Developmental Histology, Segmental Expression, and Nutritional Regulation of Somatotrophic Axis Genes in Small Intestine of Prewaned Dairy Heifers

B. T. Velayudhan,* K. M. Daniels,* D. P. Horrell,* S. R. Hill,* M. L. McGilliard,* B. A. Corl,*
H. Jiang,† and R. M. Akers*¹

*Department of Dairy Science, and

†Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg 24061

ABSTRACT

Components of the somatotrophic axis and nutrition regulate intestinal development and maturation of enterocytes. We measured gene expression in the mucosal layer of small intestine of preweaned dairy heifers to test the hypothesis that feeding increased amounts of protein and fat alters expression of somatotrophic axis genes. Twenty-four newborn Holstein heifers were randomly assigned to 1 of 4 milk replacer (MR) diets: (1) 20% CP, 20% fat MR (DM basis) fed at 450 g/d (CON); (2) 28% CP, 20% fat MR fed at 970 g/d (HPLF); (3) 28% CP, 28% fat MR fed at 970 g/d (HPHF); and (4) 28% CP, 28% fat MR fed at 1,460 g/d (HPHF+). Dry calf starter (20% CP, 1.43% fat) was offered free choice. At 64 ± 3 d of age heifers were killed and intestinal tissues were harvested for RNA isolation and histological examination. We measured the mRNA expression of growth hormone receptor (GHR), insulin-like growth factor-I (IGF-I), IGF-I receptor (IGF-IR), and IGF binding proteins (IGFBP)-1 to -6 in duodenum, jejunum, and ileum by quantitative real-time reverse transcription PCR. Expression of IGFBP-3 mRNA was lowest in the duodenum of HPHF+ and greatest in the ileum of the CON group, whereas expression of IGFBP-4 mRNA was greatest in the jejunum of the HPHF+ group. Expression of IGFBP-5 mRNA was greatest in the CON and lowest in the HPHF+. However, overall diet did not affect expression of GHR, IGF-I, IGF-IR, or IGFBP-1, -2, and -6. Expression of somatotrophic axis genes differed among small intestinal locations. The GHR, IGF-IR, IGFBP-1, and IGFBP-5 mRNA were greatest in the ileum. Duodenum produced less IGF-IR, IGF-I, and IGFBP-5 mRNA. Villi were shortest in the ileum, but there was no difference in villus height between the duodenum and jejunum. There was no difference in crypt depth or villus circumference between locations.

In conclusion, some components of the somatotrophic axis in preweaned dairy heifers are differentially expressed in regions of the small intestine, and the gene expression tended to be affected by dietary protein and fat.

Key words: preweaned heifer, intestinal mucosa, somatotrophic axis, gene expression

INTRODUCTION

The change in the mode of nutrition in neonatal animals from a parenteral to enteric type produces striking changes in morphology and function of the gastrointestinal tract (GIT). Proliferation and maturation of enterocytes are also affected by the components of the somatotrophic axis (MacDonald, 1999; Menard et al., 1999). Transcripts of IGF-I, IGF binding proteins (IGFBP), growth hormone receptor (GHR), and IGF-I receptors (IGF-IR) are abundant in intestinal mucosa in neonatal calves (Hammon and Blum, 2002; Pfaffl et al., 2002). Insulin-like growth factor-I is mainly associated with crypt cell proliferation, whereas IGF-II and insulin impact epithelial cell differentiation (Jehle et al., 1999). The number of IGF-IR in small intestine decreases with age in pigs (Morgan et al., 1996). Relative expression of somatotrophic axis components was found to differ among intestinal locations in neonatal calves (Ontsouka et al., 2004a). Moreover, abundance of IGF-I and IGFBP-3 mRNA were negatively correlated with villus height but positively correlated with crypt cell proliferation and crypt depth (Ontsouka et al., 2004b). Nutrition is known to modify rates of development and the structural and functional nature of the GIT. Nutritional factors can also induce differential expression and binding capacity of IGF-I and its receptors (Ontsouka et al., 2004b). Certainly changes associated with colostrum feeding are important, and there are reports (Georgiev et al., 2003 and Georgieva et al., 2003) describing expression of IGF-I axis molecules in intestine of fetal or neonatal calves; however, to our knowledge data on gene expression of somatotrophic axis molecules in small intestine of preweaned heifers do not exist.

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¹Corresponding author: rma@vt.edu

Our hypothesis was that increased amounts of protein and fat in milk replacer alter expression patterns of GH-IGF-I axis genes in the mucosal layer of small intestine of dairy heifers and correspondingly histological development. Thus our objectives were 1) to determine the effect of increased protein and fat content of milk replacer and feeding rate on the pattern of expression of selected GH-IGF-I axis genes, 2) to compare expression of these genes between duodenum, jejunum, and ileum, and 3) to determine relationships between gene expression and mucosal morphology.

MATERIALS AND METHODS

Animals and Treatments

All animal use procedures performed were approved by the Virginia Tech Animal Care and Use Committee and are described in detail in Daniels et al. (2008). Twenty-four Holstein heifers were acquired from a commercial dairy farm within 3 d of birth (40.4 ± 2.2 kg of BW on arrival) and blocked into groups of 8 in the order acquired. At the commercial dairy, all heifers received 1.89 L of thawed colostrum soon after birth. Any additional feedings at the cooperating dairy consisted of twice-daily feedings (1.89 L) of a 20% CP, 21% fat milk replacer (MR; Land O'Lakes Inc. Animal Milk Products Co., Arden Hills, MN); morning feedings were supplemented with 30 g of Gammulin (APC Inc., Ames, IA) mixed with MR to aid in the alleviation of stress associated with transit. Immediately before and after transport to the Virginia Tech Dairy Center, all calves were fed 1.89 L of warm electrolytes (Travel-Lyte; Nouriche Nutrition Ltd., Lake St. Louis, MO).

Calves arrived in 1 of 3 groups of 8 in the fall of 2005 and were individually housed in open hutches bedded with loose gravel. Animals were grouped according to age with treatments randomly assigned within each group. Treatment diets were imposed when heifers were 4 ± 1 d of age. The MR treatments used in this trial were 1) 20% CP, 20% fat MR (DM basis) fed at 450 g/d (CON); 2) 28% CP, 20% fat MR fed at 970 g/d (HPLF); 3) 28% CP, 28% fat MR fed at 970 g/d (HPHF); and 4) 28% CP, 28% fat MR fed at 1,460 g/d (HPHF+). Analyzed values of MR and average daily intakes are reported in Table 1 (Daniels et al., 2008). All MR powders used in the experiment were obtained from Land O'Lakes Inc. Animal Milk Products Co. and were nonmedicated and included whey protein as the protein source and animal tallow as the main fat source. Animals were fed treatment diets at 0700 and 1900 h. Fresh water and starter were available at all times and were offered free choice. Starter (20% CP, 1.43% fat) was composed of ground corn (44.4%), 48% CP soybean

meal (44.4%), cottonseed hulls (11.2%), and molasses (1.0%). Starter orts were recorded daily at the evening feeding. Milk replacer refusals, if any, were recorded at each feeding.

Sample Collection and Analyses

All animals were killed at 64 ± 3 d of age and tissues for analyses were harvested. Gastrointestinal tract was ligated cranially at the abomasal-duodenal junction and caudally at the ileal-cecal junction and removed from the carcass within 10 min of exsanguination. To be consistent, the duodenum, jejunum, and ileum were separated first by severing at the duodenal-jejunal junction and jejunal-ileal junction. Sections of duodenum, jejunum, and ileum (15 to 20 cm long, consistently within the segments) were collected from each animal. Intestinal segments were then flushed with ice-cold saline and weighed individually. A portion of each segment was used for RNA extraction, and the other portion was fixed for histological examination. For total RNA isolation, intestinal lumen was cut open on an ice-cold glass plate and mucosa was scraped using a cold glass slide. For consistency the same individual preformed all of the scrapings. Mucosal scrapings were snap frozen in liquid nitrogen and stored at -80°C until RNA was isolated. Remaining portions of intestinal segments with intact mucosa were pinned on to a piece of corkboard with the luminal surface facing out and fixed in 10% neutral formalin for histological evaluation.

RNA Isolation and Quantitative Reverse Transcription-PCR

Total RNA was isolated from mucosal scrapings using TRIZOL reagent (Life Technologies, Grand Island, NY) according to the manufacturer's instructions and was re-suspended in RNase-free water treated with diethyl pyrocarbonate (Sigma-Aldrich, St. Louis, MO). Purity and quantity of RNA was determined using a Nanodrop ND-1000 Spectrophotometer (Nanodrop Technologies Inc., Wilmington, DE) and accepted samples had a ratio of optical measurements at 260 and 280 nm (OD 260 nm/OD 280 nm) greater than 1.8. To evaluate integrity of 18S and 28S ribosomal RNA, 1 μg from representative samples was electrophoresed on an agarose gel and visualized by ethidium bromide staining. Single-stranded cDNA were reverse transcribed from total RNA by using the High Capacity cDNA Archive kit from Applied Biosystems (Foster City, CA) according to the manufacturer's instructions. Briefly, 20 μg of total RNA was reverse transcribed to single-stranded cDNA in a final reaction volume of 20 μL us-

Table 1. Ingredient and nutrient composition of milk replacers varying in protein and fat content fed to Holstein heifers¹

Item	Dietary treatment			
	CON ²	HPLF ³	HPHF ⁴	HPHF+ ⁵
Milk replacer				
CP, % of DM	19.8	28.3	27.1	27.1
Fat, % of DM	21.1	19.8	27.6	27.6
Gross energy, Mcal/kg	4.69	4.90	5.24	5.24
Powder intake, g of DM/d	441	951	951	1,431
Starter ⁶				
CP, % of DM	20.3			
Fat, % of DM	1.43			
Gross energy, Mcal/kg	3.52			
Starter intake, g of DM/d	560	449	401	221
Total intake ⁷				
Gross energy, Mcal/kg	3.61	5.41	5.57	6.94
DMI, g/d	1,001	1,400	1,352	1,652

¹Data adapted from Daniels et al. (2008).²CON = control milk replacer (MR) formulated to contain 20% CP and 20% fat and be fed at 450 g of DM/d.³HPLF = high protein, low fat MR formulated to contain 28% CP and 20% fat and be fed at 970 g of DM/d.⁴HPHF = high protein, high fat MR formulated to contain 28% CP and 28% fat and be fed at 970 g of DM/d.⁵HPHF+ = hPHF to be fed at 1,460 g of DM/d.⁶Starter contained 44.4% ground corn grain, 44.4% soybean meal, 11.1% cottonseed hulls, and 1.0% dried molasses.⁷Total intake = MR + starter.

ing random primers. A control containing no reverse transcriptase was used for each sample, and this was further used in quantitative real-time PCR. The cDNA produced was then diluted 10-fold with sterile deionized water. Two microliters of diluted cDNA was used in each reaction, along with 12.5 µL of SYBR Green dye (Applied Biosystems), 9.5 µL of sterile distilled water, 0.5 µL of 10 µM forward primer, and 0.5 µL of 10 µM reverse primer. The PCR conditions were 95°C for 10 min, 95°C for 15 s, and 60°C for 1 min. Real-time PCR reactions were performed in a 7300 Series Real-Time System, and data were analyzed using SDS software (Applied Biosystems, Foster City, CA). Relative mRNA abundance of IGF-I, IGF-IR, IGFBP-1 to -6, and GHR

was expressed as the fold difference in the expression of these target genes relative to the expression of the endogenous reference gene β-actin. Fold difference in target gene expression was calculated as $2^{(-\Delta Ct)}$, where ΔCt was the difference between the cycle threshold (Ct) values of the target and the β-actin in each sample ($Ct_{\text{target}} - Ct_{\beta\text{-actin}}$). Primer sequences used for each gene are shown in Table 2. Primers were designed using the Web-based program Primer 3 version 0.4.0 (Rozen and Skaletsky, 2000). Primer efficiency was tested for the endogenous reference gene and each target gene using 5 dilutions of cDNA in triplicates. A dissociation curve was performed after each real-time PCR run to rule out formation of primer dimers, genomic DNA contamina-

Table 2. Sequence of primer pairs used for determining relative abundance of mRNA for somatotrophic axis genes in the small intestine of preweaned Holstein heifers

Gene ¹	Forward primer (5'–3')	Reverse primer (5'–3')
GHR	CGTCTCTGCTGGTGAACACA	AACGGGTGGATCTGGTTGTA
IGF-I	GTTGGTGATGCTCTCCAGT	CTCCAGCCTCCTCAGATCAC
IGF-IR	TCAAGGACGGAGTCTTCACC	GCTCAAACAGCATGTCAGGA
IGFBP-1	ACCAGCCCAGAGAATGTGTC	GTTTGTCTCCTGCCTTCTGC
IGFBP-2	CAAGGGTGGCAAACATCAC	GAGGTTGTACAGGCCATGCT
IGFBP-3	CAGAGCACAGACACCCAGAA	TGCCCCGTACTTATCCACACA
IGFBP-4	GCCGCACACACGTCTATCTA	CGCTTGTCATGATTTACACGA
IGFBP-5	GTGCGGCGTCTACACTGAG	TCACGGGAGTCTCTTTTCGAT
IGFBP-6	AAGGAGAGTAAGCCCCAAGC	AGCACGGAGTCCAGATGTTT
β-Actin	GTGTGTGACAATGGCTCTGG	ACGCTTGCTCTGAGCTTCAT

¹GHR = growth hormone receptor; IGF-IR = insulin-like growth factor-I receptor; IGFBP = insulin-like growth factor binding protein.

Figure 1. Schematic illustration showing histological measurements. Villus height (VH), villus circumference (VC), and crypt depth (CD) were completed using ImagePro Plus 6.2.0 software (Media Cybernetics, Silver Spring, MD). The VH was measured from the tip to the base of the villus; CD was measured from the tip of the crypt to the point where it meets the muscularis mucosa; VC was measured by encircling the entire surface of the villus. A minimum of 5 full villi were chosen randomly per animal and the average measurement per animal was used for statistical analyses for each variable.

tion, and other impurities in the PCR amplicons. In addition, the PCR products for each primer pair were electrophoresed on 2% agarose gel to confirm purity and size of the amplicon.

Histological Measurements

Segments from different locations of small intestine fixed in 10% neutral formalin were processed for histological evaluation. Briefly, formalin-fixed tissues were dehydrated in ascending concentrations of ethanol

(starting from 70%, ending in 100% ethanol), cleared in 2 changes of xylene, and then infiltrated with and embedded in paraffin. Paraffin blocks were then cooled and sectioned using a rotary microtome (model HM340E, Microm, International GmbH, Walldorf, Germany) to generate 5- μ m-thick tissue slices. Four to five serial sections were mounted onto individual microscope slides, and then stained with hematoxylin and eosin after clearing of paraffin in xylene and gradual hydration of the tissue in descending concentrations of ethanol. Photomicrographs were obtained using an Olympus BH2 light microscope connected to a QColor3 digital camera (Olympus America Inc., Center Valley, PA) at 4 $\times$ magnification using the Q-Capture suite software program (QImaging, Surrey, British Columbia, Canada). Villus height, crypt depth, and villus circumference for each location were determined using a computer software program (Image-Pro Plus Version 6.2, Media Cybernetics, Silver Spring, MD). Villus height was measured from the tip to the base of the villus; crypt depth was measured from the tip of the crypt to the point where it meets the muscularis mucosa; villus circumference was measured by encircling the entire surface of the villus. A minimum of 5 full villi and crypts from each intestinal location was chosen randomly per animal, and the average measurement per animal was used for statistical analyses for each variable. An illustration of the method used to make measurements of different dimensions is shown in Figure 1.

Statistical Analysis

Gene expression and histomorphometry data were analyzed using the Mixed model procedure of SAS (SAS Version 9.1.3, Cary, NC) with repeated measures. Location of small intestine was the repeated measure as different regions of small intestine from the same animal were sampled after the application of one diet. Animal within treatment and group was the subject. An autoregressive (AR1) covariance structure was assumed for the location. Normality of distribution within treatment and location was lost for gene expression data for several genes on conversion of Δ Ct to $2^{(-\Delta$ Ct)}. Therefore, we analyzed Δ Ct and $2^{(-\Delta$ Ct)} using the statistical model described below. The variables Δ Ct and $2^{(-\Delta$ Ct)} were not analyzed because there would be no variation within the control treatment. The following model statement was used:

$$Y_{ijkl} = \mu + T_i + G_j + (TG)_{ij} + H_{(ijk)} + L_l + (TL)_{il} + (GL)_{jl} + e_{(ijkl)},$$

where Y_{ijkl} = dependent variable (gene expression, Δ Ct or $2^{(-\Delta$ Ct)}); μ = overall mean; T_i = fixed effect of treat-

Table 3. Least squares means ($\pm$ SE) for yield of RNA (μ g of RNA/mg of tissue) from different locations of intestinal mucosa of preweaned heifers fed with different milk replacers¹

Location	Treatment			
	CON ²	HPLF ³	HPHF ⁴	HPHF+ ⁵
Duodenum	1.15 $\pm$ 0.31	1.24 $\pm$ 0.34	1.29 $\pm$ 0.31	1.53 $\pm$ 0.31
Jejunum	1.25 $\pm$ 0.31	1.19 $\pm$ 0.34	0.73 $\pm$ 0.31	0.85 $\pm$ 0.31
Ileum	1.89 $\pm$ 0.31	2.11 $\pm$ 0.34	1.18 $\pm$ 0.31	1.64 $\pm$ 0.31

¹Yield of RNA was not different ($P = 0.356$) between different intestinal locations in different treatment groups.

²CON = control milk replacer (MR) formulated to contain 20% CP and 20% fat and be fed at 450 g of DM/d.

³HPLF = high protein, low fat MR formulated to contain 28% CP and 20% fat and be fed at 970 g of DM/d.

⁴HPHF = high protein, high fat MR formulated to contain 28% CP and 28% fat and be fed at 970 g of DM/d.

⁵HPHF+ = HPHF to be fed at 1,460 g of DM/d.

ment ($i = 1, 2, 3, 4$); G_j = fixed effect of group ($j = 1, 2, 3$); $(TG)_{ij}$ = fixed interaction of treatment and group; $H_{(ij)k}$ = random effect of heifer within treatment and group; L_l = fixed effect of intestinal location ($k = 1, 2, 3$); $(TL)_{il}$ = fixed interaction of treatment and location; $(GL)_{jl}$ = fixed interaction of group and location; and $e_{(ijkl)}$ = residual error (assumed to be normally and independently distributed).

Significance was declared at $P \leq 0.05$ for ΔCt and $P \leq 0.01$ for $2^{(-\Delta Ct)}$ to be conservative because of normality concerns. For histological measurements significance was declared at $P \leq 0.05$. The general linear models procedure of SAS was used to determine within-animal correlations between histological measurements and fold expression of the genes evaluated.

RESULTS AND DISCUSSION

To our knowledge, this study is the first to evaluate effects of feeding high protein, high fat MR on the expression of somatotrophic axis genes in the small intestinal mucosa of preweaned Holstein heifers. Total concentrations of RNA isolated from different locations of small intestinal mucosa were not different among treatment groups (Table 3). Differences in total RNA among crypt and villus fractions of different intestinal locations were reported by Ontsouka et al. (2004b) and related to predicted differences in cell turnover and protein synthesis rates in these regions. In the current study we isolated total RNA from mucosal scrapings, which includes villus and crypt fractions. Level of expression of β -actin was not affected by treatment diets or by different intestinal locations. Hence β -actin was used as an endogenous reference gene for normalization of expression of target mRNA.

Unexpectedly, overall relative expression of GHR, IGF-I, IGF-IR, IGFBP-1, -2, and IGFBP-6 mRNA were not significantly affected by treatment diets (Table 4).

However, there was a treatment effect (Table 4; $P < 0.05$ for ΔCt) for the relative abundance of mucosal IGFBP-5 mRNA. Level of IGFBP-5 mRNA in small intestine was greatest in the CON and lowest in the HPHF+ animals. Dietary regulation of IGFBP-5 expression in the small intestine of dairy cattle is not clearly understood, although increased expressions of IGFBP-5 mRNA in response to protein-restriction were reported previously in other animals. Feeding a low-protein diet to rats increased IGFBP-5 mRNA content in proximal nephrons of the kidney (Hise et al., 1994) and in the femur (Higashi et al., 1996). In our study the CON had the lowest concentration of protein in the diet, and the DMI was less than other treatments (Table 1; Daniels et al., 2008). Therefore, the increase in IGFBP-5 mRNA expression in the intestinal mucosa may be attributed to reduced protein in the diet as in rats. There was an interaction between treatment and location for the expression of IGFBP-2, -3, and -4 mRNA (Table 4). Level of expression of IGFBP-2 mRNA was greatest in the ileum of HPLF animals. Level of IGFBP-3 mRNA expression was lowest in duodenum of HPHF+ and greatest in the ileum of CON, whereas expression of IGFBP-4 was greatest in the jejunum of HPHF+ animals (Table 4). Relative expression of IGFBP in the intestinal mucosa in different treatment groups was not related to concentrations in circulation. Serum concentrations IGFBP-2 were greater in CON and IGFBP-3 were lower in CON, whereas the concentrations of IGFBP-4 and -5 in serum were similar between treatments (Daniels et al., 2008). Differential responses between systemic and local GIT expression of somatotrophic axis components were reported previously in piglets (Tang et al., 2002) and rats (Lowe et al., 1989).

In contrast to Winesett et al. (1995), we did not find a correlation between serum IGF-I and intestinal IGF-I mRNA expression. Serum IGF-I concentration was lower in CON when compared with the average of the

Table 4. Fold difference ($2^{-\Delta Ct}$, relative to endogenous reference gene β -actin) in mRNA abundance of somatotrophic axis components the small intestine (duodenum, jejunum, and ileum) of preweaned dairy heifers fed different milk replacers

Item	Treatment and location												SEM ⁵	Trt ⁷	Loc ⁸	Int ⁹
	CON ¹			HPLF ²			HPHF ³			HPHF+ ⁴						
	Duo	Jej	Ile	Duo	Jej	Ile	Duo	Jej	Ile	Duo	Jej	Ile				
GHR	1.76	1.50	2.88	0.24	0.32	0.76	0.39	0.23	0.46	0.31	0.47	0.30	0.89	—	cycle	—
IGF-I	0.47	0.70	1.47	0.19	0.98	1.24	0.37	0.87	0.77	0.57	1.69	0.95	0.32	—	fold	—
IGF-IR	0.52	0.49	1.31	−0.01	0.54	1.17	0.22	0.84	1.02	0.20	1.66	1.88	0.64	—	both	—
IGFBP-1	1.12	0.60	4.21	−0.11	0.24	5.52	0.40	0.03	1.47	−0.23	−0.62	11.26	5.40	—	both	—
IGFBP-2	1.48	0.68	0.62	0.77 ^a	0.40 ^a	2.41 ^b	0.32	0.27	1.21	0.35	0.26	0.92	0.34	—	both	fold
IGFBP-3	1.14 ^a	2.37 ^{ab}	4.26 ^b	0.60 ^a	1.10 ^a	5.78 ^b	0.79	1.19	1.51	0.71	0.82	1.50	0.70	—	fold	fold
IGFBP-4	0.47	0.79	0.80	−0.11	3.22	0.40	0.48	4.06	0.50	0.20 ^a	4.23 ^b	1.19 ^a	0.85	—	both	cycle
IGFBP-5	1.46	4.20	5.40	2.54	2.62	3.74	0.69	1.42	1.55	0.42	1.04	1.04	1.67	cycle	cycle	—
IGFBP-6	1.00	7.58	9.61	−0.15	6.50	1.95	0.84	15.51	2.60	1.03	18.89	9.18	4.91	—	cycle	—

^{a,b}Least squares means with unlike superscripts within a common treatment are different ($P < 0.01$).

¹CON = control milk replacer (MR) formulated to contain 20% CP and 20% fat and be fed at 450 g of DM/d.

²HPLF = high protein, low fat MR formulated to contain 28% CP and 20% fat and be fed at 970 g of DM/d.

³HPHF = high protein, high fat MR formulated to contain 28% CP and 28% fat and be fed at 970 g of DM/d.

⁴HPHF+ = HPHF to be fed at 1,460 g of DM/d.

⁵Standard error of the mean for treatment $\times$ location interaction (for $n = 5$).

⁶Fold = $2^{-\Delta Ct}$; cycle = ΔCt .

⁷Main effect attributable to treatment.

⁸Main effect attributable to location.

⁹Interaction of treatment and location.

Figure 2. Fold difference in expression of mRNA of somatotrophic axis components in different locations of the small intestine of preweaned dairy heifers relative to the endogenous reference gene β -actin. Abundance of IGF-IR mRNA was lowest (A, $P = 0.005$) in the duodenum, greatest in the ileum, and intermediate in jejunum. Insulin-like growth factor-I mRNA (B, $P = 0.003$) was lower in duodenum than jejunum or ileum. Relative expression of IGFBP-6 was lower (C, $P = 0.016$) in the duodenum. Bars with different superscripts differ, $P < 0.05$. Data presented as least squares means $\pm$ SE.

other 3 treatments (Daniels et al., 2008). However, in agreement with Winesett et al. (1995), we did not find an effect of nutrient status on IGF-I mRNA expression in jejunum and ileum. To our knowledge, the only reported effect of feeding on intestinal expression of somatotrophic axis genes in bovine is the study by Ontsouka et al. (2004a). In contrast from our findings, Ontsouka et al. (2004a) reported changes in the level of intestinal expression of IGF-I, IGF-IR, and IGFBP-2 mRNA in response to feeding (colostrum vs. MR) in neonatal calves. But those animals were 5 d of age and had also received dexamethasone in addition to treatment diets. Therefore, the changes in the gene expression pattern could be due to the effect of dexamethasone alone, a combination of diet and dexamethasone, or age at the time of sampling.

Unlike treatment effect, components of the somatotrophic axis were differentially expressed (Table 4, $P \leq 0.01$ for $2^{-\Delta Ct}$ or $P \leq 0.05$ for ΔCt) in the small intestinal mucosa by location (duodenum, ileum, and jejunum). Relative abundance of GHR mRNA was greater (Table 4) in the mucosal layer of ileum compared with duodenum and jejunum. Expression of IGF-IR mRNA (Figure 2A) was lowest in the duodenum, greatest in the ileum, and intermediate in the jejunum, whereas the abundance of IGF-I mRNA (Figure 2B) was lowest in the duodenum compared with the other 2 locations. Both IGFBP-1 and -5 mRNA (Table 4) were expressed intensely in the ileum in these dairy heifers compared with the other 2 small intestinal locations. Expression of IGFBP-6 (Figure 2C) was greater in jejunum and ileum compared with duodenum. Dissimilarity in the abundance of mRNA for somatotrophic axis genes between different intestinal locations suggests an unequal rate of mRNA synthesis, degradation, or both between locations (Ontsouka et al., 2004b). Further interpretation is difficult because the regulatory mechanisms and functional roles of these somatotrophic axis components are poorly defined for small intestine in dairy cattle. Regulation of IGFBP-5 mRNA abundance in rat intestinal smooth muscle by IGF-I was reported by Hou et al. (2000). In agreement with this finding we noted a positive correlation between expression of IGF-I and IGFBP-5 mRNA ($r = 0.67$; $P = 0.002$) in the small intestine. Thus, a similar regulatory mechanism may exist to control intestinal mucosal IGFBP-5 mRNA. Kuemmerle (2000) reported that endogenous IGF-I stimulated production of IGFBP-3, -4, and -5 in cultured human intestinal smooth muscle cells through a mitogen-activated protein kinase pathway. Contrasting Kuemmerle (2000), we found that abundance of IGFBP-3 and -4 mRNA in the small intestine of dairy heifers were not correlated with either the circulating concentrations (Daniels et al., 2008) or the

Table 5. Least squares means ($\pm$ SE) of villus height, villus circumference, and crypt depth for small intestine collected from 3 locations in preweaned dairy heifers fed 1 of 4 different milk replacers

Measurement region	Dietary treatment				<i>P</i> -value ⁵
	CON ¹	HPLF ²	HPHF ³	HPHF+ ⁴	
Villus height (μ m)					0.773
Duodenum	104.9 $\pm$ 17.5	87.9 $\pm$ 17.6	131.6 $\pm$ 17.4	83.6 $\pm$ 17.4	
Jejunum	90.5 $\pm$ 17.5	91.1 $\pm$ 17.6	101.3 $\pm$ 19.9	86.1 $\pm$ 15.6	
Ileum	56.6 $\pm$ 17.5	65.7 $\pm$ 21.7	71.8 $\pm$ 15.6	79.0 $\pm$ 17.4	
Villus circumference (μ m)					0.944
Duodenum	2,878 $\pm$ 647	3,945 $\pm$ 647	3,063 $\pm$ 725	3,142 $\pm$ 646	
Jejunum	3,113 $\pm$ 654	3,413 $\pm$ 647	3,485 $\pm$ 739	3,404 $\pm$ 576	
Ileum	2,944 $\pm$ 647	2,962 $\pm$ 807	3,761 $\pm$ 576	3,378 $\pm$ 646	
Crypt depth (μ m)					0.711
Duodenum	63.5 $\pm$ 9.7	78.3 $\pm$ 9.7	88.3 $\pm$ 9.6	65.7 $\pm$ 9.6	
Jejunum	74.8 $\pm$ 9.7	65.6 $\pm$ 9.7	85.6 $\pm$ 11.2	76.9 $\pm$ 8.6	
Ileum	54.9 $\pm$ 9.7	68.0 $\pm$ 12.0	66.9 $\pm$ 8.6	68.2 $\pm$ 9.6	

¹CON = control milk replacer (MR) formulated to contain 20% CP and 20% fat and be fed at 450 g of DM/d.

²HPLF = high protein, low fat MR formulated to contain 28% CP and 20% fat and be fed at 970 g of DM/d.

³HPHF = high protein, high fat MR formulated to contain 28% CP and 28% fat and be fed at 970 g of DM/d.

⁴HPHF+ = HPHF to be fed at 1,460 g of DM/d.

⁵Treatment effect.

local expression of IGF-I in the intestine. Degree of expression of mRNA does not necessarily correspond to the concentration of protein, but this finding suggests the lack of a direct relationship. Further examination of the somatotrophic axis components at protein level would give a better understanding of the functional significance of these components in different regions of the small intestine.

We did not find any effect due to treatment on villus height ($P = 0.773$), villus circumference ($P = 0.944$), or crypt depth ($P = 0.711$) in any of the small intestinal locations (Table 5). Villus height was shortest ($P = 0.035$) in the ileum compared with duodenum and jejunum, whereas there was no difference in the height of the villi between the duodenum and jejunum (Figure 3A). There was no difference in the crypt depth (Figure 3B; $P = 0.242$) or villus circumference (Figure 3C; $P = 0.976$) between the different locations in the small intestine. Histological measurements were comparable with Buhler et al. (1998), who reported tallest villi in duodenum but no difference in the crypt depth across intestinal locations between colostrum-fed and MR-fed neonatal calves.

Unlike Ontsouka et al. (2004b) we did not find any correlation between the histological measurements and level of gene expression in the small intestine of dairy heifers. Ontsouka et al. (2004b) found a positive correlation between the crypt depth and IGFBP-3 mRNA expression as well as a negative correlation between villus height and IGF-I mRNA. Even though there was no correlation between villus height and IGF-I mRNA, ileum had greater expression of IGF-I mRNA, and shortest villi. We used small intestinal mucosal

scrapings for total RNA extraction as opposed to the entire intestinal wall as was done by Ontsouka et al. (2004b). The discrepancy between the observations of these studies could be due to this sampling difference or the distinct differences in age at sampling (5 d of age vs. 64 d of age).

Given the lack of comparable data for preweaned ruminants, interpretation of our findings is difficult. Regardless, it is well recognized that both rate of feeding and alterations of diet composition impact calf performance (Blome et al., 2003), body composition (Bartlett et al., 2006; Bascom et al., 2007), and mammary development (Sejrsen and Purup, 1997). Certainly, at least in general terms, each of these physiological processes depend on the GH-IGF-I axis and interactions of nutrients with the GI tract. Thus, the logic for our evaluation of the GH-IGF-I axis in the intestinal mucosa is evident.

We anticipated that differences in expression of GH-IGF-I axis genes in the GI tract would reflect alterations in diet-induced growth and body composition of these heifers as reported in related manuscripts (Daniels et al., 2008; Hill et al., 2008). However, this was not the case. It may be that homeostatic adjustments at the level of the GI tract were sufficiently robust to minimize the functional impact of these dietary treatments on normal mucosal development. Clearly, dietary treatments had no impact on mucosa development. Thus, there is little evidence to support the notion that differences in growth or body composition were mediated by variation in expression of these genes in the small intestine. In contrast, differences in gene expression among regions provide evidence that

Figure 3. Mean villus height (A), crypt depth (B), and villus circumference (C) in different locations of small intestine of preweaned dairy heifers. Villus height was lowest ($P = 0.035$) in the ileum, whereas crypt depth was not different ($P = 0.242$) between different locations. There was no difference ($P = 0.976$) in the villus circumference measured between the different intestinal locations. Bars with different superscripts differ, $P < 0.05$. Data presented as least squares means $\pm$ SE.

it should have been possible to detect dietary-related differences within regions had they existed.

In summary, we conclude that differences in regional morphology within the small intestine of preweaned

heifers are likely affected by variation in local expression of GH-IGF-I axis genes, but effects related to changes in fat or protein composition of MR or feeding rate of MR are not.

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