

## Growth and Development III

**W121 Effect of hay or corn silage in pre-weaned calf diets on eating behavior and rumen development.** F. X. Suarez-Mena\* and A. J. Heinrichs, *The Pennsylvania State University, University Park.*

The objective of this experiment was to study effects of including corn silage or grass hay in pre-weaned calf diets on rumen development and eating behavior. Male Holstein calves ( $n = 15$ ,  $43.60 \pm 3.91$  kg BW at birth) were housed in individual pens on rubber mats with no bedding. Water and calf starter (56% whole corn grain, 40% pelleted supplement, and 4% molasses; as is) were offered free choice, and milk replacer was fed to 12% of birth BW. Calves were randomly assigned to treatments, either calf starter as the only solid feed (S), starter plus chopped hay (H), or starter plus corn silage (CS), H and CS were also offered free choice. Solid feed was offered from d 2 of age. At  $35 \pm 1$  d of age calves were euthanized and organs were harvested, emptied, rinsed and weighed to determine treatment effects on rumen development. Total 35 d DMI (milk replacer + starter + forage; 34.23 S, 33.10 H, and 34.08 CS  $\pm 2.62$  kg) and total starter intake (33 d; 11.65 S, 9.58 H, and 10.46 CS  $\pm 2.18$  kg) were not different ( $P > 0.05$ ). Calves on H and CS treatments preferred starter over forage as total forage intake (33 d) was lower (kg;  $0.86 \pm 1.03$  H, and  $0.19 \pm 0.13$  CS, mean  $\pm$  SD). In the last week, starter intake (5.14 S, 5.08 H, and 5.27 CS  $\pm 1.10$  kg), and starter plus forage intake (5.14 S, 5.46 H, and 5.35 CS  $\pm 1.17$  kg) were not different. Mass of reticulorumen ( $1.22 \pm 0.07$ ), omasum ( $0.23 \pm 0.02$ ), abomasum ( $0.56 \pm 0.02$ ), and liver ( $2.31 \pm 0.11$ ) as a percentage of BW were not different ( $P > 0.05$ ). Papillae length ( $1.22$  S,  $1.28$  H, and  $1.18$  CS  $\pm 0.10$  mm) and width ( $0.69$  S,  $0.75$  H, and  $0.73$  CS  $\pm 0.04$  mm), rumen wall thickness ( $0.89$  S,  $0.99$  H, and  $0.80$  CS  $\pm 0.06$  mm) and rumen contents pH ( $4.97$  S,  $5.31$  H,  $5.15$  CS  $\pm 0.11$ ) were also not different ( $P > 0.05$ ). High standard errors may be in part due to poor calf health as many calves from all treatments were treated for diarrhea and/or pneumonia, which had a negative effect on intake. This study shows that when palatable calf starter is available free choice, forage intake is minimal. Forage was not a palatable feed source for calves in this study.

**Key Words:** calf, rumen development, forage intake

**W122 Exogenous palmitic and palmitoleic acids respond differently in stearoyl-CoA desaturase (SCD1) inhibited bovine adipocytes.** A. K. G. Kadegowda,\* T. A. Burns, and S. K. Duckett, *Clemson University, Clemson, SC.*

Objectives were to determine the effect of exogenous palmitoleic acid (C16:1) or palmitic acid (C16:0) with or without SCD-1 inhibitors on adipogenesis in primary bovine adipocytes. Bovine primary preadipocyte cultures were isolated from intermuscular fat of Angus crossbred heifers ( $n = 2$ ). Preadipocytes were differentiated (D0) in differentiation media [DMEM containing 10% fetal calf serum,  $2.5 \mu\text{g/mL}$  insulin,  $0.25 \mu\text{M}$  DEX,  $5 \mu\text{M}$  troglitazone,  $0.5 \text{mM}$  IBMX, and  $10 \text{mM}$  acetate] for 2 d and from D2 to D12 without DEX and IBMX. From D0 to D6, cells were treated with SCD-1 inhibitors, sterculic acid (SA) or *trans*-10, *cis*-12 conjugated linoleic acid (CLA), at 0 or  $50 \mu\text{M}$ . In addition, from D2 to D6, cells were treated with (0 or  $150 \mu\text{M}$ ) of C16:0 or C16:1 and harvested for fatty acid analysis by GLC and gene expression by RT-qPCR. The effect of the treatments on cell viability in differentiated cells was assayed using Cell Counting Kit-8. Glycerol-3-phosphate dehydrogenase (GPDH) activity was assayed to determine the effect on adipocyte differentiation. The treatment effects were analyzed by ANOVA using

Proc Mixed of SAS 9.2. The C16:0 decreased ( $P < 0.05$ ) total cellular fatty acid (FA) yield (minus supplemented FA) due to reduced cellular viability of differentiated adipocytes. In contrast, a similar decrease in total cellular FA content was seen in C16:1/CLA treatment without affecting cellular viability. Palmitic acid supplementation reduced ( $P < 0.01$ ) GPDH activity compared with controls ( $3.09 \pm 0.2$  vs.  $9.51 \pm 0.2$  nmol/mg protein.min), which was further exacerbated in SCD1 inhibited adipocytes ( $0.49 \pm 0.2$  and  $0.9 \pm 0.2$  in SA and CLA treatments, respectively). In contrast, C16:1 increased GPDH activity ( $14.81 \pm 0.2$ ) which was reduced with CLA ( $4.17 \pm 0.2$ ). Compared with SA treated cells, C16:0 and C16:1 decreased the expression of fatty acid synthase, fatty acid elongase 6 and diacylglycerol acyltransferase. Furthermore, C16:0 increased *SCD1*; whereas C16:1 increased adipose triglyceride lipase. In summary, the results showed that the responses of lipogenic enzymes in bovine adipocytes differ when supplemented with C16:0 and C16:1, and with or without SCD1 inhibitors.

**Key Words:** adipocytes, gene expression, fatty acids

**W123 Stearoyl-CoA desaturase 1 (SCD1) inhibition decreases de novo fatty acid synthesis in primary bovine adipocytes.** A. K. G. Kadegowda,\* T. A. Burns, N. Tharayil, S. L. Pratt, and S. K. Duckett, *Clemson University, Clemson, SC.*

We have previously shown that sterculic acid [8-(2-octyl-1-cyclopropenyl) octanoic acid] (SA) and *trans*-10, *cis*-12 conjugated linoleic acid (CLA) inhibit SCD1 activity at  $50 \mu\text{M}$  concentration in primary bovine adipocytes. The objectives were to determine the effect of SCD1 inhibition on de novo fatty acid synthesis and expression of lipogenic and lipolytic genes. Bovine primary preadipocyte cultures were isolated from intermuscular fat of carcasses from 18-mo-old Angus crossbred heifers ( $n = 2$ ). Preadipocytes were differentiated (D0) in differentiation media [DMEM containing 10% fetal calf serum,  $2.5 \mu\text{g/mL}$  insulin,  $0.25 \mu\text{M}$  dexamethasone (DEX),  $5 \mu\text{M}$  troglitazone,  $0.5 \text{mM}$  isobutylmethylxanthine (IBMX), and  $10 \text{mM}$  acetate] for 2 d. Cells were further differentiated from D2 to D6 in media without DEX and IBMX. From D0 to D6, cells were treated with 0 or  $50 \mu\text{M}$  of SA or t10c12 CLA and harvested for gene expression by RT-qPCR. In addition, cells were incubated with acetate ( $^{13}\text{C}_2$ ) from D4 to D6 to estimate lipogenesis using GLC-MS. The geometric mean of Eukaryotic translation initiation factor 3, subunit k (EIF3K) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used for normalization of gene expression data. In acetate ( $^{13}\text{C}_2$ ) supplemented cells, the mass isotopomer distribution analysis showed that the fractional synthesis rate [molar percent excess (MPE)/h] of  $^{13}\text{C}$ -16:0 was reduced in SA ( $0.49 \pm 0.07$ ,  $-51.2\%$ ,  $P < 0.01$ ) and CLA ( $0.43 \pm 0.07$ ,  $-43.68\%$ ,  $P < 0.01$ ) treatments compared with control ( $0.89 \pm 0.07$  MPE/h). Of the lipogenic genes, CLA treatment decreased the expression of *SCD1* ( $P < 0.01$ ), acetyl-CoA carboxylase (*ACC*;  $P < 0.05$ ), fatty acid synthase (*FASN*), whereas SA supplementation decreased the expression of *ACC* ( $P < 0.05$ ). Both SA and CLA increased the expression of hormone-sensitive lipase ( $P < 0.05$ ) known to be involved in lipolysis. The results showed that SCD1 inhibition by SA and CLA in the bovine adipocytes decreases de novo fatty acid synthesis by downregulating genes involved in lipogenesis and upregulating gene involved in lipolysis.

**Key Words:** SCD1, adipocyte, GLC-MS

**W124 Metabolic differences in hepatocytes from Iberian and Landrace pigs.** L. Gonzalez-Valero, J. M. Rodriguez-Lopez, M. Lachica, and I. Fernandez-Figares,\* *CSIC (Spanish National Research Council), Granada, Spain.*

There are important differences when Iberian are compared with modern pigs in terms of metabolic activity, energy utilization and capacity of protein and fat deposition. In an attempt to partially explain differences we have used the primary culture of hepatocytes to evaluate the hepatic function and sensitivity to hormones without the interference of circulating blood factors. Hepatocytes were isolated from pure Iberian and Landrace pigs of similar body weight ( $n = 5$ ; 25 kg BW), by collagenase perfusion and seeded into collagen-coated T-25 flasks. Monolayers were established in medium containing fetal bovine serum for 1 d and switched to a serum-free medium for the remainder of the culture period. Hepatocytes were maintained in William's E amended with  $\beta$ -mercaptoethanol (0.1 mM), glutamine (2 mM), antibiotics (gentamicin, penicillin, streptomycin and amphotericin B), DMSO (1ng/ml), dexamethasone (10–8 M), insulin (1 and 100 ng/ml) and glucagon (1 and 100 ng/ml) for 24–48h. IGF-1 synthesis, urea synthesis, gluconeogenesis, insulin and glucagon impact on glycogen content were determined. Gluconeogenesis was measured in glucose free Dulbecco's modified Eagle's medium with glucagon (100 ng/ml) and lactate (5 mM), pyruvate (5 mM) and alanine (5 mM). For urea synthesis, cells were cultured in basal medium amended with ammonium sulfate (2.5 and 5mM). All results were expressed relative to the protein content of the culture. As the metabolic activity may vary among pigs, data were analyzed using the PROC MIXED procedure of SAS. Although insulin and glucagon regulated glycogen content, the difference between breeds was not significant ( $P > 0.10$ ). Nevertheless, gluconeogenesis, urea synthesis and IGF-1 synthesis were 69, 38 and 22% lower ( $P < 0.05$ ), respectively, in hepatocytes from Iberian compared with Landrace. In conclusion, the genetic background should be considered when hepatocyte culture is used for metabolic studies.

**Key Words:** hepatocytes, metabolism, breed

**W125 Effect of betaine and conjugated linoleic acid on porcine subcutaneous adipose tissue lipolysis.** M. L. Rojas-Cano<sup>1</sup>, M. Martinez-Perez<sup>2</sup>, M. Lachica<sup>1</sup>, L. Lara<sup>1</sup>, T. Ramsay<sup>3</sup>, and I. Fernandez-Figares\*<sup>1</sup>, <sup>1</sup>*CSIC (Spanish National Research Council), Granada, Spain*, <sup>2</sup>*Instituto de Ciencia Animal, La Habana, Cuba*, <sup>3</sup>*BARC, ANRI, USDA, Beltsville, MD.*

Betaine (Bet) and CLA have the potential to alter body composition in swine by decreasing body fat. The aim of the present study was determine if Bet and CLA have an effect on lipolysis in the adipose tissue of Iberian pigs. Five Iberian pigs were used (38kg BW). Adipose tissue samples were acquired following slaughter by electrical stunning and exsanguination. The adipose tissue was diced into strips and placed in Hanks' buffer (37°C, pH 7.4). In the laboratory, the strips were dissected clean of any extraneous muscle tissue and further separated into 1-cm cubes in a laminar flow hood. Adipose tissue explants (approximately 100mg) were prepared by slicing tissue cubes with a microtome. Tissue slices were blotted free of excess liquid, weighed, transferred to 6-well tissue culture with 2 mL of basal medium per well (DMEM/F12 (50:50), 25% bovine serum albumin, 25 mM HEPES, gentamycin, amphotericin B and penicillin-streptomycin) and incubated (5% CO<sub>2</sub>, 37°C) for one hour to wash away endogenous glycerol and fatty acids. Triplicate tissue

slices were then incubated in test medium (Basal medium amended with 10mM Linoleic acid (control), 10mM 10-t, 12-c CLA, 10mM Linoleic acid + 200mM Bet and 10mM 10-trans, 12-cis CLA + 200mM Bet). To estimate the direct effects of CLA and Bet on lipolysis, incubations were performed for 2 and 72 h. To examine the ability of Bet or CLA to inhibit insulin's suppression of isoproterenol-stimulated lipolysis after acute and chronic exposure, the test medium was amended with 1  $\mu$ M isoproterenol  $\pm$  10 nM insulin. Media glycerol concentration was measured at the end of the incubations. Data were analyzed by multifactorial ANOVA and mean separated by Fisher's LSD test. No effect of Bet or CLA on acute lipolysis was observed ( $P > 0.10$ ). Nevertheless, adipose tissue supplemented with 10-t, 12-c CLA decreased (22%;  $P < 0.05$ ) chronic lipolysis challenges compared with control. As expected, chronic exposure of adipose tissue to isoproterenol increased lipolysis (83%;  $P < 0.05$ ), and insulin inhibited isoproterenol stimulated lipolysis (50%;  $P < 0.05$ ). In conclusion, CLA decreased lipolysis in adipose tissue isolated from Iberian pigs while Bet had no effect in acute or chronic lipolysis.

**Key Words:** lipolysis, conjugated linoleic acid, betaine

**W126 T-box (Tbx)-2 is required for proliferation of osteoblast cells.** N. Francis<sup>1</sup>, S. M. Tornaquindici<sup>1</sup>, S. Mohan<sup>2</sup>, and K. E. Govoni\*<sup>1</sup>, <sup>1</sup>*Department of Animal Science, University of Connecticut, Storrs*, <sup>2</sup>*Musculoskeletal Disease Center, Jerry L. Pettis VA Medical Center, Loma Linda, CA.*

Bone related diseases and injuries affect many livestock species and have an enormous impact on efficiency of production and quality of life. Transcription factors are key regulators of osteoblast function; however our understanding of this complex process is limited. We recently demonstrated that *Tbx3*, a transcription factor implicated in ulnar mammary syndrome, is required for osteoblast proliferation. However, the mechanisms by which a closely related member, *Tbx2*, regulates skeletal development is unknown. We hypothesized that *Tbx2*, like *Tbx3*, is required for proliferation of osteoblasts. We used SaoS-2 cells, osteoblast-like human osteosarcoma cell line, and mouse pre-osteoblast cells (MC3T3-E1). To evaluate the role of *Tbx2*, we knocked down *Tbx2* expression by transfecting SaoS-2 and MC3T3-E1 cells with species-specific, *Tbx2*-specific small interfering (si) RNA (66 nM) and determined cell proliferation by alamarBlue assay and bromodeoxyuridine (BrdU) incorporation. Surprisingly, although we were able to detect a significant knockdown of mRNA expression of *Tbx2* in MC3T3-E1 cells, we were unable to detect *Tbx2* protein in MC3T3-E1. However, we were able to detect *Tbx2* protein in the SaoS-2 cells, therefore the SaoS-2 cell model was used for further experiments. In SaoS-2 cells, *Tbx2* siRNA reduced *Tbx2* mRNA expression  $90 \pm 4\%$  ( $P \leq 0.01$ ) and protein by  $62 \pm 4\%$  ( $P < 0.01$ ). Cell number was reduced  $29 \pm 2\%$  and BrdU incorporation was reduced  $40 \pm 2\%$  in *Tbx2* siRNA transfected cells compared with control cells ( $P < 0.001$ ). To determine if *Tbx2* is required for differentiation, we cultured control and siRNA transfected cells in media containing  $\beta$ -glycerophosphate and ascorbic acid and determined alkaline phosphatase (ALP) activity, a known marker of osteoblast differentiation. Knockdown of *Tbx2* did not alter ALP activity ( $P \geq 0.50$ ). In conclusion, 1) similar to *Tbx3*, *Tbx2* is required for optimal proliferation of osteoblast cells, 2) *Tbx2* may not be required for differentiation of osteoblasts, and 3) *Tbx2* and *Tbx3* may have both redundant and distinct functions in regulating bone development.

**Key Words:** bone, T-box, osteoblast

**W127 Comparison of feed form (pelleted vs. textured) on growing performance and rumen papillae development of dairy steers.** J. A. Davidson<sup>\*1</sup>, T. E. Johnson<sup>1</sup>, B. L. Miller<sup>1</sup>, K. B. Cunningham<sup>1</sup>, H. C. Puch<sup>1</sup>, K. M. O'Diam<sup>2</sup>, and K. M. Daniels<sup>2</sup>, <sup>1</sup>*Land O' Lakes Research Farm, Land O' Lakes Purina Feed, Webster City, IA*, <sup>2</sup>*Ohio Agricultural Research and Development Center, The Ohio State University, Wooster*.

The objective was to determine if feed form affects the growth performance of dairy steers from 13 to 24 wk of age and alters rumen papillae surface area. At 13 wk of age, 32 Holstein steers were blocked by weight and assigned to groups of 4 per pen (4 replicates per treatment). Pens were assigned to one of 2 grain mixes: pelleted (PL) or textured (TT, coarse mix of pellets and grains). Steers were limit fed 4.55 kg/d of the grain mixes (20% crude protein and 3.0 Mcal/kg, dry basis) with mixed grass hay offered, ad libitum. Body weight (BW, kg), hip height (HH, cm), heart girth (HG, cm), body length (BL, cm) measurements, and calculated body volume (BV, cm<sup>3</sup>) were completed every 2 wk. No differences were detected for any of the growth measurements or total dry matter intake. Initial measures were 101 kg BW; 95 cm HH; 104 cm HG; 100 cm BL; and 318 cm<sup>3</sup> BV. Final measures were 212 kg BW; 116 cm HH; 133 cm HG; 135 BL; and 665 cm<sup>3</sup> BV. Average daily gain and feed to gain ratio were not different (1.3 kg/d and 3.9, respectively). At 24 wk of age, 2 steers were randomly selected from each treatment and euthanized to evaluate rumen papillae surface area. Rumen papillae of PL and TT had similar surface area for portions of the rumen identified as cranial ventral (57 and 38 mm<sup>2</sup>, SE 8.7); cranial dorsal (13 and 11 mm<sup>2</sup>, SE 3.8); and caudal dorsal (32 and 19 mm<sup>2</sup>, SE 6.4). The caudal ventral region tended to be greater for PL steers (29 mm<sup>2</sup>) compared with 15 mm<sup>2</sup> for TT fed steers (SE 3.3,  $P=0.10$ ). Empty rumen weight and content DM were 5.4 and 3.4 kg for PL; 5.0 and 2.6 kg for TT, respectively. Ruminal pH tended to be different: 6.3 vs. 6.9 for PL and TT, ( $P=0.15$ ). Histological analysis did not indicate differences by form of feed for mucosal surface length, sub-mucosa thickness, and muscle thickness measurements of the rumen determined via a digital image analysis program (CellSens Imaging Software; Olympus Corp.). Feed delivery form (pellets or textured) did not differentially influence calf growth performance, nor were there negative effects on rumen size, ruminal papillae surface area, or histology.

**Key Words:** rumen, papillae, pellets

**W128 Effect of parenteral administration of glutamine on autophagy of liver cell and immune responses in weaned calves.** Z. Hu,<sup>\*</sup> Z. Cao, and S. Li, *State Key Laboratory of Animal Nutrition, College of Animal Science and Technology, China Agricultural University, Beijing, China*.

The objectives of this study were to determine the effects of an increased jugular supply of L-Gln on postweaning growth, immune responses and autophagy of weaned calves. At 35d of age, 24 Holstein calves (initially 50 ± 2.3kg; 35 ± 2 d of age) were randomly allocated to 4 treatments, and each treatment included 5 male and 1 female calves. Holstein calves were assigned to treatments of 1) no L-Gln and milk supplementation, but infusion of 2 L of 0.85% NaCl solution, Control group [C] 2) no milk supplementation, but infusion high dose L-Gln (0.64g/kg BW/day) mixed with 2 L of 0.85% NaCl solution [H], 3) no milk supplementation, but infusion moderate dose L-Gln (0.32g/kg BW/day) mixed with 2 L of 0.85% NaCl solution [M], 4) no milk supplementation,

but infusion low dose L-Gln (0.16g/kg BW/day) mixed with 2 L of 0.85% NaCl solution [L]; each treatment was delivered 2 h/d for each of 7 consecutive days starting on d1 after weaning. The dose of L-Gln for calves were calculated from published data on i.v. infusion rates for sheep (Hoskin et al., 2001) and adjusted on the basis of metabolic BW. Feed and water were freely available to all calves. All calves were euthanized at the end of infusion 7 d for measurements of autophagy of liver cell and intestinal morphology. The ADG and growth rate increased quadratically ( $P < 0.05$ ) with increasing infusions of Gln. Infusion N increased linearly in response to the graded amount of Gln infused ( $P < 0.05$ ). At the end of infusion 7 d, a blood sample was collected into a 10 mL EDTA vacuum tube to analyze immunological assays. Infusion Gln increased the abundance of CD4 and the ratio of CD4/CD8 linearly, but decreased CD8 linearly ( $P < 0.05$ ). Characterization of blood lymphocyte populations was performed by flow cytometry analysis. The urea N, Gln in plasma increased linearly with increasing Gln loads ( $P < 0.001$ ). After harvest, Villi and their crypts were identified and measured in duodenum, jejunum, and ileum of each calf using standard histomorphometric methods. Infusion Gln also increased villus height and crypt depth of intestine quadratically ( $P < 0.05$ ). In summary, the Gln supplied by milk replacer may be inadequate to meet calf metabolic demands under weaning physiological conditions.

**Key Words:** calf, L-Gln, autophagy

**W129 Influence of hay type on ruminal papillae surface area of growing dairy steers from 13 to 22 wk of age.** J. A. Davidson,<sup>\*</sup> T. E. Johnson, H. C. Puch, and B. L. Miller, *LongView Animal Nutrition Center, Land O' Lakes Purina Feed, Gray Summit, MO*.

The objective was to determine if hay type affects ruminal papillae surface area and growth of dairy calves from 13 to 22 wk of age. At 13 wk of age, 72 Holstein steers were blocked by weight into groups of 6 per pen (6 replicates per treatment). Steers were fed 2 different hays ad libitum: grass (GR) or alfalfa (AF) along with a complete grower feed at 3.3 kg DM/d per head. GR was 9.9% CP, 35.0% ADF, and 57.1% NDF; and AF was 18.7% CP, 31.1% ADF, and 43.8% ADF, dry basis. Hay intakes of the pens were measured every 2 weeks. Measurements of full BW were completed every 2 wk, and hip heights (HH) were recorded at wk 13 and 22 of age. No significant differences were detected for any of the growth measurements. Initial BW and hip heights (HH) were 114 kg and 96 cm, and final weights were 195 kg with HH of 112 cm. The pooled feed:gain efficiency was 3.9 with an ADG of 1.2 kg BW/d. The total DMI (sum of forage and concentrate intake) was slightly greater for steers fed AF compared with GR during the final 2 weeks of the study (5.6 vs. 5.2 kg/d per head). The intake of hay was similar for each feeding rate of grower during wk 13 to 20, but 0.3 kg/d per head greater for AF during wk 20 to 22 of age. At 22 wk of age, 4 steers per treatment were euthanized to examine ruminal papillae surface area. The wet weight of the rumen contents was 26.7 vs. 19.7 kg for GR and AF, respectively ( $P < 0.05$ ). Papillae surface area was measured at 4 regions: caudal dorsal, cranial dorsal, caudal ventral, and cranial ventral. Absorptive surface area was greater for steers fed AF compared with GR fed steers: caudal ventral (21.9 vs. 14.6 mm<sup>2</sup>) and caudal dorsal (22.6 vs. 16.2 mm<sup>2</sup>) regions ( $P < 0.01$ ). No difference for the cranial dorsal region was detected, but a tendency for greater surface area of cranial ventral region ( $P = 0.10$ , 31.1 vs. 43.3, GR and AF, respectively). Overall, alfalfa hay resulted in greater papillae surface area of the rumen compared with grass hay.

Selection of the forage quality or type may have a significant impact on transitioning growing calves from all grain diets to higher forage diets.

**Key Words:** dairy calves, rumen papillae, growth

**W130 Intake and performance of dairy heifers 12 to 24 wk of age following a full potential calf feeding program.** J. A. Davidson,\* D. C. Brown, and B. L. Miller, *LongView Animal Nutrition Center, Land O' Lakes Purina Feed, Gray Summit, MO.*

The objective was to determine optimal feeding recommendations for dairy heifers from 12 to 24 wk of age during transition from complete feeds to greater forage diets and maintain growth performance. At 12 wk of age, 46 heifers were blocked by birthdate into groups of 3 to 4 heifers per pen (6 replicates per treatment). Heifers were fed 2 different rates of complete grower feed (AMPLI-Calf Grower): 3.3 or 4.1 kg DM/d. Both groups received ad libitum chopped alfalfa hay (16% CP, 41% ADF, and 49% NDF, DM basis). Hay and grower feed intakes were measured daily. Measurements of full BW, hip heights (HH), heart girths (HG), and body lengths (BL) were completed every 2 wk, and BCS were recorded every 4 wk. No significant differences were detected for any of the growth measurements. Initial BW and HH were 104 kg and 98 cm. At 24 wk of age, heifers weighed 199 kg with HH of 113 cm, HG of 133 cm, and BL of 126 cm. Calculated body volume was 345 and 605 cm<sup>3</sup> at 12 and 24 wk, respectively. The pooled feed:gain efficiency was 4.2 with an ADG of 1.1 kg BW/d. Final BCS was slightly greater for 4.1 kg DM/d grower feeding rate (3.30 vs 3.45,  $P < 0.05$ ). Even though growth measurements were similar between feeding programs, intake was significantly influenced. The total DMI (sum of daily forage and concentrate intake) was greater for heifers fed grower at 4.1 kg DM/d (pooled across day of study: 5.2 vs 4.6 kg DM/d,  $P < 0.01$ ). The intake of hay was similar for each feeding rate of grower during wk 12 to 14. However, the heifers at the reduced feeding rate of grower consumed more hay over time (treatment by day,  $P < 0.01$ ). At 24 wk of age, heifers at the reduced feeding rate of grower were consuming 2.6 kg of DM/d as hay vs 1.5 kg DM of hay/d. Feeding 3.3 or 4.1 kg DM of AMPLI-Calf Grower per d did not significantly alter body growth performance of dairy heifers. However, the greater feeding rate delayed an increased consumption of the portion of total DMI as hay. The addition of the hay to the diet was great enough quality to maintain growth performance and transition the heifers to greater forage diets.

**Key Words:** dairy heifer, intake, growth

**W131 Effects of milk feeding strategies on performance, ruminal development, and metabolic and hormonal profile of Holstein calves.** B. F. Silper,\* S. G. Coelho, A. M. Q. Lana, A. U. Carvalho, C. S. Ferreira, A. P. S. Franzoni, H. M. Saturnino, and R. B. Reis, *Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brasil.*

Brazilian dairy farms feed calves 4 L/d. Strategies between conventional and accelerated systems show an opportunity to improve development. The effects of milk replacer (MR) feeding strategies on weight gain, starter intake, ruminal development, metabolic/hormonal profile, organs and perirenal fat weights were evaluated (ethical committee - UFMG 44/2009). Male calves (n = 54) were individually bedded on tropical grass. Water and starter were provided at all times. MR (22.5% CP; 17.0% fat; 12.5% solids) was fed in 2 equal portions/d in 3 strategies: 4L-60d (4 L -500 g- MR/d during 60 d), 6L-29d/4L-60d (6 L -750 g- MR/d on the first 30 d and 4 L -500 g- MR/d from 30 to 60 d) and 6L-60d (6 L -750 g- MR/d during 60 d). Weaning was abrupt at 60 d age.

Six calves/group were euthanized at 30, 60 and 90 d age. A completely randomized split-plot design was used for statistical analysis, with animals as random and time as repeated measures, means tested with SNK ( $P = 0.05$ ). There were MR refusals in first month in all groups, but none in second month. Starter intake was not different among groups ( $P > 0.05$ ) and increased with age ( $P < 0.05$ ). Weight gain and feed efficiency were higher during first month in groups allowed more MR ( $P < 0.05$ ). From 31 to 60 d age weight gain was equal ( $P > 0.05$ ), after weaning it was higher in 6L-60d ( $P < 0.05$ ). Weight gain increased each week, except on wk 5 in the group which had MR allowance reduced at d 30 ( $P < 0.05$ ). Glucose, NEFA and IGF-1 were not influenced by MR feeding strategy ( $P > 0.05$ ). Insulin was higher in 6L-60d ( $P < 0.05$ ). Glucose and insulin increased and NEFA reduced with age ( $P < 0.05$ ). Forestomach and perirenal fat weights, papillae length, and acetate, butyrate and N-NH<sub>3</sub> concentrations were not different ( $P > 0.05$ ). Ruminal pH was higher and propionate concentration lower in 6L-60d ( $P < 0.05$ ). Mitotic index of ruminal mucosa's basal stratum was higher in groups 6L-60d and 6L-29d/4L-60, as were abomasum, pancreas and liver weights ( $P < 0.05$ ). Feeding calves 6 L MR/d during 60 d improved pre and postweaning performance without reducing starter intake or ruminal development. Research supported by FAPEMIG/APQ1839/09.

**Key Words:** starter intake, milk replacer, papillae

**W132 Ontogenic changes of hepatic glucocorticoid and  $\alpha_1$ - and  $\beta_2$ -adrenergic receptors in neonatal calves.** D. Rohrbeck, J. Steinhoff-Wagner, E. Kanitz, and H. M. Hammon,\* *Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany.*

Neonatal calves, to a greater extent in preterm born, suffer from hypoglycemia after birth and have to adapt glucose metabolism by maturation. Plasma cortisol and catecholamines are elevated around birth and both hormones affect hepatic endogenous glucose production (eGP) by stimulation of glycogen degradation and gluconeogenic activity in liver. We have tested the hypothesis that the number of glucocorticoid (GR) and  $\alpha_1$ - and  $\beta_2$ -adrenergic receptors (AR) in neonatal calves change with development and correlate with eGP. Male Holstein calves were born preterm (PT; 9 d before term) or term (T) and slaughtered 26 h after birth or were term born, fed colostrum (TC) and were slaughtered on d 4 (n = 7 per group). TC calves were fed twice daily at 8% of BW/d on d 1 and 10% of BW/d from d 2 onward. For measurement of eGP U-<sup>13</sup>C-glucose (iv) was infused in PT and T calves directly after birth and in TC on d 3 of life and enrichment of U-<sup>13</sup>C-glucose in blood plasma was measured. After slaughter, liver samples were snap frozen. For AR measurements membrane suspensions (2 mg protein/ml) were prepared and saturation binding assays were performed with increasing concentrations of (<sup>3</sup>H)-prazosin and (<sup>3</sup>H)-CGP-12177 for determination of  $\alpha_1$ - and  $\beta_2$ -AR, respectively. For GR measurement the cytosol fraction (8 mg protein/ml) was incubated with increasing concentrations of (<sup>3</sup>H)-dexamethasone. Maximal binding capacity (B<sub>max</sub>) and binding affinity (K<sub>D</sub>) were calculated. Data were analyzed by General Linear Model (GLM) of SAS with ontogenic stage as fixed. For GR, B<sub>max</sub> was higher ( $P < 0.05$ ) in TC than T and PT. B<sub>max</sub> of  $\alpha_1$ -AR significantly increased with maturation (PT < T < TC;  $P < 0.05$ ). B<sub>max</sub> of  $\beta_2$ -AR was higher ( $P < 0.05$ ) in TC than in T and PT. K<sub>D</sub> for  $\beta_2$ -AR was lowest ( $P < 0.05$ ) in PT. B<sub>max</sub> of GR and  $\alpha_1$ - but not  $\beta_2$ -AR were positively correlated to eGP (r = 0.61;  $P < 0.01$  and r = 0.78;  $P < 0.001$ ). Results demonstrated that hepatic GR as well as  $\alpha_1$ - and  $\beta_2$ -AR increase during early development in calves. GR and  $\alpha_1$ -AR may be involved in stimulation of

eGP by cortisol and catecholamines in neonatal calves. Supported by DFG, Germany.

**Key Words:** calves, ontogenic maturation, adrenergic and glucocorticoid receptors

**W133 L-Arginine regulates expression of myokines and adipokines in myoblast and adipocyte cells.** H. S. Yang, X. Xiong, Y. L. Yin,\* and X. F. Kong, *Hunan Provincial Engineering Research Center of Healthy Livestock, Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan, Changsha, China.*

It is widely accepted that skeletal muscle and adipose tissue are not only the organs for energy storage and expenditure but also endocrine organs. Factors secreted from muscle (myokines) and adipose (adipokines), have important roles in the regulation of physiological actions in various tissues and organs. This study was conducted to investigate the effect of L arginine (Arg), a functional amino acid, on the expression of myokines and adipokines. Skeletal myoblasts (C2C12) and adipoblasts (3T3-L1) were cultured in high glucose Dulbecco modified high glucose Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Differentiation was induced by incubation in DMEM containing 2% horse serum (C2C12) or l-methyl-3-isobutylxanthine + dexamethasone + insulin (3T3-L1). The differentiated cells were treated with increasing concentrations of Arg for 12 h and the mRNA expression of myokines and adipokines was measured by reverse transcription (RT)-PCR. Data was analyzed by one way ANOVA. Myokine and adipokine gene expression exhibited time and Arg dose dependence. The expression of IL-15, BDNF, FGF21, EPO, and LIF increased ( $P < 0.05$ ) during C2C12 differentiation. Leptin, adiponectin, TNF, apelin and resistin expression increased ( $P < 0.05$ ) during 3T3-L1 differentiation. When the differentiated C2C12 and 3T3-L1 cells were cultured with Arg, the expression of IL-15, BDNF, Fstl, FGF21, LIF, leptin and adiponectin increased ( $P < 0.05$ ), but the expression of TNF, IL-6 and apelin decreased ( $P < 0.05$ ). Arg-induced expression of IL-15, BDNF, Fstl, FGF21, LIF, leptin, adiponectin, TNF and IL-6 was attenuated ( $P < 0.05$ ) with 100 nM rapamycin or L-NAME. Insulin (100 nM) increased ( $P < 0.05$ ) the effects of Arg on IL-15, BDNF and leptin expression in both cell types. The inhibitory actions of Arg on TNF and IL-6 expression were

attenuated ( $P < 0.05$ ) by 100 nM insulin. Results demonstrate that Arg regulates the expression of myokines and adipokines through mechanisms involving mTOR and NO signaling pathways.

**Key Words:** pigs, amino acids, expression

**W134 Role of estrogen receptor- $\alpha$  (ER- $\alpha$ ) and insulin-like growth factor receptor-1 (IGFR-1) in estradiol-stimulated proliferation of cultured bovine satellite cells.** E. Kamanga-Sollo, M. E. White, M. R. Hathaway, and W. R. Dayton,\* *University of Minnesota, St. Paul.*

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 of cultured bovine satellite cells (BSC) and 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. Treatment of BSC with 10 nM E2 significantly ( $P < 0.05$ ) stimulates proliferation and insulin-like growth factor receptor (IGFR)-1 $\beta$  phosphorylation under conditions in which neither IGF-1 nor IGF-2 expression is increased. To further investigate the mechanisms by which E2 stimulates BSC proliferation, we have used small interfering (si) RNA to silence expression of estrogen receptor (ER)- $\alpha$  and assessed the effects on E2-stimulated proliferation and IGFR-1 $\beta$  phosphorylation in BSC cultures. Additionally, we have assessed the effect of using siRNA to silence *IGFR-1 $\beta$*  expression on E2-stimulated proliferation in BSC cultures. All experiments were repeated at least 3 times using cells from 3 different steers. Treatment of ER- $\alpha$ - or IGFR-1 $\beta$ -silenced cells with E2 does not significantly stimulate proliferation. Estradiol treatment for 30 min stimulates phosphorylation of IGFR-1 $\beta$  in control BSC cultures but does not stimulate IGFR-1 $\beta$  phosphorylation in ER- $\alpha$ -silenced cells. These results indicate that binding of E2 to ER- $\alpha$  results in phosphorylation and activation of IGFR-1 $\beta$ , which results in increased proliferation in BSC cultures. The fact that this occurs under culture conditions in which neither *IGF-1* nor *IGF-2* mRNA expression is increased strongly suggests that E2 activates IGFR-1 $\beta$  via a mechanism that does not involve increased IGF-1 or IGF-2 binding to the receptor.

**Key Words:** estradiol, satellite cells, bovine