to B.A.M. standard plating methods, however, detection was reduced to 24-48h. Each assay takes approximately 1.5 h after enrichment and can be run simultaneously with other PATH*IGEN* tests.

Key Words: Campylobacter, Diagnostic, Detection

# **116** Novel Biosensors for the Rapid Detection of Salmonella Species in Various Food Matrices. Eddie Jefferies\*, Shelia Rowe, and Jill White, *IGEN International, Inc., Gaithersburg, MD.*

Salmonella are implicated in causing 2 to 4 million cases of salmonelosis annually in the United States. Symptoms of a Salmonella infection include nausea, vomiting, abdominal cramps, diarrhea, fever and headache. S. tuphimurium and S. enteritidis represent the two main species that lead to enteric disease in humans, however, procedures to detect these Salmonella species may take as long as 4 days to complete. Therefore, more rapid methods are required to determine the presence of Salmonella in food. In this study, the utility of an ORIGEN<sup>®</sup>-based Salmonella test was evaluated on different food matrices. This technology is based on a technique called electrochemiluminescence, which permits the detection of Salmonella species within an extensive array of samples. Food matrices included, poultry products, fruits and vegetables, shellfish, cheese products, milk products, chocolate, and water samples. 100 samples were tested by pre-enrichment in buffered peptone water followed by selective enrichment in Rappaport Vassiliadis soya peptone broth and testing using the  $\mathrm{PATH}IGEN^{\mathrm{TM}}$ Salmonella test or B.A.M. standard plating methods. Food samples representative of each matrix were also inoculated with known levels of Salmonella typhimirium and tested using the Salmonella test. The Salmonella test was able to detect the equivalent of less than 1 CFU/25 g of sample. The sensitivity of the PATHIGEN test was equivalent to the B.A.M. standard plating methods, however, detection time was reduced to 24- $30~\mathrm{h}.$  Each as say takes approximately  $1.5~\mathrm{h}$  after enrichment and can be run simultaneously with other PATHIGEN tests.

Key Words: Salmonella, Diagnostic, Detection

117 Comparison of cultivation to PCRhybridization for detection of Salmonella in porcine fecal and water samples. Ingrid Feder<sup>1</sup>, Jerome C. Nietfeld<sup>2</sup>, John Galland<sup>3</sup>, Teresa Yeary<sup>2</sup>, Jan M. Sargeant<sup>3</sup>, Richard Oberst<sup>3</sup>, Mark L. Tamplin<sup>1</sup>, and John B. Luchansky<sup>1</sup>, <sup>1</sup>U. S. Department of Agriculture, Wyndmoor, PA/U.S.A., <sup>2</sup>College of Veterinary Medicine, KSU, Manhattan, KS/U.S.A., <sup>3</sup>Food Animal Health and Management Center, KSU, Manhattan, KS/U.S.A.

Salmonella in swine is an economic concern to swine producers and a human health risk. A reliable and rapid technique for Salmonella detection in swine feces and water exposed to swine feces would be useful. The purpose of this research was to compare cultivation methods and PCRbased methods for the detection of Salmonella in the feces of healthy pigs and in water. In the present study, three cultivation techniques were compared to a polymerase chain reaction (PCR)-hybridization technique for the detection of Salmonella. A total of 150 fecal and water samples were tested for the presence of Salmonella: 1) 92 fecal samples were pre-enriched overnight in tryptic soy broth (TSB) followed by overnight enrichment in Rappaport-Vassiliadis R10 (RV10) broth: 2) 34 fecal samples were enriched overnight in RV10 broth with no additional enrichment; and, 3) 24 water samples were pre-enriched overnight in 3MC broth followed by overnight enrichment in RV10 broth. For the PCR detection of Salmonella, samples were tested after the first overnight enrichment. The DNA was extracted via boiling and concentrated using a Sepharose CL-6B spin column. A total of 65 samples tested positive by both cultivation and the PCR or either method alone. Salmonella was detected by both methods in 68.8% of the positive samples preenriched in TSB, in 73.3% of the positive samples pre-enriched in 3MC, and in 24.0% of the positive samples enriched in RV10. Using the kappa statistic, agreement was 76% between cultivation with pre-enrichment and the PCR for Salmonella detection but was 5.7% when using cultivation without pre-enrichment compared to the PCR. These data provide evidence that the PCR could be used in combination with cultivation to improve Salmonella detection as the PCR worked as well or better than culture for delineating positive samples. However, the PCR detected only 72% of those samples which culture identified as positive, indicating that additional improvements are warranted before the PCR replaces cultivation as the gold standard for detection of Salmonella from swine.

Key Words: Salmonella, PCR-hybridization, Swine

### ASAS/ADSA Growth and Development: Muscle Growth and Development

**118** Cyclic stretch influences  $p21^{WAF1}$  promoter activity in myoblasts and myotubes. M.K. Webster<sup>\*1</sup> and J.M. Reecy<sup>1</sup>, <sup>1</sup>*lowa State University, Ames, IA*.

The ability of skeletal muscle to adapt to an imposed workload has been well characterized, while the molecular events underlying this process are less defined. Cyclin dependent kinase inhibitors, such as p21WAF1, play an important role in cell cycle progression. These cell cycle regulators, in conjunction with members of the MvoD family, have been shown to induce the terminal differentiation of myoblasts. In previous studies, p21 expression was dramatically increased after as little as 12 hours of overload, as well as during terminal differentiation. These results suggest that either myoblasts differentiate, thereby increasing p21 expression, or p21 expression in the existing myofibers increases in response to work overload. We hypothesized that there was a stretch-responsive element within the p21 promoter. C2C12 myoblasts, plated at 1250 cells/cm2, were transiently transfected with a p21-Luciferase construct. The construct was made by inserting 2.1 kb of 5' p21 promoter upstream from the firefly luciferase gene. Cells were cyclically stretched for 24 or 48 hours with a Flexercell-3000 machine, or no stretch as a control. The stretch protocol consisted of a 20% sine stretch for 3 seconds, followed by ten seconds of rest. This cycle was repeated three times, after which the cells were allowed to rest for 30 minutes. This series was repeated for the duration of the experiments. The promoter activity in myoblasts appears to decrease in response to cyclic stretch. Additionally, C2C12myoblasts were plated at 20,000 cells/cm2, transiently transfected, and induced to differentiate with low serum media for 15 hours. The differentiating myoblasts were then cyclically stretched for up to 48 hours. There was a 6.5-fold increase in promoter activity during differentiation. In addition, p21 promoter activity appeared to increase in response to stretch. We are currently examining the mRNA abundance of p21 under these same conditions. Based on these results, it would appear that the p21 promoter responds differentially in myoblasts and myotubes to cyclic stretch.

Key Words:  $p21^{WAF1}$ , cyclic stretch, skeletal muscle

**119** Effect of intramuscular plasmid delivery and electroporation on circulating concentration of the plasmid-encoded reporter gene in the pig. A.G. Van Kessel<sup>\*1</sup>, B.G. Goldade<sup>1</sup>, B.R. Krishnan<sup>2</sup>, M.A. Morsey<sup>2</sup>, L.D. Nelson<sup>2</sup>, and P.J. Gaynor<sup>3</sup>, <sup>1</sup>University of Saskatchewan, Saskatoon, SK, Canada, <sup>2</sup>Pfizer Global Research and Development, Groton, CT, <sup>3</sup>Pfizer Global Research and Development, Terre Haute, IN.

The effect of electroporation of skeletal muscle following plasmid injection on expression of a reporter gene was examined using 24 pigs (103.6  $\pm$  0.79 kg BW). Pigs were assigned to one of four treatment groups including a vehicle control (CON) group, a group administered 2 mg plasmid in each of 2 sites (2P), a group administered 2 mg plasmid in each of 2 sites followed by electroporation (2PEP) and a group administered 1 mg plasmid in each of 2 sites followed by electroporation (1PEP). Prior to treatment, all pigs were sedated. The plasmid encoded secreted alkaline phosphatase (SEAP) driven by the CMV promotor and was injected in the right biceps femorus muscle suspended in 1 mL Na<sub>2</sub>HPO<sub>4</sub> (150 mM, pH 7.2). Twelve electrical pulses (200 volts for 20 msec) were delivered across (1 cm gap) the injection site such that 6 pulses were delivered parallel with muscle fibres and 6 pulses were delivered perpendicular to muscle fibres. Polarity was reversed for 3 pulses delivered in each orientation. Electroporation had no adverse effects on the pigs. SEAP was detected in blood by day 2 following plasmid injection in electroporated pigs with maximal circulating SEAP activity observed at day 10 and returned to baseline by day 21. Serum SEAP activity, measured in relative light units (RLU), was not significantly increased in CON (88 137) versus 2P (459 131) pigs. Comparison of 2P and 2PEP groups indicated electroporation increased SEAP activity 3.9-fold (P < 0.001). SEAP activity was different (P < 0.05) on day 7 only, between 1PEP (657 725 RLU) and 2PEP (1 005 619 RLU) treatment groups. Electroporation enhanced plasmid-encoded gene expression and may be an alternative approach to *in vivo* protein delivery.

Key Words: Swine, Electroporation, Gene Therapy

**120** Muscle-derived insulin-like growth factor-I alters postnatal growth. J. K. Armstrong\*, P. V. Malven, A. L. Grant, and D. E. Gerrard, *Purdue University*, *1151 Smith Hall, West Lafayette, IN 47907.* 

Insulin-like growth factor-I (IGF-I) regulates postnatal growth in most mammals. The source of this growth factor, however, is a point of much debate as some suggest the primary action of IGF-I results from GHdependent release from the liver, yet the ability of peripheral tissues to produce IGF-I independently from GH may play an equally important role in growth. Therefore, we crossed three lines of mice: (1) Little mice (GH deficient), (2) IGF-I knock-out mice deficient in endogenous IGF-I (eIGF-I), and (3) mice with a muscle-specific actin promoter-driven human IGF-I transgene (tIGF-I), to evaluate the role of muscle-derived IGF-I in GH and/or IGF-I deficient mice. Body weights were recorded at 1, 10, 20, 30, 40, and 60 d postnatal. Progeny were genotyped using PCR. Serum GH and IGF-I concentrations were determined by radioimmunoassay. eIGF-I +/- mice had lower (P < .01) body weights than eIGF + / + (wild type) mice after d20. Little (-/-) mice were smaller (P < .0001) than Little wild-type or heterozygotes after d20. Presence of the tIGF-I transgene resulted in increased (P < .10) body weights in eIGF-I +/+ mice (d60), eIGF-I +/- mice (d40), Little +/- mice (d40), and Little -/- mice (d60). The tIGF-I transgene did not change serum GH or IGF-I concentrations, but eIGF-I +/- mice had lower (P < .0001) serum IGF-I than wild-type eIGF mice. These data suggest both IGF-I alleles are necessary for normal body growth and serum IGF-I concentrations in mice, and that muscle-specific production of IGF-I is capable of increasing body weights in GH and IGF-I deficient mice.

Key Words: Growth hormone, Insulin-like growth factor-I, Mice

**121** Effect of an IGF-I transgene on tissue accretion rates in pigs. VG Pursel<sup>\*1</sup>, AD Mitchell<sup>1</sup>, RJ Wall<sup>1</sup>, ME Coleman<sup>2</sup>, and RJ Schwartz<sup>3</sup>, <sup>1</sup>USDA-ARS, Beltsville, Maryland, <sup>2</sup>Valentis, Inc., The Woodlands, Texas, <sup>3</sup>Baylor College of Medicine, Houston, Texas.

The objective of this research was to determine whether directing expression of IGF-I specifically to striated muscle would alter the rate of lean and fat tissue accretion. Founder transgenic pigs were produced with a fusion gene composed of avian skeletal alpha-actin regulatory sequences and the cDNA encoding IGF-I. A founder transgenic boar (hybrid dam line) was mated to 12 non-transgenic gilts from two hybrid sire lines to produce G1 transgenic (TG) and sibling control progeny (CN). Pigs were provided feed ad libitum from weaning to 120 kg BW. At 90 and  $120~\mathrm{kg}$  BW,  $31~\mathrm{TG}$  and  $39~\mathrm{CN}$  pigs were an esthetized and scanned by dual-energy x-ray absorptiometry (DXA) to estimate fat and lean body composition of the live pigs. At 90 kg TG gilts had 16.4% less fat and 6.2% more lean tissue and TG barrows had 21.1% less fat and 8.4%more lean tissue than respective CN pigs (P < 0.01 for each). During subsequent growth from 90 to 120 kg, the rates of fat accretion were 20.6% and 23.7% lower for TG gilts and barrows than for respective CN pigs. Rates of lean tissue accretion were 30.3% and 31.7% higher for TG gilts and TG barrows, than for respective CN pigs. The rates of lean tissue accretion were 632 and 485 g/d for TG and CN gilts and 696 and 529 g/d for TG and CN barrows, respectively (TG vs. CN,  $\mathrm{P}=0.0001).$ The rates of fat tissue accretion were 242 and 305 g/d for TG and CN gilts and 300 and 393 g/d for TG and CN barrows, respectively (TG vs. CN, P = 0.0001). The IGF-I transgene affected progeny of both sire lines similarly in regard to enhancement of lean tissue accretion and reduced fat deposition. These results confirm our earlier findings that the targeting of IGF-I expression to striated muscles had a major impact on lean tissue accretion.

**122 IGF-I** and analogues can increase growth in artificially-reared neonatal pigs. F. R. Dunshea<sup>\*1</sup>, C. S. Chung<sup>2</sup>, P. C. Owens<sup>3</sup>, F. J. Ballard<sup>3</sup>, and P. E. Walton<sup>3</sup>, <sup>1</sup>Agriculture Victoria, Victorian Institute of Animal Science, Werribee, Australia, <sup>2</sup>Department of Animal Science, Chungbuk National University, Republic of Korea, <sup>3</sup>Cooperative Research Centre for Tissue Growth and Repair, Adelaide, Australia.

Exogenous insulin like growth factor-I (IGF-I) has been shown to increase growth rate in neonatal pigs while an analogue of IGF-I, LR3IGF-I, has been shown to be more potent than IGF-I in the rat. Therefore a study was conducted to determine whether IGF-I and LR3IGF-I increase growth in the artificially reared neonatal pig. Eighteen pigs (2 kg initial weight) pigs were infused with control, IGF-I (8  $\mu$ g/h) or LR3IGF-I (8  $\mu$ g/h) via osmotic pumps. After 9 days an additional pump was inserted to increase the infusion rates of each of the growth factors (16  $\mu$ /h) for a further 9 days, after which time the pigs were slaughtered. Pigs were offered bovine milk ad libitum. Neither IGF-I nor LR3IGF-I had any effect upon daily gain over the first 9 days of the study. However, over the second 9 days of the study, daily gain was increased in LR3IGF-I infused pigs (386 v. 457 g/d, P < 0.01), and tended to be increased in pigs infused with IGF-I (386 v. 413 g/d, P=0.15). As a result pigs infused with LR3IGF-I were heavier than the control pigs at slaughter. Milk intake was not different during the first 9 days of the study but was significantly greater in pigs infused with growth factors over the second half of the study (2905 v. 3407 g/d, P<0.01). Pigs treated with LR3IGF-I had a larger spleen (18.9 v. 27.5 g, P<0.001) and tended to have a larger liver (286 v. 319g, P=0.078), small intestine (329 v. 383 g, P=0.10) and combined small intestine and liver (614 v. 701 g, P=0.062) weight. Plasma IGFBP3 concentrations were highly correlated (R=0.85) with average daily gain over the 3 days preceding blood sampling. In conclusion, exogenous IGF-I and particularly LR3IGF-I can increase growth rate and visceral development in ad libitum fed artificially-reared pigs. At least part of the cause was an increase in milk consumption of infused piglets.

#### Key Words: IGF-I, Pig, Neonate

123 A GnRF vaccine (Improvac<sup>®</sup>) and porcine somatotropin have synergistic and additive effects on growth performance in group-housed boars and gilts, respectively. W. T. Oliver<sup>\*1</sup>, I. McCauley<sup>2</sup>, R. J. Harrell<sup>1</sup>, D. Suster<sup>2</sup>, and F. R. Dunshea<sup>2</sup>, <sup>1</sup>North Carolina State University, Raleigh, NC, <sup>2</sup>Agriculture Victoria, Victorian Institute of Animal Science, Werribee, Australia.

Two hundred and twenty four pigs (112 boars, 112 gilts) were used in a 2x2x2 factorial design, with the factors being vaccination with a GnRF vacccine, Improvac<sup>®</sup> (I; 0 or 2 ml at 13 and 17 wks of age), porcine somatotropin (pST; 0 or 5 mg/d from 17 wks of age), and gender. Pigs were weighed and feed disappearance was measured from 17 wks of age until slaughter at 21 wks of age. Body composition was measured by dual-energy X-ray absorptiometry at 17 and 21 wks of age. At slaughter, boars were heavier than gilts (100.7 vs 95.1 kg, P < 0.008). ADG was increased by both I (1109 vs 1244 g/d, P < 0.001) and pST (1109 vs 1199 g/d, P < 0.001). Boars treated with both I and pST gained at a faster rate (1404 g/d) than control and pST treated pigs (P < 0.001), and tended to grow at a faster rate than pigs receiving I alone ( $P{<}0.08).$ Overall, I increased ADG in gilts (1000 vs 1166 g/d, P<0.001). However, ADG for gilts treated with both pST and I was not significantly different from I or pST treated pigs. Treatment with I increased feed intake by 18 % (P < 0.01), but treatment with both I and pST resulted in feed intakes similar to pigs receiving neither treatment (P>0.5). Treatment with I increased fat gain (217 vs 301 g/d, P < 0.001), while fat gain for pST treated pigs was reduced (170 g/d, P < 0.001). Pigs receiving both I and pST had fat accretion rates similar to control pigs (P>0.4). While treatment with I or pST alone had no effect on lean gain in boars (P>0.2), boars treated with both I and pST tended to have higher lean accretion rates than control boars (707 vs 782 g/d, P < 0.09). Treatment of gilts with pST increased lean gain (485 vs 652 g/d, P < 0.001), but no difference was observed in gilts treated with I. Gilts treated with I and pST accrued lean mass at a rate similar to pST treated pigs (703 vs 652 g/d, P>0.2). In conclusion, the concomitant use of I and pST improves growth performance in boars and gilts.

#### Key Words: GnRF, pST, Swine

Key Words: Swine, IGF-I, Transgenic

124 Regulation of selection-induced growth hormone expression in porcine single trait selection lines. M.F.W. te Pas\*, J.W.M. Freriksen, A.J.H.M. van Bijnen, C.L.M. Gerritsen, T.J. van den Bosch, F.J. Verburg, A.H. Visscher, and K.H. de Greef, *Institute for Animal Science and Health, ID-Lelystad.* 

Selection for increased growth rate or decreased fat deposition in pigs is associated with changed growth hormone (GH) plasma levels. The molecular mechanisms regulating selection-induced changes remain largely unknown. This study aims at investigating selection-related changes in individual components of the GH expression. Associations between performance and GH expression parameters were evaluated in 2 pig selection lines selected for increased growth rate (F-line), or decreased back fat thickness (BFT) (L-line). We investigated changes in the mRNA levels of GH and its pituitary-specific transcription factor (Pit-1) in the pituitaries of slaughter pigs. In both lines GH and Pit-1 mRNA levels increased 50 % each generation. GH mRNA levels are pulsatile in vivo. GH mRNA levels were associated with BFT and growth rate in both lines. Pit-1 mRNA level was associated to BFT in the L-line. When GH and Pit-1 were fitted together in the model GH mBNA levels were significantly associated with growth rate and BFT. GH mRNA levels are directly associated with genetic constitution for BFT, but less or not with growth rate. GH blood plasma pulsatile pattern is characterized by (1) maximum peak value, (2) area under the curve, (3) pulse width, and (4) pulse interval. Both lines showed a declining GH peak maximum level and area under the GH curve. GH pulse width was not affected by selection. In both lines the pulse interval declined during selection, indicating that the number of pulses per day increased during selection with 1 pulse per 24 h per generation. Growth rate was associated with the GH plasma baseline value in both lines. Area under the curve and GH baseline level was significantly related to BFT. Line-specific effects were found for the baseline levels in the F-line. The results suggest that selection-induced GH synthesis is probably not sufficient for demanded blood plasma secretion resulting in smaller GH peaks and increased peak numbers per day.

Key Words: Pig, Growth Hormone, Expression

**125** Cloning, mapping, and functional analysis of porcine pituitary homeodomain transcription factor genes. S.J. Rhodes<sup>\*1</sup>, K.W. Sloop<sup>1</sup>, G.E. Parker<sup>1</sup>, T.P.L. Smith<sup>2</sup>, A.D. Showalter<sup>1</sup>, A.L. McCutchan Schiller<sup>1</sup>, J.R. Blanton Jr<sup>1</sup>, and G.A. Rohrer<sup>2</sup>, <sup>1</sup>Indiana University Purdue University Indianapolis, <sup>2</sup>US MARC Nebraska.

Hormones secreted from the pituitary gland mediate regulation of growth, lactation, reproduction, the stress response, and metabolic homeostasis. Recent studies have identified transcription factors that regulate the development and function of the pituitary gland in mice and humans. Our goal is to understand the molecular events that regulate pituitary development in swine. We have cloned, mapped, and analyzed the genes encoding the porcine Lhx3 (pLhx3) and Prop-1 (pProp-1) homeodomain transcription factors. In mice, Lhx3 is expressed in the developing nervous system, and then is restricted to the pituitary. Prop-1 expression is pituitary-specific in rodents. The pLhx3 and pProp-1 proteins are conserved within their protein/protein interaction and DNA-binding domains but exhibit less conservation outside these motifs. Assay of gene expression in whole early porcine embryos by RT-PCR revealed that the pLhx3 gene is activated at the time of neurogenesis. It then displays a biphasic expression pattern with expression increasing during the establishment of the pituitary and at the time of hormone gene activation. By contrast, pProp-1 is only observed at the times of pituitary organogenesis and then is down regulated to low levels. Northern analysis demonstrated that the pLhx3 gene produces a rare transcript in the adult pituitary. RNase protection analysis detected several pProp-1 mRNAs at low levels in the pituitary gland; expression was not found in other endocrine organs. Porcine Lhx3 activated the alpha-glycoprotein and prolactin promoters in transfection assays. Recombinant pLhx3 and pProp-1 proteins specifically bound to DNA sites in gelshift assays, and the pLhx3 protein specifically interacted with Pit-1 and other partner proteins in solution binding assays. We have mapped the protein elements required for the nuclear localization of pLhx3 and have demonstrated that pLhx3 is associated with the nuclear matrix. Genetic mapping experiments placed the pLhx3 and pProp-1 genes on the q arms of pig chromosomes 1 and 2, respectively. The mapping and analysis of swine pituitary regulatory genes will provide candidate genes for genetic analysis of growth, reproductive, and metabolic traits. Supported by USDA.

#### Key Words: Growth, Gene, Transcription

**126** Purification of porcine  $\beta$ -casein from milk by liquid chromatography, N-terminal sequencing, and antisera development. Adam C.W. Kauf\* and Ronald S. Kensinger, *Pennsylvania State University, University Park, Pennsylvania.* 

Sow 's milk is not capable of supporting the genetic potential for growth of modern piglets. A better understanding of the regulation of milk protein synthesis is desirable to improve sow milk, but is hindered by a lack of reagents to directly measure porcine milk proteins. Our objective was to purify porcine  $\beta$ -case in from sow's milk, determine N-terminal amino acid sequence, and develop specific polyclonal antisera against porcine  $\beta$ -case in. Milk was collected by hand milking from an oxytocin-treated crossbred Yorkshire sow on d 27 of lactation. Milk was defatted by centrifugation (5,000 x g, 15 min), and the case in fraction precipitated by incubation at pH 4.6. A casein-enriched fraction was isolated by centrifugation (5,860 x g, 60 min), washed three times, and lyophilized for storage at -20  $^{\circ}\mathrm{C}.$  For further fractionation, it was then dissolved (24 mg/ml) in 4 M Urea and 0.01 M Imidazole at pH 6.75. A volume of 1.5 ml was applied (1 ml/min) to a 5 ml Mono Q anion-exchange column (Bio-Rad Laboratories), and eluted by a stepwise gradient of NaCl between 0 and 500 mM in Urea/Imidazole buffer. Fractions from the 165 mM NaCl step were collected and pooled from five chromatographic runs.  $\beta$ -casein (9 mg) was isolated and assessed by electrophoresis (SDS-PAGE with 4 M Urea) to be greater than 95 % pure. Direct sequencing of the 14 N-terminal amino acids by Edman degradation yielded RA-KEELNASGETEV, which confirms the sequence predicted by Alexander and Beattie (1992) from cDNA analysis.  $\beta$ -casein (400 ug) was mixed with complete Freund's adjuvant and injected into rabbits, followed by three boosters (200 ug) with incomplete Freund's adjuvant at four week intervals. Antiserum from one rabbit on day 112 after primary immunization detected 100 ng  $\beta$ -case in by Western blot procedure when used at dilution of 1:2,000,000. A goal is to use this antiserum to detect subtle changes in  $\beta$ -case in secretion by porcine mammary tissue.

Key Words: porcine  $\beta$ -case in, an ion-exchange chromatography, polyclonal antisera

**127** Effect of dexamethasone treatment on growth in neonatal swine. J. S. Seaman<sup>\*1</sup>, E. P. Berg<sup>1</sup>, T. J. Safranski<sup>1</sup>, and J. A. Carroll<sup>2</sup>, <sup>1</sup>University of Missouri, Department of Animal Sciences, <sup>2</sup>Animal Physiology Research Unit, ARS-USDA, Columbia, MO.

We have previously reported that dexamethasone (Dex) treatment at birth enhances pre-weaning growth in piglets. The objective of the present study was to evaluate the effects of Dex treatment at birth on post-weaning piglet growth. Sixty-four crossbred piglets were assigned according to birth weight and sex to receive either sterile saline (Control; n=16 males and 17 females) or Dex (2 mg/kg body weight; n=14 males and 17 females) i.m. within 1 hour of birth. Using ANOVA, the statistical model included analysis of treatment, sex, and treatment x sex interactions for weight and ADG. Birth weights  $(1.543 \pm .270 \text{ kg})$ did not differ between Dex and Controls (P > .99) or males and females (P > .67). Weaning weights (21 days of age) were increased (P < .03)for Dex piglets (7.72  $\pm$  1.10 kg) compared to Controls (7.14  $\pm$  1.15 kg). At 7 weeks of age, piglets were moved out of the nursery. There was a treatment x sex interaction in 7-week weight (P < .07) such that no difference existed between Dex and Control females; however, Dex males (17.65  $\pm$  2.20 kg) were heavier (P < .08) than Control males (15.97  $\pm$ 2.67 kg). ADG through weaning was increased (P < .03) in Dex piglets  $(.293 \pm .048 \text{ kg/d})$  compared to Controls  $(.267 \pm .047 \text{ kg/d})$ . There was a treatment x sex interaction (P < .08) for ADG from birth to 7 weeks of age. No effect of Dex treatment was observed in females (P >.59), however, a trend was observed in males (P < .08) such that Dex increased ADG compared to Controls. Further research is underway to determine if this growth increase is maintained through the grow-finish phase, and if there are effects associated with carcass quality in Dex treatment. Given that the growth rate of the neonatal pig is considered to be less than half of its potential, the use of hormonal therapies such as Dex at birth to enhance growth rates could prove to be of significant economical importance to the swine industry.

Key Words: Dexamethasone, growth, piglets

128 Involvement of the type I and type II glucocorticoid receptors (GR) in growth hormone (GH) cell differentiation (GHDIFF) during chicken embryonic development. I. Bossis\* and T.E. Porter, University of Maryland, College Park MD USA.

GHDIFF occurs around day 14 of chicken embryonic development (e14) and is coincident with maturation of the adrenal glands. We have previously shown that corticosterone (CORT) can induce premature GHDIFF both in vitro and in vivo. In the present study, the GRs mediating the effects of CORT on GHDIFF were identified. Pituitary cells isolated from e12 embryos were cultured in the presence of vehicle (A), CORT at 1 nM (B), the type II GR antagonist ZK-98299 at 10  $\mu \rm M$  (C) and B + C in combination (D). Immunocytochemistry for GH was used to assess GHDIFF. Treatment with CORT significantly increased the population of somatotrophs, but the effect was not blocked by ZK-98299  $(1.2 \pm 0.3, 22.0 \pm 2.1, 1.0 \pm 0.2 \text{ and } 19.8 \pm 1.9 \%$  of all pituitary cells for A, B, C and D, respectively;  $P \le 0.05$ , n=4 experiments). However, treatment with ZK-98299 clearly reduced the intensity of GH staining. Subsequently, several steroids were tested for their ability to induce GHDIFF in vitro. Only glucocorticoids (CORT and dexamethasone) and aldosterone (ALDO) were effective, suggesting that both the type I (mineralocorticoid) and type II (glucocorticoid) GR might be involved. To test this hypothesis, e12 pituitary cells were cultured in the presence of vehicle (A), CORT at 1 nM (B), ALDO at 0.1 nM (C), CORT plus the mineralocorticoid receptor antagonist spironolactone (SPIRO) at 10  $\mu$ M (D), ALDO plus SPIRO (E), ALDO plus ZK-98299 at 10  $\mu$ M (F). CORT plus ZK-98299 and SPIRO at 10  $\mu$ M each (G) and ALDO plus ZK-98299 and SPIRO (H). Induction of GHDIFF by CORT and ALDO was not blocked by SPIRO or ZK-98299 alone. However, it was completely abolished in the presence of both GR antagonists (1.8  $\pm$  0.3, 23.6  $\pm$  3.1, 21.7  $\pm$  2.6, 20.9  $\pm$  2.3, 23.0  $\pm$  2.3, 22.7  $\pm$  2.9, 3.4  $\pm$  0.6 and 2.5  $\pm$  0.5 % of all pituitary cells for A, B, C, D, E, F, G and H, respectively; P < 0.05, n=4 experiments). We conclude that both the type I and type II GR mediate steroid-induced GHDIFF and that either GR type is sufficient for this response.

**Key Words:** Somatotroph differentiation, Glucocorticoid receptor, Mineralocorticoid receptor

## **129** Gene expression in sexually dimorphic muscles in sheep. R.G. Mateescu\* and M.L. Thonney, *Cornell University, Ithaca, NY.*

Testosterone is known to act differentially on skeletal muscle from different regions. Two genes likely to mediate the testosterone effect are insulin-like growth factor-I (IGF-I), an important growth regulator acting in an autocrine and paracrine way, and androgen receptor (AR), as receptor density could account for differential muscle growth. Another muscle-specific gene that may play a role in differential muscle growth is myostatin (MSTN), a member of the transforming growth factor-beta superfamily, shown to be a negative regulator of skeletal muscle mass. The objective of this study was to quantify and compare the expression of these three genes in two different skeletal muscles in sheep. Eleven Dorset rams were slaughtered after reaching puberty and total RNA was extracted from samples of semitendinosus (ST) and splenius (SP) muscles. IGF-I mRNA was measured using competitive reverse-transcription-polymerase chain reaction (RT-PCR). AR and MSTN mRNA were measured by ribonuclease protection assay (RPA) with standard curves. The means (attomoles/ $\mu$ g RNA) for SP and ST muscles were 1.39 and 1.02 (SE = 0.14), 4.05 and 2.96 (SE = 0.24), and 4.30 and 3.85 (SE = 0.37) for IGF-I, AR, and MSTN respectively. The difference between the two muscles was significant for IGF-I (P < 0.1) and AR (P<0.01) mRNA levels with higher expression in the SP but not for MSTN (P>0.4). Our results show that locally produced IGF-I and the regulation of AR expression may be important for sexually dimorphic muscle growth patterns.

Key Words: muscle, gene expression, sheep

**130** The Effect of Stage of Growth and Implant Exposure on Carcass Composition and Quality in Steers. K.W. Bruns\*, R.H. Pritchard, and T.A. Wittig, *South Dakota State University, Brookings, SD*.

Angus and Angus x Limousin steers (n=45) were used to evaluate the influence of an estradiol-trenbalone acetate implant (revalor-s) on the

relationship between percent intramuscular fat content (PIMF) and carcass composition when administered at specific stages of growth. Treatments were as follows: NI) control No Implant; EI) Early Implant, d 1, BW=295 kg; or DI) Delayed Implant, d 56, BW=385 kg. Steers were procured at weaning and were backgrounded (40 d) prior to the trial. Steers were fed a 90% concentrate diet for 150 d. Serial harvest was conducted at d 1 (n=5), d 56 (n=10) and at the end of the trial (n=30). Rib sections (9-10-11) were removed and analyzed for fat, protein, and moisture content to predict whole carcass composition. Regression equations were developed to quantify changes in carcass characteristics and composition throughout the feeding phase. At d 56 (n=10) EI increased HCW vs. NI (NI 227 vs. EI 243 kg; P<.05). Backfat, REA, YG, marbling score as well as whole carcass protein (%) and whole carcass fat (%) were not different. At harvest implants (I) increased (P< .10) final body weight (532 vs. 541 kg) and HCW (341 vs. 349 kg). Implants increased REA (75.4 vs. 82.74 cm<sup>2</sup>; P<.01) and decreased KPH (3.5 vs. 3.2%; P<.10). Yield grade components HCW, BF, REA, and KPH increased linearly. The subjective maturity and marbling scores were not affected by treatment and increased in a linear fashion. Treatment had no affect on the whole carcass proportions of fat, protein, or water. The PIMF of the L. dorsi was reduced (P < .10) by EI and was unaffected by DI (NI 5.1, EI 4.0, DI 4.8%). The PIMF content increased linearly and the slope of development was greater (P < .10) for NI vs EI. These data indicate that implanting will improve carcass weight and REA with no effect on carcass composition. Early implant exposure tended to reduce the rate of PIMF development but delaying implant until 385 kg BW increased carcass production without decreasing PIMF content.

Key Words: Beef, Implant, Carcass Composition

131 Lipogenic activity and adipose tissue cellularity in steers fed casein-formaldehyde-protected starch and(or) canola lipid. C. D. Gilbert\*, D. K. Lunt, and S. B. Smith, *Texas A&M University, College Station, TX.* 

Eighteen Brangus steers of similar live weight were assigned randomly to one of three dietary treatment groups: cracked corn (Corn), caseinformaldehyde-protected Canola Lipid (CL), or casein-formaldehydeprotected Marble Plus (MP). The purpose of the study was to determine if feeding protected starch and lipid increased lipogenic enzyme activities in i.m. adipose tissue more than in s.c. adipose tissue, thus increasing marbling scores without increasing yield grade. All diets were equally balanced for ME (291 Mcal/kg), crude protein (12.5%), and dry matter (89%). Ether extract was 3.7, 6.9, and 6.9% for the Corn, CL, and MP diets, respectively. The CL and MP diets provided equal amounts of protected lipid (3.3%). The MP also contained 3.7% protected starch. Steers were fed their respective diets for 126 to 130 d before slaughter. Beef carcasses from steers fed Corn, CL, or MP did not differ in yield grade or marbling score ( $P \ge 0.23$ ). Percentage KPH fat was higher (P < 0.05) for CL and MP carcasses than for Corn carcasses. Peak diameter (154 vs 98  $\mu \rm{m})$  and volume (160 vs 112 pL) were greater in s.c. than in i.m. adipose tissue (P < 0.05). Subcutaneous adipocyte peak diameter tended (P < 0.08) to be greater in CL steers (130  $\mu$ m) than in Corn steers (120  $\mu$ m), but peak volume did not differ (P = 0.55) among treatments. There were no treatment x tissue interactions for adipocyte volume or cells/g (P > 0.59). The addition of lipid to the diets (CL and MP combined effects) increased peak adipocyte diameter (P = 0.03). The activities of 6-phosphogluconate dehydrogenase, glucose-6-phosphate dehydrogenase, NADP-malate dehydrogenase, and fatty acid synthetase did not differ among treatment groups for either s.c. or i.m. adipose tissue (P > 0.05). We were not able to demonstrate a direct effect of protected starch and lipid on i.m. or s.c. lipogenic enzyme activities, marbling score, or yield grade.

31

Key Words: Steers, Lipogenesis, Protected Fat