

## Growth and Development III

**TH253 Fatty acids differentially regulate expression of deiodinases in differentiated pig adipocytes.** H. Yan\*, W. Hanxiao, H. Lu, O. Adeola, and K. M. Ajuwon, *Purdue University, West Lafayette, IN.*

Deiodinases regulate several aspects of thyroid hormone metabolism. Deiodinase 2 (DIO2), a selenoprotein, activates thyroid hormone by converting the prohormone thyroxine (T4) by outer ring deiodination (ORD) to bioactive 3,3',5-triiodothyronine (T3). Three deiodinases, DIO1, DIO2 and DIO3 are expressed in pig adipose tissue. However, only DIO2 is believed to be involved in thyroid hormone activation, whereas DIO3 inactivates thyroid hormone by inner ring deiodination of T3 and T4 to inactive metabolites, 3,3',5'-triiodothyronine (RT3) and 3,3'-diiodothyronine (T2), respectively. DIO2 results in activation of oxidative genes such as acyl CoA oxidase (ACO) and peroxisome proliferator-activated receptor gamma co-activator 1- $\alpha$  (PGC1 $\alpha$ ) and promotes muscle differentiation. DIO3 is an imprinted gene and a candidate gene for litter size in pigs. Because of these important functions, we investigated the expression of these genes in adipose tissue of neonatal piglets and in differentiated adipocytes. Expression of DIO2 is several fold higher than that of DIO1 and DIO3. DIO2 expression was higher (2 fold,  $P < 0.05$ ) in the adipose tissue of piglets with normal average birth weight vs. light weight littermates. Expression of DIO2, but not DIO1 and DIO3, increased during adipocyte differentiation and expression was maximal (3 fold) in fully differentiated adipocytes (D9 of differentiation) relative to preadipocytes. Differentiation of adipocytes in the presence of palmitate, oleic acid and docosahexaenoic acid (DHA) resulted in a higher (8 fold) expression of DIO2 with DHA treatment ( $P < 0.05$ ), but not palmitate nor oleic acid. The increase in DIO2 by DHA was accompanied by a higher expression of uncoupling protein 3 (UCP3). These results show that DHA can be used to regulate thyroid hormone activation to alter the direction of metabolism and growth in pigs.

**Key Words:** T3, deiodinase, lipids

**TH254 Comparison of growth performance and muscle fiber characteristics in different Japanese quail lines.** Y. M. Choi\*, S. Shin, M. P. Wick, and K. Lee, *The Ohio State University, Columbus.*

The objective of this study was to compare the growth performance and fiber characteristics of the pectoralis major muscle between the random bred control (RBC) and heavy weight (HW) Japanese quail lines to better understand the mechanisms leading to increased muscle mass. The hatch weights of the HW line were approximately 1.3 fold higher than that of the RBC line (8.15 vs. 6.43 g,  $P < 0.001$ ). After 15 d post-hatch, the body weight (BW) and pectoralis major muscle weight (PMW) exhibited remarkable differences between the 2 quail lines. At 42 d post-hatch, the HW line was about 2.3 fold greater in the BW (232.0 vs. 100.2 g,  $P < 0.001$ ), and had a higher percentage of the PMW compared with the RBC line (16.21 vs. 12.48%,  $P < 0.001$ ). Color differences were observed between the superficial and deep regions of the pectoralis major muscle, the deep region showed redder and darker surface than the superficial region in both quail lines. Smaller type IIA fibers were grouped in the central area of the muscle bundle and were surrounded by larger type IIB fibers in both lines. No significant differences were observed in the total fiber numbers of the pectoralis major muscle or the numbers of superficial and deep regions between the 2 quail lines. Moreover, there were no significant differences in the cross-sectional area (CSA) of type IIB fiber between the RBC and HW lines. The HW line exhibited a greater CSA of type IIA fiber (superficial region: 239.5 vs. 122.9  $\mu\text{m}^2$ ,  $P < 0.001$ ; deep region: 247.9

vs. 140.9  $\mu\text{m}^2$ ,  $P < 0.001$ ) and a higher area percentage of type IIB fiber (superficial region: 75.10 vs. 46.99%,  $P < 0.001$ ; deep region: 45.37 vs. 21.32%,  $P < 0.001$ ) in both regions compared with the RBC line. Therefore, greater body and muscle weights of the HW quail line are result of differences in muscle fiber characteristics, especially the proportion of type IIB fiber and the CSA of type IIA fibers, compared with the RBC line. The results of this study suggest that muscle fiber hypertrophy has more of an effect on body and muscle weights of the different quail lines than muscle fiber hyperplasia.

**Key Words:** growth performance, muscle fiber characteristic, Japanese quail

**TH255 Sequence identification of bovine microRNA in adipose tissue and their differential expression due to diet.** S. K. Duckett\*, M. D. Owens, and S. L. Pratt, *Clemson University, Clemson, SC.*

MicroRNA (miRNA) are small non-coding RNA which regulate adipocyte function via translational repression or RNA interference of targeted messenger RNA. The objectives of this study were to determine: 1) the identity of miRNA present in bovine adipose tissue using Illumina high-throughput sequencing technology, and 2) determine relative expression of specific miRNA by utilizing microarray procedures. Angus steers (18 mo of age and average BW of 289 kg) were finished on pasture only (PA; n = 3) or on a high-concentrate diet (C; 85% concentrate/15% roughage; n = 3). Subcutaneous fat samples (s.c.; 5 g per sample) were removed from the tail head area of each carcass at slaughter, rinsed with sterile saline, frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . Total cellular RNA was extracted using the mirVana miRNA Isolation Kit (Ambion, Austin, TX), with equal mass from each animal pooled and subjected to high-throughput sequencing procedures (LC Sciences, Houston, TX). MicroRNA sequences obtained from the sequencing data were used to generate custom uParaflo microfluidic arrays (LC Sciences, Houston, TX). Total cellular RNA from individual PA and C samples was used in a  $3 \times 3$  microarray to determine differences in expression levels of specific miRNA. RNA sequencing yielded 12,143,031 mappable reads of which 8,459,286 (69.7%) mapped to the bovine genome and were identified as known miRNA (n = 606). Another 123,340 (1.0%) reads mapped to the bovine genome and are predicted to form a hairpin indicating them to be previously unidentified miRNA (n = 814). Overall, sequencing identified 1,389 miRNA of which 42 are unique to cattle. The microarray contained 1,374 unique miRNA identified from RNA sequencing. Fifty-five miRNA were differentially expressed due to diet ( $P \leq 0.05$ ). Twenty-four and 31 miRNAs expression were decreased or increased, respectively, in the C group compared with the PA group. Therefore, multiple miRNA are expressed in bovine subcutaneous tissue and expression of specific miRNA can be altered due to diet further indicating a role for miRNA in lipid metabolism.

**Key Words:** microRNA, RNA sequencing, microarray

**TH256 Differential expression of multiple transcripts of Agouti-related peptide in avian species and its association with nutritional status.** C. Zhang\*, Y. M. Choi, Y. Suh, and K. Lee, *The Ohio State University, Columbus.*

Agouti-related peptide (AgRP) is an endogenous antagonist of melanocortin action. It is secreted by AgRP neuron in the arcuate nucleus, a major area of the brain to regulate food intake and energy homeostasis. Our bioinformatic

ics analysis demonstrated that there are 2 major isoforms—*a*- and *b*- isoform—in the chicken, turkey and quail. To confirm the multiple transcripts of AgRP in the avian species, we cloned the AgRP gene and localized the expression of the AgRP gene in various tissues of the chicken, turkey and quail. Both AgRP *a*- and *b*-isoform contain 3 exons in the coding sequence, but *a*-isoform has a unique exon in 5' UTR. RT-PCR analysis detected the AgRP mRNA in all tissues examined: the hypothalamus, heart, liver, kidney, skeletal muscle and adipose tissues. We detected that in quails the AgRP *a*-isoform showed the highest expression in the hypothalamus and the adipose tissue, with an undetectable expression in the other tissues examined, but the *b*-isoform was not expressed in all the tissues examined. Based on our RT-PCR analysis in the quail and the sequence alignment with chicken and turkey AgRP genomic DNA sequences, there is a deletion of about 500bp on AgRP sequences in the quail, resulting in the absence of a *b*-isoform expression. In the turkeys, both the AgRP *a*- and *b*-isoform was variously expressed in all the tissues examined except in the liver. In addition, the fast and re-feeding experiment was applied to determine how food intake regulates the expression level of AgRP mRNA in the avian species. Interestingly, the AgRP *a*- and *b*-isoform demonstrated a differential expression pattern in the peripheral tissues and the hypothalamus, and their expression pattern changed along the different nutritional status. These results imply that the varied expression pattern of AgRP *a*- and *b*-isoform is correlated with the different promoter structures of these isoforms in the 5' UTR, and might give us some hint to understand varied biological functions of both isoforms.

**Key Words:** agouti-related peptide, food intake, avian species

**TH257 Regulation of bovine  $G_0/G_1$  switch gene 2 (G0S2) and comparative gene identification-58 (CGI-58) genes.** J. Ahn<sup>1</sup>, X. Li<sup>1</sup>, Y. Suh<sup>1</sup>, S. S. Hwang<sup>2</sup>, and K. Lee<sup>1</sup>, <sup>1</sup>The Ohio State University, Columbus, <sup>2</sup>Rural Development Administration, Suwon, Republic of Korea.

Adipose triglyceride lipase (ATGL) regulates the initial step of the breakdown of stored lipid by hydrolyzing triacylglycerol (TAG). ATGL has been recently shown to be inhibited by the  $G_0/G_1$  switch gene 2 (G0S2) and co-activated by comparative gene identification-58 (CGI-58). Although regulation of ATGL-mediated lipolysis by G0S2 and CGI-58 may affect fat accretion and meat quality, G0S2 and CGI-58 have not been studied in cattle. The objectives of this study are to clone G0S2 and CGI-58 genes for cattle breeds, and to examine levels of G0S2 and CGI-58 expression in various tissues in relation to the regulation of ATGL. This study reports the cDNA and amino acid (AA) sequences of G0S2 and CGI-58 in 4 popular breeds of cattle (Angus, Holstein, Jersey, and Hanwoo;  $n = 2-3$ ). The expressions of full-length and isoforms of G0S2 and CGI-58 were detected by agarose gel electrophoresis. Total gene expressions in various tissues were quantified by real-time PCR. One-way ANOVA followed by Tukey's test was used to analyze differences in G0S2 gene expression. The results revealed that full-length bovine G0S2 is 294 base pairs (bp) coding for 98 AA (substitution of Ser in Hanwoo at residue 37 and Thr in Angus at residue 64) and bovine CGI-58 is 1,047 bp coding for 348 AA (substitution of Arg in Angus at residue 56 and Val in Hanwoo at residue 120). A more significant homology (~90%) of CGI-58 cDNA and AA sequences were found between cattle and the human and mouse than in G0S2 (~60%). Two unique alternatively spliced variants were identified in CGI-58. Because both CGI-58 isoforms lack lipid droplet (LD) binding domains, both variants may not enhance the activity of ATGL via association with LD. G0S2 was highly expressed in adipose tissue and liver, and total CGI-58 was predominantly expressed in adipose tissue and muscle ( $P < 0.05$ ). In conclusion, only full-length G0S2 and CGI-58 may be responsible for the regulation of ATGL. Considering the tissue-specific expressions, both G0S2 and CGI-58 may play important roles

in adipose tissue, but CGI-58 may be more involved in the regulation of intramuscular fat in muscle than G0S2.

**Key Words:** cattle, lipolysis, gene regulation

**TH258 Age-related changes in the expression of myogenesis-associated genes in the pig muscle.** D. Loesel<sup>\*1</sup>, A. Tuchscherer<sup>2</sup>, and C. Kalbe<sup>1</sup>, <sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Institute for Muscle Biology and Growth, Dummerstorf, Germany, <sup>2</sup>Leibniz Institute for Farm Animal Biology (FBN), Institute for Genetics and Biometry, Dummerstorf, Germany.

The number and size of skeletal muscle fibers are important aspects of postnatal growth, carcass and meat quality. Myofiber formation and hypertrophic growth depend on the proliferative activity of myogenic cells. The objective of this study was to monitor the ontogenetic expression of transcription factors associated with myogenesis. Gene expression was investigated in Semitendinosus muscle of German Landrace pigs at d 64 and 93 of gestation (dg), and at d 1, 7, 21, 28, 83, 145, 188, and 250 of age ( $n = 5$  per age) using qPCR and immunohistochemistry (assessed over an area of 0.7–1.4 mm<sup>2</sup> in the cross section). Data were analyzed using the MIXED procedure of SAS with the fixed factor age taking variance inhomogeneity at the age levels into account. Expression of satellite cell marker PAX7 decreased ( $P < 0.01$ ) from dg 64 until birth at mRNA (-82%) and protein level (-60%; given as number of positive nuclei per mm<sup>2</sup>). MYOD declined by 50% from dg 93 until birth ( $P < 0.05$ ) at the mRNA level and by 43% from dg 64 until birth ( $P < 0.05$ ) at the protein level. MYOG protein decreased ( $P < 0.05$ ), whereas mRNA expression was unchanged. From birth until d 28 PAX7 mRNA (-46%;  $P = 0.09$ ) and protein (-60%;  $P < 0.05$ ) were reduced. Both MYOD and MYOG mRNA were unchanged during this period, whereas MYOD ( $299 \pm 17$  vs.  $62 \pm 6$ ;  $P < 0.01$ ) and MYOG protein ( $321 \pm 34$  vs.  $45 \pm 11$ ;  $P < 0.05$ ) were reduced. Subsequently, the PAX7 and MYOD protein was lower at any age compared with d 28 ( $P < 0.05$ ) whereas the MYOG protein was not altered. The mRNA expression of PAX7 remained unchanged until d 250, whereas MYOD mRNA ( $P = 0.05$ ) and MYOG mRNA ( $P < 0.01$ ) were lowered at d 188 or d 145, respectively. The reduction of PAX7 and MYOD expression from the fetal stages until birth reflects the end of the 2 waves of prenatal myogenesis in pigs. In the early postnatal period the decreasing expression of PAX7 could indicate the involvement of satellite cells in the appearance of tertiary myofibers and the subsequent increase in total fiber number. After weaning muscle growth mainly results from myofiber hypertrophy. However, decreased MYOD and MYOG mRNA expression may indicate that fiber hypertrophy has stopped around d 145 of age.

**Key Words:** pig, gene expression

**TH259 Establishing lean and obese Mangalica pigs as a translational model for juvenile obesity and metabolic syndrome.** C. F. Garrett<sup>\*</sup>, R. H. Amin, C. L. Bratcher, E. P. Cambier, J. L. Bartosh, and T. D. Brandebourg, Auburn University, Auburn, AL.

Alabama is at the epicenter of an obesity epidemic precipitating increased incidences of type 2 diabetes and heart failure. However, no effective strategies exist for intervention or long-term prevention of obesity. To study the underlying mechanisms linking obesity and diabetes, the Mangalica pig was imported to Auburn University as a model of juvenile obesity given its extreme, early onset, morbidly obese phenotype. Obese or lean groups were created by either allowing ad libitum access to feed or restricting energy intake to 65% of ad libitum levels. Obese pigs exhibited 2.5-fold greater subcutaneous (SC) adipose tissue mass ( $P < 0.001$ ) but no differ-

ences in muscle mass ( $P < 0.39$ ) compared with their lean counterparts. Preliminary gene expression studies indicate leptin and TNF $\alpha$  mRNA expression levels were 2.1- and 2.54-fold higher in SC of obese versus lean pigs suggesting the development of inflamed adipose tissue ( $P < 0.01$ ). Obese pigs exhibited severe fasting hypoglycemia (29 mg/dl in obese vs 67 mg/dl in lean pigs,  $P < 0.01$ ), and altered liver enzyme levels consistent with development of hepatic dysfunction. Obese pigs also displayed impaired glucose tolerance following oral glucose challenge with 2 h glucose levels 3.2-fold higher in obese vs lean pigs ( $P < 0.01$ ), suggesting development of obesity-induced insulin resistance. Conditions have been established to image left ventricle thickness and ongoing studies are being conducted at the Auburn University MRI Research Center to characterize the effect that emerging obesity has upon ventricular wall thickening, systolic and diastolic ejection volumes and vessel wall thickness to assess obesity-induced cardiovascular phenotypes. The melanocortin-4 receptor (MC4R) gene has been cloned from Mangalica pigs and allelic variation has been analyzed across 19 unrelated pigs to date. These data provide evidence that obese Mangalica pigs indeed have a metabolic phenotype that mimics juvenile obesity-like characteristics, as evidenced by impaired glucose homeostasis and a proinflammatory shift in gene expression that is consistent with the development of frank diabetes.

**Key Words:** obesity, pig, Mangalica

**TH260 Selenium treatment promotes adipogenesis in chicken embryonic fibroblasts in vitro.** A. Lee\*, Y. Suh, and K. Lee, *The Ohio State University, Columbus.*

Poultry meat consumption has been increasing steadily worldwide due to increasing consumer demand and is of great economic value. Discovering nutritional methods to increase feed conversion to lean muscle may have great effect on the poultry industry by decreasing the cost of production. Selenium, which is a metalloid element that is necessary for proper body function, has been added to the adipogenic differentiation media to maximize the differentiation of primary pig, rat, and mouse preadipocytes into adipocytes. While it is known that selenium plays a role in preventing oxidative stress and protects against cancer, it is unknown what role selenium plays in adipogenesis. Embryonic chicken fibroblasts were cultured, grown to 80% confluence, and subsequently differentiated using an adipogenic cocktail and selenium supplementation at 0  $\mu\text{g/L}$ , 37.5  $\mu\text{g/L}$ , 75  $\mu\text{g/L}$ , 150  $\mu\text{g/L}$ , 225  $\mu\text{g/L}$ , and 300  $\mu\text{g/L}$ . Oil Red O staining demonstrated that selenium supplementation increases lipid droplet accumulation in a dose dependent manner. Quantitative Real-Time PCR analysis showed a dose dependent decrease in Pref-1 expression, and a dose dependent increase in PPAR $\gamma$  expression ( $P < 0.05$ ), indicating increasing adipogenesis with increasing selenium supplementation. Subsequent cell number counts showed that proliferation was inhibited by selenium in a dose dependent manner. These results indicate that selenium may play a role in cell cycle regulation by inhibiting proliferation and promoting differentiation of fibroblasts to adipocytes. Microarray analysis of 2 samples from the 0 $\mu\text{g/l}$  and the 225 $\mu\text{g/l}$  selenium treatments was conducted and the results analyzed. The expression levels of many genes involved in cell cycle regulation, adipogenesis, fatty acid metabolism, and oxidative stress were significantly altered ( $P < 0.05$ ). These results indicate that selenium plays a significant role in several mechanisms that lead to increased adipogenesis.

**Key Words:** selenium, adipogenesis, chicken embryonic fibroblast

**TH261 Potential role of the epidermal growth factor receptor in estradiol-induced alterations in proliferation, protein synthesis, and protein degradation in bovine satellite cell cultures.** B. C. Reiter\*,

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Estradiol (E2) enhances muscle growth in several species; however, the mechanism by which E2 enhances muscle growth is not known. Treatment with 10 nM E2 stimulates proliferation and protein synthesis and inhibits protein degradation in cultured bovine satellite cells (BSC). This is particularly significant because satellite cells are the source of nuclei needed to support postnatal muscle fiber hypertrophy and are thus crucial in determining the rate and extent of muscle growth; however the mechanism(s) responsible for these E2-induced effects is not clear. Studies in other tissues have suggested that E2 may stimulate proliferation by activating the G protein-coupled estrogen receptor (GPER-1; formerly known as G protein-coupled receptor 30). Activation of GPER-1 results in activation of matrix metalloproteinases which release heparin-binding epidermal growth factor (hbEGF) from the cell membrane and released hbEGF interacts with the epidermal growth factor receptor (EGFR) resulting in increased proliferation. As an initial step in determining if this mechanism is involved in the effects of E2 on bovine satellite cell cultures, we have examined the effect of the specific EGFR tyrosine kinase inhibitor, AG1478, on the ability of E2 to affect proliferation, protein synthesis and protein degradation in BSC cultures. Treatment of BSC cultures with 10  $\mu\text{M}$  AG1478 suppresses E2-stimulated proliferation ( $P < 0.05$ ). Similarly, AG1478 treatment suppresses E2-stimulated increases in protein synthesis rate in fused BSC cultures ( $P < 0.05$ ). In contrast, AG1478 treatment does not affect the ability of E2 to decrease protein degradation rate in fused BSC cultures. These data strongly suggest that E2-stimulated transactivation of EGFR is at least partially responsible for E2-induced increases in proliferation and protein synthesis rates in BSC cultures but is not involved in the E2-induced decrease in protein degradation.

**Key Words:** estradiol, satellite cell, EGF receptor

**TH262 Interactive effects of zinc and ractopamine hydrochloride on  $\beta$ -adrenergic receptor.** T. L. Harris\*<sup>1</sup>, A. D. Hosford<sup>1</sup>, M. J. Anderson<sup>1</sup>, C. K. Larson<sup>2</sup>, and B. J. Johnson<sup>1</sup>, <sup>1</sup>*Department of Animal and Food Sciences, Texas Tech University, Lubbock,* <sup>2</sup>*Zinpro Corporation, Eden Prairie, MN.*

$\beta$ -Adrenergic receptor ( $\beta$ AR) agonists are commonly used in the beef cattle industry to improve growth rate, feed efficiency and carcass characteristics. A commonly used growth promoter, ractopamine hydrochloride (RH), has been shown to increase muscle hypertrophy by binding to the  $\beta$ AR. When activated, the intracellular  $\beta$ AR signal causes an increase in cAMP levels, however, many factors affect the responsiveness of the  $\beta$ AR. Zinc has been shown to increase  $\beta$ AR affinity for agonists by allosterically binding to the  $\beta$ AR. The objective of this study was to determine if zinc (Zn), when added in combination with RH, would stabilize the interaction of RH with the  $\beta$ AR, indicated by altered cAMP concentration, mRNA quantity, or protein abundance. Cultured bovine muscle satellite cells were established and treated at 120 h for 6, 24, and 96 h. Treatments were applied in a  $2 \times 2$  factorial arrangement with 2 Zn levels (0 or 1  $\mu\text{M}$ ) and 2 RH levels (0 or 10  $\mu\text{M}$ ) in differentiation media. The cAMP levels were measured at 6, 24, and 96 h, while mRNA and protein were measured at 24 and 96 h. At 6 h, no differences ( $P > 0.05$ ) were detected in cAMP levels between the treatments. However, at 24 h the 10  $\mu\text{M}$  RH + 1  $\mu\text{M}$  Zn treatment had a greater concentration of cAMP ( $P < 0.05$ ) compared with all other treatments. At 96 h the 10  $\mu\text{M}$  RH + 0  $\mu\text{M}$  Zn treatment had a lower concentration of cAMP ( $P = 0.05$ ) compared with the control, but did not differ from other treatments. Using RT-QPCR analysis, no differences were detected in mRNA ( $\beta$ 1 adrenergic receptor,  $\beta$ 2 adrenergic receptor, AMPK $\alpha$ , myosin heavy chain I, myosin heavy chain IIA, and myosin heavy chain IIX) abundance between treatments for the genes evaluated. Protein abundance

was determined via Western blotting procedures to assess the quantity of  $\beta 1$  and  $\beta 2$  adrenergic receptors; however, no differences were detected in protein abundance between treatments. These results indicate that Zn in combination with a  $\beta$ -agonist, may help to sustain the response during prolonged exposure. These changes in cAMP response may prolong the biological response in skeletal muscle to  $\beta$ -adrenergic agonists, and appears to be independent of  $\beta$ AR protein abundance.

**Key Words:**  $\beta$ -agonist, cAMP, zinc

#### **TH263 Effect of castration methods on performance of beef cattle.**

A. D. Moreira<sup>1</sup>, F. D. Resende<sup>2</sup>, G. R. Siqueira<sup>2</sup>, J. F. Lage<sup>\*1</sup>, M. H. Moretti<sup>1</sup>, J. M. B. Benatti<sup>1</sup>, J. A. Alves Neto<sup>1</sup>, R. C. Silva<sup>1</sup>, and R. F. Marciel<sup>1</sup>, <sup>1</sup>Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil, <sup>2</sup>Agencia Paulista de Tecnologia dos Agronegocios, Colina, Sao Paulo, Brazil.

This objective of this study was to evaluate the effect of castration methods (surgical or immunological) on performance and body weight (BW) of beef cattle fed on pasture. Thirty animals 1/2 Angus  $\times$  1/2 Nellore with  $233.0 \pm 38.2$  kg of initial BW and 8 mo of age were fed in pasture of *Brachiaria brizantha* 'Marandu' receiving 3 g/kg BW/day of proteic-energetic supplement (25% crude protein and 60% of total digestible nutrients). Animals were supplemented for 356 d (20 d for adaptation and 336 d of experimental period), during the dry season (07/21/2011 - 11/03/2011) and the rainy season (11/04/2011 - 07/11/2012). The treatments comprised 10 replicates: non-castrated animals; surgical castrated and immunocastrated. The surgical castration was realized on last day of adaptation and the immunocastration was realized with Bopriva (anti-GnRH) being applied on the first day, 84 and 237 d after beginning of the experiment. Animals were weighted on each 42 d (maintained in collective pens and housed together in each treatment for 16 h in shrunk) to obtain the shrunk BW and average daily gain (ADG). Data were analyzed (randomly design) by the MIXED procedure of SAS and the Fisher test used considering 10% probability. Animals NC has greater ADG (0.798 kg;  $P = 0.016$ ) than those submitted to SC (0.694 kg) and IC (0.721 kg). After 168 d of experimental period, the animals NC showed greater BW and this difference were observed until the end of experiment (522.8 kg;  $P < 0.001$ ) than those animals submitted to SC (490.6 kg) or IC (482.1 kg). Expectedly, the surgical or immunocastration method has a negative effect on performance of beef cattle.

**Key Words:** anti-GnRH, beef cattle, immunocastration

#### **TH264 Effect of castration methods on tissue deposition of Angus $\times$ Nellore.**

A. D. Moreira<sup>1</sup>, J. F. Lage<sup>\*1</sup>, F. D. Resende<sup>2</sup>, G. R. Siqueira<sup>2</sup>, J. M. B. Benatti<sup>1</sup>, M. H. Moretti<sup>1</sup>, J. A. Alves Neto<sup>1</sup>, G. F. Berti<sup>3</sup>, P. H. Goncalves<sup>3</sup>, and M. A. P. Alves<sup>3</sup>, <sup>1</sup>Universidade Estadual Paulista, Jaboticabal, Sao Paulo, Brazil, <sup>2</sup>Agencia Paulista de Tecnologia dos Agronegocios, Colina, Sao Paulo, Brazil, <sup>3</sup>Centro Universitario de Barretos, Barretos, Sao Paulo, Brazil.

This objective of this study was to evaluate the effect of castration methods (surgical or immunological) on deposition of muscular and subcutaneous fat (SF) tissues of beef cattle fed on pasture. Thirty animals 1/2 Angus  $\times$  1/2 Nellore with  $233.0 \pm 38.2$  kg of initial BW and 8 mo of age were fed in pasture of *Brachiaria brizantha* 'Marandu' receiving 3 g/kg BW/day of proteic-energetic supplement (25% crude protein and 60% of total digestible nutrients). Animals were supplemented for 356 d (20 d for adaptation and 336 d of experimental period) during the dry season (07/21/2011 - 11/03/2011) and the rainy season (11/04/2011 - 07/11/2012). The treatments comprised 10 replicates: non-castrated animals (NC); surgi-

cal castrated (SC) and immunocastrated (IC). The surgical castration was realized on last day of adaptation and the immunocastration was realized with Bopriva (anti-GnRH) being applied on the first day, 84 and 237 d after beginning of the experiment. Animals were evaluated on beginning of experimental period and each 42 d, through the use of ultrasound. Images of ribeye area (REA) and SF were obtained and measured between the 12 and 13th ribs. Another image of SF were obtained in the intersection of the muscles gluteus medius and biceps femoris (P8FT) using the ultrasound ALOKA 500 V. Data were analyzed (randomly design) by the MIXED procedure of SAS and the Fisher test used considering 10% probability. Similar values of REA ( $P = 0.519$ ) were observed for animals NC (70.91 cm<sup>2</sup>), SC (66.36 cm<sup>2</sup>) and IC (70.72 cm<sup>2</sup>) and the daily deposition of REA ( $P = 0.110$ ). Animals SC has greater SF (7.72 mm;  $P < 0.001$ ) than those animals IC (5.93 mm) and animals NC (3.36 mm). Animals NC has lower P8FT (4.03 mm;  $P = 0.004$ ) than those animals IC (7.27 mm) and animals SC (8.81 mm). Animals SC showed greater daily deposition of RFT ( $P < 0.001$ ) and daily deposition of P8FT ( $P < 0.001$ ) than those animals NC and IC. The castration methods do not affect the deposition of muscle tissue, but increase the deposition of SF. The surgical method is most efficient for deposition of SF in beef cattle fed in pasture

**Key Words:** anti-GnRH, subcutaneous fat, ultrasound

#### **TH265 Effect of $\alpha$ -lipoic acid on in vitro differentiation of broiler chicks' myoblasts and adipoblasts.**

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$\alpha$ -Lipoic acid, a potent antioxidant, is an 8-carbon fatty acid. It is involved in mitochondrial biogenesis and in glucose uptake. In meat producing animals it has been reported to improve weight gain and carcass leanness, but it is not known whether the gain is through hyperplasia or hypertrophy of muscle and/or adipose tissue. The objective of this study was to evaluate the effect of  $\alpha$  lipoic acid in chicken myoblast and adipoblast differentiation in vitro, by measuring the expression of myogenin, and PPAR $\gamma$ , respectively. Seventy one-day-old broilers (Ross  $\times$  Ross strain) were slaughtered to obtain fibroblasts from muscle and adipose tissues, to start cell cultures, and differentiate them into the corresponding tissues. Cells were maintained in a proliferation medium for 4 d, until they reached a pre-confluent state (d 0). On the fifth day, the medium was changed to a differential medium (DM), and the experimental treatments were applied. One concentration (100  $\mu$ M) of lipoic acid was used in myoblasts; and 125  $\mu$ M, 250  $\mu$ M and 500  $\mu$ M into adipoblasts. At the end of differentiation, cells were harvested and total RNA was extracted from them. Single-strand cDNA was synthesized according to the manufacturer's instructions. RT was carried out using oligo (dT)12-18 primer and SuperScript II. Myogenin and PPAR $\gamma$  abundance measured by qPCR were used as an indicator of muscular or adipose differentiation, respectively. To normalize for the amount of cDNA, GAPDH and  $\beta$ -actin were amplified as the housekeeping genes, respectively. All data were analyzed using a completely randomized design. No significant differences were found ( $P > 0.1$ ) in myogenin and PPAR $\gamma$  expression. The results indicate that the doses of  $\alpha$ -lipoic acid used in this experiment did not induce myoblast or adipoblast differentiation in vitro, therefore it is suggested that the weight gain effect of lipoic acid observed in vivo was not necessarily related to cell differentiation.

**Key Words:**  $\alpha$ -lipoic acid, myoblast, adipoblast