performance of nursery pigs fed diets with or without antibiotics. Weanling pigs (n = 100; 7.44 kg BW) were assigned to one of four dietary treatments with six replications per treatment and three, four, or five pigs per pen. The ADG, ADFI, and G/F were measured at d 7, 14, and 21. Pigs fed diets containing AS had increased (P < 0.05) ADG from d 0 to 7 and d 14 to 21, and overall when compared with pigs fed diets without antibiotic.

Key Words: Pigs, Alligator, Antibiotic

M244 Post-weaning development of the microbiota composition and activity in piglets fed diets with wheat bran, wheat middlings or sugar beet pulp. F. Molist\*, A. Gómez de Segura, E. G. Manzanilla, J. Gasa, R. G. Hermes, and J. F. Pérez, *Universitat Autònoma de Barcelona, Spain.* 

To determine the effect of dietary fibrous ingredients on intestinal microbial population and activity, 48 early-weaned (24-25 days) pigs were divided into six dietary treatments: a standard diet (STD) based on corn, barley and soybean protein; or high fibrous diets obtained by replacing some major ingredients of STD with 8% Wheat bran (WB), 8% Wheat middlings (WM), 6% Sugar beet pulp (SBP), 4% Wheat bran and 3% Sugar beet pulp (WB-SBP), or 4% Wheat middlings and 3% Sugar beet pulp (WM-SBP). After 10 and 15 days receiving ad libitum the experimental diets, animals were weighed, sacrificed, and digesta samples from the caecum, colon and rectum were taken. Short-chain fatty acid (SCFA) concentration was determined and microbial counts for enterobacteria and lactobacilli were determined by quantitative-PCR. Inclusion of WB promoted increases in the weight gain of the animals at day 15 (142, 1256, 136, 1164, 700, 1187 g for STD, WB, WM, SBP, WB-SBP, WM-SBP). Wheat bran, either at 8% or 4% promoted a decrease in enterobacteria counts in the caecum (11.1, 9.9, 11.7, 10.8, 8.2 and 11.6 log 16 S rDNA gene copies/g FM) and feces (10.3, 9.2, 11.5, 10.9, 8.7, 11.9 log 16 S rDNA gene copies/g FM for STD, WB, WM, SBP, WB-SBP, WM-SBP, respectively, P≤0.001) of piglets 15 days after weaning. No significant differences were observed on the lactobacilli counts at d15 or on the microbial counts at d10. The SCFA concentrations increased significantly from d10 to d15 in the caecum and feces. Diets supplemented with SBP decreased the isoacids (isobutyrate and isovalerate) proportions in the caecum of piglets at day 10 after weaning (P $\leq$ 0.003) and in the feces at day 15 post-weaning (P $\leq$ 0.004), while the inclusion of WB significantly increased the percentage of butyrate in the caecum at d15 (7.14, 14.22, 5.39, 4.63, 12.36, 11.48 %

for STD, WB, WM, SBP, WB–SBP, WM–SBP, respectively,  $P \le 0.001$ ). Results suggest a beneficial shift in the composition and activity of the hindgut microbial population of early weaned piglets fed on diets supplemented with WB.

Key Words: Piglets, Dietary Fiber, Microbial Population

M245 Dietary preference for methionine sources in 8 to 25-kg nursery pigs. T. Ettle<sup>1</sup>, M. Rademacher<sup>2</sup>, F. X. Roth<sup>3</sup>, and R. L. Payne<sup>\*2</sup>, <sup>1</sup>BOKU University, Vienna, Austria, <sup>2</sup>Degussa, Hanau, Germany, <sup>3</sup>Technical University of Munich, Munich, Germany.

Feed intake during the nursery period of growth is crucial to a pig's growth and development. As such, there is considerable interest about how the ingredients used in a typical nursery pig diet influence feed intake, and if a pig can demonstrate a preference for a particular ingredient. Therefore, the objective of this study was to investigate the dietary preferences of nursery pigs given the choice of diets supplemented with either DL-Met (DLM, 99%) or liquid Met hydroxy analogue (MHA-FA, 88%), which are the commonly used sources of supplemental Met. Mixed-sex pigs with an initial body weight of  $8.1 \pm$ 1.0 kg were randomly subdivided into 8 groups of 12 animals each for a 5-wk choice feeding trial. Groups 1 through 4 were fed one of the following treatments: 1) a Met-deficient basal diet (0.25% Met, 0.32% Cys, and 1.36% Lys); 2-3) diet 1 plus 0.1 or 0.2% DLM; or 4) diet 1 plus 0.113% MHA-FA. Treatment groups 5 through 8 were able to choose from pairs of treatment diets, and their treatments were: 5) diet 1 plus 0.1% DLM or 0.113% MHA-FA; 6) diet 1 plus 0.1% DLM or 0.152% MHA-FA; 7) diet 1 plus 0.2% DLM or 0.225% MHA-FA; or 8) diet 1 plus 0.2% DLM or 0.305% MHA-FA. Pigs fed treatments 5 and 6 had higher (P < 0.05) ADFI than those fed the Met-deficient basal diet. Pigs in treatment groups 2 through 8 had improved (P <0.05) ADG and feed:gain compared with those fed the Met-deficient basal diet. In the non-choice groups (treatments 1 through 4), feed intake was not influenced (P > 0.05) by Met source or concentration. However, in the choice-fed groups, pigs fed treatments 5, 7, and 8 consumed a higher percentage (P < 0.05) of their total feed intake from the diets containing DLM than they did from the diets containing liquid MHA-FA. In this trial, pigs expressed a dietary preference for DL-Met when given a choice of supplemental Met sources. Furthermore, this preference may be the result of sensory properties of the diets offered to the nursery pig.

Key Words: Ingredient, Methionine, Pig

## Physiology & Endocrinology - Livestock and Poultry: Endocrinology and Metabolism

M246 Hormonal response of bulls to glucose challenge in a segregating family structure. R. Pfuhl\*, O. Bellmann, F. Schneider, C. Kühn, and K. Ender, *Research Institute for the Biology of Farm Animals (FBN), Dummerstorf, Germany.* 

Cattle of the accretion type (Charolais) and the secretion type (Holstein) differ in their hormonal regulation of nutrient utilization. To deepen the insight in the physiological backgrounds, 65 F<sub>2</sub> cross bulls from five Charolais grandfathers and Holstein grandmothers were investigated to test a potential segregation of their glucose-induced insulin response. Growing bulls showed the highest hormonal activity at the age of eight months due to the onset of puberty. Thus, bulls of five families were

subjected to a glucose challenge test at this age. Every bull received a glucose solution intravenous  $(1g/kg BW^{0.75})$  via catheter into the jugular vein. Blood samples were taken in the same way before and 7, 14, 21 and 28 minutes after glucose administration. Serum insulin and glucagon concentrations as well as the glucose concentration in the whole blood were recorded. The data were evaluated with the GLM procedure of SAS. All tested bulls showed a characteristic glucose clearance curve (P=0.9832). In contrast, the insulin response curve differed and showed high variation between and within the five families. The average serum insulin concentration of family 4 reached greatest values and decreased slower, than in the other families.

Significant differences between the families were found in the serum glucagon concentrations before (P=0.025) as well as 21 minutes (P=0.0098) and 28 minutes (P=0.0063) after glucose infusion. Family 4 showed also the highest average serum glucagon levels before (P=0.05), 7 minutes (P=0.0806), 21 minutes (P=0.0098) and 28 minutes (P=0.0063) after glucose infusion. In conclusion, glucose-challenge test revealed a segregation of energy utilizing mechanisms in  $F_2$  cross bulls. Of particular concern are the bulls of family 4, which shows criteria of a possible insulin resistance and will be subjected to further investigations.

Key Words: Glucose, Insulin, Cross Bulls

M247 Growth hormone on metabolic profile of Nellore bulls of two different ages. L. S. Amorim<sup>\*1,4</sup>, C. A. A. Torres<sup>1</sup>, E. A. M. Amorim<sup>1,4</sup>, J. M. Silva Filho<sup>2</sup>, J. D. Guimaraes<sup>1</sup>, M. M. N. F. Oliveira<sup>3</sup>, K. Zorzi<sup>1</sup>, and G. R. Carvalho<sup>1</sup>, <sup>1</sup>Federal University of Vicosa, MG, Brazil, <sup>2</sup>Federal University of Minas Gerais, Belo Horizonte, MG, Brazil, <sup>3</sup>University of Diamantina, MG, Brazil, <sup>4</sup>Colorado State University, Fort Collins.

The objective of this study was to evaluate the effects of Growth Hormone (recombinant bovine Somatotropin (r-bST)) on the profiles of blood metabolites from two different ages of Nellore bulls. Sixteen bulls were allocated in a factorial arrangement  $2 \times 2$  (ages: young and adults; and r-bST: 0 and 500 mg) with four animals per treatment. The mean age of the young and adult animals was of 13.37 and 20.62 months. Four animals per treatment received every 14 days, saline solution or 500 mg of r-bST, with a total of nine injections per animal, during a 120 day experiment. Bulls were fed corn silage and concentrated diet on the base of corn crumb and soy, twice a day, supplied in individual stalls. Blood was collected every three days for metabolic evaluation. The datas were analized by ANOVA using Tukey test for to compare medias. The statistical analyses of the data were done for three applications, considering three periods (period 1, 2 and 3). The non-esterified fatty acid (NEFA) concentrations were analyzed by week. Serum cholesterol, total protein and glucose levels were affected by period and by treatment (P<0.05). The NEFA levels were affected by the weeks of collection (P < 0.05) but not by r-bST treatment (P>0.05). The GH change blood metabolic of young and adult bulls.

Key Words: Bulls, Growth Hormone, Metabolic

**M248** Leptin expression in early- and late-maturing *Bos indicus* heifers. L. F. P. Silva<sup>\*1</sup>, A. Vaiciunas<sup>1</sup>, and L. L. Coutinho<sup>2</sup>, <sup>1</sup>University of São Paulo, Pirassununga, SP, Brazil, <sup>2</sup>University of São Paulo, Piracicaba, SP, Brazil.

The process of maturation of the hypothalamus and the metabolic signal that triggers puberty are not well understood. Clearly, body weight and adiposity influence age at puberty, and leptin has been proposed to have a permissive role on puberty. Our objective was to test whether early-maturing *Bos indicus* heifers had a higher expression of leptin mRNA in adipose tissues. Among a population of 500 heifers between 20 and 25 months of age, 100 heifers were selected based on breed attributes (Nelore), month of birth, and body weight (280 to 300 kg). These 100 heifers were scored as prepubertal or pubertal according to the presence or not of a palpable corpus luteum (CL). Ten

heifers without a CL and ten heifers with a CL received a prostaglandin injection, and according to visual observation of heat and rectal palpation, 6 prepubertal and 6 pubertal heifers were selected for the experiment. These 12 heifers were slaughtered and samples of subcutaneous, omental and perirenal adipose tissues were collected and frozen in liquid nitrogen. Expression of leptin was quantified by real-time PCR using the ribosomal protein RP-L19 as a reference gene. Heifers that attained puberty earlier had significant higher expression of leptin by the adipose tissues (P < 0.05), and there was a significant treatment by tissue interaction. On average, leptin expression was increased 14.7-fold in early-maturing heifers. Early-maturing heifers had higher leptin expression in the omental fat depot (90-fold increase) and in the subcutaneous fat depot (39-fold increase), while there was no effect on leptin expression in the perirenal fat depot (1.5-fold increase). Regardless of the treatment effects, leptin expression was higher in the perirenal fat depot than in the other two tissues (P < 0.01). Leptin expression was 115-fold higher in the perirenal fat depot then in the other two depots. These results support the idea that circulating levels of leptin is an important signal for the initiation of puberty, and suggest that heifers with higher leptin expression in the adipose tissue could attain puberty earlier, with a lighter body weight.

Key Words: Bovine, Leptin, Puberty

M249 Changes in antioxidant status in beef cows during weight loss and weight maintenance. K. M. Brennan\*, J. J. Michal, R. Collins, and K. A. Johnson, *Washington State University, Pullman*.

To investigate the impact of  $\beta$ -oxidation on the enzymes, metabolites and genes that are involved in regulation of mitochondrial antioxidant status, Angus and Angus-cross cows (n=26) were examined during weight loss and weight maintenance or small weight gain. The weight maintenance (M) period occurred after weaning. The weight loss and fat mobilization period (L) occurred during lactation. Cows were weighed and condition scored weekly during the 60d period. Tail vein blood samples were collected at both intake levels at the end of the period and immediately processed to yield serum and red blood cell lysate. Serum nonesterified fatty acid (NEFA) concentrations were measured spectrophotometrically using NEFA-C (HR) kit (Wako Chemicals). Superoxide dismutase activity (SOD), an antioxidant enzyme, in red blood cell lysate was measured spectrophotometrically using a Cayman chemical assay kit. During L, cows lost approximately 77 kg of body weight (P>.0001). At the M level of intake cows maintained or gained a slight amount of body weight (approximately 13 kg). Serum NEFA concentration was significantly higher during L than M (P <.001). Average serum NEFA levels at L and M were 0.511± 0.167 and  $0.102 \pm .031$ , respectively. SOD activity in red blood cell lysate was greater in L than M (P<.025). Mobilization of body fat, as reflected by body weight loss and serum NEFA levels, results in greater antioxidant activity in red blood cells.

Key Words: Beef Cattle, Antioxidants, Oxidative Stress

**M250** Plasma ghrelin concentrations of beef cattle consuming a similar amount of dietary energy supplied by different dietary ingredients. A. E. Wertz-Lutz<sup>\*1</sup>, J. A. Clapper<sup>1</sup>, J. S. Thurlow<sup>1</sup>, D. C. Beitz<sup>2</sup>, and A. Trenkle<sup>2</sup>, <sup>1</sup>South Dakota State University, Brookings, <sup>2</sup>Iowa State University, Ames.

Previous research demonstrated that varying caloric intake by manipulating DMI of high-grain diet influenced plasma ghrelin concentrations in cattle. To determine if dietary ingredient composition influenced plasma ghrelin, insulin, NEFA, and GH concentrations when caloric intake was similar, five steers (589±18.1kg) were used in a crossover design. Dietary treatments were 50% hay-50% concentrate (HAY) offered at an amount that met the steer's maintenance requirement plus supplied an additional 3.5 Mcals of NEg daily, or a diet composed of 10% hay-90% concentrate but limit-fed to achieve a caloric intake similar to that of the HAY steers (LFC). On d 21 following initiation of the dietary treatment, serial blood samples were collected via indwelling jugular catheter at 15-min intervals. Following period 1, steers were weighed, dietary treatments were switched between groups, and intake amounts were recalculated. Sampling period 2 was initiated as described for period 1. Plasma samples were assayed for ghrelin, insulin, GH, and NEFA, and, subsequent to analyses, data were pooled by hour for statistical analyses. Hormone data were analyzed statistically as repeated measures in time using the MIXED procedure of SAS. The NEg was similar between treatment groups  $(3.5 \pm 0.04 \text{ Mcals NEg/d})$ . However, a higher DMI (P≤0.001) was required by HAY steers compared with LFC steers (9.4 vs.  $7.2\pm 0.06$  kg) to achieve the same caloric intake. Plasma ghrelin concentrations were similar (P=0.12) for HAY and LFC steers (115 vs. 107±3.3 pg/mL), and plasma GH, NEFA, and insulin concentrations were similar regardless of dietary treatment. These data are consistent with the hypothesis that ingredient composition and quantity of DM consumed do not influence plasma ghrelin concentrations of steers when caloric intake is similar and steers are in positive energy balance.

Key Words: Beef Cattle, Energy Intake, Ghrelin

**M251** Impact of metabolic acidosis on amino acid metabolism in lambs. S. L. Greenwood<sup>\*1</sup>, T. C. Wright<sup>1</sup>, J. Gilmore<sup>1</sup>, J. E. Las<sup>1</sup>, N. E. Odongo<sup>1</sup>, O. AlZahal<sup>1</sup>, A. K. Shoveller<sup>1</sup>, J. C. Matthews<sup>2</sup>, and B. W. McBride<sup>1</sup>, <sup>1</sup>University of Guelph, Guelph, Ontario, Canada, <sup>2</sup>University of Kentucky, Lexington.

Feeding high amounts of concentrate to ruminants commonly leads to increased production of volatile fatty acids (VFAs) and lactate in the rumen. Metabolic (systemic) acidosis occurs in ruminants as a result of increased absorption of these VFAs and lactate. Furthermore, metabolic acidosis results in high levels of protein degradation of the skeletal muscle in many species, a response thought to alter the concentration of plasma L-amino acids to increase plasma buffering capacity and renal catabolism of glutamine. The objective of this study was to characterize the effect of metabolic acidosis on plasma amino acids of lambs. Ten fully fleeced Rideau-Arcott wether lambs (54.3  $\pm$ 6.7 kg body weight) were randomly allocated to balanced treatment groups that were fed diets that contained either 1) a canola meal supplement (control; CS) or 2) a HCl-treated canola meal (NutriChlor) supplement to induce metabolic acidosis (AS). Lambs were fed daily at 0700 and 1100 h. Blood samples were collected daily between 1100 and 1130 h throughout the experimental period (d 0 to d 10) via jugular catheters and were pooled within animal from d 4 to d 10 for analysis. Lambs were slaughtered on d 11 and kidney, liver, and muscle samples were collected from each animal. Plasma serine (19%), glycine (32%), and glutamine (14%) concentrations were increased (P<0.05) in acidotic lambs compared to control animals. This pattern and magnitude of altered plasma amino acid profiles suggests that protein

degradation was increased in acidotic sheep. Ongoing Northern blot analyses to identify altered expression of components of the ubiquitin protein degradation system by kidney, skeletal muscle, and liver should reveal tissue-specific mechanisms responsible for the observed shifts in intra-organ amino acid metabolism. These findings suggest that future formulation of high-starch diets need to compensate for metabolic acidosis-induced shifts in amino acid metabolism.

Key Words: Metabolic Acidosis, Ruminant, Protein Degradation

M252 Palmitate and CLA isomer effects on gene expression in MDBK cells. B. J. Thering\*, M. Bionaz, and J. J. Loor, *University* of Illinois, Urbana.

A time course experiment was conducted to evaluate effects of 150 µM of Wy-14643 (WY, PPARa specific agonist) and 16:0 on CPT1A, ACADVL, ACSL1, LPIN1, PPARGC1A, SREBF1, and RPS9 (reference gene) expression by qPCR. Two replicate cultures were harvested at 0, 6, 12, 18, and 24 h. ANOVA using MIXED was used for statistical analysis. In a second experiment cells were treated for 18 h with 16:0 (150 µM), c9t11CLA (200 µM), and t10c12CLA (200 µM) plus control (CTR). Each duplicate treatment was pooled for microarray analysis using a 13K-gene bovine oligoarray platform using a dye-swap design. Microarray data were analyzed with a ≥2-fold change cut-off relative to CTR. In the time course experiment, expression of all genes except SREBF1, increased (P < 0.05) over time with both WY and 16:0. ACSL1 and LPIN1 experienced a treatment × time effect, such that expression of both genes peaked between 6 and 12 h with 16:0, and at 18 h with WY. CPT1A and ACADVL reached peak expression at 18 h and PPARA and PPARGC1A at 24 h. Except for CPT1A and PPARGC1A, expression of all other genes was higher with 16:0 vs. WY, particularly LPIN1 (7-fold at 6 h). Compared to CTR, microarray data showed 29, 47, and 43 genes with  $\geq$ 2-fold difference due to 16:0, c9t11CLA, and t10c12CLA, respectively. Ingenuity Pathway analysis showed that genes affected by c9t11CLA were associated with lipid metabolism (6 genes, e.g. ADFP (up), SCD (down), FABP3 (down)) and cell signaling and morphology (7 genes, e.g. SPP1 (up), ITGA2 (up)). Genes affected by t10c12CLA were associated with lipid transport and metabolism (11 genes, e.g. ADFP (up), SCD (down), HMGCS1 (down), SC4MOL (down)) and cellular growth (9 genes, e.g. SPP1 (up), IGF1 (up)). Palmitate affected genes involved primarily in cellular growth and among these, SPP1 and G1P2 had the highest difference ( $\geq$ 10-fold). Results suggest 16:0 activated genes through PPARA, and had greater potency in stimulating expression than WY. Marked stimulation of LPIN1 and SPP1 by 16:0 revealed an unknown, PPARA-independent mechanism on gene expression. CLAs affected expression of unpredicted genes involved in pathways of lipid metabolism and cellular growth/morphology.

Key Words: Genomics, Nutrition, Lipids

M253 Transcriptional regulation of mammary and adipose tissue gene expression in dairy cows fed a milk fat-depressing or milk fat-enhancing diet. B. J. Thering\*, D. E. Graugnard, P. Piantoni, R. L. Wallace, R. E. Everts, S. L. Rodriguez-Zas, H. A. Lewin, and J. J. Loor, *University of Illinois, Urbana*.

To better characterize mammary (MG) and adipose (AT) tissue gene networks regulating lipid synthesis and other cellular processes that

might be responsive to dietary lipid, we biopsied MG (n = 6/diet) at 0, 7, and 21 d, and AT (n = 3/diet) at 21 d of feeding mid-lactation cows (n = 6/diet) a milk fat-depressing (MFD, fish/soybean oil (1:2) at 3.5% of DM), milk fat-enhancing (MFE, EnergyBooster100, 3.5% of DM), or control (no added lipid) diet for 28 d. Milk composition was examined daily. A 13,257 bovine oligonucleotide (70-mers) array and qPCR were used for transcript profiling. Annotation was based on similarity searches using BLASTN against human, mouse and bovine RefSeq, human, mouse, and bovine UniGene, and bovine TIGR. Cy3and Cy5-labelled cDNA from MG or AT and a reference standard were used for hybridizations. Milk yield was not affected by diets ( $29 \pm 1.7$ kg/d), but milk fat % (FP) decreased (P < 0.001) gradually (3.73% to ~2.50%) with MFD and reached a nadir essentially by d 13 of feeding. MFE did not increase FP above controls and averaged 3.71%. ANOVA (FDR-adjusted  $P \pm 0.10$ ) identified 797 differentially expressed genes in MG over time due to MFD, and 378 genes in AT due to diet. Among genes in MG, we found 74 downregulated and 171 upregulated by ≥1.5-fold on d 21 vs. 0. In AT, MFD and MFE compared with control resulted in 57 and 35 genes with  $\geq$ 2-fold up or downregulation. Ingenuity Pathway Analysis of d 21-upregulated MG genes identified cell growth/proliferation (35 genes), molecular transport (22), cell assembly/organization (18), and cell signaling (15) as modified families of related genes. Overall, results suggest that milk fat depression is associated with previously-unknown adaptations in gene networks both in MG and AT. Gene expression in AT seemed responsive, via direct or indirect mechanisms, to both dietary lipids and reduced energy output in milk.

Key Words: Genomics, Lipid, Nutrition

**M254** Effect of growth hormone on expression of metabolic genes in adipose tissue of dairy cows. M. Baik<sup>2</sup>, J. L. Liesman<sup>1</sup>, B. E. Etchebarne<sup>1</sup>, J. Bong<sup>2</sup>, and M. J. VandeHaar\*<sup>1</sup>, <sup>1</sup>Michigan State University, East Lansing, <sup>2</sup>Chonnam National University, Gwangju, South Korea.

We recently developed a new bovine specific microarray that is targeted for studies on metabolic regulation in cattle. This array (BMET) is a long oligo spotted array of 2400 genes with 4 replicate spots per gene. Our objective in this study was to determine the influence of bovine growth hormone (GH) on the expression of individual genes and gene pathways associated with metabolism and metabolic regulation in adipose tissue of lactating cows. Primiparous Holstein cows were treated with 0 or 29 mg of GH per day, and omental fat was collected at slaughter on day 60. RNA was isolated from adipose tissue of 3 cows treated with GH and from 3 matched controls. Direct comparisons of the treatments were made using two arrays for each cow comparison with a reversal of dye assignments for the second array; a total of 6 arrays were performed. Approximately 9% of the genes were altered at the P < 0.05 level, and the distribution of P-values was highly skewed toward the lower P values, indicating a significant treatment effect. Approximately 100 genes were down-regulated. These included several genes associated with glucose transport and activation, fatty acid synthesis, the TCA cycle and NADPH generation, as well as steroyl-CoA desaturase, LDL receptor, and leptin. Approximately 120 genes were up-regulated. These included several genes for protein modification for several signaling pathways, zinc fingers, as well as PPAR alpha, SOCS1 and 5, and IGFBP3. In addition, several genes associated with insulin signaling were altered by GH. Pathway analysis is ongoing. The overall effects of GH were consistent with previously

published results. GH decreased activity of many enzymes that would explain the major decrease in fatty acid synthesis and esterification that occurs in adipose tissue during GH administration.

Key Words: Gene Expression, Growth Hormone, Metabolism

M255 Growth hormone receptor expression in two dairy breeds during the periparturient period. C. S. Okamura, J. F. Bader, T. C. Cantley, and M. C. Lucy\*, *University of Missouri, Columbia*.

The somatotropic axis is implicated in the profound physiological and metabolic changes that accompany parturition and the onset of lactation in dairy cattle. In Holstein cows, the expression of the primary growth hormone receptor (GHR) mRNA in liver, GHR1A, declines dramatically within one week after calving. When Angus beef cows were sampled, however, GHR1A mRNA amount did not change after calving. The present study tested whether the decrease in GHR1A was a characteristic of the Holstein breed alone or whether a second dairy breed (Guernsey) also experienced a decrease in GHR1A after calving. Holstein (n = 9) and Guernsey (n = 9) cows were paired by parturition date. Liver biopsies were taken prepartum  $(d - 20 \pm 1)$  and postpartum on d+3, and d+14±1. Liver RNA was isolated and reverse transcribed (RT) into cDNA. A quantitative RTPCR assay was used to measure the amount of GHR1A, GHR1B and IGF-I mRNA. Heterogeneous nuclear RNA (hnRNA; precursor of mRNA) was also measured to infer rates of transcription for GHR transcripts. There was no effect of breed on GHR1A, GHR1B or IGF-I mRNA amount (P > .10). Both Holstein and Guernsey cows underwent a decrease and subsequent increase in GHR1A and IGF-I mRNA from d -20 to +14 [15.6, 2.8, and 8.4 (±1.4) for GHR1A and 12.3, 2.2, and 5.9 (±.9) for IGF-I on d -20, +3, and +14, respectively; effect of day, P<.001]. There was no effect of day on hnGHR1A. The change in GHR1B was opposite to GHR1A because GHR1B increased on d+3 [12.8, 25.8, and 15.1  $(\pm 3.8)$ ; day, P<.05]. The hnGHR1B also increased on d+3 (day, P<.05). The decrease in GHR1A expression after calving occurred in two dairy breeds that have undergone independent selection for milk production. Reduced GHR1A after calving may be an inherent characteristic of dairy cows that enables nutrient partitioning for greater milk production. The decrease in GHR1A mRNA may arise from a mechanism involving enhanced mRNA turnover because the amount of its precursor mRNA (hnGHR1A) did not change in periparturient cows.

Key Words: Growth Hormone Receptor, Dairy

M256 Effects of milk replacer composition on selected blood metabolites and hormones in pre-weaned Holstein heifers. K. M. Daniels\*, S. R. Hill, K. F. Knowlton, R. E. James, R. E. Pearson, M. L. McGilliard, and R. M. Akers, *Virginia Polytechnic Institute and State University, Blacksburg.* 

We investigated the effects of increasing dietary protein and energy on the concentrations of selected blood metabolites and hormones in Holstein heifer calves. Twenty-four heifers were fed one of four milk replacer (MR) diets (n=6/diet): 20:20 (20% CP, 20% fat MR fed at 450 g/d), 28:20 (28% CP, 20% fat MR fed at 970 g/d), 28:28L (28% CP, 28% fat MR fed at 970 g/d), and 28:28H (28% CP, 28% fat MR fed at 1460 g/d). Calves arrived at the Virginia Polytechnic Institute and State University Dairy Center August 20, September 10, or September 25, 2005. Calves were fed twice daily; water and starter (20% CP)

were available at all times, and any orts were recorded daily. Serum and plasma aliquots from blood samples collected twice weekly after a 12 h fast were analyzed for insulin-like growth factor-I (IGF-I), growth hormone (GH), insulin, glucose, NEFA, triglycerides (TRI), and plasma urea nitrogen (PUN). Calves fed 20:20 had the lowest overall glucose concentration (83, 103, 107, and 107±2 mg/dl for 20:20, 28:20, 28:28L, and 28:28H). Calves on treatments 28:20, 28:28L, and 28:28H increased linearly in blood IGF-I (53 to 126 ng/ml) and decreased in TRI (0.20 to 0.13 mmol/l) over time. Calves fed 20:20 however, demonstrated quadratic IGF-I and TRI responses over time, with lowest values reported at week 4 for IGF-I (20 ng/ml) and week 6 for TRI (0.14 mmol/l). Change in insulin over time was quadratic with the lowest value (0.16 ng/ml) occurring at week 6; GH decreased linearly from 9.8 to 3.8 ng/ml. No differences were detected in PUN concentrations (mean = 7.35 mg/dl of urea N). Plasma NEFA decreased over time in all calves (0.42 to 0.37 mmol/l). Overall, the blood parameters measured here did not depend on treatment diet composition; differences in body composition and calf growth (reported elsewhere) in these animals may likely be explained by variables not measured here.

Key Words: Blood Metabolite, Calf, Milk Replacer

M257 Circulating glucose responses in early lactation dairy cows to dietary restriction and rbST treatment. A. Basson and N. H. Casey\*, University of Pretoria, Pretoria, South Africa.

Galactopoietic effects of somatotropin are the result of IGF-I and require high-quality nutrient intake. This study investigated shortterm partitioning effects during recombinant bovine somatotropin (rbST) administration in high yielding early lactation dairy cows. Administration of rbST has been shown generally to alter results of metabolic tests in the face of unchanged basal glucose and insulin concentrations. Ten multiparous Holstein cows were subjected to rbST (LactotropinO) and/or feed intake restriction to 80% of predicted ME requirement (80% ME). Responses to insulin challenge (0.1 IU canine insulin/kg BW, 210 min) and hyperglycemic clamp (+50 mg/dL whole blood, 120 min) were tested during weeks 8 (control), 9 (rbST), 11 (80% ME) and 12 (rbST + 80% ME) post partum. Plasma and whole blood samples were assayed for glucose concentrations. The rbST treatment decreased fasting glucose concentration by 10.0% (P<0.0001), which was likely a remnant of control hyperglycemia. Maximum glucose response was 4.0 mg/dL lower (P<0.0148) and took 6.5 min longer to attain (P<0.0112). Steady state glucose infusion rate (SSGIR) decreased by 8.1% (P<0.0001). The 80% ME treatment decreased glucose availability by 4.9% (P<0.0001), while no glucose responses were affected. Restricted energy intake during treatment with rbST resulted in plasma glucose increase by 5.5% (P<0,0001). Peripheral uptake and utilization of glucose increased by 5.1% (P<0,0001). Compared to energy restriction, 80% ME + rbST did not alter effects of nutrient restriction on responses to exogenous insulin challenge. Effects were small and inconsistent. SSGIR decreased by 5% in the 80% ME + rbST compared to the 80% ME period (P<0.0001) and the change in the hyperglycemic clamp in the absence of an effect in the insulin challenge may be due to differences in endogenous insulin secretion. The conclusion was that rbST treatment resulted in altered glucose metabolic responses, even with restricted energy intake.

Key Words: Glucose, rbST, Dairy Cows

M258 Alterations in hepatic gene expression profiles in dairy cows in response to L-carnitine and feed restriction. D. B. Carlson\*, J. K. Drackley, M. Bionaz, S. L. Rodriguez-Zas, N. A. Janovick Guretzky, R. E. Everts, H. A. Lewin, and J. J Loor, *University of Illinois, Urbana.* 

Previously we showed that abomasal carnitine infusion increases hepatic fatty acid oxidation and decreases liver lipid accumulation during acute feed restriction (J. Dairy Sci. 89:4819-4834). Further, carnitine has been shown to stimulate hepatic gluconeogenesis in nonruminants. Our objectives were to determine global gene expression patterns in liver using a microarray consisting of 7,872 bovine cDNA inserts and qPCR. Eight lactating Holstein cows were used in a replicated  $4 \times 4$  Latin square design with 14-d periods. Treatments were factorial combinations of dry matter intake (DMI) restriction and abomasal carnitine (20 g/d) infusion: water infusion + ad libitum DMI (WA), water infusion + restricted DMI (WR), carnitine infusion + ad libitum DMI (CA), carnitine infusion + restricted DMI (CR). Annotation was based on similarity searches using BLASTN against human, mouse and bovine RefSeq, human, mouse, and bovine UniGene, and bovine TIGR. Cy3- and Cy5-labelled cDNA from liver tissue and a reference standard were used for hybridizations. ANOVA (FDR-adjusted  $P \le 0.10$ ) identified 248, 655, and 362 differentially expressed genes due to L-carnitine infusion, feed restriction, and their interaction, respectively. Hierarchical clustering indicated that L-carnitine infusion resulted in 26% difference and feed restriction in 37% difference in global gene expression patterns. The CR treatment affected gene expression patterns by 20% relative to WR. qPCR confirmed downregulation ( $P \le 0.05$ ) of CPT1A, ADIPOR2, and PCK1 by carnitine infusion. In addition, qPCR confirmed  $\geq$ 2-fold upregulation (P  $\leq$  0.05) of PC by feed restriction compared with ad libitum-fed cows, whereas PDK4 expression increased in WR but not CR cows. Gene expression and in vitro liver metabolism data indicate that L-carnitine altered hepatic responses to feed restriction, likely by effects on acute regulatory mechanisms of enzymes associated with fatty acid oxidation and gluconeogenesis.

Key Words: Liver, Microarray, L-carnitine

M259 Hepatic gene expression profiling in postpubertal Holstein dairy heifers. J. Doelman\*, N. G. Purdie, H. Cao, L. E. Wright, N. A. Karrow, and J. P. Cant, *University of Guelph, Guelph, Ontario, Canada.* 

The liver is central to the nutritional response of animals to nutrient supply. The objective of this study was to evaluate the effects of a negative energy balance on hepatic gene expression in Holstein heifers. One-hundred postpubertal heifers between 9 and 13 months of age were randomly assigned to a fed or a 24-hour feed withdrawal treatment under a randomized block design. Liver biopsies and blood samples were taken to obtain RNA for microarray analysis and blood plasma for metabolite and hormone analyses. Spectrophotometric assays were used to quantify plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate (BhB); statistical analysis was conducted using one-way ANOVA. Plasma NEFA concentrations were significantly higher in fasted heifers (P<0.0001), while BhB levels were significantly lower (P<0.0001) with the 24-hour feed withdrawal. A cDNA microarray consisting of 8800 oligonucleotide inserts was used to identify hepatic transcript profiles. Array elements were selected from a database of bovine ESTs. A reference design was employed to compare Cy-5 labelled RNA from liver to Cy-3 labelled

RNA from reference standard (derived from bovine liver, spleen and placenta). Gene Spring analysis software was used for LOWESS normalization procedures and statistical analysis (ANOVA, P = 0.05). Sixty-six differentially expressed genes were found using Benjamini and Hochberg's False Discovery Rate (P = 0.05). Forty-two genes were found to be 4.0 to 1.5 fold lower and twenty-four were 1.5 to 2.6 fold greater with feed withdrawal. Down-regulated genes were associated with cell cycle progression or cytolysis, endocytosis, cholesterol synthesis, TCA cycle, immune response and the protein synthetic pathway. Genes associated with gluconeogenesis and fatty acid synthesis suppression, fatty acid transport, TCA cycle, cell structure and transport, and immune response were up-regulated with restricted energy intake. These results are indicative of a hepatic gene response to energetic status in postpubertal dairy heifers.

Key Words: Liver, Gene Expression, Dairy Heifer

**M260** Effects of feed restriction on lipogenic gene expression in liver of broiler chickens. H. K. Kang, E. J. Chae, I. S. Jang, S. H. Sohn, and Y. S. Moon\*, *Jinju National University, Jinju, Korea*.

Ross male broiler chickens were used to determine the effect of either energy restriction (ER) or quantitative feed restriction (QFR) on hepatic expression of the lipogenic genes. Diet restriction in all experiments was accomplished by providing chicks with 70% or 85% energy level of control (ER70, ER85), and 70% or 85% feed intake of control (QFR70, QFR85). Diet restriction groups of chickens were restricted for 7days, starting at 8 days of age. Ad libitum feeding was resumed after the restriction periods, and continued through the end of the experiment at 35 days of age. A control group was fed ad libitum throughout the experiment. Chickens were sacrificed and samples of liver were collected at 14 and 35 days of age. After completion of feed restriction, the body weights of QFR70 and ER70 were the lowest and followed by QFR85 and ER85 (P<0.05). However, complete compensatory growth by feed restricted birds relative to controls was achieved by 35 days of age in all treatment groups. Hepatic expression of fatty acid synthase (FAS) gene from OFR70 and OFR85 was 2.7 and 2.1-fold lower than that of control at 14 days of age whereas ER groups were not different from the control group. The FAS gene expressions in QFR groups and ER70 were not completely caught up the control group at 35 days of age while its expression of ER85 was 26% higher than that of control. CEBP/ $\alpha$  and PPAR $\gamma$  were the potent transcription factors to stimulate FAS gene at 35 days of age. The expression levels of SREBP-1 were not different between treatment and control groups after compensatory growth. The results of this study indicated that feeding regimen alters expression of lipogenic genes in liver and may influence lipid metabolism of broilers.

Key Words: Feed Restriction, Hepatic Gene Expression, Broilers

**M261** Purification of Japanese quail prolactin and detection of multiple glycosylated isoforms. N. Kansaku<sup>\*1</sup>, G. Hiyama<sup>1</sup>, T. Murata<sup>2</sup>, T. Sasanami<sup>2</sup>, and D. Zadworny<sup>3</sup>, <sup>1</sup>Azabu University, Sagamihara, Japan, <sup>2</sup>Shizuoka University, Shizuoka, Japan, <sup>3</sup>McGill University, Ste. Anne de Bellevue, Canada.

Prolactin (PRL) is mainly produced in the cephalic lobe of the anterior pituitary gland and affects a variety of physiological functions in birds.

We previously cloned and sequenced PRL cDNA of Japanese quail (AB162003). Since galliform PRL has no consensus sequence for N-linked glycosylation (Asn-X-Ser), an alternative glycosylation site (Asn-X-Cys) for N-glycosylation has been proposed. In Japanese quail, this site is located at position 56 of mature PRL. In general, receptor binding and activation is greatly reduced by glycosylation. However, it is unkown if PRL is glycosylated in the Japanese quail. Accordingly, this study examined the purification of PRL from laying Japanese quail. Anterior pituitary glands were collected from 6 month old Japanese quails (n=500), homogenized and PRL was purified by affinity chromatography using monoclonal antibody against recombinant chicken PRL. The purified PRL was separated by SDS polyacrylamide gel (SDS PAGE) and visualized by Coomassie brilliant blue G-250 staining. Japanese quail PRL consisted of at least three protein bands with molecular sizes estimated to be between 23 to 25 kDa. Western blot analysis using polyclonal antibody against chicken PRL detected similar bands to Coomassie brilliant blue staining. Interestingly, different signal intensity was obtained using various lectins (ConA, DBA, LCA, PHAE4, RCA120, UEFA-I, and WGA). However, no signal was detected when using PNA. These results indicate the presence of multiple forms of glycosylated PRL in the anterior pituitary gland of Japanese quail.

Key Words: Prolactin, Quail, Isoform

M262 Developmental gene expression of preprocholecystokinin (CCK) in lines of chickens divergently selected for high or low juvenile body weight. J. C. Gould\*, C. R. Miller, P. B. Siegel, and E. A. Wong, Virginia Polytechnic Institute and State University, Blacksburg.

This study was designed to measure developmental gene expression of preprocholecystokinin (CCK), a satiety inducing peptide, in the small intestine of chickens that have undergone long-term genetic selection for high (HWS) or low (LWS) 8-wk body weight. HWS chickens are hyperphagic, while LWS chickens are hypophagic. Chicks were reared in batteries with ad libitum access to feed and water. Chicks were weighed and killed on embryonic d 20 (e20), d of hatch (DOH with no access to feed), and d 3 (D3) and 7 (D7) post hatch. RNA was extracted from duodenum, jejunum, and ileum from four males from each line and time point. The abundance of CCK mRNA was assayed by real time PCR using the relative quantification method with GAPDH as the endogenous control. LWS males had a 2.4-fold higher CCK mRNA abundance than HWS males (P = 0.009), with abundance of CCK mRNA higher in the ileum (P < 0.001) than the duodenum and jejunum. There was an effect of age (P = 0.037) with gene expression of CCK increasing through D3 and decreasing slightly by D7. These results indicate that long term selection for high or low 8-wk body weight has affected gene expression of CCK in the small intestine of chickens. Higher expression of CCK in LWS males compared to HWS males suggests a role for CCK in the hypophagia observed in LWS chickens.

Key Words: Chicken, Small Intestine, Preprocholecystokinin

M263 Incremental dietary conjugated linoleic acid (CLA) mixture inclusion has non-linear effects on atherosclerosis in cholesterol-sensitive Japanese quail. C. K. Reynolds<sup>\*</sup>, M. S. Lilburn, S. G. Velleman, V. L. Cannon, J. A. Lynch, D. L. Hartzler, and W. L. Bacon, *The Ohio State University, OARDC, Wooster*.

Our objective was to determine the incremental effect of dietary CLA on the development of atherosclerosis in Japanese quail selected for cholesterol-induced aortal plaque deposition. At 19 wks of age 120 male quail were randomly assigned to one of 10 pens (12 birds/pen) and fed for ad libitum intake one of 5 diets (2 pens/diet). Diets were a negative control containing 1.25% soybean oil and no cholesterol and 4 diets containing 0.5% cholesterol and one of 4 levels of CLA oil (40% cis-9, trans-11 CLA, 40% trans-10, cis-12 CLA; replacing soybean oil) providing 0, 0.25, 0.5, or 1.0% CLA (DM basis). After 14 wks a jugular vein blood sample was taken and quail were euthanized. A score (0 to 4) was assigned for aortal lesions and samples were frozen for later analysis of plasma cholesterol and triglyceride (TAG) concentration and fatty acid concentrations in abdominal fat. Treatments had no effects on body wt (108 g) or plasma TAG, but liver wt was increased (linear, P < 0.07) by CLA (Table). Plasma cholesterol was increased (P < 0.02) by cholesterol and decreased (linear, P <0.07) by CLA. Aortal lesions were increased by cholesterol (P <0.01) and decreased by CLA (quadratic, P < 0.02), with the greatest reduction for 0.5% CLA. Feeding cholesterol increased C18:1 and C16:0 and decreased cis-9, cis-12 C18:2 and C18:3 concentrations in abdominal fat (P < 0.05). Abdominal fat concentrations of CLA, C16:0, and C18:0 were increased and concentrations of cis-9, cis-12 C18:2 and C18:3 were reduced (linear, P < 0.01) by dietary CLA. Consumption of a CLA mixture lessened the severity of cholesterolinduced atherosclerosis in Japanese quail, in part through reduced plasma cholesterol concentration. Reasons for the reduced response at the highest CLA inclusion level are not apparent, but differential effects of the individual isomers fed should be investigated.

Table 1.

Item	Control	0% CLA	0.25% CLA	0.5% CLA	1.0% CLA	SEM
Cholesterol, mg/dl	233	1364	1216	1108	1111	62
Triglyceride, mg/dl	151	335	342	475	312	58
Liver wt, g	1.86	2.35	2.60	2.81	2.79	0.14
Aorta score	1.16	3.75	3.49	2.77	3.56	0.20

Key Words: Atherosclerosis, Conjugated Linoleic Acid, Cholesterol

M264 Effects of feeding blends of grains naturally contaminated with Fusarium mycotoxins on small intestinal morphology of turkeys. C. K. Girish\* and T. K. Smith, *University of Guelph, Guelph, Ontario, Canada.* 

An experiment was conducted to investigate the effects of feeding turkeys grains naturally contaminated with Fusarium mycotoxins on morphometric indices of duodenum, jejunum and ileum, and the possible preventative effect of feeding a polymeric glucomannan mycotoxin adsorbent (GMA, Mycosorb<sup>®</sup>, Alltech, Inc., Nicholasville, KY). Three hundred 1-d-old male turkey poults were fed wheat, corn and soybean meal-based starter (0-3 wk), grower (4-6 wk), developer (7-9 wk), and finisher (10-12 wk) diets formulated with uncontaminated grains (C), contaminated grains (CONT) and contaminated grains + 0.2 % GMA (CONT+GMA). The morphometric indices were measured at the end of each growth phase and included villus height (VH), crypt depth (CD), villus width (VW), thicknesses of submucosa and

muscularis, crypt to villus ratio and apparent villus surface area (AVSA). At the end of the starter phase, feeding CONT significantly (p=0.01) decreased the VH (968.44 vs 782.68µm) in duodenum and feeding CONT+GMA prevented this effect (782.68 vs 991.28µm) (p=0.002). The feeding of CONT also reduced VH (448 vs 380µm) (p=0.04) and AVSA (29944 vs 23604 µm2) (p=0.01) in jejunum, whereas none of the variables were affected in the ileum. VW (41.62 vs 36.23µm) (p=0.04) and AVSA (29342 vs 22057µm2) (p=0.03) of duodenum, VH (738.64 vs 582.27µm) (p=0.02) and AVSA (56346 vs 41129µm2) (p=0.01) of jejunum and submucosa thickness of ileum (31.94 vs 24.69 µm) (p=0.04) were significantly reduced when birds were fed CONT compared to C at the end of the grower phase and the feeding of CONT+GMA prevented (p<0.05) these effects in jejunum and ileum. The feeding of CONT did not significantly (p>0.05) affect the morphometric variables at the end of the developer and finisher phases. It was concluded that consumption of grains naturally contaminated with Fusarium mycotoxins results in adverse effects on intestinal morphology during the early growth phase of turkeys and GMA can prevent many of these effects.

Key Words: Fusarium mycotoxin, Duodenum, Jejunum

M265 Age-specific species variation in oxidative stress in birds. X. Guan\*, K. Gyenai, C. Larson, and E. Smith, *Virginia Polytechnic Institute and State University, Blacksburg.* 

Aging reduces the ability of livestock and poultry to sustain reproductive ability, especially egg-laying in birds. Delaying aging thus has economic benefits in poultry and other livestock species. Understanding the biochemical and molecular mechanisms that underlie the aging process could help develop approaches including selection that will slow the aging process in birds. Here, our objective was to test if birds with different longevities differ in oxidative stress levels associated with aging. The species evaluated were the 20 budgies, 15 guinea fowls, 30 Japanese quails and 30 domestic turkeys. Biomarkers used to estimate oxidative stress were thiobarbiturate acid reacting substance (TBARS), an oxidant, and plasma uric acid (PUA) and glutathione (GSH), both antioxidants. Biomarkers were determined at 10, 30, 55 and 80 wks-of-age within each species. In the Guinea fowl, PUA (from 4.78 mg/dL to 8.28 mg/dL) and TBARS (0.22 to 0.57 mg/L) increased significantly with age while GSH (1074.43 to 613.63 uM) decreased significantly over the same period. Though changes in the levels of the biomarkers with age were inconsistent in the Japanese quail, the oxidant levels increased while the antioxidants decreased with age. In the turkey, PUA (6.85 to 4.69 mg/dL) decreased significantly from 10 to 55 wks-of-age while TBARS (0.31 to 0.56 mg/L) increased. In the budgie, antioxidant level decreased with age though not as rapidly as in the other species. Though changes in the oxidative stress were inconsistent and showed no clear pattern, it appears that the decline in oxidant and antioxidant status varies with species, suggesting a genetic basis to this biologically important characteristics.

Key Words: Oxidative Stress, Poultry, Biomarkers

M266 Effect of maternal stress on the stress hormone and growth response of pigs to a lipopolysaccharide (LPS) challenge. P. N. Williams<sup>\*1</sup>, J. A. Carroll<sup>2</sup>, J. W. Dailey<sup>2</sup>, and T. H. Welsh, Jr.<sup>3</sup>, <sup>1</sup>Texas A&M University-Kingsville, Kingville, <sup>2</sup>USDA-ARS, Livestock Issues Research Unit, Lubbock, TX, USA, <sup>3</sup>Texas A&M University, College Station.

This study assessed the effect of maternal stress on the stress hormone and growth response of the progeny following an endotoxin challenge. Sows were assigned to one of two treatments (n = 10 per treatment)and subjected to either a daily 5-min restraint stress (stressed; S) from d 84 to d 112 of gestation or managed per current industry standards (non-stressed; NS). All sows were then managed similarly through farrowing and lactation. At weaning (20.0  $\pm$  0.3 d) pigs from S and NS sows (n = 40 per treatment balanced for litter and gender) were selected, transferred to a climate controlled facility where they were placed into individual pens and allowed ad libitum access to feed and water. Pigs were allowed to acclimate for 14 d before LPS challenge. On d 14 pigs were weighed and non-surgically fitted with jugular catheters. On d 15 pigs were infused i.v. with LPS (25 µg/kg BW) and blood samples collected every 30 min for 1 h prior to and 6 h following LPS challenge. Serum was analyzed for cortisol (CS), norepinephrine (NE), and epinephrine (E). Weekly weights were taken and average daily gain (ADG) prior to and following LPS challenge calculated. Baseline (pre-LPS) CS, E, and NE were not affected (P > 0.05) by maternal treatment. Consistent with previous reports, CS, E and NE increased (P < 0.01) in a time-dependent manner following LPS with peak values at 3, 1 and 0.5 h post-infusion. Although not affecting the temporal pattern, S pigs had a decreased (P < 0.01) CS response and tended (P = 0.07) to have a greater E response following LPS. Furthermore, there was a tendency (P = 0.08) for S pigs to have greater NE then NS pigs post-LPS. Maternal treatment did not affect (P > 0.40) ADG prior to or following LPS challenge. However, there was a positive relationship between ADG prior to the LPS challenge and peak NE following LPS (r = 0.29; P<0.01) and a negative relationship between peak E and ADG following LPS (r = -0.23; P < 0.05). Collectively, these results indicate that maternal stress alters the stress hormone response of the progeny to an endotoxin challenge.

Key Words: Maternal Stress, LPS, Pig

**M267** Expression of porcine intestinal alkaline phosphatase during the early postnatal development. T. Li<sup>1,2</sup>, C. Yang<sup>2</sup>, D. Lackeyram<sup>2</sup>, Y. L. Yin<sup>1</sup>, C. F. M. de Lange<sup>2</sup>, and M. Z. Fan<sup>\*2</sup>, <sup>1</sup>*The Chinese Academy of Sciences, Changsha, Hunan, China*, <sup>2</sup>*University* of Guelph, Guelph, Ontario, Canada.

The small intestinal alkaline phosphatase (IAP) is responsible for hydrolyzing phosphoric ester bonds of organic phosphorus compounds and plays a role in absorption of triglycerides. To understand factors affecting early postnatal IAP expression, we examined IAP hydrolytic kinetics (Vmax, Km), digestive capacity (Vcap), jejunal IAP protein and mRNA abundances and their associations with hormonal factors in 36 ad libitum fed pigs. Six pigs were sacrificed at 1, 4, 6, 12, 20 and 28 d of age, respectively. Vmax was significantly high in jejunum and low in duodenum and distal ileum for all the age groups. Km was significantly high in jejunum, intermediate in the duodenum and low in distal ileum for all the age groups. There were postnatal decreases (P<0.05) in Vmax for all the small intestinal segments and Vcap, while significant changes in Km were only observed in jejunum. Pearson analysis showed that Vcap was correlated (P<0.05; r=0.62 and 0.64) with jejunal Vmax, however, poorly related (P=0.13; r=0.27) to duodenal Vmax. There were up to the quartic pattern of age effects (P<0.05) on the proximal jejunal homogenate and apical membrane

IAP protein abundances, while a quadratic age effect was observed (P<0.05) in the proximal jejunal IAP relative mRNA abundance measured by real time RT-PCR. Jejunal IAP Vmax was correlated (P<0.05; r=0.59-0.65) with IAP protein abundance, however, poorly related (P=0.66; r=0.08) to IAP mRNA abundance. Furthermore, jejunal homogenate and apical IAP protein abundances were poorly correlated (P=0.91 or 0.73; r=-0.02 and -0.06) with IAP mRNA abundance. In conclusion, there were dramatic reductions in IAP maximal activity and digestive capacity during early postnatal development in the pig, likely regulated at the levels of post-transcriptional IAP protein processing and post-translational IAP affinity modifications.

Key Words: Gene Expression, Intestinal Alkaline Phosphatase, Pigs

**M268** Changes of physiological and biochemical parameters in weaned pigs. X. F. Kong<sup>\*1</sup>, Y. L. Yin<sup>1</sup>, F. G. Yin<sup>1</sup>, H. J. Liu<sup>1</sup>, F. F. Xing<sup>1</sup>, Q. H. He<sup>1</sup>, T. J. Li<sup>1</sup>, R. L. Huang<sup>1</sup>, P. Zhang<sup>1</sup>, M. Z. Fan<sup>2</sup>, S. W. Kim<sup>3,4</sup>, and G. Y. Wu<sup>1,4</sup>, <sup>1</sup>The Chinese Academy of Sciences, Changsha, Hunan, China, <sup>2</sup>University of Guelph, Guelph, Ontario, Canada, <sup>3</sup>Texas Tech University, Lubbock, <sup>4</sup>Texas A&M University, College Station.

This study was conducted to determine the values of serum biochemical parameters in piglets weaned at 21 d of age. On d 0, 7, 14 and 28 post weaning, venous blood samples were obtained randomly from 5five piglets for analysis. With increasing age, the numbers of leukocytes, lymphocytes and granulocytes increased (P < 0.05) progressively. The erythrocyte volume, the platelet distributing width, serum concentrations of phosphorus, urea, total cholesterol and glucose, as well as serum activities of lipase, creatine phosphokinase and glutamate-pyruvate transaminase decreased (P < 0.05) on d 7 and increased (P < 0.05) thereafter. Serum activity of alkaline phosphatase increased (P < 0.05) progressively between d 0 and 14 post weaning and declined thereafter (P < 0.05). Concentrations of erythrocyte hemoglobins increased (P < 0.05) progressively between d 0 and 14, and the elevated values remained until d 28. In contrast, serum concentrations of ammonia, Zn, Na and Cl increased (P < 0.05) on d 7 and decreased (P < 0.05) thereafter. Serum concentrations of triglycerides on d 14 and 28 were lower (P < 0.05) than those on d 0 and 7. Serum concentrations of all amino acids, except for glutamate, glutamine, ornithine, citrulline and arginine, increased (P < 0.05) on d 7 and decreased (P < 0.05) thereafter. The ratio of kidney weight to BW decreased (P < 0.05) progressively until d 28, the ratios of stomach, lymph nodes, and liver weights to BW were the highest (P < 0.05) on d 7, and the ratio of spleen weight to BW was the highest (P < 0.05) on d 14. These results indicate that serum metabolite concentrations and organ growth undergo marked changes in postweaning piglets. (Supported by NSFC and CAS)

Key Words: Weaned Pigs, Serum Parameters, Growth

M269 Omega-3-fatty acid supplementation and the IGF system in early pregnancy in pigs. A. Brazle\*, T. Rathbun, B. Johnson, and D. Davis, *Kansas State University, Manhattan*.

The IGF system of growth factors, receptors and binding proteins functions from early in pregnancy. Recent evidence indicates improved embryo survival in gilts fed supplemental omega-3 fatty acids beginning before conception. Here we report effects of supplementing a corn-soybean meal diet (Control) with a marine source of protected omega-3 fatty acids (PFA, 1.5% of diet) on mRNA expression for IGF-I, IGF-II, IGFBP-3 and IGFBP-5 in the porcine gravid uterus. The PFA (Fertilium<sup>™</sup>365) contained equal amounts of eicosapentanoic (EPA) and docosahexanoic (DHA) acids and replaced corn in the diet beginning when gilts were approximately 170 d old (n=13/treatment). Gilts were artificially inseminated at approximately 205 d of age. Conceptus and endometrial samples were collected at d 11, 15, and 19 of gestation. All gilts were pregnant. In the conceptus, message for IGF-II and IGFBP-3 increased (P < 0.001) from d 15 to d 19 while there was an increase (P < 0.001) in IGF-I and IGFBP-5 from d11 to 15 and a decrease (P < 0.001) to d 19. In the endometrium, message for IGF-I was stable over the interval but message for IGF-II and IGFBP-5 were increased by d 15 and IGFBP-3 by d 19 (P < 0.01). There were trends for omega-3-fatty acid supplementation to increase endometrial IGF-II (P = 0.09) and IGFBP-5 (P = 0.12) on d 15. In the d-19 conceptus, embryonic but not extraembryonic IGF-I mRNA tended (P = 0.13) to be greater for PFA compared to Control gilts. During d 11 to 19 the conceptus is elongating, attaching to the uterus, and the embryonic disc is differentiating from a homogenous tissue to form the tissues and organs of the adult. One mechanism for omega-3 fatty acid effects in early pregnancy could involve epigenetic effects on mRNA expression for the IGF and IGFBP proteins.

Key Words: Pig, IGF, Omega-3 Fatty Acids

M270 Serum and anterior pituitary (AP) concentrations of IGF-I and relative amounts of AP IGF binding proteins throughout the estrous cycle in gilts. A. R. Taylor\* and J. A. Clapper, *South Dakota State University, Brookings.* 

It has been established that components of the circulating and anterior pituitary IGF system vary in response to steroids in the pig. However, whether serum and anterior pituitary concentrations of the IGF system vary throughout the estrous cycle has not been determined. To further examine this relationship the following experiment was performed. Forty gilts of similar age and weight (180 d; 120 kg) were injected with PG 600 to induce the gilts into puberty. Fifteen days later all gilts were fed 15 mg Matrix for 15 d to synchronize estrus. Gilts were checked twice daily for expression of estrus beginning 3 d after the end of Matrix treatment and continuing for 7 d. The first day each gilt exhibited estrus was designated as d 1 of the estrous cycle. Blood samples were obtained by jugular venipuncture on d 1, 4, 7, 10, 13, 16, 19, and 22 of the estrous cycle. On d 7, 13, 19, and 22 of the estrous cycle 10 pigs were killed and anterior pituitary glands (AP) were collected. Serum concentrations of IGF-I and AP concentrations of IGF-I were determined by RIA. Relative amounts of AP IGF-binding proteins (IGFBP) were determined by Western ligand blot analysis. Serum concentrations of IGF-I fluctuated throughout the estrous cycle. Mean serum concentrations of IGF-I decreased (P<0.02) from d 1 through d 10, then increased (P<0.02) on d 13 through 16, then decreased (P<0.02) from d 19 through 22. Mean AP concentrations of IGF-I were greater (P<0.03) on d 19 compared to all other days, while no difference was detected (P>0.05) in mean anterior pituitary concentrations of IGF-I on d 7, 13, and 22. Western ligand blot analysis identified 33 kDa IGFBP-2 and 29 kDa IGFBP-5 in the AP. Mean relative amounts of AP IGFBP-2 and -5 were each greater (P<0.02) in gilts on d 19 compared to all other days while no difference was detected in mean relative amounts of AP IGFBP-2 and -5 among

pigs on d 7, 13, and 22 of the estrous cycle. Anterior pituitary gland function in the pig may be influenced by the IGF system during the estrous cycle.

Key Words: Pig, Estrous Cycle, IGF

M271 Growth performance and muscle protein, RNA and DNA content in juveniles of *Pseudoplatystoma fasciatum* (Teleostei, Pimelodidae) fed lyophilized bovine colostrum. P. Pauletti, L. Kindlein, A. R. Bagaldo, A. P. O. Rodrigues, E. F. Delgado, J. E. P. Cyrino, and R. Machado-Neto\*, *Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ/USP, Piracicaba, SP, Brazil.* 

Little information is available on the physiology and growth performance of speckled catfish, Pseudoplatystoma fasciatum, an important Brazilian freshwater food-fish. Muscle DNA, RNA, total protein (TP) contents and RNA:DNA ratios are good indexes of fish growth and condition. The IGF-I plays a central role in the growth regulation system, and the defatted lyophilized bovine colostrum (BC) is a rich source of IGF-I. The objective of this study was to evaluate effects of diets with partial replacement of protein source by BC on growth performance, body composition, and muscle TP, RNA, and DNA contents of juvenile speckled catfish  $(35.14 \pm 2.23^{-1})$  fed ad libitum for 30 or 60 days with five diets (45% crude protein; 4000 kcal kg<sup>-1</sup>) with increasing levels of BC (0, 5, 10, 15 and 20%) (n=3). BC positively influenced weight gain, specific growth rate, and feed conversion only at 30 d. Carcass lipid contents were higher above 10% dietary BC levels. DNA concentration increased (P≤0.05) between 30 and 60 d; all levels of BC elicited higher DNA concentration at 60 d, except for diets 5 and 10% BC, while the RNA concentration decreased (P≤0.05). Higher (P≤0.05) TP concentrations were registered at 30 d when dietary BC levels were 15 and 20%. At 60 d, diets 0, 5, 10 and 20% BC induced reduction of RNA:DNA ratio comparatively to 30 d (P≤0.05). Increased DNA muscle content indicate that fish fed diets containing BC present additional hyperplasic growth.

Key Words: Siluriformes, Biochemical Indicators, Growth

M272 Feeding juveniles of *Pseudoplatystoma fasciatum* (Teleostei, Pimelodidae) with lyophilized bovine colostrum: Growth and protein, RNA and DNA content in liver and intestine. P. Pauletti, L. Kindlein, A. R. Bagaldo, A. P. O. Rodrigues, E. F. Delgado, J. E. P. Cyrino, and R. Machado-Neto\*, *Escola Superior de Agricultura "Luiz de Queiroz" – ESALQ/USP, Piracicaba, SP, Brazil.* 

The knowledge about the physiology and growth performance of speckled catfish, *Pseudoplatystoma fasciatum*, is strategic to the Brazilian freshwater food-fish industry. Concentrations of DNA, RNA and total protein (TP) in body tissues provide reliable indicators of feeding regime changes. The contribution of visceral tissues, namely liver and gut, to whole-body energy expenditures is related to protein synthetic activity. The liver plays an important role in the regulation of fish energy metabolism and growth, and IGF-I plays vital role in growth regulation and tissues differentiation. The defatted lyophilized bovine colostrum (BC) is a rich source of growth factors, such as IGF-I. The objective of this study was to evaluate effects of diets with partial replacement of protein source by BC on growth and TP, RNA and DNA contents in liver and intestine of juvenile speckled catfish  $(35.14 \pm 2.23 \text{ g}^{-1})$  fed ad libitum for 30 or 60 days with five diets (45%)

crude protein; 4000 kcal kg<sup>-1</sup>) with increasing levels of BC (0, 5, 10, 15 and 20%) (n=3). Liver and intestine DNA concentrations increased (P $\leq$ 0.05) between d 30 and 60 indicating a hyperplasia process, but no significant differences (P $\geq$ 0.05) for RNA concentration in the same period were registered. Liver TP did not differ (P $\geq$ 0.05) between diets, but decreased (P $\leq$ 0.05) with time; significant effect of diet and period was observed for intestinal parameters, BC 5% being superior to others

diets. Intestine TP decreased ( $P \le 0.05$ ) between d 30 and 60. Diets did not influence RNA/DNA rations in both liver and intestine, but decreased ( $P \le 0.05$ ) markedly between 30 and 60 d. Liver and intestine growth was characterized by a major contribution of hyperplasia compared to hypertrophy, as confirmed by linear increases in total DNA and a decrease in RNA/DNA and protein:DNA ratio.

Key Words: Siluriformes, Visceral Tissues, Nucleic Acids

## Production, Management & the Environment - Livestock and Poultry I

M273 Effect of ProAgri<sup>™</sup> amendment, before and after cleanout, on broiler litter moisture, calcium, nitrogen, and total and soluble phosphorus. N. G. Zimmermann<sup>\*1</sup>, R. Angel<sup>1</sup>, and W. Saylor<sup>2</sup>, <sup>1</sup>University of Maryland, College Park, <sup>2</sup>University of Delaware, Newark.

Nutrient pollution is a serious problem whenever animal production is concentrated and too little land is available for manure application. A 60 pen broiler experiment was conducted where diet and litter management were used concurrently to reduce nutrient pollution. A single flock (Ross 308) was grown on fresh pine shavings. The experiment was a  $3 \times 2 \times 2$  factorial design with unequal replication. Main effects were sex, feeding regimen, and litter amendment. The sex main effect was female, male, or straight run; number of birds per  $1.52 \times 2.44$  m pen was 55, 45, and 50, respectively. Four and six phase diets were the feeding regimen main effect. The litter amendment main effect was ProAgri<sup>™</sup> Activator followed by ProAgri<sup>™</sup> 8-26-2 sodium silicate solutions sprayed onto litter (2.54 and 0.76 l/m<sup>2</sup>, respectively). Total number of birds was 2880. At the end of the trial samples of litter from each pen, including cake, were collected to measure percent moisture, available water (A<sub>w</sub>), N, Ca and total (tP) and soluble P (sP). Furthermore, a subsample of litter was treated with ProAgri<sup>™</sup> Activator solution, 1:1(w:v) then ProAgri™ 8-26-2 sodium silicate solution, 2.4:1 (w:v). After oven drying, N, Ca, and tP and sP were measured. Only the effects of the litter amendment are reported here except for main effect interactions. Application of the litter amendment prior to bird placement did not have an effect on percent moisture, Aw, N, Ca, tP, or sP in clean out litter. However, clean out litter treated with the amendment had reduced (P>.05) sP (486 vs 271 mg/g) and N. Furthermore, a significant interaction of litter amendment with diet regimen was observed. The litter amendment reduced (P>.05) sP in litter when diets containing higher levels of tP were fed.

Key Words: Litter Amendment, Phosphorus, Nitrogen

M274 Genotype analysis of *Campylobacter* spp. isolated from various internal organs and unabsorbed yolks of commercial broiler and roaster chickens. K. L. Hiett, R. J. Buhr\*, N. A. Cox, L. J. Richardson, P. J. Fedorka-Cray, J. S. Bailey, and J. K. Northcutt, *USDA-ARS, Russell Research Center, Athens, GA*.

*Campylobacter* spp. are presently believed to be the leading bacterial etiological agent of acute gastroenteritis in the human population. Evidence implicates poultry as a significant source of the organism for human illness; however, the pathways involved in *Campylobacter* spp. contamination of poultry flocks remain unclear. In an effort to further understand the dissemination of naturally occurring *Campylobacter* spp. through commercial broiler and roaster chickens, *Campylobacter jejuni* isolates previously recovered from the liver/gallbladder, spleen,

ceca, and unabsorbed yolks of broiler and roaster chickens were genotyped using *flagellinA* Short Variable Region (*flaA*-SVR) DNA sequence analysis. All isolates recovered from broilers were of one *flaA*-SVR subtype regardless of the site of recovery. Isolates recovered from roasters comprised two subtypes. The predominant subtype (*flaA*-SVR type 1) contained isolates recovered from all locations tested. Additionally, this same *flaA*-SVR subtype was recovered from both broilers and roasters. This investigation demonstrated that very closely related subtypes of *C. jejuni* were naturally present within the internal organs and unabsorbed yolks of commercial broilers and roasters from different flocks, companies, and breeder strains. Further investigations of these subtypes are needed to understand their involvement in intestinal tract microbiology and the subsequent contamination of the final food product.

Key Words: Campylobacter, Genotyping, Tissues

**M275** Recovery of naturally occurring *Campylobacter* from the circulating blood of market age commercial broilers. L. J. Richardson<sup>1</sup>, N. A. Cox<sup>1</sup>, R. J. Buhr<sup>\*1</sup>, and M. A. Harrison<sup>2</sup>, <sup>1</sup>USDA-ARS-PMSRU, Russell Research Center, Athens, GA, <sup>2</sup>Department of Food Science and Technology, University of Georgia, Athens.

Campylobacter species have recently been recovered from several primary and secondary lymphoid tissues and internally from the spleen of poultry. The objective of this study was to determine whether naturally occurring Campylobacter can be recovered from the circulating blood of market age commercial broilers utilizing aseptic techniques. Broilers (n=100) were acquired from two commercial processing facility's live haul area on 10 separate days. The feathers where removed from the ventral surface of the humerous and alcohol was sprayed on the skin, then Betadine was applied to the area and allowed to sit for 1 min before vena-puncture (brachial vein) with a sterile needle. Five mL of circulating blood was collected and added to 45 mL of Bolton's broth without antibiotics and incubated at 42 C in microareophilic conditions for 48 h and then streaked onto Campy-Cefex plates. For flocks 9 and 10, direct plating onto aerobic plate count agar was also performed to verify that the skin had been disinfected. Standard laboratory procedures for Campylobacter were performed on ceca contents collected from all broilers sampled. *Campylobacter* were not recovered from the blood (0/60) nor the ceca (0/60) from flocks 1-4, 6, or 7. From flocks 5 and 8-10, Campylobacter were recovered from the blood (11/40) and the ceca (28/40). From aerobic plate counts performed in flocks 9 and 10, no growth was observed suggesting that the method utilized results in aseptic sampling of the circulating blood. With Campylobacter being recovered from the circulating blood, this provides insight to a possible means by which this organism is able to rapidly disseminate to tissues within the bird and suggests that Campylobacter is not strictly limited to the