

Graduate Student Paper Competition: ADSA-ASAS Northeast Section

118 Feeding heat-treated colostrum increases IgG absorption in neonatal dairy calves. J. A. Elizondo-Salazar*, A. J. Heinrichs, R. F. Roberts, and M. R. Long, *The Pennsylvania State University, University Park.*

Newborn Holstein heifer calves were studied to compare absorption of immunoglobulins G, total serum protein levels, and T cells from unheated or heat-treated colostrum. First milking colostrum with > 50 g IgG/L (measured by colostrometer) was collected from Holstein cows and frozen at -20°C until a total of 106 L were accumulated. Once collected, colostrum was thawed at 4°C and pooled in a commercial batch pasteurizer to create a uniform batch. Colostrum was thoroughly mixed at 4°C for about 20 min. 53 L of colostrum were transferred into 1.89 L containers and frozen at -20°C until needed for feeding. The remaining 53 L were heated at 60°C for 30 min, transferred into 1.89 L containers, and then frozen at -20°C until needed for feeding. A total of 28 calves weighing ≥ 35 kg at birth were systematically enrolled into 1 of the 2 treatment groups. Calves were separated from their dams at birth before suckling occurred. Before feeding colostrum, a jugular blood sample was collected from each calf. For the first feeding, 3.8 L of colostrum were bottle fed by 1 to 2 h of age. To ensure that all calves received an equal amount of colostrum, an esophageal feeder was used in calves with reduced appetite. For the second and third feeding, pasteurized whole milk at 5% of birth BW was fed. For the remaining time, calves were fed milk replacer (20% CP, 20% fat) at 10% of birth BW in 2 daily feedings until wk 5. Then it was reduced to only a.m feeding until weaning at 6 wks of age. Blood samples were collected, at 24 and 48 h of age and at wk 1 to 6. Serum from samples was used to measure IgG levels, total serum protein and T cells. Calves fed heat-treated colostrum had significantly greater IgG₁ and total IgG concentrations compared with calves fed unheated colostrum. There was no effect of treatment on total serum protein or T cells.

Table 1. Total serum protein and IgG levels.

| Age | Total Serum | | Protein (g/L) | | IgG ₁ (g/L) | | IgG ₂ (g/L) | |
|------|-------------|----------|-------------------|-------------------|------------------------|----------|------------------------|----------|
| | Heated | Unheated | Heated | Unheated | Heated | Unheated | Heated | Unheated |
| 24 h | 5.6 | 5.5 | 21.5 ^a | 18.6 ^b | 1.1 | 1.0 | | |
| 48 h | 5.7 | 5.6 | 21.2 ^a | 18.4 ^b | 1.1 | 1.0 | | |
| 1 wk | 5.6 | 5.5 | 20.5 ^a | 17.9 ^b | 1.1 | 0.9 | | |
| 2 wk | 5.6 | 5.5 | 19.6 ^a | 17.1 ^b | 1.1 | 0.9 | | |
| 3 wk | 5.4 | 5.3 | 18.2 ^a | 15.9 ^b | 1.0 | 0.9 | | |
| 4 wk | 5.2 | 5.1 | 16.6 ^a | 14.4 ^b | 0.9 | 0.8 | | |
| 5 wk | 5.0 | 5.0 | 14.6 ^a | 12.6 ^b | 0.8 | 0.8 | | |
| 6 wk | 4.7 | 4.7 | 12.3 ^a | 10.5 ^b | 0.7 | 0.8 | | |

P < 0.05

Key Words: Colostrum, Immunoglobulin, Passive Transfer of Immunity

119 Mammary and liver lipogenic gene expression in lactating mice fed diets supplemented with trans-18:1 isomers or t10c12 CLA. A. K. G. Kadegowda*¹, E. E. Connor², B. B. Teter¹, J. Sampugna¹, L. S. Piperova¹, and R. A. Erdman¹, ¹University of Maryland, College Park, ²USDA-ARS, Beltsville, MD.

The coordinated suppression of lipogenic pathways during milk fat depression (MFD) has suggested the involvement of a global regulator of lipogenic gene expression. Recent studies (Peterson et al., 2004; Harvatine et al., 2006) have implicated SREBP1 as the central regulator of fatty acid (FA) synthesis. The objective of this study was to examine the effects of trans-18:1 isomers or t10c12 CLA on mammary and liver lipogenic gene expression in lactating mice. Thirty lactating C57Bl6J mice were randomly assigned (n=5) to 6 diets supplemented with one of the following isomers t-7-, t-9-, t-11-18:1, t10c12-CLA (CLA) or PHVO (partially hydrogenated vegetable oil) from d6 to d10 postpartum (PP). Milk, mammary and liver samples were collected on d10 PP. Expression of genes involved in de novo FA synthesis (ACACA, FASN), desaturation (SCD1, SCD2), triacylglycerol (TG) synthesis (AGPAT), FA uptake (LPL), transcriptional regulation (SREBP1, ChREBP, INSIG1, SCAP, MLX, THRSP) and nuclear receptor signaling (PPARA, PPARG, LXRA, RXR) were tested by qPCR. Milk fat percentage was decreased by CLA (44%; *P*<0.001), t-7-18:1 (27%; *P*<0.001) and PHVO (23%; *P*<0.001), compared to Control. In the mammary gland, CLA decreased (*P*<0.001) the genes related to de novo FA and TG synthesis and desaturation, and genes involved in transcriptional regulation including SREBP1, ChREBP, PPARA and THRSP (*P*<0.05). PHVO and t-7-18:1 decreased the expression of AGPAT, SCD1 and THRSP (*P*<0.05). Similar to CLA, PHVO decreased SREBP1 and PPARA (*P*<0.05), while t-7 up-regulated SCAP and MLX (*P*<0.05). The measured genes were not altered by t-9- or t-11-18:1 in mammary gland. In liver, AGPAT was down regulated (*P*<0.05) with MFD, while expression of other lipogenic genes was not altered. Opposite to mammary gland, CLA up-regulated SREBP1 and PPARA (*P*<0.05) and decreased PPARG (*P*<0.05). The results showed that, in addition to SREBP1, other transcriptional regulators could be involved in milk fat synthesis.

Key Words: Lactating Mice, Trans Fatty Acids, Gene Expression

120 Photoperiod regulates diurnal expression patterns of genes related to immune function in PBMC of heifers. L. E. Lord*, X. S. Revelo, and T. B. McFadden, *University of Vermont, Burlington.*

Photoperiod can be manipulated to enhance efficiency of animal production. Skeletal long day photoperiods (SLD) involve exposure to brief pulses of light during photosensitive periods to mimic long day photoperiods (LD). Thus, SLD could be used to alter photoperiod while minimizing energy costs. We hypothesized that exposure to SLD would elicit changes in gene expression similar to those under exposure to LD. Holstein heifers were exposed to short day photoperiod (SD; 9 h light: 15 h dark; 9L: 15D, n=10) for 3 wk, followed by LD (15L: 9D, n=5) or SLD (2L: 11D: 2L: 9D, n=5) for 4 wk to test for effects on gene expression in peripheral blood mononuclear cells (PBMC). Blood was collected at 0600, 1200, 1900 and 2400h on the last day of treatments. PBMC were isolated to quantify expression of major histocompatibility complex Class I (MHC), indoleamine 2,3-dioxygenase (IDO), and long form prolactin receptor (PRLr) genes, which are photoperiod-responsive and play a role in immune function. Under SD, expression of IDO and PRLr varied during the day (*P*<0.02). Expression of all 3 genes showed significant (*P*<0.07) diurnal variation during exposure to LD or SLD, however expression patterns differed markedly from SD. Expression of MHC and IDO did not differ (*P*>0.16) between LD and SLD treatments

whereas expression of PRLr tended to be lower in SLD compared to LD ($P = 0.09$). These data demonstrate diurnal variation in expression of these 3 genes in PBMC. The similarity of gene expression profiles on SLD or LD suggest that skeletal photoperiods could be used to alter immune function in heifers.

Table 1. Effects of photoperiod and time of day on gene expression in PBMC

| | | Treatment Means | | | | P-value |
|------|-----|-------------------|--------------------|--------------------|--------------------|--------------------|
| | | 0600h | 1200h | 1900h | 2400h | Time |
| MHC | LD | 0.73 ^a | 0.82 ^a | 1.55 ^b | 0.62 ^a | 0.004 ¹ |
| | SLD | 1.20 ^a | 0.68 ^b | 1.30 ^a | 0.45 ^b | |
| | SD | 0.67 ^a | 1.05 ^b | 0.83 ^{ab} | 0.79 ^{ab} | 0.11 |
| IDO | LD | 0.73 ^a | 0.75 ^a | 1.24 ^b | 0.83 ^{ab} | 0.007 ¹ |
| | SLD | 0.99 ^a | 1.16 ^a | 1.74 ^b | 1.08 ^a | |
| | SD | 1.61 ^a | 1.36 ^{ac} | 1.01 ^{bc} | 0.71 ^b | 0.019 |
| PRLr | LD | 1.16 ^a | 0.98 ^{ab} | 0.65 ^b | 0.93 ^a | 0.07 ¹ |
| | SLD | 0.57 ^a | 0.65 ^a | 0.54 ^a | 1.03 ^b | |
| | SD | 0.12 ^a | 0.44 ^b | 0.52 ^b | 0.18 ^a | 0.0002 |

^{a, b, c} Means within a row differ ($P \leq 0.05$); ¹Main effect of time across LD and SLD treatments.

Key Words: Photoperiod, Gene Expression, Immune Function

121 Colicin E1 and EDTA have additive antimicrobial effects against *Escherichia coli* isolates in bovine milk. J. M. Scudder^{*1}, C. H. Stahl², and M. R. Waldron¹, ¹University of Vermont, Burlington, ²North Carolina State University, Raleigh.

Colicins are antimicrobial proteins produced by some strains of *E. coli* that are lethal against other strains of *E. coli* and species of bacteria. Milk components, such as calcium and magnesium have been shown to inhibit the activity of antimicrobial peptides; however, the addition of EDTA displaces these cations and destroys the casein micelles. The objective of the current study was to determine the efficacy of purified colicin E1 (ColE1), EDTA, and a ColE1/EDTA combination against a variety of mastitis-causing bacterial strains in several media. A factorial arrangement of treatments was used to test ColE1 and EDTA at concentrations of 0, 100 and 250 μ g/ml and 0, 10 and 20mM respectively, in media, whole, and skim milk. Antimicrobial activity of the treatments was determined after quantification of bacteria at several incubation time-points by serial dilution and direct plating. ColE1 did not inhibit the growth of *Klebsiella pneumoniae*, *Staphylococcus aureus*, or *Streptococcus uberis* in Luria-Bertani media (LB) at a concentration of 250 μ g/ml ($P > 0.50$), eliminating further investigation of antimicrobial effects against those microbes. The antimicrobial effects of ColE1 were similar against both *E. coli* 25922 and *E. coli* P4:O32; thus, the results for these *E. coli* strains were pooled prior to statistical analysis. When *E. coli* were incubated in LB, combinations of ColE1 $\geq 100\mu$ g/ml and EDTA $\geq 10mM$ resulted in killing and complete growth inhibition ($P = 0.02$). In whole milk, combinations of ColE1 $\geq 100\mu$ g/ml and 20mM EDTA resulted in killing and complete growth inhibition of *E. coli* ($P < 0.01$). In skim milk, *E. coli* growth was completely inhibited by 4 hours when 250 μ g/ml ColE1 and 20mM EDTA were added to the growth cultures ($P = 0.02$). Taken together, these results show that

a ColE1/EDTA combination is effective at both killing and inhibiting growth of *E. coli* in bovine milk.

Key Words: Colicin, Antimicrobial, EDTA

122 Skeletal muscle satellite cells do not spontaneously adopt adipogenic fates. J. D. Starkey^{*}, M. Yamamoto, S. Yamamoto, and D. J. Goldhamer, University of Connecticut, Storrs.

Muscle satellite cells are known to play key roles in the growth and maintenance of skeletal muscle tissue. Accumulation of intramuscular adipose tissue is commonly observed in aged and dystrophic skeletal muscle and its abundance is a key factor in determining meat quality. The myogenic regulatory factor, *MyoD*, is involved in embryonic myogenic lineage commitment and is expressed in satellite cell precursors. Recent reports indicate that skeletal muscle satellite cells have the capacity to adopt adipogenic phenotypes in culture. We investigated the myogenic commitment state of skeletal muscle satellite cells in mice using novel Cre/loxP recombination lineage tracing technology. To facilitate the permanent labeling of the vast majority of satellite cells, we developed mice with *Cre recombinase* knocked into the *MyoD* locus (*MyoD^{Cre}*) and crossed them with Cre-dependent reporter mice (*R26R^{EYFP}*) to generate offspring carrying both alleles. Cells from these mice that have expressed the *MyoD* locus constitutively express *enhanced yellow fluorescent protein* (EYFP) from the Cre-recombined reporter locus. Single *extensor digitorum longus* myofibers were isolated from adult *MyoD^{Cre/+};R26R^{EYFP/+}* mice and cultured for 14 d followed by immunocytochemistry to detect labeled (EYFP+) cells. The adipogenic cells observed in single myofiber cultures from adult mice were not labeled, indicating that they had never expressed *MyoD* and were thus most likely not of satellite cell origin. Clonal analysis of individual satellite cells from *MyoD^{Cre/+};R26R^{EYFP/+}* mice revealed that Cre/loxP-labeled cells did not spontaneously differentiate into lipid-filled adipocytes. We did, however, observe lipid accumulation in EYFP+ satellite cell cultures following exposure to 100 μ M γ -linolenic acid for 10 d. These results indicate skeletal muscle satellite cells are committed to myogenesis and do not readily differentiate into lipid-filled adipocytes without exposure to potent adipogenesis-inducing agents.

Key Words: Skeletal Muscle, Satellite Cell, Adipocyte

123 Presence of mammary epithelium modulates expression of growth-regulating genes in stroma of bovine mammary gland. J. G. Titus^{*}, S. B. Simpson, and T. B. McFadden, University of Vermont, Burlington.

The mammary fat pad acts as a paracrine regulator of mammary growth; however, the specific action of this developmental regulation is not completely understood. We hypothesized that epithelial-stromal interaction modulates stromal expression of estrogen- and growth hormone-responsive genes known to regulate mammogenesis. An epithelium-free mammary fat pad was prepared in one gland of each of 20 Friesian calves at 1 mo of age. At 18 mo of age, heifers were assigned to one of four treatments: control (C), estrogen (E), growth hormone (GH) or estrogen plus growth hormone (GE). Heifers in E and GE groups were injected s.c. with estrogen (0.1 mg/kg BW). Heifers in GH and GE groups were injected i.m. with recombinant bovine somatotropin (Posilac[®], 500mg).

Heifers in the C group received no hormone. Heifers were slaughtered 24 h after hormone treatment and stromal tissue was collected from both the epithelium-free (CFP) and intact fat pads (MFP). Total RNA was isolated from tissues and qualitative PCR was performed to determine expression of five genes in cDNA pooled from each fat pad group. Expression of estrogen receptor (ER)- α , transforming growth factor (TGF)- β and epidermal growth factor receptor (EGFR) mRNA was greater in MFP than CFP (10, 6 and 2 fold difference, respectively). TGF- α and progesterone receptor (PR) expression was similar in CFP and MFP. Quantitative real-time PCR was used to measure the rela-

tive expression of ER α and PR in the stroma. Expression of ER α was reduced in CFP relative to MFP ($P < 0.05$), however there was no effect of hormone treatment nor was there an interaction between hormone treatment and tissue type ($P > 0.05$). Neither presence of epithelium nor hormone treatment affected PR expression ($P > 0.05$). We conclude that the presence of epithelium directly affects the stromal expression of several genes involved in regulation of mammary development.

Key Words: Epithelium-Free Fat Pad, Gene Expression, Mammo-genesis