

## Growth and Development

**109 Zfp423 promotes adipogenic differentiation of bovine stromal vascular cells.** Y. Huang<sup>\*1</sup>, A. Das<sup>1,2</sup>, Q. Yang<sup>1,2</sup>, M.-J. Zhu<sup>1,2</sup>, and M. Du<sup>1,2</sup>, <sup>1</sup>Department of Animal Science, University of Wyoming, Laramie, <sup>2</sup>Department of Animal Sciences, Washington State University, Pullman.

Intramuscular fat or marbling is critical for the palatability of beef. Intramuscular fat mainly locates inside perimysial connective tissue. In mice, very recent studies show that adipocytes and fibroblasts share a common pool of progenitor cells, with Zinc finger protein 423 (Zfp423) as a key initiator of adipogenic differentiation, while fibrogenesis is promoted by transforming growth factor- $\beta$  (TGF- $\beta$ ) signaling pathway. To evaluate the role of Zfp423 in intramuscular adipogenesis and marbling in beef cattle, we sampled Sternocleidomastoid muscle for separation of stromal vascular cells (SVC). These cells were immortalized with pCI neo-hEST2 which carries a telomerase and individual clones were selected by G418, an aminoglycoside antibiotic. A total of 288 clones (3 X 96 well plates) were isolated and induced adipogenic differentiation, and their adipogenic potential was assessed by the formation of adipocytes with Oil-Red-O staining. Three clones with the highest and lowest adipogenic potential were selected for further analyses. The expression of Zfp423 was much higher ( $307.4 \pm 61.9\%$ ,  $P < 0.01$ ) in high adipogenic cells, while TGF- $\beta$  was higher ( $156.1 \pm 48.7\%$ ,  $P < 0.05$ ) in low adipogenic cells. Following adipogenic differentiation, the expression of peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ) and CCAAT/Enhancer Binding Protein  $\alpha$  (C/EBP $\alpha$ ) were higher ( $239.4 \pm 84.1\%$  and  $310.7 \pm 138.4\%$ , respectively,  $P < 0.05$ ) in high adipogenic cells. Transfecting a plasmid overexpressing Zfp423 in both stromal vascular cells and cloned low adipogenic cells dramatically increased their adipogenic differentiation, accompanied with the inhibition of TGF- $\beta$  expression. In conclusion, data show that Zfp423 is a critical regulator of adipogenesis in stromal vascular cells, and Zfp423 may provide a molecular target for enhancing intramuscular adipogenesis and marbling in beef cattle.

**Key Words:** adipogenesis, muscle, beef cattle

**110 Agouti signaling protein abundance in cattle—Relationship with fat deposition.** E. Albrecht<sup>\*1</sup>, K. Komolka<sup>1</sup>, H. Sauerwein<sup>2</sup>, T. Gotoh<sup>3</sup>, and S. Maak<sup>1</sup>, <sup>1</sup>Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany, <sup>2</sup>University Bonn, Bonn, Germany, <sup>3</sup>Kyushu University, Kuju-cho, Oita, Japan.

The agouti signaling protein (ASIP), originally known for its role in melanogenesis, has been related to obesity in mice and humans. The potentially crucial role in adipocyte development, elucidated in cell culture models, makes it a tempting candidate for economically relevant, fat related traits in farm animals. The objective of our study was to characterize the protein abundance of ASIP in different adipose tissues, in skeletal muscle, and in blood of cattle differing in fat deposition. Japanese Black (JB;  $n = 6$ ) and Holstein (HS;  $n = 5$ ) steers were fed a high energy diet to maximize intramuscular fat (IMF) deposition, resulting in  $34.3\% (\pm 1.7)$  and  $20.4\% (\pm 2.5)$  IMF content in longissimus muscle (LM) for JB and HS, respectively, when harvested at 26 mo of age. Protein extract from subcutaneous, visceral, perirenal, intra- and intermuscular fat, and LM were investigated by Western blotting with a bovine-specific ASIP antibody. Blood plasma samples were also

investigated by Western blotting after albumin removal. By immunohistochemistry, ASIP was localized in different cell types of adipose tissues, including adipocytes, and also in skeletal muscle tissue, but never in muscle fibers. In addition to the expected cytosolic signal, a putative nuclear localization of ASIP was observed. The protein abundance was highly variable among animals, and did not show a clear relationship with fat related traits like depot weights or fat cell size. Between breeds, the protein was similarly abundant within fat depots and did not reflect the earlier described ASIP mRNA overexpression in JB compared with HS. However, we detected higher ( $P < 0.05$ ) ASIP levels in blood plasma of JB (normalized to albumin) than in HS ( $1.13 \pm 0.08$  vs.  $0.89 \pm 0.09$ , respectively), which were paralleled by higher ( $P < 0.05$ ) leptin concentrations in JB ( $18.9$  ng/ml  $\pm 3.1$ ) vs. HS ( $12.6$  ng/ml  $\pm 4.1$ ). Our results provide evidence for a widespread expression of ASIP in bovine adipose tissues at the protein level and for the first time for the secretion and circulation of this protein in the blood of cattle, suggesting a potential endocrine function.

**Key Words:** ASIP, adipose tissue, blood

**111 Blood glucose and acylated ghrelin in response to duration of maternal undernutrition during gestation in twin sheep pregnancies.** M. E. Field,<sup>\*</sup> R. V. Anthony, M. D. Veters, C. Flörcke, T. E. Engle, S. L. Archibeque, and H. Han, Colorado State University, Fort Collins.

The experimental objective was to determine the effect of duration of maternal undernutrition during gestation in twin pregnancies on maternal and lamb blood parameters. Multiparous Western white face ewes were fed 100% (Control;  $n = 8$ ), or 50% of their nutrient requirements from 28 to 78 d gestational age (dGA) and readjusted to 100% beginning at 79 dGA (50–100;  $n = 10$ ), or 50% from 28 to term (50–50;  $n = 9$ ). Lambs were birthed naturally, weaned at 10 wk postpartum (wPP) and harvested at 18 wPP. Data were analyzed by preplanned orthogonal contrasts: Control vs. 50–100 and 50–50 and 50–100 vs. 50–50. At 49 and 77 dGA GLU was greater ( $P < 0.05$ ) in Control vs. 50–100 and 50–50 ewes; the same trend ( $P < 0.10$ ) occurred at 63 dGA. At 105, 112, 126, and 147 dGA GLU was greater ( $P < 0.05$ ) in 50–100 vs. 50–50 ewes. At 2 wPP ewe GLU was greater ( $P = 0.03$ ) in 50–100 ( $3.60 \pm 0.17$  mM) and 50–50 ( $3.29 \pm 0.17$  mM) than Control ( $2.99 \pm 0.16$  mM). Ewe aGRL was greater ( $P = 0.02$ ) in 50–100 ( $237 \pm 32$  pg/mL) and 50–50 ( $239 \pm 38$  pg/mL) vs. Control ( $136 \pm 32$  pg/mL) at 140 dGA and at 4 wPP the same pattern was observed (50–100,  $388 \pm 38$  pg/mL; 50–50,  $326 \pm 45$  pg/mL; Control,  $184 \pm 38$  pg/mL). Lamb GLU was reduced ( $P = 0.02$ ) in 50–100 ( $4.12 \pm 0.09$  mM) vs. 50–50 ( $4.52 \pm 0.09$  mM) at 14 wPP. Lamb aGRL was greater ( $P = 0.06$ ) in 50–100 ( $141 \pm 47$  pg/ml) and 50–50 ( $184 \pm 48$  pg/ml) vs. Control ( $59 \pm 37$  pg/ml) at 2 wPP, but did not differ at 16 wPP. Elevated post-weaning glucose in 50–50 lambs may indicate increased insulin resistance in these lambs. Early nutrient restriction resulted in elevated aGRL, even following realimentation, which persisted into the postpartum period, suggesting potential long-lasting programming of maternal appetite control during early- to mid-gestation as a result of dietary restriction. This project was supported by National Research Initiative Competitive Grant no. 2009-35206-05273 from the USDA National Institute of Food and Agriculture.

**Key Words:** maternal undernutrition, ghrelin, sheep

**112 Delaying a bovine viral diarrhea vaccine and growth implant with metaphylaxis affects performance, but not health of feedlot heifers.** M. R. McDaniel<sup>1</sup>, M. E. Hubbert<sup>2</sup>, and C. A. Loest<sup>1</sup>, <sup>1</sup>Department of Animal and Range Sciences, New Mexico State University, Las Cruces, <sup>2</sup>Clayton Livestock Research Center, New Mexico State University, Clayton.

Stress and low nutrient intake of newly received feedlot calves may limit efficacy of both a bovine viral diarrhea (BVD) vaccine and growth implant at initial processing. Use of chlortetracycline (CTC) when delaying an initial vaccine and implant may be beneficial. This 56-d study evaluated effects of timing (TIME) of a BVD vaccine and growth implant with or without CTC on health and performance of 312 heifers (184 ± 0.7 kg BW). Initial processing of heifers included treatment with tulathromycin and vaccination for bovine respiratory disease. Heifers were sorted into 24 pens, which were randomly assigned to 4 treatments. Treatments (2 × 2 factorial) were growth implant (Synovex-C; Fort Dodge Animal Health) and BVD vaccination (Bovi-Shield Gold BVD; Pfizer Animal Health) of calves on d 1 (INITIAL) or d 28 (DELAYED) after arrival with (+CTC) or without (-CTC) 3 5-d pulse-doses of CTC (Aureomycin; Alpharma Animal Health) in their diets. Diets were based on a complete feed (RAMP; Cargill) and ground corn delivered twice daily. Performance and health were analyzed using MIXED and GLIMMIX procedures of SAS, respectively. A TIME × CTC interaction ( $P = 0.06$ ) occurred for DMI; DMI was lower for DELAYED than INITIAL when heifers received -CTC, but DMI was not different between DELAYED and INITIAL when heifers received +CTC. No TIME × CTC interactions ( $P \geq 0.21$ ) occurred for BW, ADG, or G:F. Timing of BVD vaccine and implant did not affect ( $P = 0.18$ ) BW of heifers on d 28, but BW were lower ( $P = 0.04$ ) for DELAYED than INITIAL heifers on d 56. From d 29 to 56, ADG was lower ( $P = 0.02$ ) and G:F tended to be lower ( $P = 0.07$ ) for DELAYED than INITIAL heifers. Delaying the BVD vaccine and implant also tended to lower ( $P = 0.09$ ) ADG from d 0 to 56, but did not affect ( $P = 0.27$ ) G:F. On d 28, BW of +CTC heifers tended to be lower ( $P = 0.06$ ) than -CTC heifers, but CTC did not affect ( $P \geq 0.21$ ) d-56 BW, ADG, or G:F. Morbidity and mortality during the 56 d were not affected ( $P \geq 0.17$ ) by treatments. Delaying the initial BVD vaccine and growth implant with or without CTC may not improve animal health, and may have negative implications on performance of newly received feedlot heifers.

**Key Words:** vaccine, implant, heifer

**113 The effects of intrauterine growth retardation (IUGR) due to poor maternal nutrition on adipose tissue development and metabolic status in sheep.** M. L. Hoffman<sup>1</sup>, M. A. Rokosa<sup>1</sup>, S. Neupane<sup>1</sup>, S. M. Spignesi<sup>1</sup>, J. Lee<sup>2</sup>, S. A. Zinn<sup>1</sup>, and K. E. Govoni<sup>1</sup>, <sup>1</sup>Department of Animal Science, University of Connecticut, Storrs, <sup>2</sup>Department of Nutritional Sciences, University of Connecticut, Storrs.

Poor maternal nutrition negatively affects growth and development of the fetus as well as postnatal metabolism. We hypothesized that intrauterine growth retardation (IUGR) due to poor maternal nutrition would increase adiposity and negatively affect metabolism. Ewes pregnant with twins ( $n = 24$ ) were fed 100% (CON), 60% (RES) or 120% (OVER) of NRC requirements beginning at d 85 of gestation. Offspring were euthanized within 24 h of birth (d 1;  $n = 3$ /treatment) or at 3 mo of age ( $n = 3$  to 5/treatment) at which point blood samples and renal adipose tissues were collected. Animals maintained until 3 mo were removed from the ewe and fed a control diet of milk replacer and 18% creep feed. Circulating concentrations of leptin, insulin and glucose were determined by quantitative ELISA. Total cholesterol (TC) and triglycerides (TG) were

determined by enzymatic analysis. Gene expression was determined by real time-reverse transcriptase (RT)-PCR. Data were analyzed using ANOVA with significance considered as  $P \leq 0.05$ . As reported, BW were reduced by 16% in RES lambs at d 1 and 3 mo ( $P \leq 0.05$ ) compared with CON. A significant effect of treatment was not observed for concentrations of insulin or glucose ( $P \geq 0.30$ ) or mRNA expression of markers for adipogenesis, *PPAR $\gamma$*  and *C/EBP $\alpha$* , at d 1 or 3 mo ( $P \geq 0.60$ ). However, at d 1, concentrations of leptin tended to be 34% and 61% greater in RES and OVER, respectively ( $1.28 \pm 0.16$ ,  $1.72 \pm 0.12$ ,  $2.07 \pm 0.40$  mg/dL; CON, RES, OVER, respectively;  $P \leq 0.12$ ). At 3 mo, concentrations of leptin tended to be 25% and 31% greater in RES and OVER, respectively ( $0.91 \pm 0.16$ ,  $1.14 \pm 0.11$ ,  $1.27 \pm 0.08$ ; CON, RES, OVER, respectively;  $P \leq 0.13$ ). A similar trend was observed in *leptin* mRNA expression with a  $2.60 \pm 0.70$ -fold increase in OVER at 3 mo ( $P \leq 0.13$ ). At d 1, we did not observe a significant effect of treatment on TC ( $39.52 \pm 3.09$ ,  $47.3 \pm 4.27$ ,  $46.70 \pm 2.44$ ; CON, RES, OVER respectively;  $P \leq 0.22$ ). At 3 mo, TG were 45% greater in OVER compared with CON ( $P = 0.07$ ). In conclusion, these data suggest that poor maternal nutrition during gestation alters markers of adipogenesis and metabolism of lipids during early postnatal growth.

**Key Words:** intrauterine growth retardation, adipose tissue, leptin

**114 Maternal nutrition of beef cattle on pasture mediates long-term consequences for offspring primarily through effects on growth early in life.** P. L. Greenwood,\* L. M. Cafe, and D. L. Robinson, Australian Cooperative Research Centre for Beef Genetic Technologies and NSW Department of Primary Industries, Armidale, NSW, Australia.

Long-term consequences of dam nutrition on offspring, beyond those due to variation in early-life growth, were studied. Hereford cows were mated in consecutive years to Piedmontese or Wagyu sires. When confirmed pregnant, treatments (low or high pasture quality and availability) were applied to cows ( $n = 513$ ) until parturition and/or weaning. At weaning (7mo), 4 early-life growth groups (gestation-preweaning: Low-Low, Low-High, High-Low and High-High) of offspring ( $n = 240$ ) were selected within steer and heifer cohorts for further study, resulting in multi-modal distributions of offspring growth to birth and weaning. A stage stepwise regression procedure (final exclusion parameter,  $P > 0.05$ ) examined covariates including birth (BW) and weaning (WW) weight, age at measurement (A), and effects of nutrition during pregnancy (P) and lactation (L), calf sex (S), year (Y), sire breed (G), and first order interactions of factors and with covariates. BW was affected by P; WW by L and P, over and above differences due to BW. For feedlot entry (26mo), exit (30mo) and carcass (CW) weights, there were significant linear effects of BW and WW plus effects of G, S and Y, but no effects of P or L beyond those due to differences in BW and WW. Average differences of 6kg in BW of calves due to P and 55kg in WW due to L both translated into differences of ~20kg in CW, 11–12kg in retail yield and 6kg of fat trim at 30mo, but did not affect beef tenderness or marbling. When adjusted to a constant CW, heavier WW cattle had more fat trim and less retail yield. There were few interactions, however at the same CW there was a significant G × P interaction for rib eye area: Piedmontese-sired, Low 95.7 vs. High 88.4cm<sup>2</sup>; Wagyu-sired, both groups 85.7cm<sup>2</sup>. Feedlot feed intake ( $n = 142$ ) was affected by BW and WW but not when corrected for weight at start of the intake test. FCR was affected by WW and L, and whether dams were lactating in early pregnancy. Maternal nutrition did not affect RFI. Overall, there were few long-term influences of maternal nutrition on offspring beyond those related to early-life growth within our pasture-based system. Optimal maternal nutrition that maximizes capacity to re-breed has

greater economic effect than longer-term consequences of maternal nutrition on offspring.

**Table 1.** Effects on productivity

	Mean	BW	WW	P		L		Model
		Slope/ kg	Slope/ kg	Low	High	Low	High	
BW, kg	33.7	NA <sup>2</sup>	NA	29.8	35.8*	NA	NA	33.2; P G S 62.5; L BW
WW, kg	188.8	2.26*	NA	179.1	190.6#	158.3	211.5*	A S P 67.5; BW Y WW G
CW, kg	382	2.58*	0.38*	ns	ns	ns	ns	40.8; G S
US marble score	446	ns	ns	ns	ns	ns	ns	48.8; CW Y P G
Rib eye area, <sup>3</sup> cm <sup>2</sup>	89.7	ns	ns	See text		ns	ns	95.2; CW G WW
Retail yield, <sup>3</sup> kg	249.1	ns	-0.08*	ns	ns	ns	ns	14.0; Y S
LD shear force, kg	4.05	ns	ns	ns	ns	ns	ns	

<sup>1</sup>See text.

<sup>2</sup>Not applicable.

<sup>3</sup>Constant CW.

\* $P < 0.001$ ; # $P < 0.01$ ; NS  $P > 0.05$ .

**Key Words:** birth weight, fetal programming, nutrition

**115 Lean tissue accretion and the efficiency of energy and protein retention are enhanced by intermittent bolus compared to continuous feeding.** S. W. El-Kadi,\* C. Boutry, M. C. Gazzaneo, A. Suryawan, R. A. Orellana, N. Srivastava, H. V. Nguyen, M. L. Fiorotto, and T. A. Davis, *USDA/ARS Children's Nutrition Research Center, Department of Pediatrics, Baylor College of Medicine, Houston, TX.*

Feed remains the largest non-fixed input cost in animal production systems, and dietary interventions aimed at enhancing the efficiency of nutrient utilization may provide new avenues for improving profitability. To determine the effect of feeding modality on growth and lean tissue accretion, neonatal pigs ( $n = 6/\text{treatment}$ , 6-d-old) were fed the same diet in equivalent amounts continuously (CON) or intermittently (INT; meal every 4 h) for 21 d. Body composition was measured by dual x-ray absorptiometry (DXA) before and 18 d following the initiation of feeding, and fractional rate of protein synthesis was measured using the flooding dose method. Fractional protein synthesis rates, and plasma glucose and insulin were determined on the last day of feeding. Weight gain was greater ( $P < 0.05$ ) for INT than for CON pigs and resulted in heavier body weights from 9 d of feeding onwards. Total lean tissue mass and spine length were greater ( $P < 0.05$ ) in INT than CON pigs. Glucose and arterial insulin levels were greater ( $P < 0.05$ ) for INT pigs after the meal than for CON pigs. Compared with the CON group, muscle protein synthesis was 40% higher in longissimus dorsi, and 34% higher in gastrocnemius and soleus ( $P < 0.05$ ) of INT pigs. Longissimus dorsi and soleus muscles were 56 and 35% heavier ( $P < 0.05$ ) in INT than CON pigs. Protein synthesis was 22 and 48% higher ( $P < 0.05$ ) in liver and ileum of INT compared with CON pigs. Liver, jejunum and ileum weights were 72, 40, and 35% higher ( $P < 0.05$ ) in pigs receiving the INT compared with the CON treatment. Intermittent feeding resulted in improved ( $P < 0.05$ ) feed conversion efficiency (5.1 vs. 7.2 kg milk/kg body weight gain) compared with continuous feeding. In addition, the efficiency of protein and energy retentions were both enhanced ( $P < 0.05$ ) by 30 and 44% with intermittent compared with continuous feeding. These results indicate that intermittent feeding, as compared

with continuous feeding, upregulates protein synthesis resulting in an improved efficiency of protein and energy retention, greater lean tissue accretion, and enhanced growth in the neonate. Supported by NIH AR444474 and USDA/ARS 6250-51000-055.

**Key Words:** protein synthesis, body composition, insulin

**116 Tripalmitolein infusion in finished lambs.** T. A. Burns,\* M. C. Miller, A. K. G. Kadegowda, H. M. Stowe, S. M. Calcaterra, and S. K. Duckett, *Clemson University, Clemson, SC.*

Palmitoleic acid (C16:1 *cis*-9) has been proposed to function as a lipokine, regulating lipogenesis and desaturation in murine liver. Previously, our bovine adipocyte cultures provide evidence that palmitoleic acid decreases lipogenesis and desaturation. Our objective was to determine if palmitoleic acid functions similarly in ruminant animals where the primary site of lipogenesis is adipose tissue. Eight Southdown lambs approximately 13 mo of age were assigned to 1 of 2 treatment groups [tripalmitin (control) or tripalmitolein (lipokine)] in a completely randomized design. Both jugular veins were cannulated; one was used to infuse 4  $\mu\text{M}$  triglyceride treatment at 9  $\mu\text{mol} \cdot \text{kg BW}^{-1}$  per d over a 4 d period and the other was used to sample blood throughout the study and every 30 min for 9.5 h before slaughter. On the last day of infusion, 0.7 mmol  $1\text{-}^{13}\text{C}18:0$  was administered 8 h before slaughter. Lambs were sacrificed 96 h after infusion began. Serum was analyzed for fatty acid composition and enrichment. Dorsal and sternal subcutaneous adipose tissues and liver were flash frozen for gene expression and fatty acid analysis. Lipogenic genes of interest were *fatty acid synthase (FASN)* and *stearoyl-CoA desaturase (SCD1)*. There was a main effect of depot on fatty acid composition and gene expression. In the liver, C16:0, C16:1 *cis*-9, C18:1 *cis*-9, were decreased ( $P < 0.05$ ) and C18:2 n-6 was increased ( $P < 0.05$ ) compared with adipose tissue depots. The desaturation ratios (C16:1/C16:0 and C18:1 *cis*-9/C18:0) were highest in sternal adipose tissue compared with dorsal and liver tissues. Expression of *FASN* and *SCD1* was reduced ( $P < 0.05$  and  $P = 0.07$ , respectively) in sternal adipose tissue compared with dorsal subcutaneous fat. In addition, *FASN* was downregulated ( $P < 0.05$ ) in lipokine compared with control lambs. Enrichment of serum C18:0 and C18:1 *cis*-9, detectable at 90 min following stable isotope injection, indicates a reduction in desaturase activity. The fractional synthetic rate and tracer:tracee ratio of C18:1 *cis*-9 from C18:0 was greater over time ( $P < 0.05$ ) in control compared with lipokine lambs. Therefore, intravenous tripalmitolein infusion alters on fatty acid biosynthesis in lambs.

**Key Words:** palmitoleic acid, lamb, desaturation

**117 Nutritional milieu of preadipocytes determines the differentiating capabilities of bovine primary stromal vascular cultures.** A. K. G. Kadegowda,\* M. C. Miller, T. A. Burns, A. Wright, and S. K. Duckett, *Clemson University, Clemson, SC.*

Objectives were to determine the effect of nutritional milieu of preadipocytes on adipogenesis and adipose lipid metabolism in bovine adipocytes. Bovine primary stromal vascular cultures were isolated from intermuscular fat of Angus x Hereford steers (508 kg BW,  $n = 3$  per treatment) fed alfalfa/soybeans with (LC) or without (L) corn grain supplementation (0.75% BW) for 105 d. Preadipocytes were differentiated (D0) in differentiation media [DMEM containing 10% fetal calf serum, 2.5  $\mu\text{g}/\text{mL}$  insulin, 0.25  $\mu\text{M}$  DEX, 1  $\mu\text{M}$  rosiglitazone, 0.5 mM IBMX, and 10 mM acetate] for 2 d and from D2 to D12 without DEX and IBMX. From D2 to D12, cells were treated with (0 or 100  $\mu\text{M}$ ) of linoleic acid (C18:2). Cells were harvested on D-2, D0, D2, D4, D6 and D12 for

gene expression by RT-qPCR and on D6 for fatty acid (FA) analysis by GLC. Cell viability of pre-differentiated cells was assayed using Cell Counting Kit-8. The adipocyte cell sizing was done using Cell Scepter. Glycerol-3-phosphate dehydrogenase (GPDH) activity was assayed to determine adipocyte differentiation. The treatment effects were analyzed by Proc Mixed of SAS 9.2 with steer as experimental unit. The average diameter of preadipocytes and differentiated adipocytes ( $P < 0.05$ ) were larger in LC ( $16.25 \pm 0.48 \mu\text{m}$  and  $18.08 \pm 0.37 \mu\text{m}$ ) compared with those of L ( $15.58 \pm 0.43 \mu\text{m}$  and  $16.95 \pm 0.32 \mu\text{m}$ ). However, the preadipocytes from L proliferated faster ( $P < 0.05$ ) than those from LC. The GPDH activity (nmol/mg protein per min), an indicator of adipocyte differentiation was higher ( $P < 0.01$ ) in adipocytes from L ( $4.32 \pm 0.50$ ) compared with LC ( $0.51 \pm 0.5$ ). Also, the expression of genes associated with adipocyte differentiation: *fatty acid binding protein 4*, *GPDH*, *peroxisome proliferator-activated receptor gamma*, *CCAAT/enhancer binding protein  $\alpha$*  (*C/EBP $\alpha$* ), and *stearoyl-CoA desaturase 1*, were higher ( $P < 0.05$ ) in L compared with LC. Whereas the total FA content or the FA composition (except C18:1c9,  $P < 0.05$ ) did not differ between un-supplemented L and LC, C18:2 supplementation showed higher ( $P < 0.05$ ) content of total FA, C16:0, C16:1, C18:0, C18:1 and C18:2 in L compared with LC. The results showed that the nutritional milieu determines the differentiating capabilities of bovine primary stromal vascular cultures.

**Key Words:** adipocytes, fatty acids, adipogenesis

**118 Effects of feeding different forage sources on rumen fermentation and gastrointestinal tract development in young calves.** Ll. Castells<sup>\*1</sup>, A. Bach<sup>1,2</sup>, and M. Terré<sup>1</sup>, <sup>1</sup>Department of Ruminant Production, IRTA, Caldes de Montbui, Spain, <sup>2</sup>ICREA, Barcelona, Spain.

It has been previously demonstrated that offering chopped forage to young calves increases ADG and feed intake. However, the mechanism

involved in improving calf performance is not clear. Fifteen Holstein calves (BW =  $43.6 \pm 4.37$  kg) were randomly assigned to one of the 3 dietary treatments ( $n = 5$ ) to determine the effects of feeding different forages sources on rumen fermentation and gastrointestinal tract (GIT) development. Treatments consisted on a pelleted starter concentrate (20% CP, 21% NDF) offered alone (CTR), with alfalfa hay (ALF), or with oats hay (OAT) in separate buckets. Calves received 2 L of milk replacer (MR) at 12.5% DM twice daily until 49 d of age. From 50 to 56 d of age, calves received 2 L/d of MR at 12.5% and were weaned at 57 d of age. Starter, forage, and MR intake were recorded daily and BW weekly. A rumen sample was taken by an oral tube weekly to determine rumen pH and VFA concentrations. Three weeks after weaning, animals were harvested and each anatomical part of the GIT was dissected and weighed. Data were analyzed with a mixed-effects model with repeated measures, except for harvest data that had no repeated measures. Animals in OAT treatment tended ( $P = 0.10$ ) to eat more starter concentrate than the other treatments after weaning ( $2612$  vs  $1990 \pm 270.8$  g/d, respectively), and ALF animals ate more ( $P = 0.05$ ) forage than OAT animals ( $147$  vs  $47 \pm 31.0$  g/d, respectively). Rumen pH was lower ( $P < 0.01$ ) in CTR compared with OAT and ALF calves ( $5.5$  vs  $6.2 \pm 0.16$ , respectively). Furthermore, acetate proportion in the rumen tended to be greater ( $P = 0.10$ ) in ALF than in CTR and OAT treatments ( $51.8$  vs  $46.4 \pm 1.91\%$  respectively), and butyrate proportion was numerically ( $P = 0.12$ ) lower in ALF compared with the others 2 treatments ( $9.9$  vs  $14.7 \pm 1.77\%$ , respectively). Total GIT weight expressed as a percentage of BW tended ( $P = 0.07$ ) to be greater in ALF compared with the other 2 treatments ( $22.4$  vs  $20.1 \pm 0.75\%$ , respectively). In conclusion, feeding forages to young calves increases rumen pH, although the rumen fermentation profile differs depending on the forage source.

**Key Words:** calves, forage, gastrointestinal development