post-partum Holstein and Jersey calves at the Clemson University Dairy. Plasma was collected following centrifugation and stored at -20°C until assayed. Protein values were determined by placing one drop (50°μl) on a refractometer window (Model RHC-200 ATC clinical refractometer, Westover Scientific) and reading value at g/100mL. IgG values were determined by a sandwich ELISA procedure using a monoclonal antibody against bovine IgG (Sigma Chem). Plasma samples were diluted at 1:20,000 with PBS containg 0.2% ovalbumin. The data obtained were analyzed by regression procedure for correlation and by regression analysis. Mean values (n=148) for IgG was 1947 mg/100mL (S.E.=±47). Mean value (n=148) for protein was 6.13g/100mL (S.E.=±0.07). Mean IgG values (n=97) for Holsteins was 1889 (S.E.=±55.8) and for Jersey (n=51) was 2059 (S.E = ±86.2). Mean protein values (n=97) for Holsteins was 6.06 (S.E.=±0.09) and for Jerseys (n=51), 6.20 (S.E.=±0.13). Data analysis by the above statistical procedures revealed an r value of 0.26 with extensive skewness at 2000 mg/100mL IgG. Within an assay there appeared to be a good positive correlation; increased protein similarly increased IgG. However, assay to assay variation was too great to result in an overall significant correlation between protein and IgG. In addition, comparison of refractometer protein readings to a standard protein analysis procedure could possibly suggest an insensitivity of the refractometer to major protein changes in plasma.

Key Words: IgG, Dairy calves, Refractometer

Growth & Development

681 Effects of conjugated linoleic acid (CLA) and trans- $C_{18:1}$ fatty acids (TFA) on energetic metabolites and subcutaneous adipose tissue fatty acid composition. L. H. Baumgard*[1], S. R. Sanders[1], C. Davis[1], B. A. Corl[2], J. W. Perfield, II[2], D. E. Bauman[2], and G. C. Duff[1], [1] *The University of Arizona, Tucson,* [2] *Cornell University, Ithaca NY.*

Finishing beef cattle (n=30, 359 60 kg BW), which were studied in an immune trial were also utilized in this experiment. Cattle were fed isoenergetic diets (steam-flaked sorghum based) supplemented (top dressed) with rumen protected (RP) palm oil (559 g/d; EnerGII®[EII]; control), RP TFA (594 g/d) or RP CLA (609 g/d) for 35d. Each treatment provided 475 g lipid/d and RP TFA consisted of 17.2% italicizetrans-6-8, 8.7% *trans*-9, 8.8% italicizetrans-10, 5.8% *trans*-11 and 7.3% italicizetrans-12 $C_{18:1}$ and the RP CLA contained 6.5% *cis*-9, *trans*-11, 5.4 % c/t-8, c/t-10, 8.25% c/t-11, c/t-13 and 7.9% italicizetrans-10, *cis*-12 CLA. All bull calves were weighed and blood collected on d 0, 7, 13, 21, 28 and 35. Subcutaneous adipose biopsies were taken from the tail head on d 35. Overall, CLA supplementation decreased DMI (P = 0.04; 7.6, 7.4 and 6.1 kg/d for EII, TFA and CLA, respectively) and did not effect G:F or ADG. CLA supplementation tended (P = 0.10) to increase NEFA concentrations (196^a, 213^{ab} and 258^b μmole/L, for EII, TFA and CLA, respectively) and this was not dependent upon time. Supplementing CLA reduced (P=0.04) plasma glucose levels (5.4%) compared to EII and there was no trt x time interaction. Compared to EII cattle fed TFA had increased (P<0.01) concentrations of *trans*-6-8 (120%), *trans*-9 (113%), italicizetrans-11 (30%) and *trans*-12 (62%) $C_{18:1}$ fatty acids, but did not change italicizetrans-10 $C_{18:1}$ (64 mg/g fat) and also increased *cis*-9, *trans*-11 CLA (10 %). Irrespective of treatment the content of italicizetrans-10 was 3.8 fold more than italicizetrans-11 $C_{18:1}$. CLA supplementation did not alter the trans-C18:1 profile but increased italicizecis-9, *trans*-11 and *trans*- 10, *cis*-12 CLA content by 8 and 50% respectively. There was no treatment effect on total unsaturated fatty acid content (54%) or on the $Delta^9$-desaturase index (42.5) nor any of the specific Δ^9-desaturase ratios. These data indicate the Δ^9-desaturase system contributes to the *cis*-9, *trans*-11 CLA content in beef adipose tissue.

Key Words: CLA, Δ^9-desaturase

682 Effect of conjugated linoleic acid on DNA fragmentation in cultured adipocytes. K. M. Hargrave* and J. L. Miner, *University of Nebraska.*

Dietary conjugated linoleic acid (CLA) causes body fat loss and DNA fragmentation in adipose tissue of mice. DNA fragmentation is an indication that CLA may cause apoptosis, either in preadipocytes or adipocytes. We recently reported that CLA promoted DNA fragmentation in cultured preadipocytes. The present study was designed to determine if mature 3T3-L1 adipocytes are susceptible to this effect of CLA. 3T3-L1 preadipocytes were seeded into 12-well plates with DMEM plus 10% calf serum, grown to confluence, and stimulated to differentiate with dexamethasone, IBMX, insulin, and fetal bovine serum. Differentiated cells were maintained in DMEM plus 10% fetal bovine serum for 7 to 9 d and then 0, 50, 100, or 200 μM linoleic acid (LA) or trans-10, cis-12 CLA, complexed to albumin (6.6:1), or 50, 500, or 1 nM staurosporine. Media were changed every 2 d. Cells were collected on d 2, 4, and 6 of treatment. Attached and detached cell number, DNA fragmentation, cellular triglyceride content, and glycerol content of media were determined. CLA did not increase DNA fragmentation compared to either the control or LA on any day. CLA, at the 50 and 100 μM doses, reduced ($P < 0.01$) cell number on d 2 but not on d 4 or 6. LA and CLA did not alter cellular triglyceride content on any day. CLA also had no effect on glycerol content of the media. However, 200 μM LA increased ($P < 0.05$) glycerol in the media on d 2 (790 vs 1370 μg for control vs 200 μM LA, respectively). Staurosporine reduced ($P < 0.05$) total DNA on each day by 20 to 90% and appeared to increase DNA fragmentation on d 2. Additionally, 50 and 500 nM staurosporine reduced ($P < 0.05$) cellular triglyceride content on d 2 by 42 and 65%, respectively, and increased the glycerol content of the media on each day, compared to the control. In conclusion, CLA did not cause an increase in DNA fragmentation in mature 3T3-L1 adipocytes. This indicates that the DNA fragmentation observed in fat pads of mice fed CLA may be attributed to preadipocytes and not to adipocytes.

Key Words: Conjugated linoleic acid, Adipocytes, DNA Fragmentation

683 IGF-I infusion alters gene expression profile of prepubertal bovine mammary parenchyma. B. E. Etchebarne*, L.F.P. Silva, G.J.M. Rosa, P. M. Coussens, M. S. Weber Nielsen, and M. J. VandeHaar, *Michigan State University.*

Insulin-like growth factor-I (IGF-I) stimulates proliferation of bovine mammary epithelial cells in vitro and in vivo. Our objective was to identify key genes that mediate the IGF-I mitogenic response in prepubertal mammary parenchyma. IGF-I was infused via the streak canal into two quarters of six prepubertal Holstein heifers at 10 μg/quarter per d; other quarters received saline plus BSA. After 7 d, heifers were killed and mammary parenchymal tissue was collected. IGF-I increased the percentage of epithelial cells in the S-phase by 30%, as reported in a separate abstract. To date, gene expression profiles of total parenchymal mRNA from each quarter of 2 animals have been examined using a bovine-specific cDNA microarray system containing 796 unique expressed sequence tags and 539 amplicons representing known genes. A loop design was used with cDNA from each quarter of each cow labeled with Cy3 or Cy5 dyes prior to microarray hybridization. Gene expression data were normalized for dye intensity using control genes. Significance levels of differential gene expression among treatments were assessed using a mixed model approach with the procedures LOESS and MIXED of SAS. IGF-I increased expression of several genes. Of particular interest, IGF-I upregulated nuclear receptor coactivator 6 interacting protein, an activator of the STAT3 pathway; beta-1,4-N- acetylglucosaminyltransferase IV, which influences cell cycle progression and susceptibility to apoptotic stimuli; MHC Ovar-DR-alpha, which interacts with the STAT1 pathway; and nickel-specific induction protein (Cap43), a marker for rapidly proliferating breast cancer cells. Expression of these 4 genes was increased 70 to 100% (P < 0.008). We are currently evaluating the other 4 animals in the study, verifying changes with real-time PCR, and employing laser capture microdissection to measure expression profiles of epithelial and stromal cell types separately. We conclude that IGF-I infusion into prepubertal bovine mammary glands induces changes in expression of genes affecting STAT signaling, mammary cell apoptosis, and cell cycling.

Key Words: IGF-I, Microarray, Mammary development

684 Leptin intramammary infusion alters the gene expression profile of prepubertal bovine mammary parenchyma. B. E. Etchebarne*, L.F.P. Silva, G.J.M. Rosa, P. M. Coussens, M. S. Weber Nielsen, and M. J. VandeHaar, *Michigan State University*.

Increased body fatness in prepubertal heifers is associated with impaired mammogenesis. Leptin, a hormone produced by adipocytes, reduces proliferation of bovine mammary epithelial cells in vitro and in vivo. Our objective was to identify key genes mediating this inhibition. Leptin was infused via the streak canal into 2 quarters of 6 prepubertal Holstein heifers at 100 μg/quarter per d; control quarters received saline plus BSA or saline plus IGF-I. After 7 d, heifers were killed and mammary parenchymal tissue was collected. Leptin decreased the percentage of epithelial cells in the S-phase by 48% in IGF-I-treated and 19% in saline-treated quarters, as reported in a separate abstract. To date, the gene expression profile of total parenchymal mRNA from each quarter of 2 animals has been examined using a bovine-specific cDNA microarray containing 796 unique expressed sequence tags and 539 amplicons representing known genes. A loop design was used with cDNA from each quarter of each cow labeled with Cy3 or Cy5 dyes prior to microarray hybridization. Gene expression data were normalized for dye intensity biases using control genes. Significance levels of differential gene expression among treatments were assessed using a mixed model approach with the procedures LOESS and MIXED of SAS. Leptin upregulated 50 genes at $P < 0.01$; at least 30 of these had clear links to pathways mediating cell proliferation. The upregulated genes included 3 promoters of apoptosis: dynamin 2, CCAAT/enhancer-binding beta protein, and ribosomal protein S3A; 3 cell cycle regulators: nucleoporin p62, ubiquitin-like protein NEDD, and protein kinase CDK9; several transcription factor regulators; and several cellular reorganization proteins, all with fold changes from 1.5 to 4.5 ($P < 0.008$). We are currently evaluating the other 4 animals in the study, verifying changes with real-time PCR, and employing laser capture microdissection to measure expression profiles of epithelial and stromal cell types separately. We conclude that leptin infusion into prepubertal bovine mammary parenchyma induces changes in expression of genes regulating apoptosis, cell cycling and transcriptional machinery. These molecular changes might help explain the impaired mammogenesis of fat heifers.

Key Words: Leptin, Mammary development, Microarray

685 Intramammary infusion of leptin decreases proliferation of mammary epithelial cells in prepubertal heifers. L.F.P. Silva*[1], J. S. Liesman[1], M. S. Weber Nielsen[1], and M. J. VandeHaar[1], [1]*Michigan State University*.

Excessive body fatness is associated with impaired mammogenesis in prepubertal heifers. Leptin, a hormone produced by adipocytes, reduces IGF-I-stimulated proliferation of bovine mammary epithelial cells in vitro. Our objective was to determine if leptin also reduces proliferation of mammary epithelial cells in vivo before puberty. Recombinant ovine leptin (>98% purity) was infused via the streak canal into two quarters of six prepubertal Holstein heifers at 100 ug/quarter per d with or without rhIGF-I at 10 mg/quarter per d. The N-terminus of roLeptin was homologous to native oLeptin. Contralateral quarters were used as controls and received saline plus BSA with or without IGF-I. After 7 d of treatment, bromodeoxyuridine (BrdU) was infused intravenously at 5 mg/kg BW, and heifers were killed 2 h later. Samples from three parenchymal regions (proximal, intermediate, and distal to the teat) were collected, fixed, sliced, and incubated with BrdU monoclonal antibody to identify cells in the S-phase of the cell cycle. Total number of epithelial cells and BrdU-labeled cells were quantified in three microscopic fields from each slide section so that 2700 cells were counted in each quarter. Leptin infusion decreased BrdU-labeling 48% in IGF-I-treated quarters (4.1 vs. 7.9%, $P < 0.01$), and 19% (5.0 vs. 6.2%, $P = 0.01$) in saline-treated quarters. Treatment effects were likely not associated with an immune response as we used sterile technique, mammary tissue was visually normal, and endotoxin was not detected in the infusates using a commercial kit sensitive to 0.006 ng/ml. We conclude that intramammary infusion of leptin inhibits proliferation of mammary epithelial cells in prepubertal heifers and completely blocks the stimulatory effect of IGF-I on mammary epithelial cells. These results suggest that leptin may mediate the inhibitory effects of high-energy intake on mammary gland development in heifers. If so, perhaps we can prevent nutritional impairment of mammogenesis by simply managing body condition of young heifers.

Key Words: Heifer, Mammary development, Leptin

686 Compensatory growth during late gestation and its effects on metabolic status and health of transition heifers. M. S. Laubach*, D. B. Carlson, W. L. Keller, and C. S. Park, *North Dakota State University, Fargo ND/USA*.

Ten pregnant Holstein heifers averaging 499 kg of body weight and 60 d of gestation were divided into two treatments to determine if a one stair-step gestational nutrition regimen affects metabolic status and lactation potential during the transition period. The control group was fed a diet containing 14.0% crude protein and 22.4 Mcal of ME per d for the entire 210 d of the trial. During the restriction period, the treatment group was fed a diet containing 18.5% crude protein and 14.5 Mcal of ME per d until d 180 of gestation; the diet was changed to 14.0% crude protein and 29.0 Mcal of ME per d for the realimentation period. Control heifers were restricted-fed to obtain an ADG of 0.68 kg per d, while treatment heifers were restricted to less then 0.1 kg per d of gain during the restriction period. During realimentation, treatment heifers were allowed to gain 0.91 to 2.26 kg per d. Heifers were weighed for three consecutive days at the start, at 180 d of gestation, and within one wk before calving; BW was not different at initiation, at 180 d of gestation, or one week before parturition. Blood was drawn on d 14, 11, 9, 7, 5, 4, 3, 2, and 1 before parturition; within 3 h of calving; and on d 1, 2, 3, 4, 5, 7, 9, 11, and 14 after parturition to monitor glucose, insulin, triglycerides, NEFA, and the immune status. Glucose, insulin, triglycerides, and NEFA were not different between groups before or after parturition. Total leukocytes were significantly higher ($P = 0.05$) in the treatment group before parturition; however, after parturition there was no difference between groups. Milk production was not different (control, 8,571 kg; treatment, 8,453 kg). Milk fat percentage was increased in the treatment group (4.2% vs 4.1%, $P = 0.05$); however, milk protein percentage was the same in both groups (2.9%). The results suggest that compensatory growth during the last trimester of gestation does not affect the metabolic status of prepartum heifers or subsequent milk production.

Key Words: Heifer, Transition, Blood metabolites

Production, Management, & the Environment

653 A system to characterize feeding behavior of dairy cows and feeding behavior of periparturient and mid-lactation cows. M. A. DeGroot* and P. D. French, *Oregon State University, Corvallis*.

The objectives of the following research were to develop a system to describe feeding behavior of group housed dairy cows and characterize feeding behavior of periparturient and mid-lactation cows. In experiment 1, 8 periparturient Holstein and Jersey cows were used to determine feeding behavior for the three weeks before and after parturition. Cows were group housed and fed individually via Calan® doors. Behind each door was a feed tub that rested on a digital scale, equipped with a RS232 bi-directional interface. Scales were connected to a computer and a software program collected date, time, and weight events. Variables measured were meals/day, total meal duration/day, individual meal duration, daily DMI, DMI/meal, and meal efficiency (kg DM/min). Data were analyzed using the MIXED procedure of SAS. From 21 to 1 d prepartum, total meal duration decreased linearly ($P<0.01$) from 284 to 136 min/d, individual meal duration decreased linearly ($P<0.07$) from 32 to 17 min/d, DMI decreased linearly from 11.5 to 7.3 kg/d, and meal DMI decreased linearly ($P<0.01$) 1.4 to 0.9 kg. From 1 to 21 d postpartum, total meal duration and individual meal duration increased linearly ($P<0.05$) from 100 to 278 min/d and 12 to 26 min/meal, respectively. Total meal duration was positively correlated with prepartum DMI (r=0.62) and postpartum DMI (r=0.80). Meals (10.2 meals/d) and efficiency (0.06 kg DM/min) were similar for the prepartum and postpartum periods. In experiment 2, 8 Holstein and Jersey cows were used to characterize feeding behavior of mid-lactation cows (17334 days in milk). Overall, cows consumed 8.5 meals/d, total meal duration was 250 min/d, and efficiency was 0.09 kg DM/min. Daily DMI, DMI/meal,