BARRIER FUNCTION OF THE GASTROINTESTINAL TRACT OF STEERS WHEN EXPOSED TO RUMINAL ACIDOSIS AND LOW FEED INTAKE

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ABSTRACT

Ruminal acidosis and low feed intake are two nutritional challenges present on beef and dairy operations that result in reduced health and performance. The objective of this study was to determine the regional susceptibility of the gastrointestinal tract (GIT) to ruminal acidosis (RA) or low feed intake (LFI). Twenty-one Holstein steers, aged 6 to 8 mo, were blocked by body weight (BW) and within block randomly assigned to 1 of 3 treatments: 1) control (CON), 2) RA, and 3) LFI. All steers were fed a diet containing a 50:50 forage to concentrate ratio (F:C) and were exposed to a 5-d measurement period. The CON steers were provided the basal diet for ad libitum intake, the LFI steers were fed at 25% of ad libitum intake for 5 d before humane killing while the RA steers were restricted to 25% intake for 1 d and then were fed 30% of dry matter intake (DMI) in pelleted barley followed by their regular total mixed ration (TMR) allocation. The RA steers were killed 1 d after the RA challenge. After killing, tissues from the rumen, omasum, duodenum, jejunum, ileum, cecum, proximal, and distal colon were collected. Digesta from those regions were assessed and tissues were mounted in Ussing chambers in order to assess permeability using the mucosal-to-serosal flux of inulin ($J_{MS\text{-}inulin}$) and mannitol ($J_{MS\text{-}mannitol}$) and tissue conductance (Gt). Digesta pH were less for RA than CON in the rumen, cecum, and proximal colon while LFI had greater pH in the rumen and proximal colon compared to CON. Ruminal short-chain fatty acid (SCFA) concentrations in the rumen were less for the LFI ($P = 0.010$) and RA ($P = 0.007$) compared to CON steers. In the proximal colon, there was a greater proportion of butyrate ($P = 0.025$ and $P = 0.022$) and isobutyrate ($P = 0.019$ and $P = 0.019$), and a lower proportion of acetate ($P = 0.028$ and $P = 0.028$) for LFI and RA, respectively, when compared to CON steers. The $J_{MS\text{-}mannitol}$ differed between the CON and LFI steers in the proximal colon ($P = 0.041$) and in the distal colon ($P = 0.015$) with the LFI steers having a lower flux rate in both regions. Increases in gene expression for genes related to barrier function (in the rumen, jejunum, and distal colon), mucosal immunology (rumen and jejunum), and adaptive immunity (jejunum) were observed indicating compensatory mechanisms may have been stimulated. It was concluded from this study that an acute RA challenge had no effect on tissue permeability throughout the GIT within 1 d of the challenge, while steers that were on LFI for 5 d had reduced tissue permeability in the

proximal and distal colon while other regions of the GIT were unaffected. Further research is needed to analyze different management strategies proposed to mitigate the negative impact of LFI shown in production animal agriculture.

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What a wholesome and rewarding graduate degree I have had. To all, stay classy and God bless.

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LIST OF ABBREVIATIONS

ACTB	Beta-actin
ADF	Acid detergent fiber
AJ	Adherens junctions
BHBA	β-hydroxy butyrate
BW	Body weight
CLDN	Claudin 1
CP	Crude protein
CON	Control
DM	Dry matter
DMI	Dry matter intake
EE	Ether extract
F:C	Forage:concentrate ratio
FCAR	Immunoglobin A receptor
FR	Feed restriction
IGF	Insulin-like growth factor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GIT	Gastrointestinal tract
HF	High forage
$J_{MS\text{-}inulin}$	Mucosal-to-serosal inulin flux
$J_{MS\text{-}mannitol}$	Mucosal-to-serosal mannitol flux
JAM	Junctional adhesion molecules
LFI	Low feed intake

LPS	Lipopolysaccharide
MF	Medium forage
NCBI	National Center for Biological Information
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acid
OCLN 1	Occludens 1
OM	Organic matter
RA	Ruminal acidosis
RPLP0	60S acidic ribosomal protein P0
SAA	Serum amyloid A
SARA	Subacute ruminal acidosis
SCFA	Short chain fatty acids
SEM	Standard error of mean
TCJ	Tight junction
TLR	Toll-like receptor
TMR	Total mixed ration
ZO-1	Zonula-occludens 1
ZO-2	Zonula-occludens 2

1.0 GENERAL INTRODUCTION

For beef and dairy cattle, there are certain physiological states, management practices, and environmental conditions that induce stress on cattle. Whether these factors occur in isolation or as a combination, they often alter nutrient metabolism and reduce animal health, and productivity. Examples of stressors include parturition (Bertics et al., 1992), weaning (Haley et al., 2005; Blanco et al., 2009), adverse environmental conditions such as heat stress (Rhoads et al., 2009), transportation (Grandin, 2001; González et al., 2012a,b), and the dietary transition to more fermentable diets (Brown et al., 2006). Management practices have been developed to minimize the impact of the above-mentioned situations by implementing low stress handling techniques, providing adequate rest with access to feed and water during extended transportation events, replacing abrupt weaning with alternative lower stress weaning strategies, and implementing gradual step-up rations to enable cattle to adapt. However, even with these practices in place, stressors will never be completely avoidable on farm and often result in at least a transient reduction in dry matter intake (DMI). It should be noted that while a reduction in DMI reduces nutrient supply, it also increases the risks for digestive upset, such as ruminal acidosis, upon return to normal DMI.

How the ruminant gastrointestinal tract (GIT) reacts to nutritional challenges (e.g. the role of barrier function) has mainly been evaluated for the rumen, or the total GIT without any knowledge of individual regions (Zhang et al., 2013a,b; Albornoz et al., 2013a,b). That said, previous work in monogastrics has evaluated individual regions of the GIT under normal and stressed conditions (Novosad et al., 2013), but only 3 studies have evaluated responses in ruminants (Sun et al., 2013; Penner et al., 2014; Wood et al., 2015). Investigating the regional response is critical to advance our understanding as the absorptive function and capability (Novosad et al., 2013, Aschenbach et al., 2009), microbiota populations (Malmathuge, 2011), and defense mechanisms (Jutfelt, 2011; Peterson and Artis, 2014) all differ among regions of the GIT in both ruminant and monogastric animals.

Low feed intake and ruminal acidosis are two nutritional challenges that are highly prevalent on farm during stressful conditions. Research evaluating the impact of low feed intake (using a feed restriction model) has shown reduced SCFA absorption

across the reticulo-rumen, independent of diet being fed (Albornoz et al., 2013; Gäbel et al., 1993). Zhang et al. (2013a) showed that SCFA absorption through the reticulo-rumen tended to decrease with increasing severity of feed restriction (FR), but overall there was an effect of FR to reduce absorption (Zhang et al., 2013b). It was also reported in that study that severe FR (25% of ad libitum intake) is needed to compromise total tract GIT permeability. Feeding diets that contain a greater proportion of concentrate is a strategy to provide a greater energy density to promote growth and milk production, but also increases the risk of ruminal acidosis occurring. Cattle affected with ruminal acidosis have been shown to have reduced SCFA absorption across the reticulo-rumen (Penner et al., 2009; Wilson et al., 2012; Schwaiger et al., 2014) and the structural integrity of the rumen epithelium has been shown to be compromised during higher grain feeding (Steele et al., 2011).

The barrier function of the GIT is responsible for allowing adequate nutrient absorption while preventing entry of non-desired molecules, as well as providing the ability to detect and mount an immune response when necessary (Peterson and Artis, 2014). It is important for the host to establish tolerance towards the commensal microbial population residing within the lumen so the immune system is not over stimulated. When cattle face a nutritional challenge, understanding the regions of the GIT that are most compromised provides an opportunity to develop management or feeding strategies, such as feeding a higher forage diet or a feed additive during certain times of stress, that could mitigate the negative effect on health and performance by either reducing impact or accelerating recovery from low feed intake and/or an episode of ruminal acidosis.

2.0 LITERATURE REVIEW

2.1 Regional morphology, histology, and functional significance of the GIT in ruminants

Each region of the ruminant GIT differs in structure, absorptive function and capacity, microbial population, and immune function. This allows for regional specialization throughout the GIT that is specifically adapted for ruminants (Church, 1988). The classical role of the GIT has largely focused on feed consumption, digestion of feed, absorption of nutrients, and elimination of the residue. These essential processes provide the energy, amino acids, vitamins, minerals, and water that are needed by the host for the various physiological states throughout life (growth, reproduction, gestation, lactation, and maintenance), but the role of the GIT is more complex and includes luminal sensing, barrier function, and systemic communication via neuropeptide and hormone release (Sherwood et al., 2013). In addition, the GIT must function as the first line of defense for the immune system, which includes maintaining adequate barrier function. The immunological barrier function is completed through immune cell infiltration, mucus secretion, tissue permeability, luminal secretions, and epithelial cell renewal (Peterson and Artis, 2014). Both roles of the GIT are vital for a ruminant to maintain optimal health and performance.

The digestive tract has evolved over time to reflect the diet of the host. Carnivores tend to have a shorter GIT length with a faster passage rate, as they consume a diet consisting of feedstuffs that provide readily available nutrients that require less energy to extract (Dukes, 1977). Herbivores have a longer GIT with a longer digesta residence time, as they consume a diet consisting of feedstuffs that require more time and energy for nutrient extraction (Dukes, 1977). Other structural features include out-pouching (ex. cecum), submucosal folds, ruminal papillae (ruminants only), villi, and crypts, which are used to increase the surface area of the GIT to enable greater absorptive and secretory capacity (Peterson and Artis, 2014).

The GIT consists of 4 layers (Figure 2.1): the mucosa (epithelium, lamina propria, and muscularis mucosae), the submucosa (supportive connective tissue for the mucosa), the muscularis externa (circular and longitudinal smooth muscle layers used for

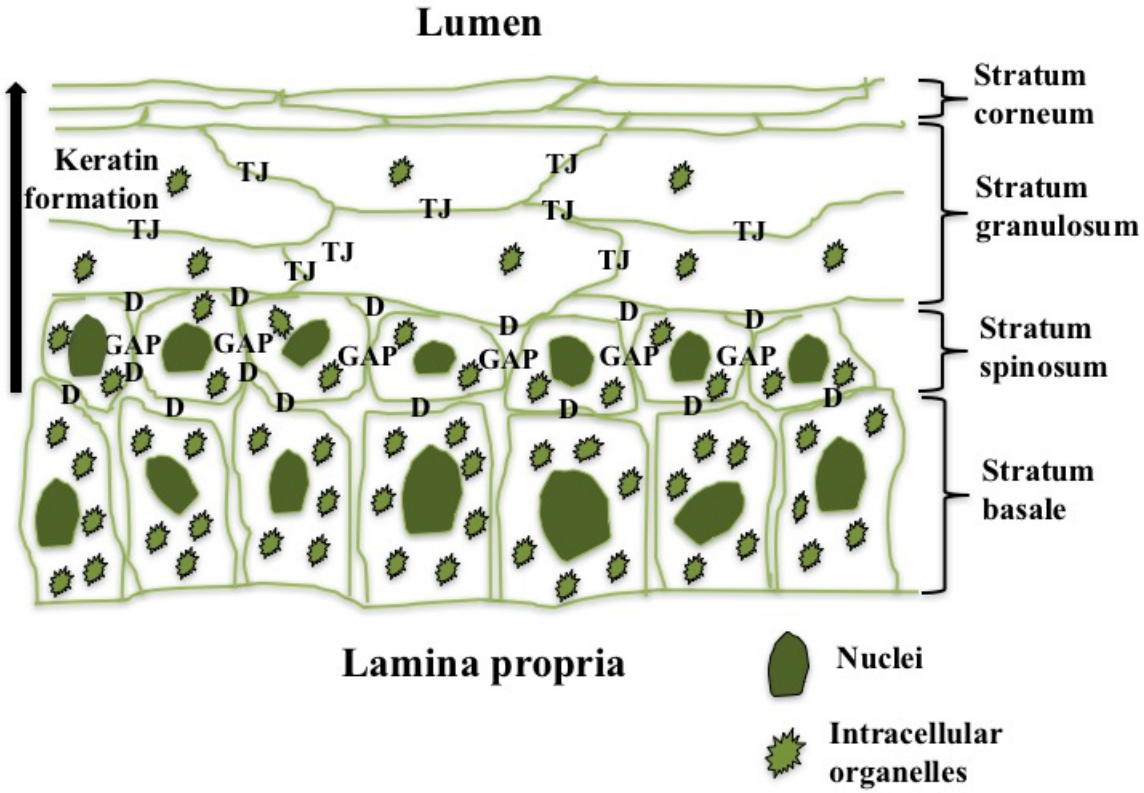

Figure 2.1. A histological representation of the ruminal epithelium with TJ= tight junction, D=desmosomes, and GAP= gap junctions.

propulsion) and the serosa (outermost connective tissue layer; Eurell and Frappier, 2006). When specifically focusing on the epithelium, the reticulum, rumen and omasum have unique morphology and histology when compared to the other GIT regions in ruminants, but each region is specifically structured for its own function.

2.1.1. Oral cavity and esophagus. The bovine oral cavity is lined with a cornified cutaneous mucus membrane, which is underlain with serous glands. The oral cavity is in close relation with the three types of salivary glands: parotid, mandibular, and buccal gland (Church, 1988), which are important for lubrication and buffering capacity through saliva. The oral cavity contains the teeth (for mechanical digestion) and the tongue. Church (1988) described the tongue as plump and piston-like muscle with a stratified squamous epithelium that forms densely packed filiform papillae. The mucus membrane that covers the tongue is different from the lower aspect (thin and delicate) when compared to the dorsal aspect (thick and cornified; Church, 1988). The lateral boundary of the oral cavity is the cheeks while the roof is the hard palate and the bottom is the sublingual floor between incisor teeth and frenulum (Church, 1988). The esophagus is composed of two concentric membranous tubes (inner mucosa and outer muscular tunic) that transport feed from the pharynx down to the reticulum, and allows for rumination to occur (Church, 1988). The mucus membrane of the esophagus is lined with a stratified squamous epithelium (Church, 1988). The esophagus ends at the cardia, which is the junction between the reticulum and the rumen, and opens directly over the reticulum cavity (Sherwood et al., 2013). The end is encircled by smooth muscle strands that form the reticular groove extending to the reticulo-omasal surface (Sherwood et al., 2013). The reticulo-omasal groove is important during suckling as the milk bypasses the rumen and enters directly into the omasum thereby avoiding fermentation. In mature ruminants, the esophagus facilitates rumination and is partially under voluntary control of the host. Rumination is the postprandial regurgitation of digesta for further mastication, followed by bolus reformation, and swallowing enabling continued fermentative digestion in the reticulo-rumen (Van Soest, 1982). Rumination is an important activity practiced by ruminants in order to complete the mechanical grinding of ingested plant material and helps to expose additional binding sites for ruminal microbes. Rumination is thought to

have been an evolutionary adaptation for ruminants, normally a prey species, to be able to mechanically process their feed at a later time if more appropriate (Sherwood et al., 2013).

2.1.1. Reticulum and rumen. The main feature making a ruminant distinct from a monogastric is the presence of a fermentative foregut (anaerobic environment). The reticulum and rumen (as well as omasum) are sites of anaerobic microbial fermentation and function to absorb the fermentative end products (SCFA; Church, 1988). Both the reticulum and the rumen have a stratified squamous epithelium containing 4-cell strata that are layered from the serosal side to the luminal side in the order of stratum basale, stratum spinosum, stratum granulosum, and stratum corneum (Graham and Simmons, 2005).

The stratum basale cells are columnar in shape and border the lamina propria (Graham and Simmons, 2005). They are undifferentiated, but contain high metabolic activity, as supported by large nuclei and an abundant amount of mitochondria and other intracellular organelles (Graham and Simmons, 2005; Steele et al., 2011) suggesting this strata is the primary metabolic region (Leighton et al., 1983). The main transporter that utilizes cellular energy to create the asymmetric ion gradients across the rumen epithelium is the Na^+/K^+ ATPase and the density of this transporter is greatest in the stratum basale relative to the other strata. Cells in the stratum basale use the ion and electrochemical gradients to energize polarized secondary active solute transport across the epithelial tissue (Graham and Simmons, 2005). It is hypothesized that the location of the Na^+/K^+ ATPase is related to the high amount of mitochondria found in this region, also indicating a large role played by Na^+/K^+ ATPase in the electrochemical gradient found throughout the ruminal epithelium (Graham and Simmons, 2005). The stratum basale cells are joined to the neighbouring stratum spinosum cells via desmosomes (Graham and Simmons, 2005) that likely facilitate nutrient movement among cell strata.

The stratum spinosum cells are cuboidal in shape and are the location of gap junctions as shown through expression of connexin 43, an integral gap junction protein (Graham and Simmons, 2005). They are desmosome-rich, which connects the stratum spinosum cells together, and they contain intracellular organelles (nuclei, mitochondria,

ribosomes, endoplasmic reticulum, golgi apparatus, and vesicles) enabling metabolic activity (Graham and Simmons, 2005). There is expression of the tight junction (TCJ) proteins (claudin-1 and zonula occludens 1 (ZO-1)) but not as great as in the stratum granulosum, as discussed below (Graham and Simmons, 2005).

The stratum granulosum cells are squamous in shape with a reduction in intracellular organelles as they contain developing cytoplasmic keratin aggregates as they migrate and differentiate into stratum corneum cells (Graham and Simmons, 2005). They are connected to each other via TCJ, which primarily contain the two proteins claudin-1 and ZO-1 (Graham and Simmons, 2005). The TCJ play a major role in barrier function by preventing the infiltration of non-desired molecules such as antigens (histamine and lipopolysaccharide; Emmanuel et al., 2007, Khafipour et al., 2009; Gozho et al., 2005, 2006) and pathogens (Klevenhusen et al., 2013) thereby promoting transcellular passage of small molecules such as water, and monosaccharides (Graham and Simmons, 2005). Compared to the stratum spinosum, there is a reduction of intracellular organelles and reduced metabolic activity, as the cells are gradually differentiate and migrate into the stratum corneum (Graham and Simmons, 2005).

The stratum corneum cells are highly keratinized squamous cells that are in direct contact with the ruminal lumen (Lavker and Matoltsy, 1970, Steven and Marshall, 1970). These cells have no intracellular organelles, are shown to have no metabolic activity, and are continuously sloughed and replaced (Lavker and Matoltsy, 1970; Graham and Simmons, 2005). With these characteristics and no evidence of junctional protein membrane expression that would form functional occluding junctions, it is thought that the function of the stratum corneum is for protection against feed particles in the ruminal lumen rather than absorption, metabolism, or barrier function (Graham and Simmons, 2005).

The reticulum is the first fermentative chamber, located at the end of the esophagus. It is non-glandular and has a honeycomb structure, making it distinct from the rumen, with several small papillae (Church, 1988). The rumen is a compartmentalized fermentative chamber covered with leaf like papillae (10 to 15 mm in a mature cow; Graham and Simmons, 2005) that contain a heterogenous microbial population in the trough between the papillae ridges (Graham and Simmons, 2005). The rumen papillae are

shaped for optimal absorptive capacity by the papillae thickness being large relative to the length (Graham and Simmons, 2005). Most of the time, the reticulum and rumen will be acknowledged as one unit called the reticulo-rumen (Hansen et al., 2004), as the digesta contents between the two can flow freely, and generally consists of 62% of the total stomach size in cattle (Van Soest, 1982). The microbial community that populates the reticulum and rumen includes archea, bacteria, protozoa, and fungi. In addition, a small number of viruses and fungi are also present (Sherwood et al., 2013).

The ruminal papillae increase the surface area of the rumen epithelium, increasing the absorptive capacity and secretory (bicarbonate and urea) capacity (Graham and Simmons, 2005). The papillae will also adapt in size depending on the diet being fed and the length of time the diet is fed. Work by Shen et al. (2004) showed that a diet with a greater proportion of concentrate (without induction of ruminal acidosis) provided a greater concentration of propionate and butyrate in the rumen. These SCFA are thought to regulate IGF-1 production, thereby stimulating ruminal papillae growth, as it was shown that the newly proliferated rumen papillae had increased IGF-1 receptors present. Increased papillae surface area is thought to enhance absorptive capability although others have suggested that ruminal contractions may have a greater role than surface area enlargement (Storm et al., 2010; JDS).

The reticulo-ruminal epithelium is no longer considered an inert fermentative chamber and transport of numerous ions has been described. This includes active transport and passive diffusion of SCFA (Bergman et al., 1990; Aschenbach et al., 2009), active transport of sodium (Gäbel et al., 1991b; Martens, 1994; Abdoun et al., 2005; Graham and Simmons, 2005), chloride (Graham and Simmons, 2005; Aschenbach et al., 2009), potassium (Harrison et al., 1975; Martens and Gäbel, 1988), magnesium (Martens et al., 1987; Leonhard-Marek and Martens, 1996; Leonhard-Marek et al., 1998), calcium (Höller et al., 1988; Schröder et al., 1997, 1999), passive transport of water facilitated via aquaporins (Rögen et al., 2011), ammonia (Bödeker and Kemkowski, 1996;) and urea (Houpt and Houpt, 1968; Stewart et al., 2005).

2.1.2. Omasum. The epithelium of the omasum is similar to the reticulum and rumen as it is non glandular while possessing a stratified squamous epithelium (Graham

and Simmons, 2005). It is also a site of anaerobic fermentation and is compartmentalized via laminae with papillae present on the laminae (Church, 1988). The laminae increase the surface area of the fermentative organ, and it has been named “the book” or “the bible” because of this feature (Stumpff et al., 2011). The omasum generally contributes to 24% of the total stomach size in cattle (Van Soest, 1982) and approximately 40-69% of SCFA that enter the omasum are absorbed (Van Soest, 1982). The omasum is mainly responsible for water (30-60% absorbed that enters the omasum; Van Soest, 1982), SCFA, bicarbonate, calcium, magnesium, sodium, chloride, amino acid, and peptide absorption (Xu et al., 2014), and has also been shown to possess a stronger ability to absorb small peptides than the rumen (Matthews and Webb, 1995). Interestingly, while the omasum and ruminal epithelium both absorb SCFA, the rumen epithelium transport SCFA in an electro-neutral anion exchange with bicarbonate (e.g. bicarbonate secretion; Bilk et al., 2005) while bicarbonate and SCFA are absorbed in the omasum (Çaushi, 2014). Another one of the main functions of the omasum is to pump digesta from the rumen to the abomasum (Van Soest, 1982).

2.1.3. Abomasum. The abomasum in a ruminant is equivalent to that in monogastrics, only with large spiral folds in the ruminant abomasum (Church, 1988). There are 4 major areas in the abomasum: the stratified squamous epithelium region, the cardiac region, the fundic region, and the pyloric region (Duke, 1977). The fundic region and the pyloric region near the abomasum and small intestine junction are much larger in ruminants than monogastrics (Sherwood et al., 2013). The stratified squamous epithelium region is non glandular (non secretory) and is the first area where digesta enters after leaving the omasum. The cardiac region consists of a single columnar epithelium with mucus secreting cardiac pits. The fundus region consists of a glandular single columnar epithelium, gastric pits that secrete hydrochloric acid, pepsinogen (enzyme for protein digestion), and mucus, and gastric glands, which are the site where proliferation and differentiation from stem cells occur (Sherwood et al., 2013). The pyloric region is where the alkaline secreting glands are located and also contains mucus-secreting pits. A type of enteroendocrine cell, called G cells, are present in the pyloric region for secreting gastrin, which is a hormone that stimulates parietal cells to secrete hydrochloric acid from the

fundic region. The hydrochloric acid is used for protein denaturation, partial enzymatic digestion, and partial triglyceride digestion (Sherwood et al., 2013). The pH of the stomach is regulated around 2 to 4, while very little absorption occurs in this region (Sherwood et al., 2013). The tissue permeability is very low, showing strong barrier function in the highly acidic organ (Caron et al., 2015).

2.1.4. Small intestine. The small intestine consists of three separate regions known as the duodenum, jejunum, and ileum and contributes approximately 80% towards total intestinal length in cattle (Duke, 1977). This region has structural adaptations to facilitate absorption of amino acids, monosaccharides, and triglycerides, including folds, villi, and microvilli (Sherwood et al., 2013). The microvilli are hair-like projections that make up the brush border membrane (Sherwood et al., 2013). The liver and the pancreas secrete bicarbonate (for buffering capacity) and digestive enzymes into the small intestine to aid with carbohydrate, lipid, and protein digestion (e.g. amylase, lipases, and trypsinogen), that will mix with digesta entering from the acidic stomach (Sherwood et al., 2013).

Throughout the small intestine, tissue permeability is greater due to this increase in nutrient absorption (Krug et al., 2014; Turner et al., 2014; Penner et al., 2014). The majority of amino acids are absorbed as single amino acids or dipeptides in the duodenum and jejunum, while most of the di- and polysaccharides are broken down with brush border enzymes (ex. maltase, lactase and sucrase) in the jejunum (Sherwood et al., 2013). The duodenum contains Brunner's glands in the submucosa layer, which secrete an alkaline mucus to neutralize the acidic digesta from the stomach, stimulate enzyme activity, and act as lubrication. The ileum is involved with enterohepatic recirculation of bile and is used as reserve capacity, considering nutrient absorption has already occurred in the duodenum and jejunum. There are several different cells present in the small intestine including enterocytes (digestive and absorptive roles), goblet cells (mucus secretion), enteroendocrine (GIT hormone secretion), paneth cells (antimicrobial secretions), and stem cells (Sherwood et al., 2013). The epithelium is shaped into villi (discussed above) and crypts, which secrete ions for water secretion, and are the initial area for cell development (Sherwood et al., 2013). The epithelia cells from the crypts

proliferate and differentiate as they migrate to the tips of the villi and have a single columnar histological arrangement (Sherwood et al., 2013; Eurell and Frappier, 2006).

2.1.5. Cecum. The cecum is a blind pouch located at the end of the ileum and is the first region of the hindgut (Church, 1988). It contributes to 1% of total intestinal length in cattle (Duke, 1977). It has been shown by Faichney (1968) that the cecum accounts for 12% of total SCFA production in sheep, while Ulyatt et al. (1975) reported between 8.6 and 16.8%. Work by Siciliano-Jones and Murphy (1989) showed that cecal concentrations of total SCFA, acetate, propionate, and butyrate increased when Holstein steers were fed a high grain diet but the contribution of cecal SCFA to diet metabolizable energy was unaffected by diet (low or high grain diet) and averaged 8.6%. This may have been due to the majority of the SCFA being metabolized in the rumen. There has been a lack of research on the microbial fermentation process and intestinal homeostasis within the hindgut, contrary to the implied importance for animal health and production (Gressley et al., 2011). The cecum lacks micro villi and villi, but has a single columnar epithelium with crypts. The hindgut has a different buffering capacity than the rumen, as there are no salivary buffers provided in this region, and a different epithelial arrangement. Therefore a great increase in SCFA production can put the cecum at risk due to weaker regulation of pH and the opportunity for an unhealthy microbial population to develop (Metzler-Zebeli et al., 2013). Mucus secretion that occurs in the cecum, but not in the rumen, reduces the negative effect of a lower pH that can occur in this region (Church, 1988). More research is needed in this area to understand how the hindgut functions as a fermentative region.

2.1.6. Colon. The colon is the next region of the GIT after the cecum and also is highly fermentative. It is structured differently in a ruminant compared to a monogastric as it is much more elongated and organized into coils (Sherwood et al., 2013), contributing to 19% of total intestinal length in cattle (Duke, 1977). There is a great amount of water and ion absorption as well as SCFA and nitrogen absorption (ammonia and urea) due to the presence of the microbial population that resides in this region (Duke, 1977; Van Soest, 1986). It has been calculated that up to 9% of metabolizable

energy requirements in cattle are from SCFA produced in the total hindgut (cecum and colon; Bergman, 1990). The colon consists of a single columnar epithelium and there are an abundant crypts present, but no microvilli or villi (Eurell and Frappier, 2006). The types of cells present in the colon include colonocytes (absorptive for mainly sodium and other ions, water, and SCFA and secretory for water, potassium and other ions), goblet cells (mucus secretion), and enteroendocrine cells (GIT hormone secretion; Sherwood et al., 2013).

2.2 Barrier function

The GIT is the largest physical and biochemical barrier separating the host from the external environment (Peterson and Artis, 2014). The purpose of GIT barrier function is to prevent entry of non-desired molecules (e.g. pathogens and antigens) while allowing optimal nutrient, ion, and water uptake from the lumen into the epithelium and systemic circulation. Most aspects of barrier function are regulated to promote health while optimizing nutrient absorptive capacity (Jutfelt, 2011). The morphological and functional differences between each region of the GIT result in differences among regions for barrier function. Generally, regions with high absorptive capacity (e.g. jejunum) will have a greater tissue permeability and reduced barrier function (Krug et al., 2014; Turner et al., 2014) compared to regions with less tissue permeability and greater barrier function. Barrier function also seems to be related to the activity of the microbial population with regions having extensive fermentation having tighter epithelium (Jutfelt, 2011; Penner et al., 2014). For example, the colon has a tight epithelium that is perhaps related to the extensive microbial community and risk for pathogen translocation.

Barrier function is mainly regulated through desmosomes, gap junctions, adherens junctions (AJ), and tight-cell junctions (TCJ; Daugherty and Mrsny, 1999; Groschwitz and Hogan, 2009). Desmosomes are a type of junctional complex that connect epithelial cells together to protect from mechanical stress (Stahley et al., 2014). This is done by the desmosomal cadherins of adjacent cells joining to the intermediate filament cytoskeleton (Stahley et al., 2014). Desmosomes are arranged on the lateral sides of cells in both simple and stratified squamous epithelium (Stahley et al., 2014). Gap junctions are composed of a variety of connexin proteins (mainly connexin 43 in the rumen epithelium;

Graham and Simmons, 2005) and directly connect the cytoplasm from two adjacent cells through the intercellular space via multiple protein sub-units formed together with a gated channel around 2 nm in the center (Sáez et al., 2003). The function of gap junctions is to allow paracellular movement of small molecules such as ions (potassium), SCFA, sugars, and signaling molecules (ex. cyclic nucleotide signaling molecule; Bruzzone et al., 1996; Sáez et al., 2003; White et al., 2003). Adherens junctions and desmosomes have been proposed to be more important for joining adjacent cells (Hartsock and Nelson, 2008) rather than transport of molecules between cells like gap junctions (Bruzzone et al., 1996) or the regulation of paracellular transport through the GIT epithelium like TCJ (Itallie and Anderson, 2014). The AJs are protein complexes located on the lateral side of the cell that develop through cadherin-catenin interactions between transmembrane proteins, intracellular adaptor proteins, and the cytoskeleton (Groschwitz and Hogan, 2009). Tight-cell junctions are also protein complexes, but they contain more types of proteins and are the most apical located adhesional junction (Groschwitz and Hogan, 2009). There is also no 15-20 nm gap between the cell membranes where TCJ are located compared to AJ and desmosomes (Groschwitz and Hogan, 2009). An expansion on TCJ and their role with barrier function is discussed below.

2.2.1. Defining barrier function. There are three different types of barriers that can be subdivided within the GIT (Jutfelt, 2011). The first is the extrinsic barrier, consisting of luminal secretions and the commensal microbial population within the GIT. The commensal microbes throughout the GIT provide competition for attachment and resources against invading pathogens. Commensal microbes can also secrete bactericidal substances such as peptide bacteriocins, hydrogen peroxide, and lactic acid, which limit the growth of pathogenic microbes in the GIT lumen (Jutfelt, 2011). Goblet cells that are located within the GIT epithelium secrete mucus, which is used to remove harmful molecules from the epithelial cells as well as reduce mechanical wear on the epithelium (Jutfelt, 2011). Other luminal secretions to control pathogens include antimicrobial factors (antimicrobial peptides such as defensins (Jäger et al., 2010)), reactive oxygen species, and hydrogen peroxide (Jutfelt, 2011).

The second is the intrinsic barrier. The intrinsic barrier consists of the epithelial cells along with cell junctions as a physical and biochemical barrier against non-desired molecules (toxins, pathogens, and the commensal microbial population) while promoting limited interference with the nutrient, ion, and water absorptive capacity. The intrinsic barrier is the physical barrier that separates host from the external environment. The cell junctions within the intrinsic barrier are discussed above, which regulate paracellular movement. In regards to transcellular movement of molecules, lipophilic molecules can cross freely across the epithelial cell membrane, while ions and hydrophilic molecules mainly use membrane bound transport systems (Jutfelt, 2011). Although both types of movement are important, barrier function consists of only paracellular movement of molecules across the GIT epithelium.

The third is the immunological barrier. Immune cells contribute by mounting an appropriate immune response when necessary, including both the innate and adaptive immune systems within the epithelium and lamina propria (Jutfelt, 2011). The microfold cells of the follicle-associated epithelium of the mucosal-associated lymphoid tissue and dendritic cells within the epithelium will sample the lumen via endocytosis, phagocytosis, or transcytosis for early detection of harmful substances that have entered the GIT and the opportunity to produce antibodies specific for the threatening molecule (Man et al., 2004). Another reason for luminal sampling is for the host to build tolerance to the commensal microbial population in order to avoid mounting an immune response when unnecessary (Jutfelt, 2011). In humans, compromised barrier function has been linked to autoimmune and inflammatory disease such as irritable bowel syndrome and colitis (Peterson and Artis, 2014). In ruminants and other monogastrics involved in livestock production, barrier function is important in order for the animal to maintain high health status by preventing disease formation resulting from pathogen infiltration through the GIT epithelium and to attain optimal production by allowing adequate nutrient digestion and absorption throughout the GIT. Indicators of barrier function that can be evaluated include tissue permeability, mucus secretions, commensal microbial populations, which can be measured through the Ussing chambers and digesta analysis. The use of these tools has been imperative to furthering barrier function research.

2.2.2. The role of tight-cell junctions. Tight-cell junctions are adhesive protein-protein junctions between epithelial cells that are located closest to the apical side of the cell. They form a continuous ring that seals around the epithelial cells and contain 4 different transmembrane proteins: claudins, occludens, junctional adhesion molecules (JAM), and tricellulin (Groschwitz and Hogan, 2009). Tight-cell junctions regulate the paracellular transport of ions and solutes and also contribute to regulating cell proliferation, polarization, and differentiation by interactions with scaffolding proteins, adaptor proteins, and signaling complexes via their intracellular domains (Groschwitz and Hogan, 2009). Both homophilic (between identical proteins) and heterophilic (between non identical proteins) interactions occur within TCJ complexes. These also occur in both cis and trans conformations (Groschwitz and Hogan, 2009). The flexibility in TCJ interactions is important considering their vital protective role of the GIT from pathogen invasion. The expression of TCJ proteins depends on what region of the GIT they are located in (as discussed above), villus/crypt localization (absorption at villi versus secretion in the crypts), whether they are located on the apical, basolateral, or lateral side of the epithelial cell, and the phosphorylation state of the protein as phosphorylation can stimulate both TCJ formation and destabilization (Groschwitz and Hogan, 2009).

Amino acids have been shown to influence TCJ integrity. Acetaldehyde has shown to disrupt barrier function by redistributing TCJ proteins (specifically occludens and ZO-1) from the intercellular junction (Seth et al., 2004). Work by Atkinson and Rao (2001) showed that acetaldehyde inhibited the protein tyrosine phosphatase in Caco-2 monolayer cells, which indicated reduced TCJ integrity by increased phosphorylation of tyrosine in the occluden and ZO-1 proteins. The amino acid $_{L}$-glutamine has shown to reduce the negative effects of acetaldehyde on barrier function by reducing tissue permeability to inulin and lipopolysaccharide (LPS), and by inducing tyrosine phosphorylation on the epidermal growth factor receptor, which is required for the $_{L}$-glutamine-mediated protection from acetylaldehyde (Seth et al., 2004). The amino acids $_{D}$-glutamate, $_{L}$-asparagine, $_{L}$-arginine, $_{L}$-lysine, and $_{L}$-alanine did not show protective effects for TCJ integrity (Seth et al., 2004). Amino acids have shown to influence TCJ gene expression as well. An example of a stimulator of TCJ protein gene expression is $_{L}$-glutamate. Work by Jiao et al. (2015) showed that $_{L}$-glutamate, which is one of the major

amino acids in milk and post-weaning diets for mammals, can stimulate enhanced gene expression of the TCJ proteins occludin, claudin-3, zonula occludens (ZO)-2, and (ZO)-3 in intestinal porcine epithelial cells when being challenged with diquat (an oxidant). It was also shown that $_{L}$-glutamate increased cell growth and transepithelial electrical resistance through enhanced mRNA expression of $_{L}$-glutamate transporter solute carrier family 1 member 1 and increased protein abundance of excitatory amino acid transporter 3. These results indicate that $_{L}$-glutamate enhances mucosal barrier function and plays a role in maintaining epithelial integrity when exposed to oxidative stress. There is a paucity of research in this area for ruminants. Tight-cell junctions are a vital component to effective barrier function within the GIT as shown through the relationship between TCJ and tissue permeability, cell proliferation, and cell differentiation.

2.2.3. Host-microbial communication and regulation. The commensal microbial population found throughout the GIT assists with digestion, nutrient availability, and in the development and functionality of the mucosal immune system located within the GIT epithelium (Peterson and Artis, 2014). Tolerance to the commensal microbial population is achieved in the host so the immune system is not over stimulated. If there is a disruption in the barrier function of the GIT epithelium, the commensal microbial population could become opportunistic and lead to infection or inflammation. Maintaining a physical barrier is important to protect the host by allowing the symbiotic relationship between the host and GIT microbial populations to function properly (Peterson and Artis, 2014). The commensal microbial population provides products for the host to use as a source of nutrition (e.g. SCFA as an energy source from microbial carbohydrate fermentation), and they also allow for competition of attachment space and resources for invading pathogens (Jutfelt, 2011). The microbial population also inhibit the growth of pathogens by secreting bactericidal substances (peptide bacteriocins, hydrogen peroxide, and lactic acid; Jutfelt, 2011). The relationship between commensal bacteria and the host operates effectively and efficiently if regulated through barrier function. Although the rumen and fecal microbial population in ruminants has been studied and used as an indicator of animal health (Dowd et al., 2008; Lettat et al., 2012; Van Baale et al., 2004; Van Donkersgoed et al., 1999), there has been a paucity of research in

characterizing the type of microbial population that is present in the ruminant hindgut. According to Valério de Oliveira et al. (2013), there is a lower amount of phylogenetic diversity in the hindgut compared to the foregut in cattle (Valério de Oliveira et al., 2013), with the main species of microbes being *Bacteroidetes* in the foregut and *Firmicutes* dominating the hindgut. There is regional GIT tolerance to the commensal microbiota to account for this variation of microbial species present.

2.3 Nutritional challenges that may compromise GIT barrier function.

Despite best management approaches, there is the potential for cattle to be exposed to nutritional challenges. These challenges, *inter alia*, may include constraints to the amount of feed available, access to available feed, physiological changes that induce a reduction in feed intake, and production settings where cattle are exposed to large changes in the proportion of fermentable carbohydrate. Two nutritional challenges that affect the beef and dairy industry are low feed intake and ruminal acidosis. A common factor with these challenges is that they alter the luminal conditions and have direct impacts on the GIT.

2.3.1. Low feed intake. There are situations on farm that can cause cattle to reduce feed intake for varying periods of time. Depending on the cause, the interruption in feeding can range from days to weeks, leading to negative impacts on health and performance (Rhoads et al. 2009). How the GIT reacts to low feed intake can vary depending on the physiological status of the animal (transition cow v.s. feedlot steer), the type of diet being offered, and whether it is voluntary reduction in feed intake (when the animal makes the choice not to consume feed due to stress or other factors) or restrictive reduction in feed intake (when the animal is restricted from feed such as during transportation).

*2.3.1.1. Practical examples of situations causing low feed intake***.** There are situations on farm that cause animals to reduce feed intake (Table 2.1). Some examples include parturition (Bertics et al., 1992; Hayirli et al., 2002), weaning (Boland et al., 2008; Haley et al., 2005), heat stress (Rhoads et al., 2009; Huber 1996, Pearce et al.,