### **Growth and Development I**

**T223** Exogenous estradiol decreases relative endometrial gene expression for *ERa* and *ERβ* in pre-pubertal dairy heifers. Chelsee T.L. Holloway\*<sup>1</sup>, Adam J. Geiger<sup>1</sup>, Jennifer Malkus<sup>1</sup>, Alan D. Ealy<sup>2</sup>, Rebecca R. Cockrum<sup>1</sup>, and Robert M. Akers<sup>1</sup>, <sup>1</sup>Virginia Polytechnic Institute and State University, Department of Dairy Science, Blacksburg, VA, <sup>2</sup>Virginia Polytechnic Institute and State University, Department of Animal and Poultry Sciences, Blacksburg, VA.

The objectives of this study were to determine (1) the effect of early nutrition on reproductive development and (2) response of reproductive tissues to estradiol  $(E_2)$  in pre-pubertal heifers. Holstein heifer calves (n = 23; 7 d of age) were fed either a restricted milk replacer diet (20% CP, 20% fat, 454 g/d) or accelerated diet (28% CP, 25% fat 1,333 g/d) for 8 wks. After the initial 8 wks, heifers were partitioned into a 2 × 2 factorial design and treated for an additional 2 wks accordingly: restricted diet + placebo implant (CON; n = 6), accelerated diet + placebo (ACC; n = 5), restricted diet +  $E_2$  implant (CONEST; n = 6) and accelerated diet +  $E_2$  (ACCEST; n = 6). Estrogen receptor  $\alpha$  (*ER* $\alpha$ ) and  $\beta$  (*ER* $\beta$ ) gene expression was determined from RNA isolated from harvested endometrial, ovarian, and hypothalamic tissues using Real-Time qPCR. Fold changes were analyzed using the GLM procedure in SAS with treatment used as a fixed effect and initial body weight used as a covariate. The main effect of diet did not differ ( $P \ge 0.127$ ) for any of the tissues. However,  $E_2$  treatment differed for the endometrium (P  $\leq 0.001$ ), but not for the ovary or hypothalamus ( $P \geq 0.528$ ). In the endometrium, ERa was upregulated ( $P \le 0.024$ ) in ACC heifers (8.44  $\pm$  1.21) as compared with ACCEST (2.69  $\pm$  1.43), and CONEST heifers  $(2.63 \pm 1.22)$ . Gene *ER* $\beta$  was also upregulated more ( $P \le 0.052$ ) in ACC heifers  $(4.16 \pm 0.52)$  than ACCEST  $(2.11 \pm 0.61)$  or CONEST heifers  $(1.46 \pm 0.52)$  in the endometrium. Additionally, *ER* $\beta$  was upregulated (P < 0.001) in CON heifers  $(4.56 \pm 0.52)$  as compared with CONEST heifers, and tended (P = 0.071) to be upregulated in ACCEST heifers. Overall, results indicated that diet alone did not affect expression of  $ER\alpha$ or  $ER\beta$  in reproductive tissues of pre-pubertal heifers. However, treatment with  $E_2$  in pre-pubertal heifers affected relative gene expression of key genes within the hypothalamic-gonadal pathway. Determining the effect of estrogen on the development of reproductive tissues is key to understanding signaling pathways that activate the onset of puberty.

Key Words: estradiol, gene expression, nutrition

**T224** Plane of nutrition affects muscle fiber hypertrophy and satellite cell activity in neonatal bull calves. Jennifer S. Bradley\*, Meghan E. MacGhee, Sarah R. McCoski, Amanda M. Reeg, Alan D. Ealy, and Sally E. Johnson, *Virginia Polytechnic Institute and State University, Blacksburg, VA*.

Muscle growth in newborn calves is a product of protein accretion concomitant with addition of nuclei to the muscle fiber. The objective of the experiment was to examine the effect of diet on live animal performance, muscle fiber hypertrophy and satellite cell activity in young calves. Neonatal Holstein bull calves were fed either a high nutritional plane (HNP; 27% CP, 10% fat) or low nutritional plane (LNP; 20% CP, 20% fat) milk replacer. Daily intake of GE for the LNP group was 50% of HNP, but exceeded the NRC requirement for NEm and NEg. Groups of HNP (n = 4) and LNP (n = 4) were slaughtered at 2, 4 and 8 wk. The LM was removed for fiber morphometric evaluation and satellite cell isolations. Data were analyzed by ANOVA using PROC GLM for the main effects of diet and time and their interaction. Calves

fed HNP gained more (P < 0.05) BW at 2, 4 and 8 wks than LNP with the largest difference at 8 wks (HNP = 749 g/d, LNP = 134 g/d, pooled SEM = 0.05). Fiber cross-sectional area was larger (P < 0.05) in HNP than LNP at 8 wk ( $1502 \pm 242 \ \mu\text{m}^2 \text{ vs.} 356 \pm 25 \ \mu\text{m}^2$ ). The numbers of Pax7 immunopositive satellite cells per fiber did not differ (P > 0.05) between the groups with time or diet. Satellite cell isolates were cultured in growth media and pulsed for 2 h with EdU, a thymidine analog, to measure proliferation rate. Isolates from HNP at 2 wks contained a greater (P < 0.05) percentage of proliferating cells during log phase growth than LNP at equal cell densities (51.7 and 35.2%, respectively, SEM = 2.1). At 4 wks, the percentage of proliferating HNP satellite cells was less (P < 0.05) than that of LNP isolates (21.5 vs. 39.1%, SEM = 1.6) and tended (P = 0.10) to remain lower at 8 wk. No differences were observed in the numbers of myofibers formed from HNP or LNP satellite cell isolates. These results demonstrate that calves fed a higher plane of nutrition gain more weight and experience greater muscle fiber hypertrophy than calves fed at a lower level. The larger HNP fiber may be attributed to greater satellite cell proliferation and their subsequent fusion into the adjacent fiber.

Key Words: muscle fiber, hypertrophy, Pax7

**T225** Effects of heat-treatment of colostrum on absorption and gut development in neonatal bull calves during the first 12 hours of life. Coral Kent-Dennis<sup>1</sup> and Thomas McFadden\*<sup>2</sup>, <sup>1</sup>University of Alberta, Edmonton, AB, Canada, <sup>2</sup>University of Missouri, Columbia, MO.

Heat-treatment of colostrum reduces transmission of pathogens to neonatal calves but may alter the bioactivity of colostrum. The objective was to determine the effects of heat-treating colostrum on absorptive function and gut development in neonatal calves. First-milking colostrum was collected in batches and stored at -20°C. Batches were thawed, pooled, mixed thoroughly, and divided into equal volumes. Half was re-frozen in 1L aliquots and the other half was heat-treated at 60°C for 60 min then re-frozen in 1L aliquots. Holstein bull calves were enrolled in the study as they were born and received 2L of either heat-treated (n = 9)or unheated (n = 8) colostrum via esophageal tube feeder within 1.5h of birth. Blood samples were taken at 0 (pre-colostrum), 3, 6, 9 and 12h after birth for analysis of IgG and β-lactoglobulin (BLG) concentrations. Six calves from each treatment group were euthanized at 12h after birth and ileal tissue was collected for analysis of villus height, crypt depth, and goblet cell count. Statistical analysis was conducted using PROC MIXED of SAS with batch as a random effect and repeated measures where appropriate. Colostral IgG concentration did not differ between heated and unheated pools. Plasma IgG concentration tended to be higher in calves fed unheated versus heated colostrum at 9 h and the difference was significant at 12h ( $15.8 \pm 1.4$  versus  $12.9 \pm 1.4$  mg/mL; P = 0.02). Plasma BLG concentrations were significantly higher in calves fed unheated versus heat-treated colostrum at 6 h (324.3  $\pm$  52 versus  $239.9 \pm 51.0$  mg/mL; P = 0.04) and 9h ( $258.2 \pm 52.0$  versus  $126.3 \pm$ 51.9 mg/mL; P = 0.002). By 12 h, there was no difference between the 2 treatment groups. These data suggest that different colostral proteins have different rates of absorption and biological half-lives. Villus height and crypt depth did not differ significantly between treatment groups. The average number of goblet cells on ileal villi was higher in calves fed heat-treated versus unheated colostrum (63.4  $\pm$  3.3 versus 48.8  $\pm$ 3.3; P = 0.02). Results indicate that heat-treatment of colostrum altered

absorption of colostral proteins and stimulated development of mucusproducing cells but did not affect mucosal growth.

Key Words: colostrum, pasteurization, calf health

## **T226** Direct-fed microbial efficacy and milk plan: Effect on heifer growth in an automated feeding system. Melissa C. Cornett\*

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Automated feeding systems (AF) offer flexible nutrition programs for pre-weaned dairy calves. The technology offers the option of changing daily milk allotment as calves grow and providing feed additives, such as direct-fed microbial (DFM). This study determined the effect of DFM efficacy and 2 milk plans (SLW, FST) on growth. Holstein heifers (n = 104) from a commercial Wisconsin herd were enrolled at birth in a  $2 \times 2$  factorial design. Factor 1 was a DFM protocol versus a placebo (PLAC) from birth to weaning. Factor 2 compared 2 feeding plans (SLW and FST) offering 543L pasteurized whole milk over 53 feeder days (FD). The SLW initially offered (7L/d), and peaked milk at 28 FD (13L/d). The FST initially offered 11L/d, and peaked milk at 21 FD (15L/d). Both plans stopped milk at 53 FD. Rate of passive transfer was 86%. Calves were housed individually before AF. Ability of calf to drink milk unassisted triggered AF move. During AF, ad libitum Ampli-Calf (Land O' Lakes, MN) calf starter was provided in a bunk. No individual starter intakes were recorded. Calf weight (WT)

was measured  $2 \times / \text{wk}$  from age  $3 \pm 2 \text{ d}$  (Mean  $\pm$  SD) until 1 wk postweaning. The effect of treatments on WT was evaluated using PROC MIXED in SAS, controlling for initial WT, age, and study period, study month was a random effect and calf nested in treatment for repeated measure. Study period was divided into 4 times: Period 1 birth to AF (P1), Period 2 FD1 - FD28 when FST offered more milk/d than SLW (P2), Period 3 FD29-FD53 (P3), and Period 4, the 7 d post-weaning (P4). Average milk consumption was  $430.9 \pm 89.0$  L for SLW and 378.5 $\pm$  78.7L for FST. Initial feeder enrollment age was 7  $\pm$  2 d. Milk plan, DFM, and study period interacted (P = 0.03). In P2, FST DFM calves weighed  $3.6 \pm 1.5$  kg (LSM  $\pm$  SEM) more than FST PLAC (P < 0.02). In P3, FST DFM calves weighed  $3.2 \pm 1.5$  kg more than FST PLAC (P < 0.04). In P3, SLW PLAC weighed  $3.0 \pm 1.5$  kg more than FST PLAC (P < 0.05). In P4, FST DFM tended to weigh  $2.9 \pm 1.6$  more than FST PLAC (P = 0.07). These results suggest DFM effectively improves growth in dairy calves on a rapid increase milk plan. Calves provided peak milk at 28FD had higher growth than calves with peak milk at 21FD. Growth can be optimized by AF and DFM.

Key Words: calves, growth, probiotic

# **T227** Anti-oxidative status and inflammatory response in neonatal calves fed quercetin with or without colostrum. Harald M. Hammon<sup>\*1</sup>, Jeannine Gruse<sup>1</sup>, Manfred Mielenz<sup>1</sup>, and Siegfried Wolffram<sup>2</sup>, <sup>1</sup>Leibniz Institute for Farm Animal Biology (FBN), Dummerstorf, Germany, <sup>2</sup>Christian Albrechts University, Kiel, Germany.

Flavonoids such as quercetin are known to exert anti-inflammatory and anti-oxidative effects that could improve neonatal health. We hypothesized that quercetin feeding during the first week of life may improve anti-inflammatory and antioxidative status in neonatal calves and that quercetin feeding compensates for an inadequate colostrum supply. Twenty-eight new-born male Holstein calves were randomly assigned to 2 feeding groups receiving same amounts of either colostrum (C; n = 14) or a milk-based formula (F; n = 13) with same nutrient density as C, but no biologically active factors (e.g., growth factors), during the first 2 d of life. From d 3 to d 7, all calves were fed milk replacer at

12% of BW. From d 2 on, groups were subdivided each into a treatment group receiving 150  $\mu$ mol quercetin/(kg BW × d) as quercetin aglycone with milk and a control group without quercetin. For characterization of anti-oxidative status, trolox equivalent antioxidative capacity (TEAC), ferric reducing ability of plasma, thiobarbituric acid reactive substances (TBARS) and prostaglandin-like substances were analyzed in blood plasma taken on d 1, 4, and 7. Liver biopsy was taken 2 h after morning meal on d 8 to determine mRNA abundances of anti-oxidative enzymes (catalase; glutathione peroxidase; superoxide dismutase) and inflammation markers (tumor necrosis factor, TNFα; serum amyloid A2, SAA2; C-reactive protein, CRP) using quantitative real-time PCR. Data were evaluated by Proc Mixed (blood) or Proc GLM (mRNA) of SAS with milk and quercetin as fixed effects. TEAC and TBARS were greater in C- than in F-fed calves (P < 0.05). Relative mRNA abundance of TNF $\alpha$ was greater in quercetin-fed groups (P < 0.05); CRP was greater (P <0.05) and SAA2 tended to be greater (P < 0.1) in F- than in C-fed calves. Absence of C feeding was associated with a reduced anti-oxidative status and increased hepatic mRNA abundance of acute phase proteins, which underlines the importance of C feeding for neonatal health. Results further indicate that quercetin supplementation barely affects the antiinflammatory and antioxidative status in neonatal calves.

Key Words: calf, quercetin, antioxidative status

#### **T228** Effect of milk aroma flavor on starter consumption, growth, and feed conversion in female Holstein calves. Yaqi Wu, Yanliang Bi, Shengli Li\*, Yajing Wang, and Zhijun Cao, *College* of Animal Science and Technology, China Agriculture University, Beijing, China.

Milk aroma flavor (Luctarom1866, Lucta, Spain) was added to calf starter to investigate its effects on starter consumption (SC), growth and feed conversion in dairy calves. Forty-four female Holstein calves (6 wk of age;  $56 \pm 6$  kg of BW) were randomly assigned to 2 groups, which were fed with 2 g or 0 g milk aroma flavor per kg starter. All calves consumed the same milk and starter from birth to the beginning of the experiment (wk 0-6). The experiment lasted 4 weeks (wk 7-10) and calves were provided same milk from the beginning of the experiment (wk 7) and weaned at 8 wk of age. SC was recorded daily and each calf was weighed weekly. The data were analyzed by ANOVA and LSD test at 5% and 1% probability utilizing SAS statistical program. The results indicated that SC of calves fed flavor had no significant differences in wk 7, 8, 9 and during the entire experimental period, while it increased by 0.38 kg per day (P < 0.05) in wk 10. Compared with the calves fed starter with no flavor, the calves fed flavor gained more weight in wk 9 (0.2 kg per day (P < 0.05)), wk 10 (0.3 kg per day (P < 0.01)), and during the whole experimental period (0.1 kg per day (P < 0.05)). From wk 9 to wk 10, the ratio of weight gain to SC was 0.42 and 0.38 for calves fed with and without flavor (P > 0.05). Overall, milk aroma flavor in starter could improve SC and average daily gain (ADG) in dairy calves and tend to encourage feed conversion.

**Key Words:** milk aroma flavor, average daily gain, weight gain:starter consumption

**T229** Caloric restriction reduces protein accretion in skeletal muscle by attenuating IGF-I signaling in young calves. Yue Lu<sup>1</sup>, Jennifer S. Bradley<sup>1</sup>, Sarah R. McCoski<sup>1</sup>, John M. Gonzalez<sup>2</sup>, Adam J. Geiger<sup>1</sup>, R. Michael Akers<sup>1</sup>, Alan D. Ealy<sup>1</sup>, and Sally E. Johnson<sup>\*1</sup>, <sup>1</sup>Virginia Polytechnic Institute and State University, Blacksburg, VA, <sup>2</sup>Kansas State University, Manhattan, KS.

Caloric restriction decreases skeletal muscle mass in mammals, principally due to a reduction in fiber size. This study examined the consequences of reduced caloric intake on the metabolic properties of skeletal muscles in young Holstein heifer calves. Newborn heifers were assigned to a control (CON, n = 5; 28% CP, 25% fat) or caloric restricted (CR, n = 5; 20% CP, 20% fat) milk replacer diet from birth to 8 wks of age. Grain (25% CP, 4% fat) was pair-fed beginning at wk 5. Diets supported ADG of 771 g/d and 220 g/d for CON and CR calves, respectively. Heifers were euthanized at 8 wks and the LM was collected for muscle morphometry, gene expression and metabolic signal transduction measurements. The effect of diet was exained using by Student t-test with  $\alpha = 0.05$ . Results revealed that CR heifers had smaller (P < 0.05) LM fiber cross-sectional area and a greater percentage of oxidative type I and IIA fibers. The oxidative fiber type of the CR LM was not associated with elevated expression of  $PGC1\alpha$ , a mitochondria biogenesis factor. Reverse transcription-qPCR revealed PGC1a mRNA levels were 40% lower in CR than CON LM. Western blot analysis detected reduced (P < 0.05) amounts of PGC1 $\alpha$ 4, a PGC1a splice variant, in CR LM. The PGC1 $\alpha$ 4 target gene, *IGF-I*, was 40% lower (P < 0.05) in CR than CON. Downstream mediators of autocrine IGF-I signaling are attenuated in CR by comparison to CON. The amount of phosphorylated AKT was lower (P < 0.05) in CR than CON. However, activated components of the AKT/mTOR signaling axis, p70 S6 kinase, ERK1/2 and 4E-BP1, were equivalent or unaffected (P > 0.05) by CR. Activation of the energy sensor, AMP kinase, was not detected in either CON or CR lysates indicating that a block to mTOR-controlled protein synthesis is absent. Protein lysates from CR LM contained less (P < 0.05) total and phosphorylated, inactive GSK3<sup>β</sup> than CON LM. Elevated GSK3<sup>β</sup> activity is associated with inhibition of protein synthesis as well as induction of atrogin. Reverse transcription-qPCR demonstrated no differences (P < 0.05) in atrogin expression levels between CON and CR. These results indicate that CR suppresses a hypertrophic PGC1a4/IGF-I/AKT pathway in young heifer calves through an undiscovered mechanism.

Key Words: skeletal muscle, metabolism, IGF-I

## **T230** Use of ultrasound for assessment of muscle area and depth in postmortem preweaned Holstein calves. Jessica H.

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The prewean period is one of the most important stages of dairy calf development. A tool is needed to more accurately assess calf growth, specifically muscle development. The objective was to determine if ultrasound can be used to predict longissimus dorsi (ribeye) linear depth and external carpi radialis (front) and semitendinosus (hind) area in postmortem preweaned Holstein calves. Postmortem bull and heifer calves (n = 191, age  $17.3 \pm 20.67$  d, body weight  $37.9 \pm 19.07$ kg) were obtained from 2 calf ranches between April and July 2013. Ultrasound images of the ribeye, front, and hind muscles were collected using an Aloka 500V equipped with a 5-cm 7.5-MHz linear transducer. Ultrasound ribeye linear depth and front and hind areas were calculated using the Ultrasound Image Capture System. The ribeye was dissected and measured for linear depth. The front and hind muscles were dissected and the cross-sectional planes were traced onto transparency paper. The transparency paper was photocopied and individual paper muscle tracings were cut out and weighed. The weights of the paper muscle tracings were then converted to areas using the known area of a standard 8.5 × 11 inch paper. Means were calculated using PROC GLM in SAS (version 9.2). Mean dissected values for the ribeye, front, and

hind muscles  $(1.65 \pm 0.44 \text{ cm}, 6.23 \pm 1.83 \text{ cm}^2, 9.05 \pm 2.37 \text{ cm}^2)$  were greater than the respective mean ultrasound values  $(1.46 \pm 0.37 \text{ cm}, 5.41 \pm 1.49 \text{ cm}^2, 8.60 \pm 2.40 \text{ cm}^2)$  indicating ultrasound underestimated the true linear depth and area values consistently. The relationship between the dissected and ultrasound measurements was tested using Pearson correlation coefficient (PROC CORR). Overall, there was a strong, positive relationship between both the dissected and ultrasound measurements for the ribeye (r = 0.55, P < 0.01), front (r = 0.65, P < 0.01), and hind muscle (r = 0.80, P < 0.01). The weight, age, and sex of the calf and the operator of the ultrasound may explain some of the variability not accounted for by the correlation coefficient. The *semitendinosus* muscle displayed the highest correlation coefficient and may be used in future studies to assess calf muscle growth and guide implementation of dynamic feeding changes on both dairy farms and calf ranches.

Key Words: ultrasound, dairy calf, muscle

**T231** Increasing the metabolizable protein supply enhanced growth performance and humoral immune response of preconditioning beef steers. Philipe Moriel<sup>1</sup>, Luis Felipe Arelaro<sup>\*1</sup>, Matt Poore<sup>2</sup>, Rodrigo Marques<sup>3</sup>, and Reinaldo Cooke<sup>3</sup>, <sup>1</sup>North Carolina State University, Waynesville, NC, <sup>2</sup>North Carolina State University, Raleigh, NC, <sup>3</sup>Oregon State University, Burns, OR.

We evaluated the effects of metabolizable protein (MP) supply on growth performance, and post-vaccination measurements of humoral immune response of beef steers. Angus steers (n = 36;  $231 \pm 21$  kg; 184  $\pm$  18 d) were weaned on d 0, stratified by weight and age on d 7, and randomly assigned into 1 of 18 drylot pens (2 steers/pen). Treatments were assigned to pens (6 pens/treatment), and consisted of isocaloric, corn silage-based diets formulated to provide 85, 100 or 115% of the daily MP requirements. Diets were offered at 2.1% of BW (DM basis) from d 7 to 49. Steers were vaccinated on d 21 and 35 with modified-live and killed vaccines (Select-Vac, Zoetis). Blood samples were collected from jugular vein on d 0, 21, 35 and 49 to determine the plasma cortisol concentrations, and serum titers against bovine viral diarrhea virus-1b (BVDV1b), infectious bovine rhinotracheitis (IBR) and Mannheimia haemolytica leukotoxin. Data were analyzed using GLIMMIX procedure of SAS, and pen(treatment) as random effect. Body weight differed only on d 49, and was greatest ( $P \le 0.05$ ) for 115MP, least for 85MP and intermediate ( $P \ge 0.21$ ) for 100MP steers (297, 278 and 290  $\pm$  7 kg, respectively). From d 7 to 49, 100MP and 115MP steers had similar ADG (P = 0.13), but both had greater (P < 0.001) ADG than 85MP steers (1.20, 1.35 and  $0.83 \pm 0.068$  kg/d, respectively). Plasma cortisol concentrations were greater ( $P \le 0.03$ ) for 85MP vs. 100MP and 115MP steers on d 21 and 35. Serum BVDV1b titers were greatest  $(P \le 0.02)$  for 115MP, and similar (P = 0.37) between 85 and 100MP steers (5.8, 3.7 and  $3.0 \pm 0.60$  base 2 log, respectively). Mean serum *M. haemolytica* leukotoxin titers were greater (P = 0.05) for 85MP vs. 100MP and 115MP steers (3.12, 2.39 and  $2.49 \pm 0.21$  ng/antibody bound, respectively). Hence, a 15% MP deficiency increased plasma cortisol, and both factors may have suppressed innate immune system leading to greater multiplication of endogenous M. haemolytica and a compensatory greater production of serum M. haemolytica leukotoxin titers. In contrast, 15% MP excess enhanced growth performance and increased serum BVDV1b titers of beef steers.

Key Words: metabolizable protein, steer, immune

#### T232 Characterization of key factors constraining lamb

**survival in marginal environments.** Francisco A. Sales<sup>\*1</sup>, Sue A. McCoard<sup>2</sup>, and Raúl J. Lira<sup>1</sup>, <sup>1</sup>Instituto de Investigaciones Agropecuarias, Punta Arenas, Chile, <sup>2</sup>AgResearch, Palmerston North, New Zealand.

Sheep farming normally occurs in areas with limited vegetation. Climate change is placing more pressure on farming in these challenging environments. There is limited research on factors limiting lamb survival in marginal environments. The objective of this study was to characterize fetal and maternal factors associated with low (<80%) lamb survival in Patagonia. Corriedale ewes, synchronized, superovulated and mated to Suffolk rams and managed under commercial grazing conditions were used. Single- (n = 8) and twin-bearing (n = 8) ewes were euthanized at d 140 of gestation and mass of the fetal and placental components and ewe carcass traits were obtained. The effect of litter size (single vs twin) on maternal and fetal characteristics was analyzed using ANOVA. Maternal live weight was similar between twin- and single-bearing ewes ( $60.4 \pm 1.6 \text{ vs } 61.4 \pm 1.6 \text{ kg}$ , P = 0.7), while twin-bearing ewes had lower carcass weight compared with single-bearing ewes (18.9)

 $\pm$  0.6 vs 20.7  $\pm$  0.6 kg, P = 0.05), associated with a trend for reduced loin area (11.8  $\pm$  0.7 vs 13.8  $\pm$  0.7 kg, P = 0.07), but similar carcass fat depth (Fat GR:  $4.5 \pm 1.1$  vs  $4.12 \pm 1.1$  mm, P = 0.8; Fat C:  $3.8 \pm 0.6$  vs  $3.8 \pm 0.6$  mm, P = 1.00). Twin fetuses were lighter ( $3.24 \pm 0.11$  vs 4.18 $\pm$  0.14 kg, P < 0.01), with proportionally lighter organ and leg muscle weight (P < 0.05), a tendency for less perirenal fat (9.15 ± 0.64 vs.  $10.99 \pm 0.78$ , P = 0.07) and disproportionately lighter adrenal glands (P = 0.05), M. adductor (P = 0.02) and M. gluteus (P = 0.03) compared with singletons. Total placentome weight  $(256.2 \pm 10.6 \text{ vs } 391.5 \pm 14.9 \text{ vs})$ g, P < 0.01) and number (49.6 ± 4.0 vs 75.6 ± 5.7, P < 0.01) were lower in twin- comparer to single-bearing ewes. Reduced placental mass and reduced fetal weight in twins compared with singletons is consistent with prior studies. The greater mobilization of skeletal muscle but not fat in twin-bearing ewes is likely a mechanism to enhance protein supply to the fetuses. Compared with studies in less marginal environments, the fetuses (both singles and twins) in this study had a higher percentage of perirenal fat (0.3% vs 0.2%), which may indicate an adaptive response, to enhance fetal survival.

Key Words: sheep, fetal, growth