ABSTRACTS
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*** Author Presenting Paper**

Physiology: Metabolism, growth, and stress

W1 Identification and initial characterization of the adipocyte hormone adiponectin in Holstein bull calves. R. C. Cheatham*[1], P. C. Gentry[1], G. C. Duff[1], and R. J. Collier[1], [1]*University of Arizona*.

The importance of adipose as a secretory organ has become apparent in recent years. Adipose tissue plays an important regulatory role in energy metabolism and nutrient partitioning. Adiponectin, a hormone exclusively secreted by differentiated adipocytes, promotes fatty acid utilization by liver and skeletal muscle and reduces plasma glucose without influencing insulin or glucagon concentrations in mice. To date, adiponectin has only been identified in humans and rodents. The objective of our study was to determine if adiponectin is expressed in bovine adipose and to characterize its expression in growing calves. An 880 bp adiponectin cDNA was created by RT-PCR of bovine adipose total cellular RNA and verified by sequence analysis. The resulting PCR product indicated 93% and 95% sequence identity with mouse and human adiponectin, respectively. Adiponectin expression was characterized in abdominal adipose tissue collected from Holstein bull calves sacrificed at 4 d (n=3), 4 wk (n=6) and 12 wk (n=5) of age. All calves were fed colostrum for at least three feedings, then fed a commercial milk replacer. Beginning on d 12, calves were offered a corn-based starter feed free choice. At slaughter, tissues were collected and snap frozen in liquid nitrogen, then stored at -80C until RNA was isolated. Adiponectin mRNA was detected on all days, indicating a likely role for this hormone in both preruminant and ruminant animals. As in other species, adiponectin appears to be adipose-specific. Adiponectin was not detected in muscle, liver or pooled RNA representing all regions of the bovine digestive tract. Amplification of the housekeeping gene G3PDH positive control verified integrity of the template and PCR reaction. Experiments to assess adiponectin expression in adult animals under different dietary conditions are underway, as is further work characterizing adiponectin in growing calves.

Key Words: Adiponectin, Adipose, Calves

W2 Effect of physiological state and somatotropin on the response to lipolytic and antilipolytic signalling in ovine adipose tissue. M. H. Carvalho, E. F. Delgado, D.P.D. Lanna, R. Machado Neto, and I. Susin, *Universidade de Sao, Piracicaba SP/Brazil*.

Bovine somatotropin (bST) treatment in vivo alters adipose tissue metabolism by enhancing lipolytic response to adrenergic agonists. We examined the impact of bST and lactation on basal and stimulated lipolytic rates with isoproterenol (ISO; 10^{-5}nM), adenosine deaminase (ADA; 0.75 U/mL), ISO plus ADA in short-term (2h) incubations of ovine adipose tissue. The anti-lipolytic effect of phenylisopropyladenosine (PIA; non-hydrolyzable adenosine analog) was evaluated at various concentrations (0.5, 1.5, 3, 100 nM). Sixteen lactating Santa Ines ewes were randomly assigned to two groups. They received two s.c. injections, with a 14 day interval, starting at d 13 postpartum with either bST (160 mg) or Vitamin E (control). Eight similar nonlactating ewes received vitamin E. Omental adipose tissue biopsies were taken on d 8 after the second bST or vitamin injection. The lipolytic rate was determined by NEFA release in media as μEq of oleic acid.$2h^{-1}g^{-1}$ tissue. Basal lipolytic rates did not change with lactation or with bST treatment in vivo (P>0,05). ISO stimulated lipolytic rate increased compared to basal and was higher for the adipose tissue from lactating ewes treated with bST (P<0,05). The lipolytic rate for adipose incubated with ADA was higher than basal for lactating ewes, with the greater response for the control. Maximum lipolytic rate with ISO+ADA was also higher for lactating ewes treated with bST (P<0.01), and there was no difference between lactating and nonlactating ewes. The PIA effects were evaluated by the inhibition of ISO+ADA lipolysis, and adipose tissue from lactating ewes treated with bST showed a reduced response to PIA. The results demonstrate that in vivo somatotropin treatment increases maximal lipolytic rates and decreases the antilipolytic effect of PIA in omental adipose tissue in ewes.

Key Words: Somatotropin, Adenosine, Lipolysis

W3 Feeding Holstein cows anionic and cationic diets prepartum coupled with short dry periods and bST. M. S. Gulay*, M. J. Hayen, and H. H. Head, *University of Florida, Department of Animal Sciences*.

Eighty-four Holstein cows were used to evaluate effects of prepartum anionic (-10 to -15 mEq/100g DM) or cationic (+20 mEq/100g DM) diets with low K (1.14 % of DM) on prepartum and postpartum DMI, BW and BCS, and subsequent MY. Treatments were in a 3x2x2 factorial arrangement that included dry period (30 d dry, 30 d dry+ECP, and 60 d dry), diet, and prepartum and postpartum bST (POSILAC®,10.2 mg/d). No interactions of bST or dry period length with prepartum diet were detected for any measure. No significant effects of prepartum diet on prepartum DMI, BW or BCS were observed. During the postpartum period (wk 1 through 14), no differences in mean BW or BCS were detected between prepartum diets fed. Decreases in BW and BCS were seen during the first 6 wk postpartum. Mean DMI during the first 28 d postpartum were similar for cows fed anionic and cationic diets prepartum (25.5 kg/d and 26.1 kg/d, respectively). No differences due to prepartum diet were observed for mean milk or 3.5 % FCM yields or for milk composition during the first 10 wk of lactation. Similarly, mean MY of cows during the first 21 wk did not differ significantly due to prepartum diet fed (anionic diet=38.6 kg/d vs cationic diet=38.5 kg/d). Feeding the anionic diet did not significantly improve either prepartum or postpartum concentrations of Ca. Cows fed the prepartum anionic and cationic diets had similar mean serum concentrations of Ca (9.35 mg/dL vs. 9.34 mg/dL), and only 8 cows fed each diet had serum concentrations of Ca less than 7 mg/dL the day following calving. No cases of clinical hypocalcemia were observed irrespective of diet fed. In conclusion, it appears that cationic diet with low K during prepartum period did not cause detrimental effects on DMI, BW or BCS changes, MY or health problems before or after calving.

Key Words: Anionic-cationic, Milk yield, Transition period

W4 Milk production of dairy cows injected with low dose of bovine somatotropin (bST) during the transition period and lactation. M. Liboni*, M. S. Gulay, T. I. Belloso, M. J. Hayen, and H. H. Head, *Department of Animal Sciences - University of Florida*.

Objective was to evaluate effects of injecting a low dose of bST (0.4 mL, 10.2 mg/d, POSILAC®) during prepartum and/or postpartum periods on milk yield (MY) and composition, BW and BCS. Multiparous Holstein cows were assigned randomly to a 2x2 factorial arrangement of treatments (TRT) to give four groups (1=no bST, n=26; 2= bST postpartum, n=25; 3=bST prepartum, n=27; 4=bST prepartum and postpartum, n=25). Bi-weekly injections of bST were in left or right ischiorectal fossa and began 3 wk before expected calving date and continued through 70 DIM; beyond 70 DIM all cows were injected biweekly with POSILAC®(500 mg/14 d). Significant effects of bST ($P<0.0579$) were detected on mean daily MY through 70 DIM; means for the four TRT groups were 33.93, 36.48, 37.76 and 40.33 kg/d, respectively; all TRT means differed ($P\leq0.063$) except for 2 vs. 3. No effects of calving season (SEA) were detected on MY (P=0.6656), nor TRT or SEA effects on BW (P=0.2817 and P=0.4297) or BCS (P=0.4315 and P=0.5158). Mean BW and BCS for the four TRT groups were 659.1 and 3.14, 659.6 and 3.00, 659.5 and 3.10, 680.4 and 3.16, respectively. No effects of bST were detected on percentages of milk fat (P=0.8825) or protein (P=0.5336); mean percentages for TRT groups during first 70 DIM were 3.82 and 2.99, 3.78 and 2.95, 3.85 and 2.88, 3.72 and 2.91, respectively. No significant effects of TRT were detected on somatic cell count (SCC, P=0.5333); TRT means were 540, 608, 326 and 576 x 103 cells/mL milk. During 70-150 DIM, when all cows were injected with full dose of POSILAC®, increases in MY still were detected but magnitude of effects were reduced to about one-half of previous differences. Means for the four TRT groups were 36.97, 38.08, 39.61 and 40.63 kg/d, respectively. We concluded that injecting bST during prepartum and/or postpartum periods increased MY without adverse effects on milk composition or on health and differences were not lost completely during later lactation.

Key Words: Milk Yield, Dairy cow transition period, bST

W5 Use of bST in transition diary cows: Effects on dry matter intake, body weight, BCS and milk yields. M. S. Gulay*, M. J. Hayen, T. I. Belloso, M. Liboni, and H. H. Head, *University of Florida*.

Objective was to determine whether injections of bST during transition period improved DMI, BW, BCS and MY. Eighty four multiparous Holstein cows were assigned to a 3x2x2 factorial design that included prepartum and postpartum bST, dry period (30 d dry, 30 d dry+ECP, and 60 d dry), and prepartum anionic or cationic diet. Biweekly injections of bST began ±21 d (±3 d) before expected calving date and through 42 d (±2 d) postpartum (C, n=42 vs I, n=42; 0 vs 10.2 mg bST/d, POSILAC®). At 56 d (±2 d), cows in both groups were injected with 500 mg bST/14 d. No interactions of dry period length or prepartum diet with bST treatment were detected for any measure. No significant effects of prepartum diet fed or dry period length were observed for DMI, BW, BCS, or MY. During the prepartum period no differences were detected between treatment groups for mean BW (C=688 vs I=682 kg) or BCS (C=3.38 vs I=3.42). Birth weights of calves did not differ between groups (C=38.3 vs I=36.5 kg). Mean BW (C=688 vs I=682 kg) and BCS (C=688 vs I=682 kg) were not affected by treatment during postpartum period. bST did not affect mean DMI during prepartum (C=16.1 vs I=16.9 kg/d) or first 28 d postpartum (C=25.7 vs I=25.9 kg/d). Mean energy status of cows during the first 4 wk postpartum was negative and did not differ between groups (C=-18.25 vs I=-16.07 Mcal/d). During first 10 wk bST-injected cows had greater mean milk, 3.5 % FCM, and SCM yields (39.6 kg/d, 42.1 kg/d and 40.5 kg/d, respectively) than non-injected cows (36.7 kg/d, 38.9 kg/d and 37.5 kg/d, respectively). No differences were observed in percentages of protein (2.86 vs 2.87%) or fat (3.93% vs 3.96 %) due to bST, but non-injected cows had greater SCC than bST- injected during the first 10 wk of lactation (527 vs 323x103). When both injected and non-injected cows received a full dose of bST at d 60 the increase in milk production was maintained better through 21 wk in the bST-injected cows (C=37.5 vs I=40.5 kg/d; $P<0.03$). No prepartum or postpartum health problems or apparent calving problems were associated with bST.

Key Words: Transition cows, bST, Milk yield

W6 Effect of low dose of bovine somatotropin (bST) on hormone, IGF-I and metabolite concentrations during the transition period. M. S. Gulay*, M. J. Hayen, and H. H. Head, *University of Florida* .

Experiment was designed to evaluate concentrations of hormones (ST and INS), growth factor (IGF-I), metabolites (glucose and NEFA) and Ca in plasma of 80 Holstein cows injected biweekly with bST during the transition period (C, n=41 vs. I, n=39; 0 vs 10.2 mg bST/d, POSILAC®). Biweekly injections of bST were started prepartum 21 d (±3 d) before expected calving date and through 42 d (±2 d) postpartum. No differences were detected for mean concentrations of glucose (I=70.6 vs C=69.3 mg/dL) or NEFA (I=265.0 vs C=273.5 μEq/L) between bST treatment groups during the overall prepartum period (d -21 to d -1), but ST (I=8.19 vs C=5.51 ng/mL; $P<0.01$), IGF-I (I=318.7 vs C=235.2 ng/mL; $P<0.01$), and INS (I=0.85 vs C=1.06 ng/ml; $P<0.02$) did differ during prepartum period. No differences were detected for mean concentrations of INS (C=0.62 vs I=0.58 ng/mL), glucose (C=61.9 vs I=62.1 mg/dL or NEFA (C=584.3 vs I=634.2 μEq/L) due to bST treatment during the overall postpartum period (d 1 to 28). Cows in non-bST group had lower mean concentrations of ST in plasma (5.52 ng/mL) than cows in bST-injected group (10.33 ng/mL; $P<0.01$). Mean concentrations of IGF-I during the overall postpartum period also were greater for the bST treated group (I=150.2 vs C=117.4 ng/mL; $P<0.01$) and they were maintained greater (+27.9%) than non-bST group throughout the early postpartum period. Concentrations of Ca during the 2 wk before through 2 wk after calving did not differ significantly due to treatment (I=9.28 vs C= 9.41mg/dL). Although serum Ca concentrations were least the day following calving, only 16 of the 80 cows had concentrations of Ca less then 7 mg/dL at that time (I=5 and C=11 cows). Changes in concentrations of metabolic hormones, IGF-I and blood metabolites due to injections of 10.2 mg bST/d during transition period likely improve metabolic status of the cows during early lactation without causing calving or health problems prepartum or postpartum.

Key Words: bST, Hormones, Transition period

W7 Nutritional modulation of hepatic growth hormone responsiveness in late-lactating dairy cows. R. P. Rhoads*[1], L. H. Baumgard[2], M. E. Van Amburgh[1], and Y. R. Boisclair[1], [1]*Cornell University, Ithaca, NY,* [2]*University of Arizona, Tucson, AZ.*

The ability of recombinant bovine somatotropin (rbST) to enhance milk yield is compromised during periods of undernutrition, such as the period immediately following parturition. This has been attributed, in part, to decreased growth hormone (GH) dependent production of IGF-I in liver. Our goal was to develop a chronic animal model to study the basis of this impairment. Six non-pregnant, late-lactating dairy cows were subjected to two 14 d periods when they were offered a high or low plane of nutrition. The high plane of nutrition provided 120% of predicted energy requirements whereas the low plane provided only 33% of maintenance requirements. During each feeding period, excipient or rbST (40 mg IM, daily) was administered in a single reversal design with 4-d periods separated by a 2-d interval. Blood samples and liver biopsies were obtained on the fourth day of excipient or rbST treatment. The shift from the high to the low plane of nutrition resulted in lower plasma IGF-I (157 vs 69 ng/ml, $p<0.01$) even though the plasma concentration of GH increased (5.9 vs 9.8 ng/ml, $p<0.01$). The plasma concentration of insulin was also reduced by underfeeding. More importantly, administration of rbST increased the plasma concentration of IGF-I to a greater extent during the high plane of nutrition (104 vs 210 ng/ml, $p<0.01$) than during the low plane of nutrition (57 vs 81 ng/ml, $p<0.01$), suggesting impaired hepatic GH responsiveness. Surprisingly, rbST caused similar increases in IGF-I gene expression in liver at both feeding levels. We conclude that in late-lactating dairy cows, a severe nutritional insult is not sufficient to completely block GH-dependent IGF-I synthesis.

Key Words: GH resistance, Liver, IGF-I

W8 Mammary gene expression analysis in peripartal dairy cows using a bovine cDNA microarray. J. J. Loor*, J. K. Drackley, H. M. Dann, R. E. Everts, S. L. Rodriguez-Zas, and H. A. Lewin, *University of Illinois, Urbana, IL.*

We used cDNA microarray technology to study mammary gene expression in dairy cows. Mammary tissue was collected by percutaneous biopsy at -14, +1, and +14 d relative to parturition from 2 multiparous Holstein cows fed according to current NRC recommendations throughout the dry period and the first 49 d postpartum. A microarray consisting of 7,872 cDNA inserts was constructed from a collection of clones selected from placenta and spleen cDNA libraries. Annotation was based on similarity searches using BLASTN and TBLASTX against the human and mouse UniGene databases. A total of 6,626 sequences (84%) have significant similarity to human or mouse genes and could be assigned as putative orthologs. Gene Ontology terms were annotated to the sequences and putative functions assigned. Cy3- and Cy5-labelled cDNA from tissue and a universal control sample (derived from a mixture of cattle tissues not including liver or mammary tissue) were used for hybridization. Three exogenous plant genes were used as spiking controls for data normalization. A parametric test using the cross-gene error model with log-transformed ratios in GeneSpring was used. Preliminary data analysis demonstrated clear increases over time in the expression (fold-change expressed as tissue/universal control) of genes with known or unknown functions associated with metabolism that accompanies copious milk synthesis. Large fold-changes in mRNA expression were detected between -14 and +14 d for *stearoyl-CoA desaturase*, *xanthine dehydrogenase*, *fatty acid binding proteins-3* and *-5*, *fatty acyl-CoA ligase-2*, *transport proteins* (ABCG2, ABCA1, TAP1), GLUT1, IGFBP3, *Lipin-1*, SPP1, kinases (*Janus*, *pyruvate dehydrogenase-4*, *myosin light-chain*), PPAR-γ, *aminoacyl tRNA synthases*, *leucine aminopeptidase*, and *β-1,4 galactosyl transferase*. Expression of *immunoglobulin lambda* and *kappa* mRNA also was markedly upregulated by d 14 postpartum. Results demonstrate the power of microarrays to study patterns of gene expression in the bovine mammary gland.

Key Words: Microarray, Mammary gland, Dairy cow

W9 Hepatic gene expression analysis in peripartal dairy cows using a bovine cDNA microarray. J. J. Loor*, J. K. Drackley, H. M. Dann, R. E. Everts, S. L. Rodriguez-Zas, and H. A. Lewin, *University of Illinois, Urbana, IL.*

We used cDNA microarray technology to study hepatic gene expression in periparturient dairy cows. Five Holstein cows fed according to current NRC recommendations throughout the prepartum period and the first 49 d postpartum were used. Liver was biopsied at -65, -30, -14, +1, +14, +28, and +49 d relative to calving. A microarray consisting of 7,872 cDNA inserts was constructed from a collection of clones selected from placenta and spleen cDNA libraries. Annotation was based on similarity searches using BLASTN and TBLASTX against the human and mouse UniGene databases. A total of 6,626 sequences (84%) have significant similarity to human or mouse genes and could be assigned as putative orthologs. Gene Ontology terms were annotated to the sequences and putative functions assigned. Cy3- and Cy5-labelled cDNA from tissue and a universal control sample (derived from a mixture of cattle tissues not including liver or mammary tissue) were used for hybridization. Three exogenous plant genes were used as spiking controls for data normalization. A parametric test using the cross-gene error model with log-transformed ratios in GeneSpring was used. Preliminary analysis of data from four cows each at -14, +1, and +14 d showed clear increases over time in the expression (fold-change expressed as tissue/universal control) of genes with known functions associated with various aspects of hepatic metabolism at the onset of lactation. Fold-change for transcripts encoding *fatty acid transporter-2* (SLC27A2), *fatty acyl-CoA ligase-2*, *carnitine-palmitoyl transferase-1* and *-2*, *acyl-CoA oxidase*, and *acetyl-CoA acyltransferase-2* increased over time. Among genes involved in glucose metabolism, the *pyruvate dehydrogenase complex* (PDHB), *PDH kinase-4*, and two *dihydrolipoamide dehydrogenases* also increased over time. mRNA expression by +14 d of genes associated with cholesterol synthesis (*sterol-C4 methyl oxidase*), steroidogenesis (*sterol carrier protein-2*), IGF-1 and -2 metabolism (IGFBP4), and antioxidant activities (*catalase*, *selenoprotein P*) was markedly upregulated. Results demonstrate the power of microarrays to study patterns of gene expression in bovine liver.

Key Words: Microarray, Liver, Dairy cow

W10 Preliminary evaluation of a sustained-release delivery system of porcine (p) somatotropin (ST) in pigs. H. S. Ringrose*[1], K. E. Govoni[1], T. A. Hoagland[1], S. Martinod[2], and S. A. Zinn[1], [1]*University of Connecticut,* [2]*Smart Drug Systems, Inc.*

To begin evaluation of sustained-release of porcine (p) somatotropin (ST) from covered-rod implants (CRI), 3 experiments (Exp.) were conducted. In Exp. 1, 6 formulations of CRI were individually incubated in 0.05 M PBS (3 mL; pH 7.5; 20^oC) for 37 d. PBS was collected at regular intervals and analyzed (Biuret) for protein released into media. One CRI formulation was identified that secreted about 4 mg pST/d for 16 d and was used in Exp. 2 and 3. Yorkshire-crossbred pigs (Exp.2: n=38; Exp.3: n=40) were housed (2 pigs/pen) in a climate-controlled barn and given ad libidum access to a pelleted feed (16% CP, 1% lysine) and water. Pigs were blocked by BW, and within block, assigned to 1 of 4 treatment groups in Exp. 2 (control, implanted at 50 kg, implanted at 75 kg, implanted at 50 and 75 kg BW), and 1 of 3 treatment groups in Exp. 3 (control, first implanted at 50 kg, first implanted at 75 kg BW, and reimplanted every 10 d). Feed intake (FI) was measured daily and BW was measured weekly. Loin eye area (LEA) and back fat (BF) were measured every 4 wk. Blood (20 mL) was obtained by venipuncture of a vena cava weekly and concentrations of ST and IGF-1 were quantified by RIA. In Exp. 2, no difference ($p > 0.10$) was observed between control and treated pigs for ADG, FI, LEA or BF. Following implantation, treatment average concentrations of ST (2.7 ng/mL) and IGF-1 (496 ng/mL) were increased ($p < 0.05$) relative to control (1.5ng/mL, 300ng/mL). However, 14 d after implantation, ST and IGF-1 returned to control values. We concluded that ST was released from CRI and stimulated IGF-1 release, but perhaps the duration of elevated ST was not sufficient to stimulate ADG. To potentially maintain sufficient release of pST, pigs were reimplanted every 10 d in Exp. 3. Average concentrations of IGF-1 (425 vs 270 ng/mL) were greater ($p < 0.05$) in treated compared with control pigs for the duration of treatment. However, we did not observe an effect of treatment on ADG or FI ($p > 0.10$) in these pigs. We conclude that ST is released from CRI and has biological activity, but not at the concentration and(or) duration needed to stimulate ADG. We are currently working on further modifications of our CRI to increase the duration of release of pST to improve growth rate, feed efficiency and carcass composition.

Key Words: Somatotropin, Growth, Pigs

W11 Actions of lipopolysaccharide, prostaglandin-F2α, and the nitric oxide generator, sodium nitroprusside dihydrate, on oocyte maturation and embryonic development in cattle. P. Soto, R. P. Natzke, and P. J. Hansen*, *Dept. of Animal Sciences, University of Florida*.

Mastitis and immunization against constituents of organisms causing mastitis can reduce fertility of cattle and sheep, respectively. Here, it was hypothesized that these effects are mediated via actions of lipopolysaccharide (LPS), prostaglandin-F2α (PGF), and nitric acid on oocyte maturation and embryonic development. To evaluate effects on oocyte maturation, oocytes were matured with various concentrations of LPS, PGF, or the nitric oxide generator, sodium nitroprusside (SNP). Following maturation, oocytes were fertilized and cultured until d 8 after insemination. To test effects on embryo growth, oocytes were matured and fertilized and cultured after fertilization with LPS, PGF, or SNP. Addition of 100 and 1000 ng/ml LPS and 50 and 100 ng/ml PGF to oocyte maturation medium reduced ($P<0.05$) the proportion of oocytes that became blastocysts at d 8 after insemination. For example, the least-squares means for percent oocytes that became blastocyst was 29, 26, 26, 21, 24, 14, and 13% (pooled SEM=4.7%) for oocytes cultured with 0, 0.01, 0.1, 1.0, 10.0, 100.0, and 1000.0 ng/ml LPS, respectively (n=80-136 oocytes/group in 4 replicates). When added after fertilization, in contrast, neither LPS nor PGF reduced development to the blastocyst stage. Addition of SNP during oocyte maturation was without effect on the proportion of oocytes that became blastocysts. However, addition of 10 mM SNP to culture medium after fertilization blocked development to the blastocyst stage ($P<0.001$) while 0.1 and 1 mM SNP did not affect development (percent oocytes to blastocyst at d 8 after insemination = 15, 15, 15, and 0%; pooled SEM=2.2%; n=144-151 oocytes/group in 5 replicates). Results indicate that LPS, PGF, and NO can have adverse effects on oocyte function (LPS, PGF) and embryonic development (NO). It is concluded that increased local synthesis of PGF and NO may mediate effects of mastitis or immune activation on fertility in cattle. It is unlikely, however, that the direct action of LPS on the oocyte is an important cause of infertility in mastitis becase effects of LPS on oocyte maturation occurred at concentrations higher than seen in peripheral circulation during mastitis.

Key Words: Oocyte, Embryo, Mastitis

W12 Postpartum changes in hormones and metabolites during early lactation in summer and winter calving Holstein cows. L. I. Nordbladh*, A. E. Sweetman, and C. S. Whisnant, *North Carolina State University, Raleigh, NC*.

Heat stress is known to decrease milk production and reproductive performance in dairy cattle. The mechanism of action is uncertain. The purpose of the current experiment was to compare levels of metabolic hormone and metabolites in early lactation dairy cows in heat stress or cool environments. A total of 18 Holstein cows (Summer (S) n = 11; Winter (W) n = 7) were used. Maximum and minimum temperatures and relative humidity were collected daily for calculation of temperature humidity index (THI). Blood samples were collected within 24 hours after calving and then weekly thereafter for 12 weeks during both seasons. Plasma concentrations of progesterone (P4), thyroxine (T4) and beta-hydroxybutyrate (BHBA) were determined using radioimmunoassay (P4, T4) or commercial kit (BHBA, Sigma). Differences between seasons in concentrations of hormones and BHBA were determined using ANOVA with the GLM procedure of SAS for repeated measures. THI was greater during the period of sampling for S cows compared with W cows. The THI during S (70.2) was classified as mild heat stress. Based on serum P4 cows calving during S had a longer interval to first ovulation (47.3 $\pm$ 4.5 d) than those calving during W (32.5 $\pm$ 2.3 d). Serum T4 concentrations were higher ($P< 0.01$) in W calving cows (3.5 $\pm$ 0.3 ug/dL) than in S calving cows (1.8 $\pm$ 0.3 ug/dL) for the first 8 weeks postpartum but were not different for weeks 9 through 12. Concentrations of BHBA did not differ between S calving (9.4 $\pm$ 3.5 mg/dL) and W calving (8.1 $\pm$ 2.9 mg/dL) cows but did decline over time postpartum ($P< 0.05$) during both seasons. Reduced T4 concentrations in heat stressed early lactation dairy cows appeared to be associated with a delay in first postpartum ovulation.

Key Words: Heat Stress, Thyroxine, Dairy Cow

W13 Differences in sensitivity to heat-shock between preimplantation embryos from heat-tolerant (Brahman and Romosinuano) and heat-sensitive (Angus) breeds. J. Hernández-Cerón*[1], C. C. Chase Jr[2], and P. J. Hansen[3], [1]*Dept. de Reproduccin, Universidad Nacional Autnoma de Mxico, Mxico D.F.,* [2]*USDA-ARS Subtropical Agricultural Research Station, Brooksville, FL,* [3]*Dept. of Animal Sciences, University of Florida, Gainesville, FL 32611-0910*.

Certain heat-tolerant breeds of cattle have acquired mechanisms to protect cells against damage from high temperature. Exposure of embryos to 41°C reduced development more for Holstein and Angus (An) embryos than for Brahman (Br) embryos. The Romosinuano (Ro) is a Bos taurus from Colombia. Like Br, Ro is a tropically-adapted breed. It is not known, however, whether this breed, distinct in origin from Br, has evolved to possess cellular adaptations to heat shock. A study was performed to test whether Br and Ro embryos survive heat-shock better than An embryos. Cows (n=14 An, 17 Br, and 15 Ro) were slaughtered in groups of 2-3 per breed (5-6 replicates). For each replicate, ovaries were pooled within breed and oocytes harvested and fertilized with semen from a pair of bulls of the specific breed. A different pair of bulls was used for each replicate. At d 4 after insemination, embryos $\geq$ 8 cells were randomly assigned to control (38.5 °C) or heat shock (41°C for 6 h) treatments. Development to blastocyst was determined on d 8. The proportion of oocytes that cleaved at d 4 tended to be highest for Ro (54 $\pm$ 8.4%, 50 $\pm$ 7.7%, and 70 $\pm$ 7.7% for An, Br and Ro; Ro vs others, P=0.07). The proportion of cleaved embryos that were $\geq$ 8 cells at d 4 was lowest (P=0.05) for Br (76 $\pm$ 8.1%, 55 $\pm$ 7.4%, and 77 $\pm$ 7.4% for An, Br, and Ro). Heat shock caused a reduction in the proportion of embryos that became blastocysts at d 8 ($P<0.001$). At 38.5°C, there were no significant differences in development between breeds. Among embryos exposed to 41°C, however, development was lower ($P<0.05$) for An than for Br and Ro. Furthermore, there was a An vs (Br + Ro) x temperature interaction (P=0.09) because heat shock reduced development more for An (30.3 $\pm$ 4.6 % at 38.5°C vs 4.9 $\pm$ 4.6 % at 41°C) than for Br (25.1 $\pm$ 4.6 % vs 13.6 $\pm$ 4.6 %) and Ro (28.3 $\pm$ 4.1 % vs 17.5 $\pm$ 4.1 %). There were no effects on cell number of d 8 blastocysts. Results demonstrate that embryos from thermotolerant breeds (Br and Ro) are more resistant to elevated temperature than embryos from a thermosensitive breed (An). Thus, the process of adaptation of Br and Ro breeds to hot environments resulted in both cases in selection of genes controlling thermotolerance at the cellular level. (USDA IFAFS 2001-52101-11318 and TSTAR 2001-34125-11150).

Key Words: Embryo, Heat shock, Breed

W14 Differences in sensitivity to heat-shock between preimplantation embryos from heat-tolerant (Brahman and Romosinuano) and heat-sensitive (Angus) breeds. J. Hernández-Cerón*[1], C. C. Chase Jr[2], and P. J. Hansen[3], [1]*Dept. de Reproducción, Universidad Nacional Autónoma de México, México D.F.,* [2]*USDA-ARS Subtropical Agricultural Research Station, Brooksville, FL,* [3]*Dept. of Animal Sciences, University of Florida, Gainesville*.

Certain heat-tolerant breeds of cattle have acquired mechanisms to protect cells against damage from high temperature. Exposure of embryos to 41°C reduced development more for Holstein and Angus (An) embryos than for Brahman (Br) embryos. The Romosinuano (Ro) is a Bos taurus from Colombia that, like Br, Ro is a tropically-adapted breed. It is not known whether this breed, distinct in origin from Br, has evolved to possess cellular adaptations to heat shock. A study was performed to test whether Br and Ro embryos survive heat-shock better than An embryos. Cows (n=14 An, 17 Br, and 15 Ro) were slaughtered in groups of 2-3 per breed (5-6 replicates). For each replicate, ovaries were pooled within breed and oocytes harvested and fertilized with semen from a pair of bulls of the specific breed. A different pair of bulls was used for each replicate. At d 4 after insemination, embryos $\geq$ 8 cells were randomly assigned to control (38.5 °C) or heat shock (41°C for 6 h) treatments. Development to blastocyst was determined on d 8. The proportion of oocytes that cleaved at d 4 tended to be highest for Ro (54 $\pm$ 8.4%, 50 $\pm$ 7.7%, and 70 $\pm$ 7.7% for An, Br and Ro; Ro vs others, P=0.07). The proportion of cleaved embryos that were $\geq$ 8 cells at d 4 was lowest (P=0.05) for Br (76 $\pm$ 8.1%, 55 $\pm$ 7.4%, and 77 $\pm$ 7.4% for An, Br, and Ro). Heat shock reduced the proportion of embryos that became blastocysts at d 8 ($P<0.001$). At 38.5°C, there were no significant differences in development between breeds. Among embryos exposed to

41°C, however, development was lower (P<0.05) for An than for Br and Ro. Furthermore, there was a An vs (Br + Ro) x temperature interaction (P=0.09) because heat shock reduced development more for An (30.3 ± 4.6 % at 38.5°C vs 4.9 ± 4.6 % at 41°C) than for Br (25.1 ± 4.6 % vs 13.6 ± 4.6 %) and Ro (28.3 ± 4.1 % vs 17.5 ± 4.1 %). There were no significant effects on cell number of d 8 blastocysts. Results demonstrate that embryos from thermotolerant breeds (Br and Ro) are more resistant to elevated temperature than embryos from a thermosensitive breed (An). Thus, the process of adaptation of Br and Ro breeds to hot environments resulted in both cases in selection of genes controlling thermotolerance at the cellular level. (USDA IFAFS 2001-52101-11318 and TSTAR 2001-34125-11150).

Key Words: Embryo, Heat shock, Breed

W15 Heat shock protein-70 is upregulated in retained testicles of cryptorchid stallions. J. N. Oyarzo*[1], P. C. Gentry[1], G. R. Dawson[1], R. L. Ax[1], and R. J. Collier[1], [1]*University of Arizona, Tucson AZ.*

Heat shock proteins (HSP) are a class of molecular chaperones that protect the three-dimensional structure of proteins subjected to thermal stress. Heat shock proteins have been implicated in fertility of several species, but have not, to date, been characterized in stallions. Lower levels of testicular HSP70 have been associated with decreased semen quality in boars. In the desert southwest US, environmental heat stress could further potentiate the decline in stallion sperm quality typical of summer months. Our objectives were to assess HSP expression in ejaculated sperm and testicular tissue. Heat shock protein 25, HSP40, HSP70 and HSP90 were assessed by Western blot using commercially available anti-human HSP antibodies validated for use with equine samples. In Exp. 1, HSP expression was determined in archived semen samples from four fertile stallions collected in January (low temperature, short photoperiod) and July (high temperature, long photoperiod), as well as in fresh and fresh frozen semen samples collected in August and September. Heat shock proteins were not detected in any semen sample, indicating that ejaculated stallion sperm do not express HSP at levels detectable by this method. In stallions, testosterone production is correlated with daylength, decreasing with decreasing photoperiod. Experiment 2 was designed to assess the effect of testis temperature independent of testosterone levels. Heat shock protein profiles were assessed in testicular tissue from three cryptorchid stallions castrated in January and February. HSP40 and HSP70 were detected in both normal descended and retained testes, however, HSP70 were increased two fold in retained testes relative to paired descended testicles, indicating that maintenance at core body temperature upregulates HSP70. Immunochemical detection of HSP in the retained testes suggest a role for HSP in maintenance of stallion sperm quality in vivo and may be useful in assisted reproductive techniques in horses.

Key Words: Stallion, Testes, Heat shock protein

W16 Nucleotide and predicted amino acid sequence of equine *bmal1*: a key biological clock component showing high homology to human *bmal1*. B. A. Murphy* and B. P. Fitzgerald, [1]*University of Kentucky, Lexington, Kentucky.*

The mammalian master circadian clock resides in the suprachiasmatic nucleus (SCN) of the hypothalamus and drives daily variations in many physiological, biochemical and behavioral processes. The SCN receives photoperiod signals from the environment and supplies the principle timing cues for synchronizing the daily oscillations in peripheral tissues. Interacting positive and negative feedback loops, of which rhythmic regulation of *Bmal1* transcription provides the positive driving force, control the internal molecular time-keeping mechanism. We report the isolation of equine *Bmal1* cDNA from a prepared lymphocyte library and subsequent sequencing of 2Kb, incorporating 94% of the entire open reading frame (ORF) based on comparison with published human *Bmal1* sequence (GenBank Accession # D89722). NCBI Blast analysis of the equine sequence demonstrated 96% homology with human *Bmal1* mRNA. The deduced amino acid sequence of the equine BMAL1 protein shows 99% homology with that of the human. Interspecies phylogenetic analysis using the Align X program of VectorNTI shows the equine *Bmal1* gene clustering more closely with the human gene than that of the hamster, rat or mouse on a phylogeny tree. This sequence data can now be used to analyze expression profiles of the transcript in peripheral tissues, specifically peripheral blood mononuclear cells (PBMCs). Isolation of the transcript from a lymphocyte cDNA library signifies the likelihood of its detection in peripheral circulation and encourages an attempt to isolate further clock genes. Small mammals have historically been used as molecular models to investigate circadian function and disorders in humans. The particularly high homology observed between equine and human *Bmal1* provides incentive to consider a large mammal such as the horse as a molecular model for assessing human peripheral circadian systems, especially now that the impracticality of sacrificing the experimental animals may be overcome by non-invasive peripheral detection of molecular clock components.

Key Words: Circadian, Bmal1, Equine

W17 Characterization of soluble CD14 in bovine milk. J.-W. Lee*[1], X. Zhao[1], and M. J. Paape[2], [1]*Department of Animal Science, McGill University,* [2]*IDRL, USDA-ARS, Beltsville, MD.*

Soluble CD14 (sCD14) has been shown to prevent death induced by "septic shock", inhibit dissemination of pathogens, stimulate lymphocyte proliferation, and facilitate phagocytosis of bacteria. It has been proposed that sCD14 in human milk plays a role not only in breast-feeding associated benefits, but also in protecting the mammary gland from bacterial infections. However, sCD14 in bovine milk has not been well documented. In the present study, milk samples from 100 lactating cows (396 functional quarters) were assayed for sCD14 to determine whether stage of lactation (0-4, 5-99, 100-200 and >200 d), somatic cell count (SCC) or intramammary infection affect concentration of sCD14 in milk. The average concentration of sCD14 was 6.90 0.17 μg/ml, was the highest 0–4 d postpartum (11.39 ± 0.49 μg/ml) and the lowest 100–200 d postpartum (4.56 ± 0.27 μg/ml). The concentration of sCD14 was lower in milk with a SCC < 250,000/ml (6.13 ± 0.28 μg/ml) than that of milk with a SCC > 750,000/ml (7.65 ± 0.29 μg/ml). No difference was found between non-infected and infected quarters. Results indicate that the concentration of sCD14 in bovine milk is close to the range of sCD14 in body fluids from other species, and can be affected by stage of lactation and SCC. The high content of sCD14 in milk 0-4 d postpartum may be associated with the known protective role of colostrum for the calf.

Key Words: CD14, Bovine, SCC

W18 Effects of recombinant bovine growth hormone on levels of the bacteria *Edwardsiella ictaluri* in channel catfish (*Ictalurus punctatus*). B.C. Peterson* and A.L. Bilodeau, [1]*USDA/ARS.*

Research was conducted to examine the immunoregulatory role of recombinant bovine growth hormone (rbGH, Posilac) in channel catfish challenged with *Edwardsiella ictaluri* (*E. ictaluri*). A total of 240 fish (3.7 ± .7 g) were assigned randomly to four treatments with three replicates each. The treatments were 1.) Con-Exposed (Sham injected by needle puncture and challenged with *E. ictaluri*), 2.) Con-Nonexposed (Sham injected by needle puncture and not challenged), 3.) rbGH-Exposed (Posilac, injected at 30 μg/g BW and challenged with *E. ictaluri*), and 4.) rbGH-Nonexposed (Posilac, injected at 30 μg/g BW and not challenged). Treatments were then randomized to one of each sampling day (1 and 5). Fish were maintained in 24, 120-L tanks (10 fish/tank) for three weeks prior to challenge. During this time, fish were injected (rbGH or sham) and specific growth rates were assessed. Fish were reinjected (rbGH or sham) two days prior to challenge with *E. ictaluri*. A genetic assay utilizing real-time PCR was used for detection and quantification of *E. ictaluri* and mortality was recorded daily. Specific growth rate was higher (P = 0.06) in rbGH-treated fish compared to sham injected controls (3.5 vs 3.0) prior to challenge. All non-exposed fish tested negative for the presence of *E. ictaluri* throughout the trial. On days 1 and 5, rbGH-Exposed fish exhibited lower (P < 0.05) levels of *E. ictaluri* when compared to Con-Exposed fish (0 vs 3,250 ± 2,927) (cell-equivalents/100 mL whole blood) and (10,230 ± 5,813 vs 62,294 ± 34,315) (cell-equivalents/100 mL whole blood), respectively. Mortality was similar between rbGH-Exposed and Con-Exposed throughout the study. Reduced levels of *E. ictaluri* in rbGH-Exposed fish suggest an immunoregulatory role for rbGH in channel catfish.

Key Words: rbGH, *Edwardsiella ictaluri*, Catfish

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