# EFFECT OF THE SEVERITY OF SHORT-TERM FEED RESTRICTION ON GUT FUNCTION

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## ABSTRACT

The objective of this study was to evaluate whether different severities of short-term feed restriction (FR) affect the absorptive function of the reticulo-rumen and total tract barrier function in beef cattle and also to determine the timeline for recovery of these functions. Eighteen ruminally cannulated and ovariectomized Angus × Hereford heifers were blocked by BW and randomly assigned to 1 of 3 treatments differing in the severity of FR. Feed offered was restricted to 75, 50 and 25% of the ad libitum feed intake measured during FR relative to that measured during the 5-d baseline period (BASE). Feed restriction (FR) was imposed for 5 d followed by 3 consecutive wk of recovery in which cattle were fed ad libitum (REC1, REC2 and REC3). Throughout the study, heifers were housed in individual pens (9 $m^2$) and were fed the same diet (60% forage: 40% concentrate) with free access to water. Dry matter intake (DMI) was measured daily and ruminal pH was measured every 2 min throughout the study. Ruminal fluid and blood samples were collected on d 3 of the BASE and FR and on d 5 of REC1 and REC3. The temporarily isolated and washed reticulo-rumen technique (WRR) was used to evaluate short-chain fatty acid (SCFA) absorption on the last day of BASE, FR, REC1 and REC3. Total tract barrier function was evaluated starting on d 2 of the BASE and FR and on d 4 of REC1 and REC3 using a pulse dose of chromium ethylenediaminetetraacetic acid (Cr-EDTA) followed by 48-h of total urine collection. Dry matter intake did not differ among treatments during BASE but, as imposed by the experimental model, DMI during FR relative to BASE equated to 70, 49, and 25%, which was close to the targeted values of 75, 50, and 25% (treatment × period, $P < 0.001$). A treatment × period interaction ($P < 0.001$) was also detected for SCFA concentration with the concentration decreasing as the severity of FR increased, whereas there were no differences during BASE. Absorption of SCFA across the reticulo-rumen tended to decrease with increasing severity of FR ($P = 0.080$). Acetate absorption (mmol/h) decreased ($P = 0.050$) by almost 70 mmol/h at 25 and 50% feed relative to BASE (322 mmol/h). Heifers restricted to 25% feed had greater urinary Cr recovery during FR than BASE while no changes were detected for those restricted to 75 and 50%. After FR, DMI (% BW) increased rapidly in REC1 for heifers restricted to 75 and 50%; however heifers restricted to 25% needed at least 2 wk to recover (treatment × period, $P < 0.001$). Regardless of the severity of FR, the duration that pH < 5.5 was highest during REC1 (period $P < 0.001$). A treatment × period interaction was observed for the absolute

absorption rate of total SCFA ($P$ = 0.009) where the total SCFA absorption rate was not different for heifers restricted to 75 and 50% across periods while an increase from FR and REC1 to REC3 was detected for heifers restricted to 25% of ad libitum intake. A treatment effect was observed for urinary Cr output ($P$ = 0.027) indicating that heifers previously restricted to 25% of ad libitum intake had greater Cr excretion in urine during recovery. This study indicates that moderate short-term FR has negative effects on the absorptive function of the reticulo-rumen but more severe FR is required to compromise total tract barrier function in beef cattle. Severe FR also negatively affects the time required for recovery of reticulo-rumen absorptive function and total tract barrier function. Regardless of severity, FR increases risk for ruminal acidosis when heifers have free access to feed after FR.

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## TABLE OF CONTENTS

## LIST OF TABLES

## LIST OF FIGURES

## LIST OF ABBREVIATIONS

| | |
|---|---|
| AE | Anion exchanger |
| BASE | Baseline |
| BCS | Body condition score |
| BHBA | β-hydroxy butyric acid |
| BW | Body weight |
| cAMP | Cyclic adenosine monophosphate |
| CP | Crude protein |
| Cr-EDTA | Chromium ethylenediaminetetraacetic acid |
| DCAD | Dietary cation-anion difference |
| DM | Dry matter |
| DMI | Dry matter intake |
| DRA | Down-regulated in adenoma |
| FR | Feed restriction |
| IGF-1 | Insulin like growth factor 1 |
| ipH | Intracellular pH |
| MCT | Monocarboxylate transporter |
| NDF | Neutral detergent fiber |
| NEFA | Non-esterified fatty acids |
| NHE | $Na^+/H^+$ exchanger |
| OM | Organic matter |

| | |
|---|---|
| PAT | Putative anion transporter |
| pCMBA | p-chloromercuribenzoic acid |
| REC | Recovery |
| SCFA | Short-chain fatty acid |
| WRR | Wash reticulo-rumen |
| ZO | Zonula occluden |
| 3-OMG | 3-O-methyl-α-D-glucose |

## 1.0. GENERAL INTRODUCTION

In beef and dairy production, a number of stressors cause a reduction in DMI. For example, weaning beef calves is usually accomplished via abrupt separation and stressors such as separation from the dam, change in diet, and re-mixing with other calves (Stookey and Watts, 2007) may induce a reduction in DMI. In addition, many behavioral changes occur after weaning including increased vocalization and walking time, and decreased eating and lying time (Price et al, 2003; Haley et al., 2005). Although there are no data showing the difference in DMI before and after weaning, a loss of weight 3 to 5 d after weaning likely implies that FR occurs (Stookey and Watts, 2007).

Another example is transportation induced short-term FR. González et al. (2012) conducted a survey showing that current commercial long haul transport (≥ 400 km) of beef cattle from Alberta was on average 16 h. Although cattle are usually without feed and water during transportation, the short duration of the transport indicates that cattle are likely exposed to short-term FR associated with the overall process of transportation, rather than complete feed deprivation. Multiple reasons such as crowded conditions during transportation and the stress associated with the exposure to a new feedlot environment further contribute to FR for newly received feedlot cattle (Grandin, 1997). In fact, newly arrived feeder calves typically only consume 0.5 to 1.5% of their BW during the first wk, 1.5 to 2.5% of their BW in the second wk, with normal intakes (2.5-3.5%) being achieved somewhere around the 3$^{rd}$ to 4$^{th}$ wk after arrival (Hutcheson and Cole, 1986).

For dairy cows, a 30% reduction in DMI has been observed during the final week before calving (Bertics et al., 1992). Similar results were found using a data set including 699 Holstein cows fed a variety of diets (49 in total) showing that DMI decreased by 32% during the final 3 wk of calving with 89% of that decline occurring in the final week before calving (Hayirli et al, 2002). Additionally, in the same study, a much greater DMI suppression was observed in the dairy cows having higher body condition score (BCS). Digestive disorders (e.g. acidosis; Owens et al., 1998; Kleen et al., 2003) or metabolic diseases (e.g. ketosis; Goldhawk et al., 2009) also cause short-term FR for beef and dairy cattle. Based on the data presented above, short-term FR

is associated with weaning, transportation, parturition, and various disease states for beef and dairy cattle.

In monogastric animals, long-lasting impacts of short-term FR have been shown on the absorptive and barrier functions of the gastrointestinal tract (Spreeuwenberg et al., 2001; Boudry et al., 2004). However, the effects of short-term FR on the function of the gastrointestinal tract have not been studied extensively in ruminants. As previous studies have shown that the severity of FR ranges from a reduction of intake to 68% on d 1 pre-partum relative to 21 d pre-partum for dairy cattle (Hayirli et al., 2002) to only 25% of expected intake during the first wk of feeding for highly-stressed newly received beef calves (Hutcheson and Cole, 1986), the current study was designed to evaluate the impact of different severities of short-term FR (75, 50 and 25% of ad libitum DMI) on ruminal absorptive function and total tract barrier function, and the time needed to recover after cattle return to ad libitum feeding.

## 2.0. LITERATURE REVIEW

### 2.1. Rumen Physiology

#### 2.1.1. Structure of the Ruminal Epithelium

The ruminal epithelium consists of four cell strata. These strata, from luminal to basolateral, are the: 1) stratum corneum; 2) stratum granulosum; 3) stratum spinosum; and 4) the stratum basale (Steven and Marshall, 1970). Under light microscopy, the outermost layers of the stratum corneum appear to be loosely associated with the microflora and are intensely keratinized and flattened. This layer consists of squamous cells and is thought to form an important barrier protecting the underlying strata (Graham and Simmons, 2005). The localization of claudin-1 and zonula occludin-1 (integral proteins of tight-cell junctions) are present at the stratum granulosum of bovine ruminal epithelia which suggests that the permeability barrier of ruminal epithelia is most likely regulated by this strata rather than by the stratum corneum (Graham and Simmons, 2005). The expression of the gap junction protein, connexin 43, was found to be localized in the stratum granulosum, stratum spinosum and stratum basale in bovine ruminal epithelia by Graham and Simmons (2005). The authors proposed that the expression of connexin 43 in the three layers was to form of a functional syncytium facilitating intercellular communication within and between the cells of the stratum granulosum, stratum spinosum and stratum basale. The stratum basale is characterized as having columnar cells with the long axis lying perpendicular to the basal membrane (Graham and Simmons, 2005). Graham and Simmons (2005) also found that $Na^+$-$K^+$-ATPase was predominately expressed in the stratum basale with decreasing density toward the stratum spinosum and stratum granulosum, and no expression in the stratum corneum. The $Na^+$-$K^+$-ATPase transporter maintains a gradient of $Na^+$ across the epithelium providing the electrochemical driving force for $Na^+$-coupled transport such as occurs for glucose and amino acids (Lingrel and Kuntzweiler, 1994) as well as apical uptake of $Na^+$ (Leonhard-Marek et al., 2010). The polarized localization of the $Na^+$-$K^+$-ATPase characterizes the ability of transepithelial $Na^+$ transport in all epithelial cells of the bovine rumen epithelium with the main absorptive and metabolic function in the stratum basale.

Overall, the ruminal epithelium ranges in thickness between 8 and 10 cells (Lavker et al., 1969) with the ruminal epithelial surface area greatly increased by dense leaf-like shaped

extensions called papillae. Papillae are extensions of the lamina propria covered with stratified squamous epithelium (Goodlad, 1981). While the change of papillae surface area is often used as an indicator for an adapted rumen epithelium (Dirksen et al., 1985), the absorptive surface area includes papillae density as well as the three-dimensional surface area. This poses a problem in interpreting results for many of the previous studies as papillae density is rarely reported (Hofmann, 1985). Despite this, numerous studies have been conducted to evaluate strategies to enhance papillae proliferation (Sakata and Tamate, 1978; Gaebel et al., 1987; Mentschel et al., 2001). It is well established that papillae proliferation and hypertrophy occur in response to dietary energy-dependent alternations via butyrate and propionate regulation of insulin like growth factor 1 (IGF-1) production (Gaebel et al., 1987; Shen et al., 2004). Under extreme conditions, large papillae in the ventral sac can exceed 15 mm in length in cattle (Dirksen et al., 1985; Reynolds et al., 2004; Graham and Simmons, 2005).

Despite promoting proliferation, rumen papillae surface area is not uniform throughout the rumen with lowest papillae surface area in the dorsal sac and largest papillae surface area observed in the ventral sac; the location most commonly used to measure papillae surface area (Kamler, 2001). However differences in the pattern of blood flow cannot be detected between the adjacent regions (Barnes et al., 1983) and cell activity does not differ across locations (Waldron et al., 2002). The difference in the papilla surfaces in different regions of the rumen may be due to the different concentrations of SCFA.

### 2.1.2. Absorptive and Secretory Functions of the Ruminal Epithelium

For ruminants, the reticulo-rumen contributes extensively to the absorption, and in some cases secretion, of ions and non-charged molecules including SCFA (Bergman et al., 1990; Gäbel et al., 1991a; Aschenbach et al., 2009), ammonia ($NH_3/NH_4^+$; Bödeker and Kemkowski, 1996; Abdoun et al., 2005), urea (Houpt and Houpt, 1968; Stewart et al., 2005), peptides and amino acids (Broderick and Wallace, 1988; Dirienzo, 1990; Matthews and Webb , 1995), glucose (Zhao et al., 1998; Aschenbach et al., 2000; Gäbel and Aschenbach, 2002), minerals like $Na^+$ (Gäbel et al., 1991b; Martens, 1994; Abdoun et al., 2005), $Mg^{2+}$ (Martens et al., 1987; Leonhard-Marek and Martens, 1996; Leonhard-Marek et al., 1998), $Ca^{2+}$ (Höller et al., 1988; Schröder et al., 1997 and 1999), $K^+$ (Harrison et al., 1975; Martens and Gäbel, 1988; Leonhard-

Marek et al., 2010) and $Cl^-$ (Aschenbach et al., 2009; Leonhard-Marek et al., 2010), and buffers ($HCO_3^-$; Gäbel et al., 1991a; Kramer et al., 1996; Penner et al., 2009a). As this thesis focuses on SCFA absorption, the subsequent section will describe the current knowledge regarding SCFA absorption. Mineral absorption and secretion will be emphasized in context with SCFA absorption.

#### 2.1.2.1. Mechanisms of Short-Chain Fatty Acid Absorption

Ruminal SCFA concentration generally ranges between 60 and 120 m*M* in bovine ruminal contents with proportions of approximately 60 to 70% acetate, 15 to 20% propionate and 10 to 15% butyrate (Titus and Ahearn, 1992). These SCFA are produced by microbial fermentation of dietary carbohydrates (Demeyer, 1981; Wolin and Miller, 1983; Bergman, 1990) and constitute up to 80% of the total energy required for maintenance of the host and 65-75% of the total metabolizable energy supply (Bergman, 1990). Few studies have quantified total SCFA production in the rumen but based on studies using multiparous lactating Holsteins in mid-to-late lactation, SCFA production can exceed 90 mol/d (Sutton et al., 2003). Interestingly, accumulation (concentration) and production are generally positively correlated, but it should be cautioned that production and concentration are not equivalent (Sutton et al., 2003). Based on previous studies, it appears that approximately 88% of the SCFA produced are absorbed from the rumen with only 12% appearing in the omasum (Bergman, 1990).

Over the last two decades, the knowledge on mechanisms involved in SCFA absorption has greatly increased. The apical absorption of SCFA can occur when SCFA are in either the protonated form (H-SCFA) via simple passive permeation or in the ionized form via electroneutral transport pathways ($SFCA^-$; Ash and Dobson, 1963; Gäbel and Aschenbach, 2006). However, because the pKa of acetate, propionate and butyrate are 4.75, 4.87 and 4.82 respectively (Cistola et al., 1982), according to the Henderson-Hasselbalch equation, approximately 90 to 99% of SCFA present in the rumen are in the dissociated form under physiological ruminal pH (5.8 to 6.8; Aschenbach et al., 2011). This suggests that passive diffusion of SCFA has been over-emphasized as the dominant mechansism for SCFA absorption. Supporting this, Penner et al. (2009a) showed that, for acetate and butyrate, only approximately 28 to 60% and 31 to 72%, respectively cross the epithelium via passive diffusion.

With passive diffusion, the absorbed SCFA will rapidly dissociate in the cytosol. This results in the release of a free proton that can lead to challenge to intracellular pH (ipH) homeostasis (Müller et al., 2002). To help regulate ipH, $Na^+/H^+$ exchangers (NHE) are used. The NHE family members are found in the ruminal epithelium with NHE1 mainly found in the stratum granulosum and NHE2 mainly located on the basal side (Graham et al., 2007). Sodium-hydrogen exchangers import $Na^+$ into epithelial cells and export $H^+$ to the lumen of the rumen thereby stabilizing ipH by utilizing the electrochemical gradient generated via the $Na^+$-$K^+$-ATPase. Proton secretion has also been hypothesized to maintain an acidic micro-climate on the apical side thereby promoting passive diffusion of protonated SCFA (Gäbel et al., 1991b). However, there have been several constraints to this theory including detection of a basic micro-climate on the ruminal epithelium (Leonhard-Marek et al., 2006) likely due to $HCO_3^-$ secretion, and that the expected rates of SCFA do not equate to SCFA lipophilicity (Walter and Gutknecht, 1986).

Apical uptake of runimal $Na^+$ occurs via electroneutral and electrogenic pathways (Gäbel et al., 1991b; Martens, 1994; Abdoun et al., 2005). The electroneutral pathway is mainly via NHE with $Na^+$ being absorbed into the cells and $H^+$ being extruded to the rumen (Martens et al., 1991). Nine isoforms of NHE have been identified in different tissues (Lam et al., 2009) and according to Graham et al. (2007) NHE1, NHE2, NHE3 and NHE8 can be detected in the bovine rumen epithelium. The NHE pathway is stimulated by ruminal fermentation and helps to maintain cellular pH homeostasis by removing protons associated with SCFA and $NH_4^+$ absorption (Leonhard-Marek et al., 2010). The electrogenic pathway for $Na^+$ uptake is via a non-selective cation channel that can be blocked by divalent cations such as $Ca^{2+}$ and $Mg^{2+}$ (Leonhard et al., 1990; Schultheiss and Martens, 1999). This pathway is driven by the negative electrical potential between cytosol and the rumen: thus to reduce the electrical gradient, positively charged ions from outside of the epithelial cells are absorbed (Leonhard-Marek et al., 2010).

Many previous studies have shown that the electroneutral pathway for $Na^+$ absorption predominates when the $Na^+$ concentration is high or when the ruminal pH is low. In contrast, at low $Na^+$ concentrations or high pH the electrogenic pathway is the primary mechanism (Martens et al., 2004; Abdoun et al., 2005; Leonhard-Marek et al., 2006). The $Na^+$-$K^+$-ATPase is found

predominately expressed on the basoleteral side of the rumen epithelium (Granham and Simmons, 2005). Once $Na^+$ is moved across the multiple layers of the rumen epithelium, $Na^+$ will be released into portal circulation via the $Na^+$-$K^+$-ATPase (Harrison et al., 1975; Martens and Gäbel, 1988). That said, $Na^+$ movement can occur in both directions although the serosal-to-mucosal movement is much smaller than the mucosal-to-serosal movement with the former likely including cotransport with $HCO_3^-$ and the paracellular pathway (Leonhard-Marek et al., 2010).

Although rates of absorption for SCFA cannot be predicted based on their lipophilic properties, absorption rates do differ among SCFA. Given the same concentration of SCFA, the absorption rate of butyrate is nearly twice as that of acetate and propionate (Rechkemmer et al., 1995). Furthermore, the pathway of absorption also differs. A recent study by Penner et al. (2009a) showed that the proportion of butyrate uptake via passive diffusion ranged from 31 to 72% while passive diffusion only accounted for 28 to 60% of acetate flux. This implies that passive permeation may be the major pathway for apical butyrate uptake because, relative to acetate, butyrate has higher lipophilicity, and greater rates and extents of intra-epithelial metabolism which will help to promote a favorable concentration gradient (Sehested et al., 1999; Kristensen et al., 2000a; Gäbel et al., 2002; Gäbel and Aschenbach, 2006).

#### 2.1.2.2. Short-Chain Fatty Acid Transporters in the Ruminal Epithelium

The family of anion exchangers (AE), down-regulated in adenoma (DRA) and putative anion transporter (PAT) are found in the intestinal epithelium and function as $HCO_3^-/Cl^-$ exchangers through which the $HCO_3^-$ is exported and $Cl^-$ is transported inside the epithelial cells in human and monogastric animals (Hoglund et al. 1996; Rajendran et al. 2000; Wang et al. 2002). Recently AE2, DRA and PAT1 expression has been confirmed for ruminal epithelial tissue and have been suggested to be potential candidate transporters for $SCFA^-$ (Bilk et al., 2005). Studies have shown that $HCO_3^-$ secretion is strongly correlated with $SCFA^-$ absorption (Gäbel et al., 1991a; Kramer et al., 1996; Gäbel and Sehested, 1997) and that $Cl^-/HCO_3^-$ exchangers are likely involved. The apparent competition between $Cl^-$ and $SCFA^-$ absorption has been demonstrated using the temporarily isolated and washed reticulo-rumen (WRR) technique (Aschenbach et al., 2009). In that study, the authors demonstrated that the $Cl^-$ absorption rate

decreased by about 50% when the total SCFA concentration was doubled with acetate and propionate having the main inhibitory effects on $Cl^-$ absorption (Aschenbach et al., 2009). However, that study also showed that $Cl^-$ can inhibit the absorption rates of acetate and propionate. This evidence proved the existence of a competition between $Cl^-$ and $SCFA^-$ in terms of their absorption rate, likely mediated via $Cl^-/HCO_3^-$ exchangers.

In vitro studies using Ussing chambers have also shown that omission of $HCO_3^-$ significantly decreases acetate uptake which proves that at least part of the SCFA absorption is $HCO_3^-$ dependent (Aschenbach et al., 2009). Addition of nitrate ($NO_3^-$) also decreases ruminal absorption of acetate, with or without the presence of $HCO_3^-$, to a similar extent indicating that part of the acetate absorption is $HCO_3^-$ independent but nitrate sensitive (Aschenbach et al., 2009). Similar results were found in a study by Penner et al. (2009a) showing that acetate uptake by the isolated ruminal epithelia includes $HCO_3^-$ dependent, $HCO_3^-$ independent nitrate sensitive, and passive diffusion mechanisms, while butyrate absorption was only mediated via $HCO_3^-$ dependent and passive diffusion mechanisms.

Following SCFA uptake into ruminal epithelial cells, approximately 30% of acetate, 50% of propionate, and 90% of butyrate are metabolized in the ruminal epithelia (Bergman, 1990). Previous studies have also shown that butyrate is the major SCFA metabolized within the ruminal epithelia (Britton and Krehbiel, 1993; Kristensen et al., 2000a,b; Kristensen and Harmon, 2004). The ketone bodies and lactate produced during SCFA metabolism are secreted into portal circulation by monocarboxylate transporters (MCT) which have 14 family members (MCT1 - MCT14) with MCT1 and MCT4 being functionally characterized and suitable for transporting a variety of monocarboxylates (Halestrap and Price, 1999; Halestrap and Meredith, 2004). Localization of MCT1 has been confirmed in the stratum basale of sheep ruminal epithelium using immunocytochemical methods and is important in aiding in intracellular pH homeostasis (Müller et al, 2002). Similar results have been found in bovine ruminal epithelium indicating that MCT1 is intensively distributed in the stratum basale with weak distribution in the stratum spinosum with little-to-no florescence in the stratum corneum and stratum granulosum (Graham et al., 2007). Recent in vivo and in vitro functional studies have led to the suggestion that MCT1 has a direct role in SCFA transport as the acetate absorption rate decreases by up to 55% when a MCT inhibitor, p-chloromercuribenzoic acid (pCMBA), was applied to the caprine rumen

epithelia (Kirat et al, 2006). However, this study did not prove whether the effect of pCMBA was due to inhibition of basolateral secretion, ipH regulation, or apical uptake, but it did prove that MCT1 was involved in SCFA transport across the rumen.

For MCT2, limited quantities of protein have been detected in ruminal tissue from adult bovine and no discernible detection in reindeer rumen (Graham et al., 2007; Koho et al., 2005). In contrast, MCT4 has been detected in the ruminal epithelium and it is mainly confined to the stratum corneum and cell boundaries of the stratum granulosum (Kirat et al, 2007). Since the stratum corneum is highly keratinized, functionality of MCT4 in this stratum is questionable. Furthermore, adding MCT inhibitors like pCMBA or phloretin to the mucosal side had no effect on acetate uptake with the Ussing chamber technique (Aschenbach et al., 2009). This indicates that SCFA extrusion is mediated by MCT on the basal side but uptake of SCFA is likely not mediated via MCT. Monocarboxylate transporters have been proven to be important in terms of regulating ipH (Müller et al., 2000; Gäbel et al., 2002).

In summary, the major energy substrates for ruminants are SCFA which are absorbed via passive diffusion of protonated SCFA or via transporters for the anion ($SCFA^-$). A figure modified from Aschenbach et al. (2011) demonstrating SCFA absorption in regulation with other molecules has been shown in this section (Figure 1.1). Future work is needed to elucidate how diet and feeding management affect the relative impact of various mechanisms involved in SCFA transport.

### 2.1.3. Barrier Function of the Ruminal Epithelium

#### 2.1.3.1. Stucture of the Ruminal Epithelial Barrier

Barrier function can be thought of as the regulation of paracellular movement. This regulation is mainly accomplished by tight junctions, zonula adherens junctions, desmosomes and gap junctions (Daugherty and Mrsny, 1999). On the apical side (predominantly, the stratum granulosum) of the epithelium, tight junctions serve as a gatekeeper of paracellular pathways with a continuous belt-like structure at the end of the luminal side of the intercellular space (Farquhar and Palade, 1963). The major components of tight junction proteins include transmembrane proteins (occludins, claudins, and junctional adhesion molecules), peripheral

membrane proteins (zonula occludins) and cytoplasmtic proteins (spectrin, cingulin, symplekin; Daugherty and Mrsny, 1999). To form the "kiss" region between two adjacent cells, the

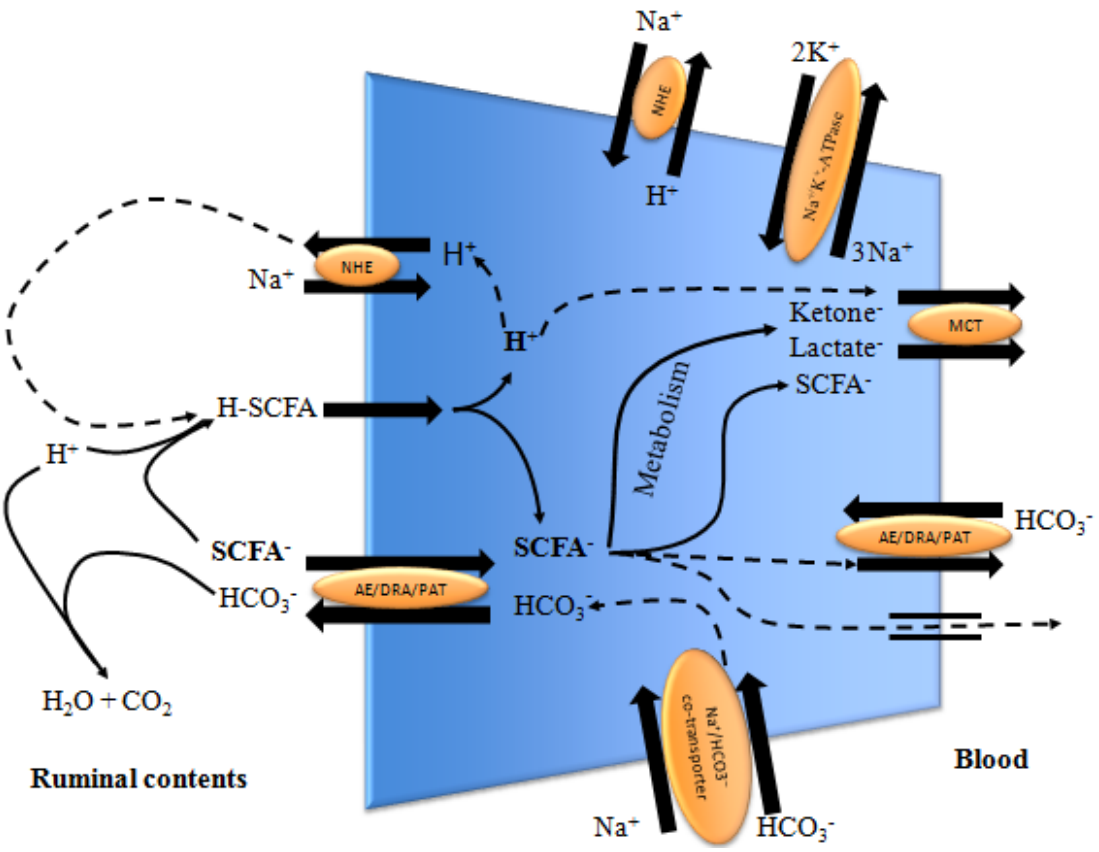


Figure 2.1. Model for SCFA transport across the ruminal epithelium. For clarity, one cell is presented although the ruminal epithelia consist of 4 strata with multiple cell layers for each stratum. This figure is modified from Aschenbach et al. (2011).

extracellular domains of the transmembrane proteins form contacts with the proteins of adjacent cells. This allows for interaction of the cytoplasmic tails of the transmembrane proteins and the peripheral membrane proteins (Zheng et al., 2006). The function of the cytoplasmic proteins is not well elucidated so far, however the interactions between the cytoskeleton and occludins have

suggested occurring through spectrin. This implies that the cytoplasmic proteins form the linkage between the peripheral proteins of tight junctions and the cytoskeleton (Daugherty and Mrsny, 1999).

Tight-cell junctions are permeable to small non-charged solutes (hydrophilic), and are capable of discriminating between ions with similar charge. This allows for selective permeability depending on the concentration of non-charged molecules and surrounding pH (Tang and Goodenough, 2003; Yu et al, 2003). Although tight-cell junctions form the primary physical barrier protecting cells and the host animal from infectious agents, some tight-cell junction proteins like claudins and zonula occludins are targets for factors expressed or released by bacteria, viruses, and parasites (Berkes et al., 2003; Schneeberger and Lynch, 2004). Once the infectious agents gain access to the tight-cell junction proteins, it can cause the failure of epithelial barrier function by disruption of tight junction structure. Using immunofluorescence, tight-cell junction proteins claudin-1 and zonula occluden 1 (ZO-1) have been shown to be localized in the stratum granulosum with decreasing intensity through the stratum spinosum to the stratum basale and a lack of expression in the stratum corneum (Furuse et al., 2002; Tsukita and Furuse, 2002; Graham and Simmons, 2005).

Gap junctions are mediated by the connexin gene family and the ubiquitously expressed gap junction protein is connexin 43 (Sáez et al., 2003). Recently, connexin 43 localization has been demonstrated with primary expression in the stratum spinosum and granulosum with decreasing density in stratum basale of the ruminal epithelium (Graham and Simmons, 2005). Gap junctions serve as a channel between cells for small ions like $Na^+$ and $K^+$, intracellular signalling molecules like cyclic nucleotides (e.g. cyclic adenosine monophosphate, cAMP), and other small molecules like metabolites, sugars, lactate, and potentially butyrate (Sáez et al., 2003; White, 2003).

#### 2.1.3.2. Interruption of Ruminal Barrier Function

Feeding cattle highly fermentable diets induces low ruminal pH with an increase in the concentration of endotoxins (Nagaraja et al., 1978a; Keunen et al., 2002; Penner et al., 2010). According to Aschenbach and Gäbel (2000), luminal acidity increased the absorption of histamine which was due to acid-induced damage of the ruminal epithelum. Ruminal epithelial

barrier function can also be damaged by luminal hyperosmolality which is mainly determined by SCFA concentration (Bennink et al., 1978). Increasing the osmolality by adding mannitol induced an increase in tissue conductance and the flux of $^{51}$Cr-EDTA across the isolated ruminal epithelium (Schweigel et al., 2005). Although the precise mechanism of barrier function disruption is not clear, the accumulation of SCFA resulting from rapid fermentation of carbohydrates can damage the protective surface of the rumen epithelium (Steele et al., 2009) and induce consequent systemic responses such as ruminitis and liver abscesses (Jensen et al., 1954; Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998; Nagaraja et al., 2005).

From the previous studies, it is clear that among the four strata of the ruminal epithelium, the stratum granulosum plays the primary role in barrier function. Under low ruminal pH or high osmolality, the barrier function can be damaged which may be due to the disruption of the structure of tight junctions.

## 2.2. Industry Relevance of Short-Term Feed Restriction

### 2.2.1. Relevance to Beef and Dairy Industries

#### 2.2.1.1. Weaning and Transportation Induced Feed Restriction in the Beef Industry

For beef cattle, short-term FR events occur in association with weaning and transportation. Weaning in beef production is usually done abruptly and ultimately exposes the calf to a range of physical and social stressors such as separation from the dam, dietary changes, environmental changes, and social changes which occur when they are mixed with calves from other herds (Stookey and Watts, 2007). Abrupt weaning is considered by some to be one of the most stressful practices for beef cattle (Loerch and Fluharty, 1999). In response to abrupt weaning, calves commonly increase vocalization and walking time, and decrease eating and lying time (Price et al, 2003; Haley et al., 2005). This results in a reduction in weight gain for weaned calves 3 to 5 d after separation from their dams (Stookey and Watts, 2007) suggesting that transient exposure to FR occurs. However, it should be acknowledged that there is no specific data showing the difference in DMI before and after weaning.

A second example where short-term FR occurs in beef cattle production is during transportation. This may include when cattle are moved between pastures, during marketing, and

when being transported to a feedlot for backgrounding or finishing. As could be expected, the the severity of the FR event associated with transportation is dependent on the distance and time in transport. A recent study evaluating current commercial practices associated with long haul transport (≥ 400 km) of beef cattle in Alberta showed that the average time cattle spent on the truck was 16 h with only 5% in transport for longer than 30 h (González et al., 2012). From this data, it is more relevant to suggest that although cattle may be without feed or water during transportation, the relative short duration of the transport indicates that cattle are likely to be exposed to short-term FR in association with the overall process of transportation. However, this study only included transport time and did not consider the timeline required for marketing and the number of times cattle are transported over several days.

Feed restriction may be further compounded after transportation. Multiple reasons such as crowded conditions during transportation and exposure to a new feedlot environment contribute to the stress after transportation. This stress, along with transportation stress, may cause newly received feedlot calves to have low levels of DMI (Grandin, 1997). In fact, newly arrived feeder calves typically only consume 0.5 to 1.5% of their BW during the first wk, 1.5 to 2.5% of their BW in the second wk, with normal intakes (2.5-3.5%) being achieved somewhere around the 3$^{rd}$ to 4$^{th}$ wk after arrival (Hutcheson and Cole, 1986). In the same study, they also reported that less than 40% of cattle were eating on the first d after arriving in the feedlot. The findings of Hutcheson and Cole (1986) are supported by those of Fluharty et al. (1994a) who found that on the 1$^{st}$ day in the feedlot newly arrived cattle only consumed 62% of DMI relative to that consumed on d 7 after arrival. Moreover, the severity of the FR event or feed deprivation event prior to arrival can have a negative effect on feed intake. For example, after 48 or 72 h of transportation, DMI for newly weaned calves decreased by approximately 48 and 74% on the 1$^{st}$ day in the feedlot compared with weaned but not transported calves (Fluharty et al., 1996). The information above proves that in the beef industry short-term FR occurs in association with weaning and transportation, and highlights the importance to understand how FR impacts the physiology of the animal.

#### 2.2.1.2. Parturition Induced Feed Restriction in the Dairy Industry

For dairy cattle, the transition period which is defined as 3 wk prior to parturition until 3 wk post-partum (Drackley, 1999) and the corresponding changes from a pregnant non-lactating state to a non-pregnant lactating state imposes enormous stress. According to Bertics et al. (1992) a 30% reduction for DMI is observed during the final week before calving. Similar results have also been reported in previous studies (Coppock et al., 1972; Lodge et al., 1975; Journet and Remond, 1976). Based on more recent data from 699 Holsteins that were fed a variety of diets (49 in total) during the last 3 wk of gestation, it was found that DMI decreased by 32% during the final 3 wk of calving with 89% of that decline occurring in the final week of calving (Hayirli et al, 2002). Dairy cows with higher body condition score (BCS) may also experience a much greater suppression in feed intake compared those with lower BCS (Hayirli et al, 2002). As many factors change during the transition period, the effect of the reduction in DMI is not currently known.

#### 2.2.1.3. Diseases and Heat Stress Induced Feed Restriction for Both Beef and Dairy Cattle

In feedlot cattle, approximately 25 to 30% of the deaths are induced by digestive disorders (Galyean and Rivera, 2003). The National Animal Health Monitoring Service in the U.S. (NAHMS; USDA, 2000) reported that 1.9% of cattle placed in feedlots had digestive disorders. The incidence of digestive disorders varied with feedlot size with higher levels (2%) for feedlots with a capacity larger than 8000 and 1.1% for feedlots with a capacity between 1000 and 8000 (NAHMS; USDA, 2000). According to Galyean and Eng (1998), several nutritional factors (diet type, feed intake and feeding behaviour) can contribute to the occurrence of digestive and metabolic disorders such as ruminal acidosis and ketosis. Variation (high-low-high) in the amount of feed consumed, even when fed the same diet can increase the incidence of ruminal acidosis and have negative effects on feedlot cattle performance (Bauer et al., 1995). Ruminal acidosis, in turn, depresses DMI thereby inducing short-term and re-occurring FR (Fulton et al., 1979; Carter and Grovum, 1990; Owens et al., 1998).

Subacute ruminal acidosis (SARA) is also a key disorder for dairy cows. A survey of 14 dairy farms in Wisconsin showed that 20.1% of early and peak lactation cows had SARA (Oetzel et al., 1999). In 2004, Oetzel found a similar result where 20% of cows from commercial dairy

herds, sampled by ruminocentesis, had ruminal pH less than 5.5 during clinical herd investigations. They found a negative association between DMI and SARA and suggested it could be used as a partial factor to detect SARA under commercial settings (Nocek, 1997; Kleen et al., 2003; Oetzel, 2003). Several studies have reported that grain-induced SARA can cause inflammation as indicated by increased acute phase proteins found in blood (Gozho et al., 2005, 2006, and 2007). This suggests that SARA can also induce short-term FR which may lead to greater risk for subsequent bouts of SARA (Dohme et al., 2008).

Numerous other factors including infectious disease and metabolic disorders can also lead to short-term FR. Under these conditions, the immune system can mediate eating behavior and DMI (Weingarten, 1996). Three mechanisms were given in that study indicating that the immune system can; 1) have neuroimmunological control of behavior, 2) be involved in the eating pathology, and 3) affect energy intake and expenditure. For dairy cows, another major disease is ketosis which affects approximately 30% of dairy cows during the first 2 wk of lactation (Duffield et al., 1998; Duffield, 2000). A recent study showed that compared with healthy cows, DMI of cows with subclinical ketosis was 26% lower during the 1$^{st}$ wk and 20% lower during the 2$^{nd}$ wk after calving (Goldhawk et al., 2009). A similar trend was found during the week before calving, indicating that DMI for cows with sub-acute ketosis was 18% lower compared with healthy cows in the same study. This further confirms that metabolic disorders may induce or exacerbate the short-term FR occurring during the transition period. Other diseases such as liver abscesses (Brink et al., 1990), bovine respiratory disease (Hutcheson and Cole, 1986) displaced abomasum (Van Winden et al., 2003), milk fever (Marquardt et al., 1977) and mastitis (Bradley, 2002) also cause a reduction in DMI. In addition, when beef and dairy cattle experience heat stress, DMI is often reduced (Maust et al., 1972; Knapp and Grummer, 1991; Holter et al., 1996). These findings point to the fact that FR occurs in both the beef and dairy sectors and unfortumately may not be preventable. Thus, strategies to accelerate recovery following a period of FR are important so that animal health, welfare, and productivity can be optimized.

## 2.3. Effects of Feed Restriction and Feed Deprivation

### 2.3.1. Effects on Animal Performance and Behaviour

It is well documented that mild FR (above maintenance DMI) can lead to compensatory weight gain for cattle during realimentation (Lopez-Saubidet and Verde, 1976; Drouillard et al., 1991a; Connor et al. 2010). However, in some cases, shortages of feed may cause prolonged moderate or severe FR. Under this scenario, unlike mild FR, low performance is expected.

#### 2.3.1.1. Effects of Feed Restriction on Performance and Behaviour of Weaned Calves

The effect of short-term FR on performance has not been studied extensively. Examples from weaning and transportation induced FR highlight the challenge of quantifying performance changes for short-term FR as compensatory gain often occurs upon realimentation. For example, weaning induces marked changes in behaviour (Veissier et al., 1989). In fact, calves on pasture that were abruptly separated from their dams during the first 3 d post weaning spent less time eating and lying down, and more time walking and vocalizing than the non-weaned ones (Price et al., 2003). Compared with the non-weaned calves, the weight gain of weaned calves was 33% less in the first 2 wk after weaning, with differences detectable untill 10 wk following weaning, after which no differences were observed (Price et al., 2003). Arthington et al. (2003) reported that calves lost BW on the first day postweaning and calves transported for 3 h after weaning lost more BW than those not transported (5.3% compared with 3.3%). In the same study, within the first week after weaning, calves that were not transported increased BW by 0.7% while the transported calves lost 3.4%. When those same researchers increased transportation time from 3 h to 6 h, BW loss for transported and non-transported calves during the first day postweaning increased (7.6% vs. 4.5%) and BW was still less than initial BW after 3 wk for both transported and non-transported calves. While the effect of weaning cannot solely be attributed to short-term FR, it does support the underlying hypothesis that weaning induces short-term FR, which further causes poor performance for weaned beef calves.

#### 2.3.1.2. Effects of Feed Deprivation and Feed Restriction on Performance and Behaviour of Newly Received Feedlot Cattle

With respect to industry practices associated with feedlot production (e.g. marketing), feed and water deprivation has been shown to cause BW loss at a rate of 0.75% of initial BW/d (Shorthose and Wythes, 1988). When deprivation of feed and water and transportation are combined together, cattle weight loss ranges from 3 to 11% of initial BW during the first 24 h

(Warriss, 1990). This has been confirmed by Knewles et al. (1999). In that study, cattle were transported for 14, 21, 26 and 31 h and the authors reported an average of 7% of live BW was lost during transportation without differences in weight loss among transportation times. During transport cattle are deprived of feed and water, even long-haul transport times are, on average, less than 24 h suggesting that cattle are feed restricted but not totally feed deprived considering the opportunity for feed consumption upon arrival (González et al., 2012). Despite this, newly received feedlot calves only consume 0.5 to 1.5% of their BW during the 1st week in the feedlot (Hutcheson and Cole, 1986) with only 38.9% of calves consuming feed on the first day upon arrival in the feedlot (Hutcheson and Cole, 1986). If cattle are transported long distances it may take more than 4 d for all cattle to begin eating (Hutcheson and Cole, 1986).

#### 2.3.1.3. Effects of Feed Deprivation and Feed Restriction on Performance and Behaviour of Transition Dairy Cows

For dairy cows, 2 d of feed deprivation caused a 13% reduction in BW and approximately 4 d was required to return to initial BW after re-feeding (Agenäs et al., 2003). Furthermore, feed deprivation for 24 and 48 h respectively caused milk production to decrease by 25 and 44% (Baird et al., 1972; Athanasiou and Phillips, 1978). A rapid reduction of milk yield was also found during feed deprivation for dairy cows by McGuire et al. (1995) and Samuelsson et al. (1996). Milk composition was influenced with fat content increasing (Hartmann and Lascelles, 1965; Reid et al., 1977; Agenäs et al., 2003) and lactose concentration decreasing (Dahlborn et al., 1995; Agenäs et al., 2003) when dairy cows were feed deprived. The decreased DMI before calving may have a negative effect on the performance with responses similar to the effect of feed deprivation. While there have been some studies evaluating feed deprivation, few studies have investigated the effects of FR (voluntary or induced reduction in feed intake) on performance. Velez and Donkin (2005) found a 50% FR for 5 d caused decreased milk production and a negative energy balance for lactating dairy cows.

### 2.3.2. Effects on Feed Digestion

Many previous studies have showed that programed DMI restriction can improve the efficiency of feed utilization (Murphy and Loerch, 1994; Sainz et al., 1995; Rossi et al., 2001; Schmidt et al., 2005) and increase dietary digestibility (Edionwe and Owen, 1989; Fiems et al.,

2007; Galvani et al., 2010). However, feeding sheep at 50% of maintenance energy and nitrogen requirements for 4 wk resulted in similar digestibility compared to sheep fed at maintenance (Perrier et al., 1994). Steers restricted to 80% of ad libitum DMI but not restricted in terms of net energy or metabolizable protein intakes (25 d) had greater diet digestibility compared with ad libitum fed steers (Clark et al., 2007). The inconsistent results may be due to different restriction levels, the restriction of DMI compared with energy and protein restriction, and the duration of the restriction event.

As total DMI is an important determinant of ruminal pH (Krause and Oetzel, 2006), an increase in ruminal pH during fasting and a rapid ruminal pH decline after re-feeding have been observed for beef cattle (Rumsey, 1978; Galyean et al., 1981). Welch and Smith (1968) found that a 36 h period of feed deprivation caused a decline in rumination activity and Clark et al. (2007) reported a reduction in the acetate proportion and greater propionate proportion in restricted steers (to 80% of ad libitum DMI) compared with those fed ad libitum. During fasting, both the volume of rumen contents and rumen content DM coefficient decrease (Warriss, 1990). Additionally, the total number of ruminal bacteria may be reduced by up to 90% after a 48 h period of feed and water deprivation (Baldwin, 1967). Galyean et al. (1981) reported a reduction in the total count of rumen bacteria after a 36 h fast coupled with transportation relative to fasted but not transported steers. They further showed that it required 104 h following re-feeding to return to basal levels. Rumen protozoa numbers also decrease sharply during fasting (Galyean et al., 1981) and ruminal fermentative activity (mg fermentation acid produced/d) was drastically reduced with 48-h starvation (Baldwin, 1967). Other studies have confirmed this response showing a 74% decrease in ruminal fermentative capacity (mL gas produced/2 h) during a 48-h feed deprivation period with the fermentative capacity returning to pre-experimental levels within 7 d of realimentation (Cole and Hutcheson, 1985).

Transportation coupled with feed withdraw may have even more severe consequences compared with only fasting. At the end of fasting (32 h), cattle that were both fasted and transported had higher total ruminal SCFA concentrations relative to those that were only fasted (Galyean et al., 1981). They also found that even after re-feeding for 3 d, the fasted and transported steers tended to have higher SCFA concentration than only fasted steers. Ruminal ammonia concentration followed a similar trend as ruminal SCFA concentrations in that study.

Thus, greater SCFA and ammonia concentration may reflect the reduced rumen motility, compromised absorptive function, or the slow passage rate of the digesta. Fluharty et al. (1996) conducted 2 studies using newly weaned calves and adapted feedlot cattle with 0, 48, and 72 h feed and water deprivation coupled with 8 h of transportation. They found that ruminal volume, total content weight, DM percentage, and protozoa number decreased as duration of feed and water deprivation increased while total rumen bacteria number and viable cellulolytic bacteria number were not influenced. Collectively, these data show that short-term FR and feed deprivation have negative effects on ruminal fermentation and that this disruption may last for a short period of time after re-feeding.

### 2.3.3. Effects on Gastrointestinal Function

Gastrointestinal development and function are strongly dependent on feed intake. Drouillard et al. (1991b) found that 14 d of FR, to about 40% of ad libitum DMI, with protein or energy deficient diets reduced stomach and intestinal weight in lambs. In contrast, increasing feed intake causes hypertrophy of intestinal cells from sheep (Burrin et al., 1992). Changes in the ovine intestine and stomach mass caused by feeding different levels of feed were observed as soon as 5 d after changing feed intake and can last for as long as 3 wk (Rompala and Hoagland, 1987). A lower mitotic index for ruminal papillae was found in both bovine and ovine epithelium after 3 d of fasting (Resend-Júnior et al., 2006). Although in the same study there were no changes in papillae size between fed and fasting states, the area to length ratio of intact papillae decreased rapidly during fasting. These studies prove that FR or total feed withdrawl can decrease the proliferation or even cause degradation of the gastrointestinal epithelia which may influence the absorptive and barrier function.

Sakata and Yajima (1984) reported that infusion of SCFA increased ruminal epithelial cell proliferation. In vivo studies of intraruminal infusion of butyrate proved that butyrate was the most potent, but indirect (Galfi et al., 1991), stimulator of ruminal epithelial proliferation (Sakata and Tamate, 1978; Sakata et al., 1980). Along this line, feeding sheep at 50% of maintenance energy and nitrogen requirements for 4 wk decreased the SCFA absorption rate compared with the sheep fed at a maintenance level (Perrier et al., 1994). Similar results were observed by Doreau et al. (1997) for sheep that were restricted to 50% of voluntary DMI for 5

wk. This chronic FR caused a 32% reduction for the SCFA absorption rate compared with sheep fed ad libitum. Low density of dietary energy for dry cows can also cause decreased ruminal absorptive function as described by Dirksen et al. (1985). They showed that as a result of feeding the dry cow diet for 7 wk, ruminal absorptive function decreased by 50%. However, hormonal changes occurring during this time cannot be overlooked. Moreover, a previous study showed that a 48-h fasting event induced a 43 to 56% decrease in SCFA absorption rate (Gäbel et al., 1993) without changes in epithelial morphology. Consistent with those findings, Gäbel and Aschenbach (2002) reported that feed-deprived sheep (48 h) had a lower net absorption rate of 3-O-methyl-α-D-glucose (3-OMG) compared with the fed sheep. The reduction in net absorption was due to increased serosal-to-mucosal movement which may be related with compromised barrier function (Gäbel and Aschenbach, 2002). Thus, it appears that chronic FR and short-term feed deprivation both negatively affect the absorptive function of the ruminal epithelia; however, there is a paucity of data describing whether short-term FR affects the absorptive function and whether the severity of the FR event impacts the response.

### 2.3.4. Effects on Substrate Utilization and Tissue Mobilization

Fasting causes a negative energy balance in cattle, which usually induces changes in energy substrate utilization. Previous studies have showen increased non-esterified fatty acids (NEFA) and β-hydroxybutyrate (BHBA) concentrations (Hartmann and Lascelles, 1965; Baird et al., 1972; Agenäs et al., 2003) and decreased glucose and insulin levels (Baird et al., 1972; Reid et al., 1977; McGuire et al., 1995; Agenäs et al., 2003) in blood as a result of feed deprivation. Restricting feed intake, despite still providing the maintenance energy and protein requirement in sheep also induced lower blood glucose concentration (Burrin et al., 1990). When dairy cows were fed a high concentrate diet or low concentrate diet restricted to 65% of DMI of ad libitum over 2 wk, plasma glucose concentration decreased under both scenarios (Nielsen et al., 2003). In the same study they also found increased plasma BHBA and NEFA during FR. Only providing 30% of the energy requirements for maintenance caused a reduction of plasma glucose and glucagon concentrations, decreased serum insulin, and an increase in serum BHBA and NEFA concentrations for ewes (Caldeira et al., 2007). However no changes in plasma glucose, NEFA and insulin were found by restricting steers to 80 and 65% DMI of ad libitum (Ouellet et al., 2001). When fasting or FR is connected with weaning, or transportation in the beef industry,

changes in the concentration of energy substrates in circulation may be different from the situations under only fasting or FR. Blood glucose may increase during stress according to Wallach (1974). Transported and fasted cattle have higher blood glucose concentration than only fasted cattle (Galyean et al. 1981). According to Knewles et al. (1999) following transportation for 14, 21, 26 and 31 h, cattle all responded with increased blood glucose and NEFA concentrations while decreased serum BHBA was observed. After arriving in the feedlot, blood glucose and NEFA concentrations in cattle both decreased to the initial levels with a sharp decline during the first 12 h while blood BHBA was increasing constantly during the 72-h recovery period (Knewles et al. 1999). These results provide support that similar to fasting, FR coupled with transportation can also cause changes in blood metabolites which indicates that regulation of energy balance of cattle is compromised. However whether this change is caused by transportation or by FR is unknown.

### 2.4. Conclusions

In conclusion, SCFA are the most important energy substrates for ruminants and that the gastrointestinal epithelia play a key role in protecting underlying tissues from damage arising from the luminal environment. However under current production systems short-term FR occurs for beef and dairy cattle. Specific scenarios of short-term FR occur include weaning, transportation, and marketing in beef industry and during the transition period for dairy cattle. According to the literature, the duration of episodes of FR in beef and dairy production appears to be relatively short (approximately 5 to 7 d) with the severity ranging FR to about 20% to 70% of DMI ad libitum. Additionally, digestive and metabolic disorders as well as heat stress and infectious disease can also cause FR for both beef and dairy cattle. Negative effects of chronic FR (> 4 wk in duration) and very short-term feed and water deprivation (for 48 h) on ruminal absorptive and barrier function have been documented; however the effect of short-term FR (5 d) which is much more relevant to the beef and dairy industry hasn't been studied yet.

### 2.5. Hypotheses

Short-term FR will decrease the absorptive function of ruminal epithelia and compromise the barrier function of the total gastrointestinal tract with more detrimental effects induced by more severe FR. The absorptive and barrier functions of the gut will recover when cattle are fed

ad libitum after the shot-term FR with the timeline needed for recovery increasing as the short-term FR severity increases.

## 2.6. Objectives

This study was designed to evaluate the effect of different severities of short-term FR on the absorptive function of the reticulo-rumen and the barrier function of the total gastrointestinal tract in cattle and to evaluate the time needed for recovery of the gut function based on the severity of FR imposed.

# 3.0. SHORT-TERM FEED RESTRICTION IMPAIRS THE ABSORPTIVE FUNCTION OF THE RETICULO-RUMEN AND TOTAL TRACT BARRIER FUNCTION IN BEEF CATTLE

## 3.1. Introduction

While beef and dairy cattle are generally fed to optimize energy and protein intake, a number of stressors within the production chain may induce short-term periods of FR. For example, abrupt weaning of calves results in dramatic changes in behaviour including increased vocalization and walking time, and decreased eating and lying time (Price et al, 2003; Haley et al., 2005). This change in activity results in a loss of weight 3 to 5 d after separation from their dams which likely corresponds to short-term FR (Stookey and Watts, 2007). Marketing, transportation, and receiving at a feedlot may also induce short-term FR. This is evident as newly arrived calves typically consume 0.5 to 1.5% of their BW during the 1$^{st}$ wk, 1.5 to 2.5% of their BW in the 2$^{nd}$ wk, with normal intakes (2.5-3.5%) only being achieved between the 2$^{nd}$ to 4$^{th}$ wk after arrival (Hutcheson and Cole, 1986). For dairy cows approaching parturition, DMI has been shown to decrease by 32% during the final 3 wk of calving with 89% of that decline occurring in the final wk before calving (Hayirli et al, 2002). Other examples for when short-term FR may occur in cattle include when experiencing metabolic (Duffield et al., 1998; Duffield, 2000; Goldhawk et al., 2009) or digestive disorders such as ruminal acidosis (Fulton et al., 1979; Carter and Grovum, 1990; Owens et al., 1998), and when cattle experience heat stress (Maust et al., 1972; Knapp and Grummer, 1991; Holter et al., 1996). Despite the evidence for the occurrence of short-term FR under industry settings, few studies have evaluated the effects of short-term FR on the function of the gastrointestinal tract in ruminants.

Previous studies investigating chronic FR for sheep have shown that restricting feed intake to 50% of the maintenance requirement (Perrier et al., 1994) or 50% of ad libitum DMI (Doreau et al. 1997) decreased ruminal SCFA absorption. This negative response may occur acutely as short-term feed deprivation (48 h) has been shown to cause a reduction in SCFA absorption rates across the reticulo-rumen (Gäbel et al., 1993) in sheep and increases passive permeation of 3-O-methyl-α-D-glucose, an indicator of compromised barrier function (Gäbel and Aschenbach, 2002). While chronic FR and short-term feed deprivation have been shown to have

negative effects on the function of the gastrointestinal tract in ruminants, there is currently a lack of data describing whether short-term FR affects the absorptive and barrier function of the gastrointestinal tract in ruminants and whether the severity of the FR event affects the outcome.

The hypothesis was that short-term FR for 5 d would negatively impact the absorptive function of the reticulo-rumen and the barrier function of the gastrointestinal tract with greater effects as the severity imposed increases. Therefore, the objective was to determine whether the severity of short-term FR affects SCFA absorption across the reticulo-rumen and total-tract barrier function.

## 3.2. Materials and Methods

Animal use for this experiment was pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol number 20100021) and was in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada).

### 3.2.1. Experimental Design and Animals

Eighteen Angus × Hereford heifers that had been previously fitted with a ruminal cannula (model 9C; Bar Diamond Inc., Parma, ID) and ovariectomized at the time of surgery were used in this study. Heifers were housed at the University of Saskatchewan Livestock Research Building and were assigned into 3 blocks based on initial BW. The mean ± standard deviation values for BW at the start of each block were 384.2 ± 8.5, 412 ± 14.4, and 429.8 ± 16.5 kg for the 3 blocks. Within block, heifers were randomly assigned to 1 of 3 treatments that differed in the severity of FR. The experiment consisted of 14 d for adaptation followed by 5 d for BASE measurements and 5 d of FR. Treatments included FR to 75, 50, or 25% of voluntary intake relative to that measured during BASE. Throughout the study, heifers were fed the same diet once daily at 0800 with water available at all times. The diet consisted of 32% barley grain, 30% grass hay, 30% barley silage and 8% vitamin and mineral supplemental pellet (DM basis). The dietary DM content was 658 ± 19.7 g/kg, and OM, CP, crude fat and NDF concentrations were 923 ± 1.2, 112 ± 3.5, 18 ± 0.4, and 401 ± 4.4 g/kg on a DM basis, respectively. The diet was formulated to meet or exceed nutrient requirements for replacement heifers according to NRC (2000) except during FR.

### 3.2.2. Sample Collection and Analysis

#### 3.2.2.1. Dry Matter Intake

Feed intake was measured daily for the first 4 d during each of the BASE and FR periods by measuring the weight of the feed offered and the weight of the feed that was refused. Throughout the study, forage samples (hay and silage) were collected twice weekly and concentrate feeds (barley grain and the mineral and vitamin pellet) were collected once weekly. All feed and refusal samples were dried in a forced air oven at 55°C until achieving a constant weight, and ground to pass through a 1-mm screen (Christy & Norris 8" Lab Mill, Christy Turner Ltd. Chelmsford, UK). Dry matter intake was determined as the difference between the DM offered and the DM refused. Feed samples were composited by block for chemical analysis.

Feed ingredient samples were analyzed for DM, ash, CP, fat and NDF. The analytical DM content of feed samples was determined according to the Association of Official Analytical Chemists (1990) for DM by drying at 105ºC for 2h (AOAC, 1990; method 930.15). Ash content was determined by combustion at 600°C for at least 5 h (AOAC, 1990; method 942.05) and the OM content was calculated using the difference between the DM and ash contents. Neutral detergent fibre was determined using α-amylase and sodium sulphite (Van Soest et al., 1991) using the ANKOM digestion apparatus (model A200, ANKOM Technology Corp., Fairport, NY). The nitrogen content was determined using flash combustion (LECO FR-528 analyzer, LECO, St. Joseph, MI) and the CP content was calculated by multiplying the N concentration by 6.25. Ether extract was determined according to AOAC method 920.39 using a Goldfisch extraction apparatus (Labconco, Kansas City, MO; Rhee, 2005). Dietary composition was determined mathematically and has been previously reported in the experimental design subsection in this chapter.

#### 3.2.2.2. Ruminal pH and Short-Chain Fatty Acid Concentration

Ruminal pH was measured every 2 min for the first 4 d of each of the BASE and FR periods using the Lethbridge Research Centre Ruminal pH Measurement System (LRCpH; Dascor, Escondido, CA; Penner et al., 2006). Prior to insertion, mV readings were recorded in pH buffers 7.0 and 4.0. The LRCpH was then placed in the ventral sac of the rumen and was

maintained in that region using 2 kg of weight attached to the electrode shroud. On d 5 of each measurement period, the pH systems were removed from the rumen, rinsed, and maintained at 39°C until the data were downloaded and post-measurement standardization were complete. The shift mV readings between pre- and post-incubation standardizations were assumed to be linear over time and were used to convert the recorded mV readings to ruminal pH. The daily ruminal pH values were summarized as minimum pH, maximum pH, and mean pH. In addition, the daily duration (min/d), area (pH × min/d) and acidosis index (pH × min/ kg of DMI) that ruminal pH was below 5.5 were calculated (Penner et al., 2009b).

Ruminal fluid samples were collected every 4 h over a 24-h period starting from 0800 h on d 3 of both the BASE and FR periods. Whole ruminal digesta was collected separately from 3 different regions (250 mL/region; cranial, ventral, and caudal) and immediately strained through 2 layers of cheese cloth. Ruminal fluid was mixed well and 10 mL was added to 2 mL of 25% (w/v) meta-phosphoric acid and stored at -20°C. The SCFA concentration was determined using the method modified from Khorasani et al. (1996). Samples were thawed and centrifuged at 6,000 × g for 10 min at 4°C and the supernatant was transferred to a new vial and centrifuged at 16,000 × g at 4°C for 10 min. One mL of supernatant was mixed with 200 µL of isocapronic acid which was used as internal standard. A 1 µL of solution was injected into a Agilent 6890 Series gas chromatograph equipped with Agilent 7683 Series Auto Sampler using a split ratio of 17:1. The column (30.0 m × 320 µm × 0.25 µm; model 7HM-G009-11, Zebron, Phenomenex, Torrance, CA) flow rate was 35 mL/min. Initially the temperature was set at 90°C for 0.1 min and then increased to 170°C at 10°C/ min (injector temperature at 170°C and detector temperature at 250°C).

#### 3.2.2.3. Blood Sampling and Analysis

Catheters were placed in the jugular vein 1 d prior to blood sampling with blood collected every 4 h over a 24-h duration on the same day and at the same time as ruminal fluid sampling. Blood was collected into containers containing 158 IU units of Li-heparin or a silica gel (BD, Franklin Lakes, NJ). For plasma, blood samples were centrifuged immediately at 2,500 × g for 15 min at 4°C, whereas, samples used for serum were allowed to clot for 4 h before being centrifuged. Harvested plasma and serum were stored at -20°C until analysis.

The concentrations of glucose and BHBA were determined in plasma. Glucose concentration was determined using a glucose oxidase-peroxidase enzyme (No. P7119; Sigma-Aldrich, Oakville, ON, Canada) and dianisidine dihydrochloride (No. F5803; Sigma-Aldrich, Oakville, ON, Canada). Absorbance was determined with a plate reader (SpectraMax PLUS384; Molecular Devices Corp., Sunnyvale, CA) at a wavelength of 450 nm. Plasma BHBA concentration was measured using the enzymatic oxidation of BHBA to acetoacetate with 3-hydroxybutrate dehydrogenase (No. H6501; Roche, Mississauga, Ontario, Canada). The color change associated with the reduction of NAD to NADH was determined using a plate reader (340 nm). A commercial kit was used to determine serum NEFA concentration (NEFA- HR 2; Wako Diagnostics, Richmond, VA).

#### 3.2.2.4. Short-Chain Fatty Acid Absorption

The temporarily isolated and washed reticulo-rumen technique (WRR; Care et al., 1984) was used to evaluate the rate of SCFA absorption. One heifer of each treatment group, within block, was subjected to the WRR procedure at 0900 while the second heifer was subjected to WRR at 1300. Measurements were conducted on the last day of BASE and FR. To perform the WRR, the reticulo-rumen contents were completely evacuated and stored in an insulated container. The reticulo-rumen was then washed twice with tap water (39°C) followed by 4 consecutive washes (5 L/wash) with a pre-heated washing buffer solution (39°C, pH 6.2; Table 3.1). The reticulo-rumen was then isolated from the rest of the gastrointestinal tract by inserting occluding devices into the esophagus and omasal orifice. The esophageal occluding device (University of Leipzig, Leipzig, Germany) consisted of a PVC suction core within an expandable rubber cuff. It was connected to a vacuum pump (model N86KT45P, KNF Neuberger Inc., Trenton, NJ) such that saliva was continuously aspirated trough the several holes of the suction core. A 75-mL Foley catheter (American Diabetes Wholesale, Pompano Beach, FL) was used to occlude the omasal orifice. Placement of these occluding devices prevented saliva inflow into the reticulo-rumen and liquid outflow, and hence resulted in the temporary isolation of the reticulo-rumen from the remainder of the gastrointestinal tract. After placement of the occluding devices, the reticulo-rumen was washed again with 5 L of washing buffer and the remaining buffer solution was removed completely. Fifteen L of incubation buffer solution (Table 3.1) was poured into the rumen and gassed with 100% $CO_2$ to facilitate buffer mixing. The chemical

composition of the incubation buffer was made based on the previous studies (Kristensen and Harmon, 2004) while considering ruminal fluid composition. Samples of the incubation buffer were collected prior to infusion, and at 5 and 65 min after infusion. At each sampling time, 5 mL was collected for measurement of osmolality and 35 mL was collected and preserved in 7 mL of 25% (w/v) meta-phosphoric acid. After mixing well, samples were frozen and stored at -20°C until being analyzed for SCFA and Cr concentrations. Short-chain fatty acid concentrations were determined as previously described for ruminal fluid, and Cr concentration was determined using atomic absorption (AA) spectrometry (iCE 3000 series, Thermo Fisher Scientific Inc., Waltham, MA). The osmolality (Model 3250, Advanced Instruments Inc. Norwood, MA) of the experimental buffer (prior to infusion with no preservative) was measured in duplicate. The actual buffer volume after 5 and 65 min infusion was corrected using Cr-EDTA. Rates of SCFA absorption were calculated using the following equations:

Absolute absorption rate of SCFA (mmol/h) = $C_{5min} \times V_{5min} - C_{65min} \times V_{65min}$

Fractional absorption rate of SCFA (%/h) = $(C_{5min} \times V_{5min} - C_{65min} \times V_{65min}) / (C_{5min} \times V_{5min}) \times 100$

Where; C = SCFA concentration in the buffer, and V = incubation buffer volume.

#### 3.2.2.5. Total Gastrointestinal Tract Barrier Function

To evaluate barrier function, Cr-EDTA was used as a paracellular permeability marker (Saunders et al., 1994). Chromium-EDTA has been shown to have an approximate mass of 340 Da and size of 10 Å (García-Lafuente et al., 2001). A Foley catheter (American Diabetes Wholesale, Pompano Beach, FL) was inserted into the bladder 1 d prior to urine collection. On d 2 of BASE and FR, a 180 m*M* solution (1 L) of Cr-EDTA was pulse dosed into the rumen at 0900 h. Urine was collected for 48 h following the infusion. A 48-h duration was chosen based on a preliminary study using the same heifers and dose rate evaluating the timeline of Cr excretion in urine (data not shown). Daily (24 h) urine output was measured and representative samples of urine were collected, and stored at -20°C until being analyzed from Cr concentration as described by Vicente et al. (2004) using atomic absorption spectrometry as previously described.

Table 3.1. Chemical composition of buffers used for washing the reticulo-rumen and for measurement of SCFA absorption across the reticulo-rumen epithelia.

| Chemical | Washing buffer[1], m*M* | Incubation buffer[1,2], m*M* |
|---|---|---|
| $CaCl_2$ | - | 2 |
| $MgCl_2$ | - | 2 |
| NaCl | 105 | 5 |
| KCl | - | 5 |
| Na-acetate | 10 | 30 |
| K-acetate | - | 35 |
| Na-propionate | 20 | 35 |
| Na-butyrate | - | 8 |
| Butyric acid | - | 7 |
| L-lactic acid | - | 5 |
| $NaHCO_3$ | 25 | 25 |
| Cr-EDTA | - | 2 |

[1]Buffers were adjusted to pH 6.2.
[2]Osmolality ± SEM of the incubation buffer was 303 ± 2.9 mOsmol/kg.

### 3.3. Statistical Analysis

Statistical analysis was performed using the PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). The statistical model was designed to evaluate whether treatment, period and treatment × period effects occurred. For this model, treatment, experimental period, and block were considered as fixed effects while cow within block was treated as a random effect. Under this model, experimental period was considered as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian Information Criterion was used. For WRR data, the timing of the WRR procedure was also tested (i.e. morning vs. afternoon). In all cases, there was no effect of time for WRR data and thus, time of the WRR procedure was removed from the model. Differences were considered significant when $P \leq 0.05$ and tendencies are discussed when $0.05 < P \leq 0.10$.

### 3.4. Results

#### 3.4.1. Dry Matter Intake and Ruminal Fermentation Characteristics

A treatment × period interaction was detected for DMI ($P < 0.001$, Table 3.2). During BASE, DMI (pooled treatment means = 11.1 kg/d) was not different among treatments, but as expected, DMI during FR was lower than BASE with the lowest values observed for 25% (2.7 kg/d), intermediate for 50% (5.3 kg/d) and the highest for 75% (8.3 kg/d). Thus, DMI during FR relative to DMI during BASE equated to 70, 49, and 25%, which was close to the targeted values of 75, 50, and 25%, respectively. Although it was not anticipated, some heifers restricted to 75% of their BASE DMI refused feed within the first 2 d of the FR period. The feed refused accounted for an additional 1.7% of BASE DMI. An interaction between treatment and period was also detected for DMI when reported as a proportion of initial BW ($P < 0.001$). The average value for DMI as a proportion of BW during BASE was 2.7% and did not differ among treatments. However, during FR, DMI as a proportion of BW was lower than in BASE and the value decreased to 2.0% for 75%, 1.3% for 50% and only 0.7% for 25% feed offered. A treatment × period interaction was detected for the concentration of total ruminal SCFA ($P < 0.001$, Table 3.2). During BASE, there were no differences among treatments but total SCFA concentration decreased from BASE to FR for all treatments. Furthermore, severity of FR affected total SCFA concentration during FR with the lowest concentration observed for 25%,

intermediate for 50%, and greatest for 75%. The molar proportion of acetate and butyrate were not affected by treatment but propionate was lower for 25% than for 75 and 50% (Table 3.3). Relative to BASE, the proportion of acetate tended to be greater ($P = 0.094$) and propionate was lower ($P = 0.033$) during FR, although butyrate was not affected.

Minimum, mean, and maximum ruminal pH values were all affected by a treatment × period interaction ($P \leq 0.002$; Table 3.4). Minimum, mean and maximum pH values were not different among treatments during BASE. However, during FR, minimum pH was highest for heifers restricted to 25% of BASE feed offering, lowest for 75%, but those restricted to 50% were not different from either 25 or 75% feed offering. Mean pH during FR was highest for 25%, intermediate for 50%, and lowest for 75%, while maximal pH was higher for 25% than for 50 and 75%. In general, mean pH increased by 0.48, 0.64, and 0.79 pH units for 75, 50, and 25% during FR relative to BASE.

Treatment did not affect the duration (min/d), area (pH × min/d) or acidosis index (pH × min/ kg of DMI) based on a pH threshold of 5.5 (Table 3.5), but period effects were detected for all 3 variables ($P \leq 0.004$; Table 3.5). An average duration of 122.6 min/d below 5.5 was found in BASE which decreased to only 4.8 min/d during FR. The area that pH < 5.5 responded similarly with a greater value of 24.2 pH × min/d during BASE than 0.4 during FR ($P < 0.01$). During BASE, the acidosis index was also higher than during FR (2.1 vs. 0.1 pH × min/kg DMI; $P < 0.01$).

### 3.4.2. Reticulo-Rumen Absorptive Function

The absolute absorption rate (mmol/h) for total SCFA tended to be affected by treatment ($P = 0.080$) with the rate numerically decreasing with increasing severity of FR (Table 3.6). Acetate absorption (mmol/h) also tended ($P = 0.057$) to decrease with increasing severity of FR, although the absolute absorption rate of propionate and butyrate were not affected by treatment. The fractional rates (%/h) for total SCFA, acetate, and propionate tended to decrease with increasing severity of FR ($P \leq 0.081$), while butyrate absorption was not affected by treatment ($P = 0.252$).

Table 3.2 Interaction between the severity of short-term feed restriction (FR) and the measurement period (BASE, FR) for DMI and total ruminal short-chain fatty acids (SCFA) concentration.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value |
| DMI, kg/d | | | | | |
| BASE | 11.7[a] | 10.8[a] | 10.9[a] | 0.375 | <0.001 |
| FR | 8.2[b] | 5.3[c] | 2.7[d] | | |
| DMI, %BW | | | | | |
| BASE | 2.8[a] | 2.7[a] | 2.7[a] | 0.102 | <0.001 |
| FR | 2.0[b] | 1.3[c] | 0.7[d] | | |
| Total ruminal SCFA, m*M* | | | | | |
| BASE | 88.4[a] | 82.8[a] | 87.0[a] | 2.46 | <0.001 |
| FR | 63.0[b] | 45.8[c] | 33.1[d] | | |

[abcd]Means within a response variable (i.e. DMI, DMI %BW, total ruminal SCFA) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).
[1]Values refer to the percentage of feed offered during the FR period relative to BASE.

Table 3.3. Effects of the severity of short-term feed restriction (FR) and the measurement period (BASE, FR) on the molar proportions of acetate, propionate, and butyrate in total ruminal short-chain fatty acids (SCFA).

| | Treatment[1] | | | | | Period | | | |
|---|---|---|---|---|---|---|---|---|---|
| SCFA, mmol/100 mmol | 75% | 50% | 25% | SEM | *P* Value | BASE | FR | SEM | *P* Value |
| Acetate | 62.6 | 62.6 | 64.4 | 0.85 | 0.248 | 62.6 | 64.9 | 0.59 | 0.094 |
| Propionate[2] | 22.4 | 22.3 | 18.9 | 1.00 | 0.044 | 22.2 | 20.1 | 0.73 | 0.033 |
| Butyrate | 11.1 | 11.1 | 12.4 | 0.53 | 0.584 | 11.7 | 11.4 | 0.41 | 0.174 |

[1]Values refer to the percentage of feed offered during FR relative to that consumed during BASE.
[2]The Tukey's post-hoc mean separation test did not produce means that differed.

Table 3.4. Interaction between the severity of short-term feed restriction (FR) and measurement period (BASE, FR) for minimum, mean, and maximum ruminal pH.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value |
| Minimum pH | | | | | |
| BASE | 5.32$^{c}$ | 5.39$^{c}$ | 5.34$^{c}$ | 0.077 | 0.002 |
| FR | 5.94$^{b}$ | 6.23$^{ab}$ | 6.51$^{a}$ | | |
| Mean pH | | | | | |
| BASE | 6.14$^{d}$ | 6.17$^{d}$ | 6.16$^{d}$ | 0.047 | <0.001 |
| FR | 6.62$^{c}$ | 6.81$^{b}$ | 6.97$^{a}$ | | |
| Maximum pH | | | | | |
| BASE | 6.85$^{c}$ | 6.85$^{c}$ | 6.88$^{c}$ | 0.038 | <0.001 |
| FR | 7.07$^{b}$ | 7.18$^{b}$ | 7.37$^{a}$ | | |

$^{abcd}$Means within a response variable (i.e. minimum pH, mean pH and maximum pH) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).
[1]Values refer to the percentage of feed offered during FR relative to that consumed during BASE.

Table 3.5. Effects of the severity of short-term feed restriction (FR) and the measurement period (BASE, FR) on indicators for ruminal acidosis using pH 5.5 as a threshold.

| | Treatment | | | | | Period | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value | BASE | FR | SEM | *P* Value |
| Duration , min/d | 60.1 | 72.8 | 58.0 | 22.81 | 0.886 | 122.6 | 4.8 | 14.56 | < 0.001 |
| Area, min × pH/d | 11.6 | 12.9 | 12.4 | 5.97 | 0.987 | 24.2 | 0.4 | 3.75 | 0.004 |
| Acidosis index[1], min × pH/kg DMI | 1.0 | 1.2 | 1.1 | 0.51 | 0.963 | 2.1 | 0.1 | 0.42 | 0.004 |

[1]Used to normalize the area that pH was below 5.5 while accounting for DMI (Penner et al., 2009b).

The absolute rate of total SCFA absorption also tended ($P$ = 0.091) to decrease from BASE to FR with a numerical reduction equating to more than 100 mmol/h (Table 3.6). Acetate absorption accounted for most of this numerical reduction as the rate was nearly 70 mmol/h lower ($P$ = 0.050) during FR than BASE. The fractional rates (%/h) for total SCFA, propionate, and butyrate absorption were not affected by period, although the fractional rate of acetate absorption tended ($P$ = 0.072) to be lower during FR than BASE.

### 3.4.3. Total Tract Barrier Function

An interaction between treatment and period was observed for urinary Cr recovery ($P$ < 0.001; Figure 3.1). During BASE, there were no differences among treatments for urinary Cr recovery and Cr recovery did not differ for heifers restricted to 75 or 50% during FR. However, heifers restricted to 25% had greater urinary Cr recovery during FR than BASE with Cr excretion in these heifers being greater than those restricted to 50% but not 75% feed.

### 3.4.4. Blood Metabolites

Plasma glucose concentration was affected by a treatment × period interaction (P = 0.026; Table 3.7). Glucose concentration was higher during FR (84.4 mg/dL) than BASE (77.3 mg/dL) for heifers restricted to 75% while no differences were found between heifers restricted to 50% and 25% during BASE and FR. For serum NEFA, a treatment × period interaction was detected ($P$ < 0.001). The interaction was the result of NEFA concentrations that were not different among treatments during BASE, whereas heifers restricted to 25% had higher NEFA concentration than those restricted to 75% and 50% during FR. Serum BHBA concentration was not affected by treatment (pooled treatment means = 8.2 mg/dL) but period effects were detected ($P$ = 0.034) indicating that BHBA concentration in FR (7.7 mg/dL) was lower than in BASE (8.7 mg/dL).

Table 3.6. Effects of the severity of short-term feed restriction (FR) and the measurement period (BASE, FR) on short-chain fatty acid (SCFA) absorption across the temporarily isolated and washed reticulo-rumen.

| | Treatment | | | | | Period | | | |
|---|---|---|---|---|---|---|---|---|---|
| Item | 75% | 50% | 25% | SEM | *P* Value | BASE | FR | SEM | *P* Value |
| Absolute absorption rate[1], mmol/h | | | | | | | | | |
| Total SCFA | 622.6 | 504.1 | 479.8 | 44.11 | 0.080 | 587.9 | 483.1 | 38.84 | 0.091 |
| Acetate | 340.4 | 266.3 | 256.1 | 24.63 | 0.057 | 321.8 | 253.5 | 21.39 | 0.050 |
| Propionate | 193.8 | 162.9 | 150.1 | 14.47 | 0.124 | 182.8 | 155.1 | 12.60 | 0.161 |
| Butyrate | 88.3 | 74.9 | 73.6 | 7.01 | 0.292 | 83.3 | 74.6 | 6.27 | 0.380 |
| Fractional absorption rate[1], %/h | | | | | | | | | |
| Total SCFA | 42.9 | 34.8 | 34.1 | 2.59 | 0.058 | 40.2 | 34.3 | 2.62 | 0.195 |
| Acetate | 40.5 | 31.9 | 31.3 | 3.11 | 0.081 | 37.9 | 31.2 | 2.54 | 0.072 |
| Propionate | 45.4 | 37.9 | 36.4 | 2.58 | 0.062 | 42.5 | 37.2 | 2.74 | 0.297 |
| Butyrate | 48.1 | 40.9 | 41.8 | 3.19 | 0.252 | 45.2 | 42.0 | 3.21 | 0.552 |

[1]Disappearance from the reticulo-rumen was assumed to be equal to absorption.

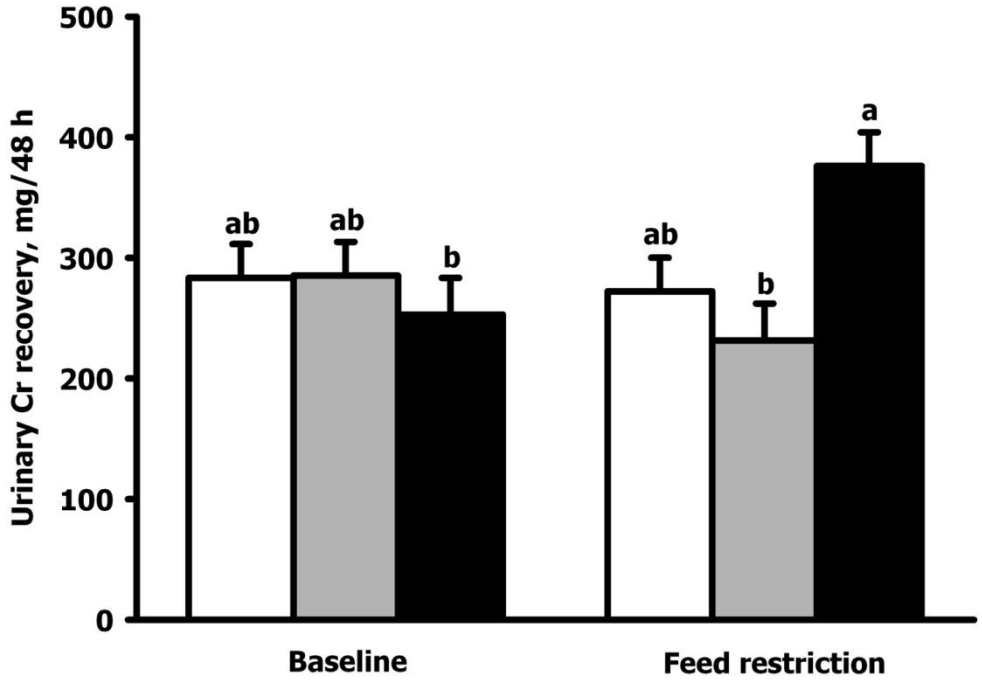


Figure 3.1. Interaction between the severity of short-term feed restriction (FR) and measurement period (BASE, FR) for 48-h urinary Cr recovery ($P < 0.001$). Heifers were fed ad libitum during the baseline period with the amount of feed offered restricted to 75% (white bars), 50% (grey bars) or 25% (black bars) during feed restriction. Means with uncommon letters differ across treatments and periods ($P \leq 0.05$).

Table 3.7. Interaction between the severity of short-term feed restriction (FR) and the measurement period (BASE, FR) for blood composition.[1]

| | Treatment | | | | |
|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value |
| Glucose, mg/dL | | | | | |
| BASE | 77.3[b] | 81.3[ab] | 81.0[ab] | 2.49 | 0.026 |
| FR | 84.4[a] | 83.7[ab] | 80.5[ab] | | |
| NEFA, μEq/L | | | | | |
| BASE | 165.9[d] | 168.6[cd] | 184.4[cd] | 24.61 | <0.001 |
| FR | 196.4[bc] | 313.4[b] | 495.5[a] | | |

[ab]Means within a response variable (glucose, NEFA) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).

[1]Plasma BHBA was not different among treatments, but the period effect was significant ($P$ = 0.034, BASE: 8.7mg/dL, FR: 7.7mg/dL).

### 3.5. Discussion

Feed restriction occurs, albeit inadvertently, in both beef and dairy production systems. Examples for when short-term FR events may occur include weaning (Price et al, 2003; Haley et al., 2005), during transportation (González et al., 2012), during the first few weeks upon arrival at a feedlot (Hutcheson and Cole, 1986; Grandin, 1997; Loerch and Fluharty, 1999), around parturition (Bertics et al., 1992; Hayirli et al, 2002), when experiencing heat stress (Maust et al., 1972; Knapp and Grummer, 1991; Holter et al., 1996), and in association with infectious diseases or metabolic and digestive disorders (e.g. displaced abomasum, ketosis; Van Winden et al., 2003; Goldhawk et al., 2009). The magnitude of the FR event varies substantially depending on the environmental (e.g. heat stress or production practices such as weaning) and host-derived factors (e.g. physiological state or disease status). Previous studies have shown that the severity of FR ranges from a reduction of intake to 68% on d 1 pre-partum relative to 21 d pre-partum for dairy cattle (Hayirli et al., 2002) to only 25% of expected intake during the first wk of feeding for highly-stressed newly received beef calves (Hutcheson and Cole, 1986). Thus, it is evident that short-term FR occurs in both beef and dairy production systems.

In swine, long-lasting impacts of even short-term FR have been shown on the absorptive and barrier functions of the gastrointestinal tract (Spreeuwenberg et al., 2001; Boudry et al., 2004). However, the effects of short-term FR on the function of the gastrointestinal tract have not been studied in ruminants. Thus, the current study was designed to evaluate the impact of the severity of short-term FR on SCFA absorption and total tract barrier function. This was accomplished by restricting feed offered over 5 consecutive d to 75, 50, or 25% of voluntary intake. The experimental model imposed was successful as actual FR severities equated to 70, 49, and 25% during FR relative to BASE.

Ruminal SCFA, especially butyrate, act as indirect stimulatory molecules promoting proliferation and the metabolic activity of cells within the ruminal epithelia (Sakata and Tamate, 1978; Nozière et al., 2000; Gorka et al., 2009; Plöger et al. 2012). Results from the current study confirm that ruminal pH and SCFA concentration are responsive to the severity of FR (Doreau et al., 1997) and level of DMI (Krause and Oetzel, 2006). Our results showed that imposing FR to 75, 50, or 25% of ad libitum intake resulted in SCFA concentrations that were 71, 55 and 38% of

that measured in BASE. Others have also reported a reduction in SCFA concentration with lower feed intake. For example, Cole et al. (1986) found that short-term feed deprivation for 13 or 46 h resulted in approximately 50 and 70% reductions in SCFA concentration. The reduction in SCFA concentration has been thought to be due to both a reduction in fermentable substrate and changes in the activity of the rumen microorganisms (Galyean et al., 1981; Cole and Hutcheson, 1985). In the current study, a period effect was detected for the acetate to propionate ratio such that during FR, it was higher than BASE (3.3 vs. 2.9; $P = 0.026$; data not shown). While a lower acetate to propionate ratio is usually detected for grains than forages (Blaxter, 1962), this response was detected despite cattle being fed a constant diet throughout the study. According to Russell (1998), although the effect of ruminal pH on the acetate to propionate ratio is more subtle than shifting the dietary forage-to-concentrate ratio, as much as 25% of the change in the acetate to propionate ratio can be attributed to a reduction in ruminal pH. In the current study, ruminal pH increased dramatically from BASE to FR, and the acetate to propionate ratio increased accordingly. The increase in acetate as a result of high pH may be a result of an inhibitory effect of high pH on the growth rate of certain populations of lactate utilizers, which are sensitive to higher ruminal pH (Mackie and Gilchrist, 1979). On the other hand, high grain diets usually induce low ruminal pH, and both in vivo and in vitro studies have shown that even a modest decline in ruminal pH can cause a negative effect on cellulose digestion (Mould and Ørskov, 1983). While we can not decipher the cause for the shift in the acetate to propionate ratio, the observed changes indicate that shifts in ruminal pH may induce changes in the acetate to propionate ratio, although changes in the microbial populations may have occurred as well.

Since SCFA, especially butyrate, act as stimuli promoting ruminal epithelial function, a reduction in the concentration may reduce the stimuli present and thus may not promote epithelial capacity and tissue integrity to the same extent. Supporting this notion, Doreau et al. (1997) showed that mature sheep exposed to chronic FR (to approximately 1% of BW for 5 wk) had an absolute absorption rate for total SCFA that was 32% lower than that of sheep provided feed at a level close to voluntary intake (approximately 1.9% of BW). Although the severity of short-term FR only tended to affect total SCFA absorption in the current study, the absolute absorption rate of total SCFA also tended to be lower during FR than BASE with extent of reduction equating to more than a 100 mmol/h. Much of this reduction (nearly 70 mmol/h) can

be accounted for by a lower absorption rate of acetate during FR than BASE. This finding was further supported with a tendency for decreasing fractional (10%/h) rate of acetate absorption as the severity of short-term FR increased.

The reason for why acetate absorption was reduced without parallel reductions for other SCFA is not currently known and differs from previous studies evaluating chronic FR (Perrier et al., 1994; Doreau et al., 1997) or short-term complete feed deprivation (Gäbel et al., 1993). In the studies of Perrier et al. (1994), Doreau et al. (1997), and Gäbel et al. (1993), the authors demonstrated that short-term feed deprivation or chronic FR, respectively, reduced the absolute rates of acetate, propionate, and butyrate absorption; however, Gäbel et al. (1993) did report a greater reduction in acetate absorption than butyrate after feed and water deprivation. Certainly, there are differences in the model used in the current study and the previous studies including the duration of the FR imposed (Perrier et al., 1994; Doreau et al., 1997), and that feed was not completely withheld as was imposed by Gäbel et al. (1993). However, recent studies have also shown that the reliance on protein-mediated transport pathways is greater for acetate than butyrate (Aschenbach et al., 2009; Penner et al., 2009a). Thus, it could be hypothesized that short-term FR has a negative effect on the activity of ruminal epithelial cells or on the transcription, translation, or post-translational modification of proteins involved in acetate absorption. This adaptation would seem to be a logical approach to reduce the energy required by the ruminal epithelia for protein synthesis when energy supply is low, and would be favoured from a physiological perspective over general reductions in selective permeability that occur with acid-induced damage (Gaebel et al. 1987; Penner et al., 2010) or complete feed deprivation (Gäbel and Aschenbach et al., 2002). Future work is needed to determine whether changes in transcript abundance and protein abundance or activity are present especially given the wide range in stratum basal cell turnover (1 to 7.9 d) and total ruminal epithelia turnover (2.3 to 17 d; Goodlad, 1981).

Candidate proteins for acetate transporters include DRA and PAT1 that have been suggested as anion exchangers on the apical side of the epithelium, or potentially AE2 which is involved in intracellular pH homeostasis and is thought to function as an anion exchange protein exporting acetate to portal circulation in exchange for bicarbonate (Bilk et al., 2005). The lack of response for butyrate suggests that passive diffusion of SCFA and intracellular metabolism of

SCFA were likely not affected by short-term FR beyond the general reduction in SCFA supply associated with FR. Future studies are needed to elucidate the underlying mechanisms causing the reduction in acetate absorption with short-term FR and to develop strategies to mitigate this response.

For ruminants, only a few studies have evaluated barrier function in either ruminal epithelia (Gäbel and Aschenbach et al., 2002; Schweigel et al., 2005; Lodemann and Martens, 2006; Penner et al., 2010; Wilson et al., 2012) or the intestinal epithelia (Emmanuel et al., 2007). However, the importance of promoting selective permeability should not be underemphasized (Penner et al., 2011; Wilson et al., 2012) as numerous studies have demonstrated that pathogenic bacteria (Narayanan et al., 2002; Nagaraja et al., 2005), and antigens such as lipopolysaccharides (Gozho et al., 2005, 2006; Zebeli and Ametaj, 2009), endotoxins (Nagaraja et al., 1978b,c; Khafipour et al., 2009), and biological amines (e.g. histamine, Irwin et al., 1979; Plaizier et al., 2008) are present in ruminal fluid. Importantly, many of these compounds have been linked to other disorders or diseases such as laminitis (Vermunt, 1992; Nocek, 1997), liver abscesses (Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998), and acute interstitial pneumonia (Loneragan et al., 2001). Thus, it is critical to understand how dietary and management factors affect the barrier function of the gastrointestinal tract; however, methodology to do so has not been proven until the current study.

To address the lack of methodology to evaluate total tract barrier function in ruminants, a pulse-dose of Cr-EDTA solution was applied into the rumen coupled with 48-h of total urine collection. Chromium-EDTA movement across the gastrointestinal epithelia is largely via paracellular processes (García-Lafuente, et al., 2001; Schwiegel et al., 2005) and Cr-EDTA appearance in urine is used as an indicator of intestinal barrier function in humans (Ten Bruggencate et al., 2006). While Cr-EDTA passage is a good indicator of paracellular permeability, Cr-EDTA (approximately 340 Da) is much smaller than most endotoxins (range in molecular mass between 2 and 70 kDa; Magalhães et al., 2007). As such, barrier function evaluated using Cr-EDTA does not directly indicate potential for endotoxin translocation; however, it is a suitable indicator of generalized barrier function. Thus, the 48-h urinary Cr appearance was used as an indicator of total tract barrier function. A 48-h duration of urine collection was selected as preliminary results showed diurnal variation in urinary Cr excretion

thereby negating collecting spot samples and that over 96% of the Cr appeared in urine 48 h following Cr-EDTA infusion into the rumen for heifers restricted to 25% of DMI ad libitum while fed the same diet as in the current study (data not shown).

The results of the current study demonstrate that barrier function was only compromised under severe short-term FR (25% of voluntary feed intake) with no negative effects observed for cattle restricted to 75 or 50% of voluntary intake. The negative effects of feed deprivation on barrier function have previously been shown using isolated ruminal epithelia incubated in Ussing chambers (Gäbel and Aschenbach et al., 2002). In that study, a 48-h duration of feed deprivation was induced in sheep and the movement of 3-O-methyl-$\alpha$-D-glucose, a small hydrophilic molecule, was evaluated. This study showed that feed deprivation drastically increased the passive movement of 3-O-methyl-$\alpha$-D-glucose across the ruminal epithelium, indicating a reduction in barrier function (Gäbel and Aschenbach et al., 2002). The reduction in barrier function could be expected to lead to greater risk for immune system activation and risk for other diseases; however, this has not been tested in vivo. Future studies should be conducted to establish the importance of selective permeability of the several segments of the gastrointestinal tract and its implications on animal health and performance in cattle.

While advances were made in terms of improving our understanding of absorptive and barrier function, it should be acknowledged that Cr-EDTA was used as a liquid volume marker for SCFA absorption in the WRR technique as well as a paracellular marker for barrier function of the total gastrointestinal tract. The use of Cr-EDTA in these two ways seems to be contradictory and thus justification for this approach is discussed below.

With the WRR technique, the non-absorbability of Cr-EDTA is a key premise in order to calculate changes in liquid volume throughout the incubation period. Based on the data from Care et al. (1984) a 99% recovery rate of Cr-EDTA was obtained after a 1 h incubation period; the same duration as used in the current study. However, the absorption of Cr-EDTA across the rumen is dependent on the osmolality of the buffer solutions used. According to Dobson et al. (1976), the recovery of Cr-EDTA ranged from 92 to 99% after an hour of incubation and that incubation with a hypertonic solution resulted in greater clearance of Cr-EDTA out of the rumen. In the current study, all heifers were exposed to a buffer solution with a similar osmolality (see

Table 3.1) and identical theoretical composition. Thus, variation in buffer composition should not bias Cr-EDTA recovery among treatments or time.

While the results have shown that barrier function was compromised for heifers restricted to 25% of DMI ad libitum, it is possible that Cr-EDTA disappeared during the WRR technique, especially for severely restricted heifers. However it is unlikely that the Cr-EDTA disappearance would be significant and very unlikely that it would impact the interpretation of the findings in chapters 3 and 4. For example, the WRR buffer only contained 2 m*M* of Cr-EDTA (15 L total volume) with this buffer solution being incubated for 1 h. A previous validation study conducted in association with the experiment presented in this thesis demonstrated that urinary Cr appearance was minimal within the first 4 h after incubation. This is especially important given the large dose of Cr-EDTA used for barrier function assessment (180 m*M* in a 1 L solution) and the prolonged incubation required for recovery in urine. Although theoretically more Cr-EDTA may have disappeared during the WRR procedure for heifers restricted to 25% during FR, little difference would be expected considering the short timeline of and low amount. Furthermore, no differences were detected in barrier function between heifers restricted to 50 and 75% thus further confirming that differences observed for absorptive function were not biased by changes in Cr-EDTA recovery.

### 3.6. Conclusions

In conclusion, regardless of the severity of short-term FR imposed, this study has demonstrated that FR reduces SCFA absorption across ruminal epithelium which is mainly because of the decreased acetate absorption rate while total tract barrier function is only affected by severe FR. Further studies on the mechanisms of acetate absorption and tight junction ultrastructure under short-term FR conditions as well as the strategies to minimize these negative effects need to be conducted.

# 4.0. RECOVERY OF ABSORPTIVE FUNCTION OF THE RETICULO-RUMEN AND TOTAL TRACT BARRIER FUNCTION IN BEEF CATTLE AFTER SHORT-TERM FEED RESTRICTION

## 4.1. Introduction

Feed restriction occurs in beef and dairy production with abrupt weaning (Haley et al., 2005), transportation (Hutcheson and Cole, 1986), parturition (Hayirli et al., 2002), and heat stress (Mader et al., 2006). It has been proven that short-term feed deprivation (Gäbel et al., 1993) and chronic FR (Perrier et al., 1994; Doreau et al., 1997) negatively affect SCFA absorption across the rumen. Moreover, short-term FR has shown to have negative effect on gut function (absorptive and total tract barrier function) in chapter 3. As it may not be possible to prevent short-term FR (e.g. occurring due to heat stress or parturition), it is important to develop a comprehensive understanding of factors affecting the recovery of absorptive and barrier function. This becomes particularly important given that SCFA absorption contributes up to 75% of the total ME supply (Bergman, 1990) and that compromised barrier function may increase the risk for ailments such as laminitis (Nocek, 1997), liver abscesses (Nagaraja and Chengappa, 1998), and acute interstitial pneumonia (Loneragan et al., 2001). Unfortunately, only two studies have evaluated barrier function showing that complete feed deprivation (48 h; Gäbel and Aschenbach, 2002) or short-term FR (see chapter 3) compromise it. The understanding of the timeline required for recovery of absorptive function is still limited (Doreau et al., 2003). Finally, no studies have evaluated whether the severity of the short-term FR affects the recovery response of absorptive and barrier function of the gut tract in ruminants.

The hypothesis was that the time required for the recovery of reticulo-rumen absorptive function and total tract barrier function would be dependent on the severity of the short-term FR imposed. Therefore, the objective was to evaluate the timeline needed for the recovery of absorptive function of the reticulo-rumen and barrier function of the total gastrointestinal tract after short-term FR of different severities.

## 4.2. Materials and Methods

This study was part of an experiment designed to evaluate the effect of the severity of FR on absorptive function of the reticulo-rumen and barrier function of the gastro-intestinal tract.

The previous chapter (chapter 3) has described the effect of the severity of FR on absorptive and barrier function, with this chapter reporting the timeline for recovery. Animal use for this experiment was pre-approved by the University of Saskatchewan Animal Research Ethics Board (protocol number 20100021) and was in accordance with the guidelines of the Canadian Council of Animal Care (Ottawa ON).

### 4.2.1. Experimental Design and Animals

Heifers and treatments used in this study have been previously reported in chapter 3. Briefly, 18 ruminally-cannulated and ovariectomized Angus × Hereford heifers were used in a randomized complete block design. Heifers were housed at the University of Saskatchewan Livestock Research Building in individual pens (9 $m^2$) throughout the study. According to initial BW, heifers were assigned into 3 blocks (mean ± STD for BW at the beginning of each block; 384.2 ± 8.5, 412 ± 14.4, and 429.8 ± 16.5 kg) and within block were randomly assigned to 1 of 3 treatments that differed in the severity of FR. The experiment consisted of a 2 wk adaptation period, 5 d of BASE, 5 d of FR and 3 consecutive wk of recovery (REC1, REC2 and REC3). The previous chapter demonstrated the effect of FR by comparing BASE and FR, and this chapter will focus on data collected during FR, REC1, REC2, and REC3. Treatments were imposed during FR such that heifers were either restricted to 75, 50, or 25% of voluntary intake measured during BASE. Throughout the study, heifers were fed the same diet once daily at 0800 h with water available at all times. The diet was formulated to meet or exceed nutrient requirements for replacement heifers except during FR (NRC, 2000). As reported in chapter 3, heifers were fed a diet containing on DM basis 32% barley grain, 30% grass hay, 30% barley silage and 8% vitamin and mineral supplemental pellets. The dietary DM (mean ± SEM) was 658 ± 19.7 g/kg, with OM, CP, crude fat and NDF concentrations of 923 ± 1.2, 112 ± 3.5, 18 ± 0.4, and 401 ± 4.4 g/kg DM respectively.

### 4.2.2. Data and Sample Collection

#### 4.2.2.1. Dry Matter Intake

Feed intake was measured daily for the first 4 d of FR and the first 6 d of REC1, REC2 and REC3 by measuring the weight of the feed offered and the weight of the feed that was

refused. Feed was analyzed for DM, ash, CP, ether extract, and NDF contents as described in chapter 3.

#### 4.2.2.2. Ruminal pH and SCFA concentration

Ruminal pH was measured every 2 min for the first 4 d of FR and the first 6 d of REC1, REC2 and REC3. Ruminal fluid samples were collected every 4 h over a 24 h duration starting at 0800 h on d 3 of FR, and d 5 of REC1 and REC3. Ruminal pH and SCFA concentration analysis was described in chapter 3.

#### 4.2.2.3. Blood Sampling and Analysis.

Blood samples were taken from the jugular vein via catheters on the same day and at the same time as ruminal fluid sampling. Blood samples were analyzed for plasma glucose and BHBA concentrations as well as serum NEFA concentration as described in detail in chapter 3.

#### 4.2.2.4. Short-Chain Fatty Acid Absorption

The WRR (Care et al., 1984) was conducted on the last day of FR, REC1, and REC3 to evaluate the rate of SCFA absorption across the reticulo-rumen epithelium. A detailed description of this technique and the method used to calculate SCFA absorption rate has been previously described (see chapter 3).

#### 4.2.2.5. Total Gastrointestinal Tract Barrier Function

Starting on d 2 of FR, and d 4 of REC1 and REC3, a 1-L solution of 180 m*M* Cr-EDTA was pulse dosed into the rumen at 0900 h. Urine was collected for the following 48 h with urine output being measured and subsamples being collected on a 24-h basis. Urine sample analysis was described in detail in chapter 3.

### 4.3. Statistical Analysis

Statistical analysis was performed using the PROC MIXED procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC). Two statistical models were used in this study. The first model was designed to evaluate whether treatment, period and treatment × period effects occurred. This model included the fixed effects of treatment, period, and block using cow within

block as the random effect. Period was included as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian Information Criterion was used. Linear and quadratic regressions were tested when the treatment × period interaction was not significant but period was significant. This was accomplished using the PROC MIXED procedure of SAS with period included as a continuous variable.

To further evaluate transient changes during the initial portion of the recovery period (i.e. REC1), the second model evaluated the treatment, day, and treatment × day effects within REC1 for DMI and ruminal pH. This model included the fixed effects of treatment, day, and block including cow within block as the random effect. Day was included as a repeated measure and the covariance error structure that yielded the lowest Akaike's and Bayesian Information Criterion were used. When day effect was significant without treatment × period interaction significance, linear, quadratic and cubic regressions were analysed using the PROC MIXED procedure of SAS using the same model as above except that day was included as a continuous variable.

For all statistical approaches, differences were considered significant when $P \leq 0.05$ and tendencies are discussed when $0.05 < P \leq 0.10$.

## 4.4. Results

### 4.4.1. Dry Matter Intake and Ruminal Fermentation

An interaction between treatment and period was detected for DMI ($P < 0.001$; Table 4.1). During FR, DMI was greatest for heifers restricted to 75%, lowest for 25% and intermediate for 50%. The DMI of all 3 treatments increased when heifers returned to ad libitum feed provision. However, there were no differences for DMI among treatments or periods during recovery although, numerically, slight increases were detected for heifers restricted to 50% and 25% from REC1 to REC2, and from REC2 to REC3. A treatment × period interaction was also detected for DMI when reported as a proportion of initial BW ($P < 0.001$, Table 4.1). Heifers restricted to 75 and 50% during FR responded similarly in that DMI as a proportion of BW increased from FR to REC1 and did not change thereafter. However, heifers restricted to 25% increased DMI as a proportion of initial BW from FR to REC1 and from REC1 to REC3 with no differences between REC1 and REC2, and REC2 and REC3. An interaction between treatment and period

was detected for STD of DMI ($P$ = 0.035, Table 4.1). Not surprisingly, numerically the highest variation was observed during REC1 compared with the other periods and heifers restricted to 25% had the numerically greater STD for DMI during REC1 compared with heifers restricted to 75 and 50%.

An interaction between treatment and period was detected for the concentration of total ruminal SCFA (P < 0.001; Table 4.2). The severity of FR affected total SCFA concentration with the lowest concentration observed for heifers restricted to 25%, intermediate for 50%, and greatest for 75% during FR. When heifers were re-fed ad libitum, the total SCFA concentration increased to similar values for all treatments from FR towards REC3. At variance to the other two groups, however, heifers restricted to 25% had numerically higher concentrations of total SCFA in REC1 compared with REC3. A treatment × period interaction was found for the molar proportion of acetate ($P$ = 0.006, Table 4.2) and propionate ($P$ = 0.050, Table 4.2). There was no change in the molar proportion of acetate for heifers restricted to 75% among periods, whereas heifers restricted to 50 and 25% of their ad libitum intake responded to ad libitum feed intake with a decrease in the molar proportion of acetate from FR to REC1 followed by an increase during REC3. The molar proportion of propionate was not different among periods for heifers restricted to 75 and 50% of ad libitum DMI while a rapid increase was found from FR to REC1 for heifers restricted to 25%. No interaction between treatment and period was found for the molar proportion of butyrate ($P$ = 0.083; Table 4.2). However the molar proportion of butyrate was affected by treatment ($P$ = 0.042) with heifers restricted to 25% of ad libitum intake during FR having a greater proportion of butyrate (12.40 mol/100 mol) across periods than heifers restricted to 75% (10.75 mol/100 mol) but heifers restricted to 50% (11.30 mol/100 mol) were not different from 25 or 75%. There was no period effect for butyrate ($P$ > 0.05).

Table 4.1. Interaction between the severity of short-term feed restriction (FR) and the measurement period (FR, REC1, REC2, REC3) for DMI and STD of DMI.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value |
| DMI, kg/d | | | | | |
| FR | 8.2$^{c}$ | 5.3$^{d}$ | 2.7$^{e}$ | 0.50 | <0.001 |
| REC1 | 11.6$^{ab}$ | 9.8$^{abc}$ | 8.2$^{bcd}$ | | |
| REC2 | 11.8$^{a}$ | 10.3$^{abc}$ | 10.1$^{abc}$ | | |
| REC3 | 11.6$^{ab}$ | 11.0$^{ab}$ | 10.7$^{ab}$ | | |
| DMI, % of BW | | | | | |
| FR | 1.97$^{c}$ | 1.30$^{d}$ | 0.67$^{e}$ | 0.122 | <0.001 |
| REC1 | 2.73$^{ab}$ | 2.38$^{abc}$ | 2.03$^{bcd}$ | | |
| REC2 | 2.82$^{ab}$ | 2.52$^{ab}$ | 2.55$^{ab}$ | | |
| REC3 | 2.80$^{ab}$ | 2.70$^{ab}$ | 2.68$^{a}$ | | |
| STD of DMI | | | | | |
| FR | 0.42$^{b}$ | 0.06$^{c}$ | 0.17$^{c}$ | 0.183 | 0.035 |
| REC1 | 0.96$^{abc}$ | 1.11$^{abc}$ | 1.53$^{ab}$ | | |
| REC2 | 0.70$^{ab}$ | 0.75$^{ab}$ | 0.84$^{a}$ | | |
| REC3 | 0.76$^{abc}$ | 0.56$^{abc}$ | 1.10$^{ab}$ | | |

[abcde]Means within a dependent variable (DMI, DMI %BW, STD of DMI) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).
[1]Values refer to the percentage of feed offered during the FR period relative to BASE.

Table 4.2. Interaction between the severity of short-term feed restriction (FR) and the measurement period (FR, REC1 and REC3) for total short-chain fatty acids (SCFA) concentration, and molar proportion of acetate, propionate and butyrate in total ruminal SCFA.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Variable[2] | 75% | 50% | 25% | SEM | *P* Value |
| Total SCFA, m*M* | | | | | |
| FR | 63.0$^{b}$ | 45.8$^{c}$ | 33.1$^{d}$ | | |
| REC1 | 72.1$^{ab}$ | 73.2$^{ab}$ | 81.2$^{a}$ | 2.69 | < 0.001 |
| REC3 | 77.2$^{a}$ | 76.2$^{a}$ | 75.3$^{a}$ | | |
| Acetate, mol/100 mol | | | | | |
| FR | 62.2$^{ab}$ | 64.5$^{a}$ | 65.0$^{a}$ | | |
| REC1 | 60.9$^{abcd}$ | 53.6$^{cd}$ | 51.6$^{d}$ | 1.2 | 0.006 |
| REC3 | 59.7$^{bc}$ | 62.1$^{ab}$ | 62.3$^{ab}$ | | |
| Propionate, mol/100 mol | | | | | |
| FR | 22.9$^{ab}$ | 20.7$^{bc}$ | 16.8$^{c}$ | | |
| REC1 | 24.8$^{abc}$ | 30.7$^{ab}$ | 31.6$^{a}$ | 1.65 | 0.0504 |
| REC3 | 24.9$^{ab}$ | 21.1$^{bc}$ | 21.8$^{abc}$ | | |
| Butyrate[3], mol/100 mol | | | | | |
| FR | 10.7 | 10.3 | 13.0 | | |
| REC1 | 10.1 | 11.1 | 12.5 | 0.88 | 0.083 |
| REC3 | 11.4 | 12.5 | 11.7 | | |

[abcd]Means within a dependent variable (total SCFA, acetate, propionate) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).
[1]Values refer to the percentage of feed offered during the FR period relative to BASE.
[2]Results are arithmetic means from 6 heifers per treatment.
[3]Treatment affected the molar proportion of butyrate ($P = 0.042$) but a period effect was not detected ($P = 0.544$).

The minimum, mean, and maximum ruminal pH values were all affected by treatment × period interactions ($P < 0.001$; Table 4.3). During FR, minimum pH was greatest for heifers restricted to 25%, lowest for 75% but those restricted to 50% were not different from either 25 or 75%. During recovery, the minimum values decreased relative to FR but were not different among treatments or wk of recovery. Mean pH during FR was higher for heifers restricted to 25 and 50% than heifers restricted to 75% with a drastic reduction in mean pH upon return to ad libitum feeding. The extent of the reduction in mean pH upon return to ad libitum feeding was dependent on the severity of the FR such that heifers restricted to 75, 50, and 25% had reductions in mean pH by 0.46, 0.82, and 1.09 unites, respectively. No differences in mean pH were detected among treatments during recovery. An interaction between treatment and period was also detected for maximum ruminal pH ($P < 0.001$; Table 4.3), where heifers restricted to 75% had no changes throughout the whole experiment, but heifers restricted to 50 and 25% had lower maximum pH during REC1 than FR with maximum pH increasing from REC1 to REC3.

Interactions between treatment and period were not detected for duration, area or the acidosis index, and only main effects were presented. Treatment had no effect on the duration (min/d), area (pH × min/d) or acidosis index (pH × min/kg of DMI) using pH 5.5 as a threshold (Table 4.4). However, period effects were detected for all 3 variables (quadratic $P < 0.001$; Table 4.4). The duration that pH was < 5.5 was less than 5 min/d during FR which increased to nearly 4 h/d during REC1 followed by a slight reduction and subsequent plateau in REC2 and REC3. Similar responses were observed for both area and the acidosis index with the lowest values during FR, highest during REC1, and slight reductions from REC1 to REC2 with no changes thereafter.

Table 4.3. Interaction between the severity of short-term feed restriction (FR) and the measurement period (FR, REC1, REC2, REC3) on minimum, mean, and maximum ruminal pH.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | 75% | 50% | 25% | SEM | *P* Value |
| Minimum pH | | | | | |
| FR | 5.94$^{bc}$ | 6.23$^{ab}$ | 6.51$^{a}$ | 0.110 | < 0.001 |
| REC1 | 5.40$^{d}$ | 5.23$^{d}$ | 5.28$^{d}$ | | |
| REC2 | 5.32$^{d}$ | 5.42$^{cd}$ | 5.37$^{d}$ | | |
| REC3 | 5.38$^{d}$ | 5.44$^{cd}$ | 5.40$^{cd}$ | | |
| Mean pH | | | | | |
| FR | 6.62$^{b}$ | 6.81$^{a}$ | 6.97$^{a}$ | 0.077 | < 0.001 |
| REC1 | 6.16$^{cde}$ | 5.99$^{ef}$ | 5.88$^{df}$ | | |
| REC2 | 6.23$^{cde}$ | 6.26$^{bcd}$ | 6.20$^{ce}$ | | |
| REC3 | 6.29$^{cde}$ | 6.32$^{bcd}$ | 6.27$^{bce}$ | | |
| Maximum pH | | | | | |
| FR | 7.07$^{bc}$ | 7.18$^{ab}$ | 7.37$^{a}$ | 0.058 | < 0.001 |
| REC1 | 6.87$^{cd}$ | 6.73$^{de}$ | 6.55$^{e}$ | | |
| REC2 | 6.93$^{bcd}$ | 6.93$^{cd}$ | 6.89$^{bcd}$ | | |
| REC3 | 6.96$^{bcd}$ | 6.96$^{c}$ | 6.96$^{bcd}$ | | |

[abcdef]Means within a response variable (minimum pH, mean pH, maximum pH) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).

[1] Values refer to the percentage of feed offered during FR relative to that consumed during BASE.

Table 4.4. Effects of the severity of short-term feed restriction (FR) and the measurement period (FR, REC1, REC2, REC3) on indicators for ruminal acidosis using pH 5.5 as a threshold.

| | Treatment[1] | | | | | Experimental period | | | | | *P* Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Variable | 75% | 50% | 25% | SEM | *P* Value | FR | REC1 | REC2 | REC3 | SEM | Linear | Quadratic |
| Duration, min/d | 92.4 | 113.3 | 161.5 | 26.93 | 0.241 | 4.8 | 233.8 | 137.1 | 114.0 | 26.09 | 0.001 | < 0.001 |
| Area, min × pH/d | 28.7 | 9.4 | 54.2 | 9.62 | 0.217 | 0.4 | 79.8 | 44.5 | 35.9 | 9.51 | 0.091 | < 0.001 |
| Acidosis index, min × pH/kg DMI | 2.6 | 4.0 | 5.7 | 0.92 | 0.107 | 0.1 | 9.0 | 4.1 | 3.2 | 0.97 | 0.361 | < 0.001 |

[1]Values refer to percentage of feed offered during FR relative to that consumed during BASE.

As dramatic changes for intake and pH were observed when transferring from FR to REC1, the response during REC1 using day as a repeated observation was further evaluated. No interactions between treatment and day during REC1 were detected for DMI (kg/d and as % of BW), pH values (minimum, mean and maximum pH), and ruminal acidosis indicators (duration and area that pH < 5.5). Overall treatment effects (Table 4.5) on DMI (kg/d and as a % BW) and minimum, mean, and maximum pH basically reflected the treatment effects already analyzed for REC1 in Tables 4.1 and 4.3. When considering the day by day changes, however, DMI and DMI as % of BW showed a cubic response over time while minimum and mean pH, as well as the indicators of ruminal acidosis (duration and area that pH was < 5.5) showed a quadratic to cubic response from day 1 to day 6. The greatest DMI was observed on d 1, coinciding with the lowest ruminal pH values (minimum and mean) and the highest indices of acidosis (Table 4.6). This was followed by a sharp reduction of DMI and DMI% on days 2 and 3, during which minimum and mean pH values increased and indices of acidosis decreased to plateau values, respectively.

The acidosis index (area pH < 5.5/kg DMI) was affected by a treatment × day interaction in REC1 ($P$ = 0.036; Figure 4.1). For heifers restricted to 75 and 50%, the acidosis index was not different in from d 1 to d 6 when heifers had ad libitum access to feed; however, the acidosis index changed drastically for heifers that were previously restricted to 25% of their ad libitum DMI with the highest value on d 1 and the lowest value on d 2.

### 4.4.2. Reticulo-Rumen Absorptive Function

An interaction between treatment and period was detected for the absolute absorption rate (mmol/h) of total SCFA, acetate, propionate and butyrate ($P \leq 0.021$; Table 4.7). For heifers restricted to 75%, the absolute rates for total SCFA, acetate, propionate and butyrate were not different among FR and recovery periods, however, numerically all the variables increased from FR to REC1 and then decreased in REC3. For heifers restricted to 50%, there were no differences among experimental periods, but numerically all variables increased from FR to REC1 with a smaller numerical increase from REC1 to REC3. However, heifers restricted to 25% during FR had an increase in total SCFA, acetate, and propionate absorption rates in REC3 relative to FR with intermediate values in REC1. The rate of butyrate absorption (mmol/h) was not different between FR and REC1 with an increase from REC1 to REC3 detected for heifers restricted to 25% of their ad libitum feed intake.

Table 4.5. Effects of the severity of short-term feed restriction (FR) on DMI, pH values and ruminal acidosis indicators using pH 5.5 as a threshold within the first week of recovery period.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Item | 75% | 50% | 25% | SEM | *P* Value |
| DMI, kg/d | 11.6[a] | 9.8[ab] | 8.1[c] | 0.57 | 0.003 |
| DMI, % of BW | 2.8[a] | 2.4[ab] | 2.0[b] | 0.13 | 0.006 |
| Minimum pH | 5.40 | 5.23 | 5.33 | 0.074 | 0.271 |
| Mean pH | 6.16 | 5.99 | 5.92 | 0.101 | 0.269 |
| Maximum pH | 6.87 | 6.73 | 6.56 | 0.080 | 0.059 |
| Duration, min/d | 125.6 | 249.2 | 326.5 | 74.01 | 0.191 |
| Area, pH × min/d | 39.8 | 98.7 | 99.7 | 10.15 | 0.218 |

[abc]Means within a row with uncommon superscripts differ ($P \leq 0.05$).

[1]Values refer to percentage of feed offered during FR relative to that consumed during BASE.

Table 4.6. The day effect on DMI, pH values and ruminal acidosis indicators using pH 5.5 as a threshold within the first week of recovery period.

| | Day within the first week of recovery | | | | | | | *P* Value | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Item | 1 | 2 | 3 | 4 | 5 | 6 | SEM | Linear | Quadratic | Cubic |
| DMI, kg/d | 10.5 | 8.4 | 9.5 | 10.4 | 10.2 | 10.0 | 0.41 | 0.361 | 0.212 | 0.001 |
| DMI, % of BW | 2.57 | 2.03 | 2.32 | 2.52 | 2.51 | 2.45 | 0.097 | 0.416 | 0.122 | 0.001 |
| Minimum pH | 4.93 | 5.48 | 5.46 | 5.43 | 5.44 | 5.17 | 0.076 | 0.939 | < 0.001 | 0.330 |
| Mean pH | 5.70 | 6.10 | 6.07 | 6.09 | 6.09 | 6.07 | 0.075 | 0.010 | < 0.001 | 0.006 |
| Maximum pH | 6.65 | 6.64 | 6.68 | 6.73 | 6.74 | 6.87 | 0.068 | NS[1] | NS | NS |
| Duration, min/d | 521.5 | 95.2 | 186.7 | 119.3 | 217.1 | 262.8 | 59.05 | 0.819 | < 0.001 | 0.004 |
| Area,pH × min/d | 259.8 | 68.2 | 40.0 | 17.5 | 30.4 | 60.5 | 24.24 | 0.035 | 0.082 | < 0.001 |

[1]Main effects of period were not significant ($P = 0.184$) and thus linear and quadratic contrasts were not determined.

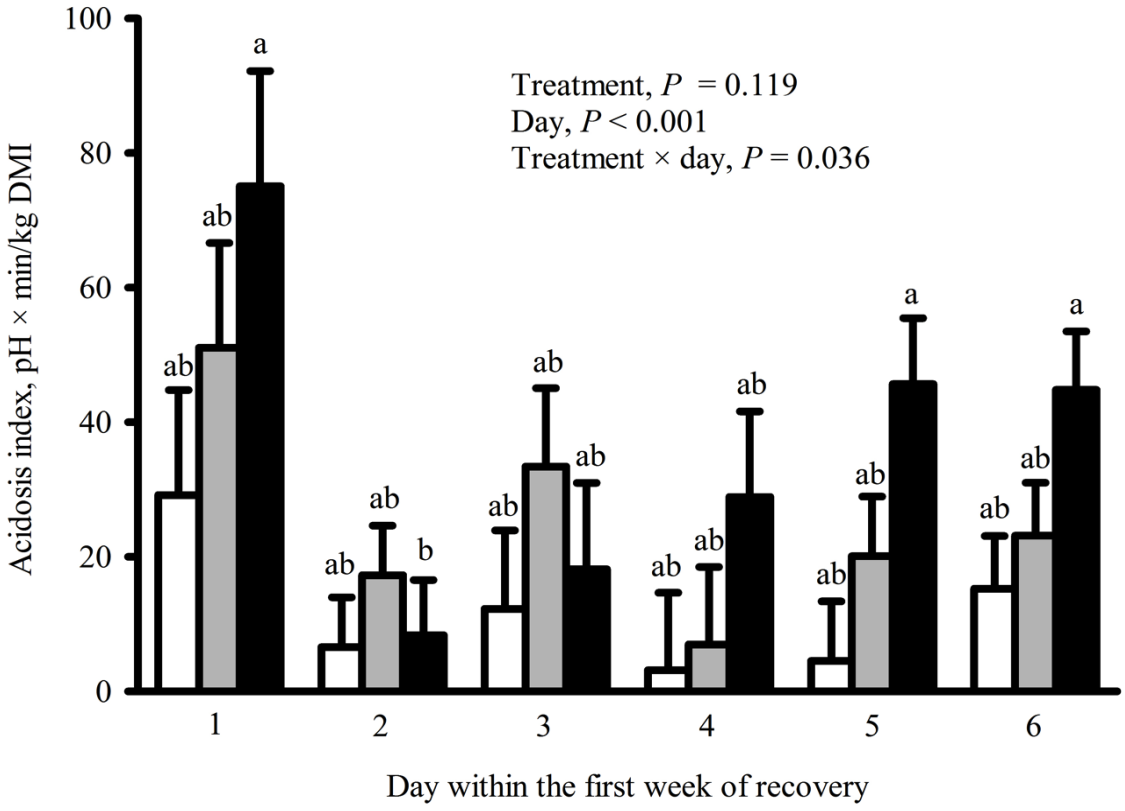


Figure 4.1. Interaction between the severity of short-term (FR) and day within the first week of recovery on ruminal acidosis index ($P$ = 0.027). Heifers were restricted to 75% (white bars), 50% (grey bars) or 25% (black bars) of ad libitum intake during FR and provided ad libitum intake during recovery. Means with uncommon letters differ across treatments and periods ($P \leq 0.05$).

Although interactions were detected for the absolute absorption rates (mmol/h), no interactions between treatment and period were detected for the fractional rates of SCFA absorption (%/h, $P > 0.05$; data not shown) and thus the main effects of treatment and time are presented (Table 4.8). There were no effects of treatment on the fractional rate of SCFA absorption; however, linear increases were detected over time ($P \leq 0.005$) for total SCFA, acetate, propionate, and butyrate.

### 4.4.3. Total Tract Barrier Function

No interaction between treatment and period was detected for Cr excretion ($P = 0.112$, data not shown). However the severity of the FR event affected urinary Cr excretion ($P = 0.027$, Figure 4.2) and thus the recovery of barrier function, although, changes in urinary Cr recovery over time was not detected (period $P = 0.993$; data not shown). Heifers restricted to 50% had the lowest Cr output and heifers restricted to 25% had the highest with heifers restricted to 75% having intermediate urinary Cr output.

### 4.4.4. Blood Metabolites

Treatment did not affect plasma glucose or BHBA concentrations while period effects were detected (Table 4.9). Plasma glucose decreased linearly from FR (83 mg/dL) to REC3 (76 mg/dL; $P < 0.001$), whereas, plasma BHBA concentration increased linearly ($P = 0.026$) over time. A treatment × period interaction was only detected for serum NEFA ($P < 0.001$, Figure 4.3). Cows restricted to 25% had higher NEFA concentration than those restricted to 75% and 50% during FR, but upon returning to ad libitum feed intake in REC1, serum NEFA concentrations decreased rapidly for all treatments with no differences observed thereafter.

Table 4.7. Interaction between the severity of short-term feed restriction (FR) and the measurement period (FR, REC1 and REC3) on the absolute absorption rate of short-chain fatty acid SCFA across the temporarily isolated and washed reticulo-rumen.

| | Treatment[1] | | | | |
|---|---|---|---|---|---|
| Variable[2], mmol/h | 75% | 50% | 25% | SEM | *P* Value |
| Total SCFA | | | | | |
| FR | 591.1[ab] | 419.4[b] | 438.7[b] | | |
| REC1 | 675.5[ab] | 606.2[ab] | 524.4[b] | 58.40 | 0.009 |
| REC3 | 588.4[ab] | 626.6[ab] | 747.4[a] | | |
| Acetate | | | | | |
| FR | 321.0[ab] | 215.9[b] | 223.5[b] | | |
| REC1 | 362.3[ab] | 317.6[ab] | 280.2[b] | 30.76 | 0.014 |
| REC3 | 317.7[ab] | 323.1[ab] | 390.6[a] | | |
| Propionate | | | | | |
| FR | 185.1[ab] | 140.2[b] | 139.9[b] | | |
| REC1 | 213.8[ab] | 198.2[ab] | 169.2[b] | 19.80 | 0.006 |
| REC3 | 183.5[ab] | 206.1[ab] | 246.3[a] | | |
| Butyrate | | | | | |
| FR | 85.1[ab] | 63.3[ab] | 75.3[ab] | | |
| REC1 | 99.4[ab] | 90.4[ab] | 74.9[b] | 9.83 | 0.021 |
| REC3 | 87.2[ab] | 97.5[ab] | 110.4[a] | | |

[ab]Means within a response variable (total SCFA, acetate, propionate, butyrate) that have uncommon letters differ among treatments and periods ($P \leq 0.05$).
[1]Values refer to the percentage of feed offered during FR relative to that consumed during BASE.
[2]Dissapearance rates from the reticulo-rumen were deemed equal to absorption rates.

Table 4.8. Effects of the severity of short-term feed restriction (FR) and the measurement period (FR, REC1 and REC3) on the fractional absorption rate of short-chain fatty acid (SCFA) across the temporarily isolated and washed reticulo-rumen.

| | Treatment[1] | | | | | Experimental Period | | | | *P* Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item, %/h | 75% | 50% | 25% | SEM | *P* Value | FR | REC1 | REC3 | SEM | Linear | Quadratic |
| Total SCFA | 42.5 | 37.9 | 38.4 | 2.66 | 0.436 | 34.4 | 38.7 | 45.9 | 2.26 | < 0.001 | 0.865 |
| Acetate | 39.7 | 34.5 | 35.0 | 2.62 | 0.329 | 31.2 | 35.8 | 42.1 | 2.10 | < 0.001 | 0.695 |
| Propionate | 45.1 | 41.7 | 41.4 | 2.91 | 0.612 | 37.2 | 41.6 | 49.4 | 2.45 | < 0.001 | 0.912 |
| Butyrate | 49.1 | 45.2 | 46.5 | 2.96 | 0.636 | 42.0 | 44.7 | 54.1 | 2.96 | 0.005 | 0.724 |

[1]Values refer to the percentage of feed offered during FR relative to that consumed during BASE.

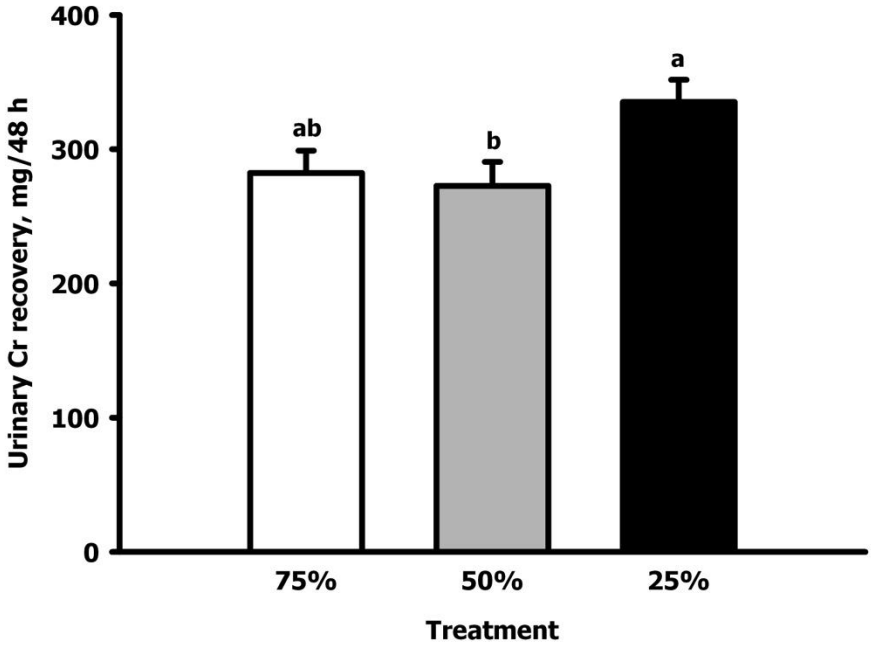


Figure 4.2. Severity of feed restriction (FR) on 48-h urinary Cr output throughout the measurement period (FR, REC1 and REC3) (treatment, $P = 0.027$). Period and treatment × period effects were not detected ($P > 0.05$). Heifers were restricted to 75% (white bars), 50% (grey bars) or 25% (black bars) of ad libitum intake during FR with ad libitum intake during recovery. Means with uncommon letters differ across treatments and periods ($P \leq 0.05$).

Table 4.9. Effects of the severity of short-term feed restriction (FR) and the measurement period (FR, REC1 and REC3) on plasma glucose and BHBA concentrations.

| | Treatment[1] | | | | | Experimental Period | | | | *P* Value | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Item | 75% | 50% | 25% | SEM | *P* Value | FR | REC1 | REC3 | SEM[1] | Linear | Quadratic |
| Glucose, mg/dL | 79.8 | 81.1 | 80.1 | 1.61 | 0.831 | 82.9 | 82.3 | 75.9 | 1.56 | 0.001 | 0.340 |
| BHBA, mg/dL | 7.96 | 7.79 | 8.13 | 0.361 | 0.797 | 7.74 | 7.42 | 8.73 | 0.361 | 0.026 | 0.144 |

[1]Values refer to the percentage of feed offered during FR relative to that consumed during BASE.

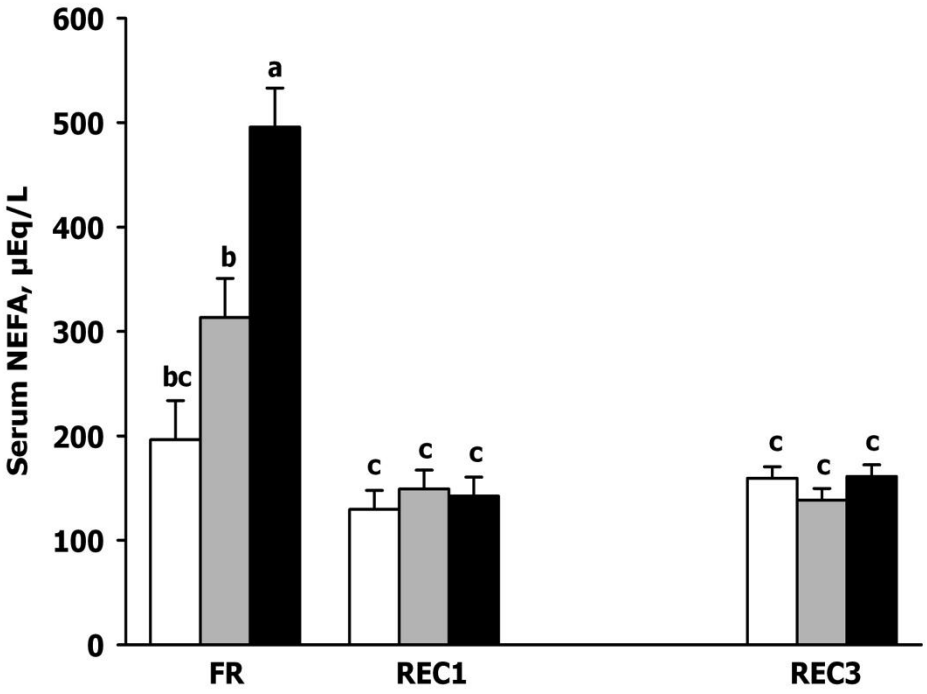


Figure 4.3. Interaction between the severity of short-term feed restriction (FR) and the measurement period (FR, REC1 and REC3) for plasma NEFA concentration ($P < 0.001$). Heifers were restricted to 75% (white bars), 50% (grey bars) or 25% (black bars) during FR. Means with uncommon letters differ across treatments and periods ($P \leq 0.05$).

## 4.5. Discussion

A growing body of evidence has demonstrated that FR, whether it be short-term feed deprivation (48 h; Gäbel et al., 1993; Gäbel and Aschenbach, 2002), chronic FR ( > 4 wk; Perrier et al., 1994; Doreau et al., 1997), or short-term FR (5 d) reduces the absorptive function of the ruminal epithelium (see chapter 3). Gäbel and Aschenbach (2002) investigated barrier function showing that feed deprivation and has a negative effect on barrier function which supports the findings in the previous chapter. While these findings have enhanced the understanding of the rapid onset of degenerative processes for the ruminal epithelia, few studies (Doreau et al., 2003) to date have evaluated the time required for the ruminal epithelia to recover. Thus, this study was designed to evaluate how the severity of short-term FR affects the timeline for recovery of ruminal absorptive function and total tract barrier function.

In the current study, it was observed that the timeline and rate of recovery for DMI (as a proportion of initial BW), following return to ad libitum intake, was dependent on the severity of the FR induced. The timeline required for DMI recovery appears to be hastened for heifers exposed to less severe episodes of short-term FR such that heifers exposed to severe FR required at least 2 wk to stabilize DMI, with those exposed to moderate FR (those restricted to 50% of their pre-FR intake) only having modest numerical increases in DMI during the 3 wk of recovery. In contrast, heifers restricted to 75% of their pre-FR DMI had resumed DMI within REC1. This finding is supported by Hutcheson and Cole (1986) as they showed that highly stressed newly received cattle require more than 2 wk for feed intake to return to expected levels. Although Hutcheson and Cole (1986) were not able to separate the effects of stressors associated with weaning, transportation, co-mingling, environmental changes, and feed deprivation on the time required to return to expected feed intake, the current study confirms their findings showing that in the absence of other stressors, FR itself has carry-over effects with heifers exposed to severe FR (those restricted to 25% of their pre-FR intake) requiring at least 2 wk to return to expected voluntary intake. This data highlights the importance of developing strategies to minimize the severity of FR associated with, e.g., long transportation.

The mechanisms for why the return to feed intake following a period of FR is delayed without external stressors is not currently known. Feed intake in ruminants is thought to be regulated via distension (Mertens, 1994) and hepatic oxidation (Allen et al., 2009). Although

digesta mass and volume were not measured in the current study, it is unlikely that physical distension hindered the return to expected feed intake, especially considering that all heifers were fed the same diet and had recently experienced a period of short-term FR.

Alternatively, Allen et al. (2009) suggested that stress-induced NEFA release may contribute to the reduction in feed intake observed for newly received calves. However, in the current study NEFA was only higher during FR for those heifers restricted to 25% of their pre-FR intake and did not differ among treatments during REC1 or REC3. Thus, it does not appear that hepatic oxidation contributed to the delayed increase in DMI for heifers restricted to 25% of ad libitum DMI, especially, considering that total SCFA concentration and molar proportion of propionate, a potent stimulator of hypophagia (Oba and Allen, 2003), did not differ among treatments during recovery. Future studies are needed to elucidate mechanisms and strategies to accelerate the recovery of DMI following a period of FR.

Provision of ad libitum access to feed following 5-d of FR induced ruminal acidosis regardless of the severity of the FR. In fact, when treatments were pooled, heifers spent more than 233 min (nearly 4 h) below pH 5.5 per day during REC1. While this was not an expected outcome given the moderate fermentability of the diet (forage-to-concentrate ratio of 60:40), numerous researchers have utilized short-term FR as part of a ruminal acidosis induction strategy (Owens et al., 1998; Momcilovic et al., 2000), and ruminal acidosis is a common occurrence following heat stress (Kadzere et al., 2002; Collier et al., 2006), likely due to short-term FR followed by large meals with alleviation of heat stress.

In an earlier study, a high correlation between feed intake and the lowest daily ruminal pH on the previous day was observed for feedlot cattle, indicating that if pH is low, cattle will regulate ruminal pH by decreasing feed intake to help regulate ruminal pH (Brown et al., 2000). Fulton et al. (1979) showed for beef cattle that DMI was depressed when ruminal pH fell below approximately 5.6. The conclusions of these previous studies are supported by the current study where the lowest ruminal pH value was observed on d 1 of REC1 (i.e. 1$^{st}$ day of ad libitum feed after 5 d of FR) followed by a reduction in DMI by 2 kg on d 2 relative to d 1. The reduction in pH caused by high DMI on d 1 of REC1 resulted in an average minimal pH value of 4.93 and a duration that pH was below 5.5 of 522 min on the 1$^{st}$ d back to ad libitum feeding.

High DMI is often associated with low pH or a greater risk for the occurrence of ruminal acidosis (Stone, 2004; Krause and Oetzel, 2006). However, DMI is not a reliable indicator of the relative risk for ruminal acidosis (Bevans et al., 2005; Penner et al., 2007, 2009b). This suggests that other factors such as acid clearance may have a larger impact on the regulation of ruminal pH (Penner et al., 2009a; Aschenbach et al., 2011). The current study confirms that DMI is not the sole risk factor as, depending on the treatment, DMI increased during recovery or was relatively constant, despite having lower pH and a greater severity of ruminal acidosis in REC1 than the subsequent periods. The period of most severe pH depression also corresponded to the period where SCFA absorption was compromised. This further emphasizes the importance of SCFA absorption on ruminal pH homeostasis (Penner et al., 2009a; Aschenbach et al., 2011).

As mentioned above, it could be expected that low rates for SCFA absorption during the 1$^{st}$ wk of ad libitum feeding (REC1) contributed to a greater risk for ruminal acidosis (Gäbel et al., 2002; Penner et al., 2009a; Aschenbach et al., 2011). Conversely, it is also possible that ruminal acidosis may have contributed to lower absorptive function of the reticulo-rumen during REC1. It has been shown in previous experiments using the WRR technique that reducing buffer solution pH from 6.8 to 4.8 led to decreases in the net absorption of $Na^+$, $Mg^{2+}$ and $Cl^-$ across the reticulo-rumen in sheep (Gaebel et al., 1987), and that approximately 5 d were necessary for the epithelium to recover to full absorptive function for these electrolytes (Gaebel and Martens, 1988). Using Ussing chambers, acidification of the mucosal side to pH 5.2 compared with pH 6.2 decreased the mucosal-to-serosal flux of butyrate in the recovery period which suggests that low ruminal pH also induces negative carry-over effects on the absorptive function of the ruminal epithelium for SCFA absorption (Wilson et al., 2012). As cause or effect can not be elucidated in the current study, future work is needed to determine the interrelationship between absorptive function of the rumen epithelium and ruminal acidosis.

As was hypothesized, the absolute SCFA absorption rate (mmol/h) increased from REC1 to REC3 for heifers restricted to 25% of ad libitum intake while heifers restricted to 75 and 50% of ad libitum intake during FR either had no change in the rate of SCFA absorption or the rate increased only numerically. This finding is important from 2 perspectives; firstly, it suggests that down-regulation of epithelial functions can occur more rapid than functional recovery of the

ruminal epithelium and, secondly, it confirms the hypothesis that the severity of the FR event impacts not only on the down-regulation of epithelial functions but, especially, on the recovery response. To my knowledge, this is the first time that the severity of FR and the resulting recovery time has been investigated.

Regarding the recovery response following FR, insight and support for the timeline observed can be provided from previous studies evaluating ruminal epithelial adaptation. A previous study (Etschmann et al., 2009) has demonstrated that functional adaptation precedes morphological adaptation and occurs rapidly with more than 70% of the total adaptive response for $Na^+$ absorption occurring in the first 7 d following an increase in diet fermentability. Moreover, Doreau et al. (2003) suggested that approximately 2 wk was required for recovery of the absorptive function of the ruminal epithelia following a 10-wk period of FR to 50% of maintenance requirement. This adaptation corresponds with a reduction in time required for epithelial turnover , with an average of 16 d when fed roughage, 11 d when fed concentrate, but being markedly reduced (turnover of 4 d) during dietary transition from roughage to concentrate (Goodlad, 1981). However, epithelial hypertrophy cannot be eliminated as a possible mechanism partially explaining the adaptive response (Burrin et al., 1992).

In the current study, although a recovery response was observed for the absorptive function, it was observed that heifers restricted to 25% of ad libitum feeding had compromised barrier function relative to heifers restricted to 50% and data is suggestive that barrier function may have been compromised relative to heifers fed 75% of ad libitum feeding prior to recovery. However, period effects or an interaction between treatment and period for total tract barrier function were not detected indicating that barrier function may not have recovered for heifers exposed to severe FR until REC3. In chapter 3, it was shown that only heifers exposed to severe FR had greater urinary Cr recovery relative to baseline measurements. Thus, the greater urinary Cr output in urine observed during recovery may be a carry-over effect of the FR event. Additionally, upon return to ad libitum feed intake, heifers experienced sustained low ruminal pH with heifers exposed to greater severities of FR having a more pronounced response. Exposure of ruminal epithelia to low mucosal pH increases the passive permeation of histamine (Aschenbach and Gäbel, 2000), mannitol (Penner et al., 2010), and increases tissue conductance (Wilson et al., 2012). Therefore, in the current study, the ruminal acidosis in the group restricted

to 25% of ad libitum intake could be the reason for the compromised barrier function during the recovery periods. Although ruminal epithelial barrier function was not measured independently, the compromised total gastrointestinal barrier function for the most severe FR may come from the acid-damaged ruminal barrier function. Research is needed to elucidate the molecular mechanisms regulating total tract barrier function during and after FR and to determine the regions that are most susceptible to barrier dysfunction.

### 4.6. Conclusions

The severity of the FR imposed affects the recovery response for DMI and SCFA absorption with more severe FR delaying recovery. Moreover, severe short-term FR compromises total tract barrier function over longer periods. Regardless of severity, short-term FR induces ruminal acidosis upon return to ad libitum feeding.

# 5.0. GENERAL DISCUSSION

## 5.1. Implications for Ruminant Production

As described in detail in chapter 3 and chapter 4, this study has contributed important and novel findings as short-term FR reduces ruminal epithelial absorptive function and, if severe, total tract barrier function. This research has also demonstrated that the rate of recovery for reticulo-rumen absorptive function is rapid upon return to feeding ad libitum following short-term FR. As the scientific merit for these findings has been previously discussed, the industry relevance of these points will be highlighted below.

As previously demonstrated short-term FR occurs in beef and dairy production settings, however unlike the real situations in industry, my study excluded factors other than FR to separate the effects of FR from other stressors such as weaning, transportation, parturition, and heat stress. Thus, while the results show the effect of FR in isolation, it is likely that these results are relevant and may actually under-estimate the negative effects of FR when it occurs in combination with other stressors. This may help to provide new insight into challenges cattle face under the previously mentioned production scenarios as not only does feed intake decrease but also gut function, from a general perspective, is compromised. The latter is important as it may not be as obvious as the reduction in DMI, but it is likely to have a more severe impact for the cattle health. For example, compromised absorptive function of the ruminal epithelium may cause severe ruminal pH depression as SCFA absorption is a key regulator of ruminal pH homostasis (Penner et al., 2009a; Aschenbach et al., 2011). Furthermore, since pathogenic bacteria (Narayanan et al., 2002; Nagaraja et al., 2005), antigens (Gozho et al., 2005, 2006; Zebeli and Ametaj, 2009) and biological amines (Irwin et al., 1979; Plaizier et al., 2008) are present in the ruminal digesta, the importance of barrier function of the gastrointestinal tract should not be underemphasized. Once barrier function is disrupted, the risk for disorders like laminitis (Vermunt, 1992; Nocek, 1997) and liver abscesses (Lechtenberg et al., 1988; Nagaraja and Chengappa, 1998) increase. Therefore, strategies need to be developed to reduce the negative effects of short-term FR, regardless of the severity.

### 5.1.1. Current Strategies to Mitigate the Effect of Feed Restriction in Industry

While FR may be mitigated by management approaches in some scenarios such as weaning, there are certainly cases such as heat stress and parturition, where it may be difficult to prevent. For example, methods to minimize FR for weaned calves include approaches to reduce the stress at weaning through the use of fenceline contact of the beef calves with their dams (Price et al., 2003) or weaning calves while having physical contact with the dam (Haley et al., 2005). The results showed that low-stress weaning approaches can reduce the negative effects of weaning including reducing the time spent walking, vocalizating, and increasing the time spent eating and resting. Consequently, low-stress weaning approaches have been shown to increase weight gain relative to abruptly weaned calves but even calves weaned using low-stress approaches still achieve less weight gain compared with non-weaned calves (Price et al., 2003). Despite changes in behavior, we can only extrapolate these findings to suggest that calves weaned with low-stress approaches reduce DMI to a lower extent. Furthermore, data are still limiting in terms of the extent of feed intake restriction as a result of weaning. Therefore, weaning induced FR may be able to be minimized but it does not appear that it is possible to eliminate it.

Another example for when FR occurs is during transportation of feedlot cattle. According to González et al. (2012), under current commercial practices long haul transport (≥ 400 km) of beef cattle in Alberta was on average 16 h. Theoretically it is possible to prevent feed deprivation during transportation by decreasing transit times without feed or by providing feed during transit. However, it is not practical to implement such strategies because it may cause other problems such as bruising and higher cost due to lower load density. In addition, when considering the stress associated with transportation, cattle may not eat even when feed is provided (Grandin, 1997). Because of the magnitude of the stress response during transportation and new stressors like environmental changes and mixing together with other cattle, low intake is further compounded with transported cattle after arriving at the feedlot (Hutcheson and Cole, 1986). Dietary strategies such as increasing protein density in diet (Cole and Hutcheson, 1988, Fluharty and Loerch, 1995), increasing the proportion of concentrate in the receiving diet (Fluharty et al., 1994b), or keeping the diet the same before and after transportation (Fluharty and Loerch, 1996) have been used to promote DMI for newly received feedlot cattle, but

research to date has not been that promising, at least in terms of mitigating the reduction in DMI and accelerating the recovery response.

For transition dairy cows, the DMI reduction before calving is also a major problem. When parturition approaches, in response to growing fetus and the onset of lactation, drastic hormonal changes are observed in transition dairy cows (Dann et al., 1999; Drackley, 1999). Dry matter intake decreases as well as many digestive and metabolic disorders may occur in the transition period (Drackley, 1999; Hayirli et al., 2002). As nutrient requirements increase leading up to and following parturition, nutritional strategies have been developed to increase energy intake. Increasing energy density is the most popular method; however the low NDF diet usually causes greater feed depression before parturition (Rabelo et al., 2003) and may increase the incidence and severity of ruminal acidosis after parturition (Penner et al., 2007).

Under high environmental temperature and humidity, dairy cows and beef cattle are both susceptible to heat stress (Maust et al., 1972; Mader et al., 2006). Decreased DMI is a common response with heat stress as cattle try to decrease heat production (Maust et al., 1972; Knapp and Grummer, 1991; Holter et al., 1996). The most obvious way to prevent or minimize heat stress is by providing enough shade, ventillation, and other cooling strategies such as misting. According to Schneider et al. (1984), feed intake under hot weather was about 23% greater when shade was provided compared with no shade. Another effective way to help heat-stressed cows is provision of extra cooling with sprinklers (Monty and Garbareno, 1978). Shifting feeding time to afternoon vs. morning was also been proved to increase DMI (Davis et al., 2003). However, these strategies may not prevent the reduction in feed intake occurring with heat stress and they may not be economically feasible, especially under extensive grazing settings. Colelctively, this data suggests that a more complete understanding of the impact of FR is needed.

To summarize, it is not possible to prevent FR in beef or dairy cattle. However, FR occuring in concert with weaning, transportation, parturition and hot weather can be predictable, allowing for the development and implementation of strategies to minimize the negative effect of FR.

### 5.1.2. Suggestions for Minimizing the Negative Effect of Feed Restriction

The approach used to mitigate the negative effect of FR will be dependent on the cause of FR. For example, with newly weaned dairy calves, the rumen is not fully developed. Strategies to promote rumen function are of great importance. According to Gorka et al. (2009), supplementation of sodium-butyrate at low doses in the milk replacer and starter could be used to enhance rumen development which may promote DMI after weaning. For newly weaned beef calves, especially newly received beef calves in the feedlot, the rumen is well adapted before weaning. However multiple stressors with weaning, transportation and re-mixing in the feedlot with other calves usually cause DMI depression for several weeks (Hutcheson and Cole, 1986). To encourage calves to eat and to provide relative higher energy at lower DMI, a more fermentable diet is recommended during a short-term period (e.g. a wk). A high forage and low concentrate diet need to be provided later to ensure a reasonable gain and also to reduce fat deposition should an increase in frame size be desired.

The situation is different for cattle experiencing heat stress and for transition dairy cows as the drive to return to voluntary feed intake or rapidly increase feed intake is present. This rapid increase in DMI following FR predisposes cattle to ruminal acidosis. To mitigate the extent and severity of ruminal acidosis as DMI increases, strategies to enhance ruminal buffering need to be considered. There are 2 ways to stimulate ruminal buffering including endogenous and dietary approaches. Because endogenous buffering is produced by the cattle via saliva and absorption of SCFA (Allen, 1997), strategies to increase salivary buffer supply are needed. This may include providing a diet to improve chewing activity through manipulation of particle size (Krause and Oetzel, 2006). According to the results from my study, even a moderate forage diet may induce ruminal acidosis upon feeding ad libitum, and thus a high forage with relative long particle size is recommended at first (e.g. a wk). Dietary buffering can also be achieved using low doses (approximately 1% of DM) of buffer (e.g. sodium bicarbonate). Sodium bicarbonate addition has been shown to help prevent ruminal pH depression in dairy cattle (Kennelly et al., 1999; Hu and Muyphy, 2005; Krause et al., 2008). In reality, for cattle after experiencing heat stress and cows after parturition, gradual re-introduction to full feed with buffer supplementation is suggested.

### 5.2. Future Work

Based on the results of the research within this thesis, it is concluded that FR has negative effects on the absorptive and barrier function of the gastrointestinal tract in beef cattle. However the mechanisms underlying this response are currently not known. Future work is needed to investigate the mechanisms of the compromised absorptive function of the rumen during FR and the recovery mechanisms after returning to ad libitum feeding. For the compromised barrier function of the total gastrointestinal tract, future work needs to evaluate whether the rumen accounts for most of the damage or whether different segments of the gastrointestinal tract may be more susceptible to passive transfer. Work is needed to determine whether the tight-cell junctions are disrupted or not during and after FR. Moreover, nutritional and management strategies need to be developed to reduce the negative effects of weaning, transportation, transition and heat stress induced FR and accelerate the recovery period after these scenarios.

## 6.0. CONCLUSIONS

The current study has shown that short-term FR has negative effects on ruminal absorptive function and severe FR compromoses the total gastrointestinal barrier function. Although the recovery of absorptive function is rapid, more than a week is needed for absorptive function recovery when cattle experience severe FR. Regardless of severity, short-term FR induces ruminal acidosis following ad libitum feeding. Based on the results, strategies to minimize the negative effects of short-term FR and accelerate the recovery response are necessary for beef and dairy cattle.

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