70 Adipose triglyceride lipase is a novel lipase in dairy cattle. D. Elkins* and D. Spurlock, *Iowa State University*, *Ames*.

Dairy cattle undergo negative energy balance during early lactation when increased energy requirements are not offset by energy intake. As a result, cows in negative energy balance mobilize adipose tissue to help meet their energy requirements. Excessive mobilization of adipose tissue has been associated with reproductive and health problems. Traditionally, phosphorylation of hormone-sensitive lipase (HSL) by protein kinase A has been viewed as the rate-limiting step of lipolysis. More recently, adipose triglyceride lipase (ATGL) has been identified as an additional lipase capable of hydrolyzing triglycerides to diglycerides. The objective of this research was to determine if ATGL is expressed in adipose tissue of lactating dairy cows, and to determine if its expression differs with stage of lactation. Adipose tissue was biopsied from 10 early [5-14 DIM] and 10 mid [176-206 DIM] lactation multiparous Holstein cows. Semi-quantiative western blots were used to evaluate HSL, phosphorylated HSL, and ATGL protein using commercially available antibodies. Blood samples were collected for analysis of lipolytic indicators, nonesterified fatty acid (NEFA) and glycerol. As expected, glycerol and NEFA were significantly greater in early compared to mid lactation cows ($P<0.0001$), confirming a significant difference in lipolytic activity between stages. Expression of phosphorylated HSL, but not total HSL, was significantly greater in early compared to mid lactation cows ($P=0.001$). Additionally, phosphorylated HSL was highly correlated with NEFA ($r=.66$; $P=0.03$) and glycerol ($r=.77$; $P=0.006$). ATGL protein was detected in adipose tissue of early and mid lactation cows, and its expression was significantly greater in mid compared to early lactation cows ($P=0.002$). ATGL expression was not significantly correlated with NEFA or glycerol. These results confirm that ATGL is expressed, but likely makes a small relative contribution to lipolysis in early lactation cows. Increased expression of ATGL in mid lactation may indicate a more significant role for ATGL in basal lipolysis during positive energy balance.

Key Words: Dairy, Lipase, Adipose

71 Peripartal changes of adiponectin, adiponectin receptor 1, adiponectin receptor 2, leptin and leptin receptor mRNA expression in subcutaneous adipose tissue of dairy cows. A. Lemor, M. Mielenz, A. Hosseini, and H. Sauerwein*, *University of Bonn*, *Germany*.

The transition from pregnancy to lactation is of critical importance for health, production, and profitability of the dairy cow. Within the homeorhetic regulation of the adaptation to the metabolic changes involved, the adipocytokine adiponectin is attracting increasing interest due to its role in lipid metabolism, insulin resistance and glucose homeostasis. Data on the expression of adiponectin and its receptors, adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2) as well as comparisons with the leptin system are mainly limited to monogastric species, whereas for ruminants corresponding data are lacking. We therefore aimed to characterize the mRNA expression for these adipokines and their receptors during the transition period in sc adipose tissue in dairy cows.

Biopsies from sc fat were taken at the tail head from 10 dairy cows (Holstein-Frisian) first between 13 - 2 d antepartum (AP) and second between 20 - 23 d postpartum (PP). Total mRNA was extracted and purified by spin columns. The mRNA expression of adiponectin, AdipoR1, AdipoR2, leptin and leptin receptor (obRb) was quantified by real-time RT-PCR. Leptin protein was measured via ELISA in blood samples collected at the biopsy dates. Data were analysed by Wilcoxson Matched-Pairs Signed-Ranks Test and Spearman Rank Order Correlation (SPSS).

For adiponectin mRNA time-related changes were not detectable, whereas for AdipoR1 and AdipoR2 the PP expression was reduced ($P\leq0.05$) to 57% of the AP values. Serum leptin was decreased ($P\leq0.05$) PP, the analogous decrease in leptin mRNA did not reach the level of significance; obRb mRNA expression was 2.6-fold higher ($P\leq0.05$) PP compared to AP. Positive correlations between AdipoR1 and AdipoR2 ($r=0.82$, $P\leq0.05$) were observed.

Our results indicate that the local effects of adiponectin and leptin in fat may differ between pregnancy and early lactation whereby the difference seems to be mediated at the receptor level rather than at the level of the ligand.

Key Words: Adiponectin, Leptin, Transition Cow

72 Propionate effects on the mRNA expression of adiponectin in two adipose depots and its receptors AdipoR1 and AdipoR2 in liver, skeletal muscle and adipose tissue of goats. M. Mielenz*, C. Seybold, A. Lemor, and H. Sauerwein, *University of Bonn*, *Germany*.

Adiponectin, an adipocyte-derived hormone, is an insulin sensitizing agent in monogastric mammals. In contrast to man, less information is available concerning the importance of the adiponectin system in ruminants. Propionate increases insulin secretion in ruminants but also acts directly through short chain fatty acid (SCFA) sensitive receptors (GPR41/43) in monogastrics. Here we characterize the effects of iv infused propionate on adiponectin and its receptors AdipoR1 and AdipoR2 in goats. Castrated male goats (Deutsche-Edelziege, 10 to 12 mo old) were allocated to infusions through jugular catheters after an over-night fast. They received propionate infusions (96 µmol/kg/min, pH 7.4; n = 4) or NaCl-solution of the equivalent Na-concentration (n = 5). Infusions were carried out for 260 min. The mRNAs for adiponectin, AdipoR1 and AdipoR2 were quantified by real-time RT-PCR after euthanasia in perirenal and subcutaneous adipose tissue. The receptor mRNAs were also tested in liver and semitendinosus muscle. Data analysis showed no effect of propionate on adiponectin mRNA in both adipose tissues. AdipoR1 and AdipoR2 tended to increase in subcutaneous ($P=0.087$ and $P=0.092$, respectively) but not in perirenal adipose tissue after propionate infusion. In liver, AdipoR1 increased ($P=0.01$) and AdipoR2 tended to increase ($P=0.068$) by stimulation with propionate. No effect was observed in skeletal muscle.

There was no effect on adiponectin mRNA in adipose tissue, at least in the small number of animals examined. With the exception of muscle, the mRNAs for both receptors were up-regulated in the different tissues analyzed. This effect might have been mediated by a general increase in energy intake, by insulin effects and/or signal transduction through SCFA sensitive receptors. The relevance of the propionate effects on the adiponectin system in ruminants has to be clarified in the future, as well as the role of the SCFA sensitive receptors in ruminants.

Key Words: Adiponectin, Propionate, Goat

73 Effect of ghrelin or obestatin continuously infused to dairy cows on grazing and ruminating behaviour and plasma hormone and metabolite concentrations. J. R. Roche*[1], A. J. Sheahan[1], D. P. Berry[2], L. Chagas[1], D. Blache[3], and J. Kay[1], [1]*DairyNZ*, *Hamilton, New Zealand*, [2]*Teagasc Moorepark*, *Fermoy, Ireland*, [3]*University of Western Australia*, *Perth, Australia*.

Fifty-one cows were randomly allocated to one of three groups; a control and cows continuously infused with either 0.74 μmol/d of ghrelin or obestatin sc. Infusions began 20 DIM and treatments continued for 8 wk. During wk 6 and 8, cows were observed over four 24-hr periods and grazing behaviour noted every 10 min. During wk 7, blood samples were collected every 4 h over 2 d, with the bleeding schedule staggered by 2 h on d 2 to ensure a blood sample was collected every 2 h of the 24 h period. Where necessary, data were transformed to be normally distributed. The effect of treatment on grazing behaviour and the concentration of blood metabolites and hormones was determined using mixed models, with cow included as a repeated effect. The concentration of metabolites varied with time. Ghrelin-infused cows had greater ($P<0.01$) plasma growth hormone concentrations than either control or obestatin cows, consistent with ghrelin′s role as a growth hormone secretagogue. However, this difference was only evident during a long period of ingestive inactivity between midnight and 0800 h, suggesting a possible role for ghrelin in growth hormone secretion during periods of negative energy balance. Leptin concentrations were greater ($P<0.001$) in ghrelin than obestatin cows, with control cows intermediate. In comparison, NEFA concentrations increased ($P<0.001$) from control to ghrelin to obestatin, although the effect is of little biological significance (0.09, 0.10, and 0.11 mmol/L, respectively). Plasma ghrelin concentrations were not affected by treatment, but plasma glucose was less in ghrelin- and obestatin-infused cows than control cows. Treatments did not affect time spent grazing or ruminating, although the length of the first grazing bout was longer in ghrelin and obestatin cows between the am and pm milking. Research is required to confirm or deny the role of ghrelin or obestatin in the feeding behaviour of grazing dairy cows.

Key Words: Ghrelin, Obestatin, Grazing Behavior

74 Evaluation of insulin-like growth factor-I and temperament as selection tools in Brahman heifers. L. C. Caldwell*[1,3], R. O. Dittmar III[1,3], T. D. A. Forbes[2], T. H. Welsh Jr.[1], and R. D. Randel[3], [1]*Texas AgriLife Research*, *College Station, TX*, [2]*Texas AgriLife Research and Extension Center*, *Uvalde, TX*, [3]*Texas AgriLife Research and Extension Center*, *Overton, TX*.

In an effort to detect feed efficient beef cattle, researchers are exploring the use of insulin-like growth factor-I (IGF-I) concentrations and temperament scoring as tools for selection. Studies have revealed that temperament can affect ADG. Reports from Johnston et al. (2002) suggest that concentrations of IGF-I may be associated with the prediction of residual feed intake (RFI) in beef cattle. However, when tested in Brangus females, Lancaster et al. (2007) failed to find a correlation between IGF-I concentration and RFI. The purpose of this study was to assess the relationship of IGF-I concentrations and temperament with RFI and ADG in Brahman heifers. Using a Calan gate system, 3 separate Brahman heifer calf crops from 2004 (n = 31; 16-19 mo), 2005 (n = 50; 10.5-13.5 mo) and 2006 (n = 56; 5-8 mo) were limit fed a complete ration at 2.2% BW in 70-d feeding trails. Performance and feed intake data were collected throughout the trials to determine feed efficiency. Temperament, determined by exit velocity and pen score, was evaluated at weaning. IGF-I concentrations were determined by RIA from samples collected on d 0 and 70. Correlations between IGF-I and RFI were weak ranging from r = -0.064 to 0.222 ($P > 0.05$). Temperament had no significant effect ($P > 0.05$) on RFI. The 2004 heifers categorized by temperament as calm, moderate or excitable showed mean RFI of 0.029 ±0.08, -0.178 ±0.16 and 0.111 ±0.09, respectively. Year 2005 heifers averaged -0.001 ±0.01, -0.004 ±0.01 and -0.007 ±0.01. The 2006 heifers averaged -0.002 ±0.02, -0.018 ±0.02 and 0.021 ±0.02. Weak correlations were seen between IGF-I and ADG (r = -0.056 to 0.311) ($P > 0.05$). Correlations between ADG and temperament were significant. The 2004 heifers categorized by temperament had ADG of 0.678 ±0.05, 0.628 ±0.04 and 0.647 ±0.04 ($P = 0.07$), respectively. Year 2005 heifers averaged 0.88 ±0.05, 0.745 ±0.03 and 0.791 ±0.04 ($P = 0.07$). The 2006 heifers averaged 0.579 ±0.03, 0.606 ±0.03 and 0.481 ±0.04 ($P = 0.03$). These results suggest that IGF-I concentration is unrelated to RFI; however, temperament may be associated with ADG in Brahman heifers.

Key Words: IGF-I, Residual Feed Intake, Brahman

75 Variation in metabolic regulation in the liver of dairy cows during the dry period and in early lactation. H. A. van Dorland*[1], S. Richter[1], I. Morel[2], and R. M. Bruckmaier[1], [1]*Veterinary Physiology, Vetsuisse Faculty, University of Bern*, *Bern, Switzerland*, [2]*Agroscope Liebefeld-Posieux (ALP)*, *Posieux, Switzerland*.

The metabolic status and simultaneously the hepatic regulation during the dry period, after partum, and in early lactation were studied in dairy cows. Liver biopsies were obtained from 28 cows in wk 10 antepartum (a.p.), and on d 1, and in weeks 4 and 14 postpartum (p.p.). Blood samples were collected every two wk during this period. Liver samples were analyzed for mRNA expression levels of hepatic factors of the carbohydrate-, lipid metabolism, and citric acid cycle (PEPCKc, PEPCKm, PC, ACSL, CPT 1A, CPT 2, ACADVL, HMGCS1, HMGCS2, PPARα, PPARγ, SREBF1, ACLY, and CS). Blood plasma was assayed for concentrations of glucose, BHBA, NEFA, cholesterol, triglycerides, insulin, IGF-1, T3, and T4. For evaluation, cows were divided into 2 groups based on the plasma concentration of β-hydroxybutyric acid (BHBA) at four weeks p.p. (group H, BHBA >0.75 mmol/L; group L, BHBA <0.75 mmol/L, respectively). In both groups, plasma parameters followed a pattern usually observed in dairy cows. However, changes were moderate and the energy balance in cows turned positive in week 7 p.p. Significant group effects were found in week 10 a.p., when plasma concentration of triglycerides was higher in L than in H, and in week 4 p.p., when plasma concentration of glucose and IGF-1 was lower in H than in L. Similarly, moderate changes in mRNA expression of hepatic factors between the different time-points were observed. One significant group difference was found in week 10 a.p., when a higher relative expression of mRNA of PEPCKc was observed in H than in L. Significant Spearman Rank Correlation Coefficients between the variables were not similar at each time-point and were not similar between the groups at each time-point, suggesting that metabolic regulation differs between cows. Conclusive, metabolic regulation in dairy cows is obviously a dynamic system, and differs between cows at different metabolic stages related to milk production.

Key Words: Metabolism, Liver, Gene Expression

76 Mild metabolic acidosis in sheep alters renal and skeletal muscle expression, but not liver, of amino acid enzymes and transporters. Y. Xue*[1], S. F. Liao[1], S. Greenwood[2], B. W. McBride[2], J. A. Boling[1], and J. C. Matthews[1], [1]*University of Kentucky*, *Lexington*, [2]*University of Guelph*, *Guelph, ON, Canada*.

In metabolic acidotic rodents and humans, the altered expression of glutamine (Gln), glutamate (Glu), and alanine (Ala) metabolizing enzymes and transporters by the kidney, skeletal muscle, and liver is important to maintain blood acid-base balance. To test the hypothesis that mild metabolic acidosis (acidosis) induces an analogous response in sheep, the relative content of mRNA, protein, or mRNA and protein, for 6 metabolizing enzymes and 5 plasma membrane transporters of Gln/Ala/Glu was determined by real-time PCR and immunoblot analyses in homogenates of kidney, skeletal muscle, and liver of control (n = 5) vs acidotic (n = 5) sheep (Las et al., 2007, J. Anim. Sci. 85:2222). In kidney, where Gln use is increased in acidotic rodents, SN1 (Gln transporter) mRNA content was 790% greater (P=0.05), whereas Gln synthetase protein and mRNA was 56 (P=0.01) and 43% (P=0.04) lower, respectively, in acidotic sheep. In contrast, no change in phosphoenolpyruvate carboxykinase (protein and mRNA), renal glutaminase (mRNA), or Glu dehydrogenase (protein) was found, although their expression is highly increased in acidotic rodents. In skeletal muscle, a net releaser of Gln and net user of Ala in acidotic sheep, the protein content of aspartate transaminase was 101% greater (P=0.03) while Glu dehydrogenase tended to be (P=0.11) 33% greater in acidotic sheep. However, no change in the content of Ala transaminase protein, SNAT2 (Ala,Gln transporter) mRNA, or 3 Glu/aspartate transport proteins was found. In acidotic rodents, the liver is a net releaser of Gln. However, the mRNA or protein content of any enzyme or transporter did not differ in control vs acidotic sheep. These results indicate that the potential for aspartate and Glu use by skeletal muscle, and Gln absorption by the kidneys, is increased in mildly acidotic sheep, whereas liver capacity for Gln, Ala, and Glu metabolism remains constant.

Key Words: Acidosis, Amino Acid Metabolism, Sheep

77 Effects of prepartum 2,4-thiazolidinedione on metabolism and performance of transition dairy cows. K. L. Smith*, W. R. Butler, and T. R. Overton, *Cornell University*, *Ithaca, NY*.

Thiazolidinediones (TZD) are potent, synthetic ligands for peroxisome proliferator activated receptor-gamma (PPAR-γ) that reduced plasma concentrations of NEFA and increased dry matter intake (DMI) during the peripartum period in a previous experiment. Data from Holstein cows (n = 31) entering second or greater lactation were used to determine whether late prepartum administration of TZD would affect periparturient metabolism and milk production. Cows were administered 0, 2.0, or 4.0 mg TZD/kg BW by intrajugular infusion once daily beginning 21 d before expected parturition until parturition. Plasma samples were collected daily from 22 d before expected parturition through 21 d postpartum and twice per week from wk 4 through 9 postpartum. With increasing doses of TZD prepartum, plasma NEFA concentrations decreased linearly during the peripartum (P = 0.01) and postpartum (P = 0.02) periods (d –7 to d +7; 268, 246, 193 ± 22 μEq/L and d 0 to d +21; 342, 296, 224 ± 35 μEq/L, respectively). Postpartum liver triglyceride content was decreased linearly (11.0, 9.1, 4.7 ± 1.9%; P = 0.02) and glycogen content increased linearly (2.16, 2.26, 2.75 ± 0.19%; P = 0.02) with prepartum TZD administration. Peripartal DMI (16.2, 17.6, 17.6 ± 0.5 kg/d) was increased linearly (P = 0.04) by TZD administration. Cows administered TZD prepartum had increased (P = 0.001) postpartum BCS (wk 1 through 9). Yields of 3.5% fat-corrected milk were affected quadratically (P = 0.04) by prepartum TZD administration (52.4, 54.1, 47.0 ± 1.8 kg/d). Generally, cows administered 4.0 mg TZD/kg BW had the lowest (P < 0.10) milk component yields. Prepartum TZD administration linearly decreased (P = 0.03) the number of days to first ovulation (28.9, 25.0, 18.1 ± 3.6 d) and increased (P = 0.05) the number of cows ovulating on or by d 21 postpartum. These results indicate that prepartum administration of TZD improves metabolic health and DMI of periparturient dairy cows, decreases reliance on body fat reserves, and may promote return of ovarian function during early lactation.

Key Words: Transition Cow, Thiazolidinedione, PPAR-γ

78 Glutamine synthetase is up-regulated in the liver of old beef cows by estradiol implants. E. D. Miles*[1], B. W. McBride[2], K. R. Brown[1], K. K. Schillo[1], J. A. Boling[1], and J. C. Matthews[1], [1]*University of Kentucky*, *Lexington*, [2]*University of Guelph*, *Guelph, ON, Canada*.

Previous research in our laboratory has shown that old beef cows have reduced hepatic expression of glutamine synthetase (GS) and alanine transaminase (ALT), two enzymes in the liver that are critical for optimal N recycling. This experiment was conducted to characterize the effect of supplemental estrogen on these two proteins and other indicators of hepatic glutamate metabolic capacity. Fourteen old (> 10 yr) nonpregnant beef cows were housed in a dry lot with ad libitum access to alfalfa hay and water for 28 d. On d 1 of the study, cows received (n = 7/group) either a sham (Control) or Compudose (25.7 mg estradiol; Elanco Animal Health) implant. On d 14 and 28, jugular blood and liver biopsy samples were collected. The effects of estrogen treatment (TRT), time after implant (DAY), and their interaction (TRT × DAY) were assessed by ANOVA, using the repeated measures option of PROC MIXED (SAS). Plasma estrogen concentration of implanted cows (5.07 pg/mL) was 222 % more (P = 0.01) than for control cows (1.5 pg/mL). Plasma ammonia, and serum urea N levels, aspartate transaminase (AST), and ALT concentrations were not affected ($P \geq 0.21$). Immunoblot analyses were performed to quantify the relative liver content of GS, ALT, AST, glutamate dehydrogenase (GDH), and two System X-AG transporters (GLT-1 and EAAC1). For GS protein content, TRT (P = 0.01), DAY (P = 0.03), and TRT x DAY (P = 0.03) effects were observed. Specifically, GS content was increased 350% by d 14 (P = 0.002) and 200% by d 28 (P = 0.05). In contrast, the protein content of ALT ($P \geq 0.37$), AST ($P \geq 0.71$), GDH ($P \geq 0.21$), GLT-1 ($P \geq 0.43$), or EAAC1 ($P \geq 0.19$) was not affected by estrogen implant. Hepatic content of GTRAP3-18, an inhibitor of EAAC1 activity, also was not altered ($P \geq 0.36$). These results indicate that the hepatic expression of GS in old beef cows is sensitive to stimulation by supplemental estrogen, whereas expression of other proteins that support hepatic glutamate metabolism is not.

Key Words: Aging, Estrogen, Nitrogen Metabolism