

Growth and Development: Adipose and Body Composition in Ruminants

243 Plane of dietary protein during late gestation in beef cows alters longissimus lumborum adipogenic gene expression in the offspring. S. Moisa*, D. Shike, D. B. Faulkner, and J. J. Loor, *University of Illinois, Urbana*.

The aim of this study was to evaluate potential carryover effects of the maternal diet on offspring performance, intramuscular adipogenic gene expression, and carcass characteristics. Two groups of pregnant Angus cows were fed a control diet (%DM = TDN 63.2, CP 9.48) or a higher-protein diet containing wet distiller's grain with solubles (WDGS; % DM = TDN 67.0, CP 13.0) during the last 96 d of gestation. Data were analyzed as a factorial design (maternal nutrition and stage of growth) with repeated measures using PROC MIXED in SAS. Seven early-weaned offspring (116 ± 6 d of age at weaning) from each group of cows were fed the same high-starch diet during a ~100-d growing phase. Subsequently, both groups of steers received the same diet during the finishing phase. Biopsies of the longissimus lumborum (LL) were collected at 0 (before start of growing phase), 100, 200 (mid-way through finishing), and 1 wk before harvest. Expression of the adipogenic transcription regulator peroxisome proliferator-activated receptor γ (PPARG), the lipogenic transcription regulators sterol responsive element binding factor 1 (SREBF1) and MLX interacting protein-like (MLXIPL), and the lipogenic enzymes fatty acid synthase (FASN) and stearoyl-CoA desaturase (SCD) was evaluated via quantitative PCR. Results showed no significant differences ($P > 0.05$) of maternal nutrition on offspring BW or ADG (1.4 and 1.6 kg/d during growing and finishing phases). At 100 d, steers from dams fed more dietary protein during pregnancy had greater (maternal diet \times time $P < 0.05$) expression of PPARG, SREBF1, FASN, and SCD. In contrast, steers from cows fed the control diet during pregnancy had an increase (maternal diet \times time $P < 0.05$) in expression between 100 and 200 d on study. Results indicated a precocious pro-adipogenic response during the growing phase in LL of steers born from cows fed a higher level of protein in the diet.

Key words: lipogenesis, marbling, gene expression

244 Oleic acid enhances G protein-coupled receptor 43 (GPR43) in cultured bovine intramuscular adipocytes. K. Y. Chung*¹, S. B. Smith², and B. J. Johnson¹, ¹Texas Tech University, Lubbock, ²Texas A&M University, College Station.

G protein-coupled receptor 43 (GPR43) is a 7-transmembrane domain receptor that can be activated by fatty acids and regulates cAMP signal pathways in bovine adipocytes. The GPR43 is highly expressed in isolated murine adipocytes but lowly expressed in stromal-vascular cells. Our previous results did not detect the GPR43 protein in bovine perirenal or subcutaneous (s.c.) adipose tissues but the protein was present in bovine intramuscular (i.m.) adipose tissue. We hypothesized that oleic acid (18:1n-9) may regulate adipogenesis of bovine i.m. adipose tissue. Primary cultures of i.m. and s.c. preadipocytes were isolated from adipose tissues dissected from bovine longissimus muscle. Data were analyzed as a completely randomized design using the MIXED model, each treatment performed in triplicate. Means were considered different at $P < 0.05$. Preadipocytes were treated with various levels of oleic acid (1 μ M, 10 μ M, 100 μ M, and 500 μ M) for C/EBP β , PPAR γ , stearoyl CoA desaturase (SCD), and GPR43 protein and mRNA analysis. Real-time quantitative PCR was used to measure mRNA contents. The mRNA concentrations of C/EBP β , PPAR γ , and GPR43 were increased ($P < 0.05$) in the i.m. adipocyte by oleic acid, but no effects

were observed in the s.c. adipocytes ($P > 0.05$). Western blot analysis revealed that treatment with oleic acid enhanced PPAR γ protein in both i.m. and s.c. adipocytes in a dose-dependent manner. Relative GPR43 per GAPDH protein levels in i.m. adipocytes tended to be increased ($P = 0.10$) by treatment with oleic acid. Interestingly, mRNA concentrations of SCD were decreased with oleic acid treatment ($P < 0.05$). These data indicate that oleic acid alters mRNA and protein concentrations of C/EBP β , PPAR γ , and SCD in bovine i.m. adipocytes, and these effects may be mediated through the GPR43 receptor.

Key words: G protein-coupled receptor 43, adipocyte, oleic acid

245 Effect of stearoyl-CoA desaturase 1 inhibitors on lipid metabolism and cellular proliferation in primary bovine adipocytes. A. K. G. Kadegowda*, T. A. Burns, S. L. Pratt, and S. K. Duckett, *Clemson University, Clemson, SC*.

Objectives were to determine the effects of stercularic acid (SA) and trans-10, cis-12 conjugated linoleic acid (t10c12 CLA), known stearoyl-CoA desaturase 1 (SCD1) inhibitors, on lipid metabolism and cellular proliferation in primary bovine adipocytes. Bovine primary preadipocyte cultures were isolated from intermuscular fat of 18 mo-old Angus crossbred heifers ($n = 3$) and differentiated (D0) in differentiation media [DMEM containing 10% fetal calf serum, 2.5 μ g/mL insulin, 0.25 μ M dexamethasone (DEX), 20 μ M troglitason, 0.5 mM isobutylmethylxanthine (IBMX), and 10 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 1 of 4 levels (0, 50, 100, or 200 μ M) of SA or t10c12 CLA. In Expt. 1, trans-11 18:1 was added at 0 or 100 μ M to the media on D4 and the cells harvested for fatty acid (FA) analysis after 48h. In Expt. 2, stearic acid-¹³C18 was added at 100 μ M on D4 and cells harvested at 0, 6, 12, 24, and 48h for FA analysis by GC-MS. The effect of SA and t10c12 CLA on cell proliferation of undifferentiated pre-confluent cells was assayed using Cell Counting Kit-8 at 24, 48, and 72h post incubation. The experimental design was 2 \times 4 factorial and data were analyzed by PROC MIXED (SAS). The total cellular FA yield (minus supplemented FA) did not change due to SA or t10c12 CLA (except 200 μ M). The desaturation of trans-11 18:1 to c9t11 CLA was decreased ($P < 0.05$) up to 95% by both SA and t10c12 CLA compared with Control (0 μ M). Stearic acid-¹³C18 was enriched up to 75% by 6h of incubation. The enrichment of labeled oleate increased ($P < 0.05$) from 4.1% at 6 h to 28.4% at 48h in the control (0 μ M SA). The SA inhibited the conversion of labeled stearate to oleate at all the supplemented levels and time points ($P < 0.05$). The SA and t10c12 CLA treatments did not affect the cell viability at the tested concentrations. Results showed both SA and t10c12 CLA inhibit SCD1 activity/expression at 50 μ M concentration without affecting adipose lipid content. Also, SCD1 inhibition does not affect bovine preadipocyte viability.

Key words: stearoyl-CoA desaturase 1, adipocyte, inhibition

246 Palmitoleic acid (C16:1), not an elongation product, decreases lipogenesis and desaturation in bovine adipocyte cultures. T. A. Burns*, C. M. Klein, S. K. Duckett, S. L. Pratt, and T. C. Jenkins, *Clemson University, Clemson, SC*.

Our objective was to confirm the identity of fatty acids elongated from C16:1 and to determine if C16:1 or an elongated fatty acid is

responsible for decreased desaturation and lipogenesis rates previously seen in cultured bovine adipocytes supplemented with C16:1. Bovine stromal vascular cultures were isolated, propagated, and tested for their capacity to differentiate into adipocytes. Cells were passaged 4 times, allowed to reach confluence, and held for 2 d. On D0, primary differentiation media [Dulbecco's Modified Eagle's Medium (DMEM) containing 10% fetal calf serum (FCS), and 2X antibiotic/antimycotic (AB/AM), insulin at 2.5 µg/mL, 0.25 µM dexamethasone, 20 µM troglitazone (TRO), 0.5 mM isobutylmethylxanthine, and 10 mM acetate] was applied for 2 d and replaced with secondary media {DMEM, 10% FCS, 2X AB/AM, insulin at 2.5 µg/mL, 5 µM TRO, 10 mM acetate, containing 1 of 4 levels of fatty acid [0 µM (CON), 150 µM C16:0, 150 µM C16:1, or 150 µM C18:1cis11]} for 4 d. On D6, cells were harvested for fatty acid analysis. In addition, cells were incubated with ¹³C₂, ¹³C_{18:0}, or ¹³C_{16:1} on D6 to estimate lipogenesis and desaturation rates and confirm elongation products of C16:1 using GLC-MS. Data were analyzed using Proc GLM of SAS 9.2. In C16:1-supplemented cells, C16:1, C18:1cis11, and C20:1 were elevated ($P < 0.05$) compared with CON cells. In C18:1cis11-supplemented cells, C18:1cis11 and C20:1 were elevated ($P < 0.05$) compared with CON, but C16:1 was not. The C18:1cis9/C18:0 ratio was decreased ($P < 0.05$) in C16:1 cells compared with all other treatments. Incorporation of ¹³C_{16:1} into cells and presence of ¹³C label in C18:1cis11 and C20:1 confirmed them as elongation products of C16:1 in bovine adipocytes. By 12 h of ¹³C_{18:0} incubation, cells supplemented with C16:1 had reduced ($P < 0.05$) ¹³C_{18:1cis9} compared with all other treatments. Similarly, ¹³C_{16:0} was reduced ($P < 0.05$) in C16:1-treated cells compared with CON and C18:1cis11-treated cells following ¹³C₂ incubation. Therefore, inhibition of desaturation and lipogenesis can be attributed to C16:1 and not its elongation products; C18:1cis11 or C20:1.

Key words: bovine, adipocyte, palmitoleic acid

247 Palmitic and stearic acids induce adipogenic gene expression in single- or co-cultures of bovine intramuscular preadipocyte and satellite cells. S. H. Choi^{*1}, K. Y. Chung², B. J. Johnson², K. H. Kim³, and S. B. Smith¹, ¹Texas A&M University, College Station, ²Texas Tech University, Lubbock, ³National Institute of Animal Science, Suwon, Gyeonggi, Korea.

We hypothesized that saturated fatty acids would stimulate lipogenic gene expression in single- and cocultured intramuscular (i.m.) preadipocytes and myoblasts. Bovine satellite cells (BSC) and i.m. preadipocytes were isolated from 14-mo-old crossbred steers. Both cell types were cultured with 10% fetal bovine serum (FBS)/Dulbecco's modified eagle medium (DMEM), and 1% antibiotics during the 3-d proliferation period. After proliferation, BSC and i.m. preadipocytes were treated with 3% horse serum DMEM or 5% FBS/DMEM with antibiotics, respectively, for 4 d. Finally, single or combined BSC and i.m. preadipocytes were cultured with 40 µM palmitic, palmitoleic, stearic, oleic, or linoleic acids for 2 h. The endogenous 40S ribosomal protein S9 (RPS9) control was used to normalize the expression of AMP-activated protein kinase- α (AMPK α), C/EBP β , carnitine palmitoyltransferase I - β (CPT1 β), peroxisome proliferator activated receptor- γ (PPAR γ), glucose transporter type 4 (GLUT4), and stearoyl-CoA desaturase (SCD). Data were analyzed as a 2 × 2 factorial ANOVA with chemical treatment and culture method as the main effects. Palmitic and stearic acids significantly stimulated C/EBP β ($P < 0.0001$) and CPT1 β ($P = 0.02$ and $P = 0.001$, respectively) gene expression in single- and co-cultured i.m. preadipocytes. Also, oleic and linoleic acids depressed SCD gene expression in single- and cocultured i.m.

preadipocytes ($P < 0.0001$). In myoblasts, palmitic acid significantly enhanced C/EBP β gene expression in both single- ($P = 0.036$) and cocultured ($P = 0.028$) myoblasts. Expression of GLUT4 in single- ($P = 0.006$) and co- ($P = 0.016$) cultured myoblasts was depressed by oleic and linoleic acids. Oleic acid and linoleic acid decreased SCD gene expression in both single- and cocultured myoblasts ($P < 0.0001$). Saturated fatty acids stimulate genes associated with differentiation of i.m. preadipocytes, but have less effect in differentiating myoblasts. Funded in part by Beef Check-off dollars.

Key words: fatty acid, preadipocyte, satellite cell

248 The effect of chromium propionate on bovine intramuscular and subcutaneous preadipocytes and muscle satellite cells. R. J. Tokach^{*1}, W. Rounds², K. Y. Chung¹, and B. J. Johnson¹, ¹Texas Tech University, Lubbock, ²Kemin Industries Inc., Des Moines, IA.

Chromium (Cr) propionate is a feed ingredient that has been used in the livestock industry to improve immune efficiency of livestock species, increase pork quality, and increase milk yield in dairy cattle. The aim of these in vitro experiments was to determine the effect of chromium propionate (Kemin Industries, Des Moines, IA) on changes in transcription factors and receptors important in adipose tissue differentiation and skeletal muscle growth. We hypothesized that Cr would increase mRNA expression of glucose transporter 4 (GLUT4) in intramuscular (IM) preadipocytes and protein expression in bovine muscle satellite cells (BSC). Intramuscular and subcutaneous (SC) preadipocytes and BSC were isolated from the semimembranosus to determine the effect of treatment on GLUT4 and peroxisome proliferator-activated receptor gamma (PPAR γ) mRNA and GLUT4 protein abundance. Preadipocyte cultures were treated with differentiation media plus 0.1 µM, 1 µM, or 10 µM concentrations of Cr or 10 µM, of sodium propionate for 96, 120, and 144 h before harvest. Data were analyzed as a randomized complete block design using the MIXED model. Real-time quantitative PCR was used to measure the relative level of mRNA. For the IM and SC preadipocyte cultures, GLUT4 mRNA abundance tended to increase ($P = 0.10$) after 144 h of treatment with differentiation media plus either 1 µM or 10 µM of Cr. In the SC preadipocyte cultures, the GLUT4 mRNA abundance increased ($P \leq 0.05$) for the average of the 3 levels of Cr as compared with the differentiation media alone and this increase tended ($P = 0.10$) to be linear. These results indicated that Cr altered glucose uptake mechanisms regardless of adipose tissue type. The mRNA abundance for PPAR γ increased ($P \leq 0.01$) when differentiation media was added to the preadipocyte cultures at 144 h. The GLUT4/GAPDH protein abundance decreased ($P \leq 0.01$) in a dose-dependent manner when Cr was added to the BSC. The results indicated that Cr may have caused a feedback mechanism in skeletal muscle, due to increased efficiency of glucose transport caused by the Cr supplementation.

Key words: chromium propionate, glucose transporter 4, preadipocyte

249 Effect of rate of gain during grazing on gene expression of adipose tissue in growing beef cattle. P. A. Lancaster^{*}, E. D. Shorman, G. W. Horn, C. R. Krehbiel, and U. DeSilva, Oklahoma Agricultural Experiment Station, Stillwater.

The stocker cattle production phase could benefit by influencing adipose tissue development before finishing. Previous research indicates that nutritional management can affect fat deposition in growing cattle. Our objective was to evaluate rate of gain to a common initial finishing BW on gene expression of adipose tissue in growing cattle. Angus

steers ($n = 72$; 259 ± 28 kg BW) were blocked by BW and sire and allotted to 4 treatments: (1) grazing dormant native range (NR) plus a protein supplement (1.0 kg/d) followed by season-long grazing NR (CON), (2) grazing dormant NR plus a corn-based supplement (1% of BW) followed by short-season grazing NR (CORN), (3) grazing wheat pasture (WP) at a high stocking rate (3.0 steers/ha) for a moderate ADG (LGWP), and (4) grazing WP at a low stocking rate (1.0 steers/ha) for a high ADG (HGWP). Supplements were fed individually 5 d/wk. Four steers per treatment were harvested at an estimated HCW of 200 kg before finishing, and subcutaneous (SC), perirenal (PR), and intramuscular (IM) adipose tissue collected. Gene expression was determined using qRT-PCR. Performance and carcass data are presented in a companion abstract (Sharman et al., 2011). MANOVA revealed a treatment \times adipose tissue interaction ($P < 0.05$) for genes involved in lipogenesis and adipogenesis. Canonical variate analysis indicated that treatment did not affect lipogenic or adipogenic gene expression in IM, but in general, lipogenic and adipogenic gene expression in SC and PR was greater in HGWP and LGWP than CON and CORN. The lipogenic genes fatty acid synthase and diacylglycerol acyltransferase 2 were highly correlated ($P < 0.01$) with the canonical variate (0.91 and 0.64, respectively). The adipogenic genes peroxisome proliferator activated receptor gamma, sterol regulatory element binding protein, and CAATT/ enhancer binding protein β were correlated ($P < 0.05$) with the canonical variate (0.65, 0.95, and 0.32, respectively). In summary, moderate to high rates of gain of cattle grazing wheat pasture significantly increased lipogenesis and adipogenesis in SC and PR, but development of IM was not affected.

Key words: adipose tissue, gene expression, stocker cattle

250 Effect of ewe body condition during mid to late gestation on mammary growth and composition of female progeny. K. E. Boesche*, A. L. Hunter, K. M. O'Diam, S. C. Loerch, and K. M. Daniels, *The Ohio State University, Ohio Agricultural Research and Development Center, Wooster.*

The foundation for functional mammary secretory tissue, parenchyma (PAR), is established early in life; amount of PAR directly relates to future milk production. Dam body condition score (BCS) during mid to late gestation may affect progeny postnatal mammary growth and composition via in utero metabolic programming events. Pregnant ewes ($n = 96$; ≈ 80 d of gestation) were allotted to treatment groups based on initial BCS of 2, 3, or 4 (on a 1 to 5 scoring system with 1 being extremely thin and 5 being extremely fat). Ewes were housed in 18 pens (6 pens per treatment) and fed a maintenance diet of limit-fed corn silage (1.1 kg DMI/d), to which whole shelled corn was supplemented at 0.12, 0.26, and 0.47 kg DMI/d for BCS groups 2, 3, and 4, respectively. Diets were adjusted every 2 wk to maintain desired BCS throughout pregnancy. Prior to weaning, lambs nursed their mothers and were fed a common starter. Lambs were weaned (≈ 56 d of age; 23.59 kg) and placed on a common finishing diet that met NRC requirements. Female progeny from the 3 BCS groups ($n = 73$) were slaughtered at similar BW (46.9 ± 0.5 kg; $P = 0.913$), and age (126.3 ± 2.8 d; $P = 0.159$). Udders were removed and mammary tissue subjected to biochemical analysis. Total mammary gland weights (179.2 , 167.4 , and 175.6 ± 8.8 g for BCS 2, 3, 4, respectively) did not differ

by treatment ($P = 0.615$). However, PAR weight of progeny from BCS 2 ewes (25.3 g) was greater ($P = 0.074$) than that of BCS 3 (18.5 g) or BCS 4 (18.8 g) progeny. Protein mass within PAR (BCS 2 = 1.43, 3 = 1.02, and 4 = 1.07 ± 0.13 g) varied by treatment ($P = 0.053$), as did DNA mass within PAR (BCS 2 = 134.8, 3 = 93.1, and 4 = 103.0 ± 13.4 mg; $P = 0.072$). Lipid mass within PAR did not differ by treatment and averaged 7.10 ± 1.17 g ($P = 0.228$). Despite detectable differences in PAR due to treatment, no differences in weight or composition of the mammary fat pad were found. Factors that promote mammary PAR growth may have a positive impact on future milk production. Our observations suggest that BCS during gestation may have important lactation performance implications for female progeny.

Key words: body condition score, sheep, mammary

251 Defining maturity of Nellore cattle based on growth and body composition. M. Marcondes*^{1,3}, L. Tedeschi², S. V. Filho^{1,3}, M. Gionbelli¹, and L. F. Silva¹, ¹Universidade Federal de Viçosa/INCT-CA, Viçosa, MG, Brazil, ²Texas A&M University, College Station, ³INCT - Ciência Animal, Viçosa, MG, Brazil.

The aim of this study was to understand the growth development and chemical composition of empty BW (EBW), soft tissues, and bone, and to determine a system to define maturity of Nellore cattle. A database containing carcass and body compositions of 249 animals from 11 studies was used. There were 63 intact males, 105 steers, and 81 heifers of Nellore breed. Allometric regressions were used to predict body water, CP, and ash; except for ether extract (EE) in which an exponential equation was used to fit the data. The maturity was defined as the point of which no significant accretion of protein in the fat-free DM (FFDM) was observed. The water in the soft tissue (STW) was regressed on Logistic, Gompertz, and brake lines equations whereas the fitting for bone chemical composition was performed using an exponential equation. Sex effect was evident on empty body water ($P = 0.057$) and EE ($P < 0.001$), therefore, this effect was not included in the analysis of fat-free DM, evidencing that maturity is more correlated with breed than sex. The fitting of the exponential equation suggested that Nellore cattle reach maturity with 445 kg and the break line analysis indicated a plateau around 429 kg. At this point, the relationship between CP and ash in the FFDM is 79.62:20.38. A high relationship between STW and EE in the soft tissue (STEE) was observed ($STEE = 0.920 - 1.147 \times STW$; $P < 0.001$), but the soft tissue was not a good predictor for maturity because it depends on the type of diet. The analysis of bone chemical composition showed that EE, water, and ash become constant between 400 and 500 kg of EBW whereas CP in the bone is always constant at 19.1%. Our data also suggested that bone composition could be a good predictor of maturity; however due to the great variability in the database, it was not possible to determine a BW at which these components become constant with a reliable precision. We concluded that Nellore cattle reach maturity between 429 and 445 kg of EBW and that CP in the FFDM and CP, water, and ash in the bone are good predictors of maturity; however soft tissue composition alone cannot be used to predict maturity.

Key words: prediction, development, composition