

429 Growth hormone signalling in ruminant adipose tissue. R. G. Vernon*¹, A. Arana², and I. M. Fleming¹, ¹Hannah Research Institute, Ayr, Scotland, ²Universidad Publica de Navarra, Pamplona, Spain.

GH acts chronically on adipocytes to inhibit lipogenesis, antagonise insulin action and accentuate lipolysis. In addition, in some species, but not sheep, GH has a transient, acute, insulin-like effect, while for some preadipocyte-like cell-lines GH acts as a commitment factor. Studies on GH signalling in adipocytes have focused on these latter two effects and have implicated STAT proteins, MAP kinase, phosphatidylinositol-3 kinase (PI3K) and p70^{s6} kinase. Explants of sheep adipose tissue respond chronically to GH in vitro, and have been used to show that the GH-induced inhibition of lipogenesis is due in part to a decreased transcription of acetyl CoA carboxylase, while the accentuation of lipolysis by GH is due partly to a decreased ability of adenosine to inhibit catecholamine-stimulated lipolysis. Effects of GH on lipogenesis and response to adenosine are prevented by actinomycin D, an inhibitor of gene transcription, and H7, an inhibitor of protein serine kinases. However, inhibitors of MAP kinase and p70^{s6} kinase did not prevent effects of GH, and GH did not activate MAP kinase in sheep adipocytes. Wortmannin, an inhibitor of PI3K mimicked the effects of GH on both lipogenesis and response to adenosine. The phorbol ester, PMA, which activates and then down-regulates some isoforms of protein kinase C (PKC) partly inhibited effects of GH on lipogenesis and mimicked GH effects on response to adenosine. The PKC isoforms involved were determined using differentiated 3T3-F442A adipocytes. Individual PKC isoforms were eliminated using antisense techniques. Loss of either PKC α , δ , ϵ or zeta had no effect on the inhibition of lipogenesis by GH, elimination of either PKC γ or μ partly decreased the effect of GH while removal of both diminished the inhibitory effects of GH by 80%. Thus the chronic metabolic effects of GH involve both gene transcription and involvement of isoforms of PKC, whereas the MAP kinase and PI3K pathways do not appear to be involved.

Key Words: Growth Hormone, Adipose Tissue, Intracellular Signalling

430 TNF induced insulin resistance in adipocytes. J. M. Stephens, Louisiana State University, Baton Rouge.

A number of studies have demonstrated that TNF α is associated with profound insulin resistance in adipocytes, and may also play a critical role in the insulin resistance of obesity and type II diabetes. Reports on the mechanism of TNF α action have been somewhat contradictory. GLUT4 down-regulation has been implicated as a possible cause of insulin resistance as has been the reduced kinase function of the insulin receptor. Here we examine the effects of TNF α on the protein components thought to be involved in insulin-stimulated glucose transport in adipocytes, namely the insulin receptor, its major substrate IRS-1, and the insulin responsive glucose transporter, GLUT4. Prolonged exposure of 3T3-L1 adipocytes to TNF α causes a substantial reduction (>80%) in IRS-1 and GLUT4 mRNA and protein as well as a lesser reduction (<30%) in the amount of the insulin receptor. Nevertheless, the remaining proteins appear to be biochemically indistinguishable from those in untreated adipocytes. Both the insulin receptor and IRS-1 are tyrosine phosphorylated to the same extent in response to an acute insulin stimulation following cellular TNF α exposure. Furthermore, the ability of the insulin receptor to phosphorylate exogenous substrate in the test tube is also normal following its isolation from TNF α treated cells. These results are confirmed by the reduced, but obvious, level of insulin-dependent glucose transport and GLUT4 translocation observed in TNF α treated adipocytes. We conclude that the insulin resistance of glucose transport in 3T3-L1 adipocytes exposed to TNF α for 72-96 hours results from a reduced amount in requisite proteins involved in insulin action. These results are consistent with earlier studies indicating that TNF α reduces the transcriptional activity of the GLUT4 gene in murine adipocytes, and reduced mRNA transcription of a number of relevant genes may be the general mechanism by which TNF α causes insulin resistance in adipocytes.

431 Insulin Action in Human Skeletal Muscle. J. R. Zierath, Karolinska Institute, Sweden.

A wide variety of common and rare clinical metabolic syndromes including obesity and non-insulin dependent diabetes mellitus (NIDDM), are associated with peripheral insulin resistance. Skeletal muscle represents the major tissue involved in insulin-stimulated glucose disposal. Intense interest is now focused on whether reduced insulin-mediated glucose uptake in muscle from NIDDM patients results from alterations in the insulin signal transduction pathway or from alterations in traffic and/or translocation of GLUT4 to the plasma membrane. Potential targets for impaired traffic/translocation of GLUT4 include defective phosphorylation of IRS-1 and reduced PI-3 kinase activity. By utilizing a novel technique for in vitro studies of human skeletal muscle, we have investigated the intercellular signaling mechanisms underlying the activation of glucose transport in muscle from healthy or NIDDM subjects. Muscle samples were obtained from the vastus lateralis by means of an open biopsy procedure. In muscle from healthy individuals, a maximal insulin concentration induced a 3-fold increase in 3-O-methylglucose transport activity, which closely paralleled the insulin-induced increase in cell surface GLUT4. The decrease in whole body insulin-mediated glucose uptake observed in NIDDM subjects can be linked to defects in insulin action in skeletal muscle. Insulin-stimulated 3-O-methylglucose transport in muscle from NIDDM patients is negatively correlated with fasting plasma glucose. Decreased insulin-stimulated 3-O-methylglucose transport can be explained by impaired translocation of GLUT4 to the plasma membrane. This defect is coupled to reduced insulin-stimulated insulin receptor substrate 1 (IRS-1) tyrosine phosphorylation and reduced activity of PI3-kinase activity in NIDDM muscle. Decreased insulin-stimulated 3-O-methylglucose transport can be normalized in vitro following exposure to 4 mM for 2 hours. In conclusion, in skeletal muscle from NIDDM patients, glucose transport and GLUT4 translocation appear to be down regulated due to diminished insulin signal transduction.

Key Words: Insulin Signaling, Glucose Transport, Skeletal Muscle

432 Signal cross-talk by beta-adrenergic receptors. Links to the regulation of protein metabolism. S. E. Mills*¹, Purdue University, Lafayette, IN.

Beta-adrenergic receptors (β AR) belong to a family of seven transmembrane domain proteins which signal through interaction with the heterotrimeric G-proteins. The well characterized signaling cascade through Gs and the activation of adenylate cyclase and protein kinase A has become more complex with recent findings of β AR interaction with other G proteins and activation of additional signaling pathways. These novel pathways are of particular interest because they are shared by a variety of growth factors and, therefore, may explain how β AR agonists promote muscle accretion in animals.

A number of hormones and their receptors which signal through G proteins regulate cell growth and differentiation. All appear to signal through the mitogen-activated protein kinase (MAPK), although the pathways leading to MAPK activation are divergent and cell specific. In the myocyte, the β AR agonist isoproterenol stimulates protein synthesis and mimics other hypertrophic agents in activating MAPK (Biochem J. 314:115). Activation of MAPK is Ca⁺⁺ dependent but not cAMP dependent, suggesting the involvement of multiple G proteins in β AR signaling. In HEK293 cells, isoproterenol activates MAPK via the $\beta\gamma$ subunit of Gi. Only the β AR kinase phosphorylated receptor activates MAPK suggesting desensitization of the Gs pathway is the signal to activate the Gi pathway (Nature 390:88). Curiously, MAPK activation in the myocyte is pertussis toxin insensitive (Gi independent), indicating that multiple signaling pathways to MAPK are possible. The MAPK pathway is shared by a variety of growth factors including insulin and growth hormone. Activation of this pathway by β AR provides a potential mechanism for muscle hypertrophy in β AR agonist-treated animals. However, the significance of this pathway in a cell exposed to the typical wide array of interacting hormonal signals is not known. Given this caveat, the availability of a large number of G proteins in cells suggest a need to broaden the view of how β AR may modify cell metabolism and growth.

433 Growth and carcass traits of normal and callipyge lambs fed different amounts of protein. R. D. Sainz*, J. S. Cabbage, F. F. Franco, and C. I. Quintero, *University of California, Davis*.

In order to determine optimal dietary protein levels for callipyge (C) vs. normal (N) sheep, 20 N and 19 C ewe lambs (initial weight 29.1 ± 4.4 kg) were individually fed diets containing 8, 11, 14, 17 or 20% crude protein for 91 d. Changes in body components were assessed by comparative slaughter, and data were subjected to ANOVA using a model that included phenotype (based upon the longissimus muscle (LD) area:carcass weight ratio), diet and the interaction. None of the interaction terms were significant. Carcass weights were similar among treatment groups, although dressing percentages tended to be greater ($P < 0.10$) for C than N lambs. C lambs had greater LD areas (19.0 vs. 13.2 cm², $P < 0.001$) and smaller backfat thicknesses (3.9 vs. 4.5 mm, $P < 0.05$) than N lambs at the same carcass weight, and tended to be leaner (22.0 vs. 24.8% fat, $P < 0.10$). Although DM intakes were similar among treatments (1.55 ± 0.13 kg/d), average daily gains (ADG, g/d) increased linearly with dietary protein content ($P < 0.01$). Carcass weight gains tended to be greater in C lambs than in N lambs (139 vs. 121 g/d, $P < 0.10$), reflecting greater rates of carcass protein deposition in those animals (21.6 vs. 18.2 g/d, $P < 0.05$). Rates of carcass protein deposition also increased linearly with dietary protein content, but these were not different among diets when analyzed using the full ANOVA model. These data indicate that C lambs partition more protein into carcass tissues (i.e., skeletal muscle) than N lambs at all dietary protein levels.

Key Words: Callipyge Lambs, Dietary Protein, Growth

434 Plasma metabolites in normal and callipyge lambs fed different amounts of protein. J. S. Cabbage*, R. D. Sainz, and F. F. Franco, *University of California, Davis*.

In order to study metabolic responses to dietary protein levels in callipyge (C) vs. normal (N) sheep, 20 N and 19 C ewe lambs (initial weight 29.1 ± 4.4 kg) were individually fed diets containing 8, 11, 14, 17 or 20% crude protein for 91 d. Blood samples were taken weekly by jugular venipuncture into evacuated tubes containing EDTA and kept on ice until centrifuged. Plasma samples were collected, aliquoted and stored at -200°C pending analyses of glucose, urea and total protein concentrations. Data were subjected to ANOVA using a model that included phenotype (P, based upon the longissimus muscle area:carcass weight ratio), diet (D), $P \times D$ and time, with $P \times D$ within-animal included to account for repeated sampling. Glucose concentrations did not vary ($P > 0.05$) among P or D groups, nor was any interaction found. Glucose levels (4.97 ± 1.72 mM) did vary by sampling day, but not in any systematic way. Urea concentrations increased linearly with dietary protein content ($P < 0.01$); this increase tended to be smaller in C than in N lambs ($P < 0.10$). Protein concentrations (11.6 ± 4.3 mg/dL) varied non-systematically among days, but were unaffected ($P > 0.05$) by P or D. These data support the notion that C lambs partition more dietary protein toward anabolism (i.e., less amino acid is catabolized) than N lambs.

Key Words: Callipyge Lambs, Metabolites

435 Changes in fat, bone and fat free lean tissue in callipyge and normal lambs across the growth curve. G. D. Snowder¹, M. B. Solomon², S. K. Duckett³, and N. E. Cockett⁴, ¹USDA-ARS, Dubois, ID ²USDA-ARS, Beltsville, MD ³University of Idaho, Moscow, ID ⁴Utah State University, Logan, UT.

The objective was to characterize the development of fat, bone and fat free lean tissue as affected by the callipyge (CLPG) gene across the growth curve of Columbia lambs. Fifty-two lambs (26 = CLPG; 26 = normal (N)) were slaughtered at live weights of 7, 20, 36, 52 and 69 kg. At 24 hr postmortem, bone and soft tissue were separated from the right side of carcass and weighed. Carcass soft tissue was mixed and ground three times before sampling. Samples were analyzed by AOAC methods for moisture, protein and fat. Ash was determined by difference. Total carcass fat and fat free lean tissue were estimated from the chemical composition profiles on each animal. The muscle to fat ratio was also derived. ANOVA statistical procedures were used to determine differences between genotypes within weight groups. Bone weight was not influenced by the CLPG gene at any live weight. At the lighter weights (7 and 20 kg), there was no difference in fat, fat free lean or their ratio. At 36 kg, CLPG lambs were leaner ($P < .001$), had more fat free lean tissue ($P = .005$), and a higher fat free lean tissue to fat ratio compared to N lambs (7.0 vs 4.6 , respectively; $P = .01$). These differences were diminished at heavier weights (52 and 69 kg) where CLPG lambs tended to have less total fat and more fat free lean tissue but these contrasts were not statistically significant. These findings imply that a rapid growth in fat free lean tissue accompanied by a decrease in fat deposition may occur between 20 and 36 kg in CLPG lambs.

Key Words: Callipyge, Lamb, Composition

436 Heterogeneity in satellite cell colony size and telomere length. P. E. Mozdziak* and E. Schultz, *University of Wisconsin, Madison*.

Telomeres consist of highly conserved repetitive TTAGGG DNA sequences that are essential for chromosomal replication. It has been suggested that telomeric DNA sequences decrease in length with each cell division, and that telomere length can be used as a reporter of proliferation potential. The objectives of these studies were to determine telomere length in turkey satellite cells, and to compare telomere length between large and small satellite cell colonies. Satellite cells were isolated from the *Pectoralis thoracicus* of one-day-old turkeys, seeded on gelatin coated plates at clonal density, and grown for 10 days. Individual colonies were selected and classified as large (240 ± 23 cells; $n=29$) or small (36 ± 5 cells; $n=29$). Each colony was subcloned and expanded for 12 days in culture. Genomic DNA was isolated from the clonally derived cultures using an EasyDNA isolation kit (InVitrogen, Carlsbad CA), digested with Hinf I, and fractionated through a 0.3% agarose gel cast on a Gel Bond Support membrane (FMC Bioproducts, Rockland ME). Each gel was dried and subsequently hybridized (overnight) with a ³²P end-labeled oligonucleotide probe (TTAGGG)₄. Gels were washed with saline-sodium citrate, and exposed to X-ray film. Autoradiograms revealed a discreet telomeric restriction fragment greater than 38 kB that had greater mobility than undigested genomic DNA. Incubation of turkey genomic DNA with the enzyme BAL31 before Hinf I digestion confirmed that the long restriction fragment (>38 kB) was at the end of the chromosomes. Satellite cells demonstrated heterogeneity in colony size, and they also exhibited heterogeneity in telomere length because autoradiograms revealed a greater proportion of telomeric restriction fragments below 25 kB in DNA from large satellite cell colonies compared to DNA isolated from small satellite cell colonies suggesting that large colonies have shorter telomeres than small colonies. The long telomeres in DNA from small colonies may reflect a mechanism to conserve satellite cell proliferation potential.

Key Words: Turkey, Southern Hybridization, Skeletal Muscle

437 Lipogenic enzyme activity in adipose tissue of lambs expressing the callipyge gene. D. C. Rule^{1*}, G. D. Snowder², G. E. Moss¹, and N. E. Cockett³, ¹University of Wyoming, Laramie, ²USDA-ARS, Dubois, ID, ³Utah State University, Logan.

Expression of the callipyge (CLPG) gene in lambs results in muscle hypertrophy and reduced fat deposition compared with normal (NORM) lambs. The objective was to determine if lipogenic enzyme activity in ovine adipose tissue was affected by expression of the CLPG gene. Five or six lambs of each phenotype were slaughtered at each serial live weight group of 41, 57, or 73 kg. Subcutaneous adipose tissue activities were determined for lipoprotein lipase (LPL; nMol oleate released/(min × g)), fatty acid synthase (FAS; nMol NADPH oxidized/(15 min × g)), acyl-CoA synthetase (ACS; nMol palmitate to palmitoyl-CoA/(10 min × g)), and glycerol-3 phosphate acyltransferase (GPAT; nMol G3P to glycerolipid/(10 min × g)). At 41, 57 and 73 kg, respectively, LPL tended to be greater for NORM than CLPG (28 vs. 38, $p=.07$; 31 vs. 47, $P=.06$; 22 vs. 29, $P=.16$). FAS tended to be greater for NORM than CLPG at 41 and 57 kg (101 vs. 271, $P=.12$; 435 vs. 668, $P=.06$). FAS was greater for NORM than CLPG at 73 kg (295 vs. 550, $P=.02$). ACS was similar at 41 kg, and tended to be greater for NORM than CLPG at 57 and 73 kg (332 vs. 430, $P=.07$; 341 vs. 406, $P=.18$). GPAT was greater for NORM than CLPG at 41 kg (300 vs. 465, $P=.03$), similar at 57 kg, and tended to be greater for NORM at 73 kg (203 vs. 266, $P=.14$). Results indicate that decreased fat deposition in CLPG may be associated with lower lipogenic enzyme activities in adipose tissue.

Key Words: Adipose Tissue, Lambs, Lipogenesis

438 Skeletal muscle α -actin mRNA is increased in callipyge lambs. J. D. Heller^{*1}, S. A. Kramer¹, M. Koohmaraie², G. W. Smith¹, and M. E. Doumit¹, ¹Michigan State University, East Lansing, and ²USDA, ARS, U.S. MARC, Clay Center, NE.

The callipyge mutation results in extreme muscle hypertrophy. Longissimus muscle (LM) mass is increased 30% in callipyge lambs compared to non-callipyge lambs, but no apparent difference in infraspinatus (IS) muscle weight exists. The objective of this study was to quantify skeletal muscle α -actin mRNA in the LD and IS muscles of 12-wk-old callipyge ($n=4$) and non-callipyge ($n=4$) lambs. Total RNA was separated by electrophoresis under denaturing conditions in 1.2% agarose gels. Relative levels of skeletal muscle α -actin mRNA were determined by Northern analysis and normalized relative to amounts of 18S rRNA. Skeletal muscle α -actin mRNA levels were greater in callipyge vs non-callipyge lambs in both the LM and IS ($P < 0.05$). The elevated levels of skeletal muscle α -actin mRNA in the IS were unexpected and may indicate that multiple mechanisms contribute to the callipyge phenotype. These results indicate that skeletal muscle hypertrophy in callipyge lambs may be due in part to increased synthesis of myofibrillar proteins. Increased skeletal muscle α -actin mRNA abundance in callipyge vs non-callipyge lambs suggests that callipyge muscle hypertrophy involves pretranslational regulation of myofibrillar protein synthesis.

Key Words: Callipyge Lamb, α -actin mRNA

439 Mixture-screening for identification of bacterial colonies containing recombinant DNA. Y-Y. Xiao^{*}, J. Purintrapiban, J. Huang, M. A. Beilstein, and N. E. Forsberg, Oregon State University, Corvallis.

A challenge faced by scientists is to screen large numbers of bacterial colonies for one which contains a successful ligation of a DNA fragment into a larger plasmid. This laborious procedure may be hastened by a new strategy to identify a successful ligation: mixture screening. Here, several bacterial colonies are screened at once, and the elements of a mixture of bacteria which harbor a successful ligation are then individually screened. A procedure to screen 100 colonies is as follows: 1. Ten individual colonies are inoculated into a single tube containing LB and incubated overnight. Each colony is also inoculated individually onto an LB plate and incubated overnight. Similar processes are used for the remaining 90 samples to yield 10 tubes each of which contain a mixture of 10 bacterial colonies. Mixtures are screened for a successful ligation using plasmid DNA mini-preps and restriction enzyme digestion. When a mixture containing a successful ligation is identified, a corresponding plate of 10 individual colonies is then screened to identify a single bacterial colony which harbors the successful ligation. In this example, materials and labor to screen 100 colonies are reduced by 80%.

Key Words: Mixture-Screening, Sub-Cloning

440 A rapid ultrasound-guided procedure to administer a DNA label into fetal sheep. P. L. Greenwood, R. M. Slepatis, J. W. Hermanson, and A. W. Bell^{*}, Cornell University, Ithaca, NY.

A novel technique was developed to deliver a bolus dose of a DNA label into the peritoneal cavity of fetal sheep in utero during mid to late gestation. The procedure was performed on 54 Suffolk × (Finn × Dorset) fetuses from 85 to 130 d gestation in 25 ewes. Ewes were anesthetized using Ketamine HCl; fetal location and orientation were determined by ultrasound. Intraperitoneal injection of a solution of the DNA label, bromodeoxyuridine (BrdU), was attempted using a spinal needle and real time ultrasonic imaging to position the needle. Dye and color-coded nylon line were also injected to allow identification of individual fetuses within litters and the injection site. Duration of the procedure was (mean ± SD) 44 ± 16 min, which declined over the duration of the study but increased with increasing litter size. Recovery from anesthesia was rapid and uneventful in all cases. The location of the markers and detection of label in fetal muscle nuclei by immunocytochemistry confirmed injection into the peritoneal cavity in 46 of the 54 fetuses (85%). Fetal weight was estimated with a high degree of precision ($R^2 = 0.93$) using predicted growth curves and/or ultrasound measurement of metacarpal bone length. Dose of label administered (110 ± 33 mg BrdU/kg fetal weight) was similar to the desired dose (100 mg/kg fetal weight) and was adequate in all cases, as judged by intensity and distribution of labeling, and repeatability of data. The results demonstrate that this procedure can be used to determine whether myonuclei have entered S-phase of the cell cycle in fetal sheep from 85 to 130 d gestation.

Key Words: Fetus, Cell Cycle, Methodology

441 Plasmid Transfection and Retroviral Transduction of Pig Muscle Cells. J. Blanton Jr.*¹, C. Bidwell¹, C. Sharkey¹, D. Sanders¹, D. McFarland², and A. Grant¹, ¹*Purdue University, West Lafayette IN.*, ²*South Dakota State University, Brookings.*

Introduction of reporter genes into pig muscle cells will facilitate studies of skeletal muscle growth and development. The objective of this study was to determine an optimal method for transient and stable incorporation of genes into porcine muscle cells using cationic liposome transfection, electroporation and retroviral transduction. Porcine myoblasts and fibroblasts were isolated from muscle of 2 wk old pigs and clonally-derived using a robotic cell manipulator. Myogenic cell lines were identified using muscle-specific monoclonal antibodies. Four liposomes (LipofectAMINE, Lipofectin, CellFECTIN and DMRIE-C) at different DNA:lipid ratios were tested for their ability to transiently transfect myoblasts and fibroblasts with an SV40 luciferase reporter plasmid. LipofectAMINE resulted in the greatest ($P < .05$) transient luciferase activity for both cell types. Porcine myoblasts were found to contain endogenous LacZ activity. Subsequently, stable transfections were conducted using a green fluorescent protein (GFP) reporter and neomycin resistance plasmid. Transfection with lipofectAMINE and G418 selection resulted in stable GFP expression in 1:16,000 myoblasts and 1:33,000 fibroblasts. Electroporation resulted in stable transfection efficiencies of only 10^{-5} cells for both cell types, and 50% death loss. Porcine muscle cells were transduced with GFP using stomatitis virus glycoprotein G pseudotyped retrovirus, and resulted in efficiencies of 1:1.2 for myoblasts and 1:1.1 for fibroblasts. In place of antibiotic selection, transduced GFP-positive cells were separated from GFP-negative cells by fluorescent activated cell sorting and GFP expression was stable for six doublings. Transfected and transduced fibroblasts and myoblasts maintained normal growth and myotube fusion characteristics. These results indicate that cationic liposomes are superior to electroporation for transient transfections, whereas, retroviral transduction produced stable reporter gene expression in $>80\%$ of porcine muscle cells.

Key Words: Pig, Transfection, Retrovirus

442 Evaluation of body composition of transgenic pigs using dual-energy x-ray absorptiometry (DXA). A. D. Mitchell*¹, V. G. Pursel¹, and G. Bee², ¹*USDA, Agricultural Research Service, Beltsville, MD.*, ²*ETH, Zurich, Switzerland.*

Dual-energy x-ray absorptiometry (DXA) was used as a non-invasive method to measure the body composition of pigs with a transgene composed of an avian skeletal alpha-actin regulatory sequence and a cDNA encoding IGF-1. Male and female pigs that were either control (C) or transgenic (TG) with respect to the IGF gene were evaluated for body composition by DXA scanning at approximately 60, 90 and 120 kg live BW. The DXA scan provided measurements of the fat and lean content of the total body. At 60 kg, TG females and males had slightly less total body fat ($P > .05$) than the respective C pigs. At 90 kg, the TG females and males had 6.8 and 6.9% less fat ($P > .05$) and at 120 kg they had 6.4% ($P > .05$) and 15.4% less fat ($P < .05$) than the respective C pigs. During growth from 60 to 90 kg, TG males deposited 9.3% less fat ($P > .05$) and females deposited 17.3% less fat ($P < .05$) compared to their respective C pigs. The rate of fat deposition (g/d) was 11% less in TG females and 14% less in TG males compared to respective controls ($P < .05$). During growth from 90 to 120 kg, TG males deposited 16.7% less fat and females 2.3% less fat compared to their respective C pigs ($P > .05$). There were no differences in lean tissue deposition between TG and C pigs during growth from 60 to 90 kg. However, during growth from 90 to 120 kg, TG males deposited 33% more lean and TG females 29% more lean than their respective C pigs ($P < .05$). During the 90–120 kg growth period the rate of lean tissue deposition (g/d) was 505 and 609 ($P < .05$) for C and TG males and 320 and 338 ($P > .05$) for C and TG females. In conclusion, the use of DXA permitted the evaluation of the composition of growth of TG and C pigs from 60 to 120 kg. The results suggest that differences in body composition were the results of decreased fat deposition between 60 and 120 kg and increased lean deposition between 90 and 120 kg in the TG pigs compared to the C pigs.

Key Words: Pigs, Carcass Composition, Dual-Energy X-ray Absorptiometry

443 Growth performance of porcine somatotropin (PST)-treated pigs immunized with plasmid DNA encoding leptin-green fluorescent protein (GFP) fusion proteins. C. M. Evock-Clover*¹, D. E. Kerr², D. Wray-Cahen¹, T. G. Ramsay¹, and N. C. Steele¹, ¹*USDA-ARS, Beltsville, MD.*, ²*University of Vermont, Burlington.*

A plasmid DNA immunization technique was employed to attempt immunoneutralization of leptin in pigs, using growth performance as a bioassay. Pigs were immunized three times at 2-week intervals starting at 40 kg BW with plasmid DNA containing the cytomegalovirus promoter directing the expression of GFP or a combination of two leptin-GFP fusion proteins in which the 5' region (encoding a 20 amino acid signal peptide and the next 38 amino acids), or the entire coding region of porcine leptin (167 amino acids), were cloned in frame and upstream of GFP. The GFP treatment group was included to determine if GFP, or the immunization process alone, could account for any observed leptin-GFP response. Additional treatments were: control (buffer-injected daily starting at 60 kg BW), PST (100 μ g PST/kg BW/d starting at 60 kg), and leptin-GFP + PST. Pigs remained on treatment for six weeks after reaching 60 kg BW. The GFP-immunized pigs did not differ (Student's t-test) from the control group for any parameter measured, so the results are reported as a 2×2 factorial design. PST administration resulted in higher ADG (1176 vs 1048 g/d; $P < .01$), and lower feed intake (118 vs 162 kg; $P < .0001$) and feed:gain ratio (2.4 vs 3.7; $P < .0001$) than controls. Our method of leptin immunization did not affect growth performance and did not lead to an expected increase in feed intake. All immunized pigs developed antibodies to GFP that were detectable in 1:4000 dilutions of serum by ELISA. However, we have not been able to detect any leptin antibodies in serum taken from these pigs prior to slaughter. Any possible leptin antibodies generated short-term may not have been manifest throughout the finishing phase of growth.

Key Words: Leptin, Immunization, Somatotropin

444 Insulin regulation of protein synthesis in sheep adipose tissue. A. Arana*¹, E. Finley², and R. G. Vernon², ¹*Universidad Publica de Navarra, Pamplona, Spain.*, ²*Hannah Research Institute, Ayr, Scotland.*

The objective of this study was to further characterise the mechanism whereby insulin stimulates protein synthesis in sheep adipose tissue. Explants of subcutaneous adipose tissue from castrated male sheep, were maintained in tissue culture in Medium 199, with 2mM acetate and antibiotics, for 24h following which the rate of protein synthesis was determined by measuring the amount of ³H leucine incorporated into protein over a 4h period. Addition of insulin (100ng/ml) during the 4h assay period had no effect on the rate of protein synthesis, but when added during the 24h culture period insulin increased the rate ($P < 0.05$) from 46.4 to 63.0nmol/4h per 10^6 cells (SED 5.4, $n=4$); the effect is thus chronic. Insulin is thought to stimulate protein synthesis via several signalling systems, including the MAP kinase and the phosphatidylinositol-3 kinase (PI3 kinase)/p70^{s6k} pathways. Insulin activated MAP kinase and PI3 kinase of sheep adipocytes. Addition of the MAP kinase inhibitor (PD 98059) during the culture period had no effect on the rate of protein synthesis in the presence or absence of insulin (rate of protein synthesis in the presence of insulin was 31.2 and 30.9nmol/4h per 10^6 cells in the presence and absence of PD 98059 respectively; SED 3.75, $n=8$). Addition of either 50nM rapamycin (rap) (inhibitor of p70^{s6k}) or 500nM wortmanin (inhibitor of PI3 kinase) during culture, decreased the rate of protein synthesis in the absence of insulin but did not prevent stimulation by insulin (46.1 and 57.0nmol/4h per 10^6 cells in the absence and presence of insulin alone respectively; 30.8 and 42.3nmol/4h per 10^6 cells in the absence and presence of insulin with rap and 30.0 and 52.8nmol/4h per 10^6 cells in the absence and presence of insulin with wortmanin; SED 4.56, $n=4$). The addition of PD 98059 along with rap had no further effect above that induced by rap alone. Thus effects of insulin on protein synthesis in sheep adipose tissue are chronic and do not appear to involve either the MAP kinase or the PI3 kinase/p70^{s6k} pathways.

Key Words: Adipose Tissue, Insulin, Protein Synthesis

445 Cloning and regulation of porcine peroxisome proliferator-activated receptor γ (PPAR γ). K. L. Houseknecht^{1*}, C. A. Bidwell¹, C. P. Portocarrero¹, M. E. Spurlock², and A. Heady¹, ¹*Purdue University, West Lafayette, IN*, ²*Purina Mills, St. Louis, MO*.

Peroxisome proliferator activated receptor γ (PPAR γ) belongs to the PPAR family of nuclear receptors that regulate adipocyte differentiation and gene expression. PPAR γ has also been implicated in the regulation of insulin action since thiazolidinediones, potent insulin sensitizing drugs, are ligands for PPAR γ . The aim of this study was to clone the porcine PPAR γ gene and investigate the expression of PPAR γ in porcine tissues. The full coding sequence of PPAR γ was cloned using the reverse transcriptase-polymerase chain reaction (RT-PCR). Three overlapping PCR products were assembled into a 1,742 bp cDNA with 1,515 bp of protein coding sequence and 227 bp of 3' untranslated sequence. The coding sequence had 91% nucleic acid identity and 98% amino acid identity to human PPAR γ . Northern blot analysis of adipose tissue indicates that the mRNA was 1.8 kb. Alternative promoter usage and differential splicing of the PPAR γ gene produces two isoforms γ 1 and γ 2. The PPAR γ 2 isoform has an additional exon encoding 30 residues extending from the amino terminal of PPAR γ 1. A 278 bp PCR product from the amino terminal end of PPAR γ was cloned into a transcription plasmid to generate a labeled RNA probe which simultaneously detects PPAR γ 1 and γ 2. This RNA probe will protect a 278 bp fragment of the PPAR γ 2 mRNA and 178 bp fragment of the PPAR γ 1 mRNA in a ribonuclease protection assay. PPAR γ 2 was highly expressed in adipose tissue and weakly in lung while γ 1 was expressed ubiquitously. Expression of both γ 1 and γ 2 were higher in subcutaneous adipose tissue of high lean gain vs moderate lean gain pigs ($p < 0.05$) but was unaltered by fasting (3d) in 60 or 150 kg pigs. PPAR γ may play an important role in adipocyte differentiation and insulin action in the pig.

Key Words: PPAR γ , Adipocyte, Pig

446 Regulation of androgen receptor mRNA expression in three different skeletal muscles during postnatal development in bulls and steers. A. M. Brandstetter^{*1}, M. W. Pfaffl², J. F. Hocquette¹, Y. Geay¹, and H. Sauerwein², ¹*INRA, Clermont/Theix, France*, ²*FML Weihenstephan, TU München, Germany*.

The effect of testicular steroids on muscle growth is evident from the sexual dimorphism in muscularity. Varying hormone sensitivities of muscles might account for the differential growth rates. The present study thus compares androgen receptor (AR) mRNA expression rates in three different muscles with known divergent growth patterns.

Seventeen Montbéliard bulls and 18 steers were assigned to 4 different slaughter dates, at 4, 8, 12 or 16 months of age. Total RNA was extracted from samples of *m. semitendinosus* (ST) *m. triceps brachii* (TB) and *m. splenius* (SP). AR mRNA in 200ng of total RNA was quantified via a competitive reverse transcription polymerase chain reaction (RT-PCR). A cRNA mutant, coding for the ligand binding domain and derived from the bovine AR sequence, was used as an internal standard. Data were analysed using levels of 18S ribosomal RNA as covariate. Results were expressed per RNA unit and per g of tissue, taking the recovery of RNA into account.

AR mRNA levels in bulls were similar to those in steers. Variations in AR mRNA expression rates with age were muscle-dependent ($P < 0.05$). In SP, AR mRNA levels per RNA unit increased 1.2-fold from 4 to 12 months of age ($P = 0.001$). AR mRNA expression rates in ST and TB were comparable to those in SP at 12 and 16 months and were not affected by age. When expressed on a g tissue basis, the AR mRNA concentrations in TB were 1.6- to 2-fold higher ($P < 0.01$) than in ST and SP at comparable ages due to highest RNA extraction yields in TB ($P < 0.001$). These data indicate that the direct effects of androgens on skeletal muscle are regulated at the ligand level rather than at the receptor level, but might depend on receptor expression varying among muscles.

Key Words: Androgen Receptor, Skeletal Muscle, Cattle

447 Developmental changes and tissue differences in the response of protein synthesis to insulin in suckling pigs. T. A. Davis^{*}, D. G. Burrin, M. L. Fiorotto, P. J. Reeds, H. V. Nguyen, D. Wray-Cahen, and P. R. Beckett, *USDA/ARS Children's Nutr. Res. Ctr., Baylor College of Medicine, Houston, TX*.

Feeding stimulates protein synthesis rates (Ks) in tissues of young animals and this response decreases with development. To determine whether this response to feeding is mediated by insulin, we performed hyperinsulinemic (0, 30, and 100 ng per kg of body weight raised to 0.66 exponent per min)-euglycemic-amino acid clamps in fasted 7- and 26-d-old pigs ($n = 5/\text{dose}$). These insulin doses reproduced the plasma insulin levels observed in fasted, fed, and re-fed pigs, respectively. Ks was determined with a flooding dose of 3H-phenylalanine.

In 7-d-old pigs, insulin stimulated ($P < 0.05$) Ks in gastrocnemius and masseter muscles and skin by 60, 40, and 45 percent, respectively, but did not stimulate Ks in liver or gut. At 7 days, the maximum response to insulin was achieved at the 30 ng insulin dose. In 26-d-old pigs, insulin stimulated ($P < 0.05$) Ks in gastrocnemius and masseter muscles by 25 and 12 percent, respectively, but had no effect in skin. Insulin suppressed ($P < 0.05$) Ks in liver and intestine by 29 and 12 percent, respectively, in 26-d-old pigs. At 26 days, the maximum response to insulin was achieved at the 30 ng insulin dose in muscle and at the 100 ng insulin dose in liver and intestine.

The results suggest that, when amino acids and glucose are maintained at fasting levels, low physiological doses of insulin stimulate protein synthesis in peripheral tissues of suckling pigs; this response decreases with development. In visceral tissues, insulin does not stimulate protein synthesis of young suckling pigs and suppresses protein synthesis in older suckling pigs. We conclude that the stimulation of protein synthesis by feeding in peripheral tissues of young animals is mediated by insulin. However, other factors are likely involved in the stimulation of protein synthesis by feeding in the viscera.

Supported by NIH AR44474 and USDA/ARS 58-6250-6-001.

Key Words: Insulin, Protein Synthesis, Growth

448 The effect of hepatocyte growth factor (HGF) on turkey satellite cell proliferation and differentiation. C. Y. Zeng^{*}, D. C. McFarland, J. E. Pesall, K. K. Gilkerson, and L. S. Vander Wal, *South Dakota State University, Brookings*.

The effect of hepatocyte growth factor (HGF) on turkey satellite cell proliferation and differentiation was examined in cell culture. Satellite cell clones established from one muscle from one individual animal were previously shown to differ in their responsiveness to IGF, FGF, PDGF, and insulin. The "Early" clone was shown to be more responsive to mitogenic stimuli than the "Late" clone. In the present study, however, the Late clone was more responsive to the mitogenic effects of HGF than the Early clone ($P < .01$). When administered HGF, the Late clone grew nearly threefold faster than controls, while Early clone proliferation increased only 16% over controls. To test whether HGF exerted its effect only during the initial treatment period, cells were administered HGF for either the first 24 h or continuously during a 96-h growth period. The results showed that greatest proliferation occurred when cells were administered HGF continuously. In fact, proliferation rates of cultures exposed to HGF only during the first 24 h were no different than controls not receiving HGF ($P < .05$). Maximal proliferation was seen in the presence of 5 ng/ml HGF in the media. At 96 h, in low serum medium, HGF depressed differentiation of the Early clone by 46%, while that of the Late clone was depressed by only 14%. The results demonstrated that HGF increases proliferation and decreases differentiation of turkey satellite cells.

Key Words: Turkey, Satellite Cell, Hepatocyte Growth Factor

449 Recombinant bovine somatotropin (BST) treatment increases plasma $\text{NO}_2^- + \text{NO}_3^-$ response to endotoxin (LPS) challenge in cattle. T. H. Elsasser*¹, S. Kahl¹, T. S. Rumsey¹, R. J. Collier², and R. Hoffman², ¹USDA, Agricultural Research Service, Beltsville, MD and ²Monsanto Co., St. Louis, MO.

Nitric oxide (NO) is a short-lived biological signaling molecule produced from the enzymatic cleavage of arginine by any of several isoforms of nitric oxide synthase (NOS). NO rapidly decays to the more stable anions NO_2^- and NO_3^- (NOx) and inferences regarding the production of NO are made by quantifying these anions. Several pathological effects of LPS are mediated by increases in NO induced by immune cytokines, and BST can modulate cytokine responses to LPS. We studied the effect of BST (.1 mg/kg BW, i.m. daily for 11 d) on NOx responses in cattle in conjunction with graded levels of LPS challenge (0, .2, 1.0, 3.0 $\mu\text{g}/\text{kg}$ BW, i.v. bolus, *E. Coli* 055:B5). Twenty eight steers and 4 heifers (Angus \times Hereford) were assigned in a factorial arrangement in two replications to treatments consisting of +/-BST (n = 16) and LPS dose (n = 8/dose). BST treatment was switched between the first and second replication. LPS or saline were injected through the jugular vein; blood samples were collected at 0, 3, 6, 8, 12 and 24 h relative to injection. Plasma was assayed for NO_2^- by the Griess reaction following conversion of NO_3^- to NO_2^- by bacterial NO_3^- reductase. Plasma NO_2^- response, measured as area under the time \times NO_2^- concentration curve (AUC), increased with LPS dose fitting a linear relationship with the log of LPS dose (13.3, 33.6, and 44.6 h \times ng/mL for .2, 1, and 3 $\mu\text{g}/\text{kg}$, respectively; SEM = 4.72, P < .01). At all doses of LPS, BST resulted in higher response in plasma NO_2^- (AUC) than those measured in calves not treated with BST (28.9 vs 15.1, SEM = 3.4, P < .01). A decrease in plasma IGF-I concentration was used as an indicator of pathology after LPS. Plasma IGF-I was minimally affected by LPS doses in nonBST calves; those treated with BST displayed significant decreases in IGF-I at 3 μg LPS/kg BW (P < .05). The data suggest that treatment with BST may modulate differences in NO production during disease stress.

Key Words: Somatotropin, Nitric Oxide, Insulin-like Growth Factor-I

450 Porcine erythropoietin receptor: Cloning of a partial cDNA and expression in embryonic/fetal liver. P.L. Pearson*, H. G. Klemcke, T. P. L. Smith, and J. L. Vallet, USDA, ARS, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE.

Previous studies have indicated a fetal loss of approximately 30% in pigs between d 25 and d 50 of pregnancy when fetuses are in a crowded uterine environment. A rapid expansion of the embryonic/fetal blood supply also occurs at this time and may be an important factor in fetal survivability and litter size in pigs. To understand this process, we have cloned a partial cDNA for the porcine (p) erythropoietin receptor (EPOR) and examined the expression of pEPOR mRNA in embryonic/fetal liver on gestational d 24, 30 and 40.

Using total RNA from d 30 embryonic pig liver, reverse transcription (RT) followed by polymerase chain reaction (PCR) with primers generated using an EPOR consensus sequence, a partial cDNA of 532 bp (corresponding to a portion of the extracellular domain) for pEPOR was subcloned and sequenced. Additional RT-PCR generated a 867 bp fragment (including most of the cytoplasmic domain). Rapid amplification of cDNA ends (RACE) procedures were used to obtain the 3' end, which was cloned and sequenced, giving a total sequence of 1450 bp, corresponding to amino acids 80-508 of the human EPOR plus the 3' untranslated region. The deduced amino acid sequence shows homology ranging from 79.7% identity with rat EPOR to 89.9% identity with bovine EPOR.

Northern analysis indicated an apparent size of 2.3 kb for pEPOR mRNA. Porcine EPOR mRNA was detected in liver (5 μg total RNA) from porcine embryos/fetuses on d 30 and 40 of gestation but not on d 24 (n = 3/d). This report is the first known cloning of a partial cDNA for pEPOR. The results indicate a high homology with other known EPOR sequences, and show increased expression of pEPOR mRNA in porcine liver during a time corresponding to a rapid expansion of the erythron and a critical time for fetal survival in a crowded uterine environment.

Key Words: Pig, Hematopoiesis, Blood

451 Determination of Protein Turnover and Ribosome Activity in Bovine Myotubes: Effects of Serum, Insulin, and Dexamethasone. T. L. Woods*, C. W. Smith, J. G. Klaasmeier, D. L. Grant, and S. J. Jones, University of Nebraska, Lincoln.

The objective of this study was to determine protein turnover in bovine embryonic myotubes incubated with either insulin (INS) or dexamethasone (DEX) and to determine changes in polysome populations in these cells in the presence or absence of serum, when treated with either INS or DEX. In the first experiment, protein synthesis and degradation were measured, when the myotubes were incubated with increasing DEX concentrations (0, 50, 100, 150, 175, 200, 250, 500, 750, or 1000 nM). Protein degradation increased quadratically (P < .01). Protein synthesis in the DEX-treated cells was not different from controls. When bovine myotubes were incubated with increasing INS levels (0, 0.5, 1, 5, 10, 50, 75, 100, 500, or 1000 ng/mL), protein synthesis increased quadratically (P < .01). As INS levels increased, protein degradation decreased linearly (P < .01) in these cells. In the next study, cells were treated with six DEX concentrations (0, 25, 50, 75, 100, or 200 nM) or six INS levels (0, 1, 5, 10, 50, or 100 ng/mL) in the presence or absence of 2% horse serum (HS), and polysome profiles were separated on 15 to 60% sucrose gradients. Monosome and polysome peak areas were greater (P < .01) in the myotubes treated with serum. Although serum did not alter (P < .13) the 40S or 60S peak heights, protein and RNA concentrations, the 80S peak height was greater (P < .01) in the serum-treated cultures. Increased INS decreased the 40S peak height quadratically (P < .01), while the polysome percent increased quadratically (P < .01). Increased DEX levels were found to increase (P < .01) the monosome peak area, while decreasing (P < .01) the polysome percent in bovine embryonic myotubes. With DEX and INS, both protein and RNA amounts were not different (P > .13) between the hormone levels studied. These results suggest that insulin and dexamethasone influence protein turnover in bovine embryonic myogenic cells via shifts in polysome populations.

Key Words: Muscle Cells, Ribosomes, Protein Turnover

452 Stability of alternatively spliced IGF-I mRNA transcripts in growth plate chondrocytes. L. A. Laugero* and A. M. Oberbauer, University of California, Davis.

Linear bone growth is driven by cellular activity of chondrocytes within the growth plate (gp) of long bones. Circulating and locally produced insulin-like growth factor (IGF-I) enhances the proliferation and hypertrophy of gp chondrocytes leading to increased gp width, thus promoting elongation. The abundance of each alternative IGF-I mRNA transcript was determined in gp chondrocytes. The most predominant transcript expressed is class 1Ea, classes 2Ea, and 1Eb are expressed in low levels, and class 2Eb is undetectable. The presence of multiple transcripts suggests differential regulation of IGF-I at the level of transcription, post-transcription, translation, and post translation levels. The present study addressed post-transcription regulation by evaluating the relative stabilities of the 1Ea, 2Ea, 1Eb mRNA classes. Cells from costochondral gps from six 28 d male Spague-Dawley rats were isolated, plated in 10% fetal bovine serum (FBS). Media were replaced 24 h later with FBS supplemented media containing actinomycin-D (Act D) (3 $\mu\text{g}/\text{ml}$). Cells were harvested 0,3,5,8,11, and 15 h post-Act D addition and total cellular RNA was isolated. Alternative IGF-I mRNAs were measured by quantitative reverse transcription polymerase chain reaction. Endogenous IGF-I mRNA was calculated based on a standard curve generated from a synthetic IGF-I cRNA standard, containing the primer sequence for each class of IGF-I mRNA. Maximal levels of alternative transcripts were 12.33 \pm 0.50, 0.012 \pm .0001, and 2.52 \pm 0.168 pg, for 1Ea, 2Ea, and 1Eb, respectively. In the presence of Act D, IGF-I mRNA for all classes decayed over time (p<0.0001). Endogenous IGF-I mRNA half-life was estimated by determining the time at which half-maximal IGF-I mRNA levels were reached. The estimated half-lives for 1Ea, 2Ea, 1Eb were not significantly different (p>0.1) and were 1.32, 0.51, and 0.65 h for class 1Ea, 2Ea, and 1Eb, respectively. These results suggest that mRNA stability may not be a major point of IGF-I regulation in gp chondrocytes, and therefore, transcription initiation may be the primary mechanism by which IGF-I mRNA abundance is controlled in these cells.

Key Words: Chondrocyte, IGF-I, Half-life

453 Serum effects on ribosomal recruitment in differentiating C2C12 myogenic cells. J. G. Klaasmeyer*, J. B. Edeal, and S. J. Jones, *University of Nebraska, Lincoln*.

During translation, ribosomes that are actively synthesizing protein attach to mRNA strands forming multi-ribosomal units called polyribosomes. A common practice to stimulate differentiation is to exchange fetal bovine serum (FBS) with a low concentration of horse serum. To determine the effects of serum on ribosome recruitment during differentiation, two studies were conducted using mouse C2C12 myogenic cells. Myogenic cells were plated at 100,000 cells per plate on 60 mm culture dishes in DMEM + 10% FBS. The first study was initiated when cells were 80–85% confluent. Media was changed to DMEM with 2% horse serum 24 h after initiation of the study to stimulate differentiation. Seven plates were removed from the incubator washed twice in PBS and frozen at -70°C at time intervals of: 0, 12, 24, 30, 36, 42, 48, 72, 96, 120h after initiation of the study and frozen. In a second study the media was changed to DMEM + 2% HS at 24 after initiation in one half of the plates the remainder received fresh media with DMEM + 10% FBS. Five plates of each treatment were removed from the incubator and washed at 0, 12, 24, 30, 36, 42, 48 and 72hr and frozen. Polyribosomes were fractionated by centrifugation on 15–60% sucrose gradients. Fractions were identified using a gradient fractionator, and monitored at 254 nm. Percent polyribosomes were determined by measuring the area under the peaks for monoribosomes and polyribosomes. In the first study polyribosome percentages decreased ($P < .05$) after the addition of HS and remained about 10 % lower throughout the study. In the second study the change to 2% HS caused a decline ($P < .05$) in polysome percent throughout the remainder of the study. The cells that remained on FBS did not show a decrease ($P < .05$) in the polyribosome percentage until 42hr after the initiation of the study. In conclusion changes that have been observed during differentiation may be caused due to the reduction of the growth factors present in the HS compared to FBS.

Key Words: Polyribosome, C2C12 Myogenic Cells, Differentiation

454 Classification of agonist and antagonist for the cloned porcine β_2 -adrenergic receptor using ligand binding. W. Liang*, H. Cao, and S. E. Mills, *Purdue University, West Lafayette, IN*.

The β -adrenergic receptor (βAR) protein exists in multiple-conformational states designated the active and inactive states. These two states of the receptor have different affinity for agonist but not antagonist, and affinity differences can be detected kinetically. Additionally, uncoupling of G-protein with GTP from βAR eliminates the affinity differences for agonists. The objective of these studies was to use differences of ligand binding to conformational states to predict whether ligands would be agonist-like or antagonist-like toward the pig $\beta_2\text{AR}$. A genomic clone of the pig $\beta_2\text{AR}$ expressed in CHO cells was the source of the receptor. The kinetics for different ligands were established by displacement of [125I] CYP from the membrane receptor. Data were analyzed using the computer program Kell. The β -agonists isoproterenol, epinephrine and norepinephrine exhibited affinity differences for the two states of the $\beta_2\text{AR}$ as evidenced by a 2-site model and slope values less than unity. Adding Gpp(NH)p, a nonhydrolyzable GTP analogue, eliminated the high affinity state and data modeled to only one site. Data for β -antagonist propranolol, alprenolol, oxprenolol and ICI 118,551, all fit a 1-site model as expected. These data confirm that binding kinetics can be used to initially classify ligands into 2 classes. We have previously suggested that ractopamine, clenbuterol and L644,969, β -agonists used to increase carcass protein accretion, are only weak agonists for pig βAR . Each modeled to only 1 site for the pig $\beta_2\text{AR}$, suggesting these compounds are more antagonist-like than agonist-like. The addition of Gpp(NH)p did not shift estimated affinities. Similar analyses in the pig $\beta_1\text{AR}$ indicated a tendency for 2-site models, suggesting these compounds may have greater efficacy toward the pig $\beta_1\text{AR}$. Terbutaline modeled to 2-sites in $\beta_1\text{AR}$ and $\beta_2\text{AR}$ and might be expected to be efficacious toward the pig βAR . Receptor signaling studies are necessary to confirm these preliminary predictions, but it appears that binding data can be used to distinguish agonist from antagonist for pig βAR .

Key Words: Pig, β -Adrenergic Receptor, Classification

455 Kinetic comparison of porcine β_1 - and β_2 -adrenergic receptor in CHO cells. H. Cao*, W. Liang, C. A. Bidwell, and S. E. Mills, *Purdue University, West Lafayette, IN*.

Genomic clones of the pig $\beta_1\text{AR}$ and $\beta_2\text{AR}$ have been expressed in CHO cells. The objectives of these studies were to compare binding kinetics of agonist and antagonist for the pig βAR s in order to: 1) determine subtype selective compounds for the pig, and 2) determine unique characteristics of the pig versus other species. Both the $\beta_1\text{AR}$ and $\beta_2\text{AR}$ exhibited characteristic relative affinities for non-selective agonist; $\beta_1\text{AR}$: isoproterenol > norepinephrine \geq epinephrine, and $\beta_2\text{AR}$: isoproterenol > epinephrine > norepinephrine. The purported β_1 antagonists, CGP 20712A and betaxolol, displayed high selectivity for pig $\beta_1\text{AR}$, and CGP 20712 had 50-100 fold higher affinity for pig $\beta_1\text{AR}$ than reported for the cloned $\beta_1\text{AR}$ from human or rat. The $\beta_2\text{AR}$ antagonist, ICI 118,551, did not display subtype selectivity, in agreement with observations using pig adipocyte membrane (Coutinho et al., 1990, *J. Anim. Sci.* 68: 284). Oxprenolol and alprenolol, both non-subtype-selective antagonists, displayed slight β_2 -selectivity in pig. We have previously reported that BRL 37344, a purported β_3 agonist, exhibited subtype selectivity in pig adipose and muscle membranes. The selectivity is likely accounted for by a greater affinity for the $\beta_2\text{AR}$ than the $\beta_1\text{AR}$. Our results also confirm our previous conclusions that the growth-modifying β -agonist clenbuterol, ractopamine, and L 664969 exhibited little subtype selectivity, although clenbuterol did favor the $\beta_2\text{AR}$ ($10\text{--}20 \times$ lower Kd). Affinity constants determined in cloned βAR did not agree well with our previous estimates in pig membrane, but may reflect the different radioligand used. We have identified several candidate ligands that may be useful in determining βAR subtypes in pig tissues. Further, kinetic differences between cloned βAR for pig, human, and rat confirm the inappropriateness of extrapolating results across species.

Key Words: Pig, β -Adrenergic Receptor

456 Testosterone and dihydrotestosterone, but not dehydroepiandrosterone, up-regulate androgen receptors in porcine satellite cells. N. T. Mesires* and M. E. Doumit, *Michigan State University, East Lansing*.

Androgenic steroids increase skeletal muscle mass. We have previously determined that androgen receptors (AR) are up-regulated in porcine skeletal muscle satellite cells treated with 10^{-7} M testosterone. However, the mechanisms by which androgenic steroids regulate AR levels in skeletal muscle remain unclear. Our objective was to determine the effects of testosterone (T), dihydrotestosterone (DHT), and dehydroepiandrosterone (DHEA) on porcine satellite cell AR abundance. Cultured porcine satellite cells were grown to approximately 70% confluence in minimum essential medium containing 10% fetal bovine serum. Cells were then exposed to either T, DHT, or DHEA at concentrations of 0 and 10^{-10} to 10^{-6} M for 24 h. Cells were harvested in electrophoresis sample buffer and proteins were resolved on 8% polyacrylamide gels. Rabbit polyclonal anti-androgen receptor antibodies (Affinity Bioreagents) were used to detect porcine AR by Western immunoblot analysis. T and DHT up-regulated the AR in a dose-dependent manner. At concentrations above 10^{-9} M, T up-regulated AR. DHT up-regulated AR at all concentrations tested. DHEA did not affect AR levels in porcine satellite cells. Cycloheximide, (1 and 10 $\mu\text{g}/\text{mL}$) partially inhibited AR up-regulation induced by 10^{-7} M T. These results indicate that DHT and T effectively up-regulate the AR by a mechanism which involves both synthesis of new AR protein and stabilization of existing AR. We further conclude that this mechanism by which AR levels are regulated is more sensitive to DHT.

Key Words: Satellite Cell, Androgen Receptor, Dihydrotestosterone

457 β -adrenergic stimulation increases skeletal α -actin mRNA in C₂C₁₂ muscle cells. S. A. Kramer*, M. E. Doumit, and W. G. Bergen, *Michigan State University, East Lansing.*

We have previously reported that feeding a synthetic β -adrenergic agonist (BAA) to pigs increases skeletal α -actin (SKA) mRNA abundance in finishing pigs. The objectives of this study were to characterize SKA expression during differentiation and to determine the effect of β -adrenergic stimulation on expression of SKA in C₂C₁₂ myotubes. Cells were seeded in 60 mm-diameter dishes, grown to confluence in DMEM containing 10% FBS, then induced to differentiate in DMEM containing 2% FBS. Cells were harvested at various stages of development from rapidly dividing myoblasts to fully differentiated myotubes. Myosin accumulation was measured by immunoblot analysis using an anti-sarcomeric myosin antibody (NA4). A PCR generated probe homologous to 109 bp of the 3' untranslated region of the mouse SKA gene was characterized and used to quantify SKA mRNA abundance. SKA mRNA abundance parallels sarcomeric myosin accumulation in developing C₂C₁₂ cells. Differentiated cells were exposed to isoproterenol (ISO) at 10⁻⁹M to 10⁻⁵M for 48 h, then harvested for RNA isolation. ISO treatment resulted in a dose dependent increase in SKA mRNA abundance with maximal expression at 10⁻⁵M. These data indicate that expression of SKA mRNA parallels sarcomeric myosin accumulation in differentiating C₂C₁₂ myoblasts and that SKA mRNA abundance increases upon β -adrenergic stimulation.

Key Words: α -actin mRNA, β -agonist, Myoblast Differentiation

458 The role of C/EBP α in mediating the antilipogenic effect of pST in finishing pigs. T. D. Brandebourg^{1*}, M. E. Spurlock², and S. E. Mills¹, ¹*Purdue University, West Lafayette, IN* and ²*Purina Mills, Inc., St Louis, MO.*

Somatotropin (pST) promotes muscle protein accretion and decreases lipid accretion in adipose tissue in growing pigs. The antilipogenic effect of pST is proposed to result from alterations in lipogenic gene expression although the mechanism for this effect is unclear. Similarly fatty acids down regulate lipogenic genes through an unknown mechanism. C/EBP α has been implicated in the coordinate regulation of lipogenic genes during adipocyte differentiation. To investigate the potential involvement of C/EBP α in the antilipogenic action of pST and fat feeding, 48 barrows were randomly assigned to a 2x2 factorial design. Pigs were given two levels of pST (0 and 40ug/kg BW/day) and two levels of safflower oil (0 and 10%) added to a standard corn-soybean ration. Diets were fed for 15 days with daily injections of pST beginning on day 6. Blood was sampled on days 12 and 15 and analyzed for urea nitrogen and glucose. Total RNA was extracted from subcutaneous adipose tissue and the abundance of C/EBP α and fatty acid synthase (FAS) quantified by northern analysis. pST decreased plasma urea nitrogen ($p < .01$) and significantly increased plasma glucose ($p < .01$) confirming the effectiveness of the pST treatment. Safflower oil had no effect on plasma urea nitrogen but increased plasma glucose ($p < .01$). pST decreased C/EBP α mRNA abundance by 66% ($p < .04$) and FAS mRNA by 50% ($p < .03$) in the standard diet, but pST had no effect in fat-fed pigs. Fat feeding alone decreased FAS mRNA abundance ($p < .04$) which may have obscured the pST response. The regression of C/EBP α against FAS for all pigs was significant ($r^2 = .21$) suggesting the two genes were coordinately regulated. However, the association was much better for the corn-soybean diet ($r^2 = .64$) than the safflower-supplemented diet ($r^2 = .02$). While it is tempting to suggest a role for C/EBP α in the regulation of FAS, it will first be necessary to clarify how fat supplementation appears to interfere with the pST response.

Key Words: pST, Lipogenesis, C/EBP α

459 Physiologic responses to a novel growth hormone-releasing tripeptide (EP51389) in yearling horses. R. J. Bynum^{*1}, D. L. Thompson, Jr.¹, L. R. Gentry¹, S. R. Morris¹, P. J. Burns², and R. Deghenghi³, ¹*LSU Agricultural Center, Baton Rouge, Louisiana*, ²*Thorn BioScience LLC, Lexington, KY*, ³*Europeptides, Argenteuil (France).*

Three experiments were performed to determine the physiologic responses to a novel growth hormone (GH) releasing tripeptide (EP51389) in yearling horses. In Exp. 1, fillies and geldings were used in two 4 x 4 Latin squares in which EP51389 was injected i.v. at 0, 4, 8, and 16 μ g/kg BW. Exp. 2, performed 60 d later, was a 2 x 2 factorial with dose of EP51389 (2 or 4 μ g/kg BW) and mode of injection (i.v. or i.m.) as factors; there was one filly and gelding per treatment. Exp. 3, performed 30 d later, was a randomized block design with gender as a block; horses were treated i.m. twice daily (0700 and 1900) for 21 d with saline or EP51389 (4 μ g/kg BW). On d 1, 4, 7, 14, and 21, frequent blood samples were drawn to characterize the GH responses. On d 18, an i.v. glucose tolerance test was conducted, and on d 22, the tripeptide was administered to all horses. In Exp. 1, there was a consistent GH response ($P < .001$) to all doses of EP51389, with only minor increases in response with increasing dose. In Exp. 2, equivalent GH responses were obtained with all treatments except the 2 μ g/kg BW administered i.m., which was less than the others. In Exp. 3, EP51389 increased ($P < .001$) plasma GH concentrations consistently after each injection, with only minor variations from a.m. to p.m. and over the 5 d of monitoring. Plasma IGF-I, glucose, non-esterified fatty acids, urea nitrogen, and insulin in morning samples collected during the experiment were not affected by treatment ($P > .1$), nor were glucose and insulin concentrations in response to i.v. glucose. When EP51389 was given to all yearlings on d 22, the GH response at 10 min after injection was greater ($P < .01$) for treated animals than controls. In conclusion, EP51389 is an effective secretagogue for GH in horses. However, its twice daily administration did not alter the glucose, protein, or lipid metabolic characteristics studied.

Key Words: Growth Hormone, Horses, Metabolism

460 *In vitro* muscle cell response to ractopamine. N. W. Shappell^{*1}, D. J. Smith¹, V. J. Feil¹, G. L. Larsen¹, and D. C. McFarland², ¹*USDA-ARS Biosciences Research Laboratory, Fargo, ND*, ²*South Dakota State University, Brookings.*

The effects of ractopamine (β -adrenergic agonist, RAC) and ractopamine stereoisomers (RR, RS, SR, SS) on cyclic AMP (cAMP) response, and total protein and DNA concentrations in mouse muscle cells (C₂C₁₂) were evaluated. RAC (10 μ M) caused an ~30% increase in protein and DNA concentrations in myoblasts after 48 h; no differences were found in myotubes. At a later passage number myoblasts failed to exhibit an increase in protein or or DNA upon exposure to RAC. Both myoblasts and myotubes increased cAMP production in response to 10 μ M RAC (4-7 fold control, producing 20-50 pmoles/mg protein in a 10 min incubation). RAC isomers ranked RR \gg SR \gg RS \gg SS in ability to stimulate cAMP production, with essentially no response to SS, while SR produced about 50% of the RR response. Propranolol (a β -adrenergic antagonist, 40 μ M) did not completely ablate ractopamine-stimulated cAMP production (~40% of RAC alone). Turkey satellite cells (derived from *biceps femoris* of 12-week old Nicholas 88 toms) were tested under similar conditions and produced essentially no increased cAMP when exposed to 10 μ M RAC isomers. The RR isomer raised cAMP slightly above control levels. The turkey cells were responsive to forskolin (10 μ M) showing a 90-fold enhancement over control cells in a 10 min incubation, similar to that found in C₂C₁₂ cells. The stability of RAC was evaluated under lab storage and culture conditions. RAC was stable for more than 4 mo when stored in deuterated DMSO (99.9% purity) at room temperature, as determined by sequential NMR studies. Radiolabeled RAC was incubated for 72 h in the presence of serum-containing medium, with or without C₂C₁₂ cells. Radioactivity in the medium (95% of total dose) remained as the parent compound through 72 h.

Key Words: C₂C₁₂ cells, β -Agonist, Turkey

461 Cytokines and leptin: Regulation in growth and disease. K. L. Houseknecht, *Purdue University, West Lafayette, IN.*

Leptin, the adipocyte product of the *ob* gene, has been implicated in the regulation of food intake, energy expenditure, and whole-body insulin action in rodents and humans. Leptin is a cytokine; the leptin receptor is a member of the IL-6 family of cytokine receptors. Rodent studies provide evidence that leptin gene expression is regulated by inflammatory cytokines, and changes in leptin gene expression may be responsible for the anorexia associated with an acute cytokine challenge. Specifically, IL-1 and TNF- α appear to play a major role in regulating leptin gene expression in hamsters and mice. Studies recently conducted with pigs indicate that leptin expression may be differentially regulated with endotoxin challenge; highlighting species differences in the regulation of leptin gene expression. Growth hormone, another member of the cytokine family, has also been implicated in the regulation of leptin gene expression in humans, but not rodents. However, data reported in human studies are confounded by changes in adipose tissue mass, a known predictor of leptin expression. Studies in growing cattle suggest that short-term growth hormone treatment regulates leptin gene expression prior to major changes in adiposity.

Key Words: Leptin, Cytokine, Growth Hormone

462 Anabolic cytokines for muscle. L. S. Quinn^{*/1}, K. L. Haug¹, S. E. Damon¹, L. H. Macaluso¹, and J. M. Argiles², ¹VA Puget Sound and Univ. Washington ²University of Barcelona.

The widely accepted model for skeletal muscle differentiation is that of negative control by mitogens. This model holds that muscle differentiation is controlled by growth factors and cytokines which inhibit differentiation by effects on muscle-specific transcription factor expression and Rb phosphorylation. However, recent evidence indicates that positive external regulation of myogenic differentiation by the insulin-like growth factors (IGFs) and by interleukin-15 (IL-15) is also important. Experiments involving overexpression of IGF binding protein-4 (IGFBP-4) which demonstrate an absolute requirement for IGF in myogenic differentiation will be presented. Additionally, recent work involving the in vitro and in vivo anabolic effects of IL-15 on skeletal muscle will be presented. In summary, this recent work indicates that growth factors and cytokines can have important differentiation-stimulating and/or anabolic effects on muscle in addition to well-known mitogenic effects on muscle precursor cells.

Key Words: Muscle, Cytokines, IGF

463 Regulation of muscle mass by myostatin. A. C. McPherron* and S.-J. Lee, *The Johns Hopkins University.*

The mechanisms involved in regulating tissue mass are poorly understood. We have identified a novel TGF-beta family member, myostatin (GDF-8) that appears to be essential for proper regulation of skeletal muscle mass. Myostatin is expressed in developing mouse embryos beginning at day 9.5 in the myotome compartment of developing somites. Later in development and in adult animals, myostatin is expressed specifically in skeletal muscle tissue. Myostatin-deficient mice generated by gene targeting showed a dramatic and widespread increase in skeletal muscle mass with individual muscles weighing 100-200% more than those of wild-type littermates. Naturally-occurring mutations in the myostatin gene also appear to be responsible for the double muscling phenotype seen in certain breeds of cattle. The myostatin gene has been cloned from a variety of vertebrates, including mammals, birds and fish, and the protein coding sequence has been highly conserved across species. Based on these findings, we believe that regulating myostatin function may have important applications both for agriculture and for the treatment of muscle wasting disease in humans.

Key Words: Myostatin, Muscle, Double-muscling

464 Physiological roles of leptin, myostatin, and other cytokines. M. E. Spurlock, *Purina Mills, Inc., St. Louis, MO.*

Cytokines, including those typically referred to as proinflammatory cytokines, perform a wide array of physiological functions that are now recognized as normal components of the regulation of growth, development, and metabolism in multiple tissues. Several cytokines, including leptin, myostatin, some interleukins, and tumor necrosis factor influence preadipocyte and(or) myoblast proliferation, differentiation, or metabolism. Furthermore, multiple cytokines are present in colostrum and milk and are thought to play critical roles in gut development and in the regulation of intestinal lymphocyte populations and function. A thorough understanding of the biochemical activities of many cytokines may lead to development of technologies that enhance food animal production.

Key Words: Cytokines, Myostatin, Leptin

465 “Leaping Lords and Leptin”: A partitioning agent? T. G. Ramsay, *USDA-ARS, Beltsville, MD.*

Leptin is a small peptide secreted by fat that can affect reproduction, immune function, feed intake and metabolism. This experiment was designed to determine if porcine leptin has the function of a partitioning agent by assessing the actions of recombinant porcine leptin on adipose tissue versus skeletal muscle (C2C12 cells) in vitro. Recombinant porcine leptin was synthesized using the PGEX fusion system (Pharmacia). Stromal vascular (SV) cells derived from neonatal pig adipose tissue were isolated and used in a primary culture system as previously described (Ramsay et al., *J. Anim. Sci.* 1989). Confluent SV cells were induced to differentiate into fat cells (48 hour exposure to 0.5 mM IBMX, 1 μ M Dexamethasone, 2% pig serum, DMEM:F12). Fat cells generated in these cultures were used after one week of exposure to insulin (10 nM insulin, DMEM:F12). Adipocyte containing cultures were washed with serum free medium and incubated overnight in a basal medium (0.5% pig serum in DMEM:F12). 12 hours after exposure to basal medium, cells were exposed to porcine leptin (0–1000 ng/ml medium) + 10 nM insulin for 1 week with media changes every 2 days. On the 7th day of exposure, fresh recombinant porcine leptin-containing medium was supplemented with 1 μ Ci/ml 14 C glucose. Glucose metabolism was monitored for 4 hours. Chronic exposure to porcine leptin resulted in a stepwise reduction in glucose utilization with a maximal effect at ~50–100 ng leptin/ml medium ($p < .01$). Exposure of C2C12 myotubes to similar conditions produced a dose responsive increase in myotube glucose metabolism with a maximal effective dose in the range of ~50–100 ng leptin/ml medium ($p < .01$). Recombinant porcine leptin could inhibit the breakdown of myotube proteins as assessed by release of 3 H tyrosine from C2C12 myotubes, previously pulse labeled ($p < .05$). In addition, recombinant porcine leptin reduced dexamethasone-induced proteolysis in these C2C12 myotubes by 34% ($p < .01$). These data suggest that leptin may function as a partitioning agent to spare muscle from breakdown by redirecting energy destined for adipose utilization to muscle utilization and by reducing proteolysis.

Key Words: Leptin, Fat Cell, Myotube

467 Comparative effects of pST and a growth hormone releasing hormone analog, rismorelin, on metabolic parameters in swine. D. R. Mulvaney¹, D. B. Anderson^{*2}, A. J. Wuethrich², J. L. Roth², R. C. Smith², J. D. Muegge², L. F. Richardson², and D. H. Mowrey², ¹*Auburn University, AL*, ²*Elanco Animal Health Research and Development, Greenfield, IN.*

Administration of recombinant porcine somatotropin (rpST) to gilts during early gestation has resulted in increased myofiber number and altered composition of offspring postnatally but the effect of growth hormone releasing hormone (GHRH) is unknown. Effects of pST and rismorelin, a new GHRH analog, on serum parameters were compared. In experiment I, 64 PIC terminal line crossbred barrows were assigned to one of eight treatments (TRT): non-injected control, vehicle-injected control, pST injected (3, 6, or 9 mg/hd/d) and RIS injected (1.5, 3.0 or 4.5 mg/hd/d). Pigs were bled 5 h after morning feedings on d -2, 4, 7, 10 and 14 of TRT and serum analyzed for glucose, insulin, IGF-I and BUN. In experiment II, 31 pregnant gilts were assigned to TRT consisting of subcutaneous injections with 9.0 mg/hd/d of pST or RIS along with a non-injected TRT. Injections were given from d 15–30 of gestation. Blood samples were taken on day -1, 3, 7, 14 and 17 relative to drug administration and corresponded to d 13, 17, 21 and 31 of gestation, respectively. Serum was analyzed as in Expt. I as well as, NEFA. Data were analyzed using GLM, Proc GLM, SAS, and mixed model, Proc Mixed, SAS, procedures. Both RIS and pST increased ($p < .05$) weight gain, decreased ($p < .05$) BUN, increased ($p < .05$) serum glucose, insulin and IGF-I over the 14 d periods of treatment to barrows and bred gilts in a quadratic fashion. In Expt. II, relative changes in serum glucose, insulin and IGF-I in RIS-treated gilts were less than that observed in pST-treated gilts, suggesting a different potency for RIS and pST. Upon cessation of injection, RIS treatment BUN, insulin, FFA and glucose values remained elevated compared to controls while pST tended to decline rapidly. These data suggest that the metabolic effects of rismorelin are similar to those of pST but not as large.

Key Words: Swine, GHRH Analog, Somatotropin

468 Evaluation of a substituted piperidine growth hormone (GH) secretagogue L-163,540 on GH and IGF-1 responses in swine. C. H. Chang^{*}, L. A. McNamara, E. Rickes, E. Smith, E. Frazier, H. Y. Chen, G. Morriello, L. Yang, A. Patchett, R. G. Smith, and G. J. Hickey, *Merck Research Labs, Rahway NJ.*

The objectives were to determine the GH and IGF-1 responses to a GH secretagogue L-163,540 (540) and subsequent GH responses following 7-day in-feed treatment. Crossbred swine (~50 kg) were fitted with indwelling jugular catheters. In Study 1, 540 were given i.v. bolus ($n=3$ or 4/dose) at 0, 3, or 10 μ g/kg or orally (p.o.) at 0, 10, and 33 μ g/kg ($n=4$ to 6/dose). Blood was collected and plasma GH profiled over 180 min. When administered i.v. at 0, 3, and 10 μ g/kg, 540 resulted in peak GH (ng/ml) of 4 ± 1 , 37 ± 11 and 91 ± 9 , respectively. At 0, 10, and 33 μ g/kg p.o. 540 resulted in peak GH of 3 ± 2 , 67 ± 21 and 86 ± 23 . In Study 2, we determined initial GH responses and IGF-1 responses to 540 provided in-feed *ad libitum* over 7 days, and GH responses to subsequent i.v. boluses of 540 at 3 μ g/kg. Swine ($n=5$ /group) received 540 at 0, 1, and 20 ppm. Plasma GH were monitored over the first 240 min of 540 treatment. Peak GH were 11 ± 2 , 11 ± 2 , and 35 ± 5 ($p < 0.01$) for 0, 1, and 20 ppm groups, respectively. At 8 hours post 7-day in-feed treatment, all pigs received an iv bolus dose of 540. Peak GH post iv 540 were 61 ± 13 , 17 ± 4 ($p < 0.01$), and 5 ± 1 ($p < 0.01$) for 0, 1, and 20 ppm groups, respectively. By 3 and 10 days post in-feed treatment GH responses to 540 iv were not different among 3 dose groups. Plasma IGF-1 (ng/ml) increased from 80 (day 0) to 144 (day 7; SE=12), 97 to 195, and 95 to 237 for the 0, 1, and 20 ppm groups, respectively. By 5 days post in-feed treatment group differences in plasma IGF-1 were not significant. Results of these studies demonstrate that 540 is a potent, orally active GH secretagogue that increases IGF-1 with chronic in-feed treatment. The decreased GH response to iv 540 after chronic in-feed treatment appeared to be associated with increased IGF-1.

Key Words: L-163,540, GH, IGF-1

466 WITHDRAWN.

469 Growth hormone (GH) secretagogue activity of various nonpeptidyl GH secretagogues in chicks. E. L. Rickes¹, C. H. Chang¹, L. McNamara¹, R. G. Smith¹, J. A. Proudman², and G. J. Hickey¹, ¹Merck Research Laboratories, Rahway, NJ, ²USDA-ARS, Beltsville, MD.

The objective of this study was to investigate the activity of various GH secretagogues in male chicks. The GH secretagogues were selected on the basis of their EC₅₀ as determined by an in-vitro rat pituitary cell assay. The EC₅₀ of L-163,255, L-692,585 and L-692,429 are 1.5, 3.0 and 60.0 nM, respectively. Ten day old Peterson x Arbor Acre male broilers or male H.L. W36 layer chicks were administered via i.v. injection, saline vehicle, L-692,585 at 10 or 50 µg/kg, L-692,429 at 50µg or 1mg/kg. Blood samples were taken at 15 and 30 minutes and plasma samples assayed for GH levels. Intravenous administration of either L-692,585 or L-692,429 did not alter GH levels. L-163,255 at 50 µg and 1mg/kg was administered orally because of the high oral efficacy of this compound in other species. Fifteen minutes after oral administration L-163,255 at 50µg/kg or 1 mg/kg significantly increased GH in broilers (28.5±3.6, 33.7±3.6 ng/ml, P < 0.05, respectively) when compared to vehicle control (17±3.6). L-163,255 in layers caused an increase in GH at 1 mg/kg, 72.7±5.4 ng/ml (P < 0.01), when compared to the vehicle control (50±5.4 ng/ml). Results of this study suggests that both L-692,429 and L-692,585 at the doses given were not efficacious in stimulating GH response, while L-163,255 at the doses given was effective in stimulating GH response in male broiler and layer chicks.

Key Words: Growth Hormone Secretagogue, Chickens

470 Porcine somatotropin (pST) treatment increases protein balance by lowering body protein degradation in young swine. R. Vann¹*, H. Nguyen¹, D. Burrin¹, N. Jahoor¹, N. Steele², P. Reeds¹, and T. Davis¹, ¹USDA/ARS Children's Nutrition Research Center, Houston, TX, ²USDA-ARS Growth Biology Laboratory, Beltsville, MD.

The objective of the study was to quantify the effect of pST on whole body and tissue protein turnover in young, rapidly growing swine. Two groups of six weight-matched (15 kg) barrows were randomized to receive subcutaneous injections of pST (150 ug/kg per d) or diluent for 1 wk. pST pigs were fed ad libitum a 23% protein diet. Control animals were pair-fed the intake of the pST group. After 5 d, carotid and jugular catheters were implanted. On d 8, pigs were fed hourly meals of 1/24 of their preceding daily intake and infused intravenously with ¹³C-bicarbonate (0-2 h), ¹³C-leucine (2-8 h) and ¹⁵N₂-urea (0-8 h). Arterial and breath samples were taken throughout. Animals were sacrificed after 8 h of infusion. Leucine and α-ketoisocaproic acid labeling was measured by gas chromatography mass spectrometry and breath carbon dioxide labeling by gas isotope ratio mass spectrometry. Whole body carbon dioxide production was calculated from breath carbon dioxide labeling over 0-2 h. Leucine flux was calculated from the isotopic enrichment of α-ketoisocaproate, and leucine oxidation from the labeling of breath carbon dioxide from 6-8 h of infusion. pST treatment increased (P<0.05) gain:feed (g gain/g feed) from 0.61 (control) to 0.75 (pST)(pooled SD(PSD) 0.12). Plasma urea flux (g N/kg/d) was lowered 70 percent(P<0.001) by pST treatment (control, 0.79; pST, 0.24; PSD, 0.08). Leucine catabolism was lowered 62 percent (P<0.001). The urea kinetic data suggested that pST treatment had increased protein deposition by 40 percent. Whole body protein degradation (g protein/kg/d) calculated from leucine kinetics fell (P<0.02) from 15.1 (control) to 10.1 (pST)(PSD, 0.8) with no significant change in whole body protein synthesis (control, 18.3; pST, 18.7). We conclude that in young pigs fed high-protein diets, pST promotes growth primarily by lowering whole body proteolysis and amino acid catabolism.

Key Words: Somatotropin, Protein Turnover, Pigs

471 Meal-feeding reduces activity of somatostatin neurons but does not alter activity of growth hormone-releasing hormone neurons in steers. C. D. McMahon*, L. T. Chapin, K. J. Lookingland, and H. A. Tucker, Michigan State University, East Lansing.

Release of growth hormone (GH) from the anterior pituitary gland is regulated by two hypothalamic hormones: growth hormone-releasing hormone (GHRH) stimulates release, while somatostatin (SS) inhibits release. Meal-feeding of steers for 2 h per day reduces concentrations of GH for at least 1 h after feeding. We hypothesize that GHRH neurons are more active before than after feeding, while SS neurons are more active after than before feeding. Hypothalami were collected from four Holstein steers (4-months old) killed 1 h before feeding and four killed 1 h after feeding. Neuronal activity was measured as the percent of immunoreactive GHRH neurons in the arcuate nucleus and SS neurons in the periventricular nucleus with immunoreactive FOS and FOS-related antigens (FOS/FRAs; immediate-early gene proteins) detected in their nuclei. Basal concentrations of GH in serum averaged 4.9 ng/ml at slaughter before feeding, but then concentrations declined to 0.8 ng/ml at slaughter after feeding (pooled SEM = 0.2; P < 0.001). The percent of SS neurons containing FOS/FRAs decreased from 26.8 at 1 h before feeding to 12.7 at 1 h after feeding (pooled SEM = 1.7; P < 0.01), while percent of GHRH neurons containing FOS/FRAs was not different before (25.8) versus after feeding (29.8) (pooled SEM = 3.9; P > 0.05). These data show that concentrations of GH in serum of meal-fed steers are associated with activity of SS neurons, but not with activity of GHRH neurons.

Key Words: Growth Hormone-Releasing Hormone, Somatostatin, FOS and FOS-related Antigens

472 Effects of the growth hormone secretagogue L-163,255 on GH and IGF-1 levels when administered intravenously or orally to horses. L. McNamara¹, C. H. Chang¹, E. Rickes¹, E. Frazier¹, H. Chen¹, K. Barakat¹, R. Nargund¹, A. Patchett¹, R. G. Smith¹, K. Malinowski², and G. J. Hickey¹, ¹Merck Research Laboratories, Rahway, NJ and ²Dept. Animal Science, Rutgers - The State University of New Jersey.

The objective of these studies was to evaluate the efficacy of the GH secretagogue, L-163,255 on GH and IGF-1 levels in horses. In study 1, four thoroughbred (TB) geldings (500±100 kg, ages 7 to 11) received L-163,255 (255) i.v. in saline at 1 mg/kg, or orally, at 5 mg/kg in a crossover design. Blood samples were taken from -120 min, to 24 hours, at various intervals, for GH and IGF-1 determination. Animals were dosed with vehicle at -60 min, and treated with 255 at 0 min. Treatment with 255 at 1 mg/kg i.v., immediately increased GH levels, the mean peak concentration and AUC were 58±15 ng/ml, and 7,488±1,414 ng.min/ml, respectively. Levels returned to baseline after 120 min. Treatment with 255 given at 5 mg/kg orally, also increased GH, reaching a peak of 53±13 ng/ml and AUC of 6,634±1,253 ng.min/ml, and returning to baseline around 240 min. IGF-1 levels increased to 1.10 fold over baseline in the i.v. dosed animals, and a 1.08 fold increase in the orally dosed group. Peak IGF-1 concentration was reached by 8 hours, and remained elevated at 24 hours. In study 2, horses were dosed i.v. once daily with 255 at 1 mg/kg for 3 days to determine if IGF-1 levels could be further increased. Six TB geldings (500±100 kg, ages 7 to 11) were dosed with either saline vehicle or 255 at 1 mg/kg i.v. once daily for 3 days. Samples for IGF-1 levels were taken at 0, 8, 24, 48 and 72 hours. Treatments were crossed over 14 days later. After the initial treatment of 255, IGF-1 levels increased steadily over the study period; at 72 hours IGF-1 had significantly increased (p<0.05) 1.5 fold over baseline. These studies demonstrate that L-163,255 is an effective GH secretagogue in horses when administered i.v. or orally, and IGF-1 levels can be significantly increased by a once daily i.v. administration over a three day period.

Key Words: L-163,255, GH, Horses

473 The effect of dietary energy source on fat metabolites, insulin-like growth factor-I and insulin in growing horses. J. K. Baldock*, J. E. Minton, R. H. Raub, J. D. Weber, and C. M. Hill, *Kansas State University, Manhattan.*

Feeding diets high in soluble carbohydrates to young growing horses has been implicated in the development of orthopedic diseases, and the substitution of dietary fat for soluble carbohydrates has received some attention. Because insulin-like growth factor-I (IGF) is integral in the development of growth cartilage, and is believed to be nutritionally regulated, we designed an experiment to evaluate the effect of isocaloric addition of fat on metabolic endpoints in growing horses. Twelve quarter horse weanlings, 4 female and 8 male, ranging in ages from 151–226 d, were blocked by sex and age and assigned to one of two treatment groups. Group 1 (G1) (n=6) was fed a concentrate (CONC) containing 33.9% starch and 2.21% fat, and Group 2 (G2) was fed a CONC containing 24.0% starch and 10.3% fat. Both CONC were formulated to be isocaloric and isonitrogenous (2.8 mcal/kg DE, 16% CP). In addition to CONC, brome hay (89.0% DM, 1.9 mcal/kg DE, 7.4% CP) was fed. Body weight was recorded weekly and diets were adjusted to achieve a predicted ADG of .91 kg. Diets were fed at 0800 h and 1600 h for 60 d. On d 0, 30 and 60, blood samples were obtained via a jugular vein catheter at frequent intervals from 1 h before until 5 h after morning feeding. Serum was analyzed for insulin, IGF, NEFA, and total cholesterol (CHOL). ADG was the same between treatments (.83 ± .01 kg). There was a consistent increase in insulin response to feeding on d 0, 30 and 60 for both groups. On d 60 the insulin response to feeding was lower (P<.05) over time in G2 compared to G1, however, there was no treatment x time effect on d 0 or 30. CHOL concentrations were similar between groups on d 0. However, G2 had elevated CHOL concentrations on d 30 and 60 compared to G1 (P<.01). There were no differences in NEFA or IGF concentrations on d 0, 30 or 60. These results suggest that energy source, at least at the level used in this study, does not affect serum IGF, but does affect serum insulin and CHOL concentrations. Also, energy source had no effect on ADG.

Key Words: Horse, Dietary Fat, IGF-I

474 Growth hormone response to growth hormone releasing factor by Holstein calves from genetic lines selected for milk yield. L. H. Baumgard*¹, W. J. Weber¹, H. Chester-Jones¹, L. B. Hansen¹, G. W. Kazmer², S. A. Zinn², and B. A. Crooker¹, ¹University of Minnesota, St. Paul, and ²University of Connecticut, Storrs.

Holstein bull and heifer calves (n = 101) from select (S) and control (C) lines that differed by more than 4,500 kg milk/305 d lactation were administered a growth hormone (GH) releasing factor (GRF) analog (4 µg/100 kg BW) on 10, 56, 140, 196, 252, and 364 ± 3 days of age. Blood plasma samples (n = 15) were obtained from a jugular catheter from - 30 to 120 min relative to GRF administration. Area under the GH response curve (0 to 60 min, AUC) was quantified after subtracting mean pre-challenge (PREC) GH concentrations. Data were analyzed for effects of line, age, gender, season and their interactions with PROC MIXED of SAS for repeated measures and incorporated the spatial power law SP(POW) for unequally spaced data with age as the repeated affect. Means were different when P < 0.05. PREC GH concentrations did not differ between lines, were greater in bulls than heifers (4.4, 3.4 ng/ml), decreased with age (7.0^w, 4.6^x, 3.6^y, 3.4^y, 3.1^y, 1.8^z ng/ml), and were greater in summer than fall, winter, or spring (4.7^x, 3.3^y, 3.6^y, 3.9^y ng/ml). AUC decreased with age and did not differ between lines. Heifers tended (P = 0.10) to respond more than bulls (1537, 1365 ng/ml per min) to GRF. Peak GH concentration decreased with age (99.2^w, 63.7^x, 41.6^z, 55.8^{x^y}, 49.6^{y^z}, 44.4^{y^z} ng/ml) and did not differ between lines or genders or among seasons. Time from GRF administration to GH peak was greatest in young calves (11.8^{x^y}, 13.9^x, 10.1^{y^z}, 10.0^{y^z}, 9.3^z, 10.9^{y^z} min) and did not differ between lines or genders or among seasons. GH clearance was more rapid in heifers than bulls (2.1, 1.7 ng/ml per min), varied among ages, and did not differ between lines or among seasons. Although plasma GH has been implicated as a heritable trait, we conclude the GH variables measured in this study were not useful in predicting genetic merit of calves from these substantially divergent lines of cows.

Key Words: Selection, GH, GRF

475 Endocrine and metabolic effects of growth hormone and exercise in mares. M. J. Garber*, N. J. Hughes, B. C. Peterson, T. C. Bramble, R. A. Battaglia, G. T. Schelling, and R. A. Roeder, *University of Idaho, Moscow.*

Eight non-pregnant mares were used in a 2 × 2 factorial arrangement to determine effects of growth hormone (GH) and/or exercise (EX) on serum metabolic and endocrine factors. Mitogenicity of sera and of extract from crushed (C) or uncrushed (U) muscles was also determined. EX mares worked 10 min/d, 5 d/wk for 4 wk on an inclined (10%) treadmill. EX protocols were adjusted weekly based on improvements in V_O2max. GH mares received s.c. injections of 30 mg/d recombinant porcine GH. Sera were collected on d 0 and 30 for glucose (GLU), insulin (INS), insulin-like growth factor I (IGF-I), non-esterified fatty acids (NEFA), and cell culture. In EX mares, samples were taken prior to exercise, then at 5, 10, 20, 30, 45, 60, and 90 min post-exercise (PE) for GLU and INS and at 10, 20, 30, 60, and 90 min PE for NEFA. On d 31 the mares were sacrificed and the extensor digitorum longus muscles were removed; one from each mare was gently crushed. Each muscle was rocked in PBS (1 L/kg) for 2 h at 4°C. GH increased resting concentrations of serum IGF-I (P<.011), INS (P<.008), and NEFA (P<.02) by 505, 3147, and 153%, respectively, regardless of EX. Only sedentary GH mares experienced an increase (16%, P<.044) in resting GLU. In EX mares, GH did not change blood GLU PE. However, GH increased (P<.03) INS at all times PE. There were GH x period (d 0 or d 30) interactions (P<.05) for NEFA at 20, 30, and 90 min PE; d 30 PE NEFA decreased for GH and increased for non-GH mares. Proliferation and protein synthesis rates of C2C12 mouse satellite cells were increased (P<.025 and P<.027, respectively) and protein degradation was decreased (P<.001) by sera from GH-treated mares. Proliferation of cells cultured in C or U muscle extract was unaffected (P>.05) by crush, EX or GH except at the highest culture concentration (80 µg/mL muscle protein), where extract from GH-treated mares increased (P<.043) proliferation. In summary, GH, but not EX, increased serum metabolic and endocrine factors and improved myoblast growth.

Key Words: Growth Hormone, Exercise, Horse

476 Organization and regulation of the gene encoding the sheep acid labile subunit of the 150 kDa IGF-binding protein complex. R. P. Rhoads*, A. W. Bell, P. L. Greenwood, and Y. R. Boisclair, *Cornell University, Ithaca, NY.*

Circulating insulin-like growth factor (IGF)-I and II play a major role in the growth and productivity of agricultural animals. In adult animals, most circulating IGF is sequestered in a 150 kDa complex composed of a molecule each of IGF, IGF binding protein-3 (IGFBP-3) and a serum protein called acid labile subunit (ALS). This complex is thought to extend the half-lives of IGFs and prevent non-specific activation of the insulin receptor by IGF. To study the biology of ALS in ruminants, we have isolated the sheep ALS gene and determined its organization. The gene covers ~3.3 kb of chromosomal DNA and consists of two exons interrupted by an ~1000 bp intron. The sheep ALS cDNA sequence is 81% and 75% identical to those of human and mouse, respectively. Overall, the sheep ALS gene encodes a protein of 606 amino acids which is 76% and 72% identical with human and mouse ALS, respectively. Regulation of the sheep ALS gene was analyzed by Northern blotting. A single transcript of 2.2 kb was detected in adult liver, but not in kidney, spleen, heart, lung, muscle, or brain. Expression in liver was not detected during fetal life and was barely detectable at birth. At 7 d of age, the ALS gene was clearly expressed and increased little afterward. This contrasts with earlier expression of the IGFBP-3 gene at 85 d of fetal life, and of the growth hormone receptor gene at birth. To determine if the sheep ALS gene is modulated by nutrition, weaned lambs (3 mo) were fed daily 3.6 Mcal ME and 178 g CP (H, n=4) or 1.9 Mcal ME and 122 g CP (L, n=4). After 6 wk, lambs were slaughtered and levels of expression of ALS and IGFBP-3 were analyzed in liver. The abundance of ALS or of IGFBP-3 mRNAs was not altered, despite significant differences in growth rate (394 vs 243 g/d, H vs L, P< 0.05). In conclusion, expression of the sheep ALS gene occurs only in liver soon after birth and is not modulated by nutrition in growing sheep.

Key Words: IGF System, Acid Labile Subunit, Sheep

477 Somatotropin induced advancement of embryonic growth and rate of somitogenesis in porcine embryos. D. R. Mulvaney¹, R. C. Smith², D. B. Anderson², J. L. Wallace-Evers², J. D. Muegge², and A. J. Wuethrich², ¹Auburn University, AL, ²Elanco Animal Health Research and Development, Greenfield, IN.

Administration of recombinant porcine somatotropin (rpST) to gilts during early gestation has resulted in increased muscle growth yet the mechanisms are unknown. To examine these effects more directly, sixty PIC Camborough-15 maternal line gilts once bred were randomly assigned to be noninjected controls (C) or injected BID with 7.5 mg/hd/d rpST (P) from gestation d 10–15. Two h post-feeding on d 14, gilts were bled and serum prepared for use in a Porcine Embryo Culture (PEC). On d 15, gilts were sacrificed, embryos (EMB) harvested via gentle flushing of the uteri with warm PBS, sorted, scored, and then placed in Minimum Essential Medium (MEM) prior to culturing in MEM plus pig serum (PS) and antibiotics in 30 ml glass bottles on a roller apparatus. A 2X2 factorial PEC experiment with embryo (C-EMB vs P-EMB) and PS sources (C-PS vs P-PS) was conducted when at least 4 EMB/litter of similar stage of development were isolated. Embryos were cultured in an environment of 95% O₂ and medium containing 50% PS. Following PEC, EMB were evaluated for developmental parameters (somite number, crown trunk length, etc.) and composite morphological score (MS). Somitogenesis and development in d 15 C-EMB cultured in P-PS medium occurred at a 22% (.27 vs .33 somite pairs/h; P<.07) and 44% faster rate (8.5 vs 12.2 MS/h; P<.005) compared to C-PS. EMB's from pST treated gilts and all EMB's cultured in medium containing P-PS had higher MS (P<.01) compared to C. These data suggest: 1) positive effects of rpST treatment on rate of embryonic development both in vivo and in vitro; and 2) the potential utility of the PEC system to study mechanisms influencing porcine myogenesis, and EMB growth and viability during the peri-implantation period.

Key Words: Porcine Embryo Somitogenesis, Whole Embryo Culture, Somatotropin

478 Comparative efficacy of administration of rismorelin and pST during early gestation on muscle growth in newborn pigs. D. R. Mulvaney¹, D. B. Anderson², A. J. Wuethrich², R. C. Smith², J. L. Evers², J. D. Muegge², L. F. Richardson², J. L. Roth², and D. H. Mowrey², ¹Auburn University, AL, ²Elanco Animal Health Research and Development, Greenfield, IN.

Treatment of gilts with recombinant (rpST) during early gestation has resulted in increased myofiber number and muscle growth of progeny (Rehfeldt et al., 1996) yet effects of GHRH are unknown. Using a randomized block design, fifty-six gilts were utilized to study the efficacy of pST and rismorelin, a new GHRH analog, injection during gestation for altering progeny muscle growth. In experiment I, gilts received no injection (CTL) or subcutaneous injections BID 9.0 mg/hd/d of RIS or pST from d 15–30 of gestation (GEST). In experiment II, gilts received no drug, 4.5 mg/hd/d RIS or 7.5 mg/hd/d pST. Gilts were fed 2.7 kg/d of a 16% CP coarse ground, corn-soy diet prebreeding through breeding (day 0). On d 1 of GEST, gilts were fed 2.27 kg through d 12, then 2.73 kg from d 13 throughout GEST. Within 24 h of birth, pigs were weighed (BWT), ranked within litter by percentile (PCT). Pigs representing the 12.5, 50 and 87.5 PCT were bled by venipuncture before affixation with carbon dioxide. Weights and crown rump lengths (CRL) were measured. After exsanguination, semitendinosus and peroneus longus muscles were excised, weighed, sub-sampled and frozen to enable enumeration of myofibers by morphometric analyses of alkaline ATPase-stained sections. Serum was analyzed for glucose, FFA, creatine, insulin, IGF-I and BUN. Data were subjected to least squares ANOVA using GLM and mixed model techniques for determination of main effects of treatment (TRT). No effect of TRT on BWT or CRL was found. Pigs from the 12.5 PCT had lighter muscles than those of the 50th and 87.5 PCT. Both PL and ST muscles from pigs of litters treated with RIS or pST expressed as a % of BWT were heavier (P<.05) than pigs from CTL. No consistent differences due to TRT were detected for serum parameters. RIS and pST injection during d 15-30 of GEST were effective in altering muscle development of progeny.

Key Words: Pigs, rpST, Rismorelin

479 Effect of progressive cachectic parasitism and growth hormone treatment on hepatic 5'-deiodinase activity in calves. S. Kahl^{1*}, T. H. Elsasser¹, J. L. Sartin², and R. Fayer¹, ¹USDA, Agricultural Research Service, Beltsville, MD, ²Auburn University, Auburn, AL.

Conversion of thyroxine (T₄) to the metabolically active hormone, triiodothyronine (T₃), is catalyzed by 5'-deiodinase (5'D). The objective of this study was to examine the effect of protozoan parasitic infection with *Sarcocystis cruzi* on hepatic 5'D activity and plasma concentrations of T₃ and T₄ in placebo- or bovine GH (bGH)-injected calves. Holstein bull calves (127.5 ± 2.0 kg BW; n=3-5/group) were assigned to control (C, ad libitum fed), infected (I, 250,000 *S. Cruzi* oocyst per os, ad libitum fed), and pair-fed (PF, non-infected, fed to intake of I treatment) groups noninjected, and three similar groups injected daily with bGH (USDA-B-1, .1 mg/kg, i.m.) designated as C_{GH}, I_{GH} and PF_{GH}. GH injections were initiated on d 20 postinfection (PI), 3 to 4 d prior to the onset of clinical signs of the acute phase response (APR), and were continued to d 56 PI at which time calves were euthanized for liver collection. Blood samples were collected on d 0, 28, and 55 PI. Nutrition did not affect type I 5'D in liver. Treatment with bGH increased (P < .05) 5'D activity (nmol I⁻ × h⁻¹ × mg protein⁻¹) in C (6.58 ± .24 vs 5.28 ± .29) and PF (6.21 ± .38 vs 4.95 ± .63) but not in I calves; compared to PF calves, infection with *S. Cruzi* reduced 5'D 25% (P < .05) and 47.8% (P < .01), respectively, in placebo and bGH injected calves. Neither nutrition nor bGH treatment significantly affected plasma concentrations of T₄ and T₃ on d 28 and 55 PI. On day 28 PI, the average plasma concentration of T₃ and T₄ was reduced in infected calves (I+I_{GH}), respectively, 36.4% (P < .01) and 29.6% (P < .05) compared to pair-fed calves (PF+PF_{GH}: T₃, 1.75 ± .12; T₄, 81 ± 6 ng/mL). However, infection did not affect plasma thyroid hormones on d 55 PI. The data suggest that chronic parasitism in growing calves inhibits basal and bGH-stimulated generation of T₃ in liver and, consequently, changes the local thyroid status of this tissue.

Key Words: 5'-Deiodinase, Growth Hormone, Protozoan Infection

480 Somatotropin regulates lipoprotein lipase and fatty acid synthase activity in adipose tissue from neonatal swine. Y. X. Wang^{*}, S. K. Fried, R. N. Petersen, and P. A. Schoknecht, Cook College, Rutgers University, New Brunswick, NJ.

Lipoprotein lipase (LPL) is the major enzyme responsible for hydrolysis of triacylglycerol in lipoproteins to provide free fatty acids for tissue utilization or storage, while fatty acid synthase (FAS) is the major enzyme for de novo fatty acid synthesis in adipose tissue. Somatotropin (ST) inhibits lipid accretion in neonatal pig adipose tissue. This inhibition may be through regulation of LPL and/or FAS activity. To test this hypothesis, four 7-d crossbred pigs were used. Subscapular adipose tissue fragments were cultured for 24h with or without ST (4.5 nM) in the absence or presence of insulin (7 nM) in M199 medium. LPL activity was measured using radiolabelled triolein with 1 U of LPL activity defined as catalyzing the release of 1 μmol free fatty acid per h. FAS activity was assayed spectrophotometrically by following NADPH conversion to NADP⁺ with 1 U of FAS activity defined as oxidizing 1 nmol NADPH to NADP⁺ per min. ST significantly inhibited LPL activity regardless of the presence of insulin. ST inhibited FAS only in the presence of insulin. These data suggest that ST inhibits lipid deposition in neonatal pig adipose tissue by decreasing LPL activity directly and by decreasing insulin-stimulated LPL and FAS activity. Since lipid is a major component of the neonatal diet, ST may have an important role in nutrient partitioning in the neonate.

Activity	-ST/-Ins	+ST/-Ins	-ST/+Ins	+ST/+Ins
LPL (U/g tissue)	3.5 ± 0.9 ^a	1.8 ± 1.0 ^c	7.0 ± 1.2 ^b	1.8 ± 0.6 ^c
FAS (U/g tissue)	44.8 ± 10.6 ^a	44.1 ± 11.4 ^a	77.0 ± 7.5 ^b	60.0 ± 9.3 ^c

Mean ± SEM. Values within a row with different superscripts were significantly different at p<0.05.

Key Words: Growth Hormone, Lipid Accretion,

481 Muscle and organ specific regulation of IGF-1 mRNA expression in growing steers: Quantification with an internally standardized competitive RT-PCR. M. Pfaffl, F. Schwarz, H. H. D. Meyer, H Sauerwein*, *TU Muenchen-Weihenstephan, Germany.*

To investigate the role of local IGF-1 expression within the auto/paracrine regulation of allometric growth, we developed a method which allows for a sensitive and reliable quantification of IGF-1 mRNA: an internally standardized competitive RT-PCR. A 184 b competitive standard IGF-1 cRNA was constructed recombinantly by deleting 56 b out of a hepatic wild-type IGF-1 fragment. Known quantities of the competitor IGF-1 cRNA were co-amplified with series of 250 ng tissue-RNA. The resulting RT-PCR products of IGF-1cRNA (184 bp) and wild type IGF-1 mRNA (240 bp) were quantified by HPLC-UV. The assay has a detection limit of 1600 IGF-1 cRNA molecules, an assay variation of 7.4% (n=5) and assay linearity was given (R=0.997). Tissue IGF-1 mRNA expression was investigated in growing steers which were fed either intensively (I; n=8) or kept on pasture (P; n=9) or underwent compensation (C; n=9). Animals were slaughtered at 570±12.5 kg and samples were collected from liver, heart and 4 muscles (m. splenius (SP), m. soleus (SO), m. cutaneus trunci (CT) and m. semispinalis capitis (SC), which were selected in order to represent maximal differences in fiber composition as well as in growth impetus. The different growth velocities led to significant differences in IGF-1 mRNA expression rates in all organs and m. splenius:

group	SP	SO	CT	SC	liver	heart
I	7.9 ¹ (1.3) ² a ³	8.4(2.1)	2.5(1.0)	4.8(0.6)	2872(643) a	32.3 (4.4)a
C	1.6(0.5) b	13.9(2.6)	5.2(1.9)	6.9(1.2)	480(110) b	16.6(3.8) b
P	8.7(2.2) a	13.5(2.8)	1.3(0.3)	4.1(0.6)	2236(171) a	19.9(3.9) b

¹ × 10¹⁰ IGF-1 mRNA molecules/g tissue = mean; ² SEM of samples are in parenthesis; ³ different superscripts indicate differences between feedings (p<0.05)

These differences were not attributable to alterations in DNA/RNA ratio. The results indicate that IGF-1 mRNA expression is regulated tissue specifically not only in different organs but also within skeletal muscles.

Key Words: IGF-1 mRNA, Skeletal Muscle, Competitive RT-PCR

482 Effects of time point of first colostrum intake on plasma insulin-like growth factor I (IGF-I), IGF binding proteins (IGFBPs), and insulin in neonatal calves. I. Zanker, H. Hammon*, and J. W. Blum, *University of Berne, Switzerland.*

The somatotropic axis in the neonatal calf is influenced by the amount of colostrum intake after birth, but colostrum IGF-I is not absorbed. This study was planned to investigate effects of delayed colostrum intake in neonatal calves on growth hormone (GH), IGF-I, IGFBPs, and insulin concentrations in blood plasma. Calves received 1st colostrum 2 h (group A), 6 h (group B), 12 h (group C), and 24 h (group D) after birth. Thereafter, milkings 2 to 5 were fed every 12 h followed by milk replacer up to d 7. Blood samples before and 2 h after feeding were taken at 1st and 3rd colostrum intake and on d 7. GH, IGF-I, and insulin were measured by RIA, IGFBP-2 and -3 by ligand blots. Plasma IGF-I concentrations decreased during wk-1 in groups A and B and increased after each feeding in group D (P <.05). IGF-I concentrations before 1st colostrum intake were higher (P <.05) in group A than in groups C and D and higher (P =.05) in group B than in group D. Before 3rd colostrum intake IGF-I concentrations were higher (P <.05) in groups A and B than in group D. Plasma IGFBP-2 concentrations before 1st colostrum feeding were higher (P <.05) in group D than in groups A and C and before 3rd colostrum feeding were higher (P <.05) in group D than in group A. Plasma IGFBP-3 concentrations before 1st colostrum intake were higher (P =.05) in group A than in group D and before 3rd colostrum feeding were higher (P <.05) in groups A and B than in group D. Plasma GH concentrations showed no time or group differences in wk-1 of life. Plasma insulin concentrations increased (P <.05) after 1st colostrum intake in groups A, B, and C, after 3rd colostrum intake and on d 7 in groups B and C, but never in group D. The postprandial insulin rise after 1st colostrum intake was greater (P <.05) in group A than in groups C and D. The data demonstrate that plasma IGF-I and insulin levels are influenced by delayed colostrum intake. Plasma IGF-I decreases with delayed colostrum intake were associated with decreased IGFBP-3/IGFBP-2 ratios.

Key Words: Neonatal Calf, IGF-I, IGFBPs

483 The influence of exogenous GH on age related muscle accretion of entire Holstein-Friesian male calves. Z. Holzer¹, Y. Aharoni¹, A. Brosh¹, A. Orlov¹, and F. Buonomo², ¹ARO, *Institute of Animal Science, Neve Ya'ar Research Center, Israel* ²Protiva, *a unit of Monsanto Company.*

The decrease in rate of protein deposition with increasing age and liveweight, in steers, indicates the existence of a biological limit for daily protein growth (Byers, 1982, Fed. Proc.,41:2562). The objective of this study was to assess the effect of rbST (Posilac[®]) in overcoming this biological limitation, on protein deposition, in Holstein-Friesian intact male calves, fed on two levels of metabolizable energy concentration. Fifty six male calves, about 185 days old and of an average weight of 210 kg at the start of the experiment, were arranged, at random, in an experiment of a 2x2 factorial design (n=14), with 2 levels of exogenous growth hormone (0; rbST) and two diet metabolizable energy concentrations (2.5 and 2.7 Mcal/kg DM). Thus, four sub-treatments were formed: low energy, rbST treated (LT), and control (LC) and high energy rbST treated (HT) and control (HC). The rbST treated and the control animals received biweekly injections of 500mg Posilac[®], and placebo, respectively. Hormone and metabolites concentration, and meat qualities, will be reported elsewhere. ADG during the whole experiment, which lasted 224 days in average, was 1115, 1060, 1388 and 1290 g (P<0.09 for rbST and P<0.001 for diet energy concentration main effects) for LT, LC, HT and HC, respectively. Total fat in the large depots, expressed as % of the carcass, was: 2.12, 3.03, 3.28 and 4.35 (P<0.001 for rbST and for energy main effects). On the low energy diet, there were no differences in performance until the age of about 240 days. Since then a tendency to an advantage in rate of gain to the rbST treated animals was observed. On the high energy concentration diet, an advantage to the rbST treated animals was evident throughout the span of the experiment. This can be attributed to a change in the energy content of the live weight gain.

Key Words: Male Calves, Bovine Somatotropin, Fat Deposition

484 Effect of growth factors on insulin-like growth factor binding proteins (IGFBPs) in media conditioned by primary porcine satellite cells. Z. Yi*, M. Hathaway, W. Dayton, and M. White, *University of Minnesota, St. Paul.*

Muscle satellite cells are myogenic cells which are critically important for postnatal muscle growth. The insulin-like growth factors (IGFs) are believed to play an important role in satellite cell growth and development and the biological activity of the IGFs is regulated by a family of IGFBPs. Other growth factors, which affect satellite cell growth, may work in part through the IGF-IGFBP system. The objective of this study was to determine the individual and combined affects of IGF-1, basic fibroblast growth factor (bFGF), transforming growth factor-beta (TGF-β) and platelet derived growth factor (PDGF) on IGFBP level in media conditioned for 24 hr by primary porcine satellite cells (PSC). IGFBP level in media conditioned by PSC and muscle-derived fibroblasts were measured using ¹²⁵I-IGF-1 ligand blotting and compared in order to determine which IGFBPs were specific to satellite cells. PSC were isolated from the hind limb muscle of 7 wk old crossbred pigs, plated on fibronectin-coated plates and exposed to various growth factors in a serum-free medium. IGFBP-2, -3, -4 and -5 were observed in PSC conditioned media (CM). However, IGFBP-2 and -4 were also secreted by muscle-derived fibroblasts. Thus, IGFBP-3 and -5 were secreted only by PSC. IGFBP-3 has not been shown to be produced by myogenic cell lines. IGFBP-3 levels in PSC CM were increased 1.9, 2.9, 1.5 and 2.3 fold by IGF-1(20 ng/ml), TGF-β (0.5 ng/ml), bFGF (50 ng/ml), or PDGF-BB (15 ng/ml) respectively (P<0.05) over levels in basal CM. IGFBP-5 levels in these cultures were increased 2.5, 1.9, 2.3 and 1.5 fold by IGF-1, TGF-β, bFGF, or PDGF-BB respectively (P<0.05) over basal CM. Combining bFGF or TGF-β with IGF-1 did not affect the level of IGFBP-3 whereas IGFBP-5 was increased 1.4 or 2 fold respectively (P<0.05). These data indicate that PSC produce IGFBP-3 and -5 and that the levels of these IGFBPs can be regulated by IGF-1 and other growth factors.

Key Words: Satellite Cells, Porcine, IGFBP

485 Decreased steady-state insulin-like growth factor binding protein-3 (IGFBP-3) mRNA level is associated with differentiation of cultured porcine myogenic cells. B. J. Johnson, F. Yang, M. E. White, M. R. Hathaway, and W. R. Dayton*, *University of Minnesota, St. Paul.*

Insulin-like growth factor binding proteins (IGFBP) have been shown to affect the biological activity of IGF-I in several cell types, including cultured muscle cells. Additionally, IGFBP-3 has been shown to have IGF independent effects on cell proliferation. Unlike immortalized muscle cell lines, primary cultures of porcine embryonic myogenic cells (PEMC) express IGFBP-3 mRNA and secrete a protein that is immunologically identifiable as IGFBP-3. Additionally, steady-state (SS) IGFBP-3 mRNA levels change significantly during differentiation. Here we report that differentiation of PEMC in an IGFBP-3-free medium, as evidenced by increased SS myogenin mRNA, fusion and creatine phosphokinase (CPK) activity, is accompanied by reduced SS IGFBP-3 mRNA levels. SS levels of IGFBP-3 mRNA decreased approximately seven fold ($P < .05$) during differentiation and then increased to pre-differentiation levels once differentiation was complete. Addition of TGF- β_1 (0.5ng/ml) to PEMC suppressed fusion and resulted in a seven-fold increase in SS IGFBP-3 mRNA and a 1.8 fold increase in IGFBP-3 protein levels as compared to untreated control cultures ($P < .05$). In contrast addition of IGF-I to proliferating PEMC enhanced differentiation as evidenced by a 4 fold increase in SS myogenin mRNA level and decreased SS IGFBP-3 mRNA level (8 fold, $P < .05$) and protein (3 fold, $P < .02$). Results show that reduction in IGFBP-3 mRNA level is coincident with increased expression of myogenin mRNA in PEMC, suppression of differentiation by TGF- β causes increased IGFBP-3 protein and steady-state IGFBP-3 mRNA levels, and enhancement of differentiation by IGF-I results in decreased IGFBP-3 protein and SS mRNA levels that are coincident with increased expression of myogenin mRNA. Viewed together these data suggest that reduced production of IGFBP-3 may play a role in regulating differentiation of PEMC.

Key Words: Pig, IGFBP-3, Muscle

486 Conjugation methods affect anti-somatostatin antibody production, but do not affect growth response during active immunization against somatostatin in rats. Y. S. Kim*, K. H. Kim, and Y. J. Choi, *University of Hawaii at Manoa, Honolulu, and Seoul National University, Suwon, Korea.*

Active immunization against somatostatin (SS) has resulted in inconsistent effects on animal growth, possibly due to differences in antibody production against SS resulting from differences in conjugation methods or carrier proteins used in conjugation. To test this hypothesis, in Exp. 1, 175 g male rats ($n=48$) were divided into 6 groups and immunized at 0, 21, and 42 d against phosphate buffer, 0.2 mg BSA, 0.2 mg key-hole limpet hemocyanin (KLH), 0.2 mg histone, 0.2 mg SS-KLH, and 0.2 mg SS-histone conjugates, then sacrificed at 56 d. Glutaraldehyde was used to conjugate SS-14 to the carrier proteins. In Exp. 2, SS-14 was conjugated to KLH using carbodiimide. 150 g female rats ($n=40$) were divided into 4 groups and immunized at 0, 14, and 28 d against phosphate buffer, 0.2 mg KLH, and 0.2 mg SS-KLH conjugate, then sacrificed at 44 d. Rats in one group were not immunized. Antibody titers were measured using ELISA with SS-BSA conjugate as an antigen. In Exp.1 antibody titer was detected in the KLH-SS group at up to 7 times dilution, but not detectable in the histone-SS group. No significant difference in body wt gain and organ wt was observed among the groups. In Exp2, antibody titer was detectable up to 2,000 times dilution in the serum of KLH-SS group. As in Exp 1, active immunization against SS did not affect body wt gain or organ wt. Competitive ELISA results demonstrated that the anti-SS antibody is specific to SS-14 but not to SS-28 or 3 other SS analogues tested. In conclusion, this study demonstrated that amount of anti-SS antibody production was affected by the carrier proteins, SS-carrier protein conjugation method; and active immunization against SS did not improve rat growth and body composition regardless of the extent of anti-SS antibody produced.

Key Words: Active Immunization, Somatostatin, Rat Growth

487 Effect of cysteamine hydrochloride on secretion of growth hormone (GH) in boars. K. V. McElwain^{1*}, M. J. Estienne¹, T. G. Hartssock², and C. R. Barb³, ¹*University of Maryland Eastern Shore, Princess Anne,* ²*University of Maryland, College Park,* ³*USDA/ARS, Athens, GA.*

Depending on the dose administered, cysteamine hydrochloride, an intermediate of cysteine metabolism, has been shown to either increase or decrease secretion of GH in sheep (McLeod et al., *Comp. Biochem. Physiol.* 112(3):523-533; 1995). The purpose of this study was to determine the effect of cysteamine hydrochloride on GH secretion in boars. Twelve Poland China \times Yorkshire boars, weighing 103.4 ± 3.0 kg and fitted with indwelling jugular vein catheters, were individually penned in an environmentally controlled room. Boars received i.v. injections of either 0, 25, 50, or 75 mg cysteamine hydrochloride/kg BW at h 0 ($n = 3/\text{treatment}$). Blood samples were collected every 15 min from h 0 to h 4. Serum concentrations of GH were determined by RIA. There was an effect of treatment ($P < .05$) on mean GH concentrations. Mean GH concentrations (ng/ml) were $1.97 \pm .46$, $2.24 \pm .59$, $.91 \pm .06$, and $.62 \pm .08$ for boars receiving 0, 25, 50, and 75 mg cysteamine hydrochloride/kg BW, respectively. The cysteamine hydrochloride-mean GH response had a linear ($P < .01$) component. Cysteamine hydrochloride at the 75 mg/kg BW dose decreased mean GH concentrations ($P < .05$) compared to the 0 and 25 mg/kg BW groups. The frequency and amplitude of GH pulses were unaffected ($P > .1$) by treatment. Overall, GH pulse amplitude was $2.35 \pm .58$ ng/ml and GH pulse frequency was $.75 \pm .07$ pulses/h. Results from this experiment indicate that cysteamine hydrochloride suppresses circulating GH concentrations in a dose dependent fashion in boars.

Key Words: Cysteamine Hydrochloride, Growth Hormone, Boars

488 Post-natal function of the somatotrophic axis in piglets born naturally or by Caesarian-Section. J. A. Carroll^{*1}, J. A. Daniel², J. A. Sterle², D. H. Keisler², and R. L. Matteri¹, ¹*Animal Physiology Research Unit, Agricultural Research Service, USDA,* ²*University of Missouri, Columbia.*

The objective of this study was to determine what effects the natural birth process would have on the development of the somatotrophic axis in the neonatal pig. Eight crossbred sows were selected for the study ($n=4$ each for natural birth and Caesarian-Section). Blood and tissue samples from 38 piglets were collected at birth to establish baseline values for various blood parameters and expression of specific mRNAs associated with the somatotrophic axis. All remaining piglets were sustained with the natural birth sows until 2 wk of age at which time 39 piglets were sacrificed for blood and tissue sample collection. Gestational age at birth did not differ ($P > .16$) between the natural birth and C-Section piglets ($113.6 \pm .14$ and $113.2 \pm .27$ days, respectively). Serum growth hormone (GH) did not differ ($P > 0.86$) between the two groups at birth, but was greater ($P < .038$) at 2 wk in the C-Section piglets as compared to the natural birth piglets (19.46 ± 2.63 and 12.44 ± 1.99 ng/ml, respectively). Serum insulin-like growth factor 1 (IGF-1) was greater ($P < .003$) in the natural birth piglets as compared to the C-Section piglets (28.77 ± 2.08 and 20.87 ± 1.4 ng/ml, respectively) at birth but did not differ at 2 wk of age. Pituitary content of GH mRNA and GH-releasing hormone receptor mRNA did not differ ($P > 0.14$) between the two groups; however, expression of both mRNAs declined ($P < .0002$) from birth until 2 wk of age. Liver expression of IGF-1 mRNA did not differ ($P > .80$) between natural birth and C-Section; however, there was an increase ($P < .0001$) in both groups from birth to 2 wk of age. Liver expression of GH receptor mRNA was greater in the C-Section piglets as compared to the natural birth piglets at birth ($P < .038$) and at 2 wk of age ($P = .006$). These data support our hypothesis that the natural birth process may significantly alter the post-natal function of the somatotrophic axis in the neonatal pig.

Key Words: Porcine, Growth Hormone, Birth

489 Neuropeptide Y stimulates release of growth hormone-releasing hormone and somatostatin from perfused bovine hypothalamic slices. C. D. McMahon, K. J. Lookinland*, and H. A. Tucker, *Michigan State University, East Lansing.*

Release of growth hormone (GH) from the anterior pituitary gland is regulated by two hypothalamic hormones: growth hormone-releasing hormone (GHRH) stimulates release, and somatostatin (SS) inhibits release. Hypothalamic neurotransmitters also stimulate or inhibit release of GH, but the role of one neurotransmitter, neuropeptide Y (NPY), is not well understood. We hypothesize that NPY stimulates release of GHRH. Fresh parasagittal slices (600 μm) of hypothalami were cut in oxygenated Hank's Balanced Salt Solution at 4 °C (calcium and magnesium-free). Slices were perfused at 37 °C with Minimal Essential Medium-alpha at a rate of 0.15 ml/min. Medium was gassed with a mixture of 95% O₂:5% CO₂ and collected at 20-min intervals. After perfusing with medium for 120 min, slices were perfused with either medium, or NPY at 10⁻¹⁰, 10⁻⁸ or 10⁻⁶ M for 20 min (n = twelve slices per treatment). Neuropeptide Y stimulated release of GHRH in a dose-dependent manner, which resulted in a greater net area under the GHRH curve at 10⁻⁶ M (14.1 ng·min·ml⁻¹) compared with controls (-0.3 ng·min·ml⁻¹) (pooled SEM = 3.5; P < 0.05). Similarly, NPY stimulated release of SS in a dose-dependent manner, which resulted in a greater net area under the SS curve at 10⁻⁶ M (10.6 ng·min·ml⁻¹) compared with controls (0.9 ng·min·ml⁻¹) (pooled SEM = 2.1; P < 0.01). Peak concentrations of GHRH occurred at 40 min, while peak concentrations of SS occurred 60 min after start of perfusing NPY. The data support our hypothesis that NPY stimulates release of GHRH, but there is a paradoxical release of SS. Because peak concentrations of SS occurred 20 min after peak concentrations of GHRH, we speculate that NPY initially stimulates GHRH neurons, and then GHRH, in turn, stimulates release of SS from terminals in the median eminence.

Key Words: Growth Hormone-Releasing Hormone, Somatostatin, Neuropeptide Y

490 Antimicrobial supplementation of pigs alters serum insulin-like growth factor-1 (IGF-1) concentrations within 3 days post-weaning. T. L. Henderson*, Z. Yi, W. R. Dayton, M. E. White, and M. R. Hathaway, *University of Minnesota, St. Paul.*

Recent studies aimed at increasing our understanding of antimicrobial-induced growth promotion have shown that adding subtherapeutic levels of antimicrobials to weanling pig diets for five weeks, significantly increases serum concentration of the potent mitogenic factor insulin-like growth factor-1 (IGF-1). This study was designed to assess the time course of this response. Sixteen pigs, 27 days old, were paired by litter, gender, weaning weight, and pre-weaning serum IGF-1 concentrations. Pigs were weaned on d 0 of the trial and received a corn-soybean meal based diet containing 20% crude protein. Pigs, within a pair, were randomly allotted to one of two treatment diets: 1) basal diet or 2) basal diet plus ASP-250. Serum samples were obtained twice/wk for 4 wk. Body weights were measured weekly. Serum IGF-1 concentrations decreased within 3 d of weaning for all pigs, however, the magnitude of decrease was less (P < 0.02) for pigs fed ASP-250 than for control pigs. Serum IGF-1 concentrations were 29% greater in ASP-250 supplemented pigs compared to control pigs (P < 0.001) for the entire study. Serum IGF-1 concentrations returned to pre-weaning levels by d 18 in pigs fed ASP-250, but not until d 26 in control pigs. Pigs fed ASP-250 gained weight 35% faster than control pigs over the 4 wk trial (P < 0.001). Differences in serum IGF-1 concentrations could be observed between control and ASP-250 supplemented pigs within 3 days of weaning.

Key Words: Antimicrobial, Pig, IGF-1

491 Antimicrobial-induced increases in serum insulin-like growth factor-1 (IGF-1) concentrations are not the result of increased voluntary feed intake. M. R. Hathaway*, W. R. Dayton, M. E. White, D. Young, and T. Doan, *University of Minnesota.*

Adding antimicrobials to the diets of ad libitum fed weanling pigs has been shown to increase serum insulin-like growth factor-1 (IGF-1) concentrations and increase voluntary feed intake. Since nutrition has a profound effect on serum levels of IGF-1, this study was designed to assess the effect of feed intake on antimicrobial-induced increase in serum IGF-1 levels in crossbred weanling pigs. Twenty-four pigs were allotted to groups of three by litter, gender, weaning weight, and pre-weaning serum IGF-1 concentrations. Pigs were weaned at 19 d to a corn-soybean meal based diet containing 20% crude protein. Pigs, within a group, were randomly allotted to one of three treatment diets: 1) basal diet fed ad libitum, 2) basal diet plus ASP-250, fed ad libitum or 3) basal diet plus ASP-250, limit-fed to 85% of the basal ad libitum consumption level. Feed consumption data was collected daily. Serum samples were obtained weekly. Body weights were measured weekly. Although the ASP-250 ad libitum fed pigs did not consume more feed than their control littermates, their serum IGF-1 concentrations were elevated by 22% (P < 0.01). Similarly, although the ASP-250 limit-fed pigs consumed 16% less feed than their control littermates, their serum IGF-1 concentrations were elevated by 15% (P < 0.01) but did not differ from those of their ASP-250, ad libitum fed littermates. Pigs fed ASP-250 ad libitum gained weight faster (P < 0.02) than control pigs over the 4 wk trial. Pigs fed ASP-250, either ad libitum or limit fed were 45% more efficient than control pigs in converting feed to gain (P < 0.05). Results of this study establish that an antimicrobial-induced increase in voluntary feed intake is not necessary to observe an antimicrobial-induced increase in serum IGF-1 concentrations.

Key Words: Antimicrobial, Pig, IGF-1

492 Intracerebroventricular injection of galanin, neuropeptide Y and somatostatin, alone or with GH secretagogue, L-692,585, in pigs. S.-J. Cho*¹, L. L. Anderson¹, C. H. Chang², L. A. McNamara², E. Frazier², H. Chen², and G. J. Hickey², ¹Iowa State University, Ames; ²Merck Research Laboratories, Rahway, NJ.

We investigated the effect of galanin (GAL), neuropeptide Y (NPY) and somatostatin (SRIH) with or without the nonpeptidyl GH secretagogue, L-692,585 (585), on GH secretion in the pig. In Yorkshire barrows (n=9, 40-45 kg BW) an intracerebroventricular (icv) stainless steel cannula was placed by stereotaxic coordinates; and an indwelling jugular vein (iv) cannula fitted for repeat blood sampling. SRIH (2 or 8 $\mu\text{g}/\text{kg}$ BW), GAL (4 $\mu\text{g}/\text{kg}$) or NPY (4 $\mu\text{g}/\text{kg}$) were administered icv (150 μl) alone or with 585 (30 $\mu\text{g}/\text{kg}$). Blood samples were collected and plasma GH levels monitored over a 3-h period. Average peak GH response (\pm SE, ng/ml) following icv administration of 2 (2.6 \pm .5, P>.05) or 8 $\mu\text{g}/\text{kg}$ SRIH (2.2 \pm .3, P<.05), respectively, was significantly decreased (P<.05) compared with saline alone (4.3 \pm .3). Peak GH response following 2 or 8 $\mu\text{g}/\text{kg}$ SRIH + 585 (30 \pm 5, 31 \pm 7, respectively) was significantly lower (P<.05) than 585 alone (53 \pm 9). Average peak GH response following icv administration of GAL (10 \pm 3) was significantly increased (P<.05) compared with saline control (3.8 \pm .2). GH response was similar (P>.05) following icv administration of GAL + 585 (41 \pm 5) or 585 alone (40 \pm 8). GH response following icv administration of NPY (3 \pm 1) was not different (P<.05) from that following saline injection. NPY + 585 increased GH response (24 \pm 3), however the magnitude of response was significantly (P<.05) less than GAL + 585 or 585 alone. These results suggest that endogenous GH secretion was affected by SRIH and GAL but not by NPY, and 585-stimulated GH response appeared to be modulated by all three neuropeptides. (National Pork Producers Council 96-3316)

Key Words: GH-Secretagogue, Neuropeptide Y, Galanin

493 Isolation of divergent 5'-untranslated regions of the bovine growth hormone receptor mRNA. H. Jiang* and M. C. Lucy, *University of Missouri-Columbia*.

Two variants (1A and 1B) containing different 5'-untranslated regions (5'-UTR) have been isolated for the bovine growth hormone (GH) receptor cDNA. These two variants result from the initiation of transcription by a liver-specific 1A promoter and a constitutive 1B promoter in a single GH receptor gene. In the human and the rat, 5'-UTRs in addition to those for 1A and 1B have been isolated. Therefore, there may be other 5'-UTRs for the bovine GH receptor mRNA generated by novel promoters and/or alternative splicing. As a first step in assessing this possibility, we used rapid amplification of cDNA ends (RACE) to isolate 5'-UTRs of the bovine GH receptor mRNA in liver and longissimus muscle. Amplified cDNA ends were cloned and sequenced. Besides 1B, seven new 5'-UTRs (designated bghrmv1 to bghrmv7) were obtained from bovine muscle. All eight 5'-UTRs shared the same region of exon 2 in the bovine GH receptor gene. Bghrmv1, bghrmv2, and bghrmv3 shared 59 bp identical upstream sequence from exon 2 but then diverged in their 5'-UTR. Furthermore, a 44 bp region (180 bp upstream from exon 2) in bghrmv3 was 100% identical to 1B. Bghrmv5 and bghrmv6 contained unique sequences spliced onto exon 2. Bghrmv7 was related to bghrmv6 because the bghrmv6 sequence (upstream from exon 2) was also found in the upstream region (235 bp from exon 2) of bghrmv7. The seven new 5'-UTRs isolated from bovine muscle were not found in bovine liver. In liver, two 5'-UTRs (differing at the 5' extensions for 1A) and one 5'-UTR for 1B were isolated. Except for the 1A and 1B variants, all other new bovine 5'-UTRs were not homologous to the 5'-UTRs of GH receptor mRNA for the human and the rat. These findings suggest that 5'-UTRs other than 1A and 1B are generated from alternative splicing and/or novel promoters. Furthermore, splicing of GH receptor mRNA may be tissue specific.

Key Words: Growth Hormone, Receptor, cDNA

494 Interaction of bovine somatotropin and rumen undegraded protein on growth rate in Holstein heifers. I. Bruckental¹, W. J. Painter², G. E. Dahl², and R. A. Erdman^{2*}, ¹*The Volcani Institute, Bet Dagan, Israel*, ²*The University of Maryland, College Park*.

Accelerated growth in heifers can be used as a means to reduce cost of raising replacement animals in dairy herds. The greatest potential for increased skeletal growth rate occurs during the prepubertal period. Bovine somatotropin (bST), if administered in conjunction with increased rumen undegraded protein (RUP) in the diet, may increase growth rate without fattening. The objectives of this experiment were to test the effect of RUP and the combination of bST and RUP on growth in dairy heifers. Seventeen Holstein heifers averaging 142 (\pm 31) days of age and 114 (\pm 17) kg body weight were used in a 241 day experiment. Treatments consisted of a control diet (C) containing 17.6% crude protein (CP), and 4.2% RUP, a high RUP diet (RUP) containing 17.6% CP, and 7.3% RUP and a group fed the RUP diet and injected every 14 days with bST (equivalent to 17.5 mg daily dose) (RUPbST). Body weight (291 kg) and wither height (113 cm) at puberty, and age (336 days) at puberty, as determined by serum progesterone were not affected by treatment ($P > .1$). Covariant adjusted final body weights were 321, 344, and 374 kg ($P < .001$) and final wither heights were 116, 118, and 120 cm ($P < .01$) for the C, RUP, and BST treatments, respectively. Growth curves for individual animals were generated for body weight and wither height. Average daily growth rates for weight gain were .90, .98, and 1.12 kg/day ($P < .07$) and wither height gain were .112, .122, and .128 cm/day ($P < .08$), for the C, RUP, and BST treatments, respectively. There were time by treatment interactions $P < .01$ for rates of body weight and wither height gains, where the response to both RUP and RUPbST was greatest early in the experiment while there was little response at the end. At any given body weight across treatments, wither heights were not different between treatments suggesting that increased body weight could be achieved without fattening using RUP or the combination of RUP and bST.

Key Words: Heifer Growth, Rumen Undegradable Protein, Somatotropin

495 Development and biological activity of epitope-tagged porcine IGF-I. C. L. Reichel*, A. L. Grant, C. A. Bidwell, and D. E. Gerrard, *Purdue University, W. Lafayette, IN*.

Development of an expression vector for production of porcine IGF-I that is biologically active and can be distinguished from normal endogenous porcine IGF-I would facilitate studies of IGF-I actions in porcine tissues. Construction of an IGF-I mature peptide with a T7 amino terminus tag and carboxy hexahistidine tag facilitates detection as well as purification. The objective of this study was to develop expression vectors for synthesis and secretion of biologically active epitope-tagged IGF-I. A prokaryotic expression vector was first constructed and characterized for its ability to direct synthesis of IGF-I. Porcine cDNA representing the mature peptide coding region was directionally cloned into pET24a(+) expression vector so that the T7 tag was at the amino terminus and the histidine tag was at the carboxy terminus. Tagged IGF-I (TIGF-I) was isolated and purified from *E. coli* BL21(DE3) cells using immobilized metal affinity and size exclusion chromatography. Immunoreactivity was determined by western blot analysis using T7 and IGF-I antibodies. Biological activity of purified TIGF-I was tested by measuring creatine kinase activity of cultured L6E9 and L6A1 muscle cells treated with TIGF-I. Purified TIGF-I increased ($P < .05$) creatine kinase activity at 100 and 10,000 ng/ml medium, relative to untreated cultures, similar to that observed with commercial recombinant human IGF-I and insulin. A eukaryotic expression vector, containing a constitutive promoter, the IGF-I signal peptide, TIGF-I, and carboxy peptide, was then constructed to synthesize and secrete TIGF-I. L6A1 muscle cells were stably transfected with the recombinant vector and LipofectAMINE. Cultured transfected cells exhibited creatine kinase levels greater ($P < .05$) than control cells. Transfected cells also resulted in greater creatine kinase activity than nontransfected L6A1 cells treated with TIGF-I. Results indicate that an epitope-tagged porcine IGF-I is immunologically and biologically active.

Key Words: IGF-I, Muscle, Pig

496 Somatotropin (bST) regulates leptin gene expression in growing cattle. C. P. Portocarrero*¹, K. L. Houseknecht¹, S. Ji², R. P. Lemenager¹, and M. E. Spurlock², ¹*Purdue University, West Lafayette, IN*, ²*Purina Mills, St. Louis, MO*.

Leptin is an adipocyte protein which regulates food intake and energy metabolism in rodents and man. Hormonal regulation of leptin expression in cattle is unknown. The specific aim of this study was to determine the effect of short term (3 d) bST treatment on leptin gene expression in adipose tissue of growing beef cattle. Angus cross-bred cattle ($n=12$) with initial body weight of 296.9 ± 27 kg were used; each animal served as its own control. Subcutaneous adipose tissue was collected surgically from the hip region for leptin mRNA analysis (RNase protection) following no treatment (control). Seven days later, each animal received injections of bST (200 μ g/kg BW*d; Protiva, St. Louis, MO) daily for 3 d. Approximately 15 h following the final injection, subcutaneous adipose tissue was obtained from the alternate hip (bST treatment). Induction of IGF-1 mRNA expression in adipose tissue was used as an indication of animal response to bST treatment (positive control). IGF-1 message in adipose tissue and average daily plasma growth hormone concentrations were significantly increased ($p < 0.01$) with bST treatment. Leptin mRNA abundance (corrected for 18 s mRNA) was increased with bST treatment compared to controls ($p < 0.001$). Plasma insulin was increased 2-fold with bST, with no effect on plasma cortisol. These data indicate that short-term administration of bST regulates leptin gene expression, prior to significant treatment effects on adiposity.

Key Words: Leptin, Growth Hormone, Adipocyte

497 Response to immune challenge in pigs selected for high lean gain: Role of leptin. M. T. Leininger*¹, C. P. Portocarrero¹, C. A. Bidwell¹, M. E. Spurlock², J. N. Nielsen¹, and K. L. Houseknecht¹, ¹Purdue University, West Lafayette, IN, ²Purina Mills, St. Louis, MO.

During periods of stress and sickness, food intake and growth are restricted in swine. This is especially prominent in pigs genetically selected for high lean gain. We are interested in identifying the factors that contribute to reduced growth rate, food intake, and survival rate during sickness and stress. Leptin, the adipocyte product of the *ob* gene regulates food intake in rodents. Cortisol increases expression of leptin in multiple species and also acts to repartition nutrients away from various tissues during times of stress and sickness. Eight high lean gain (HLG) genotype pigs and eight moderate lean gain (MLG) genotype pigs were raised via SEW procedures, and growth rates were recorded. At 90 kg BW, pigs were surgically fitted with jugular catheters for frequent blood sampling; each pig served as its own control. Pigs were injected with saline (control) or lipopolysaccharide (LPS, 0.025 mg/kg) and blood samples collected and body temperature measured hourly for 10 hours. Subsequently a subcutaneous adipose tissue biopsy was collected. HLG pigs had higher ADG than MLG pigs from 4 to 16 weeks of age (0.810 kg/d vs. 0.731 kg/d, $P < 0.006$). The LPS-induced peak body temperature (4 hr post injection) was greater for MLG vs. HLG ($P < 0.05$). Plasma cortisol tended to be higher in HLG vs. MLG pigs following LPS challenge. Basal leptin gene expression and leptin response to LPS challenge tended to be lower in HLG pigs vs. MLG, but differences were not significant ($P > 0.05$). Thus, alteration in leptin gene expression does not appear to play a role in the acute response to an inflammatory cytokine challenge in the pig.

Key Words: Leptin, Cytokine, Pig

498 The effect of leptin immunization on feed intake in finishing swine. A. J. Wuethrich*¹, D. L. Hancock¹, H. B. Long², J. Manetta², J. L. Roth¹, and D. B. Anderson¹, ¹Elanco Animal Health, Greenfield, IN and ²Lilly Research Labs, Indianapolis, IN.

Leptin is a protein produced by the adipose tissue, which has been shown to have effects on feed intake, weight gain, reproductive performance, and immune function in rodents. Since very little is known about the effects of peripheral administration of leptin in swine, a study was conducted to test the effect of leptin on feed intake in swine. The goal of this experiment was to immunize animals against endogenous leptin using murine leptin fragment 1–20 as the antigen. Heavier animals tend to have greater fat deposits and hence are expected to have a greater amount of endogenous leptin. Therefore, 30 heavyweight gilts (average of approximately 170 kg) were chosen to be used on this study in order to maximize the effect seen with the immunization. The gilts were fed *ad libitum* and were housed individually in 6' x 10' pens. Feed intake and animal weights were measured weekly. The treatments consisted of a sham injected control (Freunds complete and incomplete adjuvant), and two treatments of murine leptin fragment 1–20 leptin at 100 µg/animal. One group of gilts was immunized in the ear and the other group was immunized along the back, using murine leptin fragment 1–20 in Freunds complete adjuvant. Booster immunizations using murine leptin fragment 1–20 in Freunds incomplete adjuvant were given at 3 week intervals. No differences were observed in feed intake ($P > .24$) or animal weights ($P > .40$), when compared with controls in any immunization period. At 35 days after primary immunization, both non-control treatments showed a high titer against the murine leptin fragment antigen and native porcine leptin. In conclusion, antibodies were produced against porcine leptin, but did not enhance feed intake.

Key Words: Swine, Leptin, Immunization

499 Metabolic engineering with recombinant adenoviruses. C.B. Newgard*, P. Antinozzi, K. Trinh, H. Berman, R. Noel, R. H. Unger, and R. O'Doherty, *University of Texas Southwestern Medical Center and VA Medical Center, Dallas.*

A major goal of our group is to gain understanding of critical regulatory steps in metabolism through manipulation of gene expression. To this end, we have developed recombinant adenoviral vectors for *in vitro* and *in vivo* studies. These DNA viruses allow transfer of genes to primary cells in culture such as hepatocytes or pancreatic islets with 70-100% efficiency. They can also be used for efficacious transfer of genes to the liver of intact animals. Examples of the use of this technology for gaining insights into metabolic regulation and endocrine mechanisms include: 1) Expression of glycerol kinase in pancreatic islet β -cells activates glycerol metabolism and confers glycerol-stimulated insulin secretion. Glucose and glycerol are equally potent as insulin secretagogues in such cells, despite the fact that glycerol is poorly oxidized, suggesting that oxidation of carbohydrate fuels is not critical for stimulation of insulin secretion; 2) Overexpression of the catalytic subunit of glucose-6-phosphatase (G6Pase) curtails glycolytic flux and insulin secretion in β -cells, and reduces glucose utilization and storage in hepatocytes. Conversely, overexpression of glucokinase in hepatocytes stimulates glucose storage and usage. Delivery of the G6Pase gene to liver of normal rats causes glucose intolerance, hyperinsulinemia, and reductions in circulating free fatty acids. These data are consistent with a potential contributory role of dysregulated G6Pase expression in the etiology of type 2 diabetes, and with the idea that upregulation of glucokinase activity may be a means of reversing elevated hepatic glucose output in such patients; 3) Delivery of the leptin gene to liver of normal rats results in a 10-fold increase in circulating leptin levels, complete ablation of body fat, a fall in circulating triglyceride and FFA, and a sharp reduction in insulin levels that appears to be due to complete depletion of lipid stores in the pancreatic islets; 4) Specific blockade of the normal glucose-induced rise in malonyl CoA by expression of the enzyme malonyl CoA decarboxylase in β -cells reveals that this intermediate, which links glucose and lipid metabolism via its capacity to inhibit fatty acid oxidation, is not a primary signal for insulin secretion.

500 Inducible gene expression systems to assess protein function in vitro and in vivo. J. Huang, M. A. Beilstein, Y. H. Kim, Y. Xiao, and N. E. Forsberg*, *Oregon State University, Corvallis.*

Goals of our work have been to understand the functions of various proteolytic systems in skeletal muscle. To accomplish this, we adopted the LacSwitch inducible promoter system to assess the functions of calpains. L8 muscle cells were transfected with plasmids which allowed over-expression of either calpastatin inhibitory domain (CID) or dominant negative (DN) m-calpain. By over-expressing these plasmids we determined that calpains accounted for approximately 60% of total protein degradation in L8 myotubes and m-calpain accounted for roughly one-half of calpain-mediated degradation. Inhibition of calpains stabilized a variety of muscle proteins including fodrin, nebulin and poly-(ADP-ribose) polymerase (PARP). To assess the function of the proteases *in vivo*, we plan to develop transgenic mice in which we may specifically control activities of proteases in skeletal muscle. The ecdysone inducible promoter system has been modified with the use of a muscle-specific promoter (α -actin). This system will be used to specifically express a gene-of-interest inducibly in skeletal muscle of mice to assess protease function and to evaluate strategies to enhance skeletal muscle growth in domestic animals.

Key Words: Muscle, Gene Expression, Calpain

501 Expression of IGF-I in skeletal muscle of transgenic swine. V. G. Pursel^{1*}, G. Bee¹, K. D. Wells¹, A. D. Mitchell¹, T. Elsasser¹, R. J. Wall¹, M. B. Solomon¹, M. E. Coleman², and R. J. Schwartz³, ¹USDA-ARS, Beltsville, MD, ²GeneMedicine, Inc., The Woodlands, TX, ³Baylor College of Medicine, Houston, TX.

Although growth hormone is considered the primary growth-promoting hormone in mammals, many of its effects are thought to be mediated by insulin-like growth factor-I (IGF-I). The aim of this research was to determine whether directing expression of IGF-I specifically to striated muscle would enhance lean muscle growth in swine. Transgenic pigs were produced with a fusion gene composed of avian skeletal α -actin regulatory sequences and the cDNA encoding IGF-I. Founder transgenic pigs were mated to non-transgenic pigs to produce G1 transgenic and sibling control progeny. Pigs were weaned at 4 wk of age, fed a corn-soy diet at 110% of NRC requirements from 20 to 90 kg body weight and then provided feed ad libitum until they reached 120 kg body weight. At 120 kg body weight 20 transgenic and 22 control pigs were sacrificed to evaluate carcass composition. The IGF-I concentration of plasma collected at 60, 90, and 120 kg body weights were 11.7% higher in transgenic females and 9% higher in transgenic males than in control pigs ($P < .05$). This minor degree of IGF-I elevation was insufficient to alter the concentration of growth hormone in plasma. The transgenic pigs had larger loin eye areas than control pigs (38.6 vs. 32.6 cm², $P = .0001$). In contrast, the average backfat and P2 backfat measurements were lower in transgenic than control pigs (30.4 vs. 33.5 mm, $P = .02$, and 15.0 vs. 20.9 mm, $P = .001$, respectively). Organ weight for heart was higher for transgenic than control pigs (390 vs. 356 g, $P = .024$), but weights for kidney, liver, adrenal, lung and spleen did not differ for transgenic and control pigs ($P > .10$ for each). Neither average daily gain nor feed efficiency differed for transgenic and control pigs. Transgenic and control pigs did not differ in general appearance, and no gross abnormalities, pathologies, or health-related problems were encountered. Based on these results we conclude that enhancing IGF-I specifically in skeletal muscle had a positive effect on carcass composition of swine.

Key Words: IGF-I, Transgenic Swine, Carcass Composition

502 Future of Transgenic Technology for Animal Agriculture. G. E. Seidel, Jr., Colorado State University, Fort Collins.

Transgenic procedures are among the most powerful tools for studying biology. However, use of transgenic techniques for studying farm animal species has been rather limited; many of these experiments might best be described as exploratory or anecdotal. This is due partly to the low success rates and huge expense of such work, partly to inbred lines and long generation intervals, and partly to not testing appropriate hypotheses. For reasons of expense, concerns for safety, and inadequate understanding of basic biology, uses of transgenes in production animal agriculture are likely to be minimal for many years to come. However, a major use will be to produce valuable pharmaceuticals; use of animal tissues and organs for transplantation to people will not be far behind. These nonagricultural applications of transgenic farm animals will provide a windfall of information that will make applications of this technology to production agriculture more feasible, as will accumulation of information from the human genome project and similar endeavors. The ability to modify the genome of fetal fibroblasts in vitro and transplant these nuclei into oocytes to produce animals of the modified genotype will greatly increase the power of transgenic approaches in agricultural animals. Appropriately framed hypotheses plus newer transgenic approaches will provide important information that will be useful for both transgenic and nontransgenic applications to animal agriculture. A high priority is to continue the expeditious sharing of information that has made agricultural research so useful.

Key Words: Farm Animals, Transgene, Embryo

503 Activation state of muscle satellite cells isolated from steers implanted with a combined trenbolone acetate and estradiol implant. B. J. Johnson*, N. Halstead, M. E. White, M. R. Hathaway, A. DiCostanzo, and W. R. Dayton, University of Minnesota, St. Paul.

Muscle satellite cells were isolated from 7 yearling steers implanted for 31 d with a combined implant containing 120 mg of trenbolone acetate (TBA) and 24 mg of estradiol (E) and from 7 unimplanted control steers. Implanted steers had a 28% higher ADG and a 23% greater feed efficiency than did unimplanted control steers. Implanted steers had increased circulating insulin-like growth factor (IGF)-I concentrations on d 6, 14 and 31 after implantation ($P < .001$) while circulating IGF-I concentrations in control steers remained constant or decreased at these time points. ($P < .05$). Maximum fusion percentage was greater in satellite cell cultures isolated from implanted steers (ISC cultures) than in satellite cell cultures isolated from control steers (NSC cultures) (72.8% vs. 54.8%, $P < .005$). ISC cultures also contained a greater number of myotube nuclei than did NSC cultures (7998 nuclei/cm² vs. 5150 nuclei/cm², $P < .001$). After 72 h in culture, the number of cells (corrected for plating density) was 43% greater in ISC cultures than in NSC cultures ($P < .05$). ³H-thymidine incorporation rates/10⁵ cells at 24 and 34 h after plating were greater in ISC cultures than in NSC cultures ($P < .05$), however, incorporation rates did not differ at 72 h. These data suggest that TBA + E implantation may result in an in vivo activation of muscle satellite cell proliferation that can be detected in cell culture. This activation may play an important role in TBA + E-enhanced muscle growth.

Key Words: Satellite Cell, Muscle, Anabolic Steroid

504 Moderate maternal undernutrition alters glucose transporter levels in maternal insulin-responsive tissues and in the placenta. R. A. Ehrhardt*, R. M. Slepetic, and A. W. Bell, Cornell University, Ithaca, NY.

Increased insulin resistance in insulin-responsive maternal tissues and elevated placental glucose transport capacity are two major mechanisms that act to maintain fetal glucose supply during moderate maternal undernutrition. Objectives of the present study were to determine whether these mechanisms are mediated by nutrition-induced alterations in the relative abundance of facilitative glucose transporters (GLUTs) in the placenta and in maternal insulin-responsive tissues. Twin pregnant ewes were fed 60% (UF) or 100% (F) of predicted requirements for maintenance of zero energy balance in maternal tissues from day 122 to 135 post coitus (PC) ($n=5$ ewes/treatment). Maternal semitendinosus muscle, subcutaneous adipose tissue and perirenal adipose tissue, and placentomes from both placentae were collected immediately after slaughter on d 135 PC. GLUT protein expression was quantified in microsomal or solubilized membrane preparations from these tissues by Western blotting and/or by cytochalasin B equilibrium binding analysis. GLUT mRNA expression in the placenta was quantified by Northern blotting. GLUT4 protein level in perirenal adipose tissue was 58% lower ($P < 0.05$) in UF than in F ewes, but levels in subcutaneous adipose tissue were unaffected by nutrition. GLUT4 protein level in semitendinosus muscle was 33% lower ($P < 0.05$) in UF than in F ewes. GLUT1 protein level was not altered by nutrition in semitendinosus muscle; GLUT1 protein was undetectable in all adipose tissue samples. The concentration of D-glucose inhibitable cytochalasin B binding sites was elevated by 20% in UF versus F placentae ($P < 0.01$). GLUT1 protein abundance in the placenta was not altered by nutrition whereas GLUT3 protein abundance was 20% greater in UF than in F ewes ($P < 0.01$). Placental abundance of GLUT1 mRNA and GLUT3 mRNA were not affected by nutrition. These results suggest that decreased GLUT4 protein abundance in maternal skeletal muscle and perirenal adipose tissue contributes to the exaggeration of pregnancy-related insulin resistance observed during moderate maternal undernutrition. Also, increased placental GLUT3 protein abundance partly explains the elevated placental glucose transport capacity observed in vivo during moderate maternal undernutrition.

Key Words: Glucose Transporters, Undernutrition, Pregnancy

505 Accretion of amino acids in the gravid uterus during late pregnancy in Holstein cows. A. W. Bell, W. S. Burhans*, R. M. Slepetic, and D. A. Ross, *Cornell University, Ithaca, NY.*

Our objective was to determine rates of accretion of individual amino acids in the conceptus tissues of Holstein cows during late pregnancy, as a first step towards definition of amino acid requirements for conceptus growth. Multiparous, monotocous cows (n=6) were slaughtered at 190, 193, 229, 233, 268 and 270 d of pregnancy, respectively. Fetus, fetal fluids, fetal membranes, placentomes, and uterine tissues were separately weighed, ground, stored at 20°C, and freeze-dried before chemical analysis. Dried samples were defatted and hydrolyzed with HCl (6 mol/L); amino acid composition of hydrolysates was measured by HPLC (Beckman 334) with a two-buffer elution system on an ion-exchange column, post-column derivatization with o-phthalaldehyde, and norleucine (150 µmol/L) as internal standard. Sulfur amino acids were separately analyzed after preoxidation of hydrolysate with formic acid. Essential (EAA) and nonessential (NEAA) amino acid concentrations (g/100 g CP) of aggregated tissues in the gravid uterus, calculated as weighted means, are tabulated as means for 2 animals each at approximately 190, 230, and 270 d of pregnancy.

	Day of Pregnancy				Day of Pregnancy		
	190	230	270		190	230	270
EAA	0.8	1.4	1.7	ASP	7.3	6.9	6.8
MET	3.7	6.1	7.4	SER	5.0	4.3	4.1
LYS	1.1	2.1	2.6	GLU	12.4	12.1	12.1
PHE	3.1	3.3	3.5	GLY	13.2	11.6	10.8
THR	2.4	2.4	2.6	ALA	7.4	6.9	6.6
LEU	5.3	6.3	6.8	CYS	0.0	2.6	4.0
ILEU	1.1	2.6	3.3	TYR	2.4	2.6	2.6
VAL	1.9	3.6	4.4	OH-LYS	0.6	0.6	0.5
ARG	5.8	7.1	7.9	ORN	0.1	0.1	0.2

The sum of calculated accretion rates (g/d) of the above 18 individual amino acids accounted for 74%, 83%, and 88% of total CP accretion rates (62, 90, and 117 g/d) at d 190, 230, and 270 of pregnancy, respectively. These data represent the first estimates of net requirements of individual amino acids for conceptus growth in late-pregnant cows. However, they do not take account of the extensive catabolism of amino acids in conceptus tissues and its impact on disposal of metabolizable amino acid supply.

Key Words: Amino Acids, Gravid Uterus, Cow

506 Effects of initial body condition, frame size and concentration of dietary energy on increase of fat cover and ribeye area of finishing steers. A. Trenkle*, *Iowa State University, Ames.*

To study the effects of initial body condition, frame size and dietary energy on growth of Longissimus dorsi muscle area and rate of accumulation of fat cover of finishing cattle, eighty crossbred 14 month-old steers with an average weight of 400 ± 17.4 kg were sorted into two groups based on height at hips (125.6 and 132.3 cm) and these groups subdivided based on measurements with ultrasound into groups with more and less initial fat cover (.19 and .43 cm). Each of the four groups was fed corn-based finishing diets containing 1.3 and 1.4 Mcal NE/g/kg for 103 days. Steers were scanned ultrasonically between the 12th and 13th ribs before and at the end of the trial and at about 4-wk intervals. Overall means ± SEM were 1.82 ± .04 kg/d, 10.8 ± .14 kg/d, 5.95 ± .09 kg, 354 ± 3.2 kg, 87.1 ± 1.3 cm², and .95 ± .05 cm for ADG, feed/d, feed/gain, carcass weight, REA and fat cover, respectively. Shorter steers gained faster and had greater carcass fat cover (P<.05). Steers with less initial fat cover had lower initial weight, were more efficient and had less carcass fat cover (P<.05). Steers fed the lower energy diet consumed more feed and were less efficient (P<.05). Accumulation of subcutaneous fat fit an exponential growth curve: fat thickness, cm = ae^{kt}, and growth of REA fit a linear curve: REA, cm² = a + kt; where a = initial measurement, k = rate constant and t = days from first measurement. For fat cover; a = .209 & .437, and .293 & .327 and k = .0136 & .0093 and .0138 & .0098 for low & high initial fat and for short & tall hip height (r ranged from .76 to .89). For REA; a = 57.29 & 60.77 and 57.74 & 60.32, and k = .2839 & .2516 and .2645 & .2645 for the respective groups (r ranged from .82 to .89). None of the main effects had any effect on growth of REA. Shorter steers and steers with less initial fat accumulated fat at a faster rate. Concentration of dietary energy had no effect on these parameters. The results of this study indicate that accumulation of fat cover and increase in REA during the finishing period can be predicted from initial ultrasound measurements of incoming feeder cattle.

Key Words: Cattle, Muscle, Adipose

507 Unbiased estimation of the slope of unknown functions. N. St-Pierre*, *The Ohio State University, Columbus, OH.*

Frequently, measurements are taken for which the interest is in their rates of change. For example, a series of body weight measurements are taken and growth rates are estimated from the difference between consecutive weights divided by the amount of time between the two measurements. Alternatively, a polynomial curve of first or higher order is used to estimate the true unknown function. The first derivative of the polynomial function gives an estimate of the instantaneous growth rate. We have shown that both procedures yield biased estimates of growth rates. The objectives of this research was (1) to derive potential unbiased estimators of the slope of unknown growth functions, and (2) to compare the results of the proposed method with those obtained by fitting a polynomial function. Cubic spline functions are fitted to weight data. Numerical derivatives are estimated for each time points and give estimates of instantaneous growth rates. Three types of cubic splines were investigated in a Monte Carlo simulation: cubic Hermite (H), Akima (A) and the smoothing spline of Schoenberg (S). Data were generated from a complex multiphasic time-series model of mouse growth, based on the following design: (1) structure of the independent (time) variable (uniform vs. random), (2) location of estimate (at mean time, mean time ± 1 s.d., mean time ± 2 s.d.), (3) experimental error (coefficient of variation = 1, 5, and 25%), (4) number of measurements (5, 20, and 80 points), (5) range of time points (10, 50 and 90% of maximum), and (6) error structure (normal, or with 5% outliers). Results confirmed the analytical proof that current procedures yield biased estimates. The H, A, and S procedures did not always result in unbiased estimates. The magnitude of the bias was much lower when measurements were done uniformly through time. The smoothed spline had lower estimation variance and bias than other spline methods in all cases. Bias and estimation variance increased with experimental errors (P<.001), and the presence of outliers (P<.001). Cubic spline methods can yield better estimates of growth rates but are not free of bias.

Key Words: Growth Rate, Estimation

508 Dietary conjugated linoleic acid decreases back fat in finisher gilts. F. R. Dunshea*¹, E. Ostrowska², M. Muralitharan³, R. Cross², D. E. Bauman⁴, M. W. Pariza⁵, and C. Skarie⁶, ¹*Victorian Institute of Animal Science, Werribee*, ²*Swinburne University of Technology, Hawthorn*, ³*Charles Sturt University, Wagga Wagga (Australia)*, ⁴*Cornell University, Ithaca NY*, ⁵*University of Wisconsin, Madison WI*, ⁶*ConLinCo Inc., Detroit Lakes MN (USA)*.

Conjugated linoleic acid (CLA) has been shown to decrease body fat content of rodents. One constraint facing the pig industry is that *ad libitum* feeding can often result in high levels of body fat. The aim of this study was to determine whether dietary CLA supplementation can decrease body fat content in pigs. Thirty Large White x Landrace gilts (initial weight and P₂ backfat, 56.0 kg and 11.3 mm, respectively) were allocated to one of 6 levels (0, 1.25, 2.5, 5.0, 7.5 and 10.0 g/kg) of dietary CLA. The wheat-based diet was formulated to contain 3.42 Mcal DE, 177 g CP and 9.3 g available lysine per kg. The six levels of CLA were obtained by substituting soya oil with a proprietary CLA isomer mix (CLA 55, Natural Lipids, Norway). Pigs were kept in individual pens and had *ad libitum* access to water and their respective diet for 8 weeks. Dietary CLA had no effect (P=.471) on feed intake (2.61 vs 2.71 kg/d for pigs fed diets without and with CLA, respectively) whereas average daily gain (812 vs 886 g/d, P=.143) and feed conversion efficiency (.304 vs .319, P=.114) tended to be increased. Consequently, final weight also tended to be increased by CLA supplementation (100.2 vs 104.8 kg, P=.094). Dietary CLA reduced (P=.024) P₂ backfat at all levels of supplementation (21.0, 17.1, 16.1, 16.9, 15.4 and 14.6 mm for gilts fed diets containing 0, 1.25, 2.5, 5.0, 7.5 and 10.0 g/kg CLA, respectively). Carcasses from gilts fed diets without CLA supplementation had a lower water content than those from gilts supplemented with CLA (487 vs 516 g/kg, P=.029). These data suggest that dietary CLA supplementation can decrease carcass fat content and may improve growth performance without altering feed intake in finisher gilts.

Key Words: Pig, Conjugated Linoleic Acid, Body Composition

509 Blood plasma concentrations and salivary and urinary excretion of nitrite/nitrate in calves: dependency on age and nutritional effects. J. W. Blum*, B. Hüslér, C. Morel, H. Hammon, and R. M. Bruckmaier, *Univ. of Berne, Switzerland.*

Nitrate (NO₃) or nitrite (NO₂) are ingested/absorbed or endogenously produced from nitric oxide (NO). We have measured NO₂/NO₃ (NO_x) in blood plasma, saliva and urine in milk-fed calves from birth until slaughter, in calves suckling up to 3 mo old, in calves weaned if 4 mo old and in the 1st wk of life in calves fed differently (lacking or delayed colostrum feeding, feeding different amounts of colostrum), and treated with growth hormone (GH) or Long-R³-IGF-I, in heifers and cows in blood plasma, urine and in colostrum and milk. NO_x consisted primarily of NO₃. Plasma NO_x was high (300–700 μmol/L) at birth (before 1st feeding), decreased within 4–7 d to 50–120 μmol/L, remained > 50 μmol/L in male veal calves and in age-matched female calves. NO_x further decreased in heifers up to 2 y to ≤ 3 μmol/L and was no longer measurable in 3–8 y old cows, although they ingested some NO₃ with water (≤ 450 μmol/L). Salivary NO_x decreased within 3 d postnatally from 300–600 to 150–160 μmol/L and remained measurable in veal calves up to slaughter. Urinary NO_x excretion in 5 d old calves was high [400–500 μmol/L, 16–17 μmol/(kg × 24 h)], and was lower on d 10, 60 and 115 of life [6–11 μmol/(kg × 24 h)]. NO_x was not detectable in urine in heifers and 3–8 y old cows. Feeding calves with milk, to which 200 μmol NaNO₃/kg^{.75} were added, caused a rapid rise of NO₃ (but not NO₂) in plasma, saliva and urine, 400 μmol NaNO₃/kg^{.75} raised salivary NO₂ excretion and 200 μmol NaNO₂/kg^{.75} led to a marked rise of plasma NO₃ (but not NO₂) in plasma and urine. NO_x was not measurable in milk of healthy or subclinically infected udders. In conclusion, different feeding of neonatal calves and administered hormones had no effects on NO_x in plasma, saliva and urine. High NO_x levels in neonatal calves and their presence in saliva and urine in veal calves and suckling calves, which ingested no NO₃ or NO₂ with milk, indicate marked endogenous production of NO₃ which decreased with increasing age.

Key Words: Cattle, Nitrate, Nitrite

510 Influence of environmental temperature on maturation of skeletal muscle during the early postnatal period in pig. G. Lossec, P. Ecolan, P. Herpin, and L. Lefaucheur*, *INRA, Pig Research Institute, Saint-Gilles (France).*

Early after birth, piglets rely almost exclusively on muscular shivering thermogenesis to produce heat in the cold and this can possibly modulate skeletal muscle development. An experiment involving 10 individually housed Large White piglets was conducted to determine the influence of cold (24–15°C, C group) versus thermonutrality (34–30°C, TN group) between birth and 5 days of age on myosin heavy chain (MHC) polymorphism and metabolic characteristics of *longissimus* (L), *rhomboideus* (RH) and red portion of *semitendinosus* (ST) muscles. Piglets were fed artificial milk every two hours, and those exposed to cold received 43% more feed on a liveweight basis in order to achieve similar growth rate. C piglets produced 93% more heat and exhibited intense shivering during the whole experiment. Using an immunocytochemical approach, a panel of anti-MHC monoclonal antibodies (mAb) was used on serial muscle cross sections. Cold exposure increased the proportion of secondary fibers reacting with slow type I MHC mAb in L (17.8 vs 5.4%), ST (57.1 vs 27.8%) and RH (75.0 vs 55.1%) muscles, whereas all primary fibers were labeled in both groups. Proportion of both primary and secondary fibers reacting with a specific α-cardiac MHC mAb dramatically increased in the 3 muscles of C animals (100 vs 32.6 and 21.8 vs 6.2% in LD; 72.1 vs 13.4 and 69.4 vs 35.1% in ST; 25.9 vs 2.2 and 75.0 vs 32.4% in RH, respectively). To a lower extent, cold also decreased the proportion of primary and secondary fibers containing fetal MHC, denoting an accelerated postnatal maturation of muscle during cold exposure. Cold increased oxidative enzyme activities as measured by citrate synthase in L (+14%), ST (+12%) and RH (+15%) muscles, whereas the activity of lactate dehydrogenase, a glycolytic enzyme, was unchanged. Cold exposure also induced an increase in plasma levels of T3 (+30%). To what extent these changes are the result of sustained shivering or are due to the action of hormonal factors such as thyroid hormones remains to be determined.

Key Words: Skeletal Muscle, Cold Stress, Piglets

511 In vitro growth of muscle satellite cells derived from mouse lines long-term selected for different growth traits. K. Walther*, C. Rehfeldt, E. Albrecht, G. Nuernberg, and U. Renne, *Research Institute for Biology of Farm Animals, Dummerstorf (Germany).*

Animal models that vary largely in growth rate, muscle accretion, and body composition are particularly suitable to elucidate regulatory mechanisms of skeletal muscle growth. The objective of this study was to investigate the influence of long-term selection for different growth traits on *in vitro* proliferation and differentiation of satellite cells and their response to various growth factors. Primary muscle cell cultures were derived from three lines of mice selected for body weight (Du-6), protein content (Du-6P), and an index combining body weight and endurance fitness (Du-6+LB) on day 42 over 70 generations, and a control line (Du-Ks).

During 14 days of cultivation in DMEM supplemented with 8% FBS the Du-6+LB and Du-6P muscle cells synthesized more (P<.1) DNA and protein than the cells of Du-6 and Du-Ks. Initially, DNA synthesis and protein accumulation of Du-6 cells were higher too, but after 4 days of growth declined to the level of Du-Ks. To characterize differentiation the activity of creatine kinase (CK) and the extent of cell fusion were determined. After changing from 8% to 1% FBS at day 6 of cultivation CK activity tended to increase until day 8 in Du-6P and Du-6, whereas CK activity remained almost unchanged in Du-6+LB and Du-Ks, respectively. This was consistent with changes in fusion kinetics measured by image analysis. To study the extent that growth factors stimulate DNA synthesis muscle cells were treated with insulin (10 μM, 1nM), IGF-I (1μM, 10 μM), and EGF (1μM, 10 μM) and [³H] thymidine incorporation was quantified. The response of DNA synthesis rate to growth factors differed (P<.1) between lines and was growth factor-dependent. EGF stimulated (P<.01) DNA synthesis rate in all cell lines. It caused higher effects in Du-6P, Du-6, and Du-6+LB cells as compared to Du-Ks. In response to insulin and IGF-I DNA synthesis rate tended to be increased (P>.1), but not significantly, in all lines. A dose-dependent effect was not observed.

These data demonstrate that long-term growth selection induces differences in satellite cell growth kinetics and their sensitivity to growth factors.

Key Words: Cell Culture, Muscle, Growth Factor

512 Feeding plasma protein does not affect growth parameters in young pigs when controlled for food-intake. C. J. Dyer¹, K. J. Touchette², J. A. Carroll¹, G. L. Allee², and R. L. Mattern¹, ¹*Animal Physiology Research Unit, Agricultural Research Service, USDA*, ²*Dept. Animal Science, University of Missouri, Columbia.*

The addition of spray-dried plasma protein (PP) to early growing pig diets results in increased food intake and faster growth. It is unknown if the increased growth is due mainly to increased feed intake or is mediated through other physiological mechanisms affected by PP. At 14 d of age, 26 barrows were fitted with gastric cannuli and randomly assigned within a 2×3 factorial experimental design, including treatments of PP or no PP and feeding levels of 80%, 100%, or 120% of normal feed intake. Pigs were fed through gastric cannuli every two hr from 10 a.m. to 10 p.m. for 11 d, then sacrificed for tissue collection. Blood samples were drawn on d 2,5,8, and 11, and body weight (BW) was recorded daily. RNA expression was quantified by hybridization assays and serum IGF-1 concentrations were measured by radioimmunoassay. Data were analyzed by two-way ANOVA (with repeated measures where appropriate). An effect of feeding level was found in BW through time (p=.037), d 11 BW (p=.003), and ADG (p<.001). All of these endpoints were lower in the 80% feed intake groups than in the 100% or 120% feed intake groups. The slopes of the growth curves also differed by level (80%<100%<120%, p<.001). No effect of plasma on weight gain was observed. Leptin, adipose IGF-1 and liver IGF-1 RNA expression did not differ between level or plasma. Serum IGF-1 concentrations were correlated with ADG across treatments (r=.579, p=.002), as was adipose IGF-1 RNA expression with ADG (r=.482, p=.015). Leptin RNA expression was correlated with ADG only at the 100% feeding level (r=.677, p=.045). The only endpoint measured which showed significant differences due to PP was hypothalamic IGF-1 RNA expression (decreased in PP-treated pigs, p=.032). These data suggest that, when feed intake is controlled, plasma protein does not affect growth or peripheral endocrine parameters in young pigs.

Key Words: Pigs, Growth, Nutrition

513 Serum hormone concentrations using anabolic agents in grazing young bulls. O. E. Morón-Fuenmayor*¹, J. A. Aranguren², and S. Pietrosevoli¹, ¹Facultad de Agronomía-LUZ, ²FCV-LUZ, (Venezuela).

The objective of the study was to determine the effect of the implant continuous on serum hormone concentrations in young bulls under tropical conditions. Twenty young bulls with initial weight of 169.42±16.17 kg and 15 mo. of age were assigned randomly to treatments: no-implanted (NI), zeranol implanted (ZI), ATB+17β-estradiol implanted (ATBI), and the combination zeranol plus ATB+17β-estradiol (ZATBI). Reimplanting were made every 60 d. for zeranol and 120 d. for ATB+17β-estradiol. The trial lasted 472 d. Blood samples were taken before, 1 and 30 days after implanting, three times a day. Analysis of variance was performed using GLM procedure to evaluate hormonal concentrations of Testosterone (T), Cortisol (C), Insulin (I), Triiodothyronine (T₃) and Thyroxine (T₄). There were not differences (P>0.05) for I and T₄. C tended to be higher in NI and ZI than ATBI and ZATBI (8.94, 7.96 vs. 5.92, 5.66 ng/ml). The T concentrations were lowest for ZI, ATBI and ZATBI (P<0.05) than NI (0.20, 0.23, 0.10 VS. 1.58 ng/ml), respectively. However, T₃ was higher (P<0.05) for ZI than ATBI (0.98 vs. 0.78 ng/ml) respectively. In conclusion, the continuous use of implant affects the concentration of T₃, Testosterone and Cortisol.

Key Words: Implant, Hormone Concentrations, Serum

514 Continuous use of anabolic agents and breed types on growth and carcass characteristics in grazing young bulls. O. E. Morón-Fuenmayor¹, J. A. Aranguren², and S. Pietrosevoli*¹, ¹Facultad de Agronomía-LUZ, ²FCV-LUZ (Venezuela).

The objective of the study was to determine the effect that the continuous use of anabolic agents and breed type had on the growth, and the carcass characteristics of young bulls under tropical conditions. Fifty young bulls with initial weight of 169 kg and about 15 mo. of age were grouped according to their breed type in predominance *Bos indicus* (n=18) and predominance *Bos taurus* (n=32) and randomly assigned to the treatments: no-implanted (T1), zeranol implanted (T2), ATB+17β-estradiol implanted (T3), and the combination zeranol plus ATB+17β-estradiol (T4). The trial lasted 472 d. Analysis of variance-covariance was done and the initial age was used as a covariable. The variables evaluated were: daily weight gain (DWG), thoracic circumference (TC), scrotal circumference (SC), dressing percentage (DP), ribeye area (AOL12), marbling score, cover fat, muscularity, and final maturity score (FMS). Anabolic and breed types did not have influence on (P>0.05) DGW, TC, DP, AOL12, marbling, cover fat, muscularity, FMS and CLASIF. There were SC increment differences (P<0.01) between the groups (10.56 vs. 2.21, 3.58, 2.43 cm) for T1 T2, T3 and T4 respectively. T1x*Bos indicus* and T1x*Bos taurus* had bigger SC (11.96 and 9.18 cm) than the implanted groups. In conclusion, the continuous use of anabolic implants in young bulls did not affected the growth and the carcass characteristics; however, they reduced markedly the scrotal circumference.

Key Words: Anabolic, Breed Type, Carcass

515 Effects of oviductal epithelial cells from various sources on bovine embryo development *in vitro*. S. Wang¹, R. G. Holyoak¹, T. J. Bunch*², and T. D. Bunch¹, ¹Utah State University, Logan, ²University of Utah School of Medicine, Salt Lake City.

The effects of oviductal epithelial cells (OVEP) collected from ampulla and isthmus regions and during follicular and luteal phases of the estrous cycle on bovine embryo development *in vitro* were evaluated using a complete randomized block design with 4 treatments in seven blocks. Epithelial cells were recovered by flushing the oviductal lumen and cultured in TCM-199 containing 10% fetal bovine serum. Oocytes (n = 3507) were aspirated from abattoir ovaries and subjected to *in vitro* maturation (IVM) and fertilization (IVF). IVM/IVF ova were cultured (treatment, TRT) with OVEP collected from: TRT1- ampullar region at follicular phase; TRT2- ampullar region at luteal phase; TRT3- isthmus region at follicular phase; and TRT4- isthmus region at luteal phase. Cleavage rate was determined at 45 h after IVF and embryo development was evaluated on Days 6, 8 and 10 of culture (IVF = Day 0). Data were angularly transformed and analyzed by ANOVA. A set of orthogonal polynomial contrasts was used to compare the effects between ampulla and isthmus OVEP and between follicular and luteal phase OVEP. Ova cocultured with ampulla OVEP had a higher cleavage rate (75.3 vs. 68.7%, P < .01), less ova that stopped development before the 8-cell stage (35.8 vs. 43.6%, P < .05), and more blastocysts at Day 8 (20.6 vs. 16.8%, P < .05) than with isthmus OVEP. There was no difference (P > .05) between ampullar and isthmus OVEP in the percentages of morulae at Day 6 (49.6 vs. 45.5), and expanded or hatched blastocysts at Day 10 (11.2 vs. 8.9). There was no difference (P > .05) between OVEP collected during the follicular or luteal phases in oocyte cleavage and post-cleavage development. These results suggest that ampullar OVEP promotes oocyte cleavage and embryo development beyond the 8-cell stage compared with isthmus OVEP. OVEP from either follicular or luteal phases had similar effects on promoting embryo development *in vitro*.

Key Words: Oviductal Epithelial Cells, Coculture, Bovine

516 The effects of β-mercaptoethanol on bovine embryo development *in vitro*. S. Wang¹, R. G. Holyoak¹, T. J. Bunch², and T. D. Bunch*¹, ¹Utah State University, Logan, ²University of Utah School of Medicine, Salt Lake City.

β-mercaptoethanol (2-ME) is a low molecular thiol compound that is often used to promote growth of mammalian cells *in vitro*. This study investigated the effects of 2-ME on the *in vitro* development of bovine preimplantation embryos using a randomized complete block design with 2 treatments in 11 blocks. Oocytes (n = 2004) were aspirated from abattoir ovaries and procedures for *in vitro* maturation (IVM), *in vitro* fertilization (IVF), and *in vitro* culture (IVC) followed Bavister *et al.* (Theriogenology 37: 127-146, 1992). Treatments (TRT) consisted of 100 μM 2-ME (M7522, Sigma) per ml IVC medium and no 2-ME in IVC medium (Control). Cleavage rates were determined at 48 h after fertilization. Embryonic development was evaluated on Days 6, 8 and 10 of culture (IVF = Day 0). The percentage data were angularly transformed and analyzed by ANOVA. The percentage of cleaved ova was 81.3 (825/1015) and 83.4 (826/989), the percentage of morula production at Day 6 was 52.2 and 55.8, and the percentage of blastocyst production at Day 8 was 14.0 and 15.0 for TRT and Control, respectively. There was no difference (P > .05) in cleavage rates or morula and blastocyst production between treatments. However, more embryos (P < .01) cultured in IVC medium containing 2-ME developed to the expanded blastocyst stage at Day 8 (6.3% vs. 3.4%) than the Control. At Day 10, the percentages of expanded blastocysts were 13.1 and 7.5, and hatching or hatched blastocysts were 9.1 and 2.1 for TRT and Control, respectively. The differences were highly significant (P < .01). We conclude that the involvement of 2-ME in IVC medium improves the development of IVM/IVF-derived bovine embryos to the blastocyst stage, and especially to expanded and hatched blastocyst stages of development.

Key Words: Bovine, In Vitro Culture, β-mercaptoethanol

517 Measurement of muscle protein synthesis may be different depending on choice of isotope used. T. J. Wester*¹, G. E. Lobley², L. M. Birnie¹, and M. A. Lomax¹, ¹The University of Aberdeen, ²The Rowett Research Institute, Bucksburn, Scotland.

Incorporation of isotopically labeled amino acid into protein has been used extensively to measure muscle protein synthesis. Use of different isotopes can enable repeated measurements without having to account for increased background isotope in existing proteins. We examined the feasibility of using four isotopes of Phe to measure protein synthesis in two muscles. Six 30 kg fasted lambs were infused continuously for 6 h with different isotopes of Phe in each of four periods. Blood was sampled serially during each infusion, after which longissimus (LD) and vastus lateralis (VL) muscles were biopsied. Order of isotope infused was constant, as follows: L-[1-¹³C]Phe (¹³C), L-phenyl-d₅-alanine (d₅), L-[¹⁵N]Phe (¹⁵N), and L-[2,6-³H]Phe (³H). Muscle was homogenized in sulfosalicylic acid and free-pool Phe was separated from precipitated protein-bound pool Phe by centrifugation. The area × time curve of isotopic availability was also determined from plasma free Phe isotopic activity (IA). Free-pool Phe IA of ¹³C, d₅, and ¹⁵N was determined using gas chromatography-mass spectrometry (GC-MS). Protein-bound pool Phe IA was determined by GC-MS for d₅ and isotope ratio-MS for ¹³C and ¹⁵N. The IA of protein-bound and free-pool ³H was measured by fluorimetry and liquid scintillation counting after enzymatic conversion to β-phenethylamine. Calculated rate of fractional protein synthesis (Ks) differed with the isotope used where values from ¹³C=>¹⁵N>d₅=³H (P<.001) when plasma-free Phe was used as the precursor pool and ¹³C>¹⁵N=d₅=³H (P<.01) with muscle free-pool Phe. When the ratio tissue free-pool:plasma free-pool was examined, the value for ³H was lower than others (P<.001). Differences existed for Ks between muscles (VL about 60% faster than LD, P<.001), but the ratio tissue free-pool:plasma free-pool was similar. Differences in Ks and free pool ratios among isotopes probably reflect the different techniques used to measure IA and are not due to actual biological differences.

Key Words: Stable Isotopes, Precursor Pool

518 Growth curves, feed intake, water intake, organ weights, and heat production of mice selected for high or low heat loss. P. S. Miller*, M. K. Nielsen, and H.-Y. Chen, *University of Nebraska, Lincoln.*

A long-term growth experiment was conducted using mice selected for high (MH) or low (ML) heat loss (16 generations of selection). In addition, a control line (MC) that was randomly selected from the same base population was evaluated. Sixty mice (30 females and 30 males) from each line with an initial weight = 15 g were used. All mice were allowed ad libitum access to a commercial diet (CP = 24.5%) and water throughout the 18-wk experimental period. Mice were weighed and feed and water disappearance were measured weekly. During the last week of the experiment, heat loss was measured for each mouse using direct calorimetry. At wk 18, all mice were killed and liver and spleen weights were recorded. Evaluation of nonlinear growth curves (Weight = $W_{t_{max}} / (1 + (K_t / wk))$) indicated minimal differences in the parameters among lines. Males had greater (P < .0001) mature body weight as indicated by $W_{t_{max}}$ (males = 41.1 g, females = 31.4 g). Females matured at a faster (P < .001) rate than males (females K_t = 1.29 wk, males K_t = 1.74 wk). The response of cumulative feed intake vs wk of study was greater in male vs female mice. Also, the rate of cumulative feed intake was greater (P < .001) in MH vs ML mice. The rate of cumulative water intake was not different (P > .2) among lines or between males and females. The MH mice lost 48% more (P < .0003) heat than ML mice (MH > MC > ML; 178, 145, and 120 kcal/BW^{.75}). Female mice lost more (P < .0001) heat than males (154 vs 141 kcal/BW^{.75}). Liver weight was not different (P > .10) among lines or between sexes. Spleen weight was 26% greater (P < .0001) in females vs males and 24% greater (P < .02) in MH vs ML mice. Although these lines of mice differ significantly in feed intake and heat loss, the lines do not differ in growth parameters. Male mice differed from females in growth, feed intake, heat loss, and spleen weight.

Key Words: Mice, Growth Rate, Genetic Models

519 Effect of Posilac® and Revalor®-S alone and in combination on growth performance and carcass characteristics in feedlot steers. T. C. Bramble*, R. A. Roeder, B. C. Peterson, M. J. Garber, and G. T. Schelling, *University of Idaho, Moscow.*

The objective of this study was to evaluate growth performance, carcass characteristics, and levels of circulating IGF-I in 72 Angus crossbred beef steers averaging 345 kg treated with Posilac® and Revalor®-S alone and in combination. In a completely randomized block design treatments were: 1) CON (Control); 2) POS (Posilac® 320 mg recombinant bovine somatotropin s.c injection/14 d); 3) REV (Revalor®-S, 120 mg trenbolone acetate and 24 mg estradiol-17β implant); and 4) POS+REV. The steers were bled on d 0, 28, 56, and 112 for IGF-I determination. Selected treatment effects are summarized in the table below.

Item	Treatment				P-value	SE
	CON	POS	REV	POS+REV		
Feed/gain	6.35 ^a	6.07 ^{ab}	6.03 ^{ab}	5.63 ^b	**	.19
ADG, kg/d	1.42 ^a	1.38 ^a	1.56 ^b	1.63 ^b	**	.04
Feed intake, kg/d	8.99 ^a	8.34 ^b	9.40 ^a	9.16 ^a	*	.24
Hot carcass wt, kg	317.0 ^a	316.0 ^a	328.0 ^a	330.0 ^a	NS	9.2
Ribeye area, cm ²	77.7 ^a	82.6 ^{ab}	84.5 ^b	79.5 ^{ab}	*	2.4
Backfat, cm	1.1 ^a	1.0 ^a	.9 ^a	1.0 ^a	NS	.1
Marbling score	541.0 ^a	501.5 ^b	510.3 ^{ab}	490.9 ^b	*	13.3
Yield grade	2.9 ^a	2.5 ^a	2.4 ^a	2.8 ^a	NS	.2
Dressing percent	60.9 ^a	61.2 ^a	60.3 ^a	60.1 ^a	NS	.6
Serum IGF-I ^d , ng/ml	276.8 ^a	361.6 ^b	417.6 ^b	483.7 ^c	**	22.5

^a*abc* means within a row with different superscripts differ at * P<.1, ** P<.05, NS = not significant (P<.1); ^d*d* 112

These data indicate that key feedlot performance variables (ADG, feed/gain, and feed intake) were significantly increased with the use of REV and POS+REV. Furthermore, this research also demonstrates the possible negative consequence of treating feedlot steers with POS or POS+REV on carcass marbling.

Key Words: Steers, Growth, Somatotropin

520 Serum from steers treated with Posilac® and Revalor®-S alone and in combination alters protein synthesis and degradation in C2C12 myotube cultures. B. C. Peterson*, R. A. Roeder, M. J. Garber, T. C. Bramble, and G. T. Schelling, *University of Idaho, Moscow.*

The objective of this study was to examine the effects of Posilac® and Revalor®-S on protein synthesis and degradation of C2C12 myotubes incubated in serum from treated steers. In a completely randomized block design using 72 Angus crossbred feedlot steers, the treatments were: 1) CON (Control); 2) POS (Posilac®, 320 mg recombinant bovine somatotropin s.c. injection/14d); 3) REV (Revalor®-S, 120 mg trenbolone acetate and 24 mg estradiol-17β implant); and 4) POS+REV. Serum was harvested on d 0, 56, and 112 for in vitro muscle cell protein synthesis and degradation measurements and plasma was collected on d 0, 28, 56, and 112 for IGF-I determination. Cultures of C2C12 muscle cells were grown in Dulbecco's Modified Eagle Medium (DMEM) containing 10% fetal calf serum until 50% confluent. Myotube formation was induced by replacing the growth medium with DMEM containing 2% fetal calf serum. Treatment with cytosine arabinoside and microscopic examination of cultures insured that approximately 90% of the cell nuclei were contained in myotubes. Protein degradation was measured by a pulse-chase procedure using [³H] leucine and 2% test serum. Protein synthesis was determined as cpm of [³H] leucine incorporated per mg of cell protein in the presence of 2% test serum. Serum harvested from steers on d 0 had no effect (P>.05) on protein degradation or synthesis. For d 56 and d 112, POS and POS+REV serum decreased (P<.01 and P<.05, respectively) protein degradation and REV and POS+REV serum increased (P<.09 and P<.0001, respectively) protein synthesis relative to CON. IGF-I levels were not significantly different on d 0 (P>.10) but were increased (P<.05) by all treatments for d 28, 56, and 112 relative to CON. It appears that protein degradation or synthesis or both are favorably altered by POS, REV, or POS+REV combination. These observations may help explain the anabolic effects of growth promoting agents in feedlot steers.

Key Words: C2C12 Myotubes, Protein Turnover, IGF-I

521 Skeletal muscle morphology alterations due to Posilac® and Revalor®-S treatments, alone or in combination in feedlot steers. N. J. Hughes*¹, G. T. Schelling¹, M. J. Garber¹, J. S. Eastridge², M. B. Solomon², and R. A. Roeder¹, ¹University of Idaho, Moscow, ²USDA-ARS, Beltsville, MD.

The objective of this study was to evaluate changes in muscle fiber characteristics in Angus crossbred feedlot steers treated with Posilac® or Revalor®-S, alone or in combination. Using a completely randomized design, treatments were: 1)CON (Control); 2)POS (Posilac®, 320 mg recombinant bovine somatotropin s.c. injection/14 d); 3)REV (Revalor®-S, 120 mg trenbolone acetate and 24 mg estradiol-17 β implant); and 4)POS+REV. Muscle samples were removed from each steer immediately after slaughter on d 0 (BW 345 kg) and d 112 (BW 585 kg) from the longissimus (LM) and supraspinatus (SS) muscles. Transverse sections (8 μ m) were cut with a cryostat and stained for succinate dehydrogenase activity in combination with myosin ATPase (pH 9.4). Using an automated digital analyzer, muscle fiber area and fiber type percentage were evaluated. Fibers were classified as slow twitch oxidative (SO), fast twitch oxidative glycolytic (FOG), or fast twitch glycolytic (FG). Implantation with REV decreased the number of SO fibers in the LM (P<.10) and FOG fibers in the SS (P<.10), while increasing the number of FG fibers in the LM (P<.10) and SO fibers in the SS (P<.10). Steers treated with REV exhibited a larger SO (P<.10), FOG (P<.05) and FG (P<.10) fibers in the SS and FG (P<.10) in the LM. The administration of POS decreased (P<.05) the percentage of SO fibers and increased (P<.10) FG fiber distribution in the LM, and increased (P<.05) FOG fiber area in the SS. The combination of POS+REV had no effect (P>.10) on the distribution of fiber types in either muscle, but increased FOG (P<.05) and FG (P<.10) fiber area in the SS. These results indicate that growth promoting agents POS, REV, and POS+REV demonstrated their ability to increase muscle hypertrophy relative to CON, but POS+REV exhibited no additive effect.

Key Words: Growth Promoters, Muscle Morphology, Feedlot Steers

522 Estimation of prediction equation for insulin infusion rate to achieve target plasma insulin concentrations in pigs of different weights. D. Wray-Cahen*, H. V. Nguyen, P. R. Beckett, P. J. Reeds, E. O. Smith, and T. A. Davis, *USDA-ARS Children's Nutrition Research Center, Baylor College of Medicine, Houston, TX.*

When insulin (INS) is infused at the same rate into pigs of different weights, different plasma INS concentrations result. We sought to establish a mathematical relationship which would predict the INS infusion rate required to achieve a target plasma INS concentration for a given weight in young growing pigs. To achieve this, we performed INS-dose response studies in 7- and 26-d old suckling pigs, using a hyperinsulinemic-euglycemic clamp technique with (n=10) or without (n=8) clamping amino acids. The 7-d old pigs weighed 1.95 \pm 0.43 kg and 2.36 \pm 0.47 kg in the 2 studies, respectively and the 26-d old pigs weighed 7.11 \pm 1.36 kg and 8.51 \pm 1.34 kg, respectively. Target plasma INS concentrations ranged from 3 to 4000 μ U INS/ml. Each pig received infusions of 2-3 INS doses for 2-3 h/dose. We determined the mean plasma INS concentration for the last hour of each clamp period for each dose of INS. We used linear regression to define the dose response relationship between plasma INS concentration (y) and INS infusion rate (x) for each age group, with x being scaled to various exponents of body weight. The metabolic scaling factor (exponent of body weight) which gave the best fit was selected using r² as the primary criterion. Because the linear relationship and fit from the 2 studies were similar, the data were combined. The metabolic scaling factor which resulted in the best linear fit was 0.66. The equation for the relationship was y=0.55985+0.2443x, where y is expressed in μ U of INS per ml of plasma and x is expressed in ng of INS infused per min per kg^{0.66}. The r² for this equation was 0.938. However, this equation did not describe the relationship once plasma INS concentrations greater than 100 μ U/ml were achieved. At very high concentrations, the equation systematically underestimated the INS concentration and it appears that INS clearance was progressively depressed.

Key Words: Insulin, Pig, Euglycemic Clamp

523 Porcine hepatocytes display either parenchymal or bile duct morphology in culture: phenotypic and genotypic characterization. T. J. Caperna*, T. S. Sonstegard, and N. C. Talbot, *USDA-ARS, Beltsville, MD.*

Hepatocytes isolated from crossbred pigs (25 to 63 kg) were maintained *in-vitro* as co-cultures with mitomycin-C-inactivated STO embryonic mouse fibroblasts. Non-dividing hepatocytes were maintained for up to seven weeks as confluent monolayers in serum-free medium containing 1% DMSO and 1 \times 10⁻⁶M dexamethasone. Transmission electron microscopic (TEM) analysis indicated *in vivo*-like parenchymal morphology with many hepatocytes sandwiched between STO cells. Significant levels of inducible P-450 were found in hepatocyte monolayers; the cells also maintained low levels of γ -glutamyl transpeptidase (GGT). When dexamethasone was removed from the medium and DMSO was lowered to 0.5%, hepatocytes differentiated into bile duct cells which were characterized by high GGT activity, and by formation of multicellular ductal structures. TEM analysis revealed compact cells containing abundant cilia which projected into the lumens of the multicellular ductal structures. GGT was localized along apical membranes of ductal structures and total cellular GGT activity increased concomitantly with development of these structures. P-450 levels were markedly reduced in bile duct cultures. In an attempt to identify some of the changes in gene expression associated with this divergent differentiation pattern, total RNA was isolated from hepatocytes cultured as bile duct and parenchymal cells, and then subjected to analysis by RAP(RNA directed arbitrarily primed)-PCR. Using 24 different combinations of 10 nucleotide base primers for cDNA synthesis and generation of RT-PCR products, approximately 33 sequences were found that potentially represent differentially expressed RNA in these two cell types. Sequence analysis of these and other products may enable definition of specific signaling molecules operating in this hepatocyte/bile duct differentiation model and may also elucidate their potential roles in embryonic tissue development.

Key Words: Hepatocytes, Bile Duct, Differentiation

524 Evaluation of a simple blood test to predict growth performance of beef bulls. E. E. Connor*, S. M. Barao, E. Russek-Cohen, and G. E. Dahl, *University of Maryland, College Park.*

The relationships between GH response to GHRH vs growth performance, carcass fat percentage and milk production in cattle have been demonstrated. A simplified measurement of GH response to GHRH in beef bulls at weaning was evaluated to determine its ability to predict subsequent growth performance. Fifty-four Angus bulls averaging 229 d (SD = 20) of age were administered by i.v. injection two doses (1.5 and 4.5 μ g/100 kg BW) of human GHRH (1-29) analog. Blood was collected via jugular venipuncture at 0 and 10 min relative to injection of each dose of GHRH for serum GH determination. The 1.5 μ g GHRH/100 kg BW challenge was repeated 7 d after the first challenge on a random subset of bulls (n = 9) to estimate the repeatability of GH responses within animal. Pearson's ranked correlation revealed responses from the two 1.5 μ g GHRH/100 kg BW challenges were correlated (r = .70). Relationships between BW vs GH concentration 10 min post-injection of GHRH, weaning weight EPD (WWEPD), yearling weight EPD (YWEPD) and weaning weight (WW) throughout a 140-d bull test were evaluated using simple linear regression. Preliminary results show a relationship (P < .0001) between GH response to 4.5 μ g GHRH/100 kg BW vs BW through Day 112 of the bull test, using age-adjusted weaning weight as a covariate. Response to GH was a better predictor of Day 112 BW (r² = .57) than WWEPD (r² = .47) and YWEPD (r² = .14); however WW was the best predictor of Day 112 BW (r² = .90). There was no relationship between GH response to the 1.5 μ g/100 kg BW dose of GHRH vs BW through Day 112 of the bull test. Based on results of a previous study, GH response may be useful for predicting final carcass composition. Results of this study suggest that GH response to GHRH is a useful tool for identifying beef bulls with superior growth potential, but further refinement of the technique is required for application at the producer level.

Key Words: Beef Cattle, Growth Performance, GHRH

525 The immunomodulating activity of dietary 3-hydroxy-3-methylbutyrate (HMB) in weanling pigs. P. Ostaszewski¹, E. Kozłowska¹, A. K. Siwicki², J. Krzyzanowski³, J. C. Fuller, Jr.⁴, and S. Nissen⁵, ¹Dept. of Animal Physiology, Warsaw Agricultural Univ., Warsaw, Poland, ²Dept. of Epizootic with Clinic of Infectious Diseases, Olsztyn, Poland, ³Dept. of Reproduction, Agricultural Univ., Lublin, Poland, ⁴Metabolic Technologies Inc., Ames, Iowa, ⁵Iowa State Univ., Ames.

Previous studies have shown that oral administration of 3-hydroxy-3-methylbutyrate (HMB), a catabolite of the amino acid leucine, enhances immune response in sheep and broilers, but thus far this effect has not been proven in weanling pigs. A previous experiment had failed to show an effect when a high level of dietary HMB (0.4% of the ration) was fed for 6 wk. In the current study a much lower concentration of HMB (50 mg/kg BW-day⁻¹) was fed during first 21 days after weaning. Sixteen weanling pigs (avg. initial BW 6.0 kg) from the same sow were randomly assigned to either an HMB or control group (n=8 per group). Blood was collected at 0, 7, 14, 21, 28 and 35 d after the start of the experiment for the determination of respiratory burst activity (RBA) and potential killing activity (PKA) of phagocytes, lymphocyte proliferation (LP) stimulated by either concanavalin-A (Con-A) or lipopolysaccharide (LPS), plasma lysozyme activity and total Ig levels. Pigs fed HMB supplemented diets showed an increase in RBA (27%), PKA (39%*), LP-ConA (29%), LP-LPS (15%), lysozyme activity (47%*) and total Ig levels (14%) at 21 d which coincided with the end of HMB administration (*=P<0.05 vs control). At d 35, the effect of feeding HMB was still manifest two wk post HMB administration as there was an increase in RBA (37%*), PKA (54%*) and lysozyme activity (55%*) in the HMB-fed group (*=P<0.05 vs control). In conclusion, these data demonstrate that 50 mg/kg BW daily-dietary HMB given for the first 3 wk after weaning positively affects nonspecific cellular and humoral immune response of growing pigs and this stimulatory effect of HMB on the immune system is maintained for at least 2 wk following HMB treatment.

Key Words: 3-Hydroxy-3-Methylbutyrate, HMB, Immune Function

526 Effect of milk replacer composition on serum fatty acid profiles of nursing kittens. R. L. Kelley* and A. J. Lepine, The Iams Company, Lewisburg, OH.

To determine the effect of milk replacer composition on serum fatty acid (FA) profiles (FAP), thirty-seven kittens from 8 litters were randomly assigned to three diet treatments, Milk Replacer (MR) -I (n=13), MR-II (n=11) or Queen reared (CTL; n=14). MR-I was formulated to supply (dry matter basis) 40% protein by the inclusion of casein and whey protein concentrate at a 50:50 ratio and 34% fat. Fat sources (butter oil, canola oil, corn oil, arachidonic acid and docosahexaenoic acid (DHA)) were selected to provide a FAP that closely resembled queen's milk, with specific attention to the FAs, palmitate, stearate, oleate, linoleate, arachidonate and DHA. MR-II was a typical feline milk replacer which provided (dry matter basis) 42% protein and 28% fat by the inclusion of whey protein concentrate, casein, dried skim milk, vegetable oil and butter fat. When possible, all treatments were represented across litter and gender within litter and CTL litters cross fostered to a mean of 4.6 kittens/nursing queen. All kittens were allowed to nurse the queen for 24 h (d 0) to obtain adequate immune protection. MR treatments were subsequently fed every 3 h for d 1-2, every 4 h for d 3-10, every 5 h for d 11-20 and every 6 h for the remainder of the study (d 30). Blood samples were collected on d 30 via jugular puncture. All comparisons made between treatments were derived using the GLM procedure of SAS. Serum FAP were reflective of dietary treatment. MR-I fed and CTL kittens were similar in thirty-six of the forty-eight FA examined. MR-I fed kittens were slightly higher than CTL in serum DHA, however both treatments were significantly higher than MR-II fed kittens (P < 0.01). Least-square means (differences denoted by superscripts, P < 0.05) for specific FA percentage are shown in table for all treatments. Dietary FA composition was shown to influence serum FAP of nursing kittens, with MR-I more closely matching CTL. These data may in part explain the similar body composition previously reported for MR-I and CTL kittens (Lepine et al., 1998).

Fatty Acid (%)	MR-I	MR-II	CTL
Palmitic Acid	14.98 ^a	12.22 ^b	15.96 ^a
Stearic Acid	13.16 ^a	11.50 ^b	13.82 ^a
Elaidic Acid	0.26 ^a	4.10 ^b	0.22 ^a
Arachidonic Acid	7.14 ^a	2.28 ^b	8.35 ^a
Docosahexaenoic Acid	1.47 ^a	0.26 ^b	1.14 ^c

Lepine, A. J., R. L. Kelley and G. Bouchard. 1998. Effect of feline milk replacers on growth and body composition of nursing kittens. (submitted) Am. College Vet. Intern. Med.

Key Words: Feline, Milk Replacer, Fatty Acids

527 Influence of 3-hydroxy-3-methylbutyrate (HMB) on specific cellular immune response after *in vitro* and *in vivo* immunization with *Yersinia ruckeri* antigen. A. K. Siwicki¹, M. Morand², J. C. Fuller, Jr.³, S. Nissen⁴, K. I. Kazua¹, E. Glabski¹, and P. Ostaszewski⁵, ¹Conseil, Zabieniec Warsaw, Poland, ²General du Jura, France, ³Metabolic Technologies Inc., Ames, Iowa, ⁴Iowa State Univ., Ames, and ⁵Warsaw Agricultural Univ., Poland.

In practice, protection of fish against disease by immunization has been of limited effectiveness. Therefore, immunomodulating compounds, such as HMB, that can enhance the effective use of the vaccines would be of great benefit. In the present study the effect of HMB on cellular specific immune response after either *in vitro* or *in vivo* immunization of rainbow trout (*Oncorhynchus mykiss*) with anti-*Yersinia ruckeri* vaccine was studied. Fish were fed pellets containing HMB at doses of 0, 10, 25 and 50 mg/kg BW per day. After 2 wk of HMB supplementation, the fish were immunized by intraperitoneal injection of 0.2 ml of *Y. ruckeri* vaccine. The control group was similarly injected with phosphate buffered saline (PBS). At 7, 14, 18, 21, 28 and 35 d after immunization blood and spleens were taken from 10 fish in each group for testing. For *in vitro* immunization, the spleens were sectioned and placed in a 35 mm sterile well with L15 medium containing HMB at concentrations of 0, 0.1, 1, 5, 10, 25, 50 or 100 µg/ml of medium. The spleens were injected with *Y. ruckeri* vaccine and incubated at 17 C for 10 d. When analyzed by the ELISPOT assay, HMB increased the total and specific antibody secreting cells after immunization at concentrations from 10 to 100 µg/ml (P<0.01). Dietary HMB also increased the levels of total and specific antibody secreting cells when the fish were vaccinated *in vivo*. In conclusion, the results of the present study show that HMB activates the specific cellular immune response after either *in vitro* or *in vivo* immunization of rainbow trout with *Y. ruckeri* vaccine.

Key Words: 3-Hydroxy-3-Methylbutyrate, HMB, Rainbow Trout

528 *In vitro* effects of 3-hydroxy-3-methylbutyrate (HMB) on measures of immune function and immunocompetence in fish. A. K. Siwicki¹, P. Ostaszewski², J. C. Fuller, Jr.*³, S. Nissen⁴, and M. Studnicka⁵, ¹Zabieniec Warsaw, Poland ²Warsaw Agricultural Univ., Poland, ³Metabolic Technologies Inc., Ames, Iowa, ⁴Iowa State Univ., Ames, and ⁵Catholic Univ., Lublin, Poland.

3-Hydroxy-3-methylbutyrate (HMB) has been shown to counteract many of the negative effects of modern production methods and results in increased growth and protection against diseases. *In vitro* studies demonstrated that HMB enhances proliferation of macrophages in culture. In the present study the influence of HMB on the immunocompetence cell activity in rainbow trout (*Oncorhynchus mykiss*) and carp (*Cyprinus carpio*) was examined. The immunocompetence cells were separated from the pronephros and spleen after centrifugation in either Percoll or Lymphoprep gradients. HMB was prepared in RPMI-1640 medium at concentrations of 0, 0.1, 1, 5, 10, 25, 50 and 100 µg/ml of medium. The effects of HMB on the respiratory burst activity (RBA) of PMN/MN cells stimulated by PMA; potential killing activity (PKA) of phagocytes; and lymphocyte proliferation (LP) stimulated by either concanavalin-A (Con-A) or lipopolysaccharide (LPS) were examined. HMB addition increased the polymorphonuclear (PMN) and mononuclear (MN) cell activity. HMB also stimulated the phagocytic cell activity as analyzed by RBA (+84%, P<0.01) and PKA assays (+140%, P<0.01) and lymphocyte proliferation stimulated by both Con-A and LPS (P<0.01) at concentrations between 10 to 100 µg/ml. The greatest effect of HMB on the macrophage and lymphocyte activities was observed at a concentration of 50 µg/ml. HMB was also observed to have an immunostimulating influence on the macrophages and T or B lymphocyte activity in rainbow trout and carp. The results of this *in vitro* study demonstrated that HMB improved immunocompetence cell activity and that HMB enhanced the cell-mediated and nonspecific cellular defense mechanisms in fish.

Key Words: 3-Hydroxy-3-Methylbutyrate, HMB, Fish

529 Immunomodulating effect of 3-hydroxy-3-methylbutyrate (HMB) on the nonspecific cellular and humoral defense mechanisms in rainbow trout (*Oncorhynchus mykiss*). A. K. Siwicki^{1*}, M. Morand², K. Goryczko³, P. Ostaszewski⁴, J. C. Fuller, Jr.⁵, and S. Nissen⁶, ¹Zabieniec Warsaw, Poland, ²General du Jura, France, ³Rutki Gdansk, Poland, ⁴Warsaw Agricultural Univ., Poland, and ⁵Metabolic Technologies Inc., Ames, Iowa, ⁶Iowa State Univ., Ames.

Studies have shown that HMB increases the immune response in animals as measured by antibody levels to specific antigens and macrophage and T-cell function. In the present study we examined the influence of HMB on the nonspecific cellular and humoral defense mechanisms and protection against furunculosis in rainbow trout (*Oncorhynchus mykiss*). HMB was fed in a pelleted ration at either 0, 10, 25 or 50 mg/kg BW per d for 8 wk. Blood, pronephros and spleen were taken from 10 random fish from each group for analysis. The phagocytic ability of PMN cells (NBT test), respiratory burst activity of macrophages, potential killing activity of phagocytes, lymphocyte proliferation stimulated by either concanavalin-A (Con-A) or lipopolysaccharide (LPS), lysozyme activity and total Ig levels in plasma were analyzed before and at 1, 2, 3, 4, 6 and 8 wk. At 2 and 4 wk, a challenge test was performed by injection of *Aeromonas salmonicida*. HMB at doses between 10 to 50 mg/kg BW increased the phagocytic ability of neutrophils and macrophages by up to 36% ($P < 0.01$) and increased the mitogen-stimulated lymphocyte proliferation by up to 90% ($P < 0.01$) when compared with the control group. HMB also increased the lysozyme activity and total Ig levels in plasma. Additionally, reduced mortality after the in vivo challenge with *A. Salmonicida* suggested that HMB activated nonspecific protection against this bacterial disease in rainbow trout. In conclusion, when HMB is included in the diets of fish the specific and non-specific immune defenses are enhanced.

Key Words: 3-Hydroxy-3-Methylbutyrate, HMB, Rainbow Trout

530 Whole body antioxidant status in young growing rats induced by dexamethasone and the effect of 3-hydroxy-3-methylbutyrate (HMB) treatment on recovery after dexamethasone treatment. A. Orzechowski*, P. Ostaszewski, A. Brodnicka, J. Wilczak, M. Jank, B. Balasinska, A. Mrowczynska, and T. Ploszaj, Warsaw Agricultural University, Warsaw, Poland.

Over the past several years, research has shown that oxidative stress plays a role in the general process of aging and tissue damage. Reactive oxygen species (ROS) can influence the evolution of many degenerative diseases through oxidative attack and can be partially prevented by the presence of endogenous antioxidants. The most prominent and ubiquitous of these is glutathione. Blood and muscle glutathione (reduced form, GSH) were determined in young growing rats (6 weeks of age, $n=12$) after exposure to intragastric administration of dexamethasone (DEX, 2 mg/kg BW). This synthetic corticosteroid is known to induce an imbalance between the rate of generation and removal of oxidizing species. DEX was given each day for 5 d. One group of animals had a 5 d recovery period alone and another group received HMB (40 mg/kg BW) during the 5 d recovery period. DEX treatment resulted in a 40% reduction ($P < 0.001$) of blood GSH and a 16% ($P < 0.05$) reduction of muscle GSH (GSH/tissue wet weight). HMB treatment resulted in decreased ($P < 0.001$) blood GSH concentration to approximately 41% of control value, while HMB did not influence GSH content in muscle. Dexamethasone induced oxidative stress most probably through down regulation of the rat's antioxidant defense system and hence the drop in GSH was a good index of impaired whole body reaction to DEX. Muscle wet weight was also decreased by 73% ($P < 0.001$) in the DEX group compared with the control group. No differences were found between the groups in relation to protein concentration; however, DNA content was decreased after treatment with DEX. In conclusion, GSH can be used as a measure of anti-oxidant status after DEX treatment.

Key Words: 3-Hydroxy 3-Methylbutyrate, HMB, Oxidative Stress

531 Effects of age, gluconeogenic substrates, and diet on hepatic gluconeogenesis and pyruvate carboxylase expression in bovine calves. S. S. Donkin*, D. S. Black, R. B. Greenfield, and C. Agca, Purdue University, West Lafayette, IN.

The transition from the prerinuating to the ruminating state represents one of the most dramatic changes in glucose metabolism in mammals. Developmental changes in liver cell metabolism in calves, during this period, include a marked decrease in the use of lactate for gluconeogenesis. The objective of these experiments was to determine the effects of age, and diet type (solid feed vs. milk), on gluconeogenesis and expression of pyruvate carboxylase, a key enzyme in lactate metabolism to glucose. Hepatocytes were obtained from 10-14 day old prerinuating (PR) calves, 12-week old ruminating (R) calves that were weaned at 6 weeks of age, or from 12-week old milk-fed prerinuating (MFP) calves. The rates of incorporation of [$2-^{14}C$]propionate or [$U-^{14}C$]lactate into glucose (nmol per μg DNA per h), were measured in hepatocytes suspension cultures and Dulbecco's Modified Eagles Media containing 1% BSA, 2.5 mM propionate, 1.0 mM lactate and 1.0 mM pyruvate. Gluconeogenesis from lactate, in the presence of 2.5 mM propionate, decreased ($P < .05$) with age regardless of the type of diet fed (PR vs R, MFP; 3.62 vs. 1.23, 0.46). Glucose synthesis from propionate was similar between MFP and PR calves. Pyruvate carboxylase expression was determined by Northern blot analysis of total RNA from sequential liver biopsy samples, obtained every 14 days from birth to 84 days of age, using the PR and MFP calves. Pyruvate carboxylase expression was decreased ($P < .05$) by 38% from 14 days to 84 days for age, however, there was no diet x age effect on PC expression. The coincident decreases in gluconeogenesis from lactate and PC mRNA point to developmental decreases in PC expression that are reflected in the decreased use of lactate for glucose synthesis. The lack of diet effects on PC expression in the neonatal calf suggests changes during the transition to ruminating status that are independent of the appearance of end-products of rumen fermentation.

Key Words: Gluconeogenesis, Hepatocytes, Lactate

532 The effect of pre-weaning nutrient supply on post-weaning growth and efficiency. M. L. Fenton¹, T. E. Hughes¹, W. S. Pitchford¹, and P. A. Speck^{2*}, ¹University of Adelaide, Australia, and ²Agriculture Victoria, Rutherglen, Australia.

The objective of this work was to study the combined effect of the environment and genotype by examining the effect of pre-weaning nutrient supply on lines of mice selected for 7 generations for either high or low feed efficiency. Selection was for net feed intake post-weaning (NFI) defined as the variation in intake independent of variation in weight gain, weight maintained and sex. Pre-weaning nutrient supply was altered by cross-fostering within selection lines, at birth to get a range (3-17) of litter sizes. After weaning (at 24 days), 120 mice from each selection line were placed on feed intake tests, where feed intake and body weight were recorded weekly for three weeks. Following the feed intake test, body composition was measured using an EM-SCAN Small Animal Body Composition Analyser.

There were no differences between the selection lines in weight. However at larger litter sizes mice from the low feed intake line were heavier than mice from the high feed intake line. On average the low feed intake line consumed 18% less, had a 27% lower NFI and were 8% fatter than the high feed intake line. In contrast to body weight, the response to the environment was the same in both lines, mice from larger litters consumed less, and had less fat. NFI was not influenced by nutrient supply.

Trait	High Feed Intake	Low Feed Intake
Weight (g/pup weaned)	-0.50±0.06	-0.30±0.05
Feed Intake (g/day/pup weaned)	-0.05±0.02	-0.03±0.01
Fat (%/pup weaned)	-0.11±0.15	-0.08±0.07

It appears from these results that the difference between the selection lines in their response to a restriction in pre-weaning nutrient supply is a function of growth rather than intake. Selection for low NFI in young mice resulted in no change in body weight, increased post-weaning efficiency, animals that were slightly fatter, and were less responsive to changes in nutrient supply.

Key Words: Net Feed Intake, Litter Size, Mice

533 Feed efficiency and carcass traits of ram lambs actively immunized against GnRH. Z. Kiyama*¹, B. W. Hess¹, M. L. Riley¹, W. J. Murdoch¹, T. E. Adams², and G. E. Moss¹, ¹University of Wyoming, Laramie and ²University of California, Davis.

It was hypothesized that ram lambs immunized against GnRH would possess the desirable production and carcass traits of intact ram lambs. Intact male lambs weighing $32.6 \pm .5$ kg at 90-days of age received no treatment (R, n=13), were immunized with GnRH linked to keyhole Limpet hemocyanin in either Freund's complete adjuvant (I1, n=11) or ISA (SEPPIC, Inc) adjuvant (I2, n=12), or were castrated (C, n=11). Animals were then placed into individual feeding pens and fed feedlot rations. Lambs were slaughtered when they reached live body weights of $57.8 \pm .4$ kg. Testicular weights in R, I1 and I2 lambs averaged 402 ± 23 , 65 ± 8 , and 254 ± 46 g, respectively. Days on feed (121 ± 4) were similar ($P > .05$) among groups, but average daily gains (ADG, kg) were greater ($P < .05$) in R ($.24 \pm .01$) than C ($.19 \pm .01$) lambs, with I1 ($.2 \pm .01$) and I2 ($.21 \pm .01$) lambs intermediate ($P > .05$). Feed efficiency (kg gain/kg feed) for R lambs ($5.86 \pm .13$) did not differ ($P > .05$) from I2 lambs ($6.75 \pm .28$), but exceeded ($P < .05$) that of I1 ($7.31 \pm .48$) and C ($6.98 \pm .18$) lambs. Ribeye area, lean maturity and muscling score did not differ among groups ($P > .05$). R, I1, and I2 lambs, respectively, had more desirable ($P < .05$) yield grades ($1.7 \pm .1$, $1.8 \pm .1$, $1.7 \pm .1$), less ($P < .05$) back fat (mm; $3.3 \pm .3$, $3.6 \pm .3$, $3.3 \pm .3$), and less ($P < .05$) marbling (298 ± 20 , 279 ± 12 , 290 ± 22) than C ($2.7 \pm .2$, $5.8 \pm .5$, 415 ± 17) lambs. Flank streaking, color of fat and overall quality grade did not differ ($P > .05$) among groups. Body wall thickness (mm) for R ($18.8 \pm .5$) and I2 (18.8 ± 1.0) lambs were less ($P < .05$) than C lambs (24.1 ± 1.0) with I1 ($21.3 \pm .8$) being intermediate ($P > .05$). In summary, immunization against GnRH decreased testicular weight and reduced live animal performance comparable to that of castrated males. Partitioning of nutrients for growth and deposition of fat appears to differ among immunologically castrated and mechanically castrated lambs probably due to residual testicular activity.

Key Words: Rams, Immunization, Castration

534 Metabolic indicators of muscle mass in dorset sheep. S. J. Seaton¹, J. N. Clarke², W. S. Pitchford¹, and P. A. Speck³, ¹University of Adelaide, Australia, ²AgResearch, Ruakura Agricultural Research Centre, Hamilton, New Zealand, and ³Agriculture Victoria, Rutherglen, Australia.

The carwell phenotype is characterized by increases in muscle mass, and almost specifically in the loin region. Identification of the phenotype is difficult and variable. The carwell genotype appears to be related to the callipyge genotype using marker analysis. The purpose of the present study was to assess the potential of blood metabolites in discriminating the progeny from carwell sires from those of normal sires. Progeny from 150 Dorset ewes mated to 8 sires (2 carwell sires and 6 normal sires) were bled via jugular venipuncture at approximately 12 weeks of age. Plasma creatinine and urea was measured using an auto-analyser. Eye muscle characteristics were measured posterior to the 13th rib using an Aloka SSD-500 real-time ultrasound scanner. Plasma creatinine was significantly ($P < 0.05$) different between progeny from carwell and control sires, (356.4 25.3 carwell, 255.4 21.3 control μM). Plasma urea was also significantly ($P < 0.05$) different between progeny from carwell and control sires (7.09 ± 0.37 carwell, 8.92 ± 0.31 control mM). Carwell progeny had significantly ($P < 0.05$) higher eye muscle depths and areas, than control sires. The data highlights the potential usefulness of using plasma creatinine and urea to screen populations for the identification of animals which have increased carcass lean.

Key Words: Blood Metabolites, Carwell, Carcass Lean