1A mRNA expression in the liver of dairy cows at parturition is not caused by ending of pregnancy or by initiation of lactation *per se*.

Key Words: Growth Hormone Receptor, Cows, Liver

T144 A miniature condition in Brahman cattle is associated with a single nucleotide mutation within the growth hormone gene. B. L. McCormack[1], C. Agca[1], C. C. Chase, Jr.[2], T. A. Olson[3], T. H. Elsasser[4], A. C. Hammond[5], T. H. Welsh, Jr.*[6], and M. C. Lucy[1], [1]*University of Missouri, Columbia*, [2]*USDA, ARS, Brooksville, FL*, [3]*University of Florida, Gainesville*, [4]*USDA, ARS, Beltsville, MD*, [5]*USDA, ARS, Athens, GA*, [6]*Texas A&M University, College Station*.

Miniature Brahman cattle at the USDA, ARS in Brooksville, FL have normal proportioned growth but are approximately 70% of normal mature height and weight. Pedigree analyses suggest that the condition is inherited as a recessive gene. The objective was to clone the GH cDNA from miniature cattle and compare its sequence to normal cattle. Messenger RNA was isolated from pituitary and a cDNA for the protein coding region of the GH gene was amplified by reverse transcription PCR from each of two miniature cattle. The cDNA were cloned into plasmid vectors and the top and bottom strands were sequenced by automated DNA sequencing. Both cDNA clones contained a nucleotide polymorphism in which base number 641 of GenBank AF034386 (Bos indicus GH) was mutated from a cytosine (C) to a thymine (T). The C to T change encodes a mutation (threonine to methionine) at amino acid 200. The threonine is located in the fourth alpha helix of GH and is one of nine amino acids that participate directly in binding of GH to the GH receptor. Amino acid mutations at this location are associated with dwarfism in humans. Four miniature and four normal stature cattle from the Brooksville herd were tested for the polymorphism by using restriction fragment length polymorphism (RFLP) analysis of PCR-amplified GH gene with BsmBI restriction enzyme (specific for mutated nucleotide). The four miniature cattle were homozygous for the mutation (-/-). Two of the normal stature cattle were homozygous for the wild type allele (+/+) and two were heterozygous (+/-) (P<.05). Miniature Brahman cattle were homozygous for a single nucleotide polymorphism that encodes a mutation in an amino acid involved in binding of GH to the GH receptor. Normal stature cattle had at least one copy of the normal GH allele. We conclude that threonine 200 in bovine GH is required for normal growth in cattle.

Key Words: GH, Mutation, Growth

ASAS - Growth and Development

T145 Effect of gender and feeding program on productive performance and carcass quality of heavy pigs. J. Peinado*[1], M. Nieto[2], J. C. González[1], G. G. Mateos[3], and P. Medel[1], [1]*Imasde Agropecuaria Madrid, Spain*, [2]*Copese Segovia, Spain*, [3]*Universidad Politécnica de Madrid, Madrid, Spain*.

A total of 252 Duroc x Landrace*Large White pigs of 28.3 $\pm$ 2.1 kg of initial BW was used to study the influence of sex and a 10 % lysine restriction from 30 to 60 kg BW on productive performance and carcass quality. There were six treatments arranged factorially with two types of feed from 30 to 60 kg BW (LL, 0.89 % lys *vs* HL, 0.97 % lys) and three sexes (castrated females, CF; entire females, EF; castrated males, CM). Energy:lys ratio was maintained constant in all the diets, and each treatment was replicated three times (14 pigs housed together). Males were castrated at birth and females at 30 kg BW. From 60 kg BW to slaughter all pigs received a common diet *ad libitum* (2.4 Mcal NE/kg and 0.70 and 0.67 % lys from 60 to 90 and from 90 to 119 kg BW, respectively). From 30 to 60 kg BW pigs fed the LL diet had worse feed conversion than pigs fed the HL diet (2.12 *vs* 1.97 g/g; $P < 0.05$). However, from 60 to 90 kg BW pigs fed the LL diet ate more and grew faster than pigs fed the HL diet (1,982 *vs* 1,815 g/d; and 688 *vs* 619 g/d for LL and HL diet, respectively; $P < 0.05$). For the whole period, pigs fed the LL diet ate 5.5 % more feed than pigs fed HL diet (1,983 *vs* 1,879 g/d; $P < 0.05$) but gains and feed conversion were not affected. Gender did not influence productive performance. Backfat was higher for CF than for EF with CM in an intermediate position (28.3, 23.3, and 25.4 mm, respectively; $P < 0.05$). Fat thickness at *Gluteus medius* muscle (GM) was higher for CF than for EF or CM (20.2, 17.9, and 18.6 mm, respectively; $P < 0.05$). Feeding program did not affect backfat or GM fat. It is concluded that a reduction of 10 % of lysine from 30 to 60 kg BW increased feed intake in the global period without affecting growth or feed conversion. Also, castration of the females might improve some quality carcass parameters of pigs destined to the cured ham industry.

Key Words: Pig Performance, Castration, Lysine Level

T146 Effect of protein from placental bovine tissue on puberty and growth mice. F. A. Nunez[1], J. A. Garcia-Macias[1], J. A. Lopez[2], and F. G. Rios*[2], [1]*Facultad de Zootecnia - Universidad Autonoma de Chihuahua (Mexico) Periferico F. R. Almada, Mexico*, [2]*FMVZ - Universidad Autonoma de Sinaloa, Culiacan-Mazatlan, Mexico*.

Our aim was to evaluate activity of protein from placental bovine tissue partially purified by chromatographic column with Sephadex 50-40#8482. Sixty Balb/C pubertal mice, (thirty females and thirty males), were used in a randomized design experiment with 3 x 4 factorial arrangement to test two sex (females and males), two placental proteins (PI and PII) and four placental proteins (25, 50, 75 and 100 μg/mice/day of the placental protein). We compared this to a positive control (100μg/mice/day) of bovine serum albumin (BSA) and negative control group (100μL/mice/day) of ammonium bicarbonate (AB). Four groups (three males and three females) received four dose, 25, 50, 75 and 100 μg/mice/day of the placental protein PI (MW ranks 17 to 30 kDa), and four groups (three males and three females) received four dose, 25, 50, 75 and 100 μg/mice/day of the placental protein PII (MW ranks 31 to 97 kDa). Body weight and feed intake were measured daily for a ten day experimental period. The experiment was analysed as factorial design randomised with three factors; means comparison performed by contrast. Feed efficiency (FE) of female mice (0.095g:g) and male mice (0.091) were not affected (P#88050.05), by sex and treatments. The rate of growth (RG) observed a effect of sex; the male mice were heavier(P#88040.01) in 44.82% than female mice (2.61$\pm$0.23 vs. 1.17$\pm$0.23). RG in the mice group injected with AB was less (P#88040.05) than mice group injected with BSA (1.54$\pm$0.25 vs. 2.16$\pm$0.24), average daily gain (ADG) was different in male mice (P #88040.05) in 29.1% than female mice (0.51$\pm$0.01 vs. 0.36$\pm$0.09g/d). In the mice group injected with AB and the mice group injected with 50μg/mice/d of PII proteins, ADG was less than the other treatments in the same sex (P#88040.05). The female mice injected with 100 μg/mice/d were less ADG. The carcass composition was not affected (P#88050.05) by sex and treatments, 41.96$\pm$1.56 for male vs. 41.57$\pm$1.59, for female, however dreesing carcass percentage observed lineal tendency (P#88040.05) in PI and PII for male mice. It is concluded that proteins from placental bovine tissue partially purified, not improve growth in pubertal mice.

Key Words: Placental Protein, Mice, Growth

T147 Maternal undernutrition changes angiotensin type 1 and 2 receptors in ovine fetal heart. H.-C. Han*[1], K. J. Austin[1], S. P. Ford[1], P. W. Nathanielsz[2], and T. R. Hansen[1], [1]*University of Wyoming, Laramie*, [2]*New York University, New York*.

Nutrient restriction during early gestation causes compensatory growth of both the left and right ventricles of the ovine fetal heart by day 78 of gestation (Biol Reprod 69:133). Angiotensin II mediates cardiovascular pathologies through its effects on the type 1 (AT1) and type 2 (AT2) receptors. AT1 has been shown to mediate deleterious effects such as vasoconstriction, cellular growth, and endothelial cell damage in adult systems. Very little is known about fetal heart angiotensin receptors and the consequences of activation. Previously we reported that AT1 mRNA was down regulated in fetal left ventricle (LV) from nutrient restricted ewes when compared to control fed ewes. It was hypothesized that maternal undernutrition (energy and protein) would adversely affect AT1 and AT2 protein expression in the fetal LV. Pregnant ewes were randomly assigned to control (n = 8, 100% NRC requirements) or nutrient-restricted groups (n = 8, 50% NRC requirements). Ewes were maintained on diets from day 28-78 of gestation. Fetal LV was

snap frozen on day 78 of gestation. Protein was extracted in a reducing environment (beta mercaptoethanol) using Laemmli buffer. Protein (100 μg) was separated using SDS-15% 1D-PAGE and transferred to nitrocellulose membranes. AT1 and AT2 were detected using antibody against AT1 or AT2. Immunoreactive bands were detected using chemiluminescence substrate. Data represent arbitrary units. AT1 was down regulated in fetal LV from nutrient restricted when compared to control fed ewes (1630 vs 1345 $\pm$78.72, pooled standard error; $P < 0.05$). A 50-kDa form of AT2 was also down regulated in nutrient restricted LV (3290 vs 2814 $\pm$ 149.52; $P < 0.05$) while a lower molecular weight form of AT2 (22kDa) was upregulated (7672 vs 11044 $\pm$ 880.69; $P < 0.05$) when compared to control fed ewes. It is concluded that hypertrophy of fetal LV in response to maternal undernutrition is associated with decreased AT1 and 50-kDa AT2, and increased 22kDa AT2 protein expression. Fetal LV gene expression in response to changes in the angiotensin receptors may have longer-term implications in development of cardiovascular disease during post-natal life. NIH P20RR16474.

Key Words: Ruminant, Fetal Heart, Angiotensin

T148 Microarray analysis of gene expression during ovarian development in swine.

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The objective was to measure global changes in porcine ovarian gene expression by using microarray analysis. cDNA clones from porcine reproductive tissues were isolated and sequenced. The cDNA insert from individual clones was amplified by PCR and arrayed onto polylysine-coated slides (n = 20,000 clones per slide). Clones were represented either once (75%) or multiple times (25%) on individual slides. Total RNA was isolated from fetal ovary (60 d of gestation), neonatal ovary (22 d of age), prepubertal ovary (150 d of age), or adult ovary [ovary or corpus luteum(CL)]. Duplicate samples from four animals per developmental stage were used in the analysis (n = 40 microarrays). A reference sample was generated by pooling total RNA from ovarian follicles and CL. Each microarray slide was hybridized to cy5 labeled test sample and cy3 labeled reference sample. The arrays were normalized and filtered using GeneSpringTM software (Silicon Genetics, Redwood City, CA). Statistical analysis was done using Welch t-test and Bonferroni multiple testing correction with 0.01% false discovery rate. A Tukey post-hoc test was used to determine differential expression among the developmental stages. Approximately 16,500 clones passed the filtering and 1148 genes (7%) showed evidence of differential expression in ovarian tissues ($P<0.01$). Examples of genes found to be differentially expressed were: synaptonemal complex protein 3 and transportin 3 (fetal ovary); zona pellucida glycoprotein 3 and H19 (neonatal ovary); insulin-like growth factor binding protein 5 (fetal and neonatal ovary); connexin 43 (prepubertal ovary); apolipoprotein D (adult and prepubertal ovary); 3β hydroxysteroid dehydrogenase (adult ovary and CL); betamicroseminoprotein and major histocompatibility complex genes (CL). Distinct differences in gene expression across ovarian development were shown. This research was funded by the Monsanto Company.

Key Words: Ovary, Pig, Microarray

T149 Hormonal regulation of leptin receptor expression in primary cultures of porcine hepatocytes.

T. J. Caperna*, A. E. Shannon, S. M. Poch, W. M. Garrett, and M. P. Richards, *USDA, Agricultural Research Service.*

Leptin, a polypeptide hormone primarily produced by fat cells, has been shown to regulate energy metabolism in monolayer cultures of rodent hepatocytes and hepatic tumor cell lines. However, in porcine hepatocytes, we and others have demonstrated that leptin plays a minimal role, if any, in cellular energetics. The goals of this study, were to establish the presence of porcine hepatocyte leptin receptors and to determine the influence of regulatory hormones on leptin receptor gene expression. Hepatocytes were prepared from 30-70 kg pigs and seeded into T-25 flasks coated with pig tail collagen. Monolayers were established in Williams E medium containing fetal calf serum for one day and switched to serum-free medium with basal hormone conditions (1 ng/ml insulin and 10 nM dexamethasone) for an additional two days. For the final 24 hr, insulin (1 or 100 ng/ml) or glucagon (100 ng/ml), were added in the presence or absence of 100 nM T3. RNA was extracted and quantitative RT-PCR was performed with primers specific for porcine long form and total leptin receptors. Leptin receptor expression was calculated relative to 18S rRNA expression. The expression of the long form of the leptin receptor was confirmed under basal conditions. Insulin, glucagon and recombinant human proteins at 100 ng/ml (ghrelin, GLP-1 and leptin) had no influence on leptin receptor expression, however, the addition of T3 was associated with a marked increase ($P<0.001$) in total and long forms of the leptin receptor, 1.6 and 2.3 fold, respectively. Despite the presence of up-regulated leptin receptor expression in T3treated cells, addition of leptin to these cultures confirmed the lack of effect of leptin on glycogen turnover or glucagon-induced cAMP production. These data suggest that porcine hepatocytes are insensitive to leptin even when leptin receptor expression is enhanced by T3.

Key Words: Pig Hepatocytes, Leptin Receptor, T3

T150 Regulation of leptin and leptin receptor expression in porcine subcutaneous adipose tissue.

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The present study was performed to examine the response of the leptin gene to hormonal stimuli in porcine adipose tissue from finishing pigs. Ten Yorkshire gilts (approximately 150 kg BW) were used in this study. Dorsal subcutaneous adipose tissue samples were acquired and adipose tissue explants (approximately 100 mg) were prepared using sterile technique. Tissue slices were then transferred to 12 well tissue culture plates containing 1 mL of media 199 with 25 mM Hepes, 0.5 % BSA, pH 7.4 and various hormone supplements of interest. Triplicate tissue slices were incubated with either basal medium or hormone supplemented media in a tissue culture incubator at 37° C with 95% air/5% CO_2. Following 24 h of incubation, tissue samples from these incubations were blotted and transferred to microfuge tubes with subsequent freezing in liquid nitrogen and storage at -80° C prior to analysis for gene expression by RT-PCR and subsequent quantification of transcripts by capillary electrophoresis with laser-induced fluorescence detection. Media from these incubations was collected in microfuge vials and stored at -20° C prior to analysis for leptin content by RIA. Dexamethasone (1 μM) reduced leptin secretion by 25% ($p < 0.05$), while the combination of insulin and dexamethasone stimulated leptin secretion into the medium by 60% ($p < 0.05$). Porcine growth hormone (GH) inhibited leptin secretion by 33% ($p < 0.05$). Neither triiodothyronine (T3, 10 nM) nor IGF-1 (250 ng/mL medium) had an effect on leptin secretion from adipose tissue slices ($p > 0.05$). Dexamethasone produced a 35% increase in leptin mRNA expression relative to insulin ($p < 0.05$). Incubation of tissue cultures with GH, T3 or leptin for 24 h had no effect on leptin mRNA expression ($p > 0.05$). Dexamethasone induced a 34% decrease in total leptin receptor expression, relative to insulin treated adipose tissue slices, following 24 h of incubation. Porcine GH, T3 and leptin had no effect on total leptin receptor expression ($p > 0.05$). These data suggest that leptin secretion is a regulated phenomenon and that post-translational processing may be significant.

Key Words: Leptin, Leptin Receptor, Adipose

T151 Secreted and signaling genes and proteins in fetal and neonatal pig adipose tissue and stromal-vascular cells.

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Although microarray studies indicate unique and unexpected genes in human and rodent adipose tissue similar studies of meat animal adipose tissue have not been reported. Total RNA was isolated from 90 day fetal stromal-vascular (S-V) cell cultures (n=4; 2 arrays, 2 cultures / array) and subcutaneous adipose tissue from two 105 day-old fetuses and neonatal pigs. Dye labeled cDNA probes were hybridized to custom microarrays (70 mer oligonucleotides) representing 600 pig genes involved in growth and reproduction. Each of the four adipose tissue arrays represented RNA from a fetus and a pig with duplicate arrays / fetus and pig. Relative intensities of 25 irrelevant or reproductive oriented genes were averaged for both S-V cell culture arrays (150 $\pm$ 20) and all adipose tissue arrays (200 $\pm$ 40) and used to represent basal gene expression. A total of 391 and 340 genes were expressed 10 fold and 200 and 160 genes expressed 40 fold over basal in S-V cultures and adipose tissue, respectively. Relative intensities in adipose tissue arrays

(n = 4) included 20,360± 2700 for IGFBP-5, 19,800± 2,800 for relaxin, 7,360 ± 1,260 for leptin and 2,390± 355 for APO-A1. Collectively, 21 secreted protein genes and 40 signaling protein genes were expressed 40 fold in S-V cultures and adipose tissue. Additionally, the agouti gene was detected by RT-PCR in S-V cultures and adipose tissue. Proteomic analysis of adipose tissue and S-V culture conditioned media and cells identified several secreted and signaling proteins including APO-A1, APO-E, relaxin, IGFBP-5 and nitric oxide synthase. Another of the secreted proteins, PAI-1, was identified by ELISA in S-V culture media. These studies demonstrate for the first time the expression of several major secreted and signaling proteins in pig adipose tissue which may influence local and overall metabolism and growth.

Key Words: Adipocytes, Microarrays, proteomics

T152 IGF-I mRNA levels in bovine satellite cell cultures: Effects of fusion and anabolic steroid treatment.

E Kamanga-Sollo*, M. S. Pampusch, G. Xi, M. E. White, M. R. Hathaway, and W. R. Dayton, *University of Minnesota, St. Paul.*

Anabolic steroids enhance muscle growth in cattle; however, their mechanism of action is not known. The goal of this study was to determine if treatment of bovine satellite cell (BSC) cultures with 17β-estradiol (E2) or trenbolone (T) directly affects proliferation rate or level of mRNA for estrogen receptor (ER)-α, androgen receptor, and growth factors that have been shown to affect muscle growth (IGF-I, IGFBP-3, and myostatin). BSC cultures established from semimembranosus muscles of steers were treated for 48 hours with concentrations of E2 or T ranging from 0.001 nM to 10 nM. IGF-I mRNA levels in proliferating BSC cultures were significantly increased at 0.01 (1.9 times control values, $p < 0.02$) and at 0.1, 1 and 10 nM E2 (2.9, 3.5 and 3.5 times control values, respectively, $p < 0 .0001$). Both 1 and 10 nM T increased IGF-I mRNA levels to 1.7 times control values ($p < 0.02$). ER-α mRNA was detectable in BSC cultures, and levels were increased (2.3 times control levels, $p < 0.001$) in cultures treated with 0.001 nM E2 but not in cultures treated with higher concentrations of E2. Androgen receptor mRNA levels also were increased (1.5 times control levels, $p < 0.02$) in cultures treated with 0.001 nM T but not by treatment with higher concentrations of T. Levels of IGFBP-3 were increased (1.4 times control values, $p < 0.02$) by treatment with 0.001 nM E2 but not by treatment with high concentrations of E2. Myostatin mRNA levels were not affected by any concentration of either of the steroids. Although, levels of IGF-I mRNA were 10 times greater ($p < 0.02$) in fused BSC cultures than in proliferating cultures, treatment of fused cultures for 48 hours with 10 nM E2 increased IGF-I mRNA levels (2.5 times control levels, $p < 0.02$). Both E2 and T increased ^{3}H-thymidine incorporation rate (1.5 times control levels, $p < 0.001$) in BSC cultures in media containing serum from which IGFBP-3 had been removed. In summary, treatment of BSC cultures with either E2 or T increased IGF-I mRNA level and proliferation rate, thus, establishing that these steroids have direct anabolic effects on cells present in the BSC culture.

Key Words: Satellite Cell, Muscle, Steroid

T153 Production of recombinant porcine IGF-binding protein-5 (IGFBP-5) and its effect on proliferation of porcine embryonic myoblast cultures (PEMC) and L6 cells and on differentiation of L6 cells in the presence and absence of IGF-I.

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IGF-binding protein (IGFBP)-5 is produced by cultured porcine embryonic myogenic cell (PEMC) cultures and is secreted into the medium. IGFBP-5 may play some role in myogenesis and/or in changes in myogenic cell proliferation that accompany differentiation. IGFBP-5 reportedly may either suppress or stimulate proliferation or differentiation of cultured cells depending on cell type. Additionally, IGFBP-5 has been shown to possess both IGF-dependent and IGF-independent actions in some cell types but not all. The goal of this study was to produce recombinant porcine IGFBP-5 and assess its IGF-I-dependent and IGF-I-independent actions on proliferation of PEMCs and L6 myogenic cells. To accomplish this, we have expressed porcine IGFBP-5 in the baculovirus system, purified and characterized the expressed recombinant porcine IGFBP-5 (rpIGFBP-5). RpIGFBP-5 suppressed IGF-I-stimulated proliferation of both PEMCs and L6 cells in a concentration-dependent manner ($P < 0.05$). RpIGFBP-5 also suppressed Long-R3-IGF-I-stimulated proliferation of PEMCs and L6 cells ($P < 0.05$), indicating that rpIGFBP-5 possesses IGF-independent activity in these cell systems. Furthermore, rpIGFBP-5 stimulated differentiation of L6 cells as indicated by an increase in creatine phosphokinase (CPK) activity ($P < 0.05$). These data demonstrate that rpIGFBP-5 has the potential to affect proliferation and differentiation of both PEMCs and L6 myogenic cells during critical periods of muscle development that may impact ultimate muscle mass postnatally.

Key Words: Porcine, IGFBP-5, Muscle

T154 Effect of recombinant porcine IGFBP-3 on IGF-I and Long-R3-IGF-I-stimulated proliferation and differentiation of L6 myogenic cells.

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Insulin-like growth factor (IGF)-I stimulates both proliferation and differentiation of myogenic precursor cells. In vivo, IGFs are bound to one of the members of a family of six high-affinity IGF binding proteins (IGFBP 1-6) that regulate their biological activity. One of these binding proteins, IGFBP-3, affects cell proliferation via both IGF-dependent and IGF-independent mechanisms and it has generally been shown to suppress proliferation of cultured cells; however, it also may stimulate proliferation depending upon the cell type and the assay conditions. Cultured porcine embryonic myogenic cells produce IGFBP-3 and its level drops significantly immediately prior to differentiation. Additionally, IGFBP-3 suppresses both IGF-I and Long-R3-IGF-I-stimulated proliferation of embryonic porcine myogenic cells. In this study we have examined the effects of recombinant porcine IGFBP-3 (rpIGFBP-3) on IGF-I- and Long-R3-IGF-I-stimulated proliferation and differentiation of the L6 myogenic cell line. L6 cells potentially provide a good model for studying the actions of IGFBP-3 on muscle because they contain no non-muscle cells and they do not produce detectable levels of IGFBP-3. RpIGFBP-3 suppresses both IGF-I and Long-R3-IGF-I-stimualted proliferation of L6 cells, indicating that it suppresses proliferation via both IGF-dependent and IGF-independent mechanisms. Our data also show that rpIGFBP-3 causes IGF-independent suppression of proliferation without increasing the level of phosphosmad-2 in L6 cultures. Additionally, rpIGFBP-3 suppresses IGF-I-stimulated differentiation of L6 cells. In contrast, however, rpIGFBP-3 does not suppress Long-R3-IGF-I-stimulated differentiation. This suggests that rpIGFBP-3 does not have IGF-independent effects on L6 cell differentiation.

Key Words: IGF-I, IGFBP-3, Muscle

T155 Molecular cloning of untranslated regions of the porcine acid-labile subunit (pALS) gene and detection of pALS gene expression in hepatic and non-hepatic tissues.

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Tested primarily in this study was a hypothesis that there are untranslated regions including an intron in the gene of porcine acid-labile subunit (pALS) of the 150-kilodalton ternary insulin-like growth factor complex. The ALS gene consists of two exons separated by an intron in known species. Exons 1 and 2 code for the proximal region of the signal peptide and the rest of the pALS peptide, respectively. The nucleotide sequence of an exon 2 segment of pALS gene coding for the distal region of the signal peptide through the termination codon of pALS peptide has been previously reported from this laboratory. In the present study, the 5' and 3' untranslated regions (UT) of the gene were identified by rapid amplification of 5' complementary DNA end (5' RACE) and 3' RACE, respectively. A segment of the pALS gene spanning from the distal region of exon 1 through the proximal region of exon 2 coding for the proximal region of the pALS signal peptide was next amplified by polymerase chain reaction (PCR) using genomic DNA as template. The entire pALS coding sequence exhibited 85% and 83% homology to those of hALS and rALS genes, respectively, whereas 5' UT, 3' UT and intron sequences did not exhibit any significant homology to those of the human or rat. In-situ hybridization on pig liver slice using a pALS cDNA fragment revealed the gene expression in hepatocytes. Moreover, the ALS gene was also identified to be expressed in non-hepatic organs and tissues including the reproductive tract by reverse transcription-PCR using an intron-spanning primer pair.

Key Words: ALS, IGF, Gene

T156 Circulating levels of growth factors are influenced by physiological status in the horse. F. C. Buonomo*[1], D. L. Grohs[1], D. S. Ruffin[2], and J. L. Sartin[2], [1]*Monsanto Company, St. Louis, MO,* [2]*School of Veterinary Medicine, Auburn University, Auburn, AL.*

Changes in endogenous growth factor levels have been well characterized in primates, rodents and food-producing species, but less in companion animals. While it has been demonstrated that horses respond to somatotropin by increasing insulin-like growth factor-I (IGF-I), the influence of physiological status on IGF-I and IGF-II levels in horses is not well known. Thus, we examined gender, age and breed differences on IGF-I and IGF-II in intact male and female horses over the first yr of development. A significant (P<.02) age effect was observed as IGF-I levels gradually declined over the first yr of life, but more rapidly between 6 & 12 mos. of age. The decline in IGF-I was similar between sexes. Effects of age (P<.02) and agexgender interaction (P<.01) were observed for IGF-II. The IGF-II developmental pattern in both genders was characterized by a spike in the otherwise relatively stable circulating levels during the first yr. IGF-II levels increased 110% in males at 3 mos. of age, followed by a decline at 6 & 12 mos. of age. A much smaller increase (17%) in IGF-II was observed in females at 6 mos. followed by a decline at 12 mos. An overall breed effect (P<.05) was observed for IGF-I, but not IGF-II(American Miniature<Quarter Horse<Draft Horse) during the first yr of life. IGF-I and IGF-II levels were then examined under conditions associated with reduced growth in foals by measuring serum IGF-I and -II in foals suckling mares housed on either endophyte-infected or endophyte-free fescue for 4 wks. At the end of 4 wks, mares and foals were switched to alternative pastures for 4 additional wks. IGF-I and IGF-II levels were lower (P<.05) in foals of mares grazing endophyte-infected pasture compared to those grazing endophyte-free pasture. Levels of both growth factors in foals on the endophyte-infected pasture did not return to normal after being transferred to an endophyte-free pasture for a period of 4 wks. These observations suggest that IGF-II, as well as IGF-I, plays an important role in growth and development during the first yr of life in the horse. Moreover, neonatal exposure to endophyte-infected fescue may have more long term consequences on subsequent growth and development.

Key Words: Growth Factor, IGF, Horse

T157 Effects of insulin, leucine, and glucose on translation rates in primary porcine satellite cells. B. A. Creamer*, J. M. Scheffler, and S. J. Jones, *University of Nebraska, Lincoln.*

Both insulin and branched-chain amino acids have shown to increase protein synthesis in rats, perfused muscle, and *in vitro* cultures of skeletal muscle myoblasts. This increase in synthesis has been shown to occur more readily at an early age, but this response declines with time. The objective of this study was to determine if insulin, glucose, and leucine caused a shift in the amount of ribosomes in the polysome state. We hypothesized that all three work independently to increase the amount of total ribosomes forming polysomes in primary porcine satellite cells (PSC) and in porcine satellite cell derived myotubes (PDM), *in vitro*. In both PSC and PDM, the addition of insulin at or above post-feeding physiological levels caused an increase in both total RNA and the percentage of ribosomes in polysome complexes when compared to controls (p<.05). A diminution of insulin to below physiological levels, in both PSC and PDM, caused a decline in both total RNA when reduced to half-physiological levels (<.05). This reduction was mimicked in polysome formation. Leucine caused similar results in both cell types; however, the increase in total RNA was much more pronounced, at 2-times control levels, in PSC than PDM (20% and 4% increase, respectively). When only glucose was excluded from the medium, total RNA and percent polysomes were reduced (p<.05), when compared to low (1g/L) and high (4g/L) glucose treatments. However, the increase from low to high glucose was not significantly different in either total RNA or percent polysome formation (p>.05). These data indicate that 1) insulin, leucine, and glucose increase total RNA production and percent polysome formation when present in increasing amounts, and 2) the absence of glucose decreases total RNA production and polysome formation, while addition, at both low and high levels, caused similar increases in both PSC and PDM.

Key Words: Translation, Insulin, Satellite cells

T158 Development of a microarray-based ELISA for the assessment of SR-associated calcium regulatory proteins in skeletal muscle. J. S. Schulz*, S. J. Jones, and M. Zeece, *University of Nebraska, Lincoln.*

A microarray-based ELISA approach was taken for development of a novel method to assess the levels of key calcium regulatory proteins in porcine skeletal muscle. Sarcoplasmic reticulum (SR) membrane preparations were generated by homogenization of longissimus muscle samples. The microarray technique is novel and has several advantages including: very small muscle sample requirement (2g), greater sensitivity (pg), lower analyte requirement (<50 μL per array) and capability multiplexed analysis of several target proteins on the same "chip". Membranes were solubilized by homogenization with buffer containg 30% sucrose, 5 mM imidazole pH 7.4 and protease inhibitors. Extracts (4.9 nl) were spotted onto microarray slides, and incubated at 37C for 2 hr with monoclonal antibodies to several target proteins including Ryanodine receptor (RyR) and dihydropyridine receptor (DHPR). Detection of bound antibody was accomplished using laser-induced fluorescence of a labeled secondary antibody. Microarray analysis of these targets was quantitated as fluorescence intensity per ng of protein spotted. The limit of detection has been found to be 15-20 pg of target protein. Coefficient of variation for this level of analyte ranges from 14 to 33%. Data from the protein microarrays can be compared with genomic analysis of the same animals. Microarray analysis will enable rapid determination of defects that alter levels of key proteins associated with abnormal calcium regulation and the subsequent development of PSE-like conditions in the meat.

Key Words: Microarray ELISA, Calcium, Proteomics

T159 Hormonal and metabolic responses to human handling in crossbred steers. K. Uetake*[1], T. Ishiwata[1], N. Abe[2], Y. Eguchi[1], and T. Tanaka[1], [1]*School of Veterinary Medicine, Azabu University, Sagamihara, Japan,* [2]*Faculty of Agriculture, Tamagawa University, Machida, Japan.*

Hormonal and metabolic responses to human handling, which can affect skeletal muscle growth, were determined with 35 Japanese Black × Holstein steers. The steers were allocated into three pens (6.0 m × 9.5 m each) after transport at 6-10 mo of age. They were provided commercial grain feed twice a day (830, 1500). They were allowed to access dry hay or oat straw on an ad libitum basis in the early or middle fattening stage. All steers were bimonthly driven into a crush 2 h later from the morning feeding and were weighed and blood collected. The relationships between concentrations of 7 hormones and 5 metabolites were determined using Pearsons correlation coefficients. The following integrated hormonal and metabolic responses were analogized: In the early stage, increases in adrenaline (A) and noradrenalin (NA) secretion can cause an increase in plasma glucose (A: $r = 0.61$, $P < 0.001$; NA: $r = 0.53$, $P < 0.01$) and the depletion of serum vitamin A (A: $r = -0.38$; NA: $r = -0.42$, both $P < 0.05$), which could lead to an inhibition of insulin secretion ($r = 0.31$, $P < 0.10$). In addition, an increase in dopamine secretion might activate the depletion of serum triglyceride ($r = -0.29$, $P < 0.10$). In the middle stage, increases in the A and NA secretion can cause an increase in serum NEFA (A: $r = 0.31$; NA: $r = 0.32$, both $P < 0.05$) as well as a decrease in serum triglyceride (A: $r = -0.37$; NA: $r = -0.39$, both $P < 0.05$). It can also cause an inhibition of insulin secretion (NA: $r = -0.36$, $P < 0.05$) and the depletion of serum vitamin A (NA: $r = -0.33$, $P < 0.10$), which could lead to an inhibition of leptin secretion ($r = 0.38$, $P < 0.05$). Decreases in serum vitamin A and leptin could bring about a decrease in serum total cholesterol (vitamin A: $r = 0.35$, $P < 0.05$; leptin: $r = 0.30$, $P < 0.10$). In addition, an increase in cortisol secretion can cause steatolysis and an increase in serum NEFA ($r = 0.41$, $P < 0.05$). It is vitamin A that acts as intermediary between the stress responses and growth in steers.

Key Words: Cattle, Growth, Stress Response

T160 Skeletal development and productive performance of Italian Merino lambs as related to age. G. Maiorano*, F. Filetti, A. Ciarlariello, G. Gambacorta, and A. Manchisi, *University of Molise, Campobasso, Italy.*

To study skeletal development and productive performance of the Italian Merino sheep breed, 24 growing male lambs were slaughtered, in

groups equal for number, at 5, 30, 50 and 70 d of age. Lambs were naturally suckled, left to graze with their dams and allowed free access to a commercial diet. *In vivo* and *post mortem* performance were evaluated. Metacarpal (MC) and metatarsal (MT) bones were measured for length, diaphyseal diameter, weight, and moisture. MC growth plate width was also assessed after $AgNO_3$ staining. ANOVA was performed and comparisons were tested by Scheffés test. ADG was 274, 299 and 256 g/d from d 5 to 30, 30 to 50, and 50 to 70, respectively. As age increased, slaughter weight (4.6a, 11.1b, 19.5c, 23.0d kg) and carcass (with head, thoracic organs, spleen, and liver) weight (2.9a, 7.4b, 12.2c, 14.1d kg) increased ($P<0.05$), but dressing (63.6a, 67.0b, 62.5a, 61.4a %) was the highest ($P<0.05$) at d 30. Loin eye area, indicative of carcass muscularity, increased ($P<0.05$) with age (3.0a, 7.6b, 10.3c, 11.2c cm^2) up to d 50. Bones mainly grew longitudinally in the first month of age, with a following stop (9.8a, 11.0b, 11.5b, 11.7b cm for MC; 10.3a, 11.7b, 12.3b, 12.3b cm for MT; $P<0.05$). In the same period, growth plate, the site of longitudinal bone growth, has thinned ($P<0.05$) of about 35% (from 0.98 to 0.64 mm) and of another 24% in the following periods, reaching 0.40 mm at d 70. However, bone weight continued to increase ($P<0.05$) with age up to d 50, either in MC (15.5a, 24.1b, 30.2c, 32.6c g) or in MT (15.7a, 24.4b, 30.1c, 32.7c g) due to a persistence in development of transversal diameter (1.0a, 1.2ab, 1.3bc, 1.4c cm for MC; 0.9a, 1.0ab, 1.1bc, 1.2c cm for MT; $P<0.05$). Bone moisture, expression of bone chemical maturity, decreased ($P<0.05$) with age up to d 50, in both MC (49a, 38b, 29c, 25c %) and MT (49a, 38b, 27c, 23c %). The second month of age appears to be a critical period in lamb skeletal growth, which strictly related to development of muscular and adipose tissues, and influences carcass and meat quality.

Key Words: Lamb, Age, Skeletal Growth

T161 Regulation of myostatin (MSTN) and MyoD expression with acute inflammatory challenge in the channel catfish (Ictalurus punctatus).

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In mammals, MSTN is implicated in the negative regulation of skeletal muscle growth. The muscle regulatory factor, MyoD, is important for muscle regeneration in mammals. Inflammatory mediators increase the expression of MSTN and decrease the expression of MyoD in skeletal muscle of mammalian species. Environmental stressors and glucocorticoids decrease the expression of MSTN in fish. This suggests species differences in the regulation of the expression of muscle regulatory factors in response to environmental stressors. Therefore, our aim was to determine the effect of an acute inflammatory challenge on MSTN and MyoD expression in muscle tissue, and whether alterations in mRNA abundance were related to an increase in circulating cortisol. A total of 36 fish (BW 46.1 ± 3.9 g) were randomly assigned to Control (saline; n = 18) or lipopolysaccharide (LPS, injected i.p. at 1.5 mg/kg BW; n = 18) treatments. Muscle and blood samples were collected at 3, 12, and 24 h relative to injection from six fish/treatment group at each time point. The abundance of MSTN and MyoD mRNAs was evaluated using real-time reverse transcriptase PCR procedures. Plasma cortisol was measured via a time-resolved fluoroimmunoassay. The abundance of MyoD mRNA was increased in LPS injected fish at 3 h ($P = 0.05$) and 10 h ($P = 0.03$) postinjection. At 24 h postinjection there was no ($P = 0.89$) difference in MyoD mRNA abundance between treatment groups. Injection with LPS did not alter the abundance of MSTN mRNA at 3 h ($P = 0.85$) or 10 h ($P = 0.87$) postinjection. However, the abundance of MSTN mRNA was decreased ($P = 0.02$) in fish injected with LPS at 24 h postinjection. Plasma concentrations of cortisol were not affected ($P > 0.10$) by LPS injection at any of the time points measured. These data suggest that MyoD is acutely upregulated, and that MSTN is downregulated in response to inflammatory stimuli in the channel catfish. In addition, these data further indicate that there are differences in the regulation of expression of muscle regulatory factors between mammals and fish in response to inflammatory stimuli.

Key Words: Catfish, MyoD, Myostatin

T162 Expression of the anti-apoptotic gene Bcl-2 during skeletal muscle development in normal and Low Score Normal chickens.

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Cell morphology and cell-extracellular matrix interactions play key roles in determining whether cells will undergo programmed cell death, apoptosis. Cell survival requires the appropriate interaction of the cell with the extracellular matrix. Low Score Normal (LSN) myogenic cells in culture exhibit a rounded morphology whereas normal myogenic cells are elongated with maximal attachment to the extracellular substrate. The rounded LSN cells undergo apoptosis most likely due to reduced contact of the cell with the extracellular matrix through the cell surface integrin receptors. In the current study, the expression of the anti-apoptotic gene Bcl-2 was measured during embryonic and posthatch pectoralis major muscle development in normal and LSN birds. During embryonic development, pectoralis major muscle tissue was analyzed every two d beginning at d 10 through 18 d, 19, 20, and 21 d. During posthatch development muscle samples were taken every two d through d 8, and then at 2, 4, and 6 wk, and 6 mo. Expression of the mRNA was measured by semi-quantitative reverse transcriptase-PCR using primers based on the chicken Bcl-2 sequence. The results from this study have shown at embryonic d 16 through 19, and posthatch d 6 and 8 that Bcl-2 expression was significantly reduced in the LSN pectoralis major muscle ($P < 0.05$). These data suggest that the appropiate expression of Bcl-2 is important for normal muscle development to occur by preventing cell death due to apoptosis.

Key Words: Extracellular Matrix, Bcl-2, Apoptosis

T163 Proteins were differentially expressed in the plasma from high and low feed efficient broilers.

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Mitochondrial function and biochemistry have previously been linked to the phenotypic expression of feed efficiency (FE) in broilers (Bottje et al., 2002; 2003; Iqbal et al. 2004). Broilers were fed the same diet, provided same environment, and were from the same genetic line. Broilers, with low FE (g gain/g feed), though healthy, exhibit higher mitochondrial oxidative stress. In addition, proteins are differentially expressed in the mitochondria from low and high FE birds. The objective of this study was to investigate and identify proteins that expressed differentially in a readily available tissue such as plasma. Plasma from high (n=7) and low (n=7) FE broilers were initially separated using a one-dimensional sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS/PAGE). A protein with a molecular weight of 116 kDa was expressed 23% higher ($p=0.03$) in the plasma from the low feed efficient broilers compared to the high feed efficient broilers. To identity this protein the band at 116 kDa was excised from the gel and digested with trypsin into smaller polypeptides. The peptides were sequenced using matrix assisted laser desorption/ionization/time of flight (MALDI/TOF) mass spectrometry. The molecular masses obtained from the mass spectral analysis was compared to known masses in the Swiss Protein database using the program Protein Prospector. An antibody was obtained to the protein and the level of this protein was found to be significantly higher ($p=0.01$) in plasma from low feed efficient broilers in a Western blot format. The plasma proteins were also separated using a two-dimensional PAGE with the first dimension separating the proteins by charge and the second dimension by molecular weight. At least twelve plasma proteins were expressed over five fold differentially between high and low feed efficient broilers. Identification of proteins that are differentially expressed in a readily accessible tissue may aid in the development of a rapid, inexpensive, and cost effective assay to aid in progeny selection of broilers. This research was funded in part by USDA-NRI grant #2001-03443.

Key Words: Feed Efficiency, Proteomics, Breeder Selection