, ²University of Bern, Bern,

The aim of this study was to evaluate the effects of milking interval

(8 and 16 h) on milk constituents (fat, protein, lactose and dry matter

percentage, and somatic cell count) in different milk fractions in Istrian

x Awassi x East Friesian crossbreed ewes. Milk fraction samples of

20 ewes were collected during morning and evening milking in

early lactation after 25% (M25), 50% (M50), 75% (M75) and 100%

(M100) of main milk yield, and machine stripping fraction (MS) when

of identifying cows that will adapt well to 1x daily milking.

Key Words: Milking frequency, Udder capacity, Pasture

Key Words: Horse, Equine, Pasture

Lactation Biology

W99 Milk yield and udder capacity of cows with different milk concentration milked once or twice daily. D. Clark*¹, D. Dalley¹, and S. Davis², ¹Dexcel, Hamilton, New Zealand, ²ViaLactia Biosciences, Auckland, New Zealand.

Four per cent of New Zealand's dairy farmers now milk their herds once daily (1x). Jerseys are more tolerant of 1x milking than Holstein-Friesians and the hours worth of udder capacity is greater for the former. We hypothesized that Holstein-Friesian cows with more concentrated milk would be more tolerant of 1x milking than those producing less concentrated milk. Seventy-two Holstein-Friesian cows were selected as a High milk solid content (fat + protein) group (High MS) and 72 selected as a Low milk solid content group (Low MS). Within each group, 23 cows were allocated to 2x daily milking and 49 cows to 1x daily milking with grazed pasture as the sole feed. Udder capacity was determined as the total volume of milk contained in the udder 40 h after the last milking. Residual milk was removed after an intravenous injection of 10 IU oxytocin. Udder capacity was measured at approximately 90 and 150 days in milk (DIM). Cows milked 1x produced less milk, protein and fat yields and had higher SCC (P < 0.001) than those milked 2x. Cows selected for milk solids content did not differ in their milk, protein, fat yield or SCC. There was no milking frequency by milk solids content interaction. Cows milked 2x daily had greater udder capacities at 90 (P<0.05) and 150 DIM ($P \le 0.07$) than those milked 1x daily. High MS cows had lower udder capacities at 90 (P<0.07) and 150 DIM (P<0.1) than Low MS cows. Holstein-Friesian cows selected for phenotypically high milk solids content did not produce more milk, protein or fat than those selected for low milk solid content. The latter had 11-16% greater udder capacity when milked 1x daily in early-mid lactation and this

in milk (DIM). Cows yields and had higher elected for milk solids

Switzerland.

a random effect and milking time, peak flow rate, total milk yield, milking interval and milk fraction nested within milking interval defined as fixed effects. The relationships between milk fractions and constituents within milking interval were tested by using linear, quadratic and cubic contrasts. The fat content during main milking ranged from 5.81 to 6.30 % and from 3.00 to 5.70 % after the 8 and 16 h from previous milking, respectively. Compared to the main milk fractions, the MS fraction fat content was higher (P < 0.05) after both milking intervals. Protein, lactose and dry matter did not change (P >0.05) through the main milking fractions in both milking intervals. In contrast, MS fraction was lowest (P < 0.05) for protein and lactose, while highest for dry matter. Higher somatic cell count were observed in the M25 and MS fractions compared to all other milk fractions (P<0.05). Milk fat, protein, dry matter and somatic cell count were best described by a quadratic function, while lactose was described by a linear after 16 h and a cubic function after 8 h milking interval. Milking interval strongly influenced all the milk constituents in various milk fractions during ewe milking. Changes in milk constituents between and during ewe milking should be taken into account when analytical milk samples are taken.

Key Words: Milk fractions, Milking interval, Dairy ewes

W101 Effects of omitting two milkings weekly on milk yield, milk composition and udder health in Manchega and Lacaune dairy ewes. V. Castillo, X. Such, G. Caja*, E. Albanell, and R. Casals, Universitat Autònoma de Barcelona, Bellaterrra, Spain.

A total of 42 Manchega (MN) and 18 Lacaune (LC) dairy ewes were used during 15 wk (wk 7 to 22) to study the effect of omitting two milkings weekly throughout lactation on milk yield, milk composition and somatic cell count (SCC). Ewes were milked twice daily (8.00 and 18.00 h), but on Saturday and Monday once daily milking was performed. At 12 and 21 wk, milk was measured daily and sampled for composition and SCC. Friday values were used as reference to evaluate the effect of milking omission. In mid-lactation (12 wk), weekend milk yield (MN: 651 mL/d, LC: 1452 mL/d) and fat content (MN: 6.25%, LC: 5.69%) decreased (P<0.05) by 24 and 8%, respectively in MN, and by 16 and 3%, respectively in LC. In late lactation (21 wk), weekend milk yield losses were lower but still significant (MN: -18%, LC: -9%; P<0.001). However, fat content losses did not differ from mid-lactation values. In both breeds, protein content and SCC did not vary significantly as a result of weekend milking omission in mid- and late-lactation. On Monday, milk yield returned to Friday values in both breeds, but milk fat increased (MN: +14 and +7%; LC: +10 and +6%; P<0.01) in mid- and late-lactation, respectively. Fat content values reached normal level by Wednesday in MN and Tuesday in LC. In mid- and late lactation, Monday log SCC (5.01 and 4.99) was greater (P<0.05) than weekend values (4.13 and 4.63) in MN ewes, returning to the normal values on Friday. In LC ewes there were no differences in SCC between week days. Compared with estimated values for 14 milkings/week, omitting two milkings weekly in mid-lactation decreased (-17%, P < 0.05) milk yield in MN whereas it was not significant (-2%, P>0.05) in LC. In late lactation, no milk yield decrease was observed in either breed. Results suggest that omitting two milkings weekly could be an interesting management approach to reduce farm labor with no negative effects on milk yield and milk SCC values in dairy sheep. Moreover, losses in milk yield would be reduced if the milking omission was done during late lactation in MN ewes.

Key Words: Milking suppression, Milking frequency, Dairy ewes

W102 Short-term once-daily milking decreases expression of integrins and cell survival factors with no changes in apoptosis in the bovine mammary gland. K. Singh^{*1}, J. Dobson¹, C. Phyn¹, C. Prosser², V. Farr¹, and K. Stelwagen¹, ¹AgResearch Ltd., Ruakura Research Centre, Hamilton, New Zealand, ²Dairy Goat Co-operative (N.Z.) Ltd., Hamilton, New Zealand.

In ruminants, reduced milking frequency results in decreased milk yield, which is associated with a change in mammary epithelial cell

(MEC) integrity. Many cell types require anchorage to the extracellular matrix (ECM) for survival which is mediated via integrin proteins on cell membranes. The aim of this study was to examine the effect of milking frequency on MEC survival factors and the downstream apoptotic signalling events. Non-pregnant multiparous Jersey and Jersey x Friesian dairy cows (n=4) at late-lactation were milked unilaterally, either twice-daily (at 0700 and 1500h; control) or oncedaily (at 0700 h only) for 4 days, after which cows were sacrificed to collect mammary alveolar tissue. Milk yields, adjusted for pre-treatment values, were decreased (P<0.05) by 21% in once-daily compared with twice-daily milked glands. Gene expression was measured by real-time RT-PCR and the β 1-integrin, α 6-integrin, FAK and Bcl-x_{long} mRNA levels were lower (P < 0.001) in alveolar tissue from udders milked once-daily relative to those milked twice-daily by 1.9, 2.6, 2.2 and 2.2-fold, respectively. The pro-apoptotic factor Bax mRNA was also lower (P<0.001) by 2.6-fold in once-daily compared to twice-daily milked samples. The numbers of apoptotic nuclei, detected by in situ end-labelling, were low and the same in both once-daily and twicedaily milked mammary glands. These data are consistent with a loss of attachment of epithelial cells from the ECM and down-regulation of cell survival pathways during once-daily milking, although the execution phase of apoptosis has not occurred.

Key Words: Once-daily milking, Apoptosis, Integrin

W103 The association among dry period length, lactation performance and some physiological measures of Holstein cows during the following lactations. M. S. Gulay^{*1}, M. J. Hayen², K. C. Bachman², and H. H. Head², ¹Akdeniz University, Burdur, Turkey, ²University of Florida, Gainesville.

The objective of the experiment was to evaluate whether dry period (DP) length (60 d vs. 30 d) affected milk production or physiological measures of Holstein cows during the experimental (EL) and subsequent (SL) lactations. Treatments were arranged in a 3x2x2 factorial design that included DP [TRT-I=60 d dry; TRT-II=30 d dry; and TRT-III=30 d dry+estradiol cypionate (ECP)], pre- and postpartum bST (10.2 mg/d), and prepartum anionic or cationic diets. To accelerate mammary involution, ECP (15 mg) was injected (im) at dry off into cows in TRT-III. Data was collected from farm records for all cows and cohorts (TRT-IV). Across all TRT groups [(n=118); TRT-I (n=28), TRT-II (n=28), TRT-III (n=29), and TRT-IV (n=31)] the culling rates (number of culled cows divided by the total number of cows) during the experimental lactation (EL) were 25.9, 29.0, 25.0 and 28.1% (P=0.98), respectively. The pregnancy rates (number of pregnant cows divided by the total number of cows; 74.1, 67.7, 75.0 and 78.1%; P=0.99), overall culling rates (66.7, 58.8, 58.8 and 63.6%; P=0.95) or number of breedings (3.7, 3.4, 2.5 and 3.6; P=0.48) did not differ during SL. Previous lactation and EL Mature Equivalent (ME) milk yields, and percentages of milk fat (MF), milk protein (MP) and somatic cell count score (SCCs) were 9796, 9748, 9513 and 9835 kg (P=0.89) and 9748, 9796, 9513 and 9835 (P=0.89); 3.68, 3.68, 3.65 and 3.65% (P=0.98) and 3.68, 3.65, 3.69 and 3.65% (P=0.99); 2.77, 2.87, 2.83 and 2.81% (P=0.45) and 2.87, 2.83, 2.77 and 2.81 (P=0.43); and 3.85, 3.78, 3.32 and 3.23 (P=0.27) and 3.78, 3.32, 3.23 and 3.85 (P=0.29), respectively. The DP treatment did not affect respective ME milk yields (8973, 9264, 9022 and 9066kg, respectively; P=0.97) or percentages of MF (3.76, 3.80, 3.69 and 3.78%: P=0.83), MP (2.72, 2.65, 2.69, and 2.74%: P=0.58) or SCCs (5.01, 4.86, 3.95 and 4.19; P=0.20) during SL. The results indicated that shortening DP did not have negative effects on the milk production, composition or physiological measures during the EL or SL.

Key Words: Dry period length, Transition period, Subsequent ME yield

W104 Increase in stanniocalcin content in milk of cows at involution. G. Tremblay*¹, L. Delbecchi², G. F. Wagner³, B. G. Talbot², and P. Lacasse², ¹Université de Sherbrooke, Sherbrooke, QC, Canada, ²AAFC-Dairy and Swine R&D Center, Lennoxville, QC, Canada, ³University of Western Ontario, London, ON, Canada.

There are several lines of evidence indicating the existence of local control of mammary gland involution. However, the exact nature of this control has not yet been defined. We have shown that estrogen, while reducing milk production, increases the expression and concentration of stanniocalcin (STC) in the mammary gland. Since this paracrine hormone is an inhibitor of Ca transport in some tissues, we hypothesized that it may be implicated in the involution process. To further investigate this hypothesis, front right and rear left quarters of nine Holstein cows in late lactation, five multiparous and four primiparous, were unmilked for 14 days. Milk production of milked quarters was evaluated daily, while milk composition (percentage of fat, lactose, and protein, and SCC) was determined on d -7, d 1, d 2, d 7, and d 14. On the same days, milk samples were taken manually from forequarters to determine the STC content by RIA and the protease activity by zymography. Blood samples were simultaneously taken, to assay serum STC by RIA. Cessation of milking of the right forequarter and left hindquarter led to their drying off. The milk production of the two other quarters increased gradually (P<0.001) reaching about 132 % of pre-treatment level at d 14. The concentration of fat and protein in milk from milked quarters decreased slightly (P < 0.05) in the first days following the cessation of milking of the other quarters. Zymography analyses showed a constant increase in protease activity from d 2 to d 14, but only in unmilked quarters. Serum STC increased (P < 0.01) by 23 % during drying off. In milk, the STC concentration did not change in milked quarters but increased by 235 % in unmilked quarters (P < 0.001). These data support the idea that STC is implicated in the involution process.

Key Words: Mammary gland, Stanniocalcin, Involution

W105 Effects of weaning age and ambient temperature on sow endocrine status and mammary secretions around weaning. C. Farmer*¹, D. Flint², and C. Knight², ¹Agriculture and Agri-Food Canada, Dairy and Swine R & D Centre, Lennoxville, QC, Canada, ²Hannah Research Institute, Ayr, UK.

The effects of lactation length and ambient temperature on the endocrine status of sows and on indicators of mammary involution at weaning were studied. Twenty-eight first parity Yorkshire x Landrace sows were housed at 21 (CTL) or 29°C (HS) throughout lactation. Within each temperature group, half the sows were randomly assigned to a weaning age of 22 (W22: CTL, n = 7; HS, n = 8) or 44 days (W44: CTL, n = 6; HS, n = 7). Litter size was standardized to 10 or 11 on day 2 and to 9 on day 23 of lactation. Jugular blood samples and milk samples were collected on days -1, 1, 2 and 3 relative to weaning (day 0). Concentrations of prolactin (PRL), IGF-I and lactose were measured in both fluids and concentrations of Na, K and IGFBP-5 were measured in milk. Standard milk composition was also determined. Lactose was lower (P < 0.001) and protein greater (P < 0.05) in all milk samples from W44 compared to W22 sows. Percentages of milk fat and dry matter were lower (P < 0.05) in W44 than in W22 on days 2 and 3. Milk lactose was reduced (P < 0.05) with heat stress on days

2 and 3. The day before weaning, concentrations of PRL in blood (P < 0.001) and lactoserum (P < 0.01) were lower and those of IGF-I in lactoserum were greater (P = 0.01) in W44 than in W22. Values of IGFBP-5 in milk were drastically lower (P < 0.001, more than a 4-fold difference), those of Na greater (P < 0.001) and those of K unchanged (P > 0.1) for W44 compared to W22. The change in these variables following weaning differed according to lactation length (P < 0.05). On day 2, the increase in Na seen with weaning at 44 d was greater at 29 than at 21°C (P = 0.05). The greater Na/K ratio in W44 compared to W22 shows that mammary epithelial cells tight junctions become leaky as lactation advances. Weaning age therefore has an impact on milk variables which are indicative of the status of mammary involution in sows.

Key Words: Sows, Mammary involution, Lactation length

W106 Effect of bST administration during induction of lactation in 15-month-old heifers on production and health. R. S. Kensinger*, A. L. Magliaro, A. C. W. Kauf, M. L. O'Connor, and L. D. Muller, *Pennsylvania State University, University Park.*

The objective of the present study was to determine if starting bST treatment during induction of lactation would improve milk production above that of heifers induced into lactation but not treated with bST until 54 DIM. Holstein heifers (n = 32; 420 ± 28 kg BW) were randomly assigned to bST-1 or bST-54 treatment groups. They were induced into lactation at 14.9 ± 0.3 mo of age with daily sc injections of estradiol-17B and progesterone (75 and 250 ug/kg BW/d, respectively) on treatment d 1-7. In addition, heifers in the bST-1 group (n=16) received bST on treatment d 1, and this was repeated every 14 d. The remaining 16 heifers (bST-54) received their first bST dose at 54 DIM. Milking began on treatment d 18 (= d 1 of lactation). Milk yield of heifers in bST-1 (15.1 kg/d) was greater than that of bST-54 heifers (11.1 kg/d) from d 1 to 53 of lactation (P < 0.01). Milk protein, fat, and lactose averaged 18.6, 1.7, and 2.0 % on d 1 of milking and 3.9, 4.4, and 5.1 % on d 14. Milk composition was similar between groups. Mean milk production from d 54 to 248 when all heifers received bST was 23.5 and 21.1 kg/d for bST-1 and bST-54 heifers, respectively, and did not differ between groups. Overall, milk yields increased gradually and peaked at $25.6 \pm 5.3 \text{ kg/d}$ at d 149 ± 60 of lactation. Full lactation milk production was 5944 kg with 3.7 % fat, 3.3 % protein, 185,000 SCC, and 297 DIM. Heifers gained 0.87 kg BW/d during the induced lactation. They conceived with an average of 2.0 services/pregnancy, and 28 of 32 calved. Mean age at first calving was 27.6 mo, calving ease score averaged 1.9, and percent mortality of calves born was acceptable. Administration of bST from the start of induction through 53 DIM increased d 1 to 53 milk production compared to heifers induced without bST. Young heifers induced into lactation were healthy, grew normally, and produced reasonable amounts of milk with normal composition.

Key Words: Induced, Lactation, bST

W107 Characterization and regulation of the bovine stearoyl-CoA desaturase (Scd) gene promoter and effects of conjugated linoleic acid (CLA) on mammary cell growth and apoptosis. A. F. Keating^{*1,2}, F. Q. Zhao², and J. J. Kennelly¹, ¹University of Alberta, Edmonton, Alberta, Canada, ²University of Vermont, Burlington.

The bovine Scd gene plays an important role in the bovine mammary gland where stearic and vaccenic acids are converted to oleic acid and conjugated linoleic acid (CLA) respectively. This study investigated

the areas of the bovine promoter of importance in regulating this key enzyme. An area 36bp in length was identified as having a critical role in transcriptional activation and designated the Scd transcriptional enhancer element (STE). Electromobility shift assay showed that Mac-T cell nuclear protein extract forms three binding complexes on this area and mutagenesis of this area identified the binding sites for these proteins as being likely to be RFX1, SREBP and NF-Y/NF-1. The two main biologically active CLA isomers (cis-9, trans-11 and trans-10, cis-12) were shown to down-regulate the Scd gene promoter transcriptional activity significantly (~70%), with this effect occurring at the STE. Increasing doses of both isomers showed that the trans-10, cis-12 isomer had a more potent effect on transcriptional activity even at low doses with reductions of 70-80% at 15mM/ml compared to the cis-9, trans-11 isomer which showed reductions of 40-55% at this concentration. PUFA, vaccenic acid, steric acid, prolactin, dexamethasone and leptin had no effect on transcription. Transcriptional activity was up-regulated by insulin, and down-regulated by oleic acid. Statistical analysis was carried out using one way anova with significance declared at p<0.05. The effects of CLA on bovine mammary cell growth and survival were also evaluated using growth curve and TUNEL assay. Increasing doses of both CLA isomers had negative impacts on cell growth with significant effects seen at concentrations of 30uM and higher (P<0.05) and resulted in increased induction of apoptosis in the mammary cells. In addition, the expression of the GLUT1 glucose transporter protein was investigated using an immunofluoresence technique and demonstrated that GLUT1 expression was also decreased due to high doses of CLA treatment.

Key Words: Conjugated linoleic acid, Stearoyl Co-A desaturase, Bovine mammary epithelial cell

W108 Histologic aspects of gestational mammogenesis in heifers. S. Ellis* and N. Korn, *Clemson University, Clemson, SC.*

Gestational mammogenesis is critical to successful lactation and dairy production. Previous studies have shown that the proliferative cell population in prepubertal heifers consists largely of lightly staining cells distributed throughout the parenchyma. The purpose of this study was to characterize the cell populations in mammary biopsies collected from primigravid heifers to determine whether the lightly staining cell population serves as the primary proliferative population during gestational mammogenesis. Samples of mammary parenchyma from gravid Holstein heifers (n=3) were collected via routine core biopsy at three stages during gestation (d105, d150, and d250) to assess the frequency, distribution, and histologic characteristics of proliferative epithelial cells. Explants were prepared and cultured for 2h with bromodeoxyuridine (BrdU; 5µg/ml in media at 37°C) to label proliferating cells. Explants were then fixed with 4% formaldehyde, embedded in resin, sectioned at 1.5µm and stained to label cells that had incorporated BrdU. Precise quantification of parenchymal cell populations was problematic because the cytoplasmic staining heterogeneity observed was less than anticipated. A limited number of lightly staining cells were observed, but the gradation between intermediate and dark-staining cells was very difficult to reproducibly discern. Furthermore, we observed a surprising percentage of BrdUlabeled cells that had a distinct secretory morphology, complete with basally displaced nuclei, abundant euchromatin, and secretory vesicle inclusions (up to 34% at d250). The vast majority of cells with a secretory morphology and BrdU labeling were either in contact with lumenal spaces or spanned the epithelial layer to contact both the basal and lumenal spaces. These results suggest that the lightly staining cells identified as the primary proliferative population in prepubertal

animals do not constitute the main proliferative cell population during gestation. Further investigations are required to confirm these observations.

Key Words: Mammary epithelial cells, Cell proliferation, Mammogenesis

W109 Lysophosphatidic acid (LPA) stimulates mouse mammary epithelial cell growth. I. S. Yuh*¹ and L. G. Sheffield², ¹Kangwon National University, Chunchon, Korea, ²University of Wisconsin, Madison.

Lysophosphatidic acid (1-acyl-2-hydroxy-sn-glycero-3-phosphate, LPA) is a bioactive phospholipid having diverse effects on various types of tissues. When normal murine mammary gland (NMuMG) cells were cultured in the presence of 0, .01, .1, 1, 10µM LPA, cell numbers were increased with dose dependency for the 6-day culture periods (P<0.05). However, 100µM LPA appeared to be toxic. In DNA synthesis assay, 10µM LPA increased DNA synthesis 4.5 fold over control (P < 0.05). In addition, cell density was increased by LPA. To test the hypothesis that LPA-stimulated cell growth was mediated through its own receptors, NMuMG cells were grown in the presence of 10% FBS, total RNA was extracted, cDNA was synthesized by RT reaction and then LPA subtype receptor gene expressions were amplified by PCR method. NMuMG cells expressed LPA1 and LPA2 receptor genes. LPA (10µM) treatment significantly increased ERK1/ERK2 phosphorylation at 30 min after treatment and then dephosphorylated at 2 hrs after treatment. These results indicate that LPA-induced mammary epithelia growth is likely mediated through MAPK pathway. Interestingly, MAPK activation by LPA was more delayed than by other mitogens such as EGF or ATP (maximum activation at 5 min). Overall results indicate that LPA is mitogenic to normal mammary epithelia and this mitogenic effect is mediated through its own receptors and MAPK activation. It is not clear whether the mitogenic response of LPA was mediated through LPA1 or LPA2 or both type of receptors at this moment.

Key Words: Mammary, LPA, Growth

W110 Changes in mammary gland function during prolonged lactation coincide with changes in mitochondrial biogenic processes. J. George^{*1}, W. Olea¹, D. Torres¹, R. J. Collier², and D. L. Hadsell¹, ¹Baylor College of Medicine, Houston, TX, ²University of Arizona, Tucson.

Biphasic changes occur in mammary function during a lactation cycle. In early lactation, increases occur in secretory and metabolic activity, mitochondrial size, and mitchondrial number. During late lactation, these processes decrease and losses of secretory cells occur. The mechanisms driving this change are not well established. Our previous work demonstrated changes in mitochondrial oxidative damage during the lactation cycle. The hypothesis tested here is that changes in mitochondrial biogenesis and expression of anti-oxidant enzymes underly changes in mammary function that occur throughout the lactation cycle. To test this hypothesis, we measured markers of mitochondrial biogenesis within mouse mammary tissue (n=5 to 8/timepoint) collected at different times during secretory activation (days -1, 1, 2, 3, 4 and 5 postpartum) and during prolonged lactation (days 2, 8, 14, 21, 28, and 35 postpartum). Cytochrome c, COX IV, PGC1 α SOD1, SOD2, and catalase were measured by western blotting. Mitochondrial (mt) DNA copy number was measured by real-time PCR. Mitochondrial number and size were measure by morphometric analysis of electron micrographs of tissue collected on day 8 or 35 postpartum. All of the biochemical markers with the exception of COX IV demonstrated increases (P < 0.05) during early lactation. For cytochrome c, a 3-fold increase was observed between day 2 and 14 postpartum. For catalase, SOD1 and SOD2 a 1.5- to 2-fold increase occurred between days 2 and 8 postpartum. For mtDNA a 50% increase occurred between days 2 and 8. All of these changes were preceded by a dramatic upregulation of the transcriptional coactivator PGC1 α on day 1 postpartum. The prolonged lactation period was marked by decreases in cytochrome c and mtDNA copy number. In addition, a decrease in mitochondrial number approached significance (P=0.11) These results suggest that PGC1 α -dependent changes in mitochondrial biogenesis may underly variations in mammary gland function during the lactation cycle. This work support by grant # 2RO1 DK52197 and cooperative agreement #58-6250-6001.

Key Words: Mitochondria, Lactation, Persistence

W111 Characterization of Madin-Darby Bovine Kidney cell line for PPARs. M. Bionaz^{*1}, C. R. Baumrucker¹, J. P. Vanden Heuvel¹, E. Block², and G. A. Varga¹, ¹*Penn State University, University Park*, ²*Church & Dwight Co. Inc., Princeton, NJ.*

The nuclear receptors of peroxisome proliferator-activated receptors (PPARs) are critical for lipid and glucose metabolism. An in vitro bovine model could be useful for preliminary evaluation of the role of PPARs. The Madin-Darby Bovine Kidney (MDBK) cell line has not been reported to be a PPARs agonist responding cell. To test the responsiveness to PPARs agonists the MDBK cells were treated with 50 μ M of Wy-14643 (specific PPAR α agonist) and 10 μ M of rosiglitazone (specific PPARy agonist). The gene expression of PPARa, PPARy, and reported PPAR responsive genes, CPT-1 (Carnitine Palmitoyl Transferase-1), ACOX (Acyl CoA Oxidase), LPL (Lipoprotein Lipase) and GAPDH (Glyceraldehyde Phosphate Dehydrogenase) were analyzed using semiquantitative and real time PCR. Three replicates of the cultured cells were harvested at 6, 12, 18, 24 and 48 hours. Statistical analysis was performed using the REPEATED statement of the MIXED procedure of SAS. The results of gene expression were normalized using the expression of housekeeping genes. The statistical model included the effect of treatment (Wy-14643, Rosiglitazone, DMSO), time points (0, 6, 12, 18, 24 and 48), replicate (inside treatment), and treatment x time. Significance was set at P < 0.05. Semiquantitave PCR showed expression for all the genes tested, with a higher expression for PPARy compared to PPARα. With real time PCR, CPT-1 mRNA was significantly increase (3 fold) by Wy-14643 treatment at 24 hours and 48 hours. ACOX mRNA expression showed a slight response only at 48 hours (1.6 fold). The LPL gene expression was increased in response to rosiglitazone treatment by 6 hours (28 fold) and highly elevated (460 fold) increase at 24 hours. The results suggest that the MDBK cell line is a promising model to evaluate the role of PPARs for the bovine.

Key Words: PPAR, Agonists, MDBK cells

W112 Treatment of Madin-Darby Bovine Kidney cells with fatty acid PPAR agonists. M. Bionaz^{*1}, E. Shirk¹, J. P. Vanden Heuvel¹, C. R. Baumrucker¹, E. Block², and G. A. Varga¹, ¹Penn State University, University Park, ²Church & Dwight Co. Inc., Princeton, NJ.

The peroxisome proliferator-activated receptors (PPARs) are nuclear receptors activated by specific fatty acids in a dose-dependent manner. Reports describing dose dependent activation and/or expression PPARs

after fatty acid administration have been published for humans and rodents; however, no data are available on the fatty acid dose effect responses in the bovine. We have shown that the MDBK cell line expresses PPAR α and γ . Our objective was to determine if the MDBK cells are models of fatty acid regulation of PPAR activation and/or expression in vitro. Three biological replicates of MDBK cells were treated for 24 hours with 5 different fatty acids diluted in DMSO (C16:0, C18:1, C18:2, C18:3 and conjugated linoleic acid (CLA)) at 5 concentrations (10, 25, 50, 100 and 200 µM) and a vehicle control group. The total RNA was extracted and prepared for real-time PCR. The genes analyzed were two PPAR responsive genes CPT1 (Carnitine Palmitoyl Transferase-1) and LPL (Lipoprotein Lipase) and a housekeeping gene GAPDH (Glyceraldehyde Phosphate Dehydrogenase). The results of gene expression were normalized using the data of the house keeping gene. Statistical analysis was performed using the REPEATED statement of the MIXED procedure of SAS with P < 0.05 set for significance. The CPT-1 expression was significantly increased by palmitate (7 fold increase with 100 µM) with lesser responses seen with linolenic, linoleic, CLA and oleic (2.9, 1.8, 1.6 and 1.5 fold increase with 200 μ M, respectively). The LPL expression was increased significantly only by palmitate (5.4 fold increase with 100 µM). The tested unsaturated fatty acids did not increase LPL mRNA concentration with only linolenate causing an increase in the expression of the gene (1.8 fold increase with $100 \,\mu$ M). The CLA treatment showed a significant decrease in LPL mRNA at 25 µM (2.9 fold decrease). Palmitate was the most effective at affecting PPAR responsive genes in MDBK cells, followed by linolenic and linoleic while oleic and CLA fatty acids were ineffective.

Key Words: PPAR expression, MDBK cells, Fatty acids

W113 Unraveling the requirement of insulin for milk protein synthesis: A microarray perspective. K. K. Menzies^{*1,3}, C. Lefevre^{1,2}, K. L. Macmillan³, and K. R. Nicholas¹, ¹CRC for Innovative Dairy Products, University of Melbourne, Australia, ²Victorian Bioinformatics Consortium, Monash University, Clayton, Australia, ³School of Veterinary Science, University of Melbourne, Werribee, Australia.

The mammary explant culture model has been frequently used to mimic lactation and examine the endocrine control of milk protein gene expression. Murine studies show expression of the milk protein genes requires insulin with prolactin and cortisol. The role of insulin in milk protein synthesis in the dairy cow is not as clear. Culture studies using mammary explants from 4 late pregnant cows showed insulin, in the presence of prolactin and cortisol, is essential for alpha-s1-casein gene expression and plays a post-transcriptional role in synthesis of the casein proteins. To elucidate the molecular mechanisms underlying insulin action in the mammary gland a global analysis of the genes induced in the cultured explants was undertaken using Affymetrix microarray. Mammary explants from 2 groups of cows (2 cows/group) were cultured in media with prolactin and cortisol, either with or without insulin. The data was normalized and gene expression analysed using the limma package of Bioconductor. The expression of 298 genes were significantly down-regulated by insulin and 364 genes (p=0.01), including the major milk protein genes, were significantly up-regulated by up to 10-fold, by insulin. Insulin is important for a number of cellular processes essential for functional secretory alveoli. The requirement for insulin in milk protein synthesis is highlighted by its role in inducing the expression of 26 genes known to be involved in protein synthesis such as transcription and translation factors, ribosomal genes and amino acid transporters. A previous study suggests an intense selection pressure for milk yield has altered the regulation of blood glucose homeostasis such that the top quartile of Australian dairy cows (Australian Breeding Value) have unusually low concentrations of circulating insulin. Whether very low circulating insulin levels has consequent effects on the cows' efficiency for milk protein production is not known. However, the identification of key regulatory genes in the insulin-stimulated pathway of milk protein synthesis may be important to develop breeding strategies to improve the dairy cow's potential for milk protein production.

Key Words: Insulin, Milk protein synthesis

W114 Shortening the dry period from 60 to 40 days does not affect colostrum quality but decreases colostrum yield by Holstein cows. D. J. Grusenmeyer*, C. M. Ryan, D. M. Galton, and T. R. Overton, *Cornell University, Ithaca, NY.*

Holstein cows (n=334) at the end of first or greater lactation on three commercial dairy farms were used to determine whether dry period length affects colostrum quantity and quality as assessed by direct measurement of total immunoglobulin G (IgG) concentrations. Cows producing 22 kg/d of milk or more at 60 d before expected calving were assigned randomly to receive either a 60 d (actual mean = 58 d) or 40 d (actual mean = 40 d) dry period. The first milking following calving was weighed and sampled for subsequent analysis for total IgG by Single Radial Immunodiffusion. Total IgG content averaged 77.0 mg/ml and was different across the three farms (84.3, 81.6, 65.0 mg/ml; P < 0.0001), but was not affected by managing for 60 versus 40 d dry (77.6 vs. 76.4; P = 0.69). Using 50 mg/ml of total IgG as an objective threshold concentration for acceptable colostrum quality, 21% of samples had total IgG concentrations below 50 mg/ml; this proportion was not affected by dry period length but varied substantially across farms (range 10 to 36% of cows). Colostrum yield averaged 7.9 kg and varied across farms (6.7, 7.6, 9.4 kg; P < 0.001). Shortening the dry period from 60 to 40 d decreased colostrum yield (8.9 vs. 6.8 kg; P < 0.001); this difference was consistent across farms (treatment by farm, P = 0.92). Using 3.6 kg as an objective threshold for minimum successful production of colostrum, 30% of cows managed for 40 d dry produced less than 3.6 kg of colostrum compared to 13% of cows managed for 60 d dry (P < 0.001). There was no relationship (P >0.05) between colostrum yield and total IgG concentration in this dataset. Overall, results suggest that colostrum yield, but not quality, is decreased by managing cows for 40 versus 60 d dry periods. In addition, there appears to be no relationship of colostrum quantity and quantity in Holstein cows.

Key Words: Dry period, Colostrum, Transition dairy cow

W115 Effect of subclinical mastitis and breed on somatic cell counts and milk constituents and the accuracy of using pooled samples. E. L. Huether*, D. W. Holcombe, and E. R. Kretschmer, *University of Nevada, Reno.*

Accurate mastitis testing programs that allow for early detection are vital to the maintenance of a healthy, economically productive flock. Research examined the accuracy of using the somatic cell counts (SCC) of pooled samples from the right and left udder halves as well as the effect of subclinical mastitis and breed on milk constituents. Seventeen Suffolk and 66 Rambouillet multiparous and primiparous ewes were weaned at 90 ± 7 d (mean \pm SD) postpartum. A milk sample was collected from each udder half and an aliquot from each sample was then pooled. Milk samples were classified according to their SCC values as either normal (< 500,000 cells/ml-1) or as having subclinical

mastitis (\geq 500,000 cells/ml-1) and SCC values were transformed into the natural log (ln). Milk SCC averaged from the right and left udder halves did not differ (P = 0.91) from the pooled values. Fat, protein and lactose percentages were not affected ($P \ge 0.56$) by sampling method. Somatic cell counts (ln) were increased (P < 0.0001) in udder halves with subclinical mastitis when compared with non-mastitic udder halves (8.2 and 4.2 \pm 0.2 ln, respectively; mean \pm SE). Fat percentages tended to be increased (P = 0.09) in udder halves with mastitis than normal udder halves (5.7 and 4.8 ± 0.4 %, respectively). Percentage of protein was greater (P < 0.0001) in mastitic udder halves (6.5 %) than non-mastitic udder halves (5.6 %). Lactose percentages decreased (P < 0.0001) in udder halves with mastitis (3.9 %) versus normal udder halves (4.8%). Somatic cell counts and protein percentages were greater ($P \le 0.03$) for the Rambouillet versus the Suffolk breed (6.0 and $5.0 \pm 0.2 \ln$; 6.4 and $5.5 \pm 0.2 \ln$, respectively). Suffolks displayed greater ($P \le 0.04$) fat (5.7 and 4.8 %, respectively) and lactose (4.6 and 4.1 %, respectively) percentages than Rambouillets. These data indicate that pooled samples can be used to accurately determine SCC and milk constituents in ewe milk. Protein and lactose percentages were affected by mastitis and may be useful indicators of mastitis infection.

Key Words: Ewe, Mastitis, Somatic cell count

W116 Regulation of haptoglobin (Hp) mRNA expression in the bovine mammary gland parenchyma during experimental mastitis. M. A. Thielen¹, M. Mielenz¹, S. Hiss¹, W. Petzl², H. Zerbe², H. J. Schuberth³, H. M. Seyfert⁴, and H. Sauerwein^{*1}, ¹University of Bonn, Bonn, Germany, ²LMU, Munich, Germany, ³TiHo, Hannover, Germany, ⁴FBN, Dummerstorf, Germany.

Expression of Hp mRNA, one major acute phase protein in cattle, was detected in homogenates of the bovine mammary gland. The aim of this study was to localize Hp mRNA expression within the udder at the cellular level and to quantify any difference of the level of this expression caused by two major mastitis pathogens. For this purpose 3 quarters of each of 3 cows were subsequently inoculated with Escherichia coli (E. coli) at 6, 12 and 24 h pre-slaughter, the 4th quarter received saline 24 h pre-slaughter as control. Another 3 cows received Staphylococcus aureus (S. aureus) accordingly. After slaughter, tissue samples of each quarter were collected for analyses by in situ hybridization (ISH) and real-time RT-PCR. ISH allocated Hp mRNA expression to the alveolar epithelium of the mammary gland. In addition, evaluating the number of cells expressing Hp mRNA revealed a significant difference between the 4 quarters treated with E. coli (P<0.05) in contrast to the quarters exposed to S. aureus. A 17-fold rise in the number of epithelial cells expressing Hp in mammary tissues was detected during the course of E. coli infection, whereas Hp mRNA expression in response to S. aureus infection appeared unchanged. This divergence was confirmed by quantitative real-time RT-PCR: in contrast to S. aureus infected animals, Hp mRNA expression differed significantly between the quarters of *E. coli* infected animals (*P*<0.05). In the latter a 176-fold increase was observed from 0 to 24 h. In summary, this is the first study reporting the localization of Hp mRNA in mammary epithelium of cows. The lack of response to S. aureus within the first 24 h after infection is in contrast to E. coli infection and possibly highlights pathogen-species dependent modulation of the host's immune defense in the udder

Key Words: Haptoglobin, Dairy cow, Mastitis

W117 Effect of sampling day and number of lambs raised on somatic cell counts (SCC) and cell populations in ewe milk. E. R. Kretschmer*1, D. W. Holcombe¹, D. Redelman², and D. L. Garner¹, ¹University of Nevada, Reno, ²Sierra Cytometry/UNR Cytometry Center, Reno, NV.

The objective of this research was to examine the effects of number of lambs raised per ewe and day of milk sampling on live, dead and total somatic cell counts (SCC) including the percentages of specific cell types (monocytes, granulocytes, apoptotic cells and large cells) in ewe milk. Each udder half of thirty-two Rambouillet ewes (2 - 5 yr old) was sampled at 24 h after birth (d 0) and at d 10, 20, 30, 40, 50, and 70 \pm 1 d (mean \pm SD) and at d 93 \pm 10 d postpartum. Four ewes raised triplets, 16 ewes raised twins and 12 ewes raised single offspring. Somatic cell numbers in the ewe milk were quantified by flow cytometry and expressed as \log_{10} values. The number of lambs raised increased the number of live cells (P = 0.03) and total cells (P = 0.07); ewes raising triplets had more cells than ewes raising twins or singles. Day of sampling had no affect (P = 0.32) on the number

of dead cells, but did affect (P < 0.05) the number of live and total cells in milk; values decreased at subsequent sampling days after d 0 but increased again at d 93. Difference in large cell percentages were noted (P < 0.04) at d 20, 50 and 70 relative to the number of lambs raised. Total number of granulocytes were greater (P = 0.036) for triplets than for twins or single offspring. Monocyte numbers were lower (P < 0.0001) at d 0 and increased throughout lactation, whereas the total large cell population was greatest (P < 0.0001) early in lactation and continued to decrease throughout lactation. Total granulocytes for all groups were greater at d 0 (P = 0.015) than that of the subsequent sampling days, and increased again at d 93, whereas, the percentage of live granulocytes and apopotic cells increased (P < 0.04) from d 0 to d 93 for all lambing groups. In conclusion, ewes raising triplets had more live and total SCC, likely reflective of an increase in the total granulocytes. Cell populations were either increased or decreased from d 0 to d 90 depending on cell type.

Key Words: Somatic cell count, Mastitis, Sampling day

Nonruminant Nutrition: Dietary Influences on Boars, Sows and Gilt Development

W118 True calcium and phosphorus digestibility and the endogenous calcium and phosphorus outputs associated with soybean meal for multi-parity sows measured by the simple linear regression technique. K. Kuang¹, R. He¹, J. Wang², Y. L. Yin^{3,4}, and M. Z. Fan^{*4}, ¹Huazhong Agricultural University, Wuhan, Hubei Province, China, ²The Chinese National Institute of Animal Sciences, Beijing, China, ³The Chinese Academy of Sciences, Changsha, Hunan Province, China, ⁴University of Guelph, Ontario, Canada.

Six Yorkshire x Landrace dry sows, with an average initial BW 200 kg and 5-7 parity, were housed individually and fed six diets (2 kg/d) according to a 6x6 Latin square design. The diets were soybean meal (SBM)-cornstarch-glucose based and contained six graded levels of Ca (1.58, 1.69, 1.94, 2.30, 2.88 and 3.65 g/kg DMI) and P (0.79, 1.91, 2.91, 4.04, 4.86 and 5.82 g/kg DMI). Chromic oxide (0.35%) was included in the diets as a digestibility marker. Each experimental period lasted 8 d with a 5-d adaptation and 3-d collection of fecal samples. True digestibility of Ca ($27 \pm 5.0\%$) and P ($44.0 \pm 4.5\%$) and the endogenous outputs of Ca (1.19 ± 0.12 g/kg DMI) and P (0.78 ± 0.17 g/kg DMI) associated with the solvent-extracted SBM for the sows were obtained by the simple linear regression analysis technique. In conclusion, Ca associated SBM was poorly digestible in sows. The endogenous fecal Ca output associated with SBM was relatively high in sows.

Key Words: Phosphorus, True digestibility, Sows

W119 Development of procedures to assess the potential for parturient hypocalcemia in sows. C. Darriet and T. D. Crenshaw*, *University of Wisconsin, Madison.*

Parturient hypocalcemia, also known as milk fever, is a common disorder in dairy cows, but occurrence in sows is not known. An increased incidence of unexplained sow mortality near farrowing has triggered questions about potential involvement of hypocalcemic related disorders as contributors to the incidence of mortality. A long-term objective was to develop procedures that could eventually be used to assess the incidence of hypocalcemia in a large population of sows. The current experiment was designed to assess diurnal variation in serum Ca and blood gas responses of sows at farrowing. On gestation day 111, indwelling venous catheters were placed in 15 multiparous or single parity sows fed diets with either minimal (0.75%) or excess (1.50%) Ca for 4 wk prior to farrowing. Five blood samples were collected at 15-min intervals (0, 15, 30, 45, and 60 min) within each of 4 designated times (0700, 1000, 1300, and 1900) within a day on gestation day 113 (G113) and lactation day 1 (L1). On the day of farrowing (L0), 5 blood samples (at 15-min intervals) were collected within each of 2 times (6 and 9 h after birth of the first pig). Blood gas assays (pH, pO₂, pCO₂, base excess and blood Na, K and Ca (ionized) were performed on 1 sample at each designated hour within days G113, L0, and L1. Serum Ca values (mg/dL) of sows fed diets with minimal or excess Ca were not different for any of the daily collections $(9.28 \text{ vs. } 9.48 \pm 0.09, \text{G113}; 9.31 \text{ vs. } 9.83 \pm 0.11, \text{L0}; \text{ and } 9.88 \text{ vs. } 9.43$ \pm 0.06, L1) pooled across hourly times and 15 min interval samples. Within days (G113, L0 and L1) no diurnal pattern was detected (P >0.10). Blood pH and blood gas values were not affected by diet or day of sampling. These results provide evidence that single daily blood samples can be collected from a large population of sows to assess incidence of hypocalcemia relative to other production traits. Diurnal patterns are not a significant source of error in assessment of serum Ca in sows at farrowing.

Key Words: Sows, Hypocalcemia, Mortality

W120 The effect of omega-3 fatty acid addition to sow diets on milk composition. S. A. Meers*, C. R. Dove, and M. J. Azain, *University* of Georgia, Athens.

The objective of this study was to determine the effects of feeding a diet containing n-3 fatty acids during late gestation and/or lactation on sow milk composition. The study was designed as a 2 x 2 factorial arrangement with main effects of feeding n-3 fatty acids in the gestation diet and/or lactation diet. Diets were corn-SBM based diets such that the gestation diet (G) was calculated to contain approximately 3290 kcal ME, 13% CP and, 0.78% lysine, while the lactation diet (L) was calculated to contain 3242 kcal ME, 17.5% CP and, 1.15% lysine. Omega-3 fatty acids, supplemented in the form of an encapsulated product (Fertilium[®], United Feeds, Sheridan, IN), added to the G or