**W88** Tea saponins affect rumen fermentation and growth performance in Growing Boer Goats. W.-L. Hu\*<sup>1</sup>, J.-X. Liu<sup>1</sup>, Y.-Q. Guo<sup>1</sup>, Y.-M. Wu<sup>1</sup>, J.-A. Ye<sup>1</sup>, X.-W. Ye<sup>2</sup>, Y.-M. Wang<sup>2</sup>, and H.-W. Ye<sup>2</sup>, <sup>1</sup>Zhejiang University, Hangzhou, P. R. China, <sup>2</sup>Hangzhou Zhengxing Animal Industries, Lin'an, Zhejiang, P.R. China.

Two experiments were conducted to investigate the effects of tea saponins (TS) on rumen fermentation and growth performance in growing Boer Goats. In Experiment 1, the Reading Pressure Technique (RPT) system was used to investigate the effect of addition of TS (0, 0.2, 0.4 and 0.8 mg/ml) on the ruminal fermentation in vitro. The 24h gas production and methane emission were significantly decreased when the TS was included. Compared to the control, the TS had little effects on pH values and the amounts of total volatile fatty acids in the rumen fluids. However, the fermentation patterns were changed, reflective of higher proportions of propionate. Ammonia-N concentration and protozoa counts were significantly reduced, while microbial protein yield were increased by the TS addition, suggesting that the TS could modify the rumen fermentation and inhibit the release of methane. In Experiment 2, twenty-seven growing Boer goats were used to evaluate the effects of the TS addition on growth performance. The animals received the same basal diets, and added with the TS at levels of 0 (C), 31/4 T11/4% and 6 g (T2) per day. The experiment lasted for 60 days with the first 15 days for adaptation. Blood samples were obtained by jugular venipuncture before the morning feeding on the final day of the experiment. The dry matter intakes, average daily gain and feed conversion ration in group T1 were higher than in other two. Serum total protein, albumin, high density lipoprotein cholesterol, Ca and P and alkaline phosphatase levels were higher in group T1 than those in C and T2, whereas the blood urea nitrogen, creatinine and total cholesterol were lower in the TS-added groups. The concentrations of glucose, glutamic-oxaloacetic transaminase and glutamic-pyruvic transaminase were not affected by the TS. From the results obtained in this study, it is inferred that the TS could modify the rumen fermentation and that proper doses of TS may have some potentials in improving the animal growth performance, whereas at high doses, it may have adverse effects on animal production.

Key Words: Tea Saponin, Rumen Fermentation, Grow Performance

**W89** Relationship between *in vitro* gas production and cell wall compounds in the diet selected by goats grazing a poor quality rangeland in North Mexico. A. Cerrillo-Soto\*, G. Nevarez-Carrasco, R. Montoya-Escalante, and A. Juarez-Reyes, *Universidad Juarez del Estado de Durango, Durango, Dgo, Mexico.* 

The study was performed to determine the *in vitro* gas production characteristics of the diet selected by goats grazing a poor quality rangeland. Four esophageally cannulated Spanish criollo goats (33 kg BW) were used to collect extrusa samples during Spring (Apr-Jun), Summer (Jul-Sep), Autumn (Oct-Dic) and Winter (Jan-Mar). Samples were collected for two days each month, morning and evening. Samples (200 mg DM) were incubated in glass syringes using ruminal fluid from three sheep fed alfalfa hay *ad libitum*. Gas volumes were recorded at 0,3,6,9,12,24,48,72 and 96h post-inoculation. Data were fitted to the equation p = a+b (1-e<sup>-ct</sup>), where *p* represents gas volume at time *t*, *a* the intercept, a + b the potential gas production, and *c* the constant rate of gas production during incubation. Data were analyzed by ANOVA for a completely randomized design. Simple linear correlation coefficients between chemical composition and *in vitro* gas production parameters were computed by PROC REG (SAS). Higher *a+b* values (*P* < 0.05) were recorded in Spring (during the new growth period of shrub species), whereas Autumn (at the beginning of the dormant forage season) registered lower values (47.6 and 34.8 mL/200 mg DM, respectively). Differences (*P* < 0.05) were recorded in the constant rate of gas production *c*. Higher values were obtained during the regular rainy season (summer = 0.069 h<sup>-1</sup>) and lower values were recorded in winter (when vegetative species are dormant = 0.047 h<sup>-1</sup>). Negative correlations were recorded between *a+b* and NDF (r = -0.77), ADF (-0.82) and lignin (r = -0.59). On the contrary, positive correlations were obtained between the constant rate of gas production *c* and CP (r = 0.79). It is concluded that differences in the *c* parameter between seasons indicate variations in nutrient availability. Negative correlations between cell wall constituents and *in vitro* gas production parameters indicated that such compounds have a detrimental effect on gas production.

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Key Words: Grazing, Semiarid Region, North Mexico

**W90** In situ ruminal digestion kinetics and volatile fatty acid production rate in goats fed premium quality dehydrated alfalfa hay supplemented with three levels of a concentrate mix. N. E. Brown\*, J. Bing, and R. N. Corley, III, *Tuskegee University, Tuskegee, AL.* 

Three non-lactating, mature, Nubian does fitted with permanent ruminal cannulas were used in a 3 x 3 Latin Square design to examine the kinetics of in situ DM disappearance and the rate of VFA production. The diets consisted of Premium Quality Dehydrated Alfalfa Hay (US Alfalfa) supplemented with 30% (high forage diet), 50% (medium forage diet) and 70% (low forage diet) Nutrena Sweet Stuff<sup>®</sup>. Proximate analysis and measurements of NDF, ADF, were also determined. The high, medium and low forage diets, had DM (92.6, 92.6 and 92.6%), CP (20.8, 18.8, 16.7%), NDF (33.8, 37.0, 40.1%) and ADF values (26.4, 28.0, 30.0%) respectively. Ruminal digestion kinetics of the high, medium and low forage diets respectively, estimated 50, 50, 53% soluble, 24, 22, 18% was potentially degradable, 26, 29, 29%, was indigestible and the fractional rate of digestion was 10, 15 and 8%h-1. There were no differences (P>.05) in the soluble and indigestible fraction (P>.05) among diets. The low forage diet was less degradable (P<.05) than the high and medium forage diets which did not differ (P>.05). The medium forage diet had a faster fractional rate of disappearance (P<. 05) than the high or low forage diets which differed (P<.05) from each other.

Estimates of daily VFA production were 3.5 mol/day for the high forage diet, 4.5 mol/day for the medium forage and 7.8 moles/day for the low forage diet. Although not significant (P>.05) there was a numerical increase in daily VFA molar production as commercial grain mix increased in the diet. Estimates of molar proportions of the high, medium and low forage diets respectively were 64:22:10, 61:24:12 and 62:22:12 for acetate, propionate and butyrate. No differences (P>.05) were seen in the molar proportions among the diets.

Increasing the ratio of commercial grain mix to the dehydrated alfalfa diets in this study did not result in significant (P>.05) benefits, but did result in numerical increases in VFA production rates in goats.

Key Words: Goats, Zero VFA Production, Dehydrated Alfalfa

## Growth and Development: Physiology of Growth and Development

**W91** DNA regulatory activity and RNA expression of the sequence surrounding the callipyge mutation. A. Skipwith<sup>\*1</sup>, A. Perkins<sup>1</sup>, T. Shay<sup>2</sup>, S. Eng<sup>2</sup>, D. Moody<sup>1</sup>, N. Cockett<sup>2</sup>, and C. Bidwell<sup>1</sup>, <sup>1</sup>Purdue University, West Lafayette, IN, <sup>2</sup>Utah State University, Logan.

The callipyge mutation is a single base pair transition from A (normal) to G (callipyge) in an imprinted gene cluster on chromosome 18 in sheep. The muta-

tion occurred in a highly conserved 12 bp motif in the intergenic region between DLK1 and GTL2. The mutation alters the expression of several genes within the 215 kb cluster when the mutation is inherited in *cis*. Therefore, it has been hypothesized that the mutation occurred in a long range control element. In addition, a transcript named CLPG1 has been detected from the region containing the callipyge mutation. The objective of this project was to analyze the genetic regulatory role and transcriptional activity of the sequence around the

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callipyge mutation. Twenty-four Sau3AII fragments ranging from 12,115 bp upstream to 26,106 bp downstream of the callipyge mutation were screened for enhancer activity using a luciferase assay and transient transfection of C2C12 cells. Seventeen luciferase plasmids had significantly higher activity than a minimal promoter plasmid (pGL3P; P < 0.05) and 2 plasmids had significantly lower activity than pGL3P (P < 0.05). Two luciferase plasmids were constructed containing 1.2 kb of sequence flanking the mutation including the 12 bp motif with either the normal or callipyge allele. Both plasmids had significant enhancer activity (P < 0.01) indicating that the callipyge mutation does not alter that activity. The expression of CLPG1 RNA was analyzed by quantitative PCR (measured as log transcript abundance per 100 ng total RNA) in semimembranosus muscle samples for all four genotypes at three ages (2 wk prenatal, 2 wk and 8 wk postnatal). There was a significant effect of genotype at the prenatal (P = 0.024) and 2 wk (P < 0.001) ages, but no significant effect of genotype at 8 wk of age (P = 0.1834). At 2 weeks of age, animals with the three genotypes containing a callipyge allele (+Mat/ CLPGPat, CLPGMat/ + Pat and CLPGMat/  $CLPG^{Pat}$ ) had significantly higher transcript abundance (P < 0.01) than animals with the normal genotype (+/+). The expression pattern of CLPG1 makes it unlikely to be directly involved with muscle hypertrophy.

Key Words: Callipyge, CLPG1 Transcript, Enhancer Activity

**W92** Effect of dietary conjugated linoleic acid on adiposity and the adipose-transcriptome. K. M. Hargrave\*, D. Pomp, and J. L. Miner, *University of Nebraska, Lincoln.* 

Dietary conjugated linoleic acid (CLA) causes body fat loss in mice. The objective of this study was to determine CLA-induced alterations in circulating metabolic hormone levels and to identify differentially expressed genes. Twenty littermate pairs of male mice (n=40, 12-wk-old) were fed diets containing 0 or 2% CLA isomers (one littermate per diet). Feed intake and body weight were measured every 8 d. Following 16 d, mice were killed and bled. Body fat and lean mass were determined by dual x-ray densitometry, and epididymal (EPI) and retroperitoneal (RP) fat pads were weighed and collected. Blood glucose was determined using a SureStep glucose monitor. Plasma leptin, insulin, interleukin (IL)-6, and tumor necrosis factor (TNF)-a were determined using a multiplex assay (Linco) run on a Luminex analyzer. Total RNA from RP fat from five of the littermate pairs (n=10) was analyzed for mRNA expression levels with the Affymetrix Mouse 430A 2.0 Array. Message levels that changed at least 40% in all five pairs of littermates were considered significantly different. CLA reduced (P < 0.01) feed intake on d 16 but did not affect body weight. Dietary CLA caused a reduction (P < 0.001) in body fat and in EPI and RP fat pad weights of 31, 69, and 76%, respectively. Lean mass tended (P = 0.07) to be greater in CLA-fed mice. Glucose and insulin levels were not affected by CLA. CLA increased IL-6 (18 vs 40 pg/ml) and decreased leptin (P = 0.07) and TNF- $\alpha$  (P < 0.05). Although CLA did not increase mRNA levels of any genes in all five littermate pairs, Cbp/p300-interacting transactivator was increased in four of the pairs and steroidogenic acute regulatory protein was increased in three. In contrast, CLA-feeding decreased mRNA abundance of many genes across all five littermate pairs, including leptin (98% reduction), peroxisome proliferator-activated receptor  $\gamma$  (93%), and diacylglycerol acyltransferase 2 (96%). These results demonstrate that CLA-feeding alters the profile and expression of many hormones and genes involved in pathways regulating obesity. Several of these are novel and may be involved in CLA's mechanism of action.

Key Words: Conjugated Linoleic Acid, Gene Expression, Mice

**W93** Decreased expression of DLK1 in the livers of 8 wk old callipyge lambs. J. N. Fleming<sup>\*1</sup>, J. M. Smith<sup>1</sup>, T. S. Hadfield<sup>2</sup>, S. L. Eng<sup>2</sup>, D. E. Moody<sup>1</sup>, N. E. Cockett<sup>2</sup>, and C. A. Bidwell<sup>1</sup>, <sup>1</sup>Purdue University, West Lafayette, IN, <sup>2</sup>Utah State University, Logan.

The livers of callipyge sheep have been reported to be smaller than the livers of normal animals. We hypothesized that if the change in liver size was a direct effect of the callipyge mutation, the liver would exhibit differential expression of genes from the imprinted gene cluster that surrounds the mutation. In order to investigate this, liver samples were collected from 15 lambs (callipyge phenotype,  $+^{Mai}/CLPG^{Pai}$ , n = 4; maternal heterozygotes,  $CLPG^{Mai}/+^{Pai}$ , n= 4; ho-

mozygotes CLPG<sup>Mat</sup>/CLPG<sup>Pat</sup>, n= 3; and normal, +<sup>Mat</sup>/+<sup>Pat</sup>, n=4) at 8 weeks of age. The effect of inheritance of the callipyge mutation on gene expression in the liver was determined by quantitative PCR (measured as the log of transcript abundance per 100 ng total RNA). The genes that were quantified from the callipyge locus included DLK1, GTL2, PEG11AS, PEG11 and MEG8. G3PD was also quantified for sample standardization. There was no significant effect of genotype (P > 0.05) on G3PD, GTL2, MEG8, PEG11AS, or PEG11 transcript abundance. A significant effect of genotype was seen for DLK1 expression (P = 0.01) with the callipyge lambs having the lowest mean transcript abundance  $(3.155 \pm 0.140)$ , followed by maternal heterozygotes  $(3.646 \pm 0.162)$ , normals (3.705  $\pm$  0.140), and homozygotes (3.995  $\pm$  0.140). Orthogonal contrasts were used to analyze the genetic model which indicated additive (p = (0.010) and reciprocal heterozygote (p = (0.043)) models to be significant. In addition, the polar overdominance contrast was also significant (p = 0.003) for DLK1 transcript abundance. The decrease in liver DLK1 expression found only in callipyge animals suggests a direct effect of the callipyge mutation on postnatal liver growth.

Key Words: Callipyge, Liver, DLK1

W94 Salmonella enterica serovars Typhimurium and Choleraesuis provoke divergent responses in serum IGF-I in young pigs. B. L. Davis\*, J. N. Fraser, K. A. Skjolaas-Wilson, T. E. Burkey, S. S. Dritz, B. J. Johnson, and J. E. Minton, Kansas State University, Manhattan.

Salmonella enterica serovar Typhimurium (ST) and serovar Choleraesuis (SC) account for essentially all cases of salmonellosis in swine, and are among the most important bacterial pathogens in terms of negative economic effects. However, these pathogens produce very different clinical outcomes, with ST producing mainly self-limiting enteritis, whereas SC, a so-called swine host adapted pathogen, is more likely to result in more serious and occasionally fatal septicemia. In numerous prior studies, we have shown that ST transiently reduced feed intake and circulating IGF-I, but recovery was rapid. Here we sought to develop a model of chronic salmonella exposure, and compare the two serovars. Weaned pigs were housed two/pen with free access to feed and water during a 14 d experiment. On d 0, pigs were fed 108 CFU ST or SC in dough balls, and bacteria were re-fed twice weekly through the course of the experiment. Control pigs were fed dough without bacteria. Feeders were weighed daily to estimate feed intake, and serum was collected on d 0, 7, and 14 to quantify circulating IGF-I (sampled from one pig/pen; same animal on each collection day). Daily feed intakes were generally similar between control pigs and those given ST (trend for reduction on d 6 and 14; P < 0.1). However, compared to control pigs, feed intake was dramatically reduced on d 2, 3, 4, 6, 8, 9, 10, and 13 in pigs given SC (P < 0.01) and tended to be reduced on d 5 (P = 0.08) and d 11 (P = 0.06). Serum IGF-I was similar between pigs on all treatments on d 0. IGF-I remained similar between control pigs and pigs fed ST on d 7 and 14. But, IGF-I was reduced in pigs given SC on d 7 (P < 0.01 vs. control and ST) and d 14 (P = 0.07 vs. control; P < 0.05 vs. ST). Thus, clear differences exist between the serovars to disrupt normal feed intake and alter circulating IGF-I. These differences may reflect the swine host-adapted nature of SC compared to ST. Moreover, the results from the ST treatment (compared to controls) indicate that the mere presence of low levels of the enteric pathogen are not sufficient to erode feed intake and reduce circulating IGF-I.

Key Words: Pigs, Salmonella, IGF-I

W95 Newborn calves fed colostrum of cows treated with rbST. Study II: IGF-I and IGF type I receptor gene expression in the liver and small intestine. A. Bagaldo, P. Pauletti, E. Delgado, D. Lanna\*, L. Coutinho, L. Kindlein, and R. Machado Neto, *Escola Superior de Agricultura Luiz de Queiroz* - *USP*, *Piracicaba, SP, Brazil.* 

Serum IGF-I levels are highly correlated to liver IGF-I synthesis; IGF-l level is also known to regulate the amount of IGF-I receptor. However, it is still to be determined what would be the impact of higher levels of IGF-I in the colostrum on the dynamic of IGF-I, both locally and systemically. This work studied the effect of different levels of IGF-I present in the colostrum in IGF-I and IGF type I receptor gene expression in the liver and intestine of calves. Forty-two, preg-

nant Holstein cows were randomly assigned to two groups, receiving either growth hormone (rbST) or vitamin E, starting from 35 days prepartum, and every 14 days thereafter until parturition. Newborn calves were randomly slaughtered right after birth (0d) without colostrum ingestion, at two (2d) and seven days (7d) of life after colostrum ingestion, and samples were taken from liver and jejunum for quantification of mRNA of IGF-I and receptor type I. Data were statistically analyzed in a completely randomized design, 2X3 factorial arrangement. Expression of IGF-I was higher at birth in the liver of calves from rbST-treated cows (P<0.05), suggesting that rbST could have had an indirect effect in the fetus. At 7d, IGF-I mRNA was also higher in the livers of calves from rbST-treated cows. Colostrum from rbST group presented higher IGF-I concentration (+30%), which could have influenced the maturation of enterocytes and the absorption of nutrients by the calves, a condition that could explain the higher concentration found in the liver. The concentration of receptor type I mRNA decreased with calves age (P<0.05). Small intestine of calves showed a precondition for cellular response to the presence of IGF-I in colostrum at birth.

Key Words: Colostrum, IGF-I, mRNA

W96 Newborn calves fed colostrum of cows treated with rbST. Study I: Rna, dna and protein concentrations in the liver and small intestine. A. Bagaldo, P. Pauletti, E. Delgado, D. Lanna\*, L. Kindlein, and R. Machado Neto, Escola Superior de Agricultura Luiz de Queiroz - USP, Piracicaba - SP Brazil.

Uncertainty regarding the effect of feeding IGF-I to calves refer to whether it could be absorbed or not, and which impact it would have over intestinal mucosa and/or animal metabolism as a whole, especially the liver. This work aims to study the effect of different levels of IGF-I found in colostrum of cows treated with rbST during dry period, in the development of the intestinal tract of newborn calves. Forty-two, pregnant Holstein cows were randomly assigned to two groups, receiving either growth hormone (rbST) or vitamin E, starting from 35 days prepartum, and every 14 days thereafter until parturition. Newborn calves were randomly slaughtered right after birth (0d) without colostrum ingestion, at two (2d) and seven days (7d) of life after colostrum ingestion, and samples were taken from liver and jejunum for quantification of DNA, RNA and total protein. Data were statistically analyzed in a completely randomized design, 2X3 factorial arrangement. Colostrum from rbST-treated group presented higher IGF-I concentration (+30%). In the liver samples, RNA and protein concentrations (mg g-1 tissue) were higher in 2d and protein/RNA ratio was higher in 7d (P<0.05). There was interaction between dam's group and calf age regarding the concentrations of DNA, protein, and protein/RNA and RNA/DNA ratios (P<0.05) for jejunum samples. Jejunum of calves fed colostrum from rbSTtreated cows presented higher DNA concentration in 2d, which decreased to intermediate levels between 2d and 7d. This effect was also observed for protein/RNA ratio. DNA contents increased at 2d, but did not differ from 7d; protein/RNA ratio was also similar among ages of control group. Protein concentration in jejunum of rbST-treated group increased in 2d and decreased in 7d, while in calves from control group this increase was observed only in 7d. RNA/ DNA ratio decreased with age (P<0.05) for these calves. Cells in jejunum of calves from rbST-treated cows presented a different phase of maturation.

Key Words: Calves, Colostrum, Intestine

**W97** The role of muscle membrane phospholipids in the developmental decline in insulin sensitivity in the piglet. K. Bergeron<sup>\*1</sup>, J. F. Bernier<sup>1</sup>, P. Julien<sup>2</sup>, A. Myre<sup>1</sup>, T. A. Davis<sup>3</sup>, and M. C. Thivierge<sup>1</sup>, <sup>1</sup>*Nutraceutical and Functional Food Institute/Département des sciences animales, FSAA, Université Laval, Qc, Canada, <sup>2</sup>Québec Lipid Research Ctr, Laval University Medical Ctr (CHUL), Qc, Canada, <sup>3</sup>USDA/ARS Children's Nutr. Res. Ctr., Dept. Pediatr. Baylor Coll. Med., Houston, TX.* 

A decline in insulin sensitivity during postnatal development has been reported. Omega-3 long-chain polyunsaturated fatty acids (**n-3LCPUFA**) in muscle membrane phospholipids have been shown to increase insulin sensitivity in human pathologies, such as obesity and diabetes type II. The objective of the present investigation was thus to examine, in neonate piglets, the effects of enrichment of muscle membrane phospholipids with n-3LCPUFA on the decline in insulin sensitivity and protein anabolism. At 2 d of age, 24 piglets were weaned and randomly assigned to one of two semi-purified milk replacers: control or enriched with n-3LCPUFA. Milk replacers were formulated to meet piglet requirements and had a composition similar to sow's milk (lactose 31.0 %, protein 29.9 %, and fat 38.3 %). They differed in their fatty acid composition (Control: 0.82 % n-3LCPUFA; Enriched: 10.99 % n-3LCPUFA). Hyperinsulinemic-euglycemic-euaminacidemic clamp procedure (100 ng insu

lin/kg<sup>-0.66</sup>·min<sup>-1</sup>) were performed at either 9 or 26 d of age to measure insulin sensitivity. Two d later, simultaneous infusions of L-[1-<sup>13</sup> C]phenylalanine (22

 $\mu$ mol/kg·h) and NaH<sup>14</sup>CO<sub>3</sub> (1.0  $\mu$ Ci/kg·h) were conducted during a 4-h period to assess protein metabolism. Insulin-stimulated amino acid disposal rate decreased by 29% between 9 and 26 d of age (P = 0.04), and the feeding of a milk replacer enriched in n-3LCPUFA did not prevent the developmental decline in this response (P > 0.05). Whole body flux of phenylalanine tended to decrease with age (P = 0.09) but was not affected by milk replacers. Oxidation of phenylalanine tended to be reduced (P = 0.06) in n-3LCPUFA fed piglets, inducing a slight increase in phenylalanine accretion into proteins (P = 0.06). These changes of the metabolic use of amino acids in the neonate fed diets enriched with n-3LCPUFA suggest that further investigation is needed to determine whether these fatty acids can be used as a novel means of enhancing growing in farm animals.

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Key Words: Omega-3 Long-Chain Fatty Acids, Insulin Sensitivity, Piglets

**W98** Insulin-like growth factor binding protein (IGFBP)-3 and IGFBP-5 mediate TGF beta- and myostatin-induced suppression of proliferation in porcine embryonic myogenic cell cultures. E. Kamanga-Sollo, M. White, M. Hathaway\*, and W. Dayton, *University of Minnesota, St. Paul.* 

TGF-beta superfamily members myostatin and TGF-beta1 have been shown to suppress both proliferation and differentiation of myogenic cells. Treatment of cultured porcine embryonic myogenic cells (PEMC) with either TGF- beta1 or myostatin increases levels of insulin-like growth factor binding protein (IGFBP)-3 and -5 mRNA and protein. Additionally, both IGFBP-3 and IGFBP-5 cause IGF-independent suppression of proliferation in PEMC cultures. Consequently, we have examined the role of these IGFBPs in the ability of TGF- beta1 and myostatin to suppress proliferation of cultured PEMC. Treatment of PEMC cultures with either myostatin or TGF- beta1 significantly (p < 0.01) increases levels of both IGFBP-5 and IGFBP-3 mRNA. We have previously shown that immunoneutralization of IGFBP-3 decreases the proliferation-suppressing activity of TGF- beta1 and myostatin. Similarly, immunoneutralization of IGFBP-5 also significantly (P < 0.05) decreases the proliferation suppressing activity of these molecules. Simultaneous immunoneutralization of both IGFBP-3 and IGFBP-5 in TGF- beta1 or myostatin treated PEMC cultures restores both IGF-I and Long-R3-IGF-I-stimulated proliferation rates to 90% of the levels observed in control cultures receiving no TGF- beta1 or myostatin treatment (p < 0.05). Even though immunoneutralization of IGFBP-3 and -5 increased proliferation rates in TGF- beta1 or myostatin-treated PEMC cultures, phosphosmad 2 levels in these cultures were not affected. Consequently, we believe that our data strongly indicate that IGFBP-3 and IGFBP-5 mediate TGF-beta1 and myostatin-induced suppression of PEMC proliferation via IGF-independent mechanisms that do not involve phosphosmad 2 signaling.

Acknowledgements: Research supported by USDA National Research Initiative Competitive Grant Number: 00-03271

Key Words: IGFBP-3, IGFBP-5, Myostatin

**W99** Exogenous ghrelin elevates plasma growth hormone concentrations in steers allowed ad libitum intake. A. E. Wertz-Lutz<sup>\*1</sup>, J. A. Daniel<sup>1</sup>, J. A. Clapper<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.

Six steers (416  $\pm$  17.2 kg) were used in a crossover design to determine the effects of intravenous infusion of bovine ghrelin (BGHR) on plasma growth hormone (GH) concentrations. Steers were fed individually once daily (0800) and allowed to consume ad libitum until 2000 when feed was removed. Daily feed allotment was sufficient to result in ≥10% feed refusal. Serial blood samples were collected from steers fitted with an indwelling jugular catheter at 15-min intervals from 0600 through 1800. Harvested plasma was assayed for BGHR and GH. Saline (SAL) or BGHR was infused via jugular catheter at 1200 and 1400, which were times when steers usually did not eat feed. Exogenous BGHR was infused to achieve a plasma concentration of 1000 pg/mL. This dosage was chosen on the basis of previous research that indicated a peak BGHR concentration of 1000 pg/mL for fasting steers. Steers were allowed 5 d to adjust between treatment periods. Then, treatments were switched between steer groups, and the sampling period was repeated. Compared with that of SAL steers (75 and 64 ± 28.9 pg/mL, 1215 and 1415 sampling time, respectively), average plasma ghrelin concentration was elevated (P≤0.0001) at the first post-infusion sampling for BGHR-infused steers (414 and 520 ± 25.9 pg/mL, 1215 and 1415 sampling time, respectively) after both infusion times. The BGHR infusion resulted in elevated (P≤0.0001) plasma GH concentrations (16.8 and 12.7 ± 1.61 ng/mL, 1215 and 1415 sampling time, respectively) compared with SAL infusion (6.8 and  $3.6 \pm 1.80$  ng/mL, 1215 and 1415 sampling time, respectively) for both infusion times. Both plasma BGHR and GH concentrations returned to baseline by 30 min post-BGHR infusion and were similar (P≥0.05) to those for SAL steers after both BGHR infusions. Intravenous administration of BGHR to achieve a concentration in plasma similar to that of a fasting steer was sufficient to result in elevated plasma GH concentrations.

Key Words: Ghrelin, Growth Hormone, Beef Cattle

**W100** Effects of constitutive expression of porcine IGFBP-3 on proliferation and differentiation of L6 myogenic cells. G. Xi\*, E. Kamanga-Sollo, M. Hathaway, M. White, and W. Dayton, *University of Minnesota, St. Paul.* 

Insulin like growth factor binding protein (IGFBP)-3 has been shown to either inhibit or potentiate proliferation of cultured cells depending on cell type and culture conditions. L6 myogenic cells do not produce detectable levels of IGFBP-3, however, we have shown that exogenous recombinant porcine IGFBP-3 (rpIGFBP-3) suppresses proliferation and differentiation of L6 myogenic cells in an IGF-I-dependent manner and also suppresses their proliferation via an IGF-I- independent mechanism. In order to assess the effects of endogenously produced IGFBP-3, we have transfected L6 myogenic cells with a vector containing rpIGFBP-3 cDNA under the control of the human EF-1α promoter and with the empty vector. We have isolated stable cell populations that constitutively produce porcine IGFBP-3 (tL6 cells) and stable mock transfected cell populations containing the empty vector (mL6 cells). Constitutive expression of IGFBP-3 does not influence expression of other IGFBPs (IGFBP-4 and IGFBP-5) that are normally produced by L6 myogenic cells. Immunoneutralization of IGFBP-3 using an anti-rpIGFBP-3 antibody increases both IGF-I- and Long-R3-IGF-I-stimulated proliferation of transfected L6 cells (39% and 23%, respectively) (p<0.01) but has no effect on mL6 cells. These data indicate endogenous porcine IGFBP-3, like exogenous rpIGFBP-3, suppresses the proliferation of L6 myogenic cells via both IGF-I-dependent and independent pathways. Immunoneutralization of IGFBP-3 also increased IGF-I-stimulated differentiation (38%, p<0.01) and caused a slight increase in Long-R3-IGF-I stimulated differentiation of transfected L6 myogenic cells but had no effect on mL6 cells. Results indicate that exogenous and endogenous IGFBP-3 affect proliferation and differentiation of L6 myogenic cells in a similar way. In addition, tL6 and mL6 cells provide a good system to further investigate the mechanisms by which IGFBP-3 affects proliferation and differentiation of myogenic cells.

Acknowledgements: Research supported by USDA National Research Initiative Competitive Grant Number 00-03271

Key Words: IGFBP-3, Stable Tansfection, L6 Myogenic Cells

W101 Transgenic over-expression of IGF-I modulates the synthesis and secretion of pig milk IGFBP-2 and -5 in the early and post-lactation periods. M. H. Monaco\*, M. B. Wheeler, and S. M. Donovan, *University of Illinois, Urbana.* 

IGF regulates mammary growth and development and its bioactivity is mediated through IGF binding proteins (IGFBP). Mammary-specific transgenic overexpression of IGF-I under the direction of the bovine  $\alpha$ -lactalbumin promoter in lactating sows resulted in a >50-fold higher milk IGF-I, 2-fold higher milk IGFBP-2 and -5 as measured by WLB in early lactation and 1.2-fold higher IGFBP-2 in postpartum secretions. Herein, free IGF-I was determined by IRMA and mammary IGFBP-2 and -5 mRNA were measured by quantitative RT-PCR to test the hypothesis that IGF-I up-regulates mammary IGFBP-2 and -5 transcription to counteract the over-expression of IGF-I. Transgenic gilts (TG; n=6) and non-transgenic gilts (CON; n=6) were mated and upon parturition, litter size was normalized to 10 piglets. Piglets were allowed to suckle until day 21 postpartum. On d110 of gestation (110g), d3 and 14 of lactation, and d4 postweaning, mammary tissues were obtained from each sow by surgical biopsy, and milk was collected. Total and free IGF-I were higher (p<0.001) in TG than CON milk. Free IGF-I accounted for 11.7±1.5% of the total IGF in TG milk during lactation, but only 3±1.2% in CON (p<0.001). In the post-weaning secretion, free IGF-I was 4-fold higher (p<0.01) in TG than CON. IGFBP-2 and -5 mRNA were affected by a transgene\*day interaction (p<0.006), but not by transgene alone (p=0.2). IGFBP-2 and -5 expression decreased 2-fold (p<0.02) and 3.5-fold (p<0.006) between d110g and d3 in CON, but not TG sows. During involution, IGFBP-2 mRNA increased 2.6-fold in CON and 8-fold in TG compared to d14 (p<0.02). Independent of transgene, IGFBP-5 mRNA increased >15-fold during involution versus d14 (p<0.05). In summary, transgenic overexpression of IGF-I prevents the early postpartum decline in mammary IGFBP-2 and -5 mRNA observed in CON sows. Despite the marked up-regulation of IGFBP synthesis and secretion, free IGF-I in milk was 3-fold higher in TG than CON. (Funded by the USDA CSREES under project NRI 00-35206)

Key Words: IGF-I, IGFBP, Mammary

W102 Small intestinal IGF-I binding protein (IGFBP)-2 and -5 and IGF receptors in piglets suckling IGF-I transgenic sows. J. L. Hartke\*, M. H. Monaco, R. H. McCusker, M. B. Wheeler, and S. D. Donovan, *University of Illinois, Urbana.* 

We produced transgenic swine (TG) that exhibit 60- to 100-fold over-expression of IGF-I in milk (600 ug/L) compared to non-transgenic sows (CON) sows (10 ug/L) and have shown that piglets suckling TG sows have greater (P < 0.05) small intestinal mucosal weight, protein and DNA content, and lactase and sucrase activities compared to piglets suckling CON sows. The cellular actions of IGF-I are exerted through type I IGF receptors and its bioactivity is regulated by IGFBP. Herein, we tested the hypothesis that IGF receptor binding and IGFBP expression would be differentially regulated in piglets suckling TG compared to CON sows. Jejunal and ileal samples were collected from piglets suckling CON or TG sows on d 7 and 21 postpartum (n = 10/d/trtmt). Intestinal IGFBP-2 and -5 mRNA expression were measured by quantitative real-time polymerase chain reaction in 3 randomly selected piglets/trtmt. Type I IGF receptor capacity (Bmax) and affinity (Kd) were determined by radioligand binding assays and Scatchard analysis (n = 4/d/trtmt). IGFBP-5 mRNA expression did not differ between CON or TG, regardless of segment or timepoint. IGFBP-2 was similar between CON and TG on d 21 and in the jejunum on d 7, however, ileal IGFBP-2 mRNA on d 7 was 30% greater (P = 0.06) in piglets suckling CON sows. Receptor affinity was unaffected by IGF-I. Receptor numbers were similar in piglets suckling CON and TG sows on d 7 and 21 in the jejunum. However, ileal receptor number was 3-fold greater in piglets suckling TG ( $61.32 \pm 10.11$ ) versus CON (19.79  $\pm$  2.93 ) on d 21 (P < 0.01). In addition, when Bmax was compared between intestinal segments, piglets suckling IGF sows had greater receptor numbers in the ileum compared to the jejunum (P < 0.02). The upregulation of IGF receptors in the ileum of piglets suckling TG sows supports previous work in our lab where we have shown that the greatest differences between piglets suckling CON versus TG sows were observed in the ileum on d 21 postpartum.

Key Words: IGF-I, Receptor, Binding Protein

W103 Growth rate, feed efficiency (FE), and IGFBP-2 and -3 in beef cattle treated with exogenous bovine (b) ST beginning at 200d, 250d and 300d of age. B. Velayudhan\*, K. Govoni, T. Hoagland, and S. Zinn, *University of Connecticut, Storrs.* 

To determine the effects of age at the start of bST treatment on the growth response to bST, 40 beef cattle (200±21d of age) were randomly assigned to one of four treatments (10 animals/treatment). Three groups received bST (33µg/ kg BW) daily beginning at 200d, 250d or 300d of age until all animals reached 400d of age. Controls did not receive bST. Animals were housed in pens (five animals/pen; two pens per treatment) and fed a diet formulated for an ADG of 1.2kg/d. Feed intake (per pen) was measured daily and BW determined weekly. Blood samples (10mL) and ultrasound measurements were collected at 200d, 250d, 300d, 350d and 400d of age. Serum concentrations of IGFBP-2 and IGFBP-3 were determined by Western ligand blot. Overall, cattle gained 262kg BW with a treatment by week interaction (P<0.01), such that during the treatment period ADG was 13.1, 8.0 and 14.2% greater (P<0.05) in cattle treated with bST beginning at 200d, 250d and 300d, respectively, compared with controls during the same length of time. ADFI was 7% less (P<0.05) in bST-treated cattle than controls. Increases in ADG coupled with a reduction in ADFI resulted in increased FE (gain/feed; P<0.01) in bST-treated cattle compared with controls. Backfat thickness increased (P<0.05) over time and was less in the bST-treated cattle (treatment by week interaction; P<0.05). Rib eye area increased (P<0.05) over time, but the increases were similar across treatment groups. Serum concentrations of IGFBP-2 decreased while IGFBP-3 increased over time. In addition, bST treatment tended to increase concentrations of IGFBP-3 and decrease concentrations of IGFBP-2 compared with controls. In conclusion, bST treatment initiated between 200d and 300d of age increased ADG and FE and decreased backfat thickness in growing beef cattle. These increases were associated with increased concentrations of IGFBP-3 and decreased concentrations of IGFBP-2. In general, the magnitude of bST-induced change was greatest when treatment was initiated at 300d of age.

Key Words: Somatotropin, Growth, IGFBP-2 and -3

W104 Expression of porcine acid-labile subunit (pALS) of the 150kilodalton ternary insulin-like growth factor complex and initial characterization of recombinant pALS protein. C. Y. Lee<sup>\*1</sup>, D. H. Lee<sup>2</sup>, C. Chun<sup>2</sup>, and S. H. Kim<sup>3</sup>, <sup>1</sup>Jinju National University, Jinju, Korea, <sup>2</sup>University of Seoul, Seoul, Korea, <sup>3</sup>Kyunghee University, Seoul, Korea.

Acid-labile subunit (ALS) is a component of the 150-kDa insulin-like growth factor-binding protein-3 (IGFBP-3) complex, which, by sequestering the majority of IGFs-I and â•"II and thereby prolonging the half-life of them in plasma, serves as a circulating reservoir of IGFs in mammalian species. A pGEX-2T plasmid and a baculovirus expression constructs harboring a coding sequence for glutathione-S-transferase (GST)-porcine ALS (pALS) fusion protein were expressed in BL21(DE3) E. Coli and Sf9 insect cells, respectively. The expressed protein was purified by glutathione or Ni-NTN affinity chromatography, followed by cleavage of the fusion protein using Factor Xa. In addition, pALS and hIGFBP-3 were also produced in small amounts in the Xenopus oocyte expression system which does not require any purification procedure. A 65-kDa pALS polypeptide was obtained following the prokaryotic expression and the enzymatic digestion, but biochemical characterization of this polypeptide was precluded because of an extremely low expression efficiency. The baculovirus- as well as Xenopus-expressed pALS exhibited the expected molecular mass of 85 kDa which was reduced into 75 and 65 kDa following deglycosylation of Asnlinked carbohydrates by Endo-F glycosidase, indicating that the expressed pALS was properly glycosylated. Moreover, irrespective of the source of pALS, the recombinant pALS and hIGFBP-3 formed a 130-kDa binary complex which could be immunoprecipitated by anti-hIGFBP-3 antibodies. Collectively, results indicate that an authentic pALS protein can be produced by the current expression systems.

Key Words: Pig, ALS, IGF

W105 Effect of ovariectomy and estradiol administration on bovine skeletal muscle insulin-like growth factor-I (IGF-I) and  $\beta$ -adrenergic receptor ( $\beta$ AR) messenger RNA (mRNA) abundance. E. K. Sissom<sup>\*1</sup>, M. J. Meyer<sup>2</sup>, Y. R. Boisclair<sup>2</sup>, M. E. Van Amburgh<sup>2</sup>, and B. J. Johnson<sup>1</sup>, <sup>1</sup>Kansas State University, Manhattan, <sup>2</sup>Cornell University, Ithaca, NY.

Insulin-like growth factor-I is a potent stimulator of postnatal skeletal muscle growth. Estradiol (E2) has been shown to affect the local production of IGF-I in skeletal muscle, as well as up-regulate the expression of the BAR subtypes in other tissues. The objective of this study was to determine the effects of E2 and/ or ovariectomy (OVX) on the steady-state mRNA levels of IGF-I and three  $\beta AR$ subtypes (\beta1, \beta2, \beta3) in semimembranosous muscle. Sixteen prepubertal Holstein heifers were used in a 2 x 2 factorial experiment with main effects of E2 and OVX. Eight heifers were ovariectomized at approximately 4.6 mo of age (150 kg BW). Estrogen or excipient was administered following a 30 d postoperative recovery period. 17β-Estradiol solubilized in corn oil (0.1 mg E2/kg BW) was given three consecutive days on 24 h intervals via subcutaneous injection. Heifers not receiving E2, received a subcutaneous injection of the corn oil excipient. Approximately 54 h post initial E2 dose, heifers were sacrificed and semimembranosous muscle was excised. Complimentary DNA was generated from total RNA isolated from these tissues. Transcriptional regulation of the genes encoding IGF-I,  $\beta$ 1,  $\beta$ 2, and  $\beta$ 3AR was determined by real-time quantitative PCR. No interactions (P > 0.10) were observed between E<sub>2</sub> and OVX. There was no main effect of  $E_2$  or OVX on the expression of the  $\beta$ AR subtypes, or of OVX on IGF-I mRNA. However, in both intact and OVX heifers, E2 administration elicited a 58% increase (P < 0.05) in muscle IGF-I mRNA compared to non-E2 treated heifers. These data suggest E2 administration can increase local muscle production of IGF-I in pre-pubertal heifers. This increase in IGF-I production may lead to an increase in postnatal skeletal muscle growth.

Key Words: β-Adrenergic Receptors, Estradiol-17β, IGF-I

W106 Effects of restricted feed intake on plasma levels of IGF-I and abundance of hepatic IGF-I and GH receptor mRNA in channel catfish. B. Peterson\* and B. Small, USDA/ARS Catfish Genetics Research Unit, Stoneville, MS.

Feed restriction and fasting of catfish are common management strategies during periods of environmental stress and disease. We have previously reported roles for GH and IGF-I in growth regulation of channel catfish, but effects of restricted feeding on the somatotropic axis are not known. Research was conducted to examine abundance of hepatic IGF-I and GH receptor (GHR) mRNA and plasma IGF-I in fed, restricted, and fasted channel catfish. One hundred and twenty fish (60.0  $\hat{A} \pm 0.2$  g) were randomly assigned to one of three treatments with four replicates each. The treatments were: 1) fed control (commercial catfish diet fed daily), 2) restricted (commercial diet fed every other day), and fasted (no feed). All fish were weighed and bled from the caudal vasculature on d 0, 21, and 42. On d 42, liver samples were excised from 8 fish per treatment (2 fish/tank), RNA was isolated, and relative abundance of hepatic IGF-I and GHR mRNA was determined by real time RT-PCR.

Final weights of the fed control, restricted, and fasted fish were 110.4, 78.8, and 40.6 g, respectively. Plasma levels of IGF-I were higher (P < 0.001) in the fed and restricted fish (7.5  $\pm$  0.8 and 5.5  $\pm$  0.6 ng/ml, respectively) compared to fasted fish (1.4  $\pm$  0.6 ng/ml) at day 21. By d 42, differences in plasma levels of IGF-I were less dramatic between treatments, but tended to remain higher in fed controls (P < 0.01). Abundance of liver IGF-I mRNA was similar (P > 0.05) among treatments. However, GHR mRNA abundance decreased 60% in the both restricted and fasted fish compared to fed controls (P < 0.001). Results showed that restricting feed intake decreased plasma IGF-I without a significant change in abundance of IGF-I mRNA. Results also demonstrated that GHR mRNA is down regulated when feed is limited. Higher plasma levels of IGF-I in the fed controls support IGF-I's role in growth regulation of channel catfish. One of the mechanisms through which growth may be inhibited in food restricted catfish is through a reduction in GHR and thus IGF-I.

Key Words: IGF-I, GH Rreceptor, Channel Catfish

W107 Zinc finger binding protein 89 (ZBP-89) is a potential transcription factor for the bovine growth hormone receptor 1A promoter. H. Jiang\*, Q. Xu, and L. Springer, *Virginia Tech*, *Blacksburg*.

Growth hormone receptor (GHR) 1A mRNA is a major GHR mRNA variant expressed in the bovine liver. The objective of this study was to identify DNA regions and transcription factors that might regulate GHR1A mRNA expression. With a deoxyribonuclease I footprint analysis, we detected a GHR1A promoter region that was able to bind to nuclear proteins from the bovine liver. Using this GHR1A promoter region as bait to screen a bovine liver cDNA library in a yeast-one hybrid analysis, we identified two cDNA clones that appeared to encode proteins binding to this GHR1A promoter region. Nucleotide sequencing and sequence analysis revealed that both cDNA clones encoded the bovine zinc finger binding protein 89 (ZBP-89), a transcription factor that has been reported to bind to several gene promoters involved in cell growth regulation. Electrophoretic mobility shift assays confirmed the ability of this GHR1A promoter region to bind to ZBP-89 protein from the bovine liver. In transient transfection analysis, cotransfection of a ZBP-89 expression plasmid enhanced (P < 0.01) reporter gene expression from a GHR1A promoter containing the identified ZBP-89-binding DNA region. These results together indicate that ZBP-89 may be a transcription factor that regulates the expression of GHR1A mRNA in the bovine liver.

Key Words: Promoter, Transcription Factor, Growth Hormone Receptor

**W108** Effects of supply of excess amino acids on leucine utilization by growing steers. M. S. Awawdeh\*, E. C. Titgemeyer, G. F. Schroeder, and D. P. Gnad, *Kansas State University, Manhattan.* 

We examined the effects of excess N supply from supplemental AA on leucine (Leu) utilization. Six ruminally cannulated Holstein steers (161 kg) were utilized in a 6x6 Latin square with an additional period. All steers received a soyhullbased diet at 2.6 kg/d DM, ruminal infusions of 200 g/d acetate, 200 g/d propionate, and 50 g/d butyrate, abomasal infusion of 300 g/d glucose, as well as abomasal infusion of an AA mixture (238 g/d) containing glutamate, glycine, and all essential AA except Leu. Treatments were abomasally infused and arranged as a 2x3 factorial with two levels of Leu (0 or 4 g/d) and three AA supplements (no additional AA, CONTROL; 100 g/d nonessential AA + 100 g/ d essential AA, NEAA+EAA; and 200 g/d essential AA, EAA). Periods lasted for 6 d, with 2-d adaptations and 4 d for excreta collection. Leucine increased (P<0.05) retained N from 24.0 to 28.0 g/d and decreased (P<0.05) urinary N from 54.9 to 51.1 g/d and urinary urea from 45.3 to 40.1 g/d. Both AA treatments increased (P<0.05) plasma urea and urinary urea N. NEAA+EAA decreased (P<0.05) retained N from 30.4 to 28.1 g/d and increased (P<0.05) urinary N from 37.8 to 59.7 g/d and urinary urea N from 28.0 to 49.3 g/d. EAA decreased (P<0.05) retained N from 30.4 to 19.5 g/d and increased (P<0.05) urinary N from 37.8 to 61.5 g/d and urinary urea N from 28.0 to 50.7 g/d. Because ruminal ammonia loading improved Leu utilization in a previous study, the decreases in retained N with the AA treatments were likely induced by AA imbalances rather than excess N, per se. Serum IGF-I concentrations were not affected (P>0.4) by treatment. Leucine increased (P<0.05) serum insulin, and both AA treatments tended (P=0.1) to increase serum insulin. Our data suggest that excess AA supply negatively impacts protein deposition by growing cattle when Leu is the limiting AA.

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Key Words: Amino Acids, Leucine, Growth

**W109** The expression of genes related to adipocytes in Lee-Sung Pigs. S. T. Ding\*, H. C. Wang, Y. H. Ko, and C. L. Chen, *National Taiwan University, Taipei, Taiwan.* 

The purpose of this study was to detect differential expression of genes related to adipocytes in Lee-Sung pigs by suppression subtraction hybridization (SSH).

the differential expression of genes between the two cell types in pigs. We found that at least 132 genes were expressed greatly in the adipocytes as compared with the S/V cells. Among these genes, 10 genes including 5 novel genes with the highest differences were selected and confirmed by Northern analysis. We found that angiotensin, calpin, stearoyl coenzyme A desaturase, and tumor necrosis factor  $\alpha$  were highly expressed in the adipocytes as compared with S/V cells. The results confirmed that the genes involved in lipid metabolism were highly expressed in porcine adipocytes. However, specific functions of the novel genes discovered in the current study await further investigation. **Key Words:** Adipocyte, SSH, Pig that RIA **W110** Role of the translational insulin signaling machinery in the anabolic effect of n-3 polyunsaturated fatty acids in growing steers. M. C. Thivierge\*1, L. Dombrowski<sup>2</sup>, A. A. Gingras<sup>1</sup>, and A. Marette<sup>2</sup>, <sup>1</sup>Université Laval, *Quebec, QC, Canada*, <sup>2</sup>Laval University Hospital Research Ctr., Quebec, QC, Canada.

Long-chain n-3 polyunsaturated fatty acids (LCn-3PUFA) in muscle membrane phospholipids are potentially involved in the regulation of protein anabolism in growing steers. Their amount is positively associated to increased muscle insulin sensitivity for amino acid utilization and altered protein metabolism. The current study focuses on the muscle cellular regulation of protein synthesis at the translational level of control. Two groups of 3 steers were used in a switchback design with 2 treatments randomized over 3 experimental periods of 5 wks. Steers were fed a basal diet composed of 44% forage and 56% concentrates. Two oil treatments were administrated continuously into the abomasum at 4% DMI: 1) control oil mixture (0.60 cotton:0.40 olive oil); or 2) refined Menhaden oil. The expression and phosphorylation state of insulin signaling components [the insulin receptor (IR), the PI3K-Akt-mTOR pathway and two modulators of translation initiation downstream of mTOR (p70S6k, 4EBP-1)] were investigated. Muscle of steers enriched with n-3LCPUFA tended to have higher amount of insulin receptors (P = 0.12). Although Akt phosphorylation on either Ser or Thr residues was not affected, mTOR phosphorylation was highly increased (P=0.06) in n-3LCPUFA enriched steers. Accordingly, phosphorylation of its downstream targets p70S6k (P = 0.14) and the (eIF)4E-binding protein 4E-BP1 (P = 0.11) both tented to be increased in steers fed n-3LCPUFA. These data strongly suggest that insulin signaling is involved in the n-3LCPUFA-mediated anabolic response. Moreover, the apparent lack of activation of the PI3K/Akt pathway suggests that the n-3LCPUFA-promoted mTORp70S6k-4EBP-1 anabolic pathway may be mediated by the increased amino acid availability at the cellular level, which would be sustained by higher whole body amino acid disposal. Supplemental energy demand required in this anabolic response was mediated through GLUT4 glucose transporters (P = 0.04).

Adipocytes and stromal vascular cells (S/V cells) from pig adipose tissues were

isolated for mRNA extraction. The SSH kit from Clontech (PCR Select) was

utilized to explore the differentially expressed genes. Subtractions were performed and the differentially expressed gene fragments were cloned into pGEM-

T Easy TA cloning vector (Promega). cDNA from adipocytes was subtracted by

the cDNA from the S/V cells. We have select 384 clones for gene sequence

determination by a genetic analyzer (ABI 3730) and for further sequence analy-

sis. These genes were subjected to a differential screening procedure to confirm

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Key Words: Insulin Sensitivity, Cellular Signaling, Steers

**W111** Effect of myostatin on avian myogenic satellite cells and embryonic myoblasts. D. McFarland\*<sup>1</sup>, S. Velleman<sup>2</sup>, J. Pesall<sup>1</sup>, and C. Liu<sup>2</sup>, <sup>1</sup>South Dakota State University, Brookings, <sup>2</sup>Ohio State University, Wooster.

Myostatin (GDF-8) inhibits the activation, proliferation, and differentiation of myogenic satellite cells. The relative importance of this growth factor is demonstrated in myostatin-null mice and cattle possessing defective myostatin genes. These defects result in greatly enhanced musculature. In the present study we

examined the effect of myostatin on turkey myogenic satellite cells and embryonic myoblasts. Compared with controls, proliferation of both turkey embryonic myoblasts and satellite cells was inhibited between 26 and 45% in serumfree medium containing 20 ng/ml myostatin. While individual turkey satellite cell clones differed in their responsiveness to myostatin (P < 0.05), there were no significant differences in the responsiveness of fast and slow growing cells as groups (P > 0.05). A slow growing clone that exhibited the greatest response to myostatin also exhibited the greatest depression of differentiation with this growth factor (P< 0.05). All other turkey satellite cell clones exhibited similar responses to the differentiation depressing effects of myostatin (P>0.05). However, myostatin had no effect on turkey embryonic myoblast differentiation (P> 0.05). When exposed to myostatin, all fast growing clones and one slow growing clone increased their expression of decorin, a growth inhibitor (P < 0.05). The present study demonstrates that myogenic cells differ in their responsiveness to myostatin and suggest a role for decorin in myostatin action in muscle development.

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Key Words: Myostatin, Muscle, Decorin

W112 A novel regulatory mechanism of muscle protein anabolism in steers. A. A. Gingras<sup>\*1</sup>, P. Y. Chouinard<sup>1</sup>, Y. Couture<sup>2</sup>, P. Julien<sup>3</sup>, P. Dubreuil<sup>2</sup>, A. Myre<sup>1</sup>, K. Bergeron<sup>1</sup>, T. A. Davis<sup>4</sup>, and M. C. Thivierge<sup>1</sup>, <sup>1</sup>Université Laval, *Qc, Canada*, <sup>2</sup>Université de Montréal, *Qc, Canada*, <sup>3</sup>Laval University Medical Ctr (CHUL), *Qc, Canada*, <sup>4</sup>Baylor College of Medicine, Houston, Texas.

Omega-3 long-chain polyunsaturated fatty acids (**n-3LCPUFA**) have been shown to improve insulin sensitivity in human pathologies, such as obesity and diabe-

tes type II. The aim of this study was to investigate the ability of n-3LCPUFA to regulate protein anabolism in growing steers. Two groups of three Simmental x Red Angus steers were used in a switch back design with two treatments assigned over three 35-d periods. Steers were fed a basal diet composed of 13% grass silage, 31% corn silage and 56% concentrates. They were fitted with chronic catheters in the abomasum and in a mesenteric artery. Oil infusions (5% DMI) were continuously administrated into the abomasum (control oil: 40% olive: 60% cottonseed; n-3LCPUFA oil: 100% Menhaden oil). On the 5th wk of each period, hyperinsulinemic-euglycemic-euaminoacidemic clamps (10, 40, 160 mU insulin/kg·h) were performed to measure insulin sensitivity. A 9-h continuous infusion (1.667 µmol/kg·h) of L[1-13C]phenylalanine (Phe) was conducted to assess protein metabolism. High dietary intake of n-3LCPUFA resulted in the incorporation of n-3LCPUFA into muscle phospholipids. With Menhaden oil infusion, n-3LCPUFA were increased (P<0.001) in muscle phosphatidylcholine from 11 to 23% (by weight). In muscle triglycerides n-3LCPUFA were increased (P<0.01) from 0.27 to 0.80%. Insulin-stimulated amino acid disposal tended to increase (+42%, P=0.09) with infusion of n-3LCPUFA. A 23% reduction (P=0.04) in whole body irreversible loss rate of Phe also occurred in n-3LCPUFA-enriched steers. Based on significant higher Phe isotopic enrichment (P=0.03) and on unaltered arterial concentrations of Phe (64 mM), it is likely that this reduction in Phe flux could result from a decrease in whole body proteolysis. These changes in hormonal or metabolic sensitivity in n-3LCPUFAenriched steers may underlie the 18% decrease (tendency: P=0.16) of feed conversion resulting from a reduction (P=0.05) in food intake. These findings suggest that muscle n-3LCPUFA could act as a potent mediator of anabolism in growing steers.

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Key Words: Omega-3 Long-Chain Fatty Acids, Insulin Sensitivity, Steers

## International Animal Agriculture

W113 Environmental factors and genetic parameters for birth weight in the indigenous Chiapas ovine breed. G. Campos<sup>1</sup>, H. Castro-Gámez<sup>1</sup>, R. López<sup>1</sup>, R. Perezgrovas<sup>2</sup>, and H. Castillo-Juárez<sup>\*3</sup>, <sup>1</sup>Universidad Nacional Autónoma de México, Ciudad Universitaria, México D.F., <sup>2</sup>Universidad Autónoma de Chiapas, Teopisca Center, Los Altos de Chiapas, México, <sup>3</sup>Universidad Autónoma Metropolitana, Calzada del Hueso, México D.F.

The aim of this study was to estimate the heritability, the permanent environment effect and the breeding values for birth weight (BW) in a sheep flock from a Mexican local indigenous ovine breed named Chiapas. Fixed effects (environmental factors) affecting BW were also determined. This information is required for breeding purposes. The flock belongs to the indigenous Tzotzil community from the mountains of Chiapas, in the south of Mexico. In 1991 a breeding program supervised by the Teopisca Center from the University Autonomous of Chiapas was introduced in order to improve the quality of this flock based on the Totzil community breeding objectives and goals. Significant fixed effects were year of lambing, sex of the lamb, number of lambing (P < 0.01). An animal model, that included significant fixed effects, and DFREML software was used to estimate heritability and the permanent environment effect. The heritability of BW was 0.27  $\tilde{A}$ ,  $\hat{A} \pm 0.10$  and the permanent environment effect was 0.26 Å, ű 0.05. The breeding values for BW ranged from Å¢â, ¬â•œ0.27 to 0.40 kg. The permanent environment effect was rather large and similar in magnitude to the additive genetic variation observed, showing that the maternal environment is very important for the variation of BW, and that BW can be used in their breeding programs.

Acknowledgements: Thanks to the indigenous Tzotzil community of Chiapas.

Key Words: Genetic Parameters, Birth Weight, Indigenous Sheep Breed

W114 Design of breeding objective including trypanotolerance for African cattle smallholders. U. Janβen-Tapken\*, Y. Li, and H. N. Kadarmideen, *Swiss Federal Institute of Technology, ETH Zentrum, Zurich, Switzerland.* 

A major disease constraint on livestock productivity in Eastern Africa is Trypanosomosis which directly affects the livelihood of poor livestock keepers. The objective of this study was to design a breeding goal including trypanosomosis to increase trypanotolerance in cattle in pastoral, agro-pastoral and crop-livestock systems of selected sites in Kenya and Ethiopia. Genetic response was compared between selection indexes with and without packed red blood cell volume (PCV) as measurement of tolerance for the disease with higher percentage indicating higher tolerance level. Selection index (SI) I without PCV used two traits, milk yield (MY) and live weight (LW) compared to selection index II including PCV additionally.

According to the findings in the field (Narok district, Kenya) the following population structure for pastoralists was assumed in this study: Number of cows is 200 over 10 age-groups with a replacement of 20 cows each year. The mating ratio of sire is 1:10 with 2 sires for each age-group. With a survival rate of 80%, 160 offspring is produced per year.

Genetic parameters used for the calculations and genetic responses were: Phenotypic standard deviation for MY, LW and PCV of 35kg, 7.4kg, 2.92% and heritabilities of 0.23, 0.30, 0.26, respectively. Phenotypic correlations between the traits were 0.15, -0.05 and 0.0, and genetic correlations were 0.01, -0.01 and 0.0.

The SI II increases MY by only 0.14kg per year compared to SI I with 2.40kg but changes PCV by 7.02% compared to a negative change of -0.02% if PCV is not included in the index. Both SI reduce live weight slightly by 0.01 and 0.02