Glucagon-like peptide 2 and its beneficial effects on gut function and health in production animals

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Abstract

Numerous endocrine cell subtypes exist within the intestinal mucosa and produce peptides contributing to the regulation of critical physiological processes including appetite, energy metabolism, gut function, and gut health. The mechanisms of action and the extent of the physiological effects of these enteric peptides are only beginning to be uncovered. One peptide in particular, glucagon-like peptide 2 (GLP-2) produced by enteroendocrine L cells, has been fairly well characterized in rodent and swine models in terms of its ability to improve nutrient absorption and healing of the gut after injury. In fact, a long-acting form of GLP-2 recently has been approved for the management and treatment of human conditions like inflammatory bowel disease and short bowel syndrome. However, novel functions of GLP-2 within the gut continue to be demonstrated, including its beneficial effects on intestinal barrier function and reducing intestinal inflammation. As knowledge continues to grow about GLP-2's effects on the gut and its mechanisms of release, the potential to use GLP-2 to improve gut function and health of food animals becomes increasingly more apparent. Thus the purpose of this review is to summarize: 1) the current understanding of GLP-2's functions and mechanisms of action within the gut; 2) novel applications of GLP-2 (or stimulators of its release) to improve general health and production performance of food animals; and 3) recent findings, using dairy calves as a model, that suggest the therapeutic potential of GLP-2 to reduce the pathogenesis of intestinal protozoan infections.

Keywords: glucagon-like peptide 2; gut health; intestinal barrier function; protozoan infection

1. Introduction

In 1902, the first factor produced in the gut and released into the bloodstream, which affected insulin secretion, was described and named 'secretin' [1]. Since that time, numerous subtypes of endocrine cells within the intestinal mucosa and their peptide products have been characterized [2]. These enteric peptides have become the subject of intensive scientific investigation regarding regulation of their release [3-5], mechanisms of action [6-8], and diverse physiological effects, including those outside of the digestive system such as bone [9], liver [10], lungs [11], and the central nervous system [12-14]. This primarily was due to the discovery that enteric peptides, such as glucagon-like peptide-1 (GLP-1), gastric inhibitory peptide (GIP), and peptide YY (PYY), play key roles in the regulation of appetite, energy metabolism [15-17], and gut function [18-20], making them targets for managing prevalent human diseases of obesity and diabetes. As knowledge has continued to grow, the potential to improve gut function and health of food animals through the manipulation of enteric peptide secretion has become increasingly more apparent [21-26].

Glucagon-like peptide 2 (GLP-2) is an enteric peptide gaining significant attention due to its roles in improving nutrient absorption, energy balance, and gut barrier function, and reducing intestinal inflammation and its subsequent impacts on human health (reviewed by [12, 27-29]) and production animals (reviewed by [22, 30]). The purpose of this review is to summarize: 1) the current understanding of GLP-2's functions and mechanisms of action; 2) the potential use of GLP-2 or stimulators of its release to improve health and production

performance of food animals; and 3) recent findings suggesting the therapeutic potential of GLP-2 to reduce the pathogenesis of intestinal protozoan infections in dairy calves.

2. Physiology of GLP-2

2.1. GLP-2 synthesis and degradation

Glucagon-like peptide 2 is a 33-amino acid peptide hormone produced primarily in the gut as a result of tissue-specific post-translational cleavage of a prohormone, proglucagon, by the enzyme prohormone convertase (PC) 1/3 in intestinal L cells [22]. The L cells comprise less than 5% of mucosal cells; however, the greatest percentage of L cells is located in the distal small intestine and colon [30]. Processing of proglucagon by PC 1/3 in L cells concurrently produces the incretin hormone, GLP-1, in addition to an appetite regulator named oxyntomodulin [31], and three peptides called glicentin, glicentin-related pancreatic polypeptide (GRPP), and intervening peptide 2 (IP-2; [32, 33]), the functions of which are not well characterized. Proglucagon is encoded by the *GCG* gene, the expression of which is regulated by a cyclic AMP-mediated pathway in intestinal cells [34], and transcription factors Pax-6 [35, 36], β -catenin/T cell factor-4 (cat/TCF-4; [37]), and factors in the Wnt signaling pathway [38]. Intestinal expression of *GCG* gene is increased by feeding and is suppressed by fasting [39]. Additionally, specific nutrients including long-chain triglycerides, protein hydrolysates, dietary fiber, and short-chain fatty acids can induce *GCG* mRNA expression [22]. For additional details regarding the transcriptional regulation of *GCG* gene, the reader is referred to Jin [38].

The half life of GLP-2 (1-33) in circulation is very short due to its removal by the kidneys and enzymatic **degradation** by dipeptidyl peptidase IV (DPP-IV) of its N-terminal His-Ala residues to a truncated GLP-2 (3-33) form [40]. The GLP-2 (3-33) form lacks *in vivo* activity [41, 42]; however it can weakly activate the GLP-2 receptor when administered at supraphysiological doses [43]. Because of GLP-2's short (approximately **7-min**) half life in humans [44], administration of DPP-IV inhibitors has been used to enhance the therapeutic effects of GLP-2 [45-47], and synthetic GLP-2 analogs that are resistant to DPP-IV degradation have been used to prolong and increase its biological activity. Modifications include replacing the Ala (2) residue with Gly [40, 42], which increases the half-life of GLP-2 to approximately **3 h** in humans [48], or conjugating the peptide to various polymers, which extends the half-life in humans to as great as **10 d**, based on theoretical projections from animal models [49-51]. Further advances in our understanding of factors that increase GLP-2 synthesis and means to enhance its biological activity will support its potential use as an enhancer of animal productivity, health, and well-being.

2.2. Regulation of GLP-2 release

Both nutrient ingestion and intestinal injury stimulate release of GLP-2 from enteroendocrine L cells whereby its actions facilitate nutrient uptake, and mucosal growth, protection, and healing [28, 52]. The signaling pathway responsible for the GLP-2 response to intestinal injury remains unknown [28]. The postprandial increase in circulating GLP-2 concentration occurs in two phases, the first of which is within **30 min** of nutrient intake and is neuronally mediated [53, 54]. Although the neuronal signals have not been completely

characterized for most species, rodent models indicate that nutrients entering the duodenum activate endocrine K cells to release gastric inhibitory peptide, which stimulates vagal afferent nerves. Vagal efferent nerves and enteric neurons then induce GLP-2 release from L cells located in the distal small intestine and colon via acetylcholine and gastrin-releasing peptide. The second peak in GLP-2 release occurs 60 to 120 min after a meal through direct nutrient stimulation of L cells [53] by activation of specialized G-protein-coupled receptors present on their apical surfaces, which act as luminal chemosensors or “taste” receptors [55, 56]. The L cells express multiple types of chemosensors that are specifically activated by nutrients and sensory factors that stimulate GLP-2 secretion, including amino acids and peptones (via receptors T1R1-T1R3, CaSR, and LPAR5), bile acids (via receptor TGR5), simple sugars and non-nutritive sweeteners (via receptor T1R2-T1R3), free fatty acids and their metabolites (via receptors GPR40, GPR41, GPR43, GPR119, and GPR120), and bitter substances (via numerous T2R receptors; [52, 57]). Through the activation of these receptors, many benefits of stimulating GLP-2 release particularly on gut function and health of production animals through dietary intervention may be possible and are discussed below.

2.3. *Physiological effects of GLP-2 on intestinal tissues*

The effects of GLP-2 on the intestinal tract are quite extensive and diverse because GLP-2 has both direct and indirect effects (via intermediate factors) on target cells, including enterocytes, goblet cells, Paneth cells, stem/progenitor cells, sensory and enteric neurons, subepithelial myofibroblasts, endothelial cells of blood vessels, and various enteroendocrine cells [30, 52, 58, 59]; Figure 1). The effects of GLP-2 most commonly reported include increased

intestinal weight and mucosal development (e.g., increased enterocyte volume, micro villus and villus heights, and crypt depth), increased mesenteric blood flow, enhanced glucose and peptide transporter expression or activity and digestive enzyme activity in the intestinal brush border, reduced gut motility, increased barrier function/reduced intestinal permeability, reduced intestinal inflammation and apoptosis of intestinal epithelial cells, and improved intestinal healing after injury (reviewed by [28, 30]). The detailed cellular and signaling mechanisms underlying many of these responses have been characterized only recently.

The GLP-2 receptor (GLP-2R), which directly responds to GLP-2 and mediates its effects, is known to be **expressed** in only 3 gut cell types: subepithelial myofibroblasts, enteroendocrine cells, and vagal afferent and enteric neurons [60-62]. The responses of other **nearby** cell types **within the gut** to GLP-2 **are then** carried out by paracrine-acting signaling molecules produced by the GLP-2R-expressing cells [58]. Activation of **GLP-2R** on subepithelial myofibroblasts releases numerous growth factors, such as insulin-like growth factor (IGF)-1, IGF-2, keratinocyte growth factor, epidermal growth factor, and transforming growth factor- β [60] that may contribute to increased crypt cell proliferation and intestinal mucosal growth observed during GLP-2 treatment [58]. Specifically, Dubé et al. [63] reported stimulated proliferation of stem cells and progenitor cells in mouse jejunum in response to GLP-2, and through the use of an *IGF-1* knockout mouse model, demonstrated that the proliferative effects of GLP-2 on crypt epithelium were IGF-1-dependent. Using a murine intestinal progenitor cell assay, Bjerknes and Cheng [64] further demonstrated that GLP-2 affects stem cell differentiation by specifically increasing the population of progenitor columnar cells, but not **mucous cell** progenitors. The same study suggested that GLP-2 stimulation of intestinal epithelial growth was mediated by an

unidentified signal from GLP-2R-expressing enteric neurons. Although mucous cell progenitor populations did not appear to be affected by GLP-2 in the study by Bjerknes and Cheng [64], increased abundance of mucin-producing goblet cells in mouse jejunum after chronic GLP-2 treatment has been reported [65].

Enteric neurons expressing GLP-2R co-express nitric oxide (NO; derived from endothelial nitric oxide synthase activity) and vasoactive intestinal peptide (VIP; [61]). These molecules have been shown to mediate many of GLP-2's effects on the gut through studies in which GLP-2 was co-administered with inhibitors of NO or VIP activities. Specifically, NO mediates increased mesenteric blood flow, amino acid uptake and intestinal protein anabolism, and increased glucose transporter expression, glucose uptake, and glucose metabolism in the small intestine of young pigs in response to GLP-2 infusion [66]. The anti-inflammatory effects of GLP-2 on the gut are mediated by VIP, including decreased expression of proinflammatory cytokines, increased expression of the anti-inflammatory cytokine IL-10, and reduced cytokine-induced apoptosis [67]. Treatment with GLP-2 increased the abundance of and activated VIP-expressing neurons in healthy and inflamed rat colon through a PI3K γ /Akt-mediated pathway [67-69]. Noticeably, GLP-2's proliferative effects on the intestinal crypts and small intestinal mucosa do not require VIP [70]. In addition to reducing inflammation, GLP-2 exhibits antioxidant activity, reduces inflammation-induced oxidative stress in intestinal tissues [71], and provides protection from gut ischemia and reperfusion injury [72]; although the mechanisms of these actions have not been fully investigated to date.

Using *GLP-2R* knockout mice, Lee et al. [73] showed that GLP-2R activation contributes to the synthesis and activity of antimicrobial peptides produced by Paneth cells in the small intestine needed to maintain normal host-bacterial balance. Antimicrobial peptides produced by Paneth cells include lysozyme, α -defensins, secretory phospholipase A2, cathelicidins, and RegIIIa (regenerating islet-derived protein IIIa), which provide a biochemical barrier of protection to the intestinal epithelial cells [74, 75]. In addition, Chen et al. [76] showed that GLP-2 increases secretory IgA, an important factor for reducing bacterial translocation through the intestinal barrier, in intestinal crypts of obstructive jaundiced rats. In the study by Lee et al. [73], *GLP-2R* knockouts were more susceptible to bacterial overgrowth in the jejunum and ileum, bacterial septicemia, and changes in the microbial population of the cecum compared to wild-type mice. Paneth cells also produce factors that support the intestinal epithelial stem and progenitor cells [74], but it is not known whether GLP-2 or activation of its receptor has effects on the expression of these factors. Thus, by activating IgA and antimicrobial peptides produced by Paneth cells, as well as increasing abundance of mucin-producing goblet cells [65], GLP-2 likely reduces exposure of the underlying mucosal cells to microbial factors that could induce an inflammatory response.

Similarly, GLP-2 treatment can improve the physical barrier of the gut against the passage of pathogens and their toxins by reducing both paracellular and transcellular permeability of the gut [77], thereby decreasing the potential for activation of the immune system and subsequent inflammatory stress [78]. Benjamin and colleagues [77] hypothesized that the reduction in cellular permeability resulted, at least in part, from changes in cellular tight junctions or modification of the epithelial cytoskeleton. In support of this hypothesis, Cani

et al. [79] found that exogenous GLP-2 treatment of *ob/ob* mice reduced plasma LPS and inflammatory tone (indicators of endotoxemia) and reduced abundance of markers of oxidative stress, numerically increased immunostaining for epithelial tight junction proteins occludin and ZO-1 in jejunum, and increased the immunolocalization of both tight junction proteins at the apical membrane of jejunal epithelium. Likewise, a recent study by Dong et al. [80] confirmed that GLP-2 treatment of mice reduced gastrointestinal permeability, as indicated by reduced gastrointestinal uptake of a fluorescently-labeled dextran and increased resistance across the jejunal membrane (evaluated via Ussing chambers). These effects were associated with increased protein expression of tight junction proteins claudin-3 and claudin-7 in jejunum. Of interest however, all of these responses to GLP-2 were absent when administered to intestinal epithelial-specific *IGF-1 receptor*-knockout mice, indicating that reductions in gut permeability induced by GLP-2 are mediated at least partly by IGF-1 receptor [80].

Therefore, due to its multifaceted effects on the gut, GLP-2 therapy has the potential to improve animal production performance (feed conversion efficiency) and animal health, primarily through its ability to increase nutrient uptake, enhance defense against intestinal pathogens, reduce damage from inflammation, and improve intestinal healing after injury. A greater understanding of factors that mediate GLP-2's effects may assist in the development of novel approaches to amplify and prolong its therapeutic effects.

2.4. *Physiological effects of GLP-2 on extra-intestinal tissues*

Although GLP-2 is primarily known for its effects on the gastrointestinal tract, expression of GLP-2R and physiological effects of GLP-2 beyond the gut have been reported, including on

pancreas, brain, bone [28], lung [11] and liver [10]. These effects of GLP-2 influence glucose metabolism, feed intake, bone resorption, and lung and liver healing post-injury, all of which could have implications for the therapeutic use of GLP-2 in livestock animal production, and will be discussed briefly.

The expression of GLP-2R has been reported in both human and rat pancreas within proglucagon-expressing α cells, but not on β and δ cells that release insulin and somatostatin, respectively [81]. Activation of GLP-2R by GLP-2 administration appears to directly stimulate pancreatic glucagon secretion and attenuate the inhibitory effects of GLP-1 on glucagon release [81]. Although studies with *GLP-2R* knockout mice indicated that GLP-2R is not required for the regulation of glucagon secretion or to maintain glucose homeostasis, it was found that loss of GLP-2R impairs maintenance of glucose homeostasis in obese mice [82]. Therefore, GLP-2 therapy could have implications for improving glucose balance in metabolically compromised animals. In support of this hypothesis, treatment of mice with a GLP-2R antagonist that were chronically fed a high-fat diet and exhibiting hyperglycemia resulted in exacerbated glucose imbalance and insulin resistance, and increased pancreatic β cell mass [83].

Within the brain, activation of GLP-2R expressed on POMC neurons in the hypothalamus by centrally produced GLP-2 has been implicated in mediating reduced feed intake, gut motility, and hepatic gluconeogenesis [12]. Although work in this area has been conducted primarily in rodents, intracerebroventricular administration of GLP-2 also reduces feed intake and circulating concentrations of glucose in 8-d-old chicks [84], indicating that similar pathways likely exist across avian and mammalian species. In addition, GLP-2 can reduce stress-induced

depression [14] and hypertension in rodents [85], and improve memory in mice with experimentally induced brain inflammation [13] through the GLP-2R expressed in the central nervous system. Although these effects were based on central administration of GLP-2, it is believed that peripheral GLP-2 (both physiological and pharmacological doses) may be able to cross the blood-brain barrier and have central effects [12]. Finally, GLP-2 has been shown to improve liver regeneration after injury in a mouse model using partial hepatectomy [10], to reduce bone resorption and improve bone mineral density in humans, particularly post-menopausal women [86], and to enhance lung recovery in mice after inflammation-related oxidative damage [11]. Thus, in the development of any GLP-2 treatment regimen, the potential effects on GLP-2R-mediated activities within the central nervous system as well as other tissues outside of the gastrointestinal tract should be considered.

3. Potential uses of GLP-2 and stimulators of GLP-2 secretion in animal production

Given the number of physiological effects of GLP-2 described, there are 2 major areas where exogenous GLP-2 treatment or stimulation of endogenous GLP-2 secretion appears useful to benefit animal production and health. These include: 1) improving gut development and digestive function, particularly in young animals, to improve animal performance and productivity; and 2) improving overall animal well-being through beneficial effects of GLP-2 on organs outside of the digestive system. Although the number of studies examining GLP-2 treatment in livestock species is limited, as observed in humans and rodent models, GLP-2 therapy (or stimulators of GLP-2 release) in livestock can enhance intestinal absorptive capacity by increasing size of segments of the small intestine and lengthening villi, increasing nutrient

249 transporter abundance, and increasing enzyme activities in the intestinal brush border. Taylor-
250 Edwards et al. [87] reported that a 10-d treatment of ruminating calves with GLP-2 increased
251 mass and tissue density (mass-to-length ratio) of the small intestine, which was due to
252 increased growth of the mucosal epithelium within the jejunum and ileum. In their study,
253 increased cell proliferation, villus height, and crypt depth were observed throughout the small
254 intestine, as well as increased amino acid use and blood flow to the small intestine to support
255 this tissue development [88]. Similar effects of GLP-2 treatment of calves, including increased
256 weight of both the small and large intestines and increased tissue density of the small intestine,
257 were reported by Connor et al. [89]. Most recently, Moran et al. [25] reported that stimulation
258 of GLP-2 release via sweet taste receptor activation by the feeding of an artificial sweetener
259 containing non-metabolizable saccharin (Sucram® Pancosma SA, Geneva, Switzerland)
260 increased activity of digestive enzymes such as maltase and alkaline phosphatase in the
261 intestinal brush border of ruminating calves, and tended to increase their feed conversion
262 efficiency measured as gain-to-feed ratio (G:F) [90]. In addition, Sucram feeding to mature,
263 lactating cows enhanced their mucosal growth through elevated intestinal glucose transporter
264 (SGLT1) expression and enhanced glucose absorption [25]. Furthermore, supplementing the
265 diet with Sucram increased energy-corrected milk production and altered nutrient partitioning
266 in cows during early lactation [91]. Combined, these studies indicate the potential to improve
267 nutrient absorption and use through GLP-2 therapy or stimulation; however, the actual benefits
268 to growth performance and feed conversion efficiency may be limited. For example, increased
269 absorptive capacity and nutrient digestion induced by GLP-2 (or stimulators of GLP-2 release)
270 may not outweigh the increased energy demands on the animal required to support greater

intestinal tissue mass. Dietary supplementation of growing, feedlot steer calves with Sucram (200 mg/kg dry matter) failed to significantly increase average daily gain and G:F during 56-d growth trials reported by McMeniman et al. [92] and Ponce et al. [93]. However, Sucram feeding did provide a short-term benefit by increasing G:F in steer calves during the first 28 d of the receiving period [92].

Similar to cattle, GLP-2 treatment of newborn pigs increased weight and villus growth of the small intestine, which appeared to occur as a result of decreased apoptosis and increased cell proliferation in the villus and crypts [94, 95]. Stimulation of GLP-2 secretion through administration of a specific bile acid (presumably through activation of the receptor TGR5) also increased size of the ileum but did not alter gut morphology or cell proliferation within the villi and crypts [26]. In this study, some associated intestinal inflammation was observed at the administered dose of bile acid, which the authors suggested may have negated other growth-promoting effects of GLP-2 on the gut. Alternatively, feeding of the sweetener Sucram, which should promote GLP-2 release [25], enhanced glucose uptake through detection by the sweet taste receptor [24] and showed the potential for improving feed intake and rate of gain in pigs at weaning [96].

However, the greatest potential for GLP-2 therapy in livestock production may be through the treatment of compromised animals to improve production, rather than enhancing productivity of those that are healthy and growing normally. For instance, Thymann et al. [59] found few effects of GLP-2 treatment on gut morphology or enzyme activity in healthy pigs raised under sanitary conditions, but found that treatment with a long-acting form of GLP-2 of

pigs with diarrhea and maintained under low-sanitary conditions exhibited a 22% increase in small intestinal weight, reduced severity of diarrhea, increased villus height and crypt depth, and substantially enhanced activity of peptidases in the brush border. Associated benefits on growth performance at weaning were not apparent, but animals were not evaluated beyond 5 d post weaning. Of interest, significant growth performance enhancement by GLP-2 has been demonstrated among both control poultry and those administered corticosterone to mimic stress [97]. Specifically, control broilers treated with GLP-2 expressed limited effects on small intestinal morphology, increased intestinal weight, increased calcium and peptide transporter mRNA expression, and improved rate of gain and feed-to-gain ratio (feed conversion efficiency). Similar benefits were also observed in “stressed” broilers, in addition to increased mRNA expression of glucose and fatty acid transporters.

In particular, the ability of GLP-2 to enhance intestinal integrity through mucin and IgA production, to improve gut barrier function through effects on tight junctions, and ultimately to reduce tissue damage from inflammation provides multiple opportunities to improve digestive health of livestock under common production scenarios. For example, heat stress is a common condition impacting farm animal production in warm climates and is estimated to cause an annual loss of \$2.4 billion across the U.S. cattle, poultry, and swine sectors [98]. Heat stress impairs gut barrier function by reducing blood flow to the gut, which can result in nitro-oxidative stress and cell death, and disrupts cellular tight junctions [99]. It is believed that intestinal cells also increase glucose absorption in response to heat stress to provide protection from apoptosis and to increase water absorption [99]. Thus, GLP-2 could be applied to reverse

many of these negative effects, to improve glucose absorption after heat stress, and to minimize injury to the intestinal mucosa.

Of interest, taste receptor T1R1-T1R3 activation by both L-glutamate and inosine 5'-monophosphate increases GLP-2 release in rat duodenum [100], and both GLP-2 administration and a combined treatment of L-alanine with inosine 5'-monophosphate increase the healing rate of intestinal ulcers in rats [47]. In addition, Bauchart-Thevret et al. [101] demonstrated that dietary monosodium glutamate supplementation of preterm pigs dose-dependently increases plasma GLP-2 concentration. Thus, stimulation of GLP-2 release via activation of T1R1-T1R3, or other L-cell taste receptors, has the potential to improve gut health of production animals. In fact, supplementing bacterial endotoxin (LPS)-challenged weaning pigs with dietary aspartate improved villus height-to-crypt depth ratio, increased activity of three mucosal enzymes responsible for digestion of disaccharides, and ultimately improved indicators of intestinal mucosal cell energy status [102]. Similarly, fish oil containing poly-unsaturated fatty acids (i.e., putative agonists of fatty acid taste receptors) increased villus height-to-crypt depth ratio, increased expression of tight junction proteins occludin and claudin-1, increased tissue diamine oxidase activity (reduced activity occurs with mucosal injury), and reduced mRNA expression of signaling molecules involved in the inflammatory response to pathogens in weaned pigs after an LPS challenge [103]. Additional studies are needed to determine whether GLP-2 is mediating these observed benefits of dietary aspartate and fish oil supplementation on gut health through activation of luminal taste receptors.

Exogenous GLP-2 therapy also has been shown to reduce protein tyrosine nitration, a marker of nitro-oxidative stress, and alter tight junction protein expression in the small intestine and cecum of calves infected with the protozoan parasite, *Eimeria bovis* [89, 104]. Both the antioxidant effects of GLP-2 and its impacts on tight junction proteins serve to reduce inflammatory damage to the intestinal mucosa during pathogen invasion. In addition, Yu et al. [105] demonstrated that cultured porcine jejunal cells treated with GLP-2 exhibit increased expression of occludin, claudin-1, and zona occludens-1, which may contribute to enhanced intestinal barrier function. Finally, pre-treatment of weaning pigs with a long-acting GLP-2 analog for 3 d prior to an intraperitoneal LPS challenge provided multiple intestinal changes that could prevent intestinal injury induced by LPS, including enhancement of the physical barrier function of the gut through increased villus height-to-crypt depth ratio in duodenum, reduced proinflammatory cytokine *interleukin-8* (*IL-8*), *interferon- γ* (*IFN- γ*), *tumor necrosis factor- α* (*TNF- α*) mRNA expression in jejunum, reduced pro-apoptotic caspase-3 activity in ileum, as well as increased digestive enzyme lactase and sucrase activities in the proximal small intestine [51, 106].

Lastly, effects of GLP-2 beyond the intestinal mucosa including those on bone, liver, and lung health could be used to alleviate other production-related conditions in livestock. As previously mentioned, subcutaneous GLP-2 injection decreases bone resorption, as indicated by reduced serum levels of a biomarker of type I collagen degradation by osteoclasts called carboxy-terminal telopeptide or CTX, and increased bone mineral density of the hip in postmenopausal women [86]. The GLP-2R is expressed on osteoclasts and its activation is believed to reduce catabolic activity of these cells [86]. Therefore, GLP-2 therapy may be useful

in livestock production to improve bone health and prevent conditions such as osteopenic disease in swine or fluorosis, which is most common among cattle, horses, and sheep in fluoride-contaminated environments [107] and increases the incidence of bone fractures in sheep [108]. Benefits of GLP-2 for these conditions include both improved calcium uptake through its effects on increasing calcium transporter expression in the intestinal tract, and its direct effects on reducing bone resorption. However, there is no published research to date on the topic of GLP-2 and bone health in livestock.

Furthermore, GLP-2 may be useful for ameliorating damage to the liver and lungs, particularly resulting from inflammation and overproduction of proinflammatory mediators during an immune challenge, based on its effects on these tissues described previously in mice [10, 11]. Although the direct effects of GLP-2 on liver and lung health of livestock have not been evaluated, feeding of aspartate for 20 d to weanling pigs was shown to affect mRNA expression of proinflammatory signaling molecules in the toll-like receptor 4 and NOD pathways that modulate innate and adaptive immune function, and to improve serum indicators of liver damage after an intraperitoneal LPS challenge [109]. Although the mechanism of action of aspartate supplementation and plasma GLP-2 were not evaluated in their study, feeding of this amino acid has the potential to promote GLP-2 release from intestinal L cells [100, 101] through activation of taste receptors (e.g., T1R1-T1R3) and could contribute to the positive hepatic effects observed. Given the potential benefits of GLP-2 on liver health, evaluation of GLP-2 for the prevention of hepatic disorders in production animals, such as fatty liver in transition dairy cows, is warranted.

In the lungs, treatment with a long-acting form of GLP-2 reduced apoptosis and oxidative-stress-induced injury in mice in response to an intraperitoneal challenge with proinflammatory TNF- α and actinomycin D [11]. These benefits have particular relevance to common respiratory diseases of livestock, such as shipping fever in cattle caused by *Pasteruella (Mannheimia) haemolytica*. This disease commonly causes an over-active proinflammatory cascade and excessive cytokine production, causing extensive damage to lung tissue [110], which may be ameliorated by GLP-2 therapy. Clearly, additional research is needed to determine the therapeutic effects of GLP-2 and stimulators of its release on extra-intestinal tissues of livestock and the expression patterns of its receptor outside of the gut.

Overall, GLP-2 therapy appears to have multiple practical applications for improving livestock production and health, such as increasing nutrient uptake (e.g., glucose absorption in piglets, calcium absorption in laying hens and post-parturient dairy cows) and feed conversion efficiency, improving bone health (e.g., reducing osteopenic disease in growing pigs), reducing the negative impacts of intestinal damage (e.g., due to parasites, diarrheal diseases, and heat stress), stabilizing gut function and health during dietary transitions (e.g., weaning), preventing or reducing lung injury during respiratory illness (e.g., shipping fever in cattle), improving glucose homeostasis and altering hepatic gluconeogenesis in metabolically challenged animals (e.g., dairy cows during the transition period), and reducing hepatic injury during various disease states.

4. Use of GLP-2 as a pre-infection therapeutic for *Cryptosporidium parvum*

Cryptosporidium parvum is a highly infectious and zoonotic protozoan parasite present in all calf populations [111]. Infection typically causes transient, mild-to-moderate diarrhea, which may impair growth performance and increase susceptibility to other infections of the gut mucosa [112]. Infection and inflammation damage enterocyte membranes, reducing nutrient absorption and barrier function, and cause disruption of tight junctions [112], microvillous atrophy, and loss of brush-border enzymes needed to facilitate digestion [113]. Many opportunities exist to apply GLP-2 as a pre- or post-infection therapeutic for infection, including GLP-2's effects on increasing mucin production, villus length, and tight junction protein expression to defend against parasitic invasion, as well as reducing nitro-oxidative stress, improving gut healing, and enhancing expression and activity of brush-border enzymes impacted by *C. parvum* infection. Of particular interest, GLP-2 treatment of cultured GLP-2R-expressing cells has been shown to reduce F-actin abundance and alter its distribution within the cytoskeleton, a process associated with cellular proliferation [114] and required for enterocyte microvillus depolarization and remodeling during intestinal healing [115]. Therefore, there is the potential for GLP-2 pretreatment to impair *C. parvum*'s ability to induce actin polymerization which is required for envelopment by host epithelial cells [116]. On the other hand, physiological effects of GLP-2 including reducing cellular apoptosis needed to eliminate parasitized intestinal cells [116] and increasing SGLT1 expression required for *C. parvum* envelopment [117], may actually benefit and support the parasite life cycle within its host.

To evaluate the protective effects of GLP-2 pretreatment on *C. parvum* infection, a trial was conducted (Connor, E.E., unpublished) wherein healthy newborn calves (n = 6) were fed normal milk replacer for 18 d and served as normal controls, or calves (n = 18) were fed milk

replacer for 18 d and orally infected with 12,500 *C. parvum* oocysts on day 8 of the trial. Among infected calves, one group (n = 6) was administered bovine GLP-2 (50 µg/kg BW, s.c. twice daily) throughout the 18-d trial (i.e., beginning 1 wk prior to infection), a second group (n = 6) received a sham injection and milk replacer supplemented with Sucram (400 mg/kg dry matter) throughout the trial, and a third group (n = 6) received sham injections and served as infected controls. All calves were sacrificed on day 10 of infection within 1.5 to 3.5 h after their last meal for tissue collection and analysis.

The *C. parvum* infection reduced villus height in ileum and increased cell proliferation in intestinal crypts, which is often observed during intestinal damage caused by cryptosporidiosis [118]. Treatment with GLP-2 improved villus height in intestinal segments, and both Sucram and GLP-2 treatments tended to reduce crypt cell proliferation to levels similar to non-infected controls. Infection also increased mRNA expression of a gene involved in cell mediated immunity and mRNA expression of a tight junction protein in jejunal epithelium, which were reversed by GLP-2 and Sucram treatments. These findings suggest minor benefits for GLP-2 and Sucram pretreatment to reduce deleterious effects of *C. parvum* infection on newborn calves.

Most interesting were potential differences observed among infected treatment groups in the quantity of *C. parvum* oocysts shed in the feces 10 d post infection. At sacrifice, oocysts quantified in fecal samples from a subsample of calves (n = 2 per treatment group) suggested that treatment of calves for 1 wk with GLP-2 or Sucram prior to an oral inoculation with *C. parvum* may reduce the number of oocysts shed in feces relative to infected controls by nearly 100-fold. The specific mechanisms contributing to these observed differences are unknown but

currently are under investigation through a larger study. Possible mechanisms include impaired ability of parasites to penetrate the mucus layer and/or attach to microvilli; impaired envelopment and/or oocyst release from host enterocytes; and enhanced immune/defensive response of the host to infection.

5. Conclusions

The physiological effects of GLP-2 on the gut have been well studied, including increasing nutrient uptake, reducing inflammation and improving healing after injury, promoting barrier function, and supporting defenses against pathogenic invasion. In recent years, additional effects of GLP-2 including its antioxidant activities, effects on tight junction proteins, and impacts outside of the intestinal tract have been described, such as promotion of bone health by decreasing bone resorption and increasing bone density, mediating glucose balance through its effects on the pancreas and hypothalamus, ameliorating inflammatory damage in the brain, liver, and lung, and promoting liver regeneration. These effects of GLP-2 have broad implications for maintaining health and productivity of production animals. Identification of factors and mechanisms that promote GLP-2 secretion and means to increase its activity in circulation will enhance its therapeutic potential in livestock species for a variety of conditions like impaired gut function during dietary transitions, heat stress, or intestinal disease, injury to liver from inflammatory stress or lungs during respiratory illness, and impaired glucose homeostasis in metabolically challenged animals. Stimulation of GLP-2 release within the gut through dietary interventions may provide additional benefit over exogenous GLP-2 administration due to its local activation and release with other co-acting factors

including GLP-1. A greater understanding of the mechanisms of GLP-2's actions within the body will support novel uses of this hormone to improve animal health and well-being.

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800 Figure 1. Effects of GLP-2 (produced by endocrine L-cells) and its mediators on target cells in the
801 gastrointestinal tract. Activation of GLP-2 receptors on enteric neurons stimulates both nitric
802 oxide (NO) and vasoactive intestinal peptide (VIP) release. The NO increases mesenteric blood
803 flow and increases amino acid and glucose uptake, and VIP reduces immune cell infiltration,
804 pro-inflammatory cytokine release, and apoptosis in enterocytes. Growth factors released from
805 subepithelial myofibroblasts by GLP-2 receptor activation, including insulin-like growth factor
806 (IGF)-1, IGF-2, keratinocyte growth factor (KGF), and epidermal growth factor (EGF)-ErbB family
807 members, mediate increased proliferation and growth of various intestinal crypt cells.
808 Expression of cellular tight junction proteins controlling gut permeability is affected by GLP-2
809 and is IGF-1 receptor-dependent. Normal synthesis and activity of antimicrobial peptides
810 produced by Paneth cells depend upon GLP-2 receptor activation. Other enteroendocrine cells
811 that secrete factors like gastric inhibitory peptide (GIP), secretin, and cholecystokinin (CCK)
812 express GLP-2 receptor and have various downstream effects.

Figure 1