

# Small intestinal growth measures are correlated with feed efficiency in market weight cattle, despite minimal effects of maternal nutrition during early to midgestation<sup>1</sup>

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**ABSTRACT:** We hypothesized that gestational nutrition would affect calf feed efficiency and small intestinal biology, which would be correlated with feed efficiency. Multiparous beef cows ( $n = 36$ ) were individually fed 1 of 3 diets from d 45 to 185 of gestation: native grass hay and supplement to meet NRC recommendations (control [CON]), 70% of CON NE<sub>m</sub> (nutrient restricted [NR]), or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON. After d 185 of gestation, cows were managed as a single group, and calf individual feed intake was measured with the GrowSafe System during finishing. At slaughter, the small intestine was dissected and sampled. Data were analyzed with calf sex as a block. There was no effect ( $P \geq 0.33$ ) of maternal treatment on residual feed intake, G:F, DMI, ADG, or final BW. Small intestinal mass did not differ ( $P \geq 0.38$ ) among treatments, although calf small intestinal length tended ( $P = 0.07$ ) to be greater for NR than NR+RUP. There were no differences ( $P \geq 0.20$ ) in calf small intestinal density or jejunal cellularity, proliferation, or vascularity among treatments. Jejunal *solute guanylyl cyclase* mRNA was greater ( $P < 0.03$ ) for NR+RUP than CON and NR. Residual

feed intake was positively correlated ( $P \leq 0.09$ ) with small intestinal mass and relative mass and jejunal RNA content but was negatively correlated ( $P \leq 0.09$ ) with jejunal mucosal density and DNA concentration. Gain:feed was positively correlated ( $P \leq 0.09$ ) with jejunal mucosal density, DNA, protein, and total cells and was negatively correlated ( $P \leq 0.05$ ) with small intestinal relative mass, jejunal RNA, and RNA:DNA. Dry matter intake was positively correlated ( $P \leq 0.09$ ) with small intestinal mass, relative mass, length, and density as well as jejunal DNA and protein content, total cells, total vascularity, and *kinase insert domain receptor* and *endothelial nitric oxide synthase 3* mRNA and was negatively correlated ( $P = 0.02$ ) with relative small intestinal length. In this study, calf performance and efficiency during finishing as well as most measures of small intestinal growth were not affected by maternal nutrient restriction during early and midgestation. Results indicate that offspring small intestinal gene expression may be affected by gestational nutrition even when apparent tissue growth is unchanged. Furthermore, small intestinal size and growth may explain some variation in efficiency of nutrient utilization in feedlot cattle.

**Key words:** feed efficiency, feed intake, fetal programming, maternal nutrition, pregnancy, small intestine

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## INTRODUCTION

In many species, maternal nutrient restriction during gestation can alter fetal growth, resulting in impaired development, low birth weight offspring, and potential long-term consequences (Wu et al., 2006; Caton and Hess, 2010; Reynolds et al., 2010). Beef cows are often undernourished during gestation due to limiting forage quality and quantity, and ruminant offspring intestinal tissues are responsive to maternal nutrition during gestation (reviewed in Meyer et al., 2012a). Because organogenesis

of the fetus occurs in early to midgestation, maternal nutrition during this period may have a more pronounced effect on small intestinal development (Fowden et al., 2006; Nathanielsz, 2006). The small intestine is not only the main site of postruminal nutrient absorption, but it also uses a large proportion of energy and nutrients for its size due to its high metabolic activity and rapid turnover (Ferrell, 1988; Johnson et al., 1990). Effects of maternal nutrition on the small intestine may therefore impact later growth and performance, including feed intake and feed efficiency.

Feed inputs are the greatest annual cost for cow-calf producers (USDA-ERS, 2014); therefore, supplementation of poor quality forages may not be practical throughout gestation. Despite this, little is known of how targeted supplementation during specific periods of gestation impacts fetal development and subsequent offspring performance. We hypothesized that nutrient restriction of beef cows during early to midgestation would affect calf feed intake and efficiency during finishing as well as small intestinal development and that supplementation with RUP during this period of restriction would mitigate these effects. Additionally, we hypothesized that feed intake and efficiency would be correlated with measures of small intestinal growth and vascularity in market weight cattle.

## MATERIALS AND METHODS

All animal procedures were approved by the University of Wyoming (UW) Institutional Animal Care and Use Committee.

### *Animals and Diets*

**Cow Management and Dietary Treatments.** Beef cows used in this study were selected from the 3- and 4-yr-old Angus × Gelbvieh cows in the UW beef herd, estrous synchronized, and artificially inseminated to a single sire. Cows were managed as a single group postbreeding and grazed native range at the UW McGuire Ranch. Pregnancy was diagnosed via transrectal ultrasonography on d 33 of gestation, at which time calves were weaned from 42 of the most uniform cows. These cows were then pen-fed (6 cows/pen) native grass hay (6.2% CP, DM basis) with a protein supplement to provide 10% dietary CP at the UW Livestock Center in Laramie, WY.

On d 40 of gestation, pregnancy was again confirmed, and 36 cows (24 diparous 3-yr-old cows and 12 tripary cows) were randomly allocated by BW and parity to receive 1 of 3 dietary treatments (Table 1) from d 45 to 185 of gestation: a control (**CON**) diet consisting of native grass hay (6.2% CP, DM basis) and a soybean meal-based supplement to meet NRC (2000) requirements for midgestation, a nutrient-restricted (**NR**) diet providing

**Table 1.** Composition of maternal dietary treatments fed from d 45 to 185 of gestation

Item	Maternal treatment <sup>1</sup>		
	CON	NR	NR+RUP
Ingredient, <sup>2</sup> % as fed			
Native grass hay	86.6	86.6	77.7
Soybean meal	8.3	8.3	7.4
Molasses	1.2	1.2	1.1
Dicalcium phosphate	2.4	2.4	—
Limestone	1.4	1.4	—
Mineral premix <sup>3</sup>	0.2	0.2	—
Fishmeal	—	—	9.5
Feather meal	—	—	3.4
Blood meal	—	—	1.1
Nutrient composition			
CP, % DM	10.0	10.0	17.1
NDF, % DM	62.7	62.7	58.8
IVDMD, %	47.2	47.2	50.9
DMI, kg/d	10.0	8.0	7.0

<sup>1</sup>Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC (2000) recommendations, a nutrient-restricted (NR) diet providing 70% of CON NE<sub>m</sub>, or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON from d 45 to 185 of gestation.

<sup>2</sup>227 g of 110,000 IU of vitamin A/kg, 27,500 IU of vitamin D/kg, and 660 IU of vitamin E/kg was added to 907 kg (as fed) of each protein supplement mixture.

<sup>3</sup>The mineral premix contained 68.3% KCl, 27.6% FeSO<sub>4</sub>, 3.1 ZnO, 0.6% MnO, and 0.4% CuSO<sub>4</sub>.

70% of the NE<sub>m</sub> of CON, or a NR diet with a RUP supplement (**NR+RUP**) to provide similar essential AA flow to the duodenum as CON. The CON diet was formulated to allow for 0.51 kg/d of BW gain in the nonlactating pregnant cows, based on the NRC (2000) recommendations for pregnant primiparous heifers to gain 0.43 kg/d BW. Minerals and vitamins were added to the CON and NR diets to provide intake similar to the NR+RUP treatment (Table 1). The RUP supplement (68.7% menhaden fish meal, 24.5% hydrolyzed feather meal, and 6.8% porcine blood meal, DM basis), designed by Scholljegerdes et al. (2005b), was provided to match the duodenal AA flow of CON using the following equation: total essential AA flow to the small intestine (g/d) = (0.055 × g of OM intake) + 1.546 (Scholljegerdes et al., 2004). This was also adjusted to account for increased RUP supplement degradation in feed-restricted cattle (Scholljegerdes et al., 2005a).

Cows were housed in dry-lot pens (6 × 20 m; n = 6/pen) and individually fed gestational treatments using individual stanchions, as previously described (Whitney et al., 2000; Lake et al., 2005). At each feeding (0600 and 1600 h daily), 50% of the daily supplement allotment was offered. After supplement consumption, within 20 min, 50% of the daily hay was offered to each cow for the remainder of each 2-h feeding period. Cows seldom had hay refusals greater than 1 kg. Hay and supplement offered daily were adjusted every 14 d for increasing NE<sub>m</sub> requirements of advancing gestation and BW change.

### Posttreatment Management and Feed Intake Test.

After treatment conclusion at d 185 of gestation, cows were managed together through calving and weaning. Calves were weaned (at approximately 200 d of age), transported to the UW Sustainable Agriculture Research and Extension Center in Lingle, WY, and backgrounded for 14 d before being placed in the feedlot and penned by sex and dam's dietary treatment. During the growing period, all animals were fed a diet containing 54.7% hay, 16.2% corn silage, 25.2% corn, and 3.9% supplement (DM basis), which was gradually transitioned to a finishing diet containing 85.8% corn, 3.0% corn silage, 6.9% hay, and 4.3% supplement (DM basis), all formulated to deliver 350 mg/d of monensin per animal·d<sup>-1</sup> of monensin.

The GrowSafe feed intake system (model 4000E; GrowSafe Systems Ltd., Airdrie, AB, Canada) was used to record individual daily feed intakes of the corn and corn silage-based finishing diet (11.2% CP, 1.98 Mcal NEm/kg, 1.33 Mcal NEg/kg, 0.55% Ca, and 0.28% P, DM basis) during the finishing period for 84 d. Two-consecutive-day BW were taken at the initiation and conclusion of the finishing period to determine ADG. From these data, expected feed intake was determined by regressing ADG and metabolic midweight on actual feed intake. Residual feed intake (**RFI**) was then calculated as the expected feed intake subtracted from the actual feed intake (Cammack et al., 2005).

### Tissue Collection and Analysis

At the conclusion of the feed intake test, steers and heifers were transported to the UW Meat Laboratory for slaughter (steers:  $n = 17$ ,  $448 \pm 1$  d of age,  $572.0 \pm 14.8$  kg BW, and 3 or 8 d postfeed intake test; heifers:  $n = 14$ ,  $466 \pm 1$  d of age,  $528.6 \pm 11.4$  kg BW, and 22 or 24 d postfeed intake test). Animals remained on the finishing diet after the feed intake test conclusion and had free access to water during a 24-h feed withdrawal before slaughter. Steers and heifers were slaughtered using standard commercial methods, and visceral organs were removed for dissection following inspection.

Viscera were removed after exsanguination, and the small intestine was dissected. A 150-cm section (not stretched and with minimal handling of the tissue) of the jejunum was sampled as described by Soto-Navarro et al. (2004) for vascular perfusion, mucosal scrape collection, and immersion fixation. This section began at a point on the mesenteric artery 15 cm caudal from its junction with the ileocecal vein and was taken by cutting around the main branches of the mesenteric arcade, including a section of the mesenteric vein. The tissue was placed in a pan of warm PBS, covered with cheese cloth, and returned to the lab for perfusion. A second 150-cm segment was taken following the first jejunal section for measurement of stripped weight and length.

Demarcations of the 3 sections of the small intestine were made using the modified methods of Soto-Navarro et al. (2004). The duodenum consisted of the pylorus to the point adjacent to the junction of the gastosplenic vein and mesenteric vein. The jejunum began after the duodenum and ended at a point adjacent to the caudal end of the last lymph node located next to the intestine. The ileum began here and ended at the ileocecal junction.

**Intestinal Tissue Preparation and Analysis.** A 15-cm segment was removed from the cranial end of the 150-cm jejunum sample and opened by cutting along the mesenteric side to expose the luminal surface. The lumen was rinsed with PBS, and the tissue sample was then weighed. Mucosal tissue was scraped from the intestine sample using a glass slide, and remaining intestinal tissue was weighed to determine mucosal density. Mucosal scrapes were flash frozen by immersion in liquid N<sub>2</sub> and then stored at -80°C for later analyses.

Another 15-cm segment was removed from the cranial end of the 150-cm jejunum sample and then rinsed by immersion in PBS. Cross-sections ( $\leq 1$  cm wide) were cut from the jejunal sample with a razor blade and immersed into Carnoy's solution (60% ethanol, 30% chloroform, and 10% glacial acetic acid) for fixation. After 6 h, fixed tissue samples were transferred to 70% ethanol for storage until embedding in paraffin. This 70% ethanol solution was changed once after approximately 12 h.

**Vascular Perfusion.** The remainder of the 150-cm jejunal section was fixed via vascular perfusion using methods of Meyer et al. (2012b). Briefly, within 10 min of sample collection, a main branch of the mesenteric artery was catheterized with polyethylene tubing, flushed with warm PBS, and pulse-infused with Carnoy's solution to fix the tissue. After allowing the tissue to fix, sections of jejunum that appeared to have the most perfusion with Carnoy's solution were removed. Small cross-sections ( $\leq 1$  cm wide) were immersed into Carnoy's solution for 6 h before being placed into 70% ethanol.

**Cellularity Estimates.** Jejunal mucosal scrapes were thawed, homogenized, and analyzed for DNA content using diphenylamine (Burton, 1956; Johnson et al., 1997), RNA content using orcinol (Kamali and Manhouri, 1969; Reynolds et al., 1990), and protein content using Coomassie brilliant blue G (Bradford, 1976) with BSA (Fraction V; Sigma Chemical, St. Louis, MO) as the standard (Johnson et al., 1997). Concentration of DNA was used to estimate cell number, and protein:DNA and RNA:DNA were used to estimate cell size.

**Proliferation and Vascularity.** Jejunal tissues immersion-fixed in Carnoy's solution were embedded in paraffin (Reynolds and Redmer, 1992), from which 5-μm tissue sections were cut, mounted on glass slides, and processed for staining procedures (Fricke et al., 1997; Scheaffer et al., 2003). Prepared tissues were treated with a blocking

buffer of PBS and 1.5% normal horse serum (Vector Laboratories, Burlingame, CA) and then incubated with mouse Ki-67 monoclonal antibody (1:100, Clone MM1; Vector Laboratories). Primary antibody was detected using a secondary antibody (ImmPress Kit) and 3, 3'-diaminobenzidine substrate (Vector Laboratories). Tissue sections were counterstained with hematoxylin to visualize unlabeled nuclei. Percentage of proliferating cells in the crypt region was then quantified (5 images per animal; Yunusova et al., 2013) using Image-Pro Plus 5.0 software (MediaCybernetics Inc., Silver Spring, MD).

Cross-sections of perfusion-fixed jejunal tissue were processed as above, and 5- $\mu\text{m}$  tissue sections were stained using periodic acid-Schiff's (Luna, 1968) staining procedures to contrast the vascular tissue and counterstained with hematoxylin. Mean capillary area, number, and circumference measurements made in the intestinal villi (5 images per animal) using the Image-Pro Plus software were used to calculate capillary area density, capillary number density, capillary surface density, and area per capillary, as described below.

**Angiogenic Factor mRNA Expression.** Quantitative real-time reverse transcription (RT-) PCR was performed on frozen jejunal mucosal samples using methods of Austin et al. (2012) to determine mRNA expression of *vascular endothelial growth factor (VEGF)*, *VEGF receptor-1 (fms-related tyrosine kinase 1 [FLT1])*, *VEGF receptor-2 (kinase insert domain receptor [KDR])*, *endothelial nitric oxide synthase 3 (NOS3; produces nitric oxide)* and *solute guanylate cyclase (GUCY1B3; nitric oxide receptor)*. Primers were designed using Primer 3 software (Rozen and Skaletsky, 2000) such that amplicons were approximately 150 bp in size; see Table 2 for primer sequences used. Total cellular RNA was extracted from frozen tissues using TriReagent (Molecular Research Center, Cincinnati, OH). The RNA pellet was resuspended in 100  $\mu\text{L}$  ribonuclease-free water and further purified using the RNeasy kit (Qiagen, Santa Clarita, CA) before purified RNA was quantified using a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Denver, CO).

Two micrograms of RNA (in 15  $\mu\text{L}$  nuclease-free water) were mixed with 4  $\mu\text{L}$  reverse transcription buffer (5x) and 1  $\mu\text{L}$  of iScript reverse transcriptase (Bio-Rad Laboratories, Richmond, CA). The mixture was placed in a thermocycler for 5 min at 25°C, 30 min at 42°C, and 5 min at 85°C and held at 4°C. The cDNA was diluted with 100  $\mu\text{L}$  nuclease-free water and stored at -20°C until semiquantitative real-time PCR was performed. Real-time PCR was performed by mixing 10  $\mu\text{L}$  of diluted cDNA with 12.5  $\mu\text{L}$  of SYBR green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA), 500 pmol each of forward and reverse primer, and 0.5  $\mu\text{L}$  of nuclease-free water in each well of a 96-well plate. Amplification was performed using an iQ5 Multicolor Real-time PCR Detection

**Table 2.** Sequence of primers used for bovine angiogenic factors and receptors

Gene of interest <sup>1</sup>	Forward primer	Reverse primer
VEGF	TCACCAAAGCCAGCACATAG	GCGAGTCTGTGTTTGAG
FLT1	GTATCACTGCAAAGCCAGCA	AGCGTTAACAGGAGCCAGAA
KDR	CCCTTCTTGAAAGCATCAGC	CGTGCTGTTCTCTGGTCA
NOS3	GTGGAGATCACCTGGCTGT	CCCTTCTTGAAAGCATCAGC
GUCY1B3	GAGGATGCCCTCGCTACTGTC	CTGCTCCGTTCCCTCTGTTTC

<sup>1</sup> Vascular endothelial growth factor (VEGF), VEGF receptor-1 (fms-related tyrosine kinase 1 [FLT1]), VEGF receptor-2 (kinase insert domain receptor [KDR]), endothelial nitric oxide synthase 3 (NOS3), and soluble guanylate cyclase (GUCY1B3).

System (Bio-Rad, Hercules, CA) and 40 cycles of 95°C for 30 s and 60°C for 30 s. Melting curve analysis was performed postamplification to ensure the quality of PCR products as noted by the presence of a single peak. Briefly, the PCR plate was heated to 95°C for 3 min and cooled to 55°C, and then the temperature was increased by 0.5°C/s up to 95°C. Bovine *glyceraldehyde 3-phosphate dehydrogenase (GAPDH)* was used as the reference gene, and all gene expression levels were quantified and reported relative to GAPDH expression using the  $2^{-\Delta\Delta CT}$  method (Livak and Schmittgen, 2001). Real-time RT-PCR was performed in duplicate for each sample/primer set.

### Calculations

Jejunal mucosal total DNA, RNA, and protein were calculated by multiplying jejunal mass by DNA, RNA, and protein concentrations to yield their total jejunal content. Total jejunal cell number was calculated by dividing the estimated total DNA (jejunal DNA concentration  $\times$  jejunal mass) by  $6.6 \times 10^{-12}$  g (Baserga, 1985). Total jejunal cell number was then multiplied by the percentage of proliferating nuclei to determine the total number of jejunal mucosal proliferating nuclei. Jejunal mucosal density was defined as mucosal weight resulting from the mucosal scrape divided by total initial jejunal sample weight. Small intestinal mass was divided by its length to determine tissue density (g/cm) for each section and its entirety.

To express vascularity as a percentage, capillary area density was calculated as the total capillary area ( $\mu\text{m}^2$ ) divided by the tissue area analyzed ( $\mu\text{m}^2$ ) and multiplied by 100 (Scheaffer et al., 2004; Soto-Navarro et al., 2004). Capillary number density was determined by dividing the total number of vessels counted by the tissue area analyzed ( $\mu\text{m}^2$ ) and multiplying this by 1,000,000 to yield capillaries per millimeter squared. The capillary surface density, or total capillary circumference per unit of tissue area, was calculated as capillary circumference ( $\mu\text{m}$ ) divided by the tissue area analyzed ( $\mu\text{m}^2$ ). Although

**Table 3.** Effect of maternal nutrient restriction with or without RUP supplementation from d 45 to 185 of gestation on offspring finishing period performance and intake<sup>1</sup>

Item	Maternal treatment <sup>2</sup>			SEM <sup>3</sup>	P-value
	CON	NR	NR+RUP		
ADG, kg/d	1.52	1.38	1.38	0.08	0.33
DMI, kg/d	9.49	8.94	9.02	0.40	0.53
Residual feed intake, kg/d	-0.021	-0.199	0.205	0.250	0.54
G:F	0.159	0.153	0.153	0.005	0.57

<sup>1</sup>Individual feed DMI, ADG, residual feed intake, and G:F are reported for 84 d in the finishing period.

<sup>2</sup>Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC (2000) recommendations, a nutrient-restricted (NR) diet providing 70% of CON NEm, or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON from d 45 to 185 of gestation.

<sup>3</sup>Standard error of the mean for CON ( $n = 11$ ), NR ( $n = 9$ ), and NR+RUP ( $n = 11$ ).

this measure represents the circumference of capillary cross-sections, it is proportional to capillary surface area (Borowicz et al., 2007). Area per capillary was also determined by dividing total capillary area ( $\mu\text{m}^2$ ) by capillary number. Total jejunal vascularity was calculated as the capillary area density multiplied by jejunal mass.

### Statistical Analysis

Two cows aborted after treatment initiation, 2 calves died immediately after birth, and 1 calf died in the feedlot, which resulted in 31 total calves at slaughter (CON:  $n = 11$ , NR:  $n = 9$ , and NR+RUP:  $n = 11$ ).

Data were analyzed in PROC MIXED of SAS 9.2 (SAS Inst., Inc., Cary, NC) with maternal treatment as a fixed effect in the model. Calf sex (slaughter date) was treated as a block. Means were separated using LSD and were considered significant when  $P \leq 0.05$  or were considered tendencies when  $P > 0.05$  and  $P < 0.10$ . Data were also analyzed using the CORR procedure of SAS to determine the relationship of DMI, RFI, and G:F with small intestinal measures.

## RESULTS

### Effects of Maternal Dietary Treatment during Gestation

Maternal dietary treatment during early and midgestation did not affect calf birth weight ( $P = 0.13$ ; data not shown) or weaning weight ( $P = 0.14$ ; data not shown) in this study. During the finishing period, ADG ( $P = 0.33$ ) and DMI ( $P = 0.53$ ) of steer and heifer offspring did not differ due to maternal dietary treatment during early and midgestation (Table 3). Additionally, there were no differences in finishing period feed efficiency measured as RFI ( $P = 0.54$ ) or G:F ( $P = 0.57$ ) due to maternal dietary treatment (Table 3).

Maternal treatment had no effect on offspring slaughter BW ( $P = 0.76$ ) or mass of the small intestine or any of its sections (g;  $P > 0.11$ ; Table 4). Although relative mass (g/kg BW) of the total small intestine did not differ ( $P = 0.38$ ), relative ileal mass tended ( $P = 0.07$ ) to differ among maternal treatments, where calves born to CON-fed cows had greater relative ileal mass ( $P = 0.02$ ) compared with calves born to NR-fed cows (Table 4). Maternal treatment affected ( $P = 0.05$ ) jejunal length and tended ( $P \leq 0.08$ ) to affect total small intestinal and ileal length (Table 4). Jejunal length was greater ( $P < 0.05$ ) for calves born to NR-fed dams than calves born to CON- or NR+RUP-fed dams, whereas calves born to CON-fed dams had greater ( $P = 0.03$ ) ileal length than calves born to NR-fed dams. Overall, total small intestinal length was greater ( $P = 0.02$ ) for calves born to NR-fed dams compared with calves born to NR+RUP-fed dams. Relative ileal length (cm/kg BW) also tended ( $P = 0.07$ ) to be affected by maternal treatment where calves born to CON-fed cows had greater ( $P = 0.03$ ) relative length than calves born to NR-fed dams (Table 4). Relative total small intestinal length did not differ ( $P = 0.57$ ), however. Despite these contradicting mass and length data, tissue density (g/cm) of the total small intestine and its individual sections did not differ ( $P > 0.19$ ) among maternal treatments (Table 4).

Jejunal concentration of DNA, RNA, and protein of steer and heifer offspring at market weight did not differ ( $P > 0.93$ ) due to maternal dietary treatment during early to midgestation (Table 5). Jejunal RNA:DNA and protein:DNA, both used to estimate differences in cell size, were not affected ( $P > 0.87$ ) by maternal dietary treatment (Table 5). Furthermore, maternal dietary treatment did not affect ( $P > 0.73$ ) total jejunal DNA, RNA, protein, and cell number. Jejunal crypt cell proliferation (%) and total proliferating cells were also not affected ( $P > 0.22$ ) by maternal treatment during gestation (Table 5).

Jejunal capillary area density, capillary number density, capillary surface density, area per capillary, and total vascularity were not affected ( $P > 0.42$ ) by maternal dietary treatment during early and midgestation (Table 5). Despite this, maternal treatment did affect ( $P = 0.03$ ) *GUCY1B3* mRNA expression (Table 6) in the jejunum, however, as calves born to dams receiving the NR+RUP treatment had greater ( $P < 0.03$ ) expression of *GUCY1B3* compared with calves born to dams receiving the CON and NR treatments. Jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, and *NOS3* did not differ ( $P > 0.33$ ) among maternal treatments (Table 6).

### Relationship of Feed Intake and Efficiency with Small Intestinal Measures

Finishing period RFI was negatively correlated with G:F ( $P = 0.001$ ) and positively correlated with DMI ( $P =$

**Table 4.** Effect of maternal nutrient restriction with or without RUP supplementation from d 45 to 185 of gestation on offspring small intestinal size at market weight

Item	Maternal treatment <sup>1</sup>			SEM <sup>2</sup>	P-value
	CON	NR	NR+RUP		
Slaughter BW, kg	552	561	541	19	0.76
Mass, g					
Small intestine	4,599	4,380	4,356	200	0.61
Duodenum	385	366	347	27	0.60
Jejunum	3,359	3,307	3,236	169	0.87
Ileum	856	707	773	53	0.12
Length, cm					
Small intestine	3,617 <sup>ab</sup>	3,754 <sup>b</sup>	3,448 <sup>a</sup>	92	0.07
Duodenum	250	245	218	26	0.64
Jejunum	2,775 <sup>a</sup>	3,093 <sup>b</sup>	2,696 <sup>a</sup>	117	0.05
Ileum	592 <sup>b</sup>	416 <sup>a</sup>	535 <sup>ab</sup>	58	0.08
Mass, g/kg BW					
Small intestine	8.35	7.79	8.04	0.30	0.38
Duodenum	0.703	0.660	0.639	0.048	0.61
Jejunum	6.10	5.87	5.97	0.25	0.80
Ileum	1.55 <sup>b</sup>	1.26 <sup>a</sup>	1.43 <sup>ab</sup>	0.09	0.07
Length, cm/kg BW					
Small intestine	6.61	6.75	6.42	0.22	0.57
Duodenum	0.458	0.441	0.406	0.049	0.74
Jejunum	5.08	5.58	5.01	0.24	0.18
Ileum	1.08 <sup>b</sup>	0.73 <sup>a</sup>	1.00 <sup>ab</sup>	0.11	0.07
Tissue density, <sup>3</sup> g/cm					
Small intestine	1.27	1.17	1.28	0.06	0.32
Duodenum	1.59	1.61	1.68	0.14	0.87
Jejunum	1.23	1.08	1.23	0.07	0.20
Ileum	1.64	1.87	1.61	0.22	0.66

<sup>a,b</sup>Within a row, means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC (2000) recommendations, a nutrient-restricted (NR) diet providing 70% of CON NEm, or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON from d 45 to 185 of gestation.

<sup>2</sup>Standard error of the mean for CON ( $n = 11$ ), NR ( $n = 9$ ), and NR+RUP ( $n = 11$ ).

<sup>3</sup>Tissue density = tissue weight (g)/tissue length (cm).

0.01; Table 7). Gain:feed was not correlated with DMI ( $P = 0.19$ ), however.

Small intestinal mass (g) was positively correlated with DMI ( $P < 0.001$ ) and tended ( $P = 0.08$ ) to be positively correlated with RFI (Table 7). Similarly, relative small intestinal mass (g/kg BW) tended ( $P = 0.06$ ) to be positively correlated with DMI and was positively correlated with RFI ( $P = 0.01$ ). Dry matter intake tended ( $P = 0.09$ ) to be positively correlated with small intestinal length but was negatively correlated with its relative length (cm/kg BW; Table 7;  $P = 0.02$ ). Small intestinal length measures were not correlated ( $P > 0.55$ ) with RFI. Relative small intestinal length was negatively correlated ( $P = 0.03$ ) with G:F, although G:F was not correlated ( $P > 0.25$ ) with any other small intestinal mass or length measure. Small intestinal density (g/cm) was positively

correlated ( $P = 0.004$ ) with DMI but not feed efficiency measures ( $P > 0.19$ ).

Jejunal mucosal density tended ( $P = 0.09$ ) to be negatively correlated with RFI but was positively correlated ( $P = 0.03$ ) with G:F (Table 8). Concentration of DNA in the jejunum tended ( $P \leq 0.09$ ) to follow this pattern (Table 8). Gain:feed during the finishing period was negatively correlated ( $P \leq 0.05$ ) with RNA concentration, RNA content, and RNA:DNA (Table 8). Conversely, protein concentration and protein content were positively correlated ( $P \leq 0.04$ ) and DNA content and total jejunal cells tended ( $P = 0.08$ ) to be positively correlated with G:F (Table 8). Residual feed intake tended ( $P = 0.08$ ) to be positively correlated with RNA content but not other measures of cellularity ( $P > 0.12$ ; Table 8). Total DNA content, protein content, and jejunal cells were also positively correlated ( $P \leq 0.004$ ) with DMI, but DMI was not correlated ( $P > 0.34$ ) with other measures of jejunal mucosal density or cellularity (Table 8).

Jejunal crypt cell proliferation (%) and total proliferating cells were not correlated ( $P > 0.13$ ) with DMI, RFI, or G:F (Table 9). Dry matter intake was positively correlated ( $P = 0.004$ ) with total jejunal vascularity but was not correlated ( $P > 0.45$ ) with capillary area density, capillary number density, capillary surface density, or area per capillary (Table 9). Residual feed intake and G:F were not correlated ( $P > 0.14$ ) with any measure of jejunal vascularity (Table 9). Similarly, jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, *NOS3*, and *GUCY1B3* were not correlated ( $P > 0.19$ ) with either efficiency measure (Table 10). Despite this, DMI was positively correlated with jejunal *KDR* ( $P = 0.05$ ) and tended ( $P = 0.06$ ) to be positively correlated with *NOS3* mRNA expression (Table 10). Jejunal *VEGF*, *FLT*, and *GUCY1B3* were not correlated ( $P > 0.10$ ) with DMI, however (Table 10).

## DISCUSSION

### Effects of Maternal Dietary Treatment during Gestation

Calf birth weight was not affected by maternal dietary treatment in the current study, suggesting that nutrient restriction and RUP supplementation during early and midgestation did not impact overall fetal growth. In previous studies, nutrient restriction in early to midgestation beef heifers or cows has generally had no effect on fetal or birth weight (Freetly et al., 2000, 2005; Meyer et al., 2010b). When ewes have been restricted in early to midgestation, fetal weight from d 78 to 90 of gestation has been reduced in some studies (Vonnahme et al., 2003), however, although weight near term has been variable (Luther et al., 2007; Carlson et al., 2009). Early- and midgestational nutrient restriction and RUP supplementation also did not affect calf weaning weight in the current

**Table 5.** Effect of maternal nutrient restriction with or without RUP supplementation from d 45 to 185 of gestation on offspring jejunal cellularity, proliferation, and vascularity at market weight

Item	Maternal treatment <sup>1</sup>				P-value
	CON	NR	NR+RUP	SEM <sup>2</sup>	
Concentration, mg/g					
DNA	6.24	6.20	6.05	0.28	0.88
RNA	6.82	6.78	6.92	0.58	0.98
Protein	39.9	39.6	38.9	2.0	0.94
RNA:DNA	0.546	0.568	0.583	0.053	0.88
Protein:DNA	0.129	0.128	0.131	0.008	0.94
Total, g					
DNA	20.9	20.7	19.5	1.5	0.78
RNA	22.2	22.0	21.7	2.3	0.99
Protein	135	133	125	10	0.74
Proliferation, %	6.65	5.46	5.35	0.61	0.23
Total jejunal cells, $\times 10^{23}$	12.7	12.6	11.8	0.9	0.78
Total proliferating jejunal cells, $\times 10^{23}$	0.839	0.697	0.659	0.100	0.38
Capillary area density, %	10.1	11.4	10.4	0.8	0.43
Capillary number density, no./per mm <sup>2</sup>	647	705	680	58	0.75
Capillary surface density, ( $\mu\text{m}/\mu\text{m}^2$ ) $\times 1,000$	0.39	0.42	0.40	0.03	0.67
Area per capillary, $\mu\text{m}^2$	1,788	1,617	1,612	142	0.57
Total jejunal vascularity, mL	336	378	335	34	0.58

<sup>1</sup>Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC (2000) recommendations, a nutrient-restricted (NR) diet providing 70% of CON NEm, or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON from d 45 to 185 of gestation.

<sup>2</sup>Standard error of the mean for CON ( $n = 11$ ), NR ( $n = 9$ ), and NR+RUP ( $n = 11$ ).

study, which is consistent with previous reports (Freetly et al., 2000, 2005). Weaning weight of offspring has been altered in other gestational protein supplementation studies, even when birth weight was unaffected (Stalker et al., 2006; Funston et al., 2010b). These studies used late gestational protein supplementation, however; therefore, its effect on preweaning growth is not surprising.

Maternal nutrient restriction with or without RUP supplementation during early and midgestation did not affect growth, feed intake, or feed efficiency of offspring during the finishing phase in the current study. Research investigating the impacts of maternal nutrition during gestation on subsequent offspring feed efficiency is limited in beef cattle but suggests that feed efficiency during the growing phase may be programmed in utero. Previously, effects of early to midgestation nutritional plane have been observed in offspring G:F during the finishing phase (Underwood et al., 2007), but protein supplementation during late gestation has both affected (Funston et al., 2010a) and not affected (Stalker et al., 2006; Larson et al., 2009) offspring G:F or RFI. Using a similar experimental design to the current study, calves from dams fed to meet NRC (2000) recommendations during early to midgestation had decreased feed intake and a negative (more efficient) RFI during the finishing phase compared with calves born to cows that were nutrient restricted, despite having similar ADG (Price et al., 2009). In another previous study, low birth weight heifers resulting from gestational undernutrition had an

improved feed conversion ratio compared with high birth weight heifers (Cafe et al., 2009). No differences in RFI or feed conversion ratio were observed in calves differing by 30% at birth when adjusted to a similar feedlot entry weight in this study, however. Similarly, birth weight explained 14.7% of the variation in feed intake of feedlot calves in another dataset but did not explain variation of feed conversion ratio or RFI (Robinson et al., 2013).

**Table 6.** Effect of maternal nutrient restriction with or without RUP supplementation from d 45 to 185 of gestation on offspring jejunal angiogenic factor and receptor mRNA relative expression at market weight

Gene of interest <sup>2</sup>	Maternal treatment <sup>1</sup>				P-value
	CON	NR	NR+RUP	SEM <sup>3</sup>	
VEGF	0.612	0.695	0.658	0.053	0.51
FLT1	2.32	2.26	1.91	0.21	0.34
KDR	2.58	2.19	2.19	0.26	0.43
NOS3	3.44	3.73	3.56	0.56	0.93
GUCY1B3	2.85 <sup>a</sup>	2.56 <sup>a</sup>	4.59 <sup>b</sup>	0.54	0.03

<sup>a,b</sup>Within a row, means with different superscripts differ ( $P < 0.05$ ).

<sup>1</sup>Beef cows were fed a control (CON) diet of grass hay and supplement to meet NRC (2000) recommendations, a nutrient-restricted (NR) diet providing 70% of CON NEm, or a NR diet with a RUP supplement (NR+RUP) to provide similar essential AA as CON from d 45 to 185 of gestation.

<sup>2</sup>Jejunal mRNA expression of vascular endothelial growth factor (VEGF), VEGF receptor-1 (fms-related tyrosine kinase 1 [FLT1]), VEGF receptor-2 (kinase insert domain receptor [KDR]), endothelial nitric oxide synthase 3 (NOS3), and soluble guanylate cyclase (GUCY1B3).

<sup>3</sup>Standard error of the mean for CON ( $n = 11$ ), NR ( $n = 9$ ), and NR+RUP ( $n = 11$ ).

**Table 7.** Partial correlation coefficients ( $r$ ) and associated  $P$ -values between DMI, residual feed intake (RFI), and G:F during finishing and small intestinal size at market weight<sup>1</sup>

Item	G:F	RFI	Small intestine, g	Small intestine, g/kg BW	Small intestine, cm	Small intestine, cm/kg BW	Small intestinal density, <sup>2</sup> g/cm
DMI	0.25 ( $P = 0.19$ )	0.46 ( $P = 0.01$ )	0.74 ( $P < 0.001$ )	0.36 ( $P = 0.06$ )	0.32 ( $P = 0.09$ )	-0.44 ( $P = 0.02$ )	0.52 ( $P = 0.004$ )
RFI	-0.58 ( $P = 0.001$ )	- ( $P = 0.001$ )	0.33 ( $P = 0.08$ )	0.45 ( $P = 0.01$ )	0.11 ( $P = 0.56$ )	0.11 ( $P = 0.59$ )	0.25 ( $P = 0.20$ )
G:F	- ( $P = 0.001$ )	-0.58 ( $P = 0.001$ )	0.18 ( $P = 0.35$ )	-0.03 ( $P = 0.88$ )	-0.12 ( $P = 0.54$ )	-0.40 ( $P = 0.03$ )	0.22 ( $P = 0.26$ )

<sup>1</sup>Individual feed intake data was collected for steers ( $n = 17$ ) and heifers ( $n = 14$ ) for 84 d in the finishing period. Slaughter and tissue collection occurred 3 (9 steers), 8 (8 steers), 22 (7 heifers), and 24 d (7 heifers) after the conclusion of the feed intake period.

<sup>2</sup>Tissue density = tissue weight (g)/tissue length (cm).

The disparity in feed efficiency effects in the current research and cited studies likely exists because of the complex nature of feed efficiency. Because of the many genetic, environmental, and genetic  $\times$  environment interaction influences on the inputs (feed intake) and output (growth in this case) of an animal, it is reasonable to assume that gestational nutrition will not affect feed efficiency in a similar manner when measured many months after a gestational nutrition insult in different studies. More research is necessary to determine the influence of dam nutrition on this highly economically relevant phenotypic trait, especially its role in the genetic  $\times$  environment interaction through epigenetics. Additionally, offspring in the current study had the opportunity for compensatory growth during both fetal and postnatal stages before reaching the finishing period. For example, placental development and uterine blood flow in beef cows increase during realimentation following a period of nutrient restriction during early gestation (Vonnahme et al., 2007; Camacho et al., 2014), giving 1 possible time period for fetal compensatory growth. Although it is unclear if fetal development was altered by maternal nutrient restriction or RUP supplementation during early and midgestation in the current study, data here suggest that calves are able to overcome poor nutrient availability during early fetal development and perform similarly to offspring born to well-nourished dams during the finishing phase.

In the current study, maternal nutrient restriction and RUP supplementation had little effect on small intestinal characteristics of market weight offspring, with only small intestinal length and *GUCY1B3* expression changes in offspring. It has been demonstrated that gestational nutrition in ruminants affects both fetal small intestinal development (reviewed in Meyer et al., 2012a) and postnatal animal health and performance (Caton and Hess, 2010; Funston et al., 2010a; Meyer et al., 2012a). Despite this, there is a general paucity of data that explores the effect of gestational nutrition on ruminant offspring small intestinal development postnatally, even though it is likely that impaired intestinal development would contribute to observed whole animal growth and health responses in these studies.

Recent research in sheep demonstrated that differences in small intestinal growth and vascularization parameters, including many of those measured here, of the neonatal (d 20 of age; Meyer et al., 2013) and market weight (d 180 of age; Yunusova et al., 2013) lamb are affected by mid- to late-gestational nutritional plane and Se supply in an artificial rearing model. This suggests that maternal nutrition can impact offspring small intestinal development postnatally, even when postnatal management is similar. In addition to species and treatment differences, the timing of maternal nutritional treatment likely contributed to the differences observed among these studies and the current study. Although fetal organogenesis and

**Table 8.** Partial correlation coefficients ( $r$ ) and associated  $P$ -values between DMI, residual feed intake (RFI), and G:F during finishing and jejunal mucosal density and cellularity at market weight<sup>1</sup>

Item	Jejunal mucosal density, %	DNA, mg/g	RNA, mg/g	Protein, mg/g	RNA:DNA	Protein:DNA	DNA, g	RNA, g	Protein, g	Total cells
DMI	0.04 ( $P = 0.84$ )	-0.08 ( $P = 0.70$ )	-0.18 ( $P = 0.35$ )	0.07 ( $P = 0.74$ )	-0.13 ( $P = 0.52$ )	0.14 ( $P = 0.48$ )	0.52 ( $P = 0.004$ )	0.11 ( $P = 0.56$ )	0.55 ( $P = 0.002$ )	0.52 ( $P = 0.004$ )
RFI	-0.33 ( $P = 0.09$ )	-0.34 ( $P = 0.07$ )	0.18 ( $P = 0.35$ )	-0.25 ( $P = 0.19$ )	0.29 ( $P = 0.13$ )	0.02 ( $P = 0.91$ )	0.06 ( $P = 0.74$ )	0.33 ( $P = 0.08$ )	0.08 ( $P = 0.69$ )	0.06 ( $P = 0.74$ )
G:F	0.42 ( $P = 0.03$ )	0.32 ( $P = 0.09$ )	-0.44 ( $P = 0.02$ )	0.40 ( $P = 0.03$ )	-0.52 ( $P = 0.004$ )	0.14 ( $P = 0.47$ )	0.34 ( $P = 0.08$ )	-0.37 ( $P = 0.05$ )	0.39 ( $P = 0.04$ )	0.34 ( $P = 0.08$ )

<sup>1</sup>Individual feed intake data was collected for steers ( $n = 17$ ) and heifers ( $n = 14$ ) for 84 d in the finishing period. Slaughter and tissue collection occurred 3 (9 steers), 8 (8 steers), 22 (7 heifers), and 24 d (7 heifers) after the conclusion of the feed intake period.

**Table 9.** Partial correlation coefficients (*r*) and associated *P*-values between DMI, residual feed intake (RFI), and G:F during finishing and jejunal proliferation and vascularity at market weight<sup>1</sup>

Item	Proliferation, %	Total proliferating cells	Capillary area density, %	Capillary number density, no. per mm <sup>2</sup>	Capillary surface density, μm/μm <sup>2</sup> , × 1,000	Area per capillary, μm <sup>2</sup>	Total vascularity, mL
DMI	-0.01 ( <i>P</i> = 0.94)	0.29 ( <i>P</i> = 0.14)	0.14 ( <i>P</i> = 0.46)	0.14 ( <i>P</i> = 0.46)	0.13 ( <i>P</i> = 0.51)	-0.07 ( <i>P</i> = 0.70)	0.52 ( <i>P</i> = 0.004)
RFI	-0.04 ( <i>P</i> = 0.84)	-0.001 ( <i>P</i> = 0.99)	0.06 ( <i>P</i> = 0.74)	0.04 ( <i>P</i> = 0.84)	0.044 ( <i>P</i> = 0.82)	-0.03 ( <i>P</i> = 0.86)	0.27 ( <i>P</i> = 0.15)
G:F	0.06 ( <i>P</i> = 0.77)	0.24 ( <i>P</i> = 0.22)	-0.13 ( <i>P</i> = 0.50)	-0.02 ( <i>P</i> = 0.92)	-0.11 ( <i>P</i> = 0.57)	0.07 ( <i>P</i> = 0.72)	-0.02 ( <i>P</i> = 0.92)

<sup>1</sup>Individual feed intake data was collected for steers (*n* = 17) and heifers (*n* = 14) for 84 d in the finishing period. Slaughter and tissue collection occurred 3 (9 steers), 8 (8 steers), 22 (7 heifers), and 24 d (7 heifers) after the conclusion of the feed intake period.

placental development occur during the period of nutritional treatments used in the current study, much of the fetal small intestinal growth as well as its final maturation occur during late gestation and into the early neonatal period. Therefore, it is possible that the plastic nature of the small intestine allows for significant compensation during late gestation and postnatal periods that corrected any disruptions in its development caused by nutrient intake of the dam during the first half of gestation. In fact, fetuses from cows that were nutrient restricted from d 30 to 125 and then realimented from d 125 to 245 of gestation had greater jejunal proliferation at d 125 and greater total intestinal vascularity at d 245 of gestation in a recent study (Meyer et al., 2010b). This suggests that the fetal intestine may have become more efficient during early- to mid-gestational nutrient restriction of the dam and then experiencing compensatory growth and allowing for greater growth and vascularization of the tissue during realimentation. Additionally, more than 18 mo elapsed between the nutritional insult during gestation and tissue collection in the current study. Because the small intestine is both incredibly plastic and heavily influenced by current and recent nutritional and environmental cues, the time between midgestation and slaughter at market weight may be enough for any potential small intestinal differences to dissipate.

Although small intestinal length was affected by maternal nutrition in this study, the small intestine elongates rapidly in early gestation (d 25 to 50 in sheep; Trahair and Sangild, 2002), establishing much of its growth in length before growth of mucosal tissue occurs. This would have been encompassed by nutritional treatment timing in this study, allowing for differences observed because small intestinal length is known to not be amenable to much change after mature length is reached (O'Connor et al., 1999).

To our knowledge, this is the first report of angiogenic factor mRNA expression in the small intestine of cattle, although it has been reported in sheep since 2008 (Holmes et al., 2008; O'Neil et al., 2008). Maternal nutrition during gestation has been demonstrated to affect jejunal *GUCY1B3* mRNA expression in both fetal (Neville

et al., 2010) and 20-d-old (Meyer et al., 2013) lambs. These data from the current and previous studies suggest that alteration of *GUCY1B3* expression is a mechanism by which gestational nutrition may impact ruminant offspring gut development and function long after a maternal nutritional insult. In this study, cows fed NR+RUP had greater circulating arginine than both cows fed CON and cows fed NR during gestational treatments (Meyer et al., 2010a). Nitric oxide was likely elevated in these dams because arginine is its precursor (substrate for NOS3); therefore, elevated nitric oxide available to the developing fetus may have stimulated *GUCY1B3* upregulation, which may have lasted into postnatal life. Because of the role of *GUCY1B3* in producing the many effects of nitric oxide, *GUCY1B3* expression may lead to greater vasodilation, angiogenesis, vascular permeability, and blood flow to the intestine to lessen other negative impacts on fetal intestinal development and/or growth. Although market weight calves in this study did not have altered intestinal mass, cellularity, or proliferation due to gestational nutrition, it is possible that increased expression of *GUCY1B3* in calves born to NR+RUP dams resulted in the lack of differences in intestinal growth. These data

**Table 10.** Partial correlation coefficients (*r*) and associated *P*-values between DMI, residual feed intake (RFI), and G:F during finishing and jejunal angiogenic factor and receptor mRNA relative expression at market weight<sup>1</sup>

Item	<i>VEGF</i> <sup>2</sup>	<i>FLT1</i>	<i>KDR</i>	<i>NOS3</i>	<i>GUCY1B3</i>
DMI	-0.30 ( <i>P</i> = 0.11)	0.02 ( <i>P</i> = 0.92)	0.37 ( <i>P</i> = 0.05)	0.35 ( <i>P</i> = 0.06)	-0.01 ( <i>P</i> = 0.96)
RFI	-0.21 ( <i>P</i> = 0.27)	0.01 ( <i>P</i> = 0.98)	0.10 ( <i>P</i> = 0.60)	0.25 ( <i>P</i> = 0.20)	0.18 ( <i>P</i> = 0.35)
G:F	-0.11 ( <i>P</i> = 0.58)	-0.10 ( <i>P</i> = 0.62)	0.02 ( <i>P</i> = 0.92)	-0.06 ( <i>P</i> = 0.76)	-0.10 ( <i>P</i> = 0.62)

<sup>1</sup>Individual feed intake data was collected for steers (*n* = 17) and heifers (*n* = 14) for 84 d in the finishing period. Slaughter and tissue collection occurred 3 (9 steers), 8 (8 steers), 22 (7 heifers), and 24 d (7 heifers) after the conclusion of the feed intake period.

<sup>2</sup>Jejunal mRNA expression of vascular endothelial growth factor (*VEGF*), *VEGF* receptor-1 (*fms-related tyrosine kinase 1 [FLT1]*), *VEGF* receptor-2 (*kinase insert domain receptor [KDR]*), endothelial nitric oxide synthase 3 (*NOS3*), and soluble guanylate cyclase (*GUCY1B3*).

also suggest that gene expression in the intestine, which may lead to functional alterations in the tissue, may result from the maternal nutritional environment even when apparent intestinal growth is unchanged.

### **Relationship of Feed Intake and Efficiency with Small Intestinal Measures**

In this study, several weak and moderate correlations were observed between feed efficiency measures (RFI and G:F) and small intestinal characteristics. Taken together, the positive correlations of RFI with small intestinal mass and relative mass and negative correlation of G:F and relative small intestinal length suggest that more efficient cattle have less small intestinal mass. The negative correlations of RFI with mucosal density and DNA concentration and positive correlations of G:F with mucosal density, DNA concentration and content, protein concentration and content, and total cells also indicate that more efficient animals had greater small intestine mucosal density. We hypothesize that the decreased mass associated with more efficient animals in this study resulted in decreased nutrient and energy requirements for tissue maintenance. Additionally, because intestinal digestive and absorptive capacity are located within the mucosal layer, we hypothesize that greater mucosal density of more efficient animals allowed for greater ability to acquire nutrients per unit of small intestinal mass.

Limited research has been conducted in which the small intestine or any gastrointestinal organs have been compared between high and low efficiency ruminants. Basarab et al. (2003) previously reported that high-RFI (low efficiency) steers had greater combined stomach and intestine (small and large) mass compared with low-RFI steers, which is in agreement with correlations observed in the current study. In another study, nuclei number was greater in the duodenum and ileum of low-RFI steers than high-RFI steers (Montanholi et al., 2013), which supports our hypothesis and current data. Conversely, Mader et al. (2009) observed no relationship of RFI and small intestinal mass or length, and Richardson et al. (2001) observed no differences in total gastrointestinal tract masses were no different between steers resulting from 1 generation of divergent selection for RFI.

The ruminant gastrointestinal tract, or portal drained viscera, is estimated to account for 20 to 25% of whole animal energy use (Ferrell, 1988; Johnson et al., 1990; Seal and Reynolds, 1993) despite visceral organ mass (including the liver and kidneys) making up only 6 to 13% of body weight (Burrin et al., 1990; Seal and Reynolds, 1993); therefore, the small intestine is a major contributor to animal energy expenditure. It has previously been concluded by Ferrell (1988) that visceral organ mass variation contributes greatly to fasting heat production and

whole animal energy use. Cant et al. (1996) estimated that for each 1 g of additional small intestinal mass, additional maintenance energy requirements are 290 to 350 cal/d. This model also estimated a possible 0.074 mmol/h increase in glucose absorption for this 1 g of tissue, which is not an inefficient conversion. Their hypothesis, however, was that there is a maximal small intestinal size after which the benefit of increased absorption possible from increased tissue mass is less than the cost of maintaining the additional tissue, and overall efficiency decreases (Cant et al., 1996). These data support that additional tissue mass associated with less efficient animals in the current study could have had more associated cost of maintenance or initial growth than benefit of additional nutrient acquisition. Because decreasing efficiency was also associated with less mucosal density, this suggests that serosal and muscularis layers, which have no digestive or absorptive capability but do require energy and nutrients for maintenance, made up a greater proportion of tissue. This would have reduced the efficiency of maintaining more tissue with presumed equal or less nutrient acquisition.

Small intestinal mass, length, DNA content, and protein content were moderately to highly positively correlated with DMI in the current study. This is in agreement with previous work in ruminants, as small intestinal mass generally increases with nutritional plane or functional workload (Ferrell, 1988; Johnson et al., 1990). The relationship of DNA and protein content were likely due to increased mass, as concentration of DNA or protein was not correlated with DMI and has been variable in previous studies with growing ruminants in a fed state (Burrin et al., 1992; Swanson et al., 2000).

The current data are the first attempt to our knowledge to better understand the relationship of intestinal vascularization, blood flow, and angiogenesis with metabolic efficiency. Although these data did not correlate VEGF and nitric oxide system expression at the mRNA level with whole animal feed efficiency, there does appear to be relationships between both systems (*KDR* and *NOS3*) and feed intake. Previous data in ewes support this concept, as nutritional plane during gestation has impacted jejunal mRNA expression of *VEGF*, *FLT1*, *KDR*, and *NOS3* (Neville et al., 2010; Meyer et al., 2012b). Blood flow to and from the intestine is important for tissue growth and nutrient flux; therefore, it is probable that alteration of blood flow to the tissue would play a role in metabolic efficiency.

Herd and Arthur (2009) suggested that individual differences in RFI can be attributed in part to tissue metabolism, turnover, and stress (37%), digestibility (10%), heat increment of feeding (9%), body composition (5%), and feeding patterns (2%), all of which have contributions from the gastrointestinal tract. Data presented here suggest that small intestinal size and growth characteristics

likely play a role in whole animal efficiency, but additional research is necessary to further explore this hypothesis.

## Conclusions

In summary, maternal nutrient restriction and RUP supplementation did not impact finishing phase performance or feed efficiency of calves in this study. Additionally, despite minimal impacts of maternal diet on small intestinal growth measures, results indicate that offspring intestinal gene expression may be affected by gestational nutrition even when apparent tissue growth is unchanged. These data support *GUCY1B3* as an important gene in small intestinal development that appears to be sensitive to maternal nutrition during gestation. Additionally, small intestinal size and growth appear to explain some variation in efficiency of nutrient utilization in feedlot cattle. More research is necessary to determine possible associations of small intestinal development and metabolic efficiency to identify specific gut-level targets for improvement of feed efficiency.

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