TH65 Characterization of putative mammary stem cells in intact and ovariectomized prepubertal heifers. N. Korn[1], L. Riggs[2], R. M. Akers[3], and S. Ellis*[1], [1]*Clemson University*, *Clemson, SC*, [2]*Louisiana State University*, *Baton Rouge*, [3]*Virginia Polytechnic Institute and State University*, *Blacksburg*.

Prepubertal ovariectomy impairs mammary development in heifers through poorly understood mechanisms that may relate to changes in stem cell physiology. A trial was conducted to determine whether ovariectomy alters the frequency or proliferative activity of mammary stem cells, as assessed by localization of retained bromodeoxyuridine (BrdU) label and detection of proliferative cell markers (Ki67) in parenchymal cell populations. At 35d of age, all heifers were injected with BrdU once per day (5mg/kg BW, iv in pH8.5 physiologic saline) for 5d to label putative stem cells. On d40, all animals underwent either an ovariectomy (OVX; n=17) or sham (INT; n=18) operation. Groups of heifers were sacrificed at 15d intervals to provide tissues for histologic analysis of parenchymal cells. At least 5 non-sequential sections per animal were stained for dual detection of Ki67 and BrdU. Statistical comparisons were complicated by heterogeneous variation and confounded effects of treatment (OVX vs. INT) on parenchymal growth. In control animals sacrificed at 40d age (n=2), BrdU- and Ki67-double positive (DP) cells were distributed throughout the basal, embedded, and lumenal strata of the epithelium. At d55, there was a marked reduction in BrdU retention and DP cells in INT animals, but distribution of DP cells through the parenchymal strata was unaltered. In samples from d70 to d160 heifers, DP cells were very rarely observed in parenchyma from INT animals, primarily due to reduced BrdU retention. In OVX animals, abundant BrdU labeling was seen at d55 and d70, but very few DP cells were observed, likely because of reduced Ki67 labeling. Between d85 and d160, BrdU retention in OVX animals declined steadily and became almost undetectable at d160. Our data suggests that ovariectomy does not abruptly alter populations of mammary stem cells. Instead, ovariectomy reduces proliferation and may allow for gradual senescence.

Key Words: Mammary Stem Cells, Prepubertal, Ovariectomy

TH66 Cloning the promoter region for bovine phosphoenolpyruvate carboxykinase gene and identification of propionate responsive region. S. L. Koser*, M. Thomas, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Cytosolic phosphoenolpyruvate carboxykinase (PCK1) is a rate-limiting enzyme for gluconeogenesis that is sensitive to nutritional and hormonal status. Bovine genomic sequence data was scanned using BLAST to determine matches to the 5′ end of mRNA for bovine PCK1 mRNA. A 1459 bp fragment of bovine PCK1 was cloned by PCR that included 221 nt corresponding to the 5′ untranslated region and 1238 nt of the proximal promoter. Segments corresponding to -815,-409, and -251 through +197 of the promoter of the bovine PCK1 gene were ligated to the firefly luciferase reporter gene. Constructs were transfected into rat hepatoma (H4IIE) cells and tested for their ability to drive gene expression. Cotransfection with renilla luciferase reporter served as a transfection control. A promoterless vector and a reporter driven by the SV40 promoter served as negative and positive controls for the experiment. Transfections were established over a 5-h period and cells were exposed to either 2.5 mM propionate, or vehicle for 23 h. Cells were harvested and the abundance of firefly and renilla luciferase were determined in cell extracts. All bovine PCK1 promoter constructs tested were capable of driving expression of luciferase. Basal expression, as the ratio of firefly to renilla luciferase activity, was enhanced ($P < 0.05$) from 0.28 to 1.09 when the size of the promoter construct was reduced from -815 to -409 but truncation to -251 did not further alter basal expression. Propionate induced ($P < 0.05$) expression of all constructs compared to controls although the response for the -215 construct was muted relative to constructs -815 and -409. When luciferase expression ratios were adjusted for basal activity the -409 promoter construct of PCK1 was most responsive to propionate and further truncation to -251 reduced ($P < 0.05$) this responsiveness (706 vs. 484 ± 98 as a percent of control). The data indicate response elements for propionate are located within -251 and -815 of the bovine PCK1 promoter. Supported by USDA-CSREES NRI Grant no. 2006-35206-16646.

Key Words: Propionate, Liver, Gene Expression

TH67 Quantification of glucose-6-phosphatase mRNA abundance in liver of transition dairy cows. E. M. Cedeño*, S. L. Koser, and S. S. Donkin, *Purdue University*, *West Lafayette, IN*.

Glucose-6-Phosphatase (G6Pase) catalyzes the final reaction for both gluconeogenesis and glycogenolysis, hydrolyzing glucose-6-phosphate (G6P) to glucose and inorganic phosphate. The activity of G6Pase appears to be largely controlled on the transcriptional level and predominantly exerted by hormonal cues and reduced feed intake. Therefore we hypothesized that G6Pase would be responsive to the onset of calving in transition dairy cows. Eleven mature Holstein cows were given ad libitum access to feed (1.61 Mcal NE/kg) beginning 28 days prior to expected calving. After calving all cows received a diet containing 1.67 Mcal NE/kg. Liver biopsy samples were obtained on days –28, +1, and +28 relative to calving. Total RNA was extracted, reverse transcribed to cDNA and used in real time PCR analysis for G6Pase and glyceraldehyde–3–phosphate dehydrogenase (GAPDH). Primers spanning exon junctions were selected based on bovine genomic sequence data. Expression of G6Pase was compared among days relative to calving and analysis accounted for the effect of day and cow x day. There was a tendency ($P = 0.08$) for G6Pase mRNA to be elevated at calving. Abundance of G6Pase mRNA relative to GAPDH was 0.8 vs. 1.1 ± 0.28 for –28 and +1 days relative to calving. G6Pase was elevated ($P < 0.05$; 1.80 ± .28) at +28 days relative to calving and precalving abundance. The data indicate that increased expression of G6Pase at calving and postcalving in liver is part of the adaptation to increased hepatic glucose release in transition cows.

Key Words: Gluconeogenesis, Liver, Transition Cows

TH68 Plasma and tissue concentrations of glucose, acetate, propionate, lactate, and hydroxybutyrate in calves subjected to conventional and accelerated milk replacer programs. H. A. Weeks*, A. G. Rius, K. M. Daniels, R. M. Akers, C. Umberger, and M. D. Hanigan, *Virginia Polytechnic Institute and State University*, *Blacksburg*.

The objective of the study was to evaluate the effects of milk replacer (MR) composition and feeding rate on plasma, liver, and muscle glucose,

lactate, acetate, propionate, and hydroxybutyrate concentrations. It was hypothesized that high rates of MR intake would cause delayed initiation of rumination resulting in reduced plasma and tissue acetate concentrations. Plasma and tissue samples previously collected from 24 Holstein heifer calves were used for the analyses. Water and starter (20% CP, 1.43% fat) were offered ad libitum and calves were fed twice daily one of four MR (n=6/treatment). Treatments were: 1) control MR (CON; 20% CP, 20% fat fed at 441 g DM/d), 2) a high protein MR (HPLF; 28% CP, 20% fat) fed at 951 g DM/d, 3) a high protein high fat MR (HPHF; 27% CP, 28% fat) fed at 951 g DM/d, and 4) treatment 3 fed at 1431 g DM/d (HPHF+). All samples were deproteinized, lyophylized to dryness, reconstituted in phosphate buffered D2O containing 0.5 mM DSS as an internal standard, and subjected to nuclear magnetic resonance spectroscopy. Spectra were phase adjusted and baseline corrected before calculation of peak integrals. All peaks were normalized to a constant DSS peak area of 100. Plasma concentrations of acetate ($P<0.001$) and lactate ($P<0.02$) increased with age reflecting onset of rumination. Increased nutrient supply from MR resulted in significant increases in plasma glucose ($P<0.001$) and decreased plasma acetate ($P<0.01$). Plasma propionate, lactate, and hydroxybutyrate were not affected by treatment. Dietary treatments did not significantly affect metabolite concentrations in liver or muscle. However concentrations of glucose ($P<0.001$), acetate ($P<0.001$), propionate ($P<0.05$), and hydroxybutyrate ($P<0.001$) were significantly greater in liver than in muscle. It was concluded that increasing nutrient intake from MR delays onset of rumination, but changes in blood concentrations of metabolites were not reflected in tissue concentrations.

Key Words: Calf, Acetate, Glucose

TH69 Effect of β-mannanase enzyme mixture addition to soy-containing milk replacers on growth and health of neonatal calves. M. E. Van Amburgh*[1], L. Nabte-Solis[1], E. B. Helmes[2], D. A. Ross[1], and T. D. Sonnenberg[1], [1]*Cornell University*, *Ithaca, NY*, [2]*ChemGen*, *Gaithersburg, MD*.

The objective of this study was to evaluate the performance of a high protein, soy protein concentrate (SPC) containing milk replacer (MR) fed to pre-ruminant calves with or without β-mannanase-based enzymes prior to and during the weaning phase of growth. Fifty-six Holstein bull calves were purchased and randomly assigned to one of four dietary treatments. Calves were 14 ± 12 d of age at the initiation of treatments and mean weight at assignment of treatments was 48 ± 5.33 kg. Milk replacer was provided at 0.28 Mcal GE/kg $BW^{0.75}$ during the first 7 days and then increased to 0.32 Mcal GE/kg BW0.75. The positive control diet consisted of a commercially available MR formulated with all milk ingredients (Excelerate, MSC Specialty Nutrition, Dundee, IL, 28% CP, 15% fat). The remaining treatments received a soy-containing MR that was formulated with 50% of the protein replaced by a SPC (Profine VF, Solae Inc., St. Louis, MO). Two different levels of a feed enzyme product, SZP (ChemGen Corp., Gaithersburg, MD), were added to two of the three SPC-containing MR at the time of mixing to provide the four treatments: all milk MR; two SPC-containing MRs with two levels of the enzyme mixture measured by β-mannanase activity; and the SPC-containing MR with no added enzymes. Calves were fed MR from day 1 to 35, then on day 36 were offered a commercially available starter and weaned by day 56. Milk replacer intake and overall DMI were not different among treatments ($P = 0.3$) and overall DMI averaged 1.65 ± 0.08 kg over the 63-day period. The enzyme treatments did not significantly affect DMI. Daily gain of calves among treatments were not different ($P = 0.3$) through the study, and averaged 0.90 kg/d. Replacing 50% of the protein in a 28% CP all-milk MR with a low antigen SPC had no significant effect on calf growth or health. The addition of β-mannanase-based enzymes to the SPC containing MR showed a trend ($P<0.08$) towards increased feed efficiency and ADG of the calves.

Key Words: Calves, Soy Protein Concentrate, Enzymes

TH70 Effects of *Bacillus subitilis* natto on performance and morphological features of ruminal papillae in dairy calves. H. T. Zhang, J. Q. Wang*, D. P. Bu, S. Y. Luan, L. F. Deng, L. Y. Zhou, H. Y. Wei, and K. L. Liu, *Chinese Academy of Agricultural Sciences*, *Beijing, China*.

Two types of *Bacillus subtilis* natto culture (Na, N1) were added to the milk/starter to evaluate effects of natto culture on the growth of calves and development of ruminal papillae. 24 Holstein bull calves of 7±1d of age were divided into three groups. Natto was provided in two ways: first, nattto cultures were mixed with milk and provided to all the calves. Calves were weaned until the intake of starter was up to 2% of their weight, and four calves were slaughtered randomly after weaning. In the second experiment, natto cultures were fed directly and the calves were slaughtered 8 weeks after weaning. Ruminal epitheliums at the area of ventral rumen sac were obtained. In the first experiment, weaning day of N1 group(49.7 d) shortened significantly when compared with the control group (57 d) ($P<0.05$). In the second experiments, average daily gain of N1 and Na group enhanced about 16.2% ($P=0.09$) and 22.1% ($P=0.02$), respectively. DMI data showed a similar pattern. Calves fed with natto culture enhanced carcase yield with remarkable difference ($P=0.01$). In respect of rumen papillae, the number and the surface of papillae per cm2 mucosa of Na and N1 groups increased remarkably compared with control. Under electron microscope, we found the shape of the papillae of control was leaf-like, narrow stalk, clumping, parakeratotic and dark-brownish color. In contrast, papillae of Na and Ni groups were flattened, tongue-shaped and straw-colored. The shape and size of the ingesta which packed into the surface of papillae were also different. Results from the present study indicated that supplementation of natto culture could promote the development of rumen papillae and the growth of calves.

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Key Words: Calf, Ruminal Papillae, Natto

TH71 Increasing levels of dietary corn oil to grazing steers alters lipogenic gene expression. S. K. Duckett*[1], S. L. Pratt[1], and E. Pavan[2], [1]*Clemson University*, *Clemson, SC*, [2]*INTA*, *Balcarce, Argentina*.

Subcutaneous adipose tissues were collected at slaughter from eighteen steers fed increasing levels of dietary corn oil [0 g/kg BW (NONE), 0.75 g/kg BW (MED) and 1.5 g/kg BW (HI)]. Steers rotationally grazed endophyte-free tall fescue pastures for 197 d and were supplemented individually with cottonseed hulls plus dietary corn oil level treatment. Subcutaneous adipose tissues were processed for RNA extraction. Relative gene expression of genes involved in lipogenesis [fatty acid synthase (FASN), acetyl Co-A carboxylase (ACC), lipoprotein lipase (LPL), stearoyl Co-A desaturase (SCD), Spot-14], transcriptional

events [peroxisome proliferators-activated receptors (PPARγ), CCAAT/enhancer-binding proteins (C/EBPα), sterol regulatory binding proteins (SREBP-1), and STAT-5], and housekeeping (GAPDH and β-actin) was determined by qRT-PCR. At the MED level of oil supplementation, FASN and SCD mRNA expression were up-regulated ($P < 0.05$) by 1.4-fold compared to NONE. In contrast, FASN and SCD mRNA expression were down-regulated ($P < 0.05$) by 0.7- and 0.8-fold for HI versus NONE. LPL mRNA expression was up-regulated ($P < 0.05$) in HI vs. NONE and unchanged ($P > 0.05$) in MED vs. NONE. For the HI oil supplementation level, Spot-14, SREBP-1 and NFY were up-regulated ($P < 0.05$) compared to NONE. Relative expression of other genes (ACC, PPARγ, C/EBPα, and STAT-5) were unaffected ($P > 0.05$) by dietary treatment. High levels of oil supplementation (8% dietary fatty acids, DM basis) to grazing steers up-regulated gene expression of key enzymes responsible for the uptake of dietary fatty acids (LPL), and down-regulated those involved in de novo fatty acid synthesis (FASN) and conversion of saturated to monounsaturated fatty acids (SCD).

Key Words: Beef, Supplementation, Gene Expression

TH72 Lipogenic gene expression in steers finished on high concentrate diets and pasture with or without energy supplementation. S. K. Duckett*[1], S. L. Pratt[1], and E. Pavan[2], [1]*Clemson University*, *Clemson, SC*, [2]*INTA*, *Balcarce, Argentina*.

Twenty-eight Angus steers (289 kg) were finished on a high concentrate diet (85% concentrate/15% roughage; C), or endophyte-free tall fescue pastures with either corn grain supplement (0.52% BW; PC), or corn oil plus soybean hull supplement (0.10% BW corn oil plus 0.45% BW soybean hulls; PO), or no supplement (pasture only; P). Subcutaneous adipose tissues were processed for RNA extraction and fatty acid composition by GLC. Relative gene expression of genes involved in lipogenesis [fatty acid synthase (FASN), acetyl Co-A carboxylase (ACC), lipoprotein lipase (LPL), stearoyl Co-A desaturase (SCD), Spot-14], transcriptional events [peroxisome proliferators-activated receptors (PPARγ), CCAAT/enhancer-binding proteins (C/EBPα), sterol regulatory binding proteins (SREBP-1), and STAT-5], and housekeeping (GAPDH and β-actin) was determined by qRT-PCR. SCD mRNA expression was up-regulated ($P < 0.001$) by 7-, 18- and 46-fold for PO, PC and C, respectively, over P. Monounsaturated fatty acid (MUFA) content (g/100g) was also increased ($P < 0.05$) by 21%, 38%, and 75% for PO, PC, and C, respectively, compared to P. FASN mRNA expression was up-regulated ($P = 0.0036$) by 4.6- and 8.8-fold for PC and C, respectively, compared to P. Palmitic acid, the end product of de novo fatty acid synthesis, was also increased by 24% and 55% for PC and C, respectively, compared to P. Finishing steers on a high concentrate diet (C) down-regulated ($P = 0.005$) STAT5 mRNA by 21-fold compared to pasture only (P). Spot-14 mRNA expression was up-regulated ($P < 0.0001$) by 3-, 2-, and 13-fold for PO, PC, and C, respectively, compared to P. Relative expression of other genes (ACC, LPL, PPARγ, C/EBPα, and SREBP-1) were unaffected ($P > 0.05$) by dietary treatment. Corn grain fed as a supplement or in a high concentrate diet up-regulated gene expression of key lipogenic enzymes responsible for the conversion of saturated fatty acids to MUFA (SCD) and de novo synthesis of fatty acids (FASN).

Key Words: Beef, Supplementation, Gene Expression

TH73 Melengestrol acetate enhances adipogenic gene expression in an *in vitro* muscle-derived cell transdifferentiation model. K. Y. Chung* and B. J. Johnson, *Kansas State University*, *Manhattan*.

We hypothesized that within postnatal skeletal muscle there was a population of muscle cells that have unique characteristics to accumulate lipid droplet. Under appropriate stimuli, these particular cells could be induced down the adipose tissue pathway, to form marbling, rather than muscle. Muscle-derived cells (MDC) were digested from semimembranosus muscle tissue of three 14-month crossbred steers. Isolated MDC were plated on pre-coated matrigel plates. Addition of insulin, oleic acid, and ciglitizone (IOC) for 7 days resulted in morphological differences in MDC compared to control cultures. Multilocular lipid droplets stained with Oil-Red-O were located not only in single MDC but in fused myotubes. Relative PPARγ mRNA levels in MDC incubated with IOC were increased ($P<0.05$). However, myogenin mRNA levels in MDC incubated with IOC were repressed ($P<0.05$) compared to non-treated MDC. Cultures of MDC treated with 10 nM estradiol (E_2) showed low lipid droplet compared with control cultures. However, cultures treated with 10 nM melengestrol acetate (MGA) resulted in cultures with highly distributed lipid droplets not only in single cell but in the myotubes. Relative C/EBPβ and PPARγ mRNA levels from MDC treated with MGA were increased ($P<0.05$) compared to control cultures. Estradiol treatment had no effect on mRNA levels. The addition of both E_2 and MGA to MDC increased ($P<0.05$) C/EBPβ mRNA level and tended ($P=0.06$) to increase PPARγ mRNA level. Relative C/EBPβ, PPARγ, and myogenin mRNA levels were investigated with C2C12, C3H 10T(1/2), and 3T3-L1 cells. Treating cultures with 10 nM MGA increased ($P<0.05$) C/EBPβ level in C2C12 myoblasts and tended ($P=0.08$) to 3T3-L1 preadipocytes. There was no difference ($P>0.05$) in relative myogenin mRNA level among control, E_2, and MGA treatments. These data indicate that indeed there are populations of cells present in postnatal skeletal muscle that under the appropriate stimuli in a culture model will differentiate into adipogenic pathway. In addition, the synthetic progestin, MGA appeared to upregulate genes necessary for the adipogenesis in the MDC.

Key Words: Melengestrol Acetate, Transdifferentiation, Muscle-Derived Cell

TH74 More selenium (Se) accumulates in whole blood, red blood cells, and liver of beef heifers when supplemented by an organic vs inorganic source. S. F. Liao*, W. R. Burris, K. R. Brown, J. A. Boling, and J. C. Matthews, *University of Kentucky*, *Lexington*.

To determine if source of dietary Se supplement differentially affects Se concentrations of blood constituents and liver tissue in growing cattle, 30 Angus heifers (261 ± 6 d of age) were fed a corn silage-based diet with no Se supplementation for 75 d. Heifers (BW 393 ± 9 kg) then were randomly assigned to 3 mineral supplement treatments (n = 10) and individually fed the supplement plus 7.8 kg/d of a basal cracked corn and cottonseed hull-based diet for 105 or 106 d, using a Calan gate system (5 heifers/pen). The basal diet supplied 0.4 mg Se/d per head, whereas the mineral supplements provided no additional Se/d (control), 3 mg inorganic Se/d as sodium selenite (**ISe**), or 3 mg organic Se/d as Sel-Plex (**OSe**; Alltech, Inc.). Selenium effects on feed efficiency and Se content of blood and liver samples were analyzed by ANOVA. Treatments did not affect ADG (0.58 to 0.67 kg/d) or feed:gain (14.4 to

12.1). Although plasma Se content was not affected, Se in whole blood, red blood cells and liver of ISe and OSe treatment animals was 18 and 59%, 31 and 62%, and 31 and 81% higher ($P < 0.0001$), respectively, than for controls (Table 1). Moreover, whole blood, red blood cells, and liver tissue of OSe animals contained 35, 24, and 38% more ($P < 0.05$) Se than did ISe, respectively. These data show that more Se accumulates in the measured Se pools (except for plasma) of growing heifers when Se is supplemented in the organic (Sel-Plex) vs inorganic (sodium selenite) form.

Table 1. Effect of dietary selenium (Se) supplementation on tissue Se concentrations

		Se supplementation			
Item	Control	Inorganic Se	Organic Se	SEM	P value[1]
Whole blood, μg/mL	0.17[a]	0.20[b]	0.27[c]	0.009	<0.0001
Red blood cells, μg/mL	0.26[a]	0.34[b]	0.42[c]	0.011	<0.0001
Plasma, μg/mL	0.05	0.11	0.08	0.029	0.289
Liver[2], μg/g	0.26[a]	0.34[b]	0.47[c]	0.027	<0.0001

[a,b,c]Means within a row lacking a common superscript letter differ ($P<0.05$). [1]P values associated with the ANOVA F test. [2]Wet weight basis.

Key Words: Bovine, Se Supplementation, Se Tissue Concentration

TH75 Basal content of sugar transporter mRNA in small intestinal epithelia of beef steers is differentially increased by abomasal vs ruminal infusion of starch hydrolysate. S. F. Liao*, D. L. Harmon, E. S. Vanzant, K. R. McLeod, J. A. Boling, and J. C. Matthews, *University of Kentucky*, *Lexington*.

Glucose and fructose are absorbed from the small intestinal lumen by SGLT1 and GLUT5, respectively, whereas GLUT2 mediated the transport of glucose and fructose across both basalateral and apical membranes. To test that mRNA content of these transporters in duodenal (**D**), jejunal (**J**), and ileal (**I**) epithelia is differentially altered by ruminal vs abomasal infusion of corn starch hydrolysate (**SH**; by α-amylase, at 20% of ME intake), 18 ruminally and abomasally catheterized Angus steers (BW ≈ 260 kg) were assigned to either water (basal), ruminal SH, or abomasal SH infusion treatments (n = 6) and fed an alfalfa-cube based diet at 1.3× NE_m requirement. After 14 or 16-d of infusion, steers were killed, small intestinal epithelia harvested, and total RNA extracted. Real-time RT-PCR analysis was conducted to quantify the relative (mRNA:18S rRNA) expression of SGLT1, GLUT5, and GLUT2. Basal expression of SGLT1 and GLUT2 mRNA was greatest ($P \leq 0.10$) by J, whereas J and D expression of GLUT5 mRNA was greater ($P \leq 0.07$) than I. GLUT5 mRNA content was not affected by SH infusion. In contrast, D SGLT1 mRNA content was increased ($P = 0.07$) 64% by ruminal SH infusion and abomasal SH infusion increased ($P \leq 0.05$) I expression of SGLT1 mRNA by 1.3-fold and GLUT2 mRNA by 6.0-fold. These results indicate that increased luminal SH increases the potential for glucose absorption across the I epithelium, increased rumen microbe-derived nutrient supply increases the potential for apical uptake of glucose (GLUT2), whereas fructose uptake potential (GLUT5) was not changed. When viewed with previous findings that SGLT1 activity is insensitive to increased luminal SH, the results also indicate that luminal substrate control of glucose uptake capacity in cattle is complex. For GLUT2 and GLUT5, the findings in this trial are novel for cattle and suggest that GLUT2 activity may respond to luminal substrate supply.

Key Words: Bovine, SLC2 and SLC5 Gene Expression, Substrate Regulation

TH76 Roles of increased IGF-I expression and the estrogen 17β, androgen and IGF-I receptors in estradiol-17β and trenbolone acetate- stimulated proliferation of cultured bovine satellite cells. E Kamanga-Sollo[1], M. E. White[1], M. R. Hathaway[1], K. Y. Chung[2], B. J. Johnson[2], and W. R. Dayton*[1], [1]*University of Minnesota*, *St. Paul*, [2]*Kansas State University*, *Manhattan*.

A combined estradiol 17β (E2)/trenbolone acetate (TBA) implant causes a significant increase in muscle IGF-I mRNA and both E2 and TBA stimulate a significant increase in IGF-I mRNA level in bovine satellite cell (BSC) cultures in media containing 10% fetal bovine serum (FBS). Even though treatment of cultured BSC with E2 or TBA in media containing 1% IGFBP-3-free swine serum results in increased proliferation there is no effect on IGF-I mRNA expression, suggesting that increased IGF-I expression may not be responsible for anabolic steroid enhanced BSC proliferation. To further examine the role of estrogen, androgen and IGF-I receptors and their respective ligands in E2 and TBA-stimulated BSC proliferation, we assessed the effects of specific inhibitors on E2 or TBA-stimulated proliferation of BSC. Both ICI 182 780 (an estrogen receptor blocker) and flutamide (an inhibitor of androgen receptor) suppressed ($p<0.05$) E2 and TBA-stimulated BSC proliferation, respectively. JB1 (a competitive inhibitor of IGF-I binding to the IGF-I receptor) reduced ($p<0.05$) both E2 and TBA-stimulated proliferation in BSC cultures. Both the Raf-1/MAPK kinase (MEK)1/2/ERK1/2, and the phosphatidylinositol 3-kinase (PI3K)/Akt pathways play significant roles in the actions of IGF-I on proliferation and differentiation of myogenic cells. PD98059, an inhibitor of the MAPK pathway, and wortmannin, and inhibitor of the PI3K pathway, both suppressed ($p<0.05$) E2 and TBA-stimulated proliferation of cultured BSC. Our data suggest that IGF-I plays a role in E2 and TBA stimulated proliferation of cultured BSC even in the absence of increased IGF-I expression.

Key Words: Anabolic Steroid, Satellite Cells, IGF-I

TH77 Effects of trenbolone acetate (TBA), Estradiol (E2) and combined TBA/E2 implants on muscle IGF-I and IGF-II mRNA levels in feedlot steers. M. S. Pampusch[1], M. E. White[1], M. R. Hathaway*[1], K. Y. Chung[2], B. J. Johnson[2], and W. R. Dayton[1], [1]*University of Minnesota*, *St. Paul*, [2]*Kansas State University*, *Manhattan*.

We have previously shown that a combined TBA/E2 implant significantly increases IGF-I mRNA levels in longissimus dorsi (LD) muscles of feedlot steers by 28 days after implantation. Here we compare the effects of E2 (24 mg), TBA (120 mg) and combined E2 (24 mg)/TBA (120 mg) implants on IGF-I mRNA levels in the LD muscles of steers implanted for 28 days. Five yearling steers per group were implanted with each implant and 5 control steers received no implant. Steers were weighed weekly starting on d0 and muscle biopsy samples were taken from each steer on d0 (prior to implantation) and on d28. RNA was prepared from each sample and real-time RT-PCR was used to determine

the levels of IGF-I and IGF-II mRNA. Total weight gain over the 28 d period of the study was significantly greater ($p<0.05$, n=5) for steroid-treated steers than for control steers. The IGF-I mRNA levels in LD muscles of control animals were not different on d0 and d28. On d28 of implantation, the IGF-I mRNA level was 60% higher ($p<0.05$, n=5) in E2/TBA implanted animals than in control steers. Similarly, on d28 the LD muscle IGF-I mRNA level was 70% higher ($p<0.05$, n=5) in E2 implanted steers than in control animals. In contrast the TBA implant did not significantly increase LD muscle IGF-I mRNA levels after 28 days of implantation. Muscle IGF-II mRNA levels were not affected by any of the implants. These data suggest that E2 is responsible for the increased muscle IGF-I mRNA level observed in steers implanted with a combined E2/TBA implant.

Key Words: IGF-I, Anabolic Steroid, Muscle

TH78 Effects of androgen and estrogen (E2) receptor blockers and E2-conjugated BSA on estrogen and trenbolone acetate-stimulated IGF-I expression in cultured bovine satellite cells. E Kamanga-Sollo[1], M. E. White*[1], M. R. Hathaway[1], K. Y. Chung[2], B. J. Johnson[2], and W. R. Dayton[1], [1]*University of Minnesota*, *St. Paul*, [2]*Kansas State University*, *Manhattan*.

Even though combined implants containing androgenic and estrogenic steroids are routinely used to enhance muscle growth in domestic meat producing animals, in particular cattle, there is no consensus concerning the mechanism by which they enhance muscle growth. A combined trenbolone acetate (TBA)/estradiol 17β (E2) implant has been shown to increase muscle IGF-I mRNA level and satellite cell number in yearling steers. Additionally, TBA or E2 treatment of cultured bovine satellite cells (BSC) resulted in increased IGF-I mRNA expression. The objective of the present study was to further evaluate the mechanisms of TBA and E2 action on BSC. Flutamide, an androgen receptor blocker, suppresses ($p<0.05$) TBA-stimulated IGF-I expression by cultured BSC indicating TBA functions through the classical androgen receptor. In contrast, ICI 182 780 (ICI), an estrogen receptor blocker, did not suppress E2-stimulated IGF-I expression in BSC cultures. In fact, treatment of cultured BSC with 100 nM ICI in the absence of E2 resulted in a 2-fold increase ($p<0.05$) in IGF-I mRNA level. BSA-conjugated-E2 (E2-BSA) binds to estrogen receptor (ER)-α and ER-β but is not able to cross the cell membrane. E2-BSA (10, 100, or 300 nM) did not stimulate proliferation of cultured BSC indicating that E2-stimulated proliferation is mediated through classical intracellular ER receptors; however, 100 nM E2-BSA did stimulate ($p<0.05$) IGF-I mRNA expression. These results raise the possibility that binding of E2 to cell surface receptors, rather than intracellular ER, is responsible for E2-stimulated IGF-I expression in BSC and suggest that the effects of E2 on IGF-I mRNA level in these cells may not be mediated through the classical genomic mechanisms involving ER-α and the AP-1 response element as it is in others tissues examined to date.

Key Words: IGF-I mRNA, Muscle, Receptor Blockers

TH79 Proglucagon and GLP-2 receptor mRNA distribution in the ruminant gastrointestinal tract. C. C. Taylor-Edwards*, D. B. Edwards, M. J. Doig, E. S. Vanzant, K. R. McLeod, J. A. Boling, J. C. Matthews, and D. L. Harmon, *University of Kentucky*, *Lexington*.

The role of glucagon-like peptide-2 (**GLP-2**) in ruminants is relatively unknown, despite its importance in the regulation of gastrointestinal growth in non-ruminants. This experiment investigated the mRNA expression pattern of proglucagon (**GCG**), the precursor of GLP-2, and the GLP-2 receptor (**GLP-2R**) in the ruminant digestive tract. Effects of nutrient supply on mRNA expression patterns and measures of gastrointestinal mass were tested using three infusion treatments: water (control) or an additional 20% of ME intake as starch hydrolysate into the rumen or abomasum. Eighteen ruminally and abomasally cannulated Angus steers (260 kg BW) were blocked by weight and randomly assigned to treatment (n = 6). Steers were fed an alfalfa-cube diet at $1.3 \times NE_M$ requirement. Steers were infused for 19 to 21 d, with the first 7 d of the period used as an adaptation period to infusion in which the amount of starch hydrolysate infused was incrementally increased each day. Steers were killed, gastrointestinal tissues weighed, and epithelial samples obtained from the forestomachs (rumen, omasum, and abomasum) and intestines (duodenum, jejunum, ileum, and colon). After extraction of total RNA from collected epithelia, real-time PCR was used to determine the expression of GCG and GLP-2R mRNA relative to 18S rRNA. Treatment had no effect on empty BW or mass of most gastrointestinal tissues except the small intestine; abomasal starch infusion increased small intestinal weight (% empty BW) by 17% ($P = 0.003$) and 18% ($P = 0.002$) compared with water or ruminal starch infusion, respectively. Expression of GCG mRNA was 5000-fold greater ($P < 0.0001$) in intestines than forestomachs. Likewise, GLP-2R expression was 49-fold greater ($P < 0.0001$) in intestines than forestomachs. Although dietary treatment did not affect expression patterns of GCG and GLP-2R mRNA, these results describe the tissue distribution of mRNA for the GLP-2 precursor and the GLP-2 receptor in ruminants.

Key Words: Glucagon-Like Peptide-2, Ruminant, mRNA Expression

TH80 Effects of overfeeding adolescent ewe lambs on progeny growth. G. J. Eckerle*, R. V. Anthony, and R. K. Peel, *Colorado State University*, *Fort Collins*.

Adolescent lambs, impregnated by embryo transfer and fed ad lib throughout gestation, are reported to produce growth restricted offspring. It was our objective to determine if ad lib feeding of peripupertal ewes following natural mating at the second observable estrus, impacted birthweight and postnatal growth characteristics of the offspring. Two replicate studies were completed, in which singleton-bearing ewe lambs were fed a complete gestational diet (11.4 MJ metabolic energy/ kg DM) at a rate which met NRC gestational age requirements (MN; n=8 year 1, n=7 year 2) or were fed ad lib (15% refusal rate) throughout gestation (HN; n=6 year 1, n=7 year 2). There was no effect ($P \geq 0.10$) of gestational intake on lamb birthweight (year 1: 5.0±0.23 vs. 4.9 ±0.20 kg; year 2: 5.01±0.42 vs. 5.68±0.40 kg; MN vs. HN respectively), lamb abdominal circumference or crown-rump length. However, during year 2, neonatal death loss was increased in HN pregnancies (0% vs. 57%; MN vs. HN), due to increased ($P \leq 0.01$) dystocia (1.0±0.0 vs. 3.5±0.7 score; 1=no assistance to 5=cesarean section). During year 1, lambs were weaned at 80 days of age and fed ad lib (11.4 MJ metabolic energy/ kg DM) until 140 days of age. Neither preweaning (0.32±0.018 vs. 0.33±0.015 kg/d, MN vs. HN) nor overall (0.28±0.009 vs. 0.29±0.010 kg/d; MN vs. HN) growth rate were effected ($P \geq 0.10$) by gestational intake. During year 2, lambs remained with their dams until they were weaned at 60d., and were fed ad lib until reaching the target slaughter weight (59 kg). Similar to year 1, no differences ($P \geq 0.10$) were observed in growth rate, target weight age, or in carcass characteristics (rib eye

area, back fat thickness and percent kidney pelvic and heart fat) collected at slaughter. Collectively, our results indicate that ad lib feeding adolescent ewe lambs, which conceived to natural service, does not impact fetal or postnatal growth rate of the progeny, but may result in increased dystocia rate and neonatal mortality.

Key Words: Peripubertal Lambs, Gestational Overfeeding, Progeny Growth Rates

TH81 Biological efficiency of crossbred beef cattle finished on feedlot and slaughtered with distinct body weights. R. Mello*[1,3], M. H. de Faria[2], A. C. de Queiroz[3], F. D. de Resende[2], D S Henrique[3], and F Maldonado[2], [1]*Universidade Federal de Roraima*, *Boa Vista, RR, Brazil*, [2]*APTA*, *Colina, SP, Brazil*, [3]*Universidade Federal de Viçosa*, *Viçosa, MG, Brazil*.

The objective in this trial was to assess the bionutritional efficiency of crossbred F1 Red Angus ± Nellore (1/2 RA 1/2 N) and F1 Blond D'Aquitain ± Nellore (1/2 BA 1/2 N) young bulls finished on feedlot and slaughtered with 480, 520 and 560 kg of shrunk body weight (SBW). A completely randomized experimental design in a 2 ± 3 (2 genetic groups ± 3 slaughter weights) factorial arrangement with six replicates was used. Data were analyzed in the SAS® software using initial SBW as a covariate. The table below shows the least-square means of ADG, DMI, gain to feed ratio (GFR), biologic multivariate nutritional index (BMNI), Kleiber ratio (KR) and residual feed intake (RFI). There was no difference among the different treatments for ADG. There was not significant effect for GG, SW and interaction on relative DMI (% BW and g/$BW^{0.75}$). As the slaughter weight rised the absolute DMI (kg/d) increased. The 1/2 BA 1/2 N young bulls slaughtered with 480 kg had lower (P<0.05) feed:gain ratio (5.2) than others (> 6.8), but GFR and KR didn't differ (P>0.05) among treatments. The 1/2 BA 1/2 N young bulls had lower (P<0.05) BMNI and RFI than 1/2 RA 1/2 N young bulls. The lighter young bulls had lower (P<0.05) BMNI in relation to the heavier young bulls. Thus, crossbred F1 Blond D'Aquitain ± Nellore young bulls and lighter animals are more bionutritionally efficient in the finishing phase on feedlot than F1 Red Angus ± Nellore and heavier animals.

Table 1. Least square means

	Genetic Group (GG)		Slaughter Weight (SW)		
	½ RA ½ N	½ BA ½ N	480	520	560
ADG, kg/d	1.490	1.614	1.636	1.475	1.544
DMI, kg/d	10.8	10.4	10.1^{b}	10.4^{ab}	11.4^{a}
DMI, % BW	2.21	2.15	2.18	2.15	2.20
DMI, g/ $BW^{0.75}$	104.8	100.8	101.5	100.9	106.1
GFR	0.139	0.156	0.161	0.142	0.140
BMNI	5.87^{B}	5.07^{A}	4.72^{b}	5.51^{ab}	6.19^{a}
KR	14.4	15.7	16.3	14.4	14.5
RFI	0.312^{B}	-0.327^{A}	-0.078	-0.085	0.140

Within a row, means followed by different capital letters and by different small letters differ (P<.05), respectively, among GG and SW by Tukey test

Key Words: Animal Performance, Feed Efficiency, Residual Feed Intake

TH82 Estimation of carcass and empty body chemical composition of Nellore and Caracu breeds. S. F. M. Bonilha*[1], L. O. Tedeschi[2], I. U. Packer[3], A. G. Razook[1], L. A. Figueiredo[1], R. F. Nardon[4], and G. F. Alleoni[4], [1]*Instituto de Zootecnia, Agência Paulista de Tecnologia dos Agronegócios*, *Sertãozinho, SP, Brazil*, [2]*Texas A&M University*, *College Station*, [3]*Universidade de São Paulo/ESALQ*, *Piracicaba, SP, Brazil*, [4]*Instituto de Zootecnia, Agência Paulista de Tecnologia dos Agronegócios*, *Nova Odessa, SP, Brazil*.

Linear regressions, based on 9-11th rib section chemical composition, were developed to estimate carcass and empty body chemical composition of 56 selected Nellore (NeS), 52 control Nellore (NeC), and 56 selected Caracu (CaS) bulls, with 20 to 24 mo of age at slaughter. Carcass composition was obtained after grinding, homogenizing, sampling, and analyzing edible fraction + bones. Empty body chemical composition was obtained after grinding, homogenizing, sampling, analyzing, and combining blood, hide, head + feet, viscera, and carcass. The percentages of water and ether extract (EE) were determined both on the rib section and on carcasses of group 1 (36 NeS; 36 NeC; and 36 CaS bulls). In group 2 (20 NeS; 16 NeC; and 20 CaS bulls) empty body percentages of water and EE were also determined. Linear regressions were developed between carcass and rib section compositions (for group 1) and between carcass and empty body compositions (for group 2). The interactions between chemical components and genetic groups in the developed equations were not significant (P > 0.05). The 9-11th rib section percentages of water (RW) and EE (RF) precisely predicted ($r^2 > 0.75$) the percentage of carcass water (CW): %CW = 29.0806 + 0.4873×%RW, $r^2 = 0.81$, SE = 1.06; %CW = 64.8316 - 0.3549×%RF, $r^2 = 0.78$, SE = 1.14. The percentages of RW and RF precisely predicted the percentage of carcass fat (CF): %CF = 10.4039 + 0.5179×%RF, $r^2 = 0.86$, SE = 1.26; %CF = 61.6067 - 0.6928×%RW, $r^2 = 0.85$, SE = 1.33. Linear regressions ($r^2 > 0.75$) between percentages of CF and CW and empty body water (BW) were found: %BW = -9.7560 + 1.1637×%CW, $r^2 = 0.88$, SE = 1.43; %BW = 73.1673 - 0.8274×%CF, $r^2 = 0.88$, SE = 1.41. Linear regressions ($r^2 > 0.75$) between percentages of CF and CW and empty body EE (BF) were found: %BF = 0.3667 + 1.0393×%CF, $r^2 = 0.98$, SE = 0.65; %BF = 101.3752 - 1.4095×%CW, $r^2 = 0.91$, SE = 1.47. Chemical composition of 9-11th rib section precisely estimated carcass percentages of water and EE. Good linear regressions were detected between carcass and empty body chemical composition.

Key Words: 9-11th Rib Section, Ether Extract, Water