The small intestinal epithelia of beef steers differentially express sugar transporter messenger ribonucleic acid in response to abomasal versus ruminal infusion of starch hydrolysate

S. F. Liao, D. L. Harmon, E. S. Vanzant, K. R. McLeod, J. A. Boling, and J. C. Matthews

Department of Animal and Food Sciences, University of Kentucky, Lexington 40546-0215

ABSTRACT: In mammals, the absorption of monosaccharides from small intestinal lumen involves at least 3 sugar transporters (SugT): sodium-dependent glucose transporter 1 (SGLT1; gene SLC5A1) transports glucose and galactose, whereas glucose transporter (GLUT) 5 (GLUT5; gene SLC2A5) transports fructose, across the apical membrane of enterocytes. In contrast, GLUT2 (gene SLC2A2) transports all of these sugars across basolateral and apical membranes. To compare the distribution patterns and sensitivity with nutritional regulation of these 3 SugT mRNA in beef cattle small intestinal tissue, 18 ruminally and abomasally catheterized Angus steers (BW ≈ 260 kg) were assigned to water (control), ruminal cornstarch (partially hydrolyzed by α-amylase; SH), or abomasal SH infusion treatments (n = 6) and fed an alfalfa-cube-based diet at 1.3 × NEm requirement. The SH infusions amounted to 20% of ME intake. After 14- or 16-d of infusion, steers were killed; duodenal, jejunal, and ileal epithelia harvested; and total RNA extracted. The relative amount of SugT mRNA in epithelia was determined using real-time reverse transcription-PCR quantification methods. Basal expression of GLUT2 and SGLT1 mRNA was greater (P < 0.09) by jejunal than by duodenal or ileal epithelia, whereas basal content of GLUT5 mRNA was greater (P ≤ 0.02) by jejunal and duodenal than by ileal epithelia. The content of GLUT5 mRNA in small intestinal epithelia was not affected (P ≥ 0.16) by either SH infusion treatment. In contrast, GLUT2 and SGLT1 mRNA content in the ileal epithelium was increased (P ≤ 0.05) by 6.5- and 1.3-fold, respectively, after abomasal SH infusion. Duodenal SGLT1 mRNA content also was increased (P = 0.07) by 64% after ruminal SH infusion. These results demonstrate that the ileum of beef cattle small intestine adapts to an increased luminal supply of glucose by increasing SGLT1 and GLUT2 mRNA content, whereas increased ruminal SH supply results in duodenal upregulation of SGLT1 mRNA content. These adaptive responses of GLUT2 and SGLT1 mRNA to abomasal or ruminal SH infusion suggest that beef cattle can adapt to increase their carbohydrate assimilation through small intestinal epithelia, assuming that altered SugT mRNA contents reflect the altered transport functional capacities.

Key words: bovine, nutrient-gene interaction, SLC2, SLC5, small intestine

INTRODUCTION

In mammals, the general model for monosaccharide absorption across small intestinal enterocytes involves at least 3 sugar transporters (SugT; Kellet et al., 2008). Sodium-dependent glucose transporter 1 (SGLT1) is capable of transporting glucose and most other monosaccharides except for fructose across the brush border membrane (BBM), whereas glucose transporter (GLUT) 5 (GLUT5; gene SLC2A5) transports only fructose across the BBM. In contrast, GLUT2 transports glucose, fructose, and most other monosaccharides and is localized to the BBM and basolateral membranes (BLM). In rodents (Ferraris, 2001), pigs (Drozdowski and Thomson, 2006), and sheep (Lescale-Matys et al., 1993; Shirazi-Beechey et al., 1997), increased luminal glucose results in increased mRNA, protein, and functional activity of SGLT1, whereas GLUT5 expression is increased by high-fructose (but not other monosaccharides) diets for rodents (Ferraris, 2001). High-carbo-
Alternatively, the expression of GLUT5, GLUT2, and SGLT1 mRNA by the small intestinal epithelium of cattle (Holstein steers) is known (Zhao et al., 1993, 1998), knowledge about the relationship between luminal nutrient challenge and SugT expression is very limited, whereas posttranscriptional regulation of SugT expression by cattle appears to be more complex than by rodents and sheep (Bauer et al., 2001a,b; Rodriguez et al., 2004).

Previously, we reported that ruminal and abomasal infusion of corn starch hydrolysate (SH) to growing steers altered the transcription profiles of nucleoside transporter genes. Using the RNA extracted from these animals, the objectives of this experiment were to test the hypotheses that 1) the relative expression of SugT mRNA differs among small intestinal epithelia and 2) the expression of SGLT1 and GLUT2 mRNA, but not GLUT5, increases in response to luminal but not ruminal SH infusion.

MATERIALS AND METHODS

Research protocols were approved by the University of Kentucky Institutional Animal Care and Use Committee.

Animal Trial Procedures

As described previously by Liao et al. (2008b, 2009), 18 Angus steers (BW = 260 ± 17 kg) were raised at the University of Kentucky Agricultural Research Center Beef Unit during February to August 2006. Because of facility and technical requirements, the animals were obtained at 3 time periods to minimize differences in initial BW at each of the 3 staggered animal trial periods. For each period, a randomized complete block experimental design was employed. Six steers in each period were blocked by BW (heavy vs. light). Within each of the 2 BW blocks, 3 steers surgically fitted with ruminal and abomasal infusion catheters (Walker and Harmon, 1995) were randomly assigned to 3 infusion treatments: 1) ruminal and abomasal infusion with water (control), 2) ruminal infusion with SH and abomasal infusion with water, and 3) ruminal infusion with water and abomasal infusion with SH. The dosage of SH infusion amounted to 20% of ME intake (approximately 800 g/d). The equalized dosage (20% of ME intake) shared by ruminal and abomasal infusion was based on an assumption that essentially all of the ruminally infused SH would be fermented in the rumen (Walker and Harmon, 1995).

The basal diet fed to all steers was blended high-quality alfalfa-hay cubes, which contained 17.8% crude protein and 1.31 Mcal of NE\textsubscript{m}/kg (DM basis), and was provided to steers at 1.33 × maintenance energy requirement calculated as 0.077 Mcal of NE\textsubscript{m}/empty BW\textsuperscript{0.75} (NRC, 1996). The DM content of the alfalfa-hay cubes was 88.1%, and the OM, NDF, ADF, and ADL contents were 90.6, 44.4, 28.1, and 7.8% (DM basis), respectively. A trace mineralized salt (92 to 96% NaCl), which contained (mg/kg) Zn (5,500), Mn (4,790), Cu (1,835), Fe (9,275), I (115), Co (65), and Se (18), was provided to steers at a dose of 40 g/d. Steers also received 20 g/d of poloxolene (Phibro Animal Health, Ridgefield Park, NJ) to minimize incidence of bloat. Steers were fed daily in 12 equally proportioned meals (once every 2 h) using automatic feeders (Ankom Co., Fairport, NJ), and had ad libitum access to fresh water throughout the trial. Steers were tethered individually in stalls (1.2 × 1.7 m) and housed in an environmentally controlled room (ambient temperature 20°C) with a 24-h light time.

The SH infusate used was a tap water solution of raw cornstarch, partially hydrolyzed by a heat-stable α-amylase (Bauer et al., 1995). The SH was chosen over raw corn starch because the digestion characteristics of SH are similar to those of native starch yet SH is more suspendable in solution and, therefore, facilitates pumping. Stock SH infusate solutions were prepared in 3 to 4 batches for each experimental period and stored at −20°C until use. Before infusion, stock solutions were diluted with tap water to a final weight of 5.5 kg for each animal and infused over 22 h per day. During infusion, the homogeneity of the SH infusate was maintained by rapid continuous mixing of the solution on 6 individual stir-plates. The SH solution or water (5.5 kg) was continuously infused at a rate of 250 mL/h to the animals to help maintain a steady-state SH supply condition for at least 14 d before tissue sample collection.

Throughout the course of experimentation, 2 steers were lost from the ruminal SH infusion group. One was due to factors unrelated to the infusion treatments, and the other gradually stopped eating during the late phase of the experimental period. No samples from these 2 animals were analyzed.

Animal Slaughter and Tissue Collection

After a 14- or 16-d SH infusion, steers were transported to a USDA-approved slaughter facility for slaughter and tissue harvesting (3 steers in a block per day). Steers were killed by stunning with a captive-bolt pistol, followed by exsanguination to allow carcass recovery for human consumption. The small intestine was removed, and its total length (from pyloric valve to ileal-cecal junction) was determined by looping the intestine across a wet stationary board that was fitted with metal pegs at 2-m increments. Detailed procedures of animal slaughter and visceral organ collection were described previously (McLeod et al., 2007; Liao et al., 2008a,b).

The sites and protocol for collection of small intestinal epithelial samples for total RNA preparation has been described previously (Howell et al., 2003). Briefly, 1-m sections of duodenum (0.5 to 1.5 m distal to the pyloric...
juncture), jejunum (middle of the first half nonduodenal small intestine), and ileum (middle of the second half nonduodenal small intestine) were taken after removing the digesta. One-half of each intestinal section was scraped to collect the epithelia and the other one-half snap-frozen in liquid nitrogen for reserve. Each section was cut in half, inverted, and rinsed with ice-cold (4°C) physiological saline (0.9% NaCl), and epithelia scraped off with a glass slide. Approximately 2 g of scraped epithelium were placed into 20 mL of TRIzol Reagent (Invitrogen Corporation, Carlsbad, CA), homogenized immediately, and stored at −80°C. Among animals, the time lapse from beginning to end of sample collection was 35 to 45 min. Within an animal, the difference between collection times among epithelial types was 1 to 2 min.

**RNA Extraction and Reverse Transcription**

**RNA Extraction and Purification.** Total RNA was extracted from the frozen epithelial homogenate (10 mL) following the instructions from Invitrogen Corporation for the TRIzol Reagent. After total RNA was recovered, a purification procedure was performed using RNaseasy Mini Kit (Qiagen, Valencia, CA) to minimize genomic DNA contamination and enrich all the mRNA longer than 200 nucleotides in molecular size. Purified RNA was then eluted with 60 µL of RNase-free distilled H2O and stored at −80°C. The integrity of the purified RNA was examined by gel electrophoresis using Agilent 2100 Bioanalyzer System (Agilent Technologies, Santa Clara, CA) at the University of Kentucky Microarray Core Facility. Visualization of the gel images and electropherograms showed that all RNA samples had high quality with RNA integrity number greater than 8.0 and 28S/18S rRNA ratio greater than 1.8. The purity and concentration of the purified RNA samples was analyzed by a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Wilmington, DE), which revealed that all the samples were of high purity with 260/280 absorbance ratios greater than 2.0 and 260/230 absorbance ratios greater than 1.75.

**Reverse Transcription.** Before reverse transcription (RT), all RNA samples were treated with DNase I enzyme (amplification grade) to remove any residual DNA, as per the manufacturer’s instructions (Invitrogen). Each RNA (3 µg) sample was combined with 1 µL of RT buffer (10×), 2 µL dithiothreitol (0.1 M), 4 µL of MgCl2 (25 mM), 1 µL of dNTP (10 mM each), and 1 µL of RNase Out then was added to the reaction. After incubation at 37°C for 2 min, the reaction was incubated with 1 µL of reverse transcriptase at room temperature for 10 min and then incubated at 50°C for 50 min. To stop the reaction, the reaction mixture was incubated at 70°C for 10 min and then chilled on ice. The resulting reaction products, cDNA, were stored at −20°C until used in real-time PCR.

**Real-Time PCR**

Before conducting real-time PCR with ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Foster City, CA), the computational predicted bovine GLUT2 and GLUT5 mRNA sequences and the actual reported bovine SGLT1 mRNA sequence were retrieved from GenBank nucleotide database (the National Center for Biotechnology Information, NIH, Bethesda, MD). The GenBank accession numbers for GLUT2 (gene name SLC2A2), GLUT5 (gene name SLC2A5), and SGLT1 (gene name SLC5A1) are XM_614140, XM_583977, and NM_174606, respectively (Table 1). The bovine 18S ribosomal RNA gene sequence was also retrieved from GenBank database with an accession number of DQ22453. The real-time PCR primer and probe sets for GLUT2, GLUT5, SGLT1, and 18S cDNA were designed and manufactured by using ABI Assays-by-Design Service (Applied Biosystems). To reduce real-time PCR “noise” from contaminating genomic DNA, all the primer and probe sets were designed to produce amplicons bridging exon-exon junctions (Cui et al., 2007). Each primer and probe set consists of 2 unlabeled PCR primers and 1 TaqMan Minor Groove Binding probe labeled with a reporter dye, 6-carboxy-fluorescein at 5’ end (FAM, Table 1). The sequences of these primers and probes, as well as the locations on their respective templates, are also shown in Table 1.

Components of a 25-µL real-time PCR reaction included an Assays-by-Design primer and probe set (1.25 µL), TaqMan Universal PCR Master Mix-No AmpErase UNG (12.5 µL), cDNA template (1.0 to 2.5 µL), and DNase/RNase free H2O (8.75 to 10.25 µL). The PCR conditions used for the amplification and quantification were an initial denaturing stage (95°C for 10 min), followed by 40 cycles of 2 amplification stages of denaturing (95°C for 15 s), and annealing/extension (60°C for 1 min), with a melting curve program (60 to 95°C), a heating rate of 0.15°C/s, and continuous fluorescence measurements.

**Development of mRNA Quantification Methodology**

**Validation of Real-time PCR Products.** To establish mRNA relative quantification methodology, first the real-time RT-PCR products need to be vali-
Regulated sugar transporter messenger ribonucleic acid expression

Table 1. Primer and probe sets used for the real-time relative quantitative PCR analyses of mRNA-derived cDNA contents of 3 sugar transporters

<table>
<thead>
<tr>
<th>Primer and probe</th>
<th>Location on template, bp</th>
<th>Sequence</th>
<th>Amplicon size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT5 (XM_583977)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>1,109–1,132</td>
<td>5′-GTGCTGTCAATGTCGTGATAACTG-3′</td>
<td>78</td>
</tr>
<tr>
<td>Probe (fwd.)</td>
<td>1,133–1,147</td>
<td>5′-FAM-TCTGCGCTTTTCTTGC-3′</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>1,167–1,186</td>
<td>5′-CCAGGAGGACAGGAATCTC-3′</td>
<td></td>
</tr>
<tr>
<td>GLUT2 (XM_614140)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>1,142–1,161</td>
<td>5′-TCATGTCCGCTGGACCTTGC-3′</td>
<td>62</td>
</tr>
<tr>
<td>Probe (fwd.)</td>
<td>1,162–1,177</td>
<td>5′-FAM-CTCCTGATAATTCTC-3′</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>1,179–1,203</td>
<td>5′-TGTCATGCTCAGTAAATTCTCAA-3′</td>
<td></td>
</tr>
<tr>
<td>SGLT1 (NM_174606)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>116–138</td>
<td>5′-GCATCTCTTCTGTCGGTGTTGAT-3′</td>
<td>69</td>
</tr>
<tr>
<td>Probe (rev.)</td>
<td>146–159</td>
<td>5′-FAM-ATGCCCAACAGCCCAC-3′</td>
<td></td>
</tr>
<tr>
<td>Reverse</td>
<td>166–184</td>
<td>5′-AAGCGTCCCCAGGTTATG-3′</td>
<td></td>
</tr>
</tbody>
</table>

1The contents in parentheses are the accession numbers for the corresponding cDNA or gene sequences recorded in the GenBank (http://www.ncbi.nlm.nih.gov) database. These sequences were retrieved for the purpose of being used as templates to design primers and probes for real-time quantitative PCR analyses. GLUT = glucose transporter; SGLT = sodium-dependent glucose transporter.

2FAM labeled at the 5′ end of the TaqMan probe is 6-carboxy-fluorescein, which is used as a reporter dye in the real-time PCR reactions.

3The custom TaqMan probes were supplied in the forward (fwd.) or the reverse (rev.) orientation as indicated in parentheses for each probe.

dated by sequence verification. To prepare PCR products for sequencing, a PureLink Quick Gel Extraction Kit (Invitrogen) was used to purify the products from the PCR mixtures, which include unincorporated nucleotides, primers, DNA polymerase, and FAM dye that was labeled to TaqMan probe by the manufacturer (Applied Biosystems). Briefly, approximately 250 µL of pooled PCR reaction mixtures were electrophoresed in a 1.2% agarose slab gel. A single PCR product band at the desired size was identified under a UV light, excised from the gel, placed into a sterile, 1.5-mL polypropylene centrifuge tube, dissolved with the gel solubilization buffer, and then filtered through an extraction column. The column-bound DNA was cleaned with the washing buffer and finally eluted out with 25 to 40 µL of DNase/RNase free H2O, and concentration determined using 1:5 and 1:15,625 dilutions of the RT product stocks, respectively.

Relative mRNA Quantification Methods. For relative quantification of SugT mRNA expression levels, the real-time quantitative RT-PCR methodology using a 2-step regimen were developed in accordance with ABI guidelines (Applied Biosystems, 2004; Liao et al., 2008b, 2009). In the first step, all the total RNA samples were reverse transcribed to cDNA as described above for RT reaction. In the second real-time PCR step, relative standard curve methods were established for each SugT cDNA, and the 18S CDNA (reverse-transcribed from 18S rRNA) was selected as an endogenous control to normalize the variations in sample preparation, mRNA inputs, and RT efficiencies. Specifically, a cDNA sample was serially diluted 2.5×, 5×, 25×, 125×, 625×, 3,125×, 15,625×, 78,125×, and 390,625×, and the linear range for target mRNA quantification was established to ascertain an appropriate amount of cDNA to be used for the standard curve method. For each cDNA sample the real-time PCR reactions (as described above) were conducted in triplicate to average out the possible pipetting, mixing, or plate setting-up errors. The minimal threshold (C_T) values detected using these dilutions were around 26 and 23 for the target and 18S cDNA, respectively. As a result, the optimal detection of SugT and 18S cDNA were achieved by using 1:5 and 1:15,625 dilutions of the RT product stocks, respectively.

The potential tissue distribution and SH infusion effects on the expression of 18S rRNA by small intestinal epithelia were evaluated by comparing the C_T values obtained from the real-time PCR reactions (Applied Biosystems, 2004). The relative quantities of SugT mRNA expression were normalized to the relative 18S rRNA quantities by calculating the SugT:18S relative quantity ratios, and these 18S-normalized quantity ratios were used for SugT tissue distribution pattern as well as SH infusion treatment effect analyses. The C_T values for 18S rRNA quantities and the normalized values for SugT tissue distribution patterns and SH infusion treatments were all subjected to statistical analysis.

Statistical Analysis

The effects of 3 small intestinal sections (duodenum, jejunum, and ileum) and the effects of SH infusion (control, ruminal, or abomasal) treatments on the expression of 18S rRNA and SugT mRNA were analyzed by a split-plot design ANOVA for a randomized complete block design using the GLM procedures (SAS Inst. Inc.,
The SH infusion treatment effects were tested in the main plot with individual steers as experimental units, and the tissue distribution effects were tested in the subplot with individual intestinal sections as experimental units (Liao et al., 2008b, 2009). Sums of squares were partitioned to period, block nested in period, treatment, intestinal section, treatment × section interaction, and the residual error. Random effects were specified for 2 model terms, period and block nested in period. The treatment × intestinal section interaction was used as the error term for the main plot, and the residual error was used as the error term for the subplot. When the interaction of treatment × section was not significant \((P > 0.10)\), the least squares means associated with infusion treatment were separated by Fisher’s LSD \((P \leq 0.10)\), and the least squares means associated with intestinal section were similarly separated within each infusion treatment. When the treatment × section interaction was significant \((P \leq 0.10)\), the least squares means associated with infusion treatment were separated by Fisher’s LSD \((P \leq 0.10)\) within each intestinal section. The probability levels of \(P \leq 0.10\) and \(0.10 < P < 0.15\) were defined as significant differences and tendencies toward differences, respectively.

**RESULTS**

**Development of mRNA Quantification Methodology**

All the real-time RT-PCR assays conducted in this study were validated first by sequencing their final products. Sequencing results revealed that the products for GLUT5, GLUT2, and SGLT1 mRNA all have 100% identities (base-pair ratios of 78/78, 62/62, and 69/69, respectively) to their corresponding GenBank template sequences (Figure 1). These product sequences now reside in GenBank database with accession numbers EF544429 [glucose transporter 5 (GLUT5)], EF544428 [glucose transporter 2 (GLUT2)], and EF544430 [sodium-dependent glucose transporter 1 (SGLT1)].

![Figure 1. Sequence comparison of the real-time reverse transcription-PCR products (product) to their respective template sequences (GenBank accession number). The symbol : indicates identical base pairs between the corresponding sequences. The underlines indicate the forward and reverse primer positions, and the highlights mark the probe positions. These product sequences now reside in GenBank with accession numbers EF544429 [glucose transporter 5 (GLUT5)], EF544428 [glucose transporter 2 (GLUT2)], and EF544430 [sodium-dependent glucose transporter 1 (SGLT1)].](image)

Numerous studies have revealed that 18S rRNA expression is very stable, and its content can be used as an endogenous control to normalize the expression of other genes in response to various stimuli (Bustin et al., 2005; Liao et al., 2008a,b, 2009). In this study, we also chose to normalize the relative expression of GLUT5, GLUT2, and SGLT1 mRNA to 18S rRNA expression. Before normalization, however, the use of 18S rRNA as a constitutive expression control was validated by testing the potential effect of SH infusion treatment on the expression levels of 18S rRNA among 3 small intestinal epithelia. No SH infusion treatment effect \((0.52 < P < 0.71)\), intestinal section distribution effect \((0.25 < P < 0.64)\), or an interaction between SH infusion treatments and small intestinal sections \((P = 0.86)\) on 18S rRNA expression was observed (data not shown). This result is consistent with those found in our previous nucleotide and cationic AA transporter research conducted with the same tissue samples (Liao et al., 2008b, 2009). Accordingly, the 18S rRNA expression levels were used to normalize the relative quantities of the target SugT mRNA expression by small intestinal epithelia.

**Basal Expression Patterns of SugT Gene Transcription by Small Intestinal Epithelia**

To characterize the normal tissue distribution patterns of GLUT5, GLUT2, and SGLT1 mRNA among 3 small intestinal sections of beef steers, the relative mRNA expression levels obtained from 6 control animals (without SH infusion) were compared among duodenal, jejunal, and ileal epithelia (Table 2). First of all, the mRNA for 3 SugT were all expressed by the epithelial tissues from all 3 small intestinal sections. However, the relative abundance for each SugT mRNA differed \((P < 0.043)\) among the 3 tissues. Ileal content of GLUT5 mRNA was 74 and 80% less \((P < 0.02)\) than for duodenal and jejunal epithelia, respectively, whereas no difference \((P = 0.31)\) was found between duode-
nal and jejunal expression. For GLUT2 mRNA, jejunal content was approximately 2.2- and 34-fold ($P < 0.001$) greater than in duodenal or ileal epithelia, respectively, whereas no difference ($P = 0.20$) was found between duodenal and ileal expression of GLUT2 mRNA. For SGLT1 mRNA, jejunal content was approximately 0.7- ($P \leq 0.09$) and 1.6-fold ($P = 0.02$) greater than in duodenal and ileal epithelia, respectively, whereas no difference ($P = 0.37$) was found between duodenal and ileal expression.

**Starch Hydrolysate Infusion Alters Basal SugT Gene Transcription Profiles**

An interaction between SH infusion treatment and small intestinal section on SGLT1 mRNA expression was detected ($P = 0.051$), but not on GLUT5 ($P = 0.42$) or GLUT2 ($P = 0.45$). Therefore, to avoid the masking of potential SH infusion effects on individual intestinal section expression of SugT mRNA, the least squares means associated with infusion treatments for all 3 SugT mRNA were separated within each small intestinal section.

Epithelial expression of GLUT5 mRNA was not affected by ruminal or abomasal SH infusion treatment at the duodenal, jejunal, or ileal section of the small intestine (Table 3). The expression of GLUT2 mRNA by duodenal or jejunal epithelia was not affected by ruminal or abomasal SH infusion treatment. Likewise, despite an apparent 95% numerical decrease in GLUT2 content, ruminal SH infusion did not alter GLUT2 mRNA expression by ileal epithelium. In contrast, abomasal SH infusion increased ($P = 0.047$) the ileal expression of GLUT2 mRNA by approximately 5.5-fold.

For SGLT1 mRNA, ruminal infusion of SH increased ($P = 0.07$) duodenal expression by approximately 64%.

**Table 2.** Tissue distribution patterns of sugar transporter mRNA among duodenal, jejunal, and ileal epithelia

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Duodenum</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT5</td>
<td>1.59</td>
<td>2.06</td>
<td>0.41</td>
</tr>
<tr>
<td>GLUT2</td>
<td>0.98</td>
<td>3.15</td>
<td>0.09</td>
</tr>
<tr>
<td>SGLT1</td>
<td>0.90</td>
<td>1.52</td>
<td>0.59</td>
</tr>
</tbody>
</table>

$a,b$Means within a row that lack a common superscript letter differ ($P \leq 0.10$).

The expression levels of sugar transporter [glucose transporter 5 (GLUT5), glucose transporter 2 (GLUT2), or sodium-dependent glucose transporter 1 (SGLT1)] mRNA for duodenal, jejunal, and ileal epithelia are from the control animals (no starch hydrolysate infusion), and the data presented are normalized to the relative 18S rRNA expression level.

$^2$The $P$-values were obtained from F-test in the ANOVA analysis ($n = 6$).

$^3$SE associated with the individual mean.

**Table 3.** Starch hydrolysate infusion treatment effect on sugar transporter mRNA expression by duodenal, jejunal, and ileal epithelia

<table>
<thead>
<tr>
<th>Transporter</th>
<th>Control</th>
<th>Ruminal</th>
<th>Abomasal</th>
</tr>
</thead>
<tbody>
<tr>
<td>GLUT5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>1.59</td>
<td>1.91</td>
<td>1.14</td>
</tr>
<tr>
<td>Jejunum</td>
<td>2.06</td>
<td>1.65</td>
<td>2.08</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.41</td>
<td>0.49</td>
<td>0.51</td>
</tr>
<tr>
<td>GLUT2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.98</td>
<td>1.37</td>
<td>1.03</td>
</tr>
<tr>
<td>Jejunum</td>
<td>3.15</td>
<td>2.68</td>
<td>3.14</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.09$^a$</td>
<td>0.004$^a$</td>
<td>0.65$^b$</td>
</tr>
<tr>
<td>SGLT1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>0.90$^a$</td>
<td>1.48$^b$</td>
<td>0.80$^a$</td>
</tr>
<tr>
<td>Jejunum</td>
<td>1.52</td>
<td>1.06</td>
<td>1.16</td>
</tr>
<tr>
<td>Ileum</td>
<td>0.59$^a$</td>
<td>0.46$^a$</td>
<td>1.32$^b$</td>
</tr>
</tbody>
</table>

$a,b$Means within a row that lack a common superscript letter differ ($P \leq 0.10$).

The relative mRNA levels of sugar transporters [glucose transporter 5 (GLUT5), glucose transporter 2 (GLUT2), or sodium-dependent glucose transporter 1 (SGLT1)] expressed by small intestinal epithelia of the 3 treatment groups (no starch hydrolysate infusion, control; starch hydrolysate infusion into rumen, ruminal; starch hydrolysate infusion into abomasums, abomasal) are presented as normalized to the relative 18S rRNA expression level.

$^2$The $P$-values were obtained from F-test in the ANOVA analysis (control, $n = 6$; ruminal, $n = 4$; abomasal, $n = 6$).

$^3$SE associated with the individual mean.
(Table 3). Similar to GLUT5, SGLT1 mRNA expression by jejunal epithelia was not affected by ruminal or abomasal SH infusion. Similar to GLUT2, the abomasal infusion of SH increased ($P = 0.046$) the ileal expression of SGLT1 mRNA by approximately 1.2-fold, whereas ruminal SH infusion did not affect ileal content of SGLT1 mRNA.

In summary, abomasal infusion of SH increased GLUT2 and SGLT1, but not GLUT5, mRNA expression by ileal epithelium, whereas ruminal SH infusion increased the expression of only SGLT1 mRNA by duodenal epithelium.

### DISCUSSION

#### Coordinated Expression and Function of SugT in Enterocytes

The mammalian genes SLC5A1, SLC2A5, and SLC2A2 encode glucose transporters SGLT1, GLUT5, and GLUT2, respectively. Detailed reviews about the kinetic and molecular properties of these proteins are available (Wright et al., 1994; Ferraris and Diamond, 1997; Ferraris, 2001; Udry and Thorens, 2004). In general, SGLT1 is responsible for the high (relatively)-affinity, concentrative uptake of most monosaccharides (including glucose and galactose) except for fructose, whereas GLUT5 functions as a low-affinity, equilibrative transporter of fructose and GLUT2 functions as a low-affinity, equilibrative transporter of most monosaccharides, including fructose. As equilibrative transporters, GLUT2 and GLUT5 transport their substrates down substrate concentration gradients, whereas SGLT1 can transport substrates against substrate concentration gradients. In the polarized absorptive epithelial cells of the small intestine (enterocytes), it is now thought that all 3 of these proteins localize and function in the BBM (Kellett and Helliwell, 2000; Kellett et al., 2008). Specifically, BBM-localized SGLT1, GLUT5, and GLUT2 transport monosaccharides into the enterocyte, whereas BLM-localized GLUT2 mediates the export of monosaccharides from enterocytes into the blood. In addition, when intracellular monosaccharide concentrations are decreased, GLUT2 also functions to supply enterocytes with blood glucose.

#### Basal Expression of SugT mRNA Along the Small Intestinal Epithelium of Beef Cattle

Relative to other species, few aspects about SugT expression by and function in the small intestinal epithelia of cattle are known (Harmon and McLeod, 2001; Harmon and Taylor, 2005). A primary objective of the present study was to determine the relative tissue expression of GLUT5, GLUT2, and SGLT1 mRNA among small intestinal epithelia of growing, forage-fed beef steers. Our findings that the duodenal, jejuna, and ileal epithelia of growing Angus steers express all 3 of these mRNA using real-time RT-PCR analysis is consistent with previous observations that the bovine small intestinal epithelium expresses these mRNA, as previously observed for Holstein cows (GLUT5, GLUT2, and SGLT1; Zhao et al., 1993, 1998) and steer calves (SGLT1; Guimaraes et al., 2007) using Northern blot analysis. In addition, our findings extend the previous observations by revealing a differential pattern of expression for GLUT5, GLUT2, and SGLT1 mRNA among duodenal, jejuna, and ileal epithelia of Angus steers.

An important understanding gained was that the relative expression of all 3 mRNA was greatest by jejunal epithelia and, numerically, was greater for duodenal than ileal epithelia. This finding is consistent with the understanding that the greatest absorption capacity for glucose is the mid-jejunum for growing steers (Krehbiel et al., 1996; Harmon and McLeod, 2001). Although adequate data are lacking to compare GLUT2 and GLUT5 protein and activity distribution patterns with that found for mRNA, Na$^+$-dependent glucose uptake activity (presumably SGLT1) in BBM vesicles was found to be intermediate in the duodenum, greatest in the jejunum, and least in the ileum of mature steers fed a fescue hay-based diet (Bauer et al., 2001b).

#### Starch Hydrolysate-Induced Regulation of SugT mRNA Content

The second objective of this study was to evaluate the effects of ruminal and abomasal SH infusion on the expression of SugT mRNA by small intestinal epithelium of growing beef steers. Ruminal supplementation of high-quality forage diets with energy concentrates (such as corn starch or SH) can increase the production of ruminal microbes, and hence the delivery of microbial-derived carbohydrates to the small intestinal lumen, whereas abomasal infusion of SH increases the supply of glucose to the small intestinal lumen (Taniguchi et al., 1995; Walker and Harmon, 1995; Huntington, 1997; Elizalde et al., 1999).

For several mammalian species, increasing dietary carbohydrate supply to the lumen of the small intestine induces SugT gene expression. The finding that GLUT5 mRNA content was insensitive to either treatment is consistent with findings from rat GLUT5 regulatory studies, which revealed that dietary glucose concentration has no effect on GLUT5 expression, whereas diets with increased in fructose stimulate GLUT5 mRNA, protein, and transport activity expression (Burant and Saxena, 1994; Shu et al., 1997; Jiang and Ferraris, 2001).

In forage-fed cattle, the luminal glucose (and other monosaccharides) concentration is reduced due to ruminal microbial fermentation, whereas the small intestinal epithelia of high-concentrate-fed dairy and beef cattle are challenged with considerable amounts of glucose (Harmon, 1992; Harmon and McLeod, 2001). For nonruminants such as rats and mice, it is known that
the mRNA abundance and protein activity of SGLT1 can be upregulated by increased dietary glucose supply (Ferraris and Diamond, 1997; Ferraris, 2001). For sheep, increasing the supply of glucose into the small intestinal lumen also results in upregulated SGLT1 mRNA content, an event that is accompanied by increased SGLT1 protein and activity (Shirazi-Beechey et al., 1991, 1992). In cattle, however, substrate regulation of steady-state SugT mRNA content has not been reported. Therefore, an important finding of this study was that GLUT2 (6.5-fold) and SGLT1 (1.25-fold) mRNA content was upregulated in response to abomasal SH infusion (hence, increased substrate supply). Interestingly, only the ileal epithelium responded to the increased luminal substrate challenge. If the regulatory mechanisms responsible for increasing SGLT1 and GLUT2 expression by ileal epithelia are also present in duodenal and jejunal epithelia, then substrate threshold response levels or substrate sensing mechanisms for these genes may differ (Dyer et al., 2007; Kellett et al., 2008).

Unexpectedly, even though increasing the ruminal supply of SH would result in an increased supply of microbial simple and complex carbohydrates to the small intestine, SGLT1 mRNA content of duodenal epithelia was increased by 64%. However, a concomitant increase in GLUT2 expression of only 40% was not statistically significant. Again, the regulatory mechanisms responsible for increasing SGLT1 expression by duodenal epithelia may differ from those present in jejuna and ileal epithelia.

If the observed increased SGLT1 and GLUT2 mRNA is positively associated with functional activity, then these results indicate that growing steers have the ability to upregulate transepithelial glucose absorption capacity in response to increased luminal substrate challenge. However, data describing alteration of SGLT1 activity in response to altered glucose supply to the small intestinal lumen of cattle appears to be too complex to draw such a definitive conclusion. Specifically, the jejunal SGLT1 activity in mature beef steers is increased in response to the increased small intestinal luminal supply of glucose derived from the abomasally infused SH, although responses in other regions of the small intestine were not measured (Bauer et al., 2001a). Other studies, however, observed no upregulation of SGLT1 activity (Bauer et al., 2001b; Rodriguez et al., 2004; Guimaraes et al., 2007) or SGLT1 protein abundance in growing beef steers (Rodriguez et al., 2004) and Holstein beef calves (Guimaraes et al., 2007) in response to increased glucose supply to the small intestinal lumen. In addition, the protein abundance of GLUT2 was not affected by the increased luminal supply of glucose to beef small intestine (Rodriguez et al., 2004; Guimaraes et al., 2007), whereas the presence of GLUT2-like activity in BBM vesicles was not evaluated.

This study has demonstrated the unique finding that mRNA encoding 3 mammalian SugT proteins (Glut5, Glut2, Sglt1) currently thought to be responsible for monosaccharide absorption from the small intestinal lumen (Harmon and Taylor, 2005) are expressed in greatest abundance in the jejunal region. This understanding appears to be consistent with the limited reports identifying the jejunum as the principle site for glucose uptake in forage-fed steers. This study also has uniquely found that increasing the small intestinal luminal supply of glucose markedly stimulates upregulation of SGLT1 and GLUT2 gene expression by the ileal epithelia. Assuming that the glucose supply-stimulated increase in SGLT1 and GLUT2 mRNA content represents a parallel increase in glucose transport activity, as it does in sheep, then these results indicate that growing forage-fed beef cattle have the capacity to adapt to increase their carbohydrate assimilation. However, additional studies that define the adaptive carbohydrate assimilation response to graded levels of luminal carbohydrate challenge are needed before the understandings from the current study can be used in diet formulation.

**LITERATURE CITED**


