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Research Article

Brief Research Report: Presence and Quantity of Glucagon-Like Peptide-2 Receptor (GLP-2R) mRNA in Bovine Rumen Epithelial and Gastrointestinal Tissue

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Abstract

The hormone Glucagon-like peptide-2 (GLP-2), acting through the Glucagon-like peptide-2 receptor (GLP-2R), is a potent stimulator of small intestine epithelial tissue proliferation. Expression of mRNA for GLP-2R has been detected in tissues of the stomach, small and large intestine, and the central nervous system of monogastric species, but investigations with ruminant tissues are lacking. The current study aimed to determine receptor presence in various bovine tissues. Four steers were used. Immediately after slaughter, tissue samples were obtained from the rumen ventral sac and dorsal sac, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas. Real-time quantitative PCR was used to determine the presence and quantity of GLP-2R mRNA in selected tissues, and the results confirm the presence of GLP-2R in the rumen ventral sac epithelium, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas of the bovine. However, there were no statistical differences among tissues in mRNA expression. Furthermore, Western blot analysis revealed the tissue with the greatest density of GLP-2R to be the epithelium of the rumen ventral sac. Thus, the mRNA for GLP-2R was present in the bovine tissues sampled, and the presence of the GLP-2 receptor in the rumen ventral sac was clear in our investigations with an indication of receptor present in the duodenum. Improved health of the rumen epithelium and intestinal mucosa could potentially improve animal performance and reduce morbidity during the transition period. Additional research regarding transition diets, GLP-2 secretion, and GLP-2R is essential to clarify the importance of this mechanism may have in promoting uneventful transition periods.

Keywords: Cattle; Hormone; Health; Performance; Western Blot; RT q-PCR

Introduction

Glucagon-like peptide-2 receptor (GLP-2R) is a 550-amino acid trans-membrane G protein-coupled receptor that mediates the response of the 33-amino acid hormone, such as glucagon-like peptide-2 (GLP-2) [14]. Expression of mRNA for GLP-2R has been detected in tissues of the stomach, small and large intestine, and the central nervous system of monogastric species such as rats, mice, humans, and pigs [17,20,26].

The hormone GLP-2, acting through the receptor GLP-2R, is a potent stimulator of small intestine epithelial tissue proliferation

as histological findings upon GLP-2 administration demonstrate cell proliferation in crypts leading to elongation of the villous epithelium [16] and increased microvilli length [3,4,7,18]. Additionally, responses to GLP-2 include increases in mucosal surface area [14,25], reduction in injury [5], restored mucosal integrity [22], and enhanced intestinal absorptive function [1,6,9,11].

Although much of this knowledge stems from studies in monogastrics, expression of GLP-2R has also been detected in tissues of ruminants [12,24], suggesting that this receptor may play a broader role in gastrointestinal adaptation. A study [19] investigated the effects of including cane molasses in the diets of dairy cows during the transition period. Their findings revealed that while cane molasses improved dry matter intake (DMI) both prepartum and postpartum, this increase in intake was not associated with higher ruminal butyrate concentrations or valerate absorption rates. Interestingly, cows that consumed molasses diets had smaller ruminal papillae, indicating that increased absorptive surface area may not be the sole determinant of volatile fatty acid (VFA) absorption. Butyrate has been linked to histone acetylation and gene regulation in gastrointestinal tissue, and in monogastric animal it enhances GLP-2 secretion [2]. These results challenged the prevailing assumption that greater rumen papillae size equates to enhanced absorption and highlighted the need to explore alternative mechanisms influencing epithelial development—particularly the potential role of GLP-2R in mediating these adaptations.

Therefore, the objective of the present study was to characterize the presence and distribution of GLP-2R across ruminant gastrointestinal tract. By identifying the anatomical distribution of this receptor, we aim to build a foundational understanding of whether GLP-2R could be involved in ruminal epithelial tissue development. We hypothesized that GLP-2R is present in bovine rumen tissue epithelium and may serve as a molecular target influenced by diet-derived metabolites such as butyrate. Understanding the spatial expression of this receptor in bovine gastrointestinal tissues could provide critical insights into nutrient-driven epithelial development and support strategies aimed at improving absorptive capacity and rumen health in dairy cattle.

Materials and Methods

All procedures were approved by the Kansas State University Institutional Animal Care and Use Committee.

Methods used in this study were based on four referenced studies [3,5,6,19].

Tissue samples

Four steers were used to evaluate the receptor presence in bovine rumen epithelial and gastrointestinal tissue. The animals were housed at the Kansas State University Dairy Teaching and Research Center (Manhattan, KS).

Samples from 4 steers were obtained from local abattoirs in Clay Center, Manhattan, KS. Immediately after slaughter, tissue samples from each animal were obtained from the rumen ventral sac and dorsal sac, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas. Harvested tissues were rinsed in 0.90% saline solution, then frozen by submerging in liquid N2 and stored at -80º C. Samples from all four steers were subjected to RT-PCR and Western blot analyses.

Protein extraction and western blot analysis

Total protein extraction from tissues was performed in the laboratory by homogenizing approximately 500 mg of sample for 30 s in 2.5 mL M-PER (78501; Pierce Biotechnology, Rockford, IL), containing 8.8 (wt/vol) g/L NaCl. Homogenate was then centrifuged at 10,000 x g for 15 min to pellet debris. Aliquots of supernatant were transferred to 2 tubes, flash-frozen in liquid nitrogen, and stored at -80ºC until further analysis. Concentration of protein was later determined at a wavelength of 260/280 nm using an ND-1000 Spectrophotometer (Nano-Drop Technologies, Wilmington, DE, USA).

Sixty micrograms of total protein were separated by gel electrophoresis using SDS-PAGE with precast Pierce Precise Protein 4-20% Gradient Gels (Pierce Biotechnology, Rockford, IL), and separated proteins were transferred onto a nitrocellulose membrane using a Trans-Blot Semi-Dry Electrophoretic Transfer system (Bio-Rad, Hercules, CA). Nitrocellulose membranes were blocked using a blocking buffer (Starting Block Buffer, Pierce Biotechnology, Rockford, IL, USA) for 15 min at 37ºC. Polyclonal rabbit primary antibody against GLP-2R (LS-A1312; Lifespan Biosciences, Seattle, WA, USA) in 5 ml blocking buffer was added to the membrane and incubated overnight at 4ºC. The nitrocellulose membrane was then washed 3x for 15 min each with phosphate buffered saline-Tween 20 (PBS-Tween). A secondary antibody, goat anti-rabbit-HRP (sc-2004; Santa Cruz Biotechnology, Inc.,

Santa Cruz, CA, USA), was added in 1 mL of blocking buffer to the membrane and incubated for 1 h. Detection of GLP-2R was completed using chemiluminescence and a Fluorchem 8800 Imaging System (AlphaInnotech, San Leandro, CA, USA).

Sample preparation and RNA isolation

Total RNA was isolated from 100 mg of tissue samples using sterile steel mortar bowls cooled by liquid N2. Tissue samples were homogenized using a sterile pestle in liquid N2. Then, 3 mL TRI Reagent (Sigma, St. Louis, MO) was added to the ground tissue sample, and 1 mL of tissue in TRI Reagent was incubated at room temperature for 5 min. Following incubation, chloroform (Sigma, St. Louis, MO) was added, and samples were centrifuged for 15 min at 12,000 x g at room temperature. Following centrifugation, the top layer was removed and transferred to a new microcentrifuge tube. Isopropanol (Sigma, St. Louis, MO) was added, and samples were centrifuged for 10 min at 12,000 x g to isolate the RNA pellet. The RNA pellet was then treated to remove any contaminating genomic DNA using the DNA-free kit (Ambion, Austin, TX). The RNA concentration was determined by absorbance at 260 nm and the integrity of RNA was determined by gel electrophoresis. Total RNA with ethidium bromide was loaded onto a 1% agarose gel to separate and visualize 28S and 18S rRNA bands.

Real-time quantitative-PCR

Real-time quantitative PCR was used to measure the quantity of glucagon-like peptide 2 receptor (GLP-2R) gene expression relative to the quantity of 18S ribosomal RNA (rRNA) in total RNA isolated from tissue. Measurement of the relative quantity of cDNA was performed using TaqMan Universal PCR Master Mix (Applied Biosystems, Foster City, CA), 900 nM of the appropriate forward and reverse primers, 200 nM of appropriate TaqMan detection probe, and 1 µL of the cDNA mixture. Bovine-specific GLP-2R forward (GTGAGACAGAGTGGCTGTCCTATG) and reverse (TGCCCACAAAGTAGTGCAAGC) primers and TaqMan (6FAM-TTGCTGCCTCCTGCCGCTCA-TAMRA) detection probes were synthesized using published GenBank sequences. Commercially available eukaryotic 18S rRNA primers and probes were used as an endogenous control (Applied Biosystems; Genbank Accession #X03205). The ABI Prism 7000 detection system (Applied Biosystems, Foster City, CA) was used to perform the assay utilizing the recommended thermal cycling variables by the manufacturer (50 cycles of 15 s at 95ºC and 1 min at 60ºC). The 18S rRNA endogenous control was used to normalize the expression of GLP-2R.

Results

Quantitative RT-PCR was performed to determine the presence and quantity of GLP-2R mRNA in selected tissues of the bovine. Our results confirm the presence of GLP-2R in the rumen ventral sac epithelium, omasum, abomasum, duodenum, jejunum, ileum, large intestine, and pancreas of the bovine. Statistical differences were not detected among tissues in mRNA expression. Quantitative distribution of GLP-2R mRNA results from RT-PCR for tissue samples obtained from steers A and B are provided in Figure 1 and from steers C and D in Figure 2. The mRNA for GLP-2R was present in all tissues sampled and appears to be numerically greater in the small and large intestines (Figure 1) for steer B and in the small intestine of steer D (Figure 2). For steers C and D, GLP-2R mRNA was numerically more concentrated in the omasum than in the abomasum and rumen ventral sac (Figure 2).

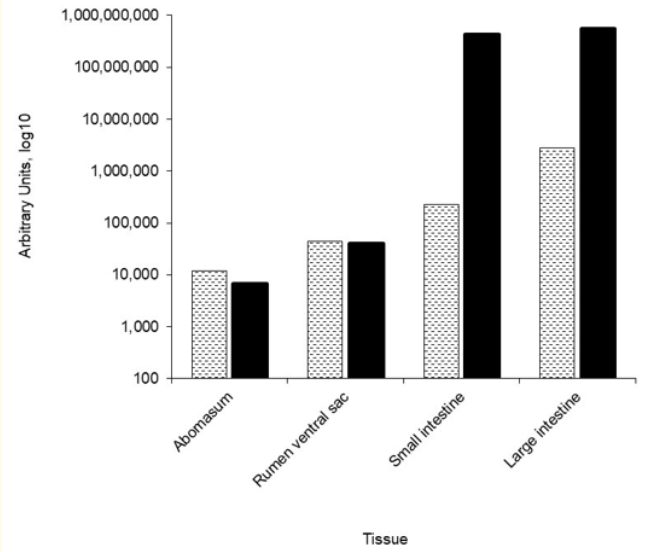

Figure 1: Glucagon-like peptide-2 receptor mRNA expression in tissues from steer A (patterned bars) and steer B (solid bars) (Pooled SEM = 1.44x108, n=2).

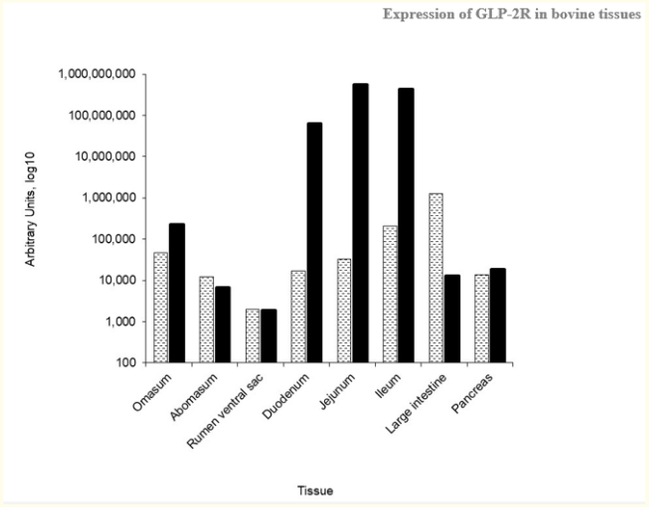

Figure 2: Glucagon-like peptide-2 receptor mRNA expression in tissues from steer C (patterned bars) and steer D (solid bars) (Pooled SEM = 747296340, n=2).

Western blot analysis was conducted to investigate the presence of the protein GLP-2R. Western blot analysis was not as conclusive as the RT-PCR results, nonetheless, it revealed the tissue with the greatest density of GLP-2R to be the epithelium of the rumen ventral sac (Figures 3 and 4). Other tissues were elusive in providing definite results using Western blot analysis with only the duodenum of steer D expressing a signal at 76kDa, indicative of the GLP-2 receptor.

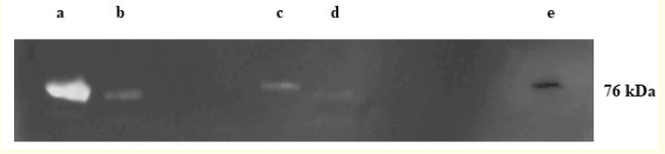

Figure 3: Representative Western blot for steer D from Manhattan, KS; (a) Steer D, Rumen Ventral Sac; (b) Duodenum; (c) Cow, Rumen Ventral Sac; (d) Cow, Rumen Ventral Sac; (e) Marker.

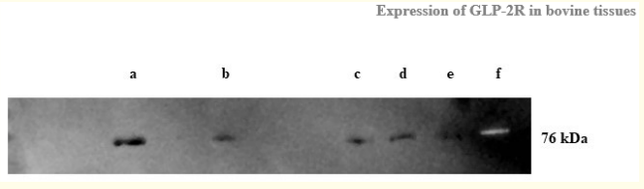

Figure 4: Representative Western blot for steer A from Clay Center, KS. (a) Steer A, Rumen Ventral Sac; (b) Cow, Rumen Ventral Sac; (c) Cow, Rumen Ventral Sac; (d) Cow, Rumen Ventral Sac; (e) Cow, Rumen Ventral Sac; (f) Marker.

Discussion

The present study was part of a broader investigation evaluating the effects of dietary supplementation with cane molasses aimed at stimulating ruminal butyrate production, thereby promoting rumen epithelial development. Additionally, this study explored a potential mechanism by which glucagon-like peptide-2 (GLP-2) and its receptor (GLP-2R) might influence epithelial growth. Although the feeding trials reported limited morphological changes in the rumen epithelium [19], the hypothesis emerged that GLP-2R might be part of the molecular signaling pathways responsible for epithelial adaptation.

Our results using RT-PCR indicate the presence of mRNA for GLP-2R in all bovine gastrointestinal tissues examined, including the rumen ventral sac, omasum, abomasum, and small and large intestines, as well as the pancreas. These findings are in agreement with previous reports in monogastric species, where GLP-2R is primarily expressed in the intestinal mucosa, particularly the small intestine [20,26]. Tissue samples from the duodenum, ileum, colon, and stomach of the rat were followed in order regarding the concentration of mRNA for GLP-2R [20]. Although some variation exists between steers in our results for mRNA concentration in sampled tissues, similarities exist between rat tissues and the sampled bovine tissues.

Importantly, the variation in mRNA levels among steers, despite standardized tissue collection, may reflect biological variability, as well as factors such as age, nutritional state, and microbial environment. The concentration of mRNA for GLP-2R can vary with age as abundance was highest in piglets at birth and significantly less for weaned piglets [21]. Similarly, nutrient intake has been shown to modulate GLP-2R expression, with enteral feeding reducing GLP-2R mRNA levels in piglets [8,21], possibly due to a feedback mechanism from luminal GLP-2 release. In ruminants, data on GLP-2/GLP-2R dynamics are scarce. However, given that short-chain fatty acids (SCFAs)—especially butyrate—can stimulate GLP-2 secretion in monogastrics [2], it is plausible that fermentation end-products in the rumen may also affect receptor expression or sensitivity. This potential interaction underscores the need for further studies examining nutrient–receptor signaling in the rumen.

Regarding Western blot analysis, detection of the GLP-2 receptor was most successful in the rumen ventral sac epithelium, while signals in other tissues were inconsistent. GLP-2R is a low-abundance receptor protein, and its detection is often technically challenging [15,27]. Incomplete glycosylation or proteolytic processing could also lead to reduced antibody binding affinity or

altered molecular weight detection [20]. Also, expression of GLP-2R at or near 76 kDa is common for the receptor in a glycosylated form [17]. Lack of signal for tissues other than the rumen ventral sac may be related to the total receptor present in the tissue. Moreover, similar difficulties in detecting GLP-2R protein have been documented in neonatal pig models, even when using the same antibodies [10]. This supports the notion that receptor abundance varies considerably by tissue and physiological context, and that mRNA expression does not always correlate with protein levels due to post-transcriptional regulation, translation efficiency, or protein turnover [13].

The detection of GLP-2R in the rumen epithelium is particularly intriguing. Although the rumen is not traditionally associated with enteroendocrine hormone action, its stratified squamous epithelium is capable of significant structural and functional adaptation in response to dietary changes [23]. The presence of GLP-2R suggests a possible role for this receptor in ruminal epithelial remodeling, potentially influencing cell proliferation, barrier integrity, or nutrient sensing. While GLP-2 has not been directly linked to the rumen in vivo, its known actions in other gut segments—such as stimulating crypt cell mitosis and inhibiting apoptosis—support a rationale for investigating its function in this context [3,25].

Conclusions

The mRNA for GLP-2R was present in the bovine tissues sampled. The GLP-2R protein was present in the rumen ventral sac, but GLP-2R protein in other tissues could not be definitively verified. It can be postulated that GLP-2 plays a role in the development of rumen epithelium and tissues of the lower gastrointestinal tract mediated through its receptor GLP-2R. Improved health of the rumen epithelium and intestinal mucosa could potentially improve animal performance and reduce morbidity during the transition period. These results provide a basis for future studies evaluating the functional role of GLP-2 signaling in ruminants and suggest that GLP-2R could be an important regulator of epithelial adaptation beyond its established role in the small intestine. Moreover, additional research regarding transition cow diets, GLP-2 secretion, and GLP-2R is essential to clarify the importance this mechanism may have in promoting uneventful transition periods.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

BJJ, WFM, and DDM contributed to the conceptualization and design of the study. WFM collected and organized the data. WFM and JMS analyzed the data. WFM wrote the original draft. All authors contributed to the interpretation of the results, and manuscript revision, and approved the submitted version.

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