

## Growth and Development II

**W212 Sodium butyrate induces adipocytic differentiation of porcine mesenchymal stem cells.** Benedetta Tugnoli<sup>1</sup>, Chiara Bernardini<sup>1</sup>, Monica Forni<sup>1</sup>, Andrea Piva<sup>1</sup>, Chad H. Stahl<sup>2</sup>, and Ester Grilli<sup>\*1</sup>, <sup>1</sup>DIMEVET, University of Bologna, Ozzano Emilia, Bologna, Italy, <sup>2</sup>Laboratory of Developmental Nutrition, College of Agriculture and Life Sciences, North Carolina State University, Raleigh, NC.

Sodium butyrate (SB) has been shown to affect the differentiation of mesenchymal stem cells (MSC) through the activation of different transcriptional pathways. The aim of this study was to investigate the effects of SB on the proliferation and differentiation potential of porcine bone marrow-derived MSC. Third passage MSC were verified by flow cytometry to be > 95% CD105<sup>+</sup>, CD90<sup>+</sup>, CD44<sup>+</sup>, CD45<sup>-</sup>, and CD34<sup>-</sup>. Cells were cultured in either a low glucose DMEM+10% FBS (BM), BM + 2.5 mM SB (SB2.5) or BM + 5 mM SB (SB5) in a randomized complete block design. Data were analyzed with 1-way ANOVA followed by linear and quadratic contrasts and the treatments had 6 independent replicates (n = 6). Cell proliferation was significantly decreased by both SB2.5 and SB5 after 48h (-55% on average,  $P = 0.001$ ) and 72h (-63% on average,  $P = 0.001$ ). To assess the impact of SB on spontaneous differentiation, MSC were cultured for 27 d, with complete media change every 3 d. At 3 d, SB treated cells showed changes in morphology compared with controls, from spindle-shaped fibroblast-like to larger sail-shaped cells with intracellular shiny droplets. At 27d, cells were stained for osteocytic (Alizarin Red), chondrocytic (Alcian Blue) and adipocytic (Oil-Red-O) differentiation. No terminal differentiation was detected in MSC cultured in the BM alone, while accumulated lipids were clearly stained by Oil-Red-O in MSC cultured in the presence of SB. The phenotypic changes observed from 3 to 27 d were supported by a change in the pattern of gene expression, analyzed by semiquantitative real-time PCR. At 3 d, both SB2.5 and SB5 increased mRNA expression of peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ;  $P = 0.05$ , linear effect) and decreased osteocalcin (OC;  $P = 0.02$ , quadratic) and aggrecan ( $P = 0.04$ , quadratic) mRNA levels compared with untreated cells. At 27d, there was an increase in PPAR $\gamma$  mRNA level ( $P = 0.02$ , linear) and decreased OC and aggrecan ( $P = 0.02$  and  $P = 0.002$ , respectively, both linear and quadratic) with SB treatment. To conclude, our data suggest that SB promotes the differentiation of porcine bone marrow-derived MSC toward an adipocytic lineage.

**Key Words:** sodium butyrate, mesenchymal stem cells, pig

**W214 Effect of butyrate on inflammatory and oxidative gene markers in porcine IPEC-J2 intestinal epithelial cells.** Hui Yan\* and Kolapo Ajuwon, Purdue University, West Lafayette, IN.

The small intestine is important for nutrient digestion and absorption as well as immune function. Newborns are sensitive to pathogen and diet-induced inflammation, which influences development of intestinal function. Butyrate has been shown to possess immune modulatory effect in various cells and tissues. But its effect on LPS-induced inflammation in the porcine IPEC-J2 intestinal cell model are unclear. Therefore, we investigated the effects of butyrate on expression of cytokines and metabolic gene markers in this cell line. A randomized complete block design was used. Main effect was the different concentrations of butyrate and replicate was used as the blocking factor. There were at least 6 replicates per treatment. Data were analyzed using PROC GLM. Gene expression was determined by RT-PCR. High concentration of butyrate (1mM) significantly increased ( $P < 0.05$ ) LPS-induced TNF $\alpha$ , IL-6, IL-8

and MCP1 expression, and this indicated that this concentration was harmful to the cells. However, lower concentrations of butyrate (10  $\mu$ M to 100  $\mu$ M) significantly decreased ( $P < 0.05$ ) LPS-induced MCP-1 and TLR4 expression (100  $\mu$ M concentration) and IL-1 $\beta$  (50  $\mu$ M) compared with control. Butyrate treatment also significantly increased expression of metabolic genes such as ACO (100  $\mu$ M) and PPAR $\alpha$  (10 to 100  $\mu$ M), indicating that butyrate increases energy expenditure. LPS treatment significantly reduced ACO expression and eliminated the effect of butyrate on ACO and PPAR $\alpha$  expression. Taken together, butyrate exhibited limited anti-inflammatory effect in IPEC-J2 cells, but strongly enhanced oxidative capacity through induction of oxidative genes.

**Key Words:** IPEC-J2, butyrate, inflammation.

**W215 MicroRNA exert a role in the process of arginine promoting rat mammary gland development.** Lianmin Chen<sup>\*1</sup>, Liangyu Hu<sup>1</sup>, Mengzhi Wang<sup>1</sup>, J. J. Loo<sup>2</sup>, Hongrong Wang<sup>1</sup>, and Lihuai Yu<sup>1</sup>, <sup>1</sup>College of animal science and technology, Yangzhou University, Yangzhou, Jiangsu Province, China, <sup>2</sup>University of Illinois, Urbana, IL.

Except for its use during milk protein synthesis, extra arginine (Arg) might exert other unknown biological or metabolic functions in the mammary gland. Previous work focused on the effect of Arg on bovine mammary epithelial cell casein production showed that 556.00 mg/L Arg (2  $\times$  the basal level) in the medium elicited the greatest stimulation of casein and mTOR-related genes. Whether Arg regulates cell development via effects on microRNA (miR) is unclear. The specific objective was to determine if miR expression in rat mammary tissue are altered by feeding 2  $\times$  Arg. Twelve pregnant littermate Wistar rats were randomly divided into 2 groups and experimental diets designed according to the AIN-93G purified diets. The Arg concentration was 12.8 g/kg in the Arg group while in the Control was 6.4 g/kg. A total of 21.62 g/kg glutamic acid was added to the Control group to keep diets iso-nitrogenous. At 17 d postpartum rats were killed and the mammary tissue harvested. Histological changes were measured by paraffin section, and mammary acinar area measured using digital imaging system software (MC30). Statistical analysis was carried out via *t*-test using SPSS16.0. Differentially expressed miR were measured using Solexa miR-Seq and verified by RT-qPCR. The miR target genes were analyzed using SBC prediction wizard (TargetScan, miRanda, PicTar, MirTarget2, PITA supported). Gene functions were analyzed via DAVID 6.7 and GO analysis. The mammary acinar area was significantly greater ( $P < 0.001$ ) due to Arg (452.71  $\mu$ m<sup>2</sup>) compared with the Control (388.68  $\mu$ m<sup>2</sup>). A total of 8 miR had greater ( $P < 0.05$ , fold-change > 2) expression in Arg compared with the Control. Among those upregulated, miR-1-3p plays a role in the control of cellular component synthesis; miR-133a-3p and miR-133a-5p play a role in the regulation of cell developmental process; miR-133b-3p exerts control of transporter activity and enzyme activity; and miR-206-3p has an important role in the regulation of cellular processes. There was no clear cellular function prediction for the upregulated miR such as miR-149-5p, miR-1b and miR-486. Results suggest dietary Arg might promote mammary tissue development through altering miR expression. The exact regulatory mechanisms need further investigation.

**Key Words:** micro RNA, arginine, development of mammary

**W216 Poor maternal nutrition decreases longissimus dorsi cross sectional area of fetal offspring at d 45 of gestation.** Joseline S. Raja\*, Sambhu M. Pillai, Amanda K. Jones, Maria L. Hoffman, Katelyn K. McFadden, Kristen E. Govoni, Steven A. Zinn, and Sarah A. Reed, *Department of Animal Science, University of Connecticut, Storrs, CT.*

Poor maternal nutrition during gestation results in long-term postnatal changes in muscle morphometrics, muscle mass and fiber number, and intramuscular adipose deposition of the offspring. Thus, we hypothesized that under- and over-feeding ewes during gestation would inhibit fetal muscle development. To test this hypothesis, 82 pregnant Western Whiteface ewes were individually housed and fed 100%, 60%, or 140% of NRC requirements for TDN beginning at d 30.2 ± 0.2 of gestation. Offspring (CON, RES, and OVER, respectively), were necropsied at d 45, 90, and 135 of gestation and within 24 h of birth. At d 45 of gestation (n = 7 ewes per diet), the triceps brachii (TB), semitendinosus (STN), and longissimus (LM) muscles were weighed. The LM were frozen and cryosectioned to determine muscle fiber cross sectional area (CSA) and the number of muscle fibers per  $\mu\text{m}^2$ . Muscle weight was expressed as percent of fetal weight and data were analyzed using the MIXED procedure of SAS, with maternal diet as the main effect. Fetal weight tended to be less in OVER compared with CON ( $P = 0.07$ ; CON: 11.0 ± 0.6 g, RES: 10.1 ± 0.5 g, OVER: 9.2 ± 0.5 g). No differences in the weight of TB, STN, or LM due to maternal diet were detected ( $P \geq 0.53$ ). The CSA of LM (n = 6 fetuses per treatment [2 fetuses per ewe]) from OVER and RES were smaller compared with CON ( $P \leq 0.008$ ; CON: 287.1 ± 3.2  $\mu\text{m}^2$ ; RES: 223.4 ± 2.9  $\mu\text{m}^2$ ; OVER: 254.9 ± 3.2  $\mu\text{m}^2$ ). However, the number of fibers per  $\mu\text{m}^2$  of LM was not different due to maternal diet ( $P = 0.47$ ). Smaller muscle fiber CSA could result from inadequate protein accretion, decreased fusion of myogenic precursor cells, or both. Similar fiber numbers suggest increased interfibrillar space, which may lead to increased connective tissue formation. In conclusion, poor maternal nutrition during early gestation alters the formation of muscle fibers as early as d45, potentially resulting in decreased muscle mass at birth.

**Key Words:** muscle, maternal nutrition

**W217 The effects of maternal under- and over-feeding on muscle development of lambs as determined by RNA-Seq analysis.** Maria L. Hoffman\*, Kristen N. Peck, Jill L. Wegryzn, Sarah A. Reed, Steven A. Zinn, and Kristen E. Govoni, *University of Connecticut, Storrs, CT.*

We previously reported that both maternal under- and over-feeding altered muscle cross sectional area and lipid content in offspring. Therefore, we hypothesized that poor maternal nutrition would alter key pathways and novel genes involved in muscle development of lambs. Ewes (n = 3/treatment) were fed 100% (CON), 60% (RES), or 140% (OVER) of NRC requirements for TDN and one twin lamb per ewe was euthanized within 24 h of birth and muscle tissue was collected for RNA-Seq analysis. RNA was prepared into cDNA libraries and sequenced using the Ion Torrent Proton. Data were aligned to the *Bos taurus* (Btau\_4.61) and *Ovis aries* (Oar\_V.3.1) reference genomes using Tophat and expression evaluated with Cufflinks. Differential gene expression was determined using the Benjamini-Hochberg multiple testing correction ( $q \leq 0.05$ ). Using the ovine and bovine references, 10 and 35 differentially expressed genes were identified, respectively. The differentially expressed genes are involved in metabolic regulation, hypertrophy, nutrient uptake, and protein turnover. Using the ovine reference, compared with CON, expression of *myogenic factor 6*, and *myostatin* increased 1.7 and 2.0-fold in OVER ( $q \leq 0.05$ ). *BTG family member 2*, *ankyrin repeat domain 1*, and *phosphoserine phosphatase*

expression decreased 1.5, 2.5, and 3.1-fold, respectively in OVER ( $q \leq 0.05$ ). Expression of *FHJ murine osteosarcoma viral oncogene homolog (FOS)* was reduced 1.6-fold in RES compared with CON ( $q = 0.05$ ). Using the bovine annotation, compared with CON, *arrestin domain containing 2* and *AT rich interactive domain 5B* expression were 3.5 and 2.9-fold greater, respectively in OVER ( $q = 0.05$ ). Expression of *PPAR $\gamma$  coactivator 1  $\alpha$* , *regulator of G-protein signaling 16*, *thrombomodulin*, and *jun B proto-oncogene* were reduced  $\geq 2$ -fold ( $q \leq 0.05$ ) in OVER. *Tripartit motif containing 63*, *FOS* and *suppressor of cytokine signal 3* expression were reduced 1.4, 1.6, and 2.8-fold, respectively in RES ( $q \leq 0.05$ ). In conclusion, although both under- and over-feeding had similar effects on offspring muscle development, based on gene expression, the mechanisms through which this occurs appear to be different.

**Key Words:** transcriptome, muscle, sheep

**W218 Muscle fiber hypertrophy is associated with increased expression of key transcriptional and epigenome regulatory genes.** Yue Lu, Jennifer S. Bradley, Sarah R. McCoski, Adam J. Geiger, R. Michael Akers, Alan D. Ealy, and Sally E. Johnson\*, *Virginia Polytechnic Institute and State University, Blacksburg, VA.*

Early neonatal nutrition affects the rate and extent of muscle growth and fiber hypertrophy in calves. The objective of the experiment was to examine the effect of caloric intake on LM, infraspinatus (INF) and semitendinosus (ST) fiber cross-sectional area (CSA), fiber type and muscle gene expression in neonatal heifers. Newborn Holstein heifers were assigned to a high nutritional plane (HNP; 28% CP, 25% fat, n = 5) or low nutritional plane (LNP; 20% CP, 20% fat, n = 5) milk replacer diet for 8 wks. Calves were pair-fed equivalent amounts of grain (25% CP, 4% fat) beginning at wk 5. Caloric intake supported ADG of 220 g/d and 771 g/d for the LNP and HNP heifers, respectively. Heifers were euthanized at 8 wks of age and samples of LM, INF and ST were collected. Gene expression was measured by RT-qPCR. Data were analyzed by ANOVA for the main effect of diet, muscle and their interaction with post-hoc Tukey separation of means. Plane of nutrition differentially affected muscle fiber hypertrophy with HNP LM and ST fiber cross-sectional area (CSA) larger ( $P < 0.05$ ) than LNP. No differences in CSA were noted between HNP and LNP INF. The smaller LNP LM and LNP ST sizes were reflected by greater ( $P < 0.05$ ) numbers of fibers/mm<sup>2</sup>. *Pax7* expression is 38% greater ( $P < 0.05$ ) in HNP LM than LNP LM and *BTG2*, *E2F6* and *myogenin* mRNA amounts are 60% greater ( $P < 0.05$ ) suggesting that muscle stem and progenitor cells are active and supplying the growing fiber with myonuclei. Messenger levels of *IGF-1* are greater ( $P < 0.05$ ) in HNP LM than LNP LM further supporting increased fiber hypertrophy. No differences ( $P > 0.05$ ) were observed in the protein degradation enzyme genes, *FOXO3*, *atrogin* or *MuRF*, indicating that protein accretion is due to elevated protein synthesis. Diet can serve as an epigenetic modifier of phenotype. Calves maintained on the LNP diet expressed lower ( $P < 0.05$ ) amounts of the histone deacetylase genes, *HDAC1* and *HDAC3*, and the lysine-specific demethylase, *KDM2A*, in the LM than their HNP counterparts. Altered expression of epigenome modifiers provides evidence for diet-induced changes in the muscle fiber chromatin architecture that may contribute to long-term metabolic responses.

**Key Words:** skeletal muscle, hypertrophy, epigenome

### W219 Myogenic regulatory factors are increased in bovine satellite cells by polyamines and their precursor amino acid ornithine.

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Skeletal muscle is unique in its growth and developmental characteristics in that normal hypertrophy is carried out through activation and fusion of satellite cells (SC) surrounding individual muscle fibers. These SC are responsive to signals from their extracellular environment especially growth factors and nutrients. Polyamines (PA) are a class of small, positively charged molecules that favorably affect growing cells through somewhat undefined physiological mechanisms. Polyamines exist in mammalian species primarily as ornithine derivatives. Abnormalities in polyamine metabolism are associated with irregular development in mammals emphasizing their critical role as regulators of growth. To test the direct effect of PA on skeletal muscle development, primary bovine SC cultures were differentiated in media containing either methionine (10 mM control 1), ornithine (10 mM), putrescine (5 mM), spermine (0.5 mM), or no supplement (control 2). SC were differentiated as a monoculture (MC) as well as in coculture (CC) alongside preadipocytes isolated from bovine intramuscular fat depots. This coculture provided a more realistic environment of that which would surround skeletal muscle and impact SC activity. Following treatment, SC were isolated from CC using laser micro-dissection technique. SC populations were analyzed for temporal expression of the myogenic regulatory factors (MRF) *MyoD*, *Myf5*, and *Myf4* (myogenin) to identify differentiating cells along with the genes *Pax7* and *Spry1* representative of quiescent cells. Protein isolation and Western Blot analyses were also performed to measure protein expression in a temporal manner. Exposure of SC's to PA in CC resulted in upregulation of *MyoD* ( $P = 0.05$ ), *Myf5* ( $P = 0.02$ ), and *Myf4* ( $P = 0.09$ ) and attenuation of *Pax7* ( $P = 0.10$ ) and *Spry1* ( $P = 0.07$ ). Statistics generated in SAS (Cary, NC) using PROC MIXED procedure. Treatment was included as a fixed effect. These results suggest that ornithine metabolism and polyamine metabolites can affect bovine skeletal muscle myogenesis, and may therefore be promising as candidates for natural means of promoting growth of lean tissue in cattle.

**Key Words:** polyamine, satellite cell, myogenic regulatory factor

### W220 Muscle gene expression patterns in finishing steers supplemented with dietary A maize (*Aspergillus oryzae* extract).

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We evaluated the effect of dietary *Aspergillus oryzae* extract on gene expression profiles in the *Longissimus lumborum* from finishing steers. Cross-bred (Simmental × Angus) yearling steers were randomly assigned to one of 2 groups ( $n = 10$ /treatment). From receiving until d21, starter and step-up diets were fed to acclimate steers to a traditional mid-west finishing diet. Steers were then fed *ad libitum* to meet or exceed NRC requirements until slaughter (d140): basal diet with or without 5 g/hd/d of *A. oryzae* extract (Amaize, Alltech Inc., Nicholasville, KY) containing  $\alpha$ -amylase (AMZ). On d90, *Longissimus lumborum* tissue was collected for gene expression analysis. Serum was collected at d 40, 90 and 140 for analysis of metabolites (BHBA, glucose, insulin, urea). Data from blood metabolites was analyzed using a mixed model. Gene expression was profiled using the Affymetrix Bovine Gene 1.0 ST Array. Performance did not differ between treatments. Hierarchical clustering indicated a treatment effect ( $P < 0.05$ ) of the AMZ-supplemented group compared with the control. A total of 1148 genes were differentially affected ( $P$

$< 0.05$ ; 430 upregulated; 718 downregulated) between treatments. The genes affected, enriched ( $P < 0.05$ ) and activated several pathways, including IGF-1 signaling, insulin receptor signaling, valine degradation, and VDR/RXR activation. The pathways activated are involved in the regulation of muscle development and growth. Blood metabolites indicated greater levels of BHBA, urea and insulin ( $P < 0.05$ ) at d40 in AMZ-supplemented steers. Only insulin remained at a greater concentration in the AMZ group than the control throughout the experiment. In conclusion, AMZ supplementation in the finishing diet affects muscle gene expression and insulin metabolisms, potentially causing a positive effect for the development of skeletal muscle in finishing steers.

**Key Words:** amylase, muscle, cattle

### W221 Investigation of effects of maternal nutrition intensification and fetal sex on development of skeletal muscle of bovine fetuses.

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This study aimed to evaluate the effects of maternal feeding level (MN) and fetal sex (FS) on skeletal muscle development of bovine fetuses at different stages of gestation (SG). Fourty-4 multiparous, dry Holstein × Gyr cows with average initial body weight of  $480 \pm 10$  kg were fed either restricted feeding at 1.15% of body weight (CO,  $n = 24$ ) or *ad libitum* (ON,  $n = 20$ ) with the same diet (93% corn silage and 7% concentrate; 111 g/kg of CP and 674 g/kg of TDN). Eleven cows of each dietary treatment were slaughtered at 139, 199, 241 and 268 d of gestation. Fetuses were necropsied to evaluate the development of skeletal muscle. Data were analyzed by MIXED procedure of SAS considering the fixed effects of MN, FS and SG ( $2 \times 2 \times 4$  factorial). Modifications in gene expression of skeletal muscle of fetuses were observed in function of MN and FS despite the lack of effect of MN ( $P = 0.330$ ) and FS ( $P = 0.518$ ) on fetal weight. The muscle mRNA expression of myogenic markers  $\beta$ -catenin and MyoD was greater in male than in female fetuses, as well the expression of all adipogenic markers evaluated (Zfp423, C/EBP $\alpha$  and PPAR $\gamma$ ), 3 of the 4 fibrogenic markers evaluated (Collagen I, Collagen III and Fibronectin) and the number of myocytes in muscle. Marginal effects of MN were observed on mRNA expression as well on the phenotypic indicators of myogenesis, adipogenesis and fibrogenesis. At the mid-gestation (139 DG)  $\beta$ -Catenin, Zfp423 and PPAR $\gamma$  expression and myocytes number were greater in ON than in CO fetuses and in males than in females, but these differences were not observed at subsequent SG. Fat content of fetal muscle was not affected by MN and FS. Almost all myogenic, adipogenic and fibrogenic markers were less expressed in late gestation than in mid-gestation, however collagen deposition, fat and crude protein content of fetal muscle were greater at late gestation than in mid-gestation. The MN changed gene expression of myogenic, adipogenic and fibrogenic markers at mid-gestation (greater in ON than in CO) but some compensatory gene expression made the effect of MN not significant in late gestation.

**Key Words:** adipogenesis, fetal programming, fibrogenesis

### W222 High-energy diet reduced myogenic gene expression of Hanwoo steers fed to three different endpoints.

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High energy diets have been used for enhancing marbling fat in high quality beef cattle. However, this has been associated with reduced yield grade when the steers were fed for long-term. The aim of this experiment was to determine the effect of additional 3% TDN (77%) diet compared with control diet (74%) on the level of adipogenic and myogenic gene expressions at 3 different endpoints. A 2 × 3 factorial arrangement (High, Control, and 26, 28, 30 mo endpoints) was used to feed 48 Hanwoo steers. Four steers were fed in same pen and 12 pens were used for treatment. Longissimus dorsi (L.D.) muscle was collected within 10 min of harvest for analysis of PPAR $\alpha$ , SCD, GLUT4, MHC1, MHC2X, and myogenin mRNA abundance. Real-Time RT-PCR was used to measure the quantity of respective mRNA relative to a ribosomal protein subunit 9 (RPS9) mRNA. Data were analyzed as a completely randomized design using the MIXED model, each treatment performed in triplicate. Difference between control and treatments were determined using the LSD procedure. Overall ADG did not differ between high-energy diet or control diet ( $P > 0.05$ ). ADG tend to increase in high-energy diet at 30 mo endpoint compared with control diet ( $P = 0.09$ ). Marbling score and carcass weight were greater at 30-mo endpoint than at other endpoints ( $P < 0.05$ ). Yield grade was not different among endpoints ( $P > 0.05$ ). Percentage of protein in L.D. muscle tend to decrease at 30 mo endpoints ( $P = 0.09$ ). Real-time quantitative PCR revealed that the mRNA content of MHC2X in muscle from high-energy diet cattle decreased (diet × endpoint,  $P < 0.05$ ) as compared with the control group. There was no interaction for MHC1 gene expression among 3 endpoints ( $P > 0.05$ ). These data indicated that high-energy diet decreased relative mRNA level of MHC2X on long-fattening periods. These fast-glycolytic genes may affect muscle protein composition in Hanwoo L.D. muscle.

**Key Words:** Hanwoo, myogenic, gene expression

#### **W223 $\alpha$ -Solanine induces myogenesis of bovine satellite cells isolated from semimembranosus and longissimus muscle tissue.**

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Solanine is a glycoalkaloid compound found in leaves, fruit and tubers of potato, tomato, and eggplant. Solanine poisoning was reported in various animal models such as mice, rabbit, and chicken. However, it is used as a treatment for human asthma. Some medicine used for treating human asthma has been used to induce myogenesis of bovine skeletal muscle. We hypothesized that  $\alpha$ -solanine may affect myogenesis of bovine satellite cells isolated from different muscle depots. Bovine satellite cells were pronase-liberated from semimembranosus (SM) and longissimus dorsi (LD) muscle tissues of 3 newborn Hanwoo calves. Bovine SM and LD satellite cells were incubated with dulbecco modified eagle medium (DMEM) with 10% fetal bovine serum for proliferation and induced differentiation with DMEM with 3% horse serum. Bovine satellite cells were treated with various levels of  $\alpha$ -solanine (control, 0.001, 0.01, 0.1, 1, and 10  $\mu$ M). mRNA abundance for myosin heavy chain 1 (MHC1), MHC2X, glucose transporter 4 (GLUT4), myogenin, G-coupled protein receptor 43 (GPR43), and  $\beta$ 2-adrenergic receptor ( $\beta$ 2-

AR) were measured by real-time quantitative PCR. Data were analyzed as a completely randomized design using the MIXED model, each treatment performed in triplicate. Means were considered different at  $P < 0.05$ . Relative MHC2X mRNA abundance was greater in both, SM and LD satellite cells with 1  $\mu$ M solanine treatments compared with the control ( $P < 0.05$ ). Relative level of MHC2X and GLUT4 were decreased in 10  $\mu$ M solanine treatments ( $P < 0.05$ ). Relative level of MHC2X and  $\beta$ 2-AR were greater in SM satellite cell compared with LD satellite cells ( $P < 0.05$ ). There was no tissue × dose interaction among MHC1 mRNA concentration ( $P > 0.05$ ). These result indicated that  $\alpha$ -solanine have a dose-dependent effect on MHC2X mRNA but did not affect to MHC1 mRNA in bovine satellite cell.

**Key Words:**  $\alpha$ -solanine, Hanwoo, semimembranosus

#### **W224 Role of epidermal growth factor receptor and erbB2 in trenbolone acetate mediated increases in bovine satellite cell proliferation and protein synthesis and decreases in protein degradation.**

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Trenbolone acetate, TBA, has been shown to increase proliferation and protein synthesis rates and decrease protein degradation rate in bovine satellite cell (BSC) cultures. Although we have previously shown that the androgen receptor is involved in these TBA effects, our current data show that 2 receptors from the erbB family, epidermal growth factor receptor (EGFR) and erbB2, also play a role in the effects of TBA on BSC proliferation, protein synthesis and protein degradation in BSC cultures. We have assessed the effects of treating BSC with AG1478, a specific EGFR tyrosine kinase inhibitor, and/or AG879, a specific erbB2 tyrosine kinase inhibitor, on the ability of TBA to affect proliferation rate (<sup>3</sup>H-Thymidine incorporation) in proliferating BSC cultures and to affect protein synthesis rate (<sup>3</sup>H-phenylalanine incorporation/mg protein) and percent protein degradation in fused BSC cultures. Statistics were done using Proc MIXED in SAS; the model included treatment as a fixed effect and experiment, BSC number and experiment replicate as random effects. Treatment with AG1478, AG879 or a combination of AG1478/AG879 significantly ( $P < 0.05$ ) suppressed TBA-induced increases in proliferation. Treatment with AG1478 significantly ( $P < 0.05$ ) suppressed specific TBA-induced increases in protein synthesis. Treatment with AG879 or a combination of AG879/1478 also decreased TBA-induced increases in protein synthesis ( $P < 0.05$ ) although the effects of AG879 or AG879/1478 may be non-specific as there was also a significant ( $P < 0.05$ ) decrease in protein synthesis rate in control cells (not treated with TBA) receiving these treatments. While treatment with either AG1478 or AG879 alone had no effect on the ability of TBA to reduce protein degradation rate in fused BSC cultures, treatment with a combination of AG879/1478 significantly ( $P < 0.05$ ) suppressed TBA-induced decreases in protein degradation rates. In summary our results show that receptors from the Erb family are necessary to support TBA-induced effects on proliferation, protein synthesis and protein degradation in BSC cultures.

**Key Words:** bovine, satellite cell, EGFR