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Secretion of glucagon-like peptide-2 responds to nutrient intake but not glucose provision in milk-fed calves

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ABSTRACT

Glucagon-like peptide 2 (GLP-2) is a peptide released by the lower gut that has potent trophic and restorative effects on the intestinal epithelium. Two experiments were conducted to assess the effects of feeding rate and either metabolizable or nonmetabolizable glucose supplementation on GLP-2 concentrations in plasma and intestinal development in Holstein calves. In the first experiment, 48 newborn calves were assigned to 12 treatments (n = 4) corresponding to the factorial combination of 4 milk feeding amounts [1.75, 1.32, 0.88, and 0.44% of body weight (BW) as dry matter (DM)] and 3 oral supplementation treatments (nonsupplemented, glucose-supplemented, and 3-O-methyl glucose-supplemented). In the second experiment 30 newborn calves (n = 10) were fed milk at a fixed rate of 1.75% of BW as DM and assigned to the same glucose supplementation treatments used in experiment 1 to investigate effects on intestinal development. In the first experiment, we found a saturating response of plasma GLP-2 to increasing feeding levels. The feeding rate at which 50% of the maximal GLP-2 release occurred was estimated to be 0.53% of BW as DM or 30.3% of the maximum feeding rate (1.75% of BW as DM), whereas maximal secretion was estimated to be about 98.6 pmol/L. In turn, feeding 75, 50, or 25% of the maximal feeding rate (i.e., 1.75% BW as DM) resulted in plasma GLP-2 concentrations 87, 72, and 49% of that in fully fed calves, respectively. Neither metabolizable nor nonmetabolizable glucose supplementation affected GLP-2 secretion and no interaction with feed intake level was detected. In the second experiment, no effect of glucose supplementation was observed on intestinal growth, mucosal cell proliferation, or expression of genes related to the actions of GLP-2. Nonetheless, we observed that a pool of genes of the GLP-2 signaling pathway was more abundantly and coordinately regulated in the colon than in the ileum of these animals, indicating an opportunity for dietary induction of the peptide in this organ. In conclusion, during this experiment, plasma GLP-2 concentrations responded in a diminishing return fashion to milk intake but not to glucose supplementation, even at milk consumption levels of only 0.4% of BW as DM.

Key words: glucagon-like peptide 2 (GLP-2), intestine, milk feeding

INTRODUCTION

Glucagon-like peptide 2 (**GLP-2**) is an intestinally secreted peptide with trophic and regenerative properties for the intestinal epithelium (Burrin et al., 2000). Genes involved in the GLP-2 pathway are expressed along the gastrointestinal tract of ruminants (Connor et al., 2010), and the GLP-2 receptor appears to be fully operative as greater epithelial development and blood flow have been recorded in response to therapeutic application of exogenous GLP-2 in ruminants (Taylor-Edwards et al., 2011; Connor et al., 2013). This peptide is known to induce proliferation of crypt cells, reduce mucosal apoptosis, decrease intestinal barrier permeability, and dampen inflammation in the gut (Drucker et al., 1996; Burrin et al., 2003; Drucker, 2005). These properties make it a potential target to maintain or enhance intestinal integrity in newborn milk-fed calves.

Secretion of GLP-2 is largely driven by luminal delivery of nutrients (Burrin et al., 2000), and its secretion has been augmented in response to greater energy intake in steers (Taylor-Edwards et al., 2010). In addition to whole-feed intake, specific nutrients such as glucose might stimulate GLP-2 (Brubaker and Anini, 2003; Shirazi-Beechey et al., 2011). Infusing the duodenum of weaned sheep with glucose for 4 d increased its rate of transport 40- to 80-fold above that in control animals (Shirazi-Beechey et al., 1991), which may have been caused by an increase in GLP-2 secretion elicited

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through activation of the sweet taste receptor located in the gut (Shirazi-Beechey et al., 2011).

Whereas a positive effect of increased energy intake and glucose infusion on GLP-2 secretion and intestinal function has been observed in ruminants (Shirazi-Beechey et al., 1991; Taylor-Edwards et al., 2010), no information is available on such responses in newborn milk-fed calves. However, knowledge regarding the effect of milk intake on endogenous GLP-2 secretion could be useful to identify when calves under usual farming circumstances (e.g., diarrhea episodes, restricted milk feeding) can benefit from exogenous GLP-2 administration or application of certain feed additive strategies with therapeutic potential that could be easier to implement under commercial schemes of animal production (Connor et al., 2015; de Diego-Cabero et al., 2015). Understanding the GLP-2 response to the enteral supply of nutrients in milk-fed calves, however, precludes the determination of the milk intake range in which GLP-2 secretion could be stimulated by nonpharmacological means (i.e., dietary interventions). Furthermore, addressing whether glucose supplementation can affect circulating GLP-2 concentrations when calves are fed variable amounts of milk will be useful to assess its potential to support intestinal integrity. Finally, given the absence of data in calves, this information will also be relevant to consider effect sizes, inter-calf variability, and adequate sample sizes in future research on GLP-2 with newborn calves where variation in food intake is to be considered.

The specific set of hypotheses tested in this work were that (1) feeding rate affects GLP-2 secretion in young calves; (2) the effect of metabolizable and nonmetabolizable glucose (i.e., 3-O-methyl glucose, **3-O-M-G**) on GLP-2 secretion depends on the feed intake level; and (3) supplementation with glucose or a nonmetabolizable glucose analog can effect organ size, epithelial morphometric dimensions, and proliferation compared with nonsupplemented calves and that this effect may differ between the small and large intestine. The aim of this work was to characterize the response of GLP-2 secretion to a range of feed intake amounts resembling the anorexic variation between intestinally healthy and diarrhea-ill calves, and to test the effect of metabolizable and nonmetabolizable glucose supplementation on GLP-2 secretion and intestinal development measures.

MATERIALS AND METHODS

Two experiments were conducted. In the first, we assessed the effect of feeding rate, glucose or 3-O-M-G supplementation, and their interaction on plasma GLP-2 concentrations. In the second experiment, we investigated the effect of glucose or 3-O-M-G supplementation on epithelial cell proliferation, histological measures, and mRNA expression of genes related to the GLP-2 signaling pathway and intestinal development and function.

Experiment 1

All procedures were conducted with approval of the University of Illinois Institutional Animal Care and Use Committee (Urbana, IL). Forty-eight newborn male Holstein calves, 4 to 5 d old, were purchased and transported to the University of Illinois Nutrition Field Laboratory research site. Calves were selected at a local farm in east-central Illinois. At time of selection, blood samples were taken from the jugular vein into a 10-mL evacuated serum separation tube (Becton Dickinson, Rutherford, NJ). Blood was centrifuged at 1,300 × *g* for 15 min, and a refractometer was used to determine total protein in the serum for all potential calves. Calves were selected based on total protein and visual health assessment and were identified with ear tags. All calves selected were given 2 mg of Se plus 100 mg of vitamin E (BO-SE, Merck Animal Health, Kenilworth, NJ), 1 mL of vitamins A and D (Sparhawk Laboratories Inc., Lenexa, KS), and 2 mL of a nasal vaccine against bovine respiratory syncytial virus (Inforce 3, Pfizer, New York, NY). If a calf was chosen for the trial and had a total protein of <5.5 g/dL, the calf was given 50 mL of antiserum (Bovisera, Colorado Serum Co., Denver, CO) and 20 mL of clostridial C and D antitoxin (Boehringer Ingelheim, Ridgefield, CT). Calves were housed in individual calf hutches with straw bedding to contain pathogen shedding and provide comfort, respectively. Upon arrival, calves were weighed. Complete blocks of calves were made based upon BW at arrival and, within each block, the 12 treatments were randomly assigned (n = 4). In both experiments, calves were fed a commercial milk replacer (Excelerate, Milk Specialties Global, Eden Prairie, MN), which contained, on average, 28.4% CP, 44% lactose, and 15.8% fat on a DM basis.

Treatment Allotment. Experiment 1 included 12 treatments from a factorial combination of 2 factors: feeding rate and supplement treatment. Feeding rate comprised 4 levels: 25, 50, 75, and 100% of a standard feeding level of 1.75% of BW as DM on experimental d 6 and 7. In turn, supplement treatments were (1) control (commercial milk replacer without supplementation), (2) commercial milk replacer plus glucose (220 mg/kg of BW daily), and (3) commercial milk replacer plus 3-O-M-G (6 mg/kg of BW daily).

Dose of glucose was based on a published study (Shirazi-Beechey et al., 1991) where activity of the sodium-glucose co-transporter 1 (SGLT1) in adult sheep