W19 Effect of Iranain Kilka fish meal on performance and some blood metabolites in early lactating dairy cows. A.R. Heravi M*[1], M. Danesh Mesgaran[1], D. Zamiri[2], and F. Eftekhary[1], [1]*Department of Animal Science, Ferdowsi University, Mashhad, Iran,* [2]*Department of Animal Science, Shiraz University, Shiraz, Iran.*

Twelve multiparous Holstein cows at 27 days in milk were used in a randomized design, with repeated measures analysis, of 8 weeks to evaluate the feed intake, milk yield and composition, blood metabolites (glucose, urea N, soluble protein and cholesterol) and progesterone when soybean meal (SBM) was replaced with different levels of Iranian Kilka fish meal, KFM, (a fish sp. located in the Caspian Sea). On a dry matter (DM) basis, the control diet (T1) consisted of alfalfa (25.2%), corn silage (15.2%), ground barley (22%), ground corn (8.4%), soybean meal (7.9%), cottonseed meal (2.5%), cottonseed (4.9%), wheat bran (6.5%), beet pulp (5.9%), urea (0.1%), limestone (0.1%), dicalcium-phosphate (0.3%), salt (0.2%) and a mineral-vitamin complex (0.8%). In T2 and T3, 28.25 and 56.50% of SBM was replaced with KFM. Dry matter intake of the cows fed T1, T2 and T3 was 23.5, 23.8 and 22.3 0.16 kg/d, respectively, and was not affected by diet (P=0.09). Milk yield (38.51, 37.7 and 39.25 0.24 kg/d); milk fat (3.06, 2.64 and 2.53 0.084%); and milk protein (2.757, 2.88 and 2.967 0.21 %) were not significantly influenced by the experimental diets. At 35 d in milk, ovarian cycles were synchronized using the Pre-Synchronization/Ovsynch protocol. Plasma cholesterol and progesterone concentrations were not affected by diets on day of first GnRH (61 d in milk) or PG injection (68 d in milk) in Ovsynch protocol. At 80 DIM, blood was collected from coccygeal vessels at 0, 1.5, 3 and 4.5 hours after the morning feed. Plasma glucose, urea nitrogen and soluble protein were not significantly affected by the diets but plasma glucose and soluble protein varied over time (P<0.01). It may be concluded that the replacing SBM with KFM in the diets designed for early lactating cows did not alter the lactational performances, blood metabolites and progesterone concentration.

Key Words: Fish meal, Ovsynch, Blood metabolites

W20 WITHDRAWN. , .

W21 The relation between milking characteristics and adrenergic receptor mRNA-expression and ligand binding in the mammary gland of dairy cows. T. Inderwies, M. W. Pfaffl, and R. M. Bruckmaier*, *Techn. Univ. Munich-Weihenstephan, Inst. of Physiology.*

Adrenergic receptor stimulation in the bovine mammary gland affects milking characteristics such as milk yield and peak flow rate. The aim of this study was to detect correlations between milkability, receptor binding capacity and receptor expression at the mRNA level. In addition, dose-response relationships of α- and β-adrenergic receptor stimulation were evaluated after application of α- and β-adrenergic agonists, respectively. Density of adrenergic receptor binding sites in the region around the large mammary ducts were investigated as well as adrenergic receptor mRNA expression. Milk flow of one quarter was recorded in 10 cows without or with additional α- and β-adrenergic receptor stimulation in 3 dosages each. After slaughter, mammary tissue was taken from the region around the large mammary ducts in the previously investigated quarters. Protein and RNA were extracted for measuring α_1-, α_2-, and β_2-adrenergic receptor binding sites and mRNA expression levels by real-time RT-PCR. Peak flow rate without additional adrenergic receptor stimulation was negatively correlated ($p<0.05$) with α_2-adrenergic receptor binding (maximal binding capacity B_{max}) and positively correlated with α_2-adrenergic receptor expression at the mRNA level ($p<0.05$). During α-adrenergic receptor stimulation, there was a negative correlation ($p<0.05$) between milkability and α_2-adrenergic receptor mRNA expression, whereas during β-adrenergic receptor stimulation no correlations were detected. Dose-response relationships existed during α-, but not during β-adrenergic receptor stimulation. Significant changes ($p<0.05$) of milk yield and peak flow rate mainly occurred after α-adrenergic receptor stimulation. In conclusion, high mRNA expression or binding levels of adrenergic receptors are not necessarily related to according changes of milk yield and peak flow rate. To influence milking characteristics, individual reactions of the cow on adrenergic stimulation have to be considered.

Key Words: Cow, Mammary gland, Adrenergic receptors

Lactation Biology

W22 Characterization of a 4,600 gene bovine microarray. C.M. Stiening*[1], J. Hoying[1], A. Hoying[1], D. Henderson[1], P. Gentry[1], Y. Kobayashi[2], and R. Collier[1], [1]*Univ. of Arizona,* [2]*Michigan State Univ.*

A cDNA microarray containing approximately 4600 ESTs was created to evaluate differential gene expression in dairy and beef cattle, with attention to mammary, pituitary and gastrointestinal tissues. Of the 4600 sequences printed, 1526 were generated from mammary tissue, with 540 of those ("Lactation" subgroup) from a subtracted cDNA library (lactating minus involuted tissue) and the remaining 986 ("Non-lactation" subgroup) from the reciprocal library (involuted minus lactating tissue). Approximately 1000 non-redundant pituitary sequences were spotted, and the majority of the remaining 2000 sequences represent the complete GI tract from esophagus to colon. The pituitary and digestive tract ESTs came from sequenced cDNA libraries that were virtually subtracted to minimize redundancy. Printing was conducted at the Univ. of Arizona Genomics Research Lab. Each sequence was spotted in triplicate in an environmentally controlled workstation using a 48-pin print head. Spot morphology and hybridization parameters were evaluated using 3 standard tests. First, SybrGreen was used to verify the presence of DNA in each spot. Second, a random Cy3-labeled oligo (9-mer) was used to validate hybridization competency. Lastly, parameters of the hybridization protocol were evaluated using a same-sample test in which half of the sample was labeled with Cy3 and the other half with Cy5. A preliminary study was next analyzed to obtain initial estimates of variance. Two cDNA arrays arranged in an incomplete block design on dye and treatment were analyzed using statistical package "R". Rough estimates of array variance (confounded with dye variance) and average pooled gene variance were calculated, with array variance = 4.1 x 10^{-7}, gene variance = 0.313, and a mean absolute difference between treatment groups of 1.02 (natural log scale). These preliminary results suggest consistency in printing and hybridization techniques and help establish confidence in our ability to produce robust microarray results with minimal extraneous (non-genetic) sources of variation.

Key Words: Microarray, Variance, Bovine

W23 Effects of varying energy intakes on the deposition of type IV collagen (Col IV) and fibronectin (FN) in the mammary tissue of pre-pubertal heifers. J. W. Forrest*[1], R. M. Akers[1], R. E. Pearson[1], E. G. Brown[2], M. J. VandeHaar[2], and M. S. Weber Nielsen[2], [1]*Virginia Tech, Blacksburg, VA ,* [2]*Michigan State University, East Lansing, MI.*

Our objective was to determine the effects of energy intake on the extracellular matrix of mammary parenchyma. At 2 wk of age, Holstein calves were assigned to 1 of 4 treatments (HH, HL, LH, and LL) with 2 levels of energy intake (High or Low) during 2 periods of growth (2 to 8 and 8 to 14 wk of age). At 14 wk, parenchyma at the stromal interface (I), mid-gland (M), and above the cistern (C) were collected from each calf, fixed, and embedded in paraffin, resulting in 30, 21, 24, and 27 samples, respectively, for each treatment. Immunocytochemical staining of sections allowed visualization of Col IV and FN. Images representing 4 increasing grades (1,2,3,4) were used to quantify protein intensities. Neither feeding level nor zone affected the frequency or intensity of Col IV staining. Average Col IV staining intensity in the basement membranes (BMs) of terminal ductular units (TDUs) and subtending ducts (SUBs) was 1.5, however, staining was observed more frequently around SUBs (75%) than around TDUs (26%). FN staining intensity adjacent to SUBs was 0.27 ± 0.15 (mean ± SEM, $p<0.1$) and 0.43 ± 0.20 ($p<0.05$) greater for HH+HL vs. LH+LL heifers and HH vs. LL heifers, respectively. FN staining intensity adjacent to TDUs was 0.55 ± 0.17 ($p<0.001$) greater in HH vs. LL heifers, 0.35 ± 0.13 ($p<0.01$) greater in HH+LH vs. HL+LL heifers, and 0.19 ± 0.13 ($p<0.1$) greater in HH+HL vs. LH+LL heifers. In addition, FN staining intensity at TDUs was 0.29

± 0.14 (p<0.05) greater in M+C vs. I zones. Similar feeding level and zone effects on staining intensity were observed for FN that was not adjacent to epithelium (i.e., interlobular). FN staining in BMs was not observed in TDUs and only in 14% of SUBs. A high rate of gain, in particular a continuous high rate, between 2 and 14 wk of age increased FN, but not Col IV, deposition throughout mammary parenchyma.

Key Words: Calves, Mammary, Extracellular matrix

W24 Regional expression of IGF-I and estrogen receptor-alpha within prepubertal bovine mammary parenchyma and fat pad.

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In cattle, prepubertal mammary development is characterized by a period of allometric growth. During this period, ductal epithelium elongate into the mammary fat pad (FP). This growth is orchestrated by signals of both local and systemic origin and likely requires the interaction between the FP and the developing parenchyma. The goal of this study was to determine whether the expression of key regulatory genes, such as IGF-I and estrogen receptor-alpha (αER), vary within each compartment during mammary development. To answer this question, we collected mammary tissues from 200 kg prepubertal Holstein heifers. Total RNA was extracted from these tissues and specific transcripts were quantified by ribonuclease protection assays. Parenchyma was collected from the cisternal region, the region adjacent to the FP boundary, and from a region equidistant to the aforementioned regions (medial). The FP was sampled near the abdominal wall (dorsal region) near the supra mammary lymph node (caudal region) and adjacent to the parenchymal boundary. αER transcript was detected in all samples and there were no differences in expression within the various parenchymal or FP regions. Similarly, expression of the IGF-I gene did not differ across the different FP regions. However, in the parenchyma, expression of this transcript tended to be lower ($P < 0.08$) in the cisternal region than in either the medial region or the region adjacent to the FP boundary. These data demonstrate that αER transcript is expressed uniformly throughout the parenchyma as well as the FP. Likewise, IGF-I expression is uniform throughout the FP. However, IGF-I transcript abundance tends to be greater in parenchymal tissues collected dorsal to the cisternal region.

Key Words: IGF-I, Estrogen receptor-alpha, Mammary development

W25 Expression of translation initiation factors in mammary glands of lactating and dry dairy cows.

C. A. Toerien*, J. P. Cant, and C. K. Stewart, *Univ. of Guelph, ON, Canada.*

Factors regulating processes and machinery involved in milk protein production were investigated using mammary glands from 12 non-pregnant dairy cows in late lactation (>250 DIM; 17 kg milk/d). For 42 d, 6 cows were milked as previously (LACT) while 6 cows were dried off (DRY). Cows were then slaughtered and mammary glands and tissue samples obtained. Quantitative histological analyses were performed on regions of interest (ROI) on micrographs of parenchymal tissue (n=4/group). Numbers of alveoli (15 ROI, magnification 40x) and lobules (9 ROI, magnification 2.5x) were similar (P>0.1) in both groups. Mammary size (P=0.07) and parenchymal weight, cell number, cell size and RNA content (P<0.05) were lower in DRY cows. Levels of main eukaryotic translation initiation factors (eIF), eIF2 and eIF4E, were also lower in DRY cows (75% and 67%; P<0.05). Together with a 44% decrease (P<0.05) in RNA:DNA from that in LACT cows, these results indicate decreased translational capacity in DRY glands. In both groups, a large percentage (48 to 60%) of intracellular eIF4E was bound to the eIF4E sequestering protein, 4EBP1, a complex that renders eIF4E biologically inactive. Active (phosphorylated) ribosomal protein S6 (rpS6) and its kinase, p70 S6K, facilitate synthesis of parts of translational machinery. In DRY cows, phospho-rpS6 and -p70 S6K were respectively maintained at 58% and 65% of that of LACT cows. This indicates a maintenance of cell signals involved in synthesis of translational machinery and mirrors the maintenance of RNA:DNA in DRY glands at 56% of LACT. In conclusion, the more pronounced decrease in expression of eIF2 than eIF4E following involution seems to indicate that eIF2 is most likely responsible for the increased translational capacity in lactation. The significant presence of the eIF4E:4EBP1 complex suggests there is an excess capacity for translation up-regulation in both lactating and dry dairy cows. Withdrawal of lactogenic and mammogenic hormones had an adverse effect on the ability of mammary glands to produce milk protein.

Key Words: Initiation factors, mRNA translation, Mammary involution

W26 Insulin-like growth factor-I (IGF-I) modulates the process of mammary apoptosis after weaning in IGF-I transgenic pigs.

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IGF-I plays a critical role in mammary cell proliferation and apoptosis. Studies in transgenic mice with mammary-specific IGF-I over-expression have reported reduced apoptotic loss of mammary cells in late lactation, with minimal effects in early lactation. The impact of mammary over-expression of IGF-I in pigs has not been previously reported. The objective of the present study was to determine the effect of mammary-specific transgenic over-expression of IGF-I on mammary epithelial cell apoptosis. IGF-I hemizygous transgenic swine over-expressing IGF-I under the direction of the bovine alpha-lactalbumin promoter (IGF) and non-transgenic (CON) gilts had litter size normalized to 10 piglets at farrowing and piglets were allowed to suckle until d 21 postpartum. On d 4 post-weaning (d 25 postpartum), animals underwent a surgical biopsy and blood and milk samples were collected. Serum IGF-I on d 4 post-weaning was not affected by mammary over-expression of IGF-I. IGF-I content in mammary secretions from d 4 post-weaning was approximately 36-fold higher (575 ± 271 μg/L) than CON (16.6 ± 1.2 μg/L) sows (p≤0.001). The predominant IGF binding proteins (IGFBP) in mammary secretions were IGFBP-2 and IGFBP-5, both of which were significantly higher in mammary secretions of IGF vs. CON sows (p≤0.05). The presence of apoptotic cells was determined in mammary tissue by Tunel assay and apoptotic cells were expressed as a percentage of total cell count. Mammary tissue from CON pigs had a significantly (p≤0.05) higher percentage of apoptotic cells than IGF transgenic sows (7.5 ± 1.7 vs. 4.6 ± 1.5 %, respectively) at d 4 post-weaning. Thus, over-expression of IGF-I results in increased IGFBP-2 and IGFBP-5 in mammary secretions during involution. However, programmed cell death in mammary tissue is lower in IGF transgenic sows, which could potentially prolong the process of involution. (Funded by the USDA CSREES under project NRICGP 00-35206.)

Key Words: IGF-I, Mammary gland, Apoptosis

W27 Changes of steroid hormone receptor expression and localization in the bovine mammary gland during different functional stages.

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Lactation can be induced in non-pregnant animals by steroid hormone treatment. These effects may be transmitted by estrogen receptors (ERa and ERb) or progesterone receptors (PR). Our aim was to study the expression of ERa, ERb and PR mRNA and protein during mammogenesis, lactogenesis, galactopoiesis (early, middle and late) and involution in the bovine mammary gland (total n=53 cows). The mRNA was assessed by means of real time RT-PCR (LightCycler) and the protein by immunohistochemistry and Western blotting. Both ERa and PR are expressed in fg/g total RNA range. Highest mRNA expression was found for ERa (285 fg/g) and PR (63 fg/g) in tissue of non-pregnant heifers followed by a significant decrease at lactogenesis (17 and 7 fg/g) with remaining low concentrations during lactation and the first 4 wk of involution. In contrast, expression of ERb was in the at/g total RNA range. Immunolocalization for ERa revealed a strong positive staining in nuclei of lactocytes in non-pregnant heifers, became undetectable during pregnancy, lactogenesis, lactation, and was again detectable 14-28 d after milking was stopped. In contrast, the PR is localized in nuclei or cytoplasm of mammary epithelial cells during all stages examined. The ERa, ERb and PR protein was found in all mammary gland stages examined by Western blotting. The signal for ERa is less abundant in tissue of heifers and at involution (4 wk). The ERb protein showed increased abundance (two isoform bands) in heifers and at 4 wk after milking was stopped. For the PR, 3 obvious isoforms (A, B and C) were found. But only the isoform B remains during the stages of lactogenesis,

galactopoiesis and involution. In conclusion, the data for ER and PR show clear regulatory changes suggesting involvement of these receptors in bovine mammary gland function.

Key Words: Steroid receptors, Mammary gland, Bovine

W28 Ontogenetic regulation of progesterone receptor (PR) expression in bovine mammary gland. E. E. Connor*, A. V. Capuco, D. L. Wood, T. S. Sonstegard, and A. F. Mota, *USDA-ARS, BARC, Beltsville, MD*.

The expression patterns of progesterone receptor (PR) mRNA and protein in the bovine mammary gland were characterized during various stages of mammary development and pregnancy. Mammary parenchyma was obtained from prepubertal heifers, pregnant heifers, non-lactating pregnant cows, lactating pregnant cows and lactating non-pregnant cows (n = 3 animals/stage). Samples were evaluated for PR mRNA by quantitative real-time RT-PCR and PR protein by western blotting and immunohistochemistry. Results indicated mean PR mRNA abundance was greatest in prepubertal heifers and lactating pregnant cows, but extremely low to non-detectable throughout most of gestation in heifers. Compared to prepubertal heifers, mean expression of PR mRNA was 6-10 times lower in non-lactating pregnant and lactating non-pregnant cows, although expression among non-lactating pregnant cows was highly variable. A similar pattern of expression was reflected in analyses of PR protein. Preliminary results of western blot analysis suggested the presence of two isoforms of PR of approximately 78 and 135 kDa, presumably representing PR-A and PR-B, respectively. Quantities of the PR-A and PR-B isoforms differed by physiological state. Our results demonstrate that PR expression in the bovine mammary gland is developmentally and hormonally regulated.

Key Words: Progesterone receptor, Mammary gland, Bovine

W29 Mammary mRNA expression of bovine haptoglobin and LPS-induced alterations. S. Hiss*[1], M. Mielenz[1], S. Schmitz[2], R. M. Bruckmaier[2], and H. Sauerwein[1], [1]*Institute of Physiology, Biochemistry and Animal Hygiene, Bonn University, Germany,* [2]*Institute of Physiology, Techn. Univ. Munich, Germany*.

Haptoglobin (Hp), an acute phase protein secreted from the liver, is discussed as a useful marker for animal health. Compared to non-ruminant species, Hp concentrations in blood are physiologically low in cattle, but the increase during inflammatory processes is more pronounced. Elevated Hp concentrations have also been reported in milk during mastitis. Our previous work using a highly sensitive Hp ELISA to characterize Hp concentrations in milk after intramammary lipopolysaccharide (LPS) challenge indicated that Hp in milk might be derived from local mammary sources. Here we demonstrate the presence of Hp mRNA in bovine mammary gland RNA extracts from the parenchymal region, from tissue around cisternal milk ducts and also from teat tissue by RT-PCR. Hp mRNA expression was then quantitatively evaluated by real-time RT-PCR during the first 12 h of LPS-induced mastitis. Results were normalized with ubiquitin mRNA expression. Six healthy lactating cows were injected in one quarter with 0.1 mg E. coli LPS (O26:B6). The contra-lateral quarter was injected with saline and served as control. Biopsies were collected from the treated and the control quarter before and 3, 6, 9 and 12 h after LPS challenge. Following LPS injections, higher Hp mRNA concentrations were observed in the treated vs control quarters at 3, 6 and 9 h ($p<0.05$). Compared to baseline values, Hp mRNA expression was increased at all times recorded after LPS challenge in treated quarters, with a peak at 9 h ($p<0.05$). In the control quarters, a less pronounced increase was observed ($p<0.05$) and might be attributed to the tissue damage induced by the biopsy procedure. In conclusion, the bovine mammary gland has to be considered at least as a partial source of milk Hp. The LPS induced increase of Hp mRNA supports a very close link between mastitis and Hp synthesis in mammary tissue and into milk and therefore emphasizes the diagnostic significance of this parameter.

Key Words: Haptoglobin, Mammary gland, Bovine

W30 mRNA expression of apoptosis-related genes in mammary tissue and milk cells in response to LPS treatment and during subclinical mastitis. A. Didier and R. M. Bruckmaier*, *Institute of Physiology, Technical University of Munich, Germany*.

Development of clinical or subclinical mastitis due to immunocompromised mammary gland physiology leads to additional costs and economical loss in the dairy industry. Our objective was to determine if induction of apoptosis in immune cells and udder tissue may contribute to impairment of immune response in the gland. In two experiments, mammary gland biopsies, udder tissue and somatic milk cells were investigated for alterations in mRNA expression of apoptosis-related genes (Caspase-3, Caspase-7 and FAS) by using real-time RT-PCR. Experiment I (6 cows) was performed on mammary gland biopsies after intramammary LPS infusion to mimic mastitis. All factors under study showed a significant increase in mRNA expression during the sampling period of 12 h in comparison to untreated control quarters ($P<0.0001$ for all genes after 3 and 6 h). FAS expression reached highest levels after 3 h of LPS infusion. Experiment II included a total of 15 cows. All control animals (n = 8) had a somatic cell count <150,000 cells/ml. Another 7 cows had partially elevated SCC with at least one quarter >150,000 cells/ml. At slaughter, milk cells and udder tissue were sampled and subjected to real-time RT-PCR. For milk cells, no significant differences in mRNA expression could be found comparing control cows with those having partially elevated SCC. In udder tissue, FAS and Caspase-3 expression was significantly higher in quarters with elevated SCC as compared to controls ($P\leq0.03$ for FAS and $P\leq0.01$ for Caspase-3). In summary, apoptosis-related gene expression is altered and may be an important factor in mammary gland immune defense under various in-vivo conditions. Increased expression of apoptosis-related genes may therefore be a factor leading to impairment of udder health.

Key Words: Apoptosis, Mammary gland immunology, Real-time RT-PCR

W31 Gene expression profiles in porcine mammary gland tissue during formation of colostrum. P. M. Schnulle and W. L. Hurley*, *University of Illinois, Urbana*.

Formation of colostrum is important for the newborn mammal. The goal of the project was to profile expression patterns of genes thought to be involved in colostrum formation in porcine mammary tissue during the peripartum period. Mammary gland tissue was collected by punch biopsy from 6 sows between 2 and 6 days prepartum, within 24 hours of parturition, and on days 3 and 6 postpartum. Total RNA was extracted, reverse transcribed, and polymerase chain reactions with primers specific for the cDNAs of interest were performed under optimized conditions. Densitometry of PCR products was standardized against 18s rRNA expression. The expression level of the neonatal Fc-gamma receptor (FcRn) was 3X higher at 2 to 3 days prepartum compared to the day of parturition ($P < 0.05$), but was not significantly different on other days. Beta2-Microglobulin is a subunit of FcRn and affects functionality of FcRn protein. Beta2-microglobulin expression increased by over 5X from lowest levels at 4 to 6 days prepartum to a peak at the day of parturition and day 3 of lactation ($P < 0.05$). Annexin II heterotetramer has low affinity binding to IgG. Annexin II light chain is necessary for functional expression of the heterotetramer. Annexin II light chain expression was 70% higher during the prepartum period and on the day of parturition than in the postpartum period ($P < 0.05$). Alpha-Lactalbumin expression, used as a marker of lactogenesis, was increased by 2.5X on the day of farrowing when compared to the prepartum period ($P < 0.05$). Polymeric immunoglobulin receptor expression also was increased on the day of farrowing (by 10X; $P < 0.001$), and was positively correlated with alpha-lactalbumin expression ($P < 0.001$). Results indicate that expression of beta2-microglobulin may affect the functional role of the FcRn expressed during colostrum formation. Furthermore, expression of annexin II light chain is associated with colostrum formation in the porcine mammary gland. Multiple IgG binding proteins may have a role in transepithelial immunoglobulin transport during colostrum formation.

Key Words: Colostrum, Immunoglobulin transport, Mammary gland

W32 Tight junction (TJ) protein expression during engorgement of rat and bovine mammary glands. C. V. Cooper*[1,2,3], K. Stelwagen[2], C. D. McMahon[2], K. Singh[2], V. C. Farr[2], and S. R. Davis[2], [1]*Dexcel Ltd., Hamilton, New Zealand,* [2]*AgResearch, Hamilton, New Zealand,* [3]*Massey University, Palmerston North, New Zealand.*

The pattern of expression of TJ proteins was investigated during engorgement of rat and bovine mammary glands. An increase in mammary TJ permeability was previously shown to occur within 24 h of milk accumulation. The expression of occludin and claudin-1, the major integral transmembrane components of TJ, was determined in two experiments. In experiment 1, Sprague-Dawley rats at peak lactation (d 16) had three abdominal inguinal glands on one side sealed to induce mammary engorgement, the remaining glands were not sealed and acted as suckled controls. Mammary tissue was collected post-mortem at 0, 6, 12, 18, 24 and 36 h after teat sealing (n = 6 rats per time point). In experiment 2, alveolar mammary tissue was collected post-mortem from 42 mid-lactation Holstein Friesian dairy cows at 0, 6, 12, 18, 24, 36 and 72 h following the last milking (n = 6 cows per time point). Immunoblotting showed a characteristic multiple banding pattern for occludin between 60 and 80 kDa. The higher molecular weight (MW) bands were highly phosphorylated and resistant to NP-40 detergent extraction, suggesting they predominantly derive from the tight junction complex. Occludin expression declined during mammary engorgement in rat and bovine glands ($P<0.05$). Claudin-1 migrated in SDS-PAGE as two bands at 22 and 28 kDa. In rats, expression of the 28 kDa band declined within 12 h of mammary engorgement ($P<0.05$), while that of the 22 kDa band, along with lower MW degradation products, increased ($P<0.05$). Both bands were expressed at low levels by 36 h of mammary engorgement. In contrast, claudin-1 protein expression did not alter with engorgement in bovine mammary glands ($P>0.05$). Occludin and claudin-1 expression showed large individual animal to animal variation. Furthermore, the response to mammary engorgement was locally regulated as no changes were detected in suckled control rat mammary glands. Between species variation in the pattern of TJ protein expression suggest that the increase in TJ permeability during milk accumulation is regulated differently between rats and dairy cows.

Key Words: Tight junction, Lactation, Mammary engorgement

W33 Developmental regulation of glucosidase II in mouse mammary gland. J. Feng* and I. K. Vijay, *University of Maryland, College Park.*

The mammary gland synthesizes and secretes large amounts of well-characterized glycoproteins of the milk fat globule membrane and α-lactalbumin during lactation. Previous studies from our laboratory have shown that several glycosyltransferases of the dolichol cycle are coordinately regulated during the growth and differentiation of the mammary gland as it cycles between dormancy and lactation. We have hypothesized that the processing glucosidases I and II would follow a similar pattern of expression in coordination with the glycosyltransferases. The developmental regulation of glucosidase II was investigated in mouse mammary gland. Glucosidase II is a heterodimer of a catalytically active subunit (α subunit) and a smaller subunit (β) that contains the signal for endoplastic reticulum (ER) retention. Mouse mammary glands at different stages of development (n=30 for virgin and post lactating glands; 20 for all the other stages) were examined for glucosidase II mRNA by RT-PCR (both α and β subunits), immunoreactive α and β subunits, and enzyme activity. All three parameters showed a similar pattern, i.e., they were low in tissues from virgin animals, increased steadily during pregnancy and lactation, reaching a peak around mid-lactation, and declined sharply in glands from post-lactating animals. At mid-lactation, glucosidase II α and β subunits mRNA level increased 4-fold relative to the virgin stage. The immunoreactive protein of the two subunits also had 5 and 7-fold increases, respectively. The glucosidase II activity increased nearly 5-fold in mid-lactation compared to virgin stage. These data suggest possible transcriptional and post-transcriptional modulation of glucosidase II during development of the mouse mammary gland. Further, the striking similarity in the regulation of this enzyme and the previously studied glycosyltransferases, when combined with the data on the developmental profile of glucosidase I, indicates that common regulatory signaling cascades may control the enzymes of the glycosylation machinery in the mammary gland. (Supported by N.I.H. grant GM59943.)

Key Words: Glycosylation, Glucosidase II, Mammary gland

Growth & Development

W34 Impact of 5α-dihydrotestosterone on musculoskeletal status of mature laying hens. T. D. Faidley*, S. E. Nicolich, and D. R. Thompson, *Merck Research Laboratories, Somerville, NJ.*

Genetic selection for improved egg production has resulted in aged laying hens that are fragile and depleted of muscle. Selective androgen receptor modulation may offer potential to improve musculature and skeletal structure of these birds. "Spent hens" have become more of a liability than an asset to the industry. We hypothesized that compounds such as 5α- dihydrotestosterone (DHT) may result in muscle and bone gain, thus improving the health and value of aged layers. Subcutaneous injections of 3 mg/kg DHT (5X weekly) were compared to saline injections in mature laying hens (n=10). Hens were housed individually in cages and allowed unlimited access to feed and water. After 3 weeks, DHT treatment decreased ($P<0.05$) egg production (0% vs. 60%), feed consumption (72 g/d vs. 126 g/d), weight gain (-13 g vs. 58 g), and breast fillet as a % of carcass weight (7.2% vs. 8.1%). DHT treatment increased ($P<0.05$) comb redness (a*, 20 vs. 13); and weights of comb (31.2 g vs. 2.2 g), heart (10.6 g vs. 8.1 g), thigh muscle (72.9 g vs. 64.9 g), and metatarsus (23.1 g vs. 21.6 g). DHT treatment had no significant effect on weight of carcass (1337 g vs. 1227 g), whole breast (302 vs. 325), or femur (9.8 g vs. 9.3 g). Breast fillet weight tended to decrease ($P<0.1$) with DHT treatment (97 g vs. 103 g). In summary, DHT treatment was successful in halting egg production and in decreasing feed consumption, however, musculoskeletal effects were inconclusive. Further research is needed to determine if anabolic treatment of aged laying hens can improve welfare and/or economics of egg production.

Key Words: Androgen, Anabolic, Laying hens

W35 Fetus growth at day 78 of gestation in nutrient restricted ewes. M. M. Schwope*, W. J. Means, A. W. Wolf, B. W. Hess, and S. P. Ford, *University of Wyoming, Laramie WY/USA.*

ABSTRACT: Under-nutrition during early gestation can affect muscle development. Our purpose was to determine if fetal growth was affected by nutrient restriction of the gestating ewe. Control (C) ewes were fed 100% of the National Research Council (NRC) recommended diet for gestating ewes. Nutrient restricted (NR) ewes were fed 50% of NRC recommendations during days 28 to 78 of gestation. Control and NR ewes were euthanized (d 78 gestation) prior to removal of gravid uteri. The head and internal organs were removed after the fetus(s) were taken from the uterus. Eviscerated ewes and fetuses were hung by the *Achilles* tendon for 24 to 34 h at 4°C or 15°C, respectively. Subsequently, ewe and fetus *Longissimus dorsi* (Ld) and *Semitendinosus* (St) were removed. Whole body, eviscerated body, Ld, and St weights were recorded. Whole body weight tended ($P = 0.07$)to be lower in NR ewes, although ewe eviscerated weight was not different($P = 0.13$). Fetal whole body ($P = 0.49$) and eviscerated weights ($P = 0.58$) were not different. However, fetal Ld weight as percentage of fetal whole body weight and as percentage of eviscerated fetal weight were different because Ld weights of NR fetuses tended to be heavier ($P = 0.10$) than C fetuses, 3.34 and 2.92 g, respectively. This relationship was not found for fetal St ($P = 0.51$). Ewe Ld and St weights were not different ($P > 0.10$) as percentage of ewe whole body and eviscerated weight. Nutrient restriction of ewes during 28 to 78 d of gestation causes differential changes in muscle development.

Key Words: Fetus, Nutrient restriction, Muscle growth