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Short communication: Glucagon-like peptide-2 and coccidiosis alter tight junction gene expression in the gastrointestinal tract of dairy calves[1]

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ABSTRACT

Tight junction (TJ) proteins are integral factors involved in gut barrier function, and therapy with glucagon-like peptide-2 (GLP-2) enhances gut integrity. Our aim was to assess effects of GLP-2 treatment on mRNA expression of 8 TJ complex proteins in the intestine of dairy calves not infected or infected with *Eimeria bovis* at 11 ± 3 d of age. Mucosal epithelium from jejunum, ileum, and cecum was collected at slaughter from Holstein bull calves assigned to 4 groups: noninfected, buffer-treated (n = 5); noninfected, GLP-2 treated (n = 4); *E. bovis*-infected, buffer-treated (n = 5); and *E. bovis*-infected, GLP-2-treated (n = 4). Infected calves were orally dosed with 100,000 to 200,000 sporulated *E. bovis* oocysts on d 0; GLP-2-treated calves received 50 μg of GLP-2/kg of body weight subcutaneously twice daily for 10 d beginning on d 18; and buffer-treated calves received an equal injection volume of 0.01 *M* Na bicarbonate buffer. All calves were killed on d 28. The mRNA expression of coxsackie and adenovirus receptor (*CXADR*), claudins 1, 2, and 4 (*CLDN1*, *CLDN2*, and *CLDN4*), F11 receptor (*F11R*), junction adhesion molecule 2 (*JAM2*), occludin (*OCLN*), and tight junction protein ZO-1 (*TJP1*) was determined by real-time quantitative PCR. In jejunum and ileum, an interaction of *E. bovis* infection and GLP-2 treatment on gene expression was noted. In jejunum of noninfected calves, GLP-2 increased *CXADR*, *CLDN2*, *OCLN*, and *TJP1* mRNA expression but had no effect on mRNA expression in infected calves. Treatment with GLP-2 also increased tight junction protein ZO-1 protein expression in jejunum of noninfected calves as determined by immunohistochemistry. In ileum, *E. bovis* decreased expression of *JAM2*, *OCLN*, and *TJP1* in buffer-treated calves, and GLP-2 increased *TJP1* expression in infected calves. In cecum, *E. bovis* infection reduced expression of *CXADR*, *CLDN4*, *F11R*, and *OCLN*, and GLP-2 therapy increased expression of *CLDN4*, *F11R*, *OCLN*, and *TJP1*. Results are consistent with studies in nonruminants showing decreased expression of TJ complex proteins in the intestinal tract during pathogen-induced diarrhea and increased TJ protein expression in intestinal tissues in response to GLP-2 treatment. In conclusion, *E. bovis* reduces gene expression of TJ proteins primarily in cecum of calves 28 d postinfection, and GLP-2 increases expression of selected TJ genes in intestinal tissues. Use of GLP-2 to improve gut barrier function in ruminants during pathogen-induced diarrhea warrants additional study.
Key words: coccidiosis, dairy calf, gene expression, tight junction protein

Short Communication

Acute diarrhea due to intestinal pathogens is a common cause of calf mortality (Uetake, 2013). Furthermore, diarrhea in early life of dairy heifers reduces milk production in the first lactation (Heinrichs and Heinrichs, 2011). Hence, benefits to calf and milk production may be achieved by enhancing intestinal health and stabilizing gut integrity and barrier function of young dairy calves. The intestinotrophic hormone, glucagon-like peptide 2 (**GLP-2**), is reported to increase tight junction (**TJ**) protein expression and enhance intestinal barrier function in cultured human intestinal cells (Moran et al., 2012) and to increase mRNA and protein expression of TJ proteins in the jejunum of pigs (Yu et al., 2014). Recent studies suggest the potential for GLP-2 therapies to benefit gut development and health in cattle (Connor et al., 2010, 2013; Taylor-Edwards et al., 2010, 2011). However, the effects of GLP-2 on intestinal epithelial barrier function of ruminants have not been investigated.

The protozoan *Eimeria bovis* is a common pathogen of young calves and causes severe diarrhea and tissue damage within the distal ileum, cecum, and colon during its life cycle within its host (Friend and Stockdale, 1980). *Eimeria bovis* infection impairs intestinal integrity (Friend and Stockdale, 1980) and, thus, could serve as an experimental model to examine the capac-

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ity for GLP-2 treatment to restore barrier function in vivo. Intestinal barrier function is regulated primarily through the action of the TJ complex, which includes transmembrane proteins and TJ-associated cytoplasmic members that control selective flux of large macromolecules, small molecules, and ions (Liang and Weber, 2014). Claudin proteins are responsible for flux of small molecules and ions through pores in the TJ (Van Itallie et al., 2008), whereas the leak of macromolucules is attributed to tight junction protein ZO-1, occludin members, and other associated proteins (Van Itallie et al., 2009). Thus, our aim was to assess the effects of *E. bovis* infection and GLP-2 therapy on gene expression of select proteins of the TJ complex in the small intestine and cecum of calves. Our hypotheses were that expression of TJ complex proteins, particularly in ileocecum, is reduced by *E. bovis* infection, and that GLP-2 treatment increases TJ gene expression and ameliorates reductions in expression of TJ complex proteins of *E.bovis*-infected calves.

To test our hypotheses, mRNA and protein expression of selected TJ complex proteins was examined in intestinal tissues from a previous study by Connor et al. (2013). Briefly, calves were assigned to 4 groups including, noninfected calves injected (s.c.) twice daily for 10 d with sodium bicarbonate buffer (n = 5), or GLP-2 (50 μg/kg of BW; n = 4), and *E. bovis*-infected calves injected (s.c.) twice daily for 10 d with sodium bicarbonate buffer (n = 5) or GLP-2 (50 μg/kg of BW; n = 4). Calves in the infected groups were orally infected with 100,000 to 200,000 sporulated *E. bovis* oocysts on study d 0, GLP-2 or buffer injections began on study d 18, and calves were killed on d 28 for tissue collection. Mean (±SD) calf age was 11 (±3) d on study d 0.

Mucosa from jejunum, ileum, and cecum was collected and stabilized in RNAlater (Life Technologies, Grand Island, NY) according to the manufacturer's instructions. Samples were stored at −80°C until RNA extraction and evaluation of mRNA expression for coxsackie adenovirus receptor (*CXADR*), *cl*audins 1, 2, and 4 (*CLDN1*, *CLDN2*, and *CLDN4*), F11 receptor (*F11R*; also known as *JAMA*), junctional adhesion molecule 2 (*JAM2*), occludin (*OCLN*), and TJ protein ZO-1 (*TJP1*) by real-time quantitative PCR. Whole tissue from jejunum of noninfected calves also was formalin fixed and stored in 70% ethanol at 4°C until sectioned for immunohistochemical analysis of TJ protein ZO-1 and occludin.

Total RNA was extracted and its integrity and quality were assessed as previously described (Connor et al., 2010). All samples had an RNA integrity number of 7.0 or greater. Reverse transcription was performed with the iScript cDNA Synthesis Kit (Bio-Rad Laboratories, Hercules, CA) using 1.0 μg of total RNA, according to the manufacturer's instructions. Real-time quantitative PCR was performed in triplicate 20-μL reactions using the iCycler iQ, SsoAdvanced Universal SYBR Green Super Mix (Bio-Rad Laboratories), and a concentration of 400 n*M* for each primer. Cycling conditions were 95°C for 3 min, followed by 45 cycles of 63°C for 30 s, 70°C for 30 s, and 95°C for 30 s. Primer sequences (Table 1) were designed using primer-BLAST (www.ncbi.nlm.nih.gov/tools/primer-blast/) except those for *OCLN*, which were based on Malmuthuge et al. (2013). Primer specificity was validated by gel purification (Qiagen, Valencia, CA) and DNA sequencing (CEQ 8000 GeXP genetic analysis system, Beckman Coulter Inc., Brea, CA) of amplicons from a standard end-point PCR reaction. Quantification of gene copy numbers in samples was based on interpolation from a standard curve analyzed simultaneously with samples as described by Connor et al. (2005). Assay amplification efficiencies ranged from 87 to 115%, and linearity (r^2) of standard curves ranged from 0.995 to 1.000. Comparisons of least squares means of gene expression level were made using the GLM procedure of SAS (SAS Institute Inc., Cary, NC). The model included the fixed effects of treatment (buffer vs. GLP-2), infection status (noninfected vs. infected with *E. bovis*), and the interaction of treatment and infection status. Further pairwise comparisons of least squares means within treatment and infection were made using the PDIFF option of SAS.

Antibodies used for immunohistochemical analysis of jejunal sections were rabbit anti-occludin (cat. no. 71–

Table 1. Primer sequences and annealing temperatures used for real-time quantitative PCR

Gene symbol	Gene name	Sense primer (5′ → 3′)	Antisense primer (5′ → 3′)
CXADR	Coxsackie and adenovirus receptor	TCCGACTCACAGAAACTGCC	CCGTACAGGTGTATGTCCCG
CLDN1	Claudin 1	AAGACGACGAGGCACAGAAG	CAGCCCAGCCAATGAAGAGA
CLDN2	Claudin 2	CCAGGCCATGATGGTGACAT	GAAGAAGACTCCGCCCACAA
CLDN4	Claudin 4	GTGCCCTCATCGTCATCTGT	TTGTTAGCCGTCCAGGACAC
F11R	F11 receptor	TCAGAGAGAGATGGCTCCCC	TCCAGTATCAGAGGCCGACA
JAM2	Junction adhesion molecule 2	CCCCATCGGAACAAGGTCAA	GACATCGCAGCTCTACCACA
OCLN	Occludin	ACGCAGGAAGTGCCTTTGGTAGC	GCAGCCATGGCCAGCAGGAA
TJP1	Tight junction protein ZO-1	AATGCATCCTGACCACCAGG	GATGGTGCCGGGTTTGTTTC

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