T255 Expression of interleukins, neuropeptides, and growth hormone receptor (GHR) and leptin receptor (LPR) genes in adipose tissue from growing broiler chickens. G. J. Hausman*[1], C. R. Barb[1], B. D. Fairchild[2], A. Hinton[1], and J. A. Cason[1], [1]*USDA-ARS*, *Athens, GA,* [2]*University of Georgia*, *Athens*.

In this study, total RNA was collected from abdominal adipose tissue samples obtained from 10 broiler chickens at 3, 4, 5, and 6 weeks of age and prepared for real time RT-PCR analysis with custom-designed primers and probes. Studies of the gene expression of cytokines and associated genes in chicken adipose tissue were initiated since the discovery of leptin has shown in many animal species that adipose tissue derived factors can dramatically influence growth and physiology. The influence of age on the expression of adipose tissue IL-15, IL-18, neuropeptide Y and GHR and LPR genes and several other cytokines was examined. Between 3 and 6 weeks of age LPR expression decreased ($P < 0.05$) with age while expression of IL-15 and GHR increased significantly ($P < 0.05$). Furthermore, IL-18 and visfatin expression increased ($P < 0.001$) between 4 and 6 weeks of age. Expression of these cytokines was detected for the first time in chicken adipose tissue. Consequently, this is the first demonstration of age related changes in cytokine gene expression in chicken adipose tissue. Gene expression of several cytokines was not detected in chicken adipose tissue including IL-6 and brain derived neurotrophic factor. Future studies are needed to elucidate the role of adipose tissue cytokines in growth and, possibly, disease resistance. Furthermore, these studies provide indirect evidence that the adipose tissue response to leptin and growth hormone change with age.

Key Words: chicken, cytokine, adipose tissue

T256 Apoptosis in different fat depots of cows treated with conjugated linoleic acids (CLA). S. Haeussler*[1], D. Germeroth[1], D. von Soosten[2], S. Dänicke[2], and H. Sauerwein[1], [1]*University of Bonn*, *Bonn, Germany*, [2]*Federal Research Institute of Animal Health*, *Braunschweig, Germany*.

Changes in adipose tissue mass may be associated with a change in adipocyte number and/or a change in adipocyte volume. For development and maintenance of homeostasis, apoptosis plays an important role within organisms. In mice, dietary CLA causes apoptosis of adipocytes (Miner et al. 2001, Obesity Res, 9:129). To investigate whether apoptosis occurs in bovine fat and if apoptosis is influenced by CLA, 25 Holstein heifers were divided in a control (CTR) and a CLA group; from d 1 postpartum (pp) until sample collection, animals from the CLA group were fed with 100 g CLA (containing 10% each of the cis-9,trans-11- and the trans-10,cis-12-CLA isomers) per day. On d 1, 42 and 105 pp, 5 animals of CTR were slaughtered; from CLA, 5 cows each were slaughtered on d 42 and 105. Retroperitoneal (RP) and subcutaneous (SC) fat from the tail head were obtained from all cows. For the detection of DNA fragmentation, deparaffinized sections (10 µm) were stained using the TUNEL method. For positive and negative controls, bovine lymph nodes were treated either with or without DNase after demasking to initiate DNA strand breaks. The apoptotic cell rate (%) was defined as mean number of TUNEL-positive cells/mean number of total cells × 100 and analyzed using the general linear model and Student's *t*-test (SPSS). We determined TUNEL-positive nuclei within bovine adipocytes. The average value for SC and RP fat in CTR animals was 12.7 ± 1.4% and 5.3 ± 1.0%, respectively. The apoptotic rate in SC depot was twice as high compared to the rate of retroperitoneal fat ($P \leq 0.001$), but did not differ with time of lactation. On d 105 pp, the apoptosis rate was increased by more than 1.5-fold in both fat depots of the CLA cows ($P \leq 0.01$) in comparison to the CTR group. Apoptosis may influence the number of adipocytes in bovine adipose tissue. SC fat as main energy store seems to be more affected by mass changes and dietary CLA, thus presumably showing a higher apoptotic rate than the RP depot.

Key Words: adipose tissue, apoptosis, cow

T257 Differences in the mRNA abundance of the adiponectin system and GPR109A in adipose tissue and liver of the F2 cows of Charolais x German Holstein crosses. M. Mielenz*[1], B. Kuhla[2], H. Sauerwein[1], and H. Hammon[2], [1]*University of Bonn*, *Bonn, NRW, Germany*, [2]*FBN Dummerstorf*, *Dummerstorf, MV, Germany*.

Adiponectin, an adipocyte-derived hormone, is described as an insulin sensitizing agent in monogastric mammals. Less information is available about influences on the regulation of the adiponectin system in ruminant species. As well, the relevance of the β-hydroxybutyrate (BHB) sensing receptor *GPR109A* with anti-lypolytic properties is not characterized yet. We herein tested for differences in diverging phenotypes of the F2 offspring from segregated Charolais x German Holstein crosses exhibiting high differences in body fat accretion (FAT (n = 9) vs. LEAN (n = 9)). The animals were slaughtered at 100 d into 2nd lactation. The mRNA abundance of adiponectin (*Adi*) and its receptors *AdiR1* and *AdiR2* as well as of *GPR109A* was analyzed via real-time PCR in 3 different adipose depots and in liver, except for *Adi*. In liver, *AMPK* and phospho-*AMPK* protein were analyzed as well. Data were analyzed using independent samples *t*-test or Mann-Whitney U-test ($P \leq 0.05$). Milk production was much lower ($P \leq 0.05$) in FAT than LEAN cows. There was a trend ($P \leq 0.1$) for a higher *Adi* mRNA content in mesenteric fat of FAT vs. LEAN cows. *AdiR1* mRNA was more abundant in perirenal and mesenteric fat of FAT cows. For *AdiR2* mRNA there was a trend ($P \leq 0.1$) for higher values in subcutaneous fat of FAT cows but lower values were observed in liver. The content of *GPR109A* mRNA was lower in perirenial fat and as a trend in liver of FAT cows compared with LEAN animals. The ratio between phospho-*AMPK* and *AMPK* did not show any difference. In conclusion, the mRNA expression profile of the *Adi* system in adipose tissue of accretion type cows (FAT cows) seems related to increased lipid accumulation. Lower *AdiR2* abundance in liver of FAT cows might be related to specific features of glucose metabolism in this family but its relevance needs further characterization, as there was no activation of *AMPK* observed. Signal transduction by BHB through *GPR109A* in perirenal fat and liver might be of more relevance for secretion type LEAN cows.

Key Words: adiponectin system, GPR109A, cattle

T258 Changes in plasma concentrations of leptin in ewes during pregnancy. J. A. Daniel*[1], A. B. Milam[1], M. E. Gafnea[1], B. K. Whitlock[2], and D. H. Keisler[3], [1]*Berry College*, *Mount Berry, GA*, [2]*University of Tennessee*, *Knoxville,* [3]*University of Missouri*, *Columbia*.

Previous research has demonstrated circulating concentrations of leptin increase in ewes during mid pregnancy then decline in late pregnancy and early lactation. This study was designed to more narrowly define the timing of changes in circulating concentrations of leptin with pregnancy in ewes. Katahdin ewes (n = 19) located at latitude 34.275 and longitude −85.183 (Mount Berry, GA) were utilized. Blood samples were collected weekly via jugular veinpuncture beginning immediately before ram exposure on September 23 and continuing until 4 weeks post-lambing.

Ewes were exposed to a ram fitted with a marking harness for a 63 d breeding season. Breeding marks were recorded daily. Lambing date and number of lambs born was recorded. Week of gestation was calculated by breeding mark. The blood sample collected before breeding was considered wk 0. Plasma concentration of leptin was determined by radioimmunoassay. Data were tested for effects of date of sample, pregnancy status, and date of sample by pregnancy status interaction using procedures for repeated measures (JMP version 7; SAS Institute Inc., Cary, NC). Data were also tested for effects of week of gestation and number of lambs. Pregnancy had an effect on plasma concentrations of leptin ($P = 0.0407$; 6.06 ± 0.19 vs 4.67 ± 0.64 ng/ml in pregnant vs non-pregnant ewes, respectively). There was also an effect of date of sample ($P < 0.0001$) on plasma concentrations of leptin. Week of gestation had an effect of plasma concentrations on leptin ($P < 0.0001$) with ewes having lower plasma concentrations of leptin during wk 12, 13, 16, and 18–21 of gestation as well as 4 weeks after lambing when compared with before breeding. Plasma concentrations of leptin were higher wk 1–12, 14, 15, and 17 of gestation than after lambing, but did not differ from values before lambing. These data confirm a decline in circulating concentrations of leptin in the last third of gestation and continuing into early lactation in ewes.

Key Words: sheep, pregnant, leptin

T259 Nutritional regulation of body condition score at the initiation of the transition period in dairy cows on grazing conditions: hepatic expression of fatty acid metabolism genes. M. Carriquiry*[1], M. L. Adrien[2], V. V. Artegoitia[2], D. Mattiauda[1], and A. Meikle[2], [1]*School of Agronomy, UDELAR*, *Uruguay*, [2]*School of Veterinary Medicine, UDELAR*, *Uruguay*.

Multiparous Holstein cows (n = 10), blocked by body weight and expected calving date, were used to investigate the effect of different body condition score (BCS) at 30 d before calving (−30 d), induced by a differential nutritional management from −100 to −30 d, on hepatic expression of peroxisome proliferator-activated receptors-α (PPARA), carnitine palmitoyl transferase-1 (CPT1A), acyl-CoA dehydrogenase-very long-chain (ACADVL), and acyl-CoA oxidase (ACO) during the transition period. From −100 to −30 d, cows were offered different planes of nutritionwith 7, 14 or 20 kg/day/cow of dry matter (DM) of a long-term pasture to achieve desired BCS −30 d. BCS (scale 1–5) was determined every 15 d, and cows had to gain 0.5 points (HI) or to maintain (LO) BCS at least in 2 subsequent observations to be included in the study. From −30 to 45 d cows were managed together. Liver biopsies were collected at −15, 15, and 45 d and mRNA abundance was determined by real time PCR using hypoxanthine phosphoribosyltransferase (HPRT) as control gene. Means from repeated measure analyses differed when $P < 0.05$. Cows had similar BCS at −100 d (2.9 ± 0.08) and differed after the nutritional treatment (3.4 vs. 2.8 ± 0.08), but groups presented similar BCS at 15 (2.9 vs. 2.7 ± 0.08) and 45 (2.8 vs. 2.7 ± 0.08) d. NEFA concentrations increased around parturition and were greater in LO than HI cows. Expressions of PPAR (2.7 vs. 1.2 ± 0.45), ACADVL (3.0 vs. 0.9 ± 0.5), and ACO (149 vs. 64 ± 28) mRNA were greater for LO than HI cows along the period evaluated. There was an effect of day on CPT1A and ACO mRNA as their abundance was increased (>2-fold) at 15 d, effect that was more evident in LO cows. There was a trend ($P < 0.09$) for an interaction of treatment by day for ACADVL mRNA as its expression was increased at 45 d only in LO cows. Results indicated nutritional plane before the transition period affected regulation of hepatic fatty acid oxidation genes, being these genes upregulated, in agreement with greater NEFA levels, in cows that maintained BCS from −100 to −30 d.

Key Words: mRNA, liver, dry period nutrition

T260 Gluconeogenic enzymes are differentially regulated by fatty acid cocktails in Madin-Darby Bovine Kidney cells. H. M. White*, S. L. Koser, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Increases in serum NEFA levels and changes in serum fatty acid profiles at calving are characteristic of the transition cow. The objective of this study was to examine the effect of 24 h exposure of Madin-Darby Bovine Kidney cells to fatty acid cocktails on expression of pyruvate carboxylase (PC), cytosolic and mitochrondrial phosphoenolpyruvate carboxykinase (PEPCK-C and PEPCK-M), and glucose-6-phosphatase. Cocktails contained C14, C16, C18, C18:1, C18:2, and C18:3 fatty acids and were designed to mimic the fatty acid profile and concentration of cows pre- and post-calving (PPCALV; 0.25 m*M*) and at calving (CALV; 0.5 m*M*). An additional cocktail mimicked the profile of cows with induced fatty liver at calving (IFL; 1 m*M*). Expression of PC mRNA tended to increase ($P < 0.1$) in cells exposed to IFL (6.0 vs. 2.8 ± 1.0 arbitrary units, control vs. IFL, respectively). Expression of PEPCK-C mRNA was increased ($P < 0.05$) in cells exposed to PPCALV compared with all other cells (5.0 vs. 1.2 ± 0.8 arbitrary units, PPCALV vs. control, respectively). Exposure to IFL increased ($P < 0.05$) the ratio of PC to PEPCK-C by 8.4 and 2.4 fold compared with PPCALV and CALV exposure. Exposure of cells to IFL tended to increase ($P < 0.1$) PEPCK-M mRNA (3.0 vs. 1.7 ± 0.5 arbitrary units, IFL vs. control, respectively) and increased ($P < 0.05$) glucose-6-phosphatase mRNA (3.2 vs. 1.5 ± 0.8 arbitrary units, IFL vs. control, respectively). To elucidate effects of fatty acid profile from concentration, cells were exposed to each profile at lower and higher concentrations. Increased concentrations of PPCALV did not increase ($P \geq 0.05$) PEPCK-C mRNA expression as observed at physiological concentrations. Increasing concentration of CALV decreased ($P < 0.05$) expression of PEPCK-C and increased ($P < 0.05$) expression of PEPCK-M mRNA. Fatty acid profile and concentration alters expression of key gluconeogenic enzymes although the magnitude and directionality of the response was not uniform. Regulation of mRNA expression for these enzymes is likely part of the coordinated response in liver during transition to calving.

Key Words: transition cow, Madin-Darby Bovine Kidney cells, fatty acids

T261 The effects of leptin on phosphorylation of mTOR and rpS6 to signal protein synthesis in bovine mammary epithelial cells. E. K. Evans*, J. A. D. R. N. Appuhamy, and M. D. Hanigan, *Department of Dairy Science, Virginia Polytechnic Institute and State University*, *Blacksburg*.

Poor nitrogen utilization efficiency of dairy cows increases the nitrogen excretion into the environment. Efficiency of milk protein synthesis is regulated through cellular signaling pathways which are responsive to hormones, cellular energy status, and cellular amino acid supply. These signals result in phosphorylation of various signaling proteins including mammalian target of rapamycin (mTOR) and ribosomal protein S6 (rpS6) which regulate initiation of protein synthesis and control polypeptide elongation. Thin cows are known to produce less milk than well-conditioned cows. It was hypothesized that this effect could be mediated through leptin actions directly on mammary epithelial cells. The purpose of this experiment was to study the effects of leptin on the phosphorylation status of mTOR and rpS6 in MAC-T cells. Cells were seeded into 6-well plates at a density of 90,000 cells per well, starved in media containing 20% of normal DMEM essential amino acid concentrations and devoid of fetal bovine serum (FBS) for 12 h and subsequently cultured for 2 h in media without or with 160 ng/mL leptin. Each treatment was replicated trice and each replicate consisted of cells in 3 wells. The cells were lysed in the presence of phosphatase and protease inhibitors

and samples were analyzed by Western immunoblotting to determine the phosphorylation status of mTOR(Ser2448) and rpS6 (Ser235/236). The membranes were first probed for the phosphorylated forms of each protein and subsequently probed for the total forms of each. Statistical analysis of immunoblotting results showed no significant difference between the phosphorylation of mTOR in cells treated with or without leptin, but leptin treatment did cause a statistically significant increase in phosphorylation of rpS6 (67%). These results suggest that leptin acts on rpS6 independent of the Ser2448 site on mTOR. Stimulation of rpS6 should stimulate ribosomal biogenesis which would lead to increased protein synthesis, thus the results are supportive of a direct role of leptin on milk protein synthesis.

Key Words: leptin, protein synthesis, signaling proteins

T262 Glucocorticoid regulation of chicken adipose triglyceride lipase in adipose tissue. J. Serr*, S. Shin, Y. Suh, M. Kim, D. Latshaw, and K. Lee, *The Ohio State University, Department of Animal Sciences*, *Columbus*.

The mechanism of adipose tissue lipolysis is one that has not been fully elucidated. Increasing our understanding of this process would allow for increased feed efficiency and reduced fat content, which would lower feeding costs for poultry production. Adipose triglyceride lipase (ATGL) is an adipose-specific enzyme which cleaves at the Sn-1 position of triglycerides, releasing non-esterified fatty acids (NEFA) into the bloodstream. Glucocorticoids have been proven to elevate the level of circulating NEFAs. To determine the regulation of ATGL by glucocorticoid, 30 Ross 308 broilers received a 200 μL intraperitoneal injection of dexamethasone (4 mg/kg). Saline was administered to an additional 12 birds to determine any effect of stress during handling and injection. Another 6 birds received no treatment and were harvested as a control. Dexamethasone-injected birds were harvested at 0.5, 1, 2, 4, and 6 h after treatment; saline-treated birds were collected at 4 and 6 h (6 per time point). Adipose tissue was collected from abdominal and subcutaneous depots. Blood samples were collected via cardiac puncture. Gene and protein expression were analyzed via quantitative real-time PCR (qRT-PCR) and Western blot, respectively. In comparison with the saline-treated group, ATGL mRNA and protein was increased in broilers injected with dexamethasone, demonstrating that any response of ATGL expression to the stress of handling was minimal compared with that of hormone treatment. When dexamethasone response was observed against the untreated group up to 2 h following injection, an increase in ATGL protein was observed as quickly as 0.5 h and increased further at 1 and 2 h, demonstrating an acute response. Additionally, plasma NEFA analysis was done to confirm the release of free fatty acids into the blood. Plasma NEFA increased gradually from 0 to 6 h, and reached statistical significance (Tukey's Test) at 4 h (concurrent with mRNA and protein expression of ATGL). These data show that ATGL expression and activity is positively regulated by glucocorticoid in a time-dependent manner.

Key Words: chicken, lipolysis, glucocorticoid

T263 Bovine acute-phase response following corticotrophin-releasing hormone (CRH) infusion. R. F. Cooke*, A. B. Scarpa, F. M. Nery, F. N. T. Cooke, and D. W. Bohnert, *Oregon State University - EOARC*, *Burns.*

The objective of this study was to evaluate plasma concentrations of cortisol, ACTH, acute-phase proteins, and pro-inflammatory cytokines in beef steers following CRH infusion. Six weaned, halter-trained Angus steers (BW = 163 ± 7.0 kg; age = 203 ± 5.8 d) were fitted with indwelling jugular catheters on d −1 of the study, and assigned to receive intravenously 0.1 μg of bovine CRH/kg of BW on d 0 of the study. Blood samples were collected every hour via jugular catheters from −2 to 8 h, and every 6 h via jugular venipuncture from 12 to 72 h relative to CRH infusion (0 h). Steer rectal temperature was assessed concurrently with each blood collection. Samples collected from −2 to 8 h relative to CRH infusion were analyzed for plasma concentrations of interleukin (IL)-1 and 6, tumor necrosis factor (TNF)-α, interferon (IFN)-γ, cortisol, ACTH, ceruloplasmin and haptoglobin, whereas samples collected from 12 to 72 h were analyzed for plasma concentrations of ceruloplasmin and haptoglobin only. Data were analyzed with the MIXED procedure of SAS. Plasma ACTH and cortisol concentrations peaked ($P \leq 0.02$) 1 h following CRH infusion, and returned to basal levels at 2 and 4 h following infusion ($P > 0.15$), respectively. Body temperature peaked at 2 and 8 h following infusion ($P < 0.01$), and returned to basal levels after 12 h ($P = 0.40$). Contrasted to all other sampling hours, plasma IFN-γ concentrations were greater ($P = 0.03$) at 1 and 5 h, plasma IL-6 concentrations were greater ($P = 0.04$) from 4 to 6 h, plasma IL-1 concentrations tended ($P = 0.12$) to be greater from 6 to 8 h, and plasma TNF-α concentrations were greater ($P = 0.03$) from 5 to 7 h following infusion. Plasma ceruloplasmin and haptoglobin concentrations increased linearly ($P \leq 0.01$), and peaked at 54 and 66 h following CRH infusion, respectively. In conclusion, infusion of CRH at 0.1 μg/kg of BW increased plasma concentrations of ACTH and cortisol, and stimulated the acute-phase response in beef steers.

Key Words: acute-phase response, corticotrophin-releasing hormone, beef cattle