THE EFFECT OF DIETARY ADAPTATION ON THE SUSCEPTIBILITY TO AND RECOVERY FROM RUMINAL ACIDOSIS IN BEEF CATTLE

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ABSTRACT

Feeding diets rich in rapidly fermentable non-structural carbohydrates can lead to the development of ruminal acidosis. This study was conducted to determine if the duration of time that cattle are fed a high-grain diet affects their absorption of short-chain fatty acids (**SCFA**) and susceptibility to, and recovery from, ruminal acidosis. Sixteen Angus heifers (BW ± SEM, 261 ± 6.1 kg) were assigned to 1 of 4 blocks, and fed a backgrounding diet consisting of 60% barley silage, 30% barley grain, and 10% supplement (DM basis). Within block, cattle were randomly assigned to 1 of 2 treatments differing in the number of days they were fed the high-grain diet prior to an acidosis challenge: 34 d for long-adapted (**LA**) and 8 d for short-adapted (**SA**). All cattle were exposed to the same 20-d dietary transition using 5 dietary steps until achieving the final diet that contained 9% barley silage, 81% barley grain, and 10% supplement (DM basis). Data were collected during an 8-d baseline period (**BASE**), on the d of the acidosis challenge (**CHAL**), and during two consecutive 8 d recovery periods (**REC1** and **REC2**). Ruminal acidosis was induced by restricting feed to 50% of DMI:BW for 24 h followed by an intraruminal infusion of ground barley at 10% DMI:BW. Cows were then given their regular diet allocation 1 h after the intraruminal infusion. The duration of time fed the high-grain diet did not affect ruminal pH, lactate, or SCFA concentrations ($P > 0.050$). However, during BASE and on the day of CHAL the SA heifers experienced greater linear ($P = 0.031$), quadratic ($P = 0.016$), and cubic ($P = 0.008$) between day change in the duration of time that pH was < 5.5 than LA heifers. Relative to BASE, inducing acidosis increased daily duration (531 to 1020 min/d; $P < 0.001$) and area (176 to 595 (min × pH)/d; $P < 0.001$) that pH was < 5.5. Inducing ruminal acidosis also increased the daily mean (0.3 to 11.4 m*M*; $P = 0.013$) and maximum (1.3 to 29.3 m*M*; $P = 0.008$) rumen fluid lactate concentrations relative to BASE, suggesting that an acute bout of ruminal acidosis was induced. In addition, a treatment × day interaction for the duration that pH was < 5.5 during REC1 suggests that LA cattle tended to recover from the CHAL more rapidly than SA cattle ($P = 0.085$). Indeed, analysis of covariance confirmed that the LA heifers experienced a quicker linear ($P = 0.019$) recovery over time from CHAL. The greater rate of recovery possibly resulted from the LA heifers having greater rates of both fractional butyrate (45 vs. 36 %/h; $P = 0.019$) and propionate absorption (42 vs. 34 %/h; $P = 0.045$), and tending to have greater rates, on an absolute basis, of butyrate absorption (94 vs. 79 mmol/h; $P = 0.087$)

and, on a fractional basis, of total SCFA absorption (37 vs. 32 %/h; $P = 0.100$). Treatment × period interactions revealed that LA heifers had greater serum D-lactate concentrations ($P = 0.003$), and fractional rates of lactate absorption ($P = 0.024$) than SA heifers, during CHAL and REC1, respectively. When treatments were pooled, the absorption (%/h and mmol/h) of acetate, propionate, butyrate, and total SCFA increased between REC1 and REC2, with intermediate values for BASE ($P \leq 0.05$). Corresponding to a reduction in absorption during REC1 (2 d post CHAL), saliva production (kg/h; $P = 0.018$) increased between BASE and REC1, with intermediate values for REC2. These results indicate that the duration of time cattle are fed a high-grain diet may stabilize rumen pH, both prior to and after an induced bout of acute ruminal acidosis, likely through increased ruminal absorptive capacity for SCFA and lactate. In addition, this study found evidence to suggest that beef cattle possess the ability to increase saliva secretion in order to compensate for decreased absorptive capacity.

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DEDICATION

This work is dedicated to

Adrian Jack O'Neill

(June 3, 1988 - March 26, 2013)

Age, you have always been supportive of my academic pursuits, even if the support was often delivered in a self-depricating manner. Your ability to brighten a room with your smile, and lift those around you will remain unparallelled. I wish I could have had one last visit, but I will forever carry the lessons that I have learned from being your friend.

Keep smiling buddy,

Schwaigz

LIST OF ABBREVIATIONS

ADF	Acid detergent fiber
AE	Anion exchanger
BASE	Baseline
BHBA	β-hydroxybutyrate
BW	Body weight
CHAL	Challenge
CoA	Coenzyme A
CP	Crude protein
d	Day
DM	Dry matter
DMI	Dry matter intake
DMI:BW	Dry matter intake-to-body weight ratio
DRA	Downregulated in adenoma
F:C	Forage-to-concentrate ratio
GC	Gas chromatography
h	Hour
HCO_3	Bicarbonate
Hp	Haptoglobin
ICP-OES	Inductively-coupled plasma optical emission spectrometry
IGF	Insulin-like growth factor
IGFBP	Insulin-like growth factor binding protein
LA	Long-adapted to the high-grain diet
LBP	Lipopolysaccharide-binding protein
LPS	Lipopolysaccharide
MCT	Monocarboxylate transporter
mRNA	Messenger ribonucleic acid
NDF	Neutral detergent fiber
NHE	Sodium-hydrogen exchanger
P	Period of sampling (BASE, CHAL, or REC)

PAT	Putative anion transporter
PCV	Packed cell volume
peNDF	Physically effective neutral detergent fiber
REC	Recovery
SA	Short-adapted to the high-grain diet
SAA	Serum amyloid A
SARA	Sub-acute ruminal acidosis
SCFA	Short-chain fatty acid
SD	Standard deviation
SEM	Standard error of the mean
T	Treatment (LA or SA)
t	Time (after feeding during BASE and REC, or challenge dose during CHAL)
TMR	Total mixed ration
wk	Week
WRR	Temporarily isolated and washed reticulorumen technique

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. GENERAL INTRODUCTION

Ruminal acidosis is often regarded as the most prevalent digestive disorder in domestic cattle (Krause and Oetzel, 2006; Nagaraja and Titgemeyer, 2007), and is primarily characterized by an accumulation of organic acids in the rumen and a subsequent decrease in ruminal pH (Penner et al., 2007, 2009a,b). However, there is no uniformly accepted pH threshold used to define the disorder (Plaizier et al., 2008), and detection is further complicated by the substantial within day (Nocek et al., 2002), between day (Aschenbach et al., 2011), and between animal (Bevans et al., 2005) variability in ruminal pH. Low ruminal pH has been shown to alter the rumen microbiome (Mackie and Gilchrist, 1979; Goad et al., 1998; Khafipour et al., 2009a) and damage the rumen epithelia leading to decreased barrier function (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012). In addition, ruminal acidosis has been associated with numerous animal welfare concerns including laminitis (Brent, 1976; Nocek, 1997), rumenitis (Jensen et al., 1954; Kleen et al., 2003; Plaizier et al., 2008), liver abcesses (Robinson et al., 1951; Nocek, 1997; Nagaraja and Chengappa, 1998), and an acute phase inflammatory response (Plaizier et al., 2008). Although the suggested mechanisms implicating ruminal acidosis with these animal welfare concerns are not yet fully elucidated, the reduction in epithelial barrier function and drastic change in the rumen microbiome brought on by low ruminal pH has been suggested (but not proven) as a contributing factor for all of these animal welfare concerns (Nocek, 1997; Emmanuel et al., 2007; Plaizier et al., 2012).

The risk for ruminal acidosis is increased by feeding diets rich in rapidly fermentable non-structural carbohydrates (Owens et al., 1998; Krause and Oetzel 2006; Kleen and Canizzo, 2012). The primary non-structural carbohydrate fed to cattle is starch that, when digested in the rumen, undergoes hydrolysis to monosaccharides that are then fermented to yield short-chain fatty acids (**SCFA**). The hydrolysis of starch occurs much more rapidly than for structural carbohydrates such as cellulose, hemicellulose, and lignin (Sniffen et al., 1992; Weisbjerg et al., 1998). With rapid rates of fermentation, the production of SCFA and the subsequent dissociation of SCFA is greater than the removal of ruminal protons from the rumen (sum of passage, absorption, and neutralization) leading to a decline in ruminal pH (Penner et al., 2007, 2009b) and an increase in osmolality (Argyle and Baldwin, 1988; Brown et al., 2000; Bevans et al., 2005).

Ruminants can regulate ruminal pH by employing both behavioural (González et al., 2012) and physiological (Aschenbach et al., 2011) mechanisms. For example, cattle have been shown to decrease DMI (Fulton et al., 1979a), and selectively consume long particles when ruminal pH is low (DeVries et al., 2008). The physiological mechanisms regulating ruminal pH primarily involve the neutralization of protons through the carbonic anhydrase reaction with bicarbonate supplied in saliva (Bailey and Balch, 1961; Erdman, 1988) and from ruminal bicarbonate secretion (Penner et al., 2009a). Ruminal bicarbonate secretion has been shown to occur in exchange for SCFA absorption (Gäbel et al., 1991; Aschenbach et al., 2009, Penner et al., 2009a), and is therefore likely the most important physiological buffering strategy (Allen, 1997), as the relative importance of bicarbonate-dependent absorption increases as pH declines (Aschenbach et al., 2009).

The risk for ruminal acidosis is increased when feeding high-grain diets (Garrett et al., 1999; Penner et al., 2007; Khafipour et al., 2009c); however, a gradual step-wise dietary transition can reduce the risk (Bevans et al., 2005; Brown et al., 2006). Implementing a gradual dietary transition (Burrin et al., 1988; Bartle and Preston, 1992) can encourage both microbial (Allison et al., 1964; Towne et al., 1990; Goad et al., 1998) and epithelial (Dirksen et al., 1985; Bannink et al., 2008) adaptation to high-grain feed, which may reduce the risk of ruminal acidosis (Penner et al., 2009a). However, there are no published studies indicating whether strategies to promote the adaptive response reduce the risk for ruminal acidosis and whether the recovery response following an episode of ruminal acidosis differs for cattle that are well adapted to the high-grain diet relative to those that are not.

2. LITERATURE REVIEW

2.1 Ruminal Acidosis

Increasing weight gain in a cost effective manner is, and will remain, an important concern for beef producers. The prevailing strategy in North America is to feed finishing feedlot cattle high-grain diets that are rich in rapidly fermentable carbohydrates (Owens, 1987; Vasconcelos and Galyean, 2007). However, this strategy can increase the risk for a digestive disorder called ruminal acidosis, which is most often characterized by a decrease in ruminal pH (Owens et al., 1998; Aschenbach et al., 2011). As the onset for, and negative consequences from, ruminal acidosis are on a continuous scale, defining a universally accepted threshold is challenging and there is currently contention surrounding the most appropriate pH thresholds used to define ruminal acidosis (Plaizier et al., 2008).

Ruminal acidosis has been associated with a decrease in the barrier function of the rumen epithelium (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012), an increase in ruminal fluid endotoxin concentration (Dain et al., 1955; Nagaraja et al., 1978a; Gozho et al., 2007) and an increase in the concentration of inflammatory proteins in blood (Brown et al., 2000; Gozho et al., 2005). Moreover, ruminal acidosis has also been implicated as a factor increasing the risk for the development of other feedlot disorders, such as laminitis and liver abscesses (Brent, 1976; Nocek, 1997; Nagaraja and Chengnappa, 1998). The following is a brief overview of the animal welfare concerns, detection, and diagnosis of this digestive disorder. Due to the various constraints surrounding the adequate detection and diagnosis of ruminal acidosis, the numerous outcomes of the disorder with a focus on animal welfare will be discussed first, followed by a detailed discussion surrounding the various definitions of ruminal acidosis and its diagnostic constraints.

2.1.1 Consequences of Ruminal Acidosis

Low ruminal pH has been shown to alter the rumen microbiome (section 2.1.1.1), decrease DMI (Brown et al., 2000; Allen 2000; Plaizier et al., 2008), and decrease both epithelial barrier function (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012) and

absorptive capacity (Gaebel and Martens, 1988; Gaebel et al., 1989; Krehbiel et al., 1995). These myriad of symptoms can not only lead to decreased energy intake and animal performance (Koers et al., 1976; Owens et al., 1998), but if severe enough can compromise animal welfare (section 2.1.1.2) and even result in death (Slyter, 1976; Nocek, 1997).

2.1.1.1 Consequences of Ruminal Acidosis on the Rumen Microbiome

In addition to the aforementioned epithelial damage caused by low ruminal pH, decreased ruminal pH also induces changes in the ruminal microbial composition (Mackie and Gilchrist, 1979; Russell et al., 1979; Nagaraja and Titgemeyer, 2007). Therefore, associations between low ruminal pH and compromised animal welfare must consider both physiological and microbiological changes brought on by decreasing ruminal pH.

As pH begins to decrease, SCFA-producing and lactate-producing bacteria, as well as lactate-utilizing bacteria, have been shown to increase in numbers in the rumen (Mackie and Gilchrist, 1979; Goad et al., 1998; Khafipour et al., 2009a). However, a progressive decline in pH to values below 5.2 can result in a large decrease in the number of cellulolytic bacteria and increase in lactobacilli and other aciduric bacteria (Slyter et al., 1970; Slyter, 1976; Nagaraja and Miller, 1989; Goad et al., 1998). These changes in the rumen microbiota result in a much more rapid rate of carbohydrate fermentation, which causes ruminal pH to plummet further. When pH falls below 5.0, the number of bacteria that produce SCFA (Slyter et al., 1970; Russell and Dombrowski, 1980) and the number of bacteria that utilize lactate (Russell and Dombrowski, 1980; Coe et al., 1999) decrease and the number of bacteria that produce lactate (Slyter et al., 1970**;** Russell and Hino, 1985; Russell, 1991) increase. In fact, a companion study (Petri et al., 2013) that examined the bacteria associated with the epithelia and solid and liquid fractions of rumen fluid of the cattle used in this study found dramatic increases in the lactate producing bacterial populations of *Lactobacillus* and *Streptococcus* in those cattle that experienced the most severe response to our induced bout of ruminal acidosis (described in Chapter 3).

The shift in the ruminal microbiome to these aciduric bacteria at low ruminal pH leads to a shift in organic acid production from SCFA to lactate (Slyter, 1976; Nagaraja and Titgemeyer, 2007). Because lactate removal from the rumen is slow (Williams and Mackenzie, 1965; Harmon et al., 1985; Møller et al., 1997), this shift in the microbiome contributes to the accumulation of

lactate, a strong proton donor, which sustains low pH (Harmon et al., 1985; McLaughlin et al., 2009; Lettat et al., 2010). In addition, low pH (Purser and Moir, 1959; Whitelaw et al., 1984; Franzolin and Dehority, 1996) and osmotic pressures above the normal 280 mOsm/L (Warner and Stacy, 1965) have been shown to decrease the number of ruminal protozoa (Quinn et al., 1962), which are thought to help stabilize ruminal pH (Mackie et al., 1978; Nagaraja et al., 1986; Newbold et al., 1986; discussed further in section 2.3.3).

2.1.1.2 Consequences of Ruminal Acidosis on Animal Welfare

High-grain feeding (Nagaraja et al., 1978b; Andersen et al., 1994a) and ruminal acidosis (Nagaraja et al., 1978a; Andersen and Jarløv, 1990; Gozho et al., 2006) have been associated with an increase in ruminal lipopolysaccharide (**LPS**) concentration. These endotoxins, which are cell wall components of all gram-negative bacteria, have thus been used as an indicator for ruminal acidosis (Gozho et al., 2005). According to Nagaraja et al. (1978b), it is likely that the reduced ruminal pH and increased osmolarity may cause bacteria to lyse and release LPS during bouts of ruminal acidosis. However, it has also been observed that LPS are shed in times of rapid growth (Wells and Russell, 1996). While ruminal LPS from gram-negative bacteria have received considerable attention as ruminal endotoxins, there is a paucity of data describing the ruminal accumulation and antigenicity of gram-positive antigens (such as lipoteichoic acids or proteins associated with the peptidoglycan cell wall) during ruminal acidosis, despite their described intestinal antigenicity (Ishii et al., 2008; Fava and Danese, 2010; Abraham and Medzhitov, 2011). Moreover, total LPS or lipoteichoic acid concentration may not provide enough detail as the virulence of these antigens varies greatly based on structure (Plaizier et al., 2012).

A total of 4 studies to date have detected lipopolysaccharides in portal circulation (Dougherty et al., 1975; Aiumlami et al., 1992; Andersen et al., 1994b; Khafipour et al., 2009c). It has been suggested that LPS likely enter arterial blood via translocation across the rumen wall and/or large intestine (Emmanuel et al., 2007; Plaizier et al., 2012); however, very little data is available to support or challenge this hypothesis. Elevated circulating LPS has been shown to reduce ruminal motility (Eades, 1997), and may induce the production of proinflammatory cytokines and subsequent hepatic production of acute phase proteins (Baumann and Gauldie,

1994; Kushner and Rzewnicki, 1994). Lipopolysaccharide binding protein (**LBP**), haptoglobin (**Hp**) and serum-amyloid A (**SAA**) are acute phase proteins that are used to indicate an acute phase inflammatory response in cattle (Alsemgeest et al., 1994; Plaizier et al., 2012). The above-mentioned acute phase proteins have been detected in ruminants subjected to bouts of ruminal acidosis on numerous occasions (Lal et al., 1991; Khafipour et al., 2009b; Gozho et al., 2007). Because circulating LPS is often not detected alongside with the increased ruminal LPS observed during a grain challenge (Andersen and Jarløv, 1990; Gozho et al., 2007; Khafipour et al., 2009b), it has been suggested that increased circulating LPS may indicate that hepatic detoxification of LPS has become overwhelmed (Plaizier et al., 2012).

In addition, ruminal acidosis has long been associated with rumenitis (Jensen et al., 1954; Kleen et al., 2003; Plaizier et al., 2008) and liver abscesses (Robinson et al., 1951; Nocek, 1997; Nagaraja and Chengappa, 1998). Liver abscesses have been proposed (but not yet proven) to occur as a result of the ruminal translocation of bacteria (Nagaraja and Chengappa, 1998; Kleen et al., 2003), likely due to the reduced barrier function that has been observed to occur at low pH (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012). Ruminal acidosis is also thought to increase the risk for laminitis (Brent, 1976; Nocek, 1997), although the mechanism linking ruminal acidosis and laminitis is not yet known. The ruminal microbial endotoxin histamine (Garner et al., 2002) has been shown to accumulate at low pH (Dain et al., 1955; Wilson et al., 1975) and translocate across the damaged ruminal wall (Aschenbach and Gäbel, 2000; Aschenbach et al., 2000). Therefore, it is thought that circulating histamine may subsequently cause laminitis through vasoconstriction of the corium (Nocek, 1997). However, laminitis in horses has been associated with increased metalloproteinase enzyme expression in basal and parabasal cells near the lamellar basement membrane of hoof tissue (Kyaw-Tanner and Politt, 2004), which may also play a role in ruminant laminitis. It has not yet been determined whether the observed relationships between ruminal acidosis and these other digestive disorders are causative or confounding, but it is clear that low ruminal pH is associated with a number of important animal welfare concerns.

2.1.2 Current Definitions and Diagnostic Constraints for Ruminal Acidosis

Ruminal pH varies substantially throughout the day (Nocek et al., 2002; Nagaraja and Titgemeyer, 2007), between days (Aschenbach et al., 2011), and between cattle even when fed the same diet (Brown et al., 2000; Bevans et al., 2005; Penner et al., 2007). Moreover, the techniques used to measure ruminal pH can influence the diagnosis of ruminal acidosis. For example, ruminal pH is not homogenous within the rumen (Wheaton et al., 1970), as pH values typically decrease in the central-ventral sac when cattle consume high-grain diets (Wheaton et al., 1970), and near the medial mat as dietary inclusion of roughages is increased (Wheaton et al., 1970; Tafaj et al., 2004; Storm and Kristensen, 2010). In addition, measuring the pH of rumen fluid collected via a ruminally canulated animal or via stomach tube have been shown to generate greater values for ruminal pH when compared to collection via rumenocentesis (Garrett et al., 1999; Duffield et al., 2004) or indwelling pH measurement systems (Dado and Allen, 1993). To capture the substantial variation in ruminal pH using spot-sampling techniques, one would need to sample numerous animals repeatedly throughout the day. The amount of training, invasiveness, and labour required to carry out this type of sampling render these techniques impractical on a large scale in a feedlot setting.

The development of systems that enable the continuous measurement of pH (Johnson and Sutton, 1968; Dado and Allen, 1993; Penner et al., 2006 and 2009b; Mottram et al., 2008) have greatly influenced our ability to detect and define ruminal acidosis in both dairy (Penner et al., 2007; Dohme et al., 2008; Khafipour et al., 2009c) and beef cattle (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011). However, the use of these instruments on a large scale is limited by the cost of the devices, as well as the labour and training required for their proper utilization and data analysis. Because continuous pH measurement is required to capture the variability in ruminal pH, the appropriate measurement of ruminal pH alone is the largest impediment to diagnosing ruminal acidosis. However, once one has collected this wealth of continuously measured pH data, another diagnostic constraint presents itself; there is considerable uncertainty surrounding how to best interpret pH data.

Among those who study ruminal acidosis, this digestive disorder is commonly classified based on the extent of the depression in ruminal pH, and the length of time that ruminal pH remains depressed (Kleen et al., 2003; Plaizier et al., 2008; Aschenbach et al., 2011). The upper

limit of ruminal acidosis is often referred to as sub-acute ruminal acidosis (Nagaraja and Titgemeyer, 2007; Penner et al., 2009a; Penner et al., 2010), and the terms acute ruminal acidosis and lactic acidosis are commonly reserved for a more severe manifestation of the disorder (Nagaraja et al., 1985; Harmon et al., 1985; Coe et al., 1999). However, a consensus has not been reached regarding the appropriate pH thresholds used to identify either (Owens et al., 1998; Nagaraja and Titgemeyer, 2007; Plaizier et al., 2008).

The most common pH thresholds suggested to identify the upper limit of ruminal acidosis (sub-acute ruminal acidosis, or **SARA**) in cattle include **6.0**, **5.8**, **5.6**, and **5.5** (Table 2.1). Upon close examination, it appears as though most researchers tend towards the lower pH thresholds of **5.6** or **5.5** to delineate SARA in beef cattle, while pH below **6.0** or **5.8** are usually reserved to indicate SARA in dairy cattle (Table 2.1).

Table 2.1. The most commonly used pH thresholds to diagnose the upper limit of ruminal acidosis in dairy and beef cattle.

pH	Dairy cattle	Beef cattle
6.0	Keunen et al. (2002) Mutsvangwa et al. (2002)	
5.8	Beauchemin et al. (2003) Penner et al. (2007) Dohme et al. (2008) Zebeli et al. (2008)	Beauchemin et al. (2001) Schwartzkopf-Genswein et al. (2003) Moya et al. (2011)
5.6	Gozho et al. (2005) Krause and Oetzel (2005) Steele et al. (2011a)	Owens et al. (1998) Cooper et al. (1999) Brown et al. (2000) Bevans et al. (2005) Nagaraja and Titgemeyer (2007)
5.5	Nocek (1997) Garrett et al. (1999) Kleen et al. (2003) Krause and Oetzel (2006)	Hibbard et al. (1995) Goad et al. (1998) Coe et al. (1999) Wierenga et al. (2010) Zhang et al. (2013a)

An approach that considers the production system is logical, as feedlot beef cattle have a much greater dietary inclusion of concentrates, and therefore are more likely to encounter lower ruminal pH (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011), and are likely

impacted to a lesser extent by decreased fiber digestibility (Erdman, 1988; Dijkstra et al., 2012). However, the physiology of the rumen is often discussed by pooling work done in sheep, dairy, and beef cattle (Aschenbach et al., 2011; Penner et al., 2011) and distinct pH thresholds have not yet received explicit attention in light of these 3 separate ruminant models (Aschenbach et al., 2011). Furthermore, some researchers that specialize with dairy cattle prefer the lower pH thresholds of **5.5** or **5.6**, and studies with beef cattle have used the pH threshold of **5.8** to define the upper limit of SARA (Table 2.1). Therefore, the following discussion will focus on the appropriateness of these thresholds for ruminants in general, using work derived from both dairy and beef cattle, as well as sheep.

Because of the variability in response to low ruminal pH, it is challenging to assign a static pH threshold to represent the complex and continuous manifestations of ruminal acidosis. However, choosing the most appropriate pH threshold should not be arbitrary; some justifications for the use of a pH threshold are better than others. As pH decreases towards and below **6.0** the growth of ruminal cellulolytic bacteria is inhibited (Russell and Dombrowski, 1980; Russell and Wilson, 1996). This is an important first step in ruminal acidosis, as the subsequent dominance of amylolytic bacteria and increase in the rate of fermentation can negatively influence ruminal pH (Hungate et al., 1952; Nagaraja and Titgemeyer, 2007), and decrease fiber digestibility (Erdman, 1988; Dijkstra et al., 2012). Fulton et al. (1979a) observed that beef cattle employ the behavioural adaptation of reducing DMI in order to help maintain ruminal pH above **5.5**. Additionally, a decrease in ruminal pH below **5.5** has been reported to decrease the absorptive capacity and barrier function of the rumen epithelium (Gaebel et al., 1989). Compromised epithelial function (defined as decreased absorptive capacity and/or barrier function) remains evident as pH is decreased below **5.5** to **5.4** (Gaebel and Martens, 1988), **5.2** (Penner et al., 2010; Wilson et al., 2012) and **5.1** (Aschenbach and Gäbel., 2000; Aschenbach et al., 2000).

Short-chain fatty acid (SCFA) absorption is regarded as the most important mechanism that regulates ruminal pH (Allen, 1997; Aschenbach et al., 2011) and damage to the physical (i.e., hyperkeratinization) and functional (i.e., decreased absorptive capacity and barrier function) integrity of the ruminal wall are some of the earliest discovered, and frequently discussed, consequences of high-grain feeding and ruminal acidosis (Bull et al., 1965; Owens et al., 1998; Plaizier et al., 2012). Therefore, the utilization of the pH 5.5 threshold may be the most

appropriate as it signifies the upper limit to losses in epithelial function (Gaebel et al., 1989), and a pH of 0.1 unit below this threshold has been shown to elicit the same response (Gaebel and Martens, 1988). However, the reduction in fiber digestibility observed to occur as pH decreases below 6.0 may increasingly justify the use of a greater pH threshold for dairy cattle, or in other production systems where high fibre digestibility is a key determinant of production.

The use of daily duration or area that ruminal pH remains below pre-defined thresholds are steadily becoming the most widely used pH parameters for quantifying severity of ruminal acidosis in cattle where pH is continuously monitored (Gozho et al., 2005; Penner et al., 2007; Aschenbach et al., 2011). Therefore, the pH threshold used is not the sole issue of contention; the duration of time that pH must remain below these thresholds has also not yet reached consensus. Gozho et al. (2005), for example, suggested that a minimum of 3 h/d must be spent between pH **5.6** and **5.2** to indicate sub-acute ruminal acidosis in adult dairy cattle, as cattle experiencing ruminal pH at or below this threshold experienced an increase in circulating acute phase proteins and decreased DMI. For beef cattle, a daily duration of at least 12 h below the pH of **5.8** (Schwartzkopf-Genswein et al., 2003), or **5.6** (Bevans et al., 2005; Wierenga et al., 2010) has been suggested to delineate SARA. However, pH depression of this magnitude below 5.6 has been associated with the accumulation of lactate during induced acidosis in beef cattle (Nagaraja et al., 1985; Coe et al., 1999), and therefore may be too severe to describe SARA (acute ruminal acidosis is defined below). In addition, although these suggested durations and thresholds are reasonable, their usefulness as an across study comparison tool is limited by the number of studies that employ them.

The pH thresholds used to identify lactic acidosis are also contentious. Most researchers either use a pH of **5.0** (Nocek, 1997; Nagaraja and Titgemeyer, 2007; Aschenbach et al., 2011), or **5.2** (Owens et al., 1998; Penner et al., 2007; Wierenga et al., 2010) as an indicator of acute ruminal acidosis. Nevertheless, the accumulation of ruminal lactate is thought by many as the standard to assess and quantify the severity acute ruminal acidosis (Nocek, 1997; Najaraga and Titegemeyer, 2007; Aschenbach et al., 2011). Not surprisingly though, once again there is a lack of consensus regarding the required accumulation in ruminal lactate necessary to define lactic acidosis. One group, led by T. Nagaraja, has suggested that ruminal lactate concentrations must exceed 50 m*M* (Goad et al., 1998; Nagaraja and Titgemeyer, 2007) to indicate acute ruminal acidosis. However, other studies (including 2 conducted by this very same group) have suggested

that a mere increase in ruminal lactate concentration can indicate acute ruminal acidosis (Harmon et al., 1985; Nagaraja et al., 1985; Coe et al., 1999). Aschenbach et al. (2011) recently suggested that a threshold of 5 m*M* of lactate accumulation may indicate risk for acute ruminal acidosis, as lactate rarely accumulates in the rumen under normal physiological conditions.

The easiest way to detect cattle that may be at risk for ruminal acidosis is through detecting an increased variability in DMI (Fulton et al., 1979a; Burrin et al., 1988; González et al., 2012). However, the most appropriate way to quantify the severity of ruminal acidosis is with the use of continuous indwelling ruminal pH measurement in combination with ruminal organic acid concentrations and osmolarity (or osmolality as reported in this study, for differences see Carter and Grovum, 1990). This presents a diagnostic constraint, as detection and diagnosis of ruminal acidosis is limited by the large variability in ruminal pH (Krause and Oetzel 2006; Bevans et al., 2005; Aschenbach et al., 2011), the lack of consensus on the pH thresholds used (Plaizier et al., 2008), and the invasive techniques required to collect reliable pH measurements. In addition, there has been comparatively less discussion on the use of other risk factors that have been associated with ruminal acidosis as diagnostic tools for assessing the prevalence and severity of ruminal acidosis; alternative risk factors that can be used in place of, or in combination with, with ruminal pH include (but are not limited to) circulating acute phase proteins (Plaizier et al., 2008), changes in the rumen microbiome (Khafipour et al., 2009a), and ruminal temperature (AlZahal et al., 2008). For the purposes of this study the severity of ruminal acidosis will be primarily assessed by the duration of time (min) and area (min × pH) below pH 5.5. Accumulation of ruminal lactate > 5m*M* will be used to differentiate between acute ruminal acidosis and SARA.

2.2 Regulation of Ruminal pH: Ruminal Acid Production and Removal

Having established ruminal acidosis as a digestive disorder primarily defined by low ruminal pH and associated with numerous animal welfare concerns, the following discussion will focus on the production and removal of ruminal acids, with a focus on how these processes are impacted by low ruminal pH. The various mechanisms for ruminal acid removal will be considered separately (as much as possible) in order to judge their efficacy as ruminal pH

decreases. The impact of high-grain feeding on acid production (Loncke et al., 2009) and removal (Bannink et al., 2012) will be discussed in detail in section 2.3.

Ruminal acidosis is caused by the rapid production of organic acids, which include lactate and short-chain fatty acids (SCFA). These weak acids are produced from microbial fermentation and readily dissociate releasing hydrogen ions at physiological pH (Aschenbach et al., 2011). Ruminal protons are primarily removed from the rumen in a condensation reaction with ruminal bicarbonate, the majority of which is supplied in exchange for SCFA across the rumen epithelium (Allen, 1997; Gäbel et al., 1991; Aschenbach et al., 2011) and through salivary secretion (Bailey and Balch, 1961; Erdman, 1988; Bannink et al., 2012).

The absorptive capacity of the reticulorumen is impressive, and alone is responsible for the majority (62-80%) of SCFA removal (Tamminga and van Vuuren, 1988; Peters et al., 1990; Dijkstra et al., 1993). As pH declines, SCFA absorption increases (Dijkstra et al., 1993) suggesting that the relative role of SCFA absorption for the regulation of ruminal pH increases with decreasing pH. Oppositely, saliva production is inhibited by high ruminal osmolarity (Bailey and Balch, 1961; Warner and Stacy, 1977; Carter and Grovum, 1990), suggesting decreased efficacy during ruminal acidosis. Other physiological buffering mechanisms thought to influence ruminal pH to a lesser degree include rumen ammonia and phosphates, liquid dilution, and passage of SCFA (Allen, 1997; Aschenbach et al., 2011).

2.2.1 Accumulation of Organic Acids

The symbiotic relationship between the ruminant animal and its rumen microbial inhabitants is essential to the digestive strategy of ruminants. Rumen microorganisms provide protein and transform what would be otherwise unavailable carbohydrates into an important source of energy for the ruminant, in exchange for a tightly regulated external environment and a supply of carbon, nitrogen, and energy (Bergman, 1990). Although highly variable, microbial digestion and fermentation of carbohydrates in the rumen has been estimated to produce approximately 4-5 moles of SCFA/kg DMI (Tamminga and van Vuuren, 1988; Bergman, 1990), and as much as 9 moles of SCFA/kg DMI when beef cattle are fed a high-grain finishing diet (Sharp et al., 1982). Estimates to predict organic acid production based on ruminally fermentable organic matter have been developed (as will be discussed in more detail in section 2.3.1);

however, based on the above mentioned rough estimations it is evident that as much as 90 moles of SCFA are produced daily in beef cattle consuming 10 kg DMI (the approximate intake of the high-grain diet used in the current study). According to the Henderson-Hasselbalch equation (discussed alongside SCFA absorption, in section 2.2.4), the dissociation of this quantity of SCFA would correspond to the daily production of approximately 89 moles of protons at a pH of 7.0 (99% dissociation) and 75 moles of protons at pH 5.5 (83% dissociation). Although these are very rough estimates, they are meant to illustrate the extensive production of ruminal organic acids and the resulting acid load in beef cattle.

The most important of the SCFA are acetate, propionate, and butyrate, which account for over 95% of all fermentation acids in the rumen (Bergman, 1990) and up to approximately 75% of the total metabolizable energy of the ruminant (Siciliano-Jones and Murphy, 1989; Bergman, 1990). Although the total concentration of SCFA in the rumen is highly variable and dependent on dietary ruminally fermentable organic matter (Bannink et al., 2008; Loncke et al., 2009), of the 60-150 m*M* of SCFA in the rumen fluid (Bergman, 1990) the majority (55-75%) is acetate, followed by propionate (15-30%), and butyrate (10-20%) (Elsden, 1945; Penner et al., 2009b; Udén, 2011). One mole of glucose can undergo fermentation into 2 moles of acetate, 2 moles of propionate, or 1 mole of butyrate (Baldwin, 1995; Bannink et al., 2006). Thus, a shift in production towards butyrate could in theory help decrease the risk for ruminal acidosis by decreasing total acid production (Aschenbach et al., 2011).

In contrast to ruminal SCFA, total rumen fluid lactate concentrations rarely rise above 5 m*M*, unless the animal is experiencing a bout of acute ruminal acidosis (Harmon et al., 1985; Nagaraja et al., 1985; Coe et al., 1999). Therefore, the low physiological concentrations of ruminal lactate limits the ability of lactate to lower rumen pH under most dietary conditions. It must be stated, however, that because lactate (pKa = 3.8) is a stronger acid than the SCFA (pKa ~ 4.8), as pH declines below 5 and SCFA concentrations decrease and lactate accumulates above 5 m*M*, the influence of lactate on ruminal pH increases (Aschenbach et al., 2011).

The negative impact of SCFA on ruminal pH has long been established (Briggs et al., 1957; Rumsey et al., 1970; Slyter, 1976). Recently, Kolver and de Veth (2002) found that total SCFA concentration explained between 67 and 80% of the within study variation in ruminal pH in dairy cattle fed a pasture-based diet. However, this negative correlation is much weaker across studies. In the same meta-analysis, Kolver and de Veth (2002) found that only 30% of the

variation in ruminal pH was explained by SCFA concentration when data were pooled across studies. Similarly, Allen (1997) found that only 13% of the variation in ruminal pH could be explained by rumen SCFA concentrations across studies. Because SCFA concentration is the result of both SCFA production and removal, and there is likely more variability inherent in SCFA removal than its production when cattle are fed similar diets, the disparity between within study and across study results suggests that there may be substantial variability in the removal of ruminal organic acids, protons, or both. Therefore, an in-depth understanding of the physiological mechanisms that regulate rumen pH is required in order to determine the factors that contribute most to the observed variability in ruminal pH.

2.2.2 Dilution and Passage

Water influx into the rumen comes primarily from saliva production (Bailey, 1961; Cassida and Stokes, 1986; Allen, 1997), but also from drinking, eating, and influx across the rumen wall when the rumen becomes hypertonic relative to the blood (Parthasarathy and Phillipson, 1953; Argyle and Baldwin, 1988). Increased rumen liquid volume was once thought to dilute the ruminal organic acids and thereby minimize the decrease in ruminal pH that occurs after feeding (Argyle and Baldwin, 1988). However, because mean daily rumen fluid osmolarity (280 mOsm/L; Warner and Stacy, 1965) is usually well below the threshold for a net influx of water (approximately 330-340 mOsm/L), the net absorption of water is expected to concentrate, rather than dilute, the organic acids in the rumen under most physiological conditions (Warner and Stacey, 1965; Willes et al., 1970; Warner and Stacy, 1972). Certainly, ruminal fluid osmolarity can reach maximal values above this threshold (Warner and Stacy, 1972; Carter and Grovum, 1990); however, incomplete mixing of introduced water with rumen digesta (Woodford et al., 1986) will lower the expected impact of dilution (Allen, 1997). The ability of an influx of ruminal water to dilute and lower ruminal proton concentrations will decrease further as rumen motility decreases due to increasing ruminal SCFA concentrations and decreasing ruminal pH (Ash, 1959; Gregory, 1987; Kezar and Church, 1979). Regardless, it is thought that dilution alone could not be responsible for significantly altering rumen pH (Allen, 1997). At a pH of 6.0, an increase in ruminal volume of 20% only changes ruminal pH by less than 0.1 pH units (Allen, 1997).

An increase in rumen volume will increase the absolute volume of liquid passage to the omasum (Dijkstra et al., 1993; Bannink et al., 2008). However, the efficacy of this buffering strategy must be questioned, as an increase in liquid volume may decrease the fractional rate of liquid passage (Dijkstra et al., 1993). In addition, even while holding the ruminal concentration of SCFA constant, an increase in initial rumen volume from 10 L to 30 L was shown to decrease SCFA absorption in dairy cattle (Dijkstra et al., 1993). In addition, Storm and Kristensen (2010) associated an increase in the size of the ruminal mat with decreased SCFA turnover from the ruminal mat, despite observing no change in the fluid flow through the ruminal mat. Together with increased pH stratification induced by the development of the ruminal mat, an increase in ruminal liquid volume may contribute to increased compartmentalization of SCFA within the rumen by increasing the distance that SCFA must travel to reach the epithelial boundry for SCFA absorption (Storm and Kristensen, 2010). A reduction in SCFA absorption would hinder rather than improve ruminal pH regulation, as SCFA absorption is widely regarded as the most important phyisological mechanism for regulating ruminal pH (Allen, 1997; Aschenbach et al., 2011; Bannink et al., 2012). However, excessive SCFA absorption rates through increased SCFA delivery to the ruminal epithelium may compromise intraepithelial pH homeostasis (Storm and Kristensen, 2010). The mechanisms linking SCFA absorption and intraepithelial pH homeostasis will be discussed in detail alongside SCFA absorption in section 2.2.4.

When water introduced into the rumen is adequately mixed with digesta, passage of water to the omasum removes free protons from the rumen, as well as those bound to dihydrogen phosphate, ammonium, SCFA, and particulate matter (Allen, 1997). This liquid passage is thought to remove approximately 15.5% of protons from the rumen (Allen, 1997). Over half of these protons (8.9%) are removed as dihydrogen phosphate in a process that is driven by salivary secretion. According to Allen (1997) the remaining 6.6% of protons that are removed by passage are bound to SCFA (3.1%), ammonium (2.1%), and particulate matter (1.4%). Because of their acid dissociation constants (Aschenbach et al., 2011), it is thought that as pH decreases the removal of protons bound to SCFA may increase, while those bound as dihydrogen phosphate and ammonium will not change (Allen, 1997). Supporting the notion that passage of H-SCFA can help to stabilize ruminal pH, it has been reported that 20-38% of total SCFA pass to the omasum (Tamminga and van Vuuren, 1988; Peters et al., 1990; Dijkstra et al., 1993). However, due to a decrease in the fractional rate of liquid passage, the importance of this buffering strategy

may decrease at low rumen pH (Dijkstra et al., 1993), and after a substantial bout of ruminal acidosis (Krehbiel et al., 1995).

2.2.3 Saliva Production

Although rates of saliva production are highly variable and impacted by diet (Bailey, 1961; Dijkstra et al., 1992; Beauchemin et al., 1994), it is believed that the majority of adult domesticated cattle secrete between 10 to 15 L (and upwards of 32 L) of saliva per kg of DMI on a daily basis (Bailey, 1961; Kaufmann, 1976; Dijkstra et al., 2012). Although the previous estimates are very crude and subject to immense variability (discussed in detail in 2.3.3), using these estimates indicate beef cattle consuming 10 kg of DM would produce 100 to 150 L of saliva daily. Ruminant saliva contains approximately 126 m*M* of bicarbonate and 26 m*M* of hydrogen phosphate. Therefore, a beef cow producing 100 to 150 L/d of saliva might expect a daily salivary contribution of approximately 13 to 19 moles of bicarbonate. Mixed saliva has pH of 8.4 (Bailey and Balch, 1961), which shifts the bicarbonate equilibrium to its ionic form, due to its effective pKa of 6.1 (Aschenbach et al., 2011). Likewise, hydrogen phosphate is also primarily unbound to hydrogen ions in saliva (pKa = 7.2; Kohn and Dunlap, 1998).

However, when saliva is mixed with ruminal contents with a pH below 6.1, both equilibriums shift so that bicarbonate and hydrogen phosphate bind with hydrogen ions. Because bicarbonate has an effective pKa that mirrors physiological pH (approximately 5.5-7.0; Krause and Oetzel, 2006), it is an ideal ruminal buffer. The bicarbonate can react with hydrogen ions leading to the consumption of hydrogen ions and production of water (condensation reaction) and carbon dioxide, in order to resist a decrease in rumen pH (Allen, 1997). For this reason, bicarbonate is thought to be the most important buffering component of saliva (Turner and Hodgetts, 1955; Allen, 1997; Aschenbach et al., 2011). On the other hand, because hydrogen phosphate has a pKa that is well above physiological pH, it is not an appropriate ruminal buffer but rather a weak base (Counotte et al., 1979; Erdman, 1988). However, as a weak base it can help neutralize protons at virtually any physiological pH. By doing so, hydrogen phosphate is thought to combine with hydrogen ions and remove them from the rumen indirectly, through passage to the omasum (Counotte et al., 1979; Allen, 1997).

Historically, saliva has received considerable attention as the most important physiological mechanism for buffering the pH of the rumen (Turner and Hodgetts, 1955; Hibbard et al., 1995; Allen, 1997). However, classic studies identified the ruminal wall as a source of bicarbonate, and even predicted that this ruminal bicarbonate secretion may be positively related to SCFA absorption (Masson and Phillipson, 1951; Ash and Dobson, 1963). It has been estimated that saliva production may be responsible for the neutralization of up to 40% of the acid produced in the rumen (Allen, 1997; Gäbel et al., 2002; Aschenbach et al., 2011). In addition, it is thought that the composition of saliva is resistant to changes caused by diet composition or feed intake (Erdman, 1988), but that buffering capacity of saliva increases as pH decreases, due to the effective pKa of bicarbonate (Allen, 1997; Aschenbach et al., 2011). However, because salivary flow is inhibited by the high rumen osmolarity observed after feeding (Bailey and Balch, 1961; Warner and Stacy, 1977; Carter and Grovum, 1990), the ability of saliva to elevate ruminal pH following a meal rich in rapidly fermentable carbohydrates may be overestimated. In addition, high-grain feeding itself has long been thought to decrease saliva production (Bailey and Balch, 1961; Owens et al., 1998), although this will be discussed in detail in section 2.3.3. Despite the early focus on saliva induced buffering of the rumen, studies have failed to prove that saliva secretion raises ruminal pH under physiological conditions (Ash and Kay, 1959; Bailey and Balch, 1961; Rumsey et al., 1972; Maewaka et al., 2002). In fact, Penner and Beauchemin (2010) found total saliva production to be positively correlated with the duration of time that pH spent below 5.8 in lactating dairy cattle. This suggests that saliva production may increase as the rumen becomes increasingly acidotic, but that it is not sufficient in elevating the pH of the rumen.

2.2.4 Short-Chain Fatty Acid Absorption

Empirical estimations indicate that SCFA absorption is responsible for the removal of approximately 52.9% of protons from the rumen at a pH of 6.0, and is therefore the single most important contributor to the removal of hydrogen ions from the rumen (Allen, 1997). Although the transport mechanisms governing the ruminal absorption of SCFA have not yet been fully elucidated, they undoubtedly include both passive diffusion and facilitated transport.

Undissociated SCFA (HSCFA) are lipid-soluble and able to passively diffuse across biological membranes. For this reason, it was once thought that there was little or no absorption of SCFA in their ionized form (Danielli et al., 1945; Masson and Phillipson, 1951; Ash and Dobson, 1963). To promote SCFA diffusion, it was speculated that the ruminal epithelium secretes carbonic acid which helps to maintain an acidified microenvironment adjacent to the ruminal epithelium (Ash and Dobson, 1963; Allen, 1997). However, Leonhard-Marek et al. (2006) used an ex vivo method consisting of a pH-sensitive fluorescent dye (5-*N*-hexadecanoyl-aminoflluorescein) to measure the average pH within 15 µm of the stratum corneum of goat and sheep ruminal epithelium, and reported that the pH of the epithelial microclimate is not acidic but rather alkaline. At the pH that they reported (approximately 7.47), 0.2 % of total SCFA would exist as lipophillic HSCFA. Although more work is required to confidently describe the pH at the epithelial boundry, rumen pH fluctuates between 5.5 and 7.0 (Krause and Oetzel, 2006). Using the Henderson-Hasselbalch equation ($[SCFA^-]/[HSCFA] = 10^{pH-pKa}$) and a pKa of approximately 4.8 (Aschenbach et al., 2011), the proportion of ionized $SCFA^-$ can be calculated for any ruminal pH. At a pH of 7.0, 99% of SCFA are anionic ($SCFA^-$), and only 1% are lipophillic (HSCFA). As pH decreases to 5.5, 83% of SCFA are anionic, and 17% are lipophillic. Thus, SCFA dissociate and decrease rumen pH to a greater extent at elevated pH, and that under phyisological conditions the majority of SCFA absorption is likely to occur in the ionized state (Aschenbach et al., 2011).

Lipophillic permeability decreases in the order of butyrate > propionate > acetate (Walter and Gutknecht, 1986), as does intraepithelial metabolism (Britton and Krehbiel, 1993; Kristensen and Harmon, 2004). Therefore, with passive diffusion as the sole absorptive model, we would expect the fractional rate of SCFA absorption to decrease in the same manner from butyrate > propionate > acetate. Appropriately, many studies have observed this pattern in the rates of fractional SCFA absorption (Danielli et al., 1945; Thorlacius and Lodge, 1973; López et al., 2003). In addition, as ruminal pH declines the fractional rate of SCFA absorption has been shown to increase in a manner that favours lipophillic diffusion (butyrate > propionate > acetate; Thorlacius and Lodge, 1973; Dijkstra et al., 1993; López et al., 2003). However, as pH decreases and ionic SCFA ($SCFA^-$) become increasingly hydrogenated, the absorption of SCFA does not increase to the extent as would be predicted by the Henderson-Hasselbalch equation (Gäbel et al., 2002; Aschenbach et al., 2011). For these reasons and because of the dominance of the

conjugate base at physiological pH, it follows that there must be $SCFA^-$ uptake. If this is the case (and it is, see below) then there must be an explanation for the observed association between ruminal carbon dioxide appearance and SCFA disappearance (Masson and Phillipson, 1951) that incorporates $SCFA^-$ uptake rather than HSCFA uptake and carbonic acid secretion, as once previously thought (Ash and Dobson, 1963).

Gäbel et al. (1991) found evidence to suggest that bicarbonate secretion across the ruminal wall is positively related to SCFA absorption. Since then, apical $SCFA^-$ uptake has been found to occur in exchange for bicarbonate (HCO_3^-) for butyrate (Sehested et al., 1999; Penner et al., 2009a), propionate (Kramer et al., 1996) and acetate (Aschenbach et al., 2009, Penner et al., 2009a). Aschenbach et al. (2009) found that bicarbonate-dependant ionic acetate and propionate uptake occurs in competition with chloride ions, suggesting that a non-specific Cl^-/HCO_3^- exchanger is increasingly responsible for SCFA uptake. Bilk et al. (2005) identified three Cl^-/HCO_3^- exchangers as possible proteinacious candidates involved in nonspecific $SCFA^-/HCO_3^-$ exchange across the sheep rumen epithelium; these include, the basolateral anion exchanger 2 (**AE2**), apical downregulated in adenoma (**DRA**), and apically located putative anion transporter (**PAT1**). According to a recent review by Penner et al. (2011), it is likely that DRA plays an important role in $SCFA^-/HCO_3^-$ exchange. In addition, apical uptake of ionic acetate has been shown to occur via a nitrate-sensitive and HCO_3^--independent transport mechanism (Aschenbach et al., 2009).

Whether generated by the nearly complete intraepithelial dissociation of HSCFA following lipophillic diffusion, or by SCFA metabolism, protons must be cleared from the epithelia (i.e., through condensation with HCO_3^-, or basolateral clearance) in order to insure that they are not transported back into the rumen (Aschenbach et al., 2011). Therefore, intraepithelial pH homeostasis is influenced by SCFA absorption, and proton clearance from the rumen epithelium also merits consideration. Countercurrent exchange of Na^+ and H^+ via the Na^+/H^+ exchanger (**NHE**) is one mechanism in which protons are cleared from the rumen epithelia (Müller et al., 2000). In addition, Stumpff et al. (2009) suggested that basolateral movement of ionic acetate occurs through large, non-specific anion channels in addition to $SCFA^-/HCO_3^-$ exchange and simple diffusion of HSCFA. The monocarboxylate/H^+ co-transporter 1 (**MCT1**) is thought to be responsible for basolateral clearance of the products of intra-epithelial SCFA

metabolism (Müller et al., 2002). This includes lactate and the ketone bodies β-hydroxybutyrate (**BHBA**), acetone, and acetoacetone, as well as the hydrogen ions generated by their production.

The rumen epithelial cells are capable of generating HCO_3^- for secretion to the rumen, as they have been found to contain carbonic anhydrase (Carter, 1971). This discovery suggests that intraepithelial oxidation can drive the production of bicarbonate for secretion into the rumen. However, Aschenbach et al. (2009) inhibited carbonic anhydrase with ethoxyzolamide and found a negligible effect on acetate absorption, suggesting that extracellular HCO_3^- is primarily imported for $SCFA^-$ exchange. Importing HCO_3^- may be a homeostatic mechanism to regulate intracellular pH, as the hydrogen ion generated by the dissociation of carbonic acid would acidify the epithelial cytosol. Indeed, Bondzio et al. (2011) found a down-regulation in the mRNA and protein expression of ovine epithelial carbonic anhydrase after six weeks that sheep were fed a high concentrate diet. Because high-grain feeding has been shown to increase SCFA absorption (Thorlacius and Lodge, 1973; Gäbel et al., 1991; Sehested et al., 2000), this observation strengthens the suggestion made by Ashchenbach et al. (2009) that extracellular HCO_3^- is preferentially linked to apical $SCFA^-/HCO_3^-$ exchange. The basolateral uptake of circulating HCO_3^- likely helps maintain intraepithelial pH homeostasis, and through apical secretion into the rumen, provides ruminal HCO_3^- to imrove ruminal pH homeostasis.

It is therefore understood that both saliva production and SCFA absorption provide the majority of ruminal bicarbonate supply (Allen, 1997; Bannink et al., 2012). As observed for saliva production (Penner and Beauchemin, 2010), it has long been known that as ruminal pH declines, SCFA (Dijkstra et al., 1993) and lactate (Williams and MacKenzie, 1965; Harmon et al., 1985) absorption increase. Thus, one might anticipate an increase in the importance of both physiological buffering strategies as ruminal pH declines. However, whereas the positive correlation observed between saliva production and pH suggests that this buffering mechanism is not sufficient in ameliorating ruminal pH, increased SCFA absorption has been associated with decreased risk for ruminal acidosis (Penner et al., 2009a). In addition, increased expression of enzymes involved in SCFA metabolism (Penner et al., 2009b) may also be associated with decreased severity of ruminal acidosis. Collectively, these findings suggest that investigations geared towards better understanding the physiology and adaptability of the rumen epithelium may provide us with the tools necessary to improve our understanding of the incredible variation in ruminal pH and susceptibility to ruminal acidosis (Allen, 1997; Bevans et al., 2005).

2.3 Dietary Adaption to High-Grain Diets

The previous sections discussed some of the microbial and physiological changes that accompany a decrease in ruminal pH (section 1.1), and the strategies employed by the animal to resist these changes (section 1.2). The objective of the current section is to discuss the microbial and physiological changes that occur when feeding high-grain diets, and how these processes might adapt to influence the regulation of ruminal pH. When possible, a focus will be directed towards these adaptive processes and how they may be influenced by conventional feeding management practices for feedlot cattle in North American feedlots.

Providing a gradual dietary transition to high-grain diets reduces the risk for ruminal acidosis by providing time for epithelial and microbial adaptation relative to rapid or abrupt dietary change. Because saliva production (Bailey, 1961; Kaufmann et al., 1980; Allen, 1997) and liquid passage rates (Owens and Goetsch, 1986; Kreikemeier et al., 1990) decrease during adaptation to high-grain feed, other pH regulatory mechanisms must increase in relative importance in order to maintain ruminal pH within physiological limits. It is currently thought that rumen epithelial adaptation (Thorlacius and Lodge, 1973; Gäbel et al., 1991; Sehested et al., 2000) compensates for the decreased function of these other pH regulatory mechanisms, and that SCFA absorption becomes increasingly important when cattle are fed diets rich in concentrates (Dirksen et al., 1985; Bannink et al., 2012).

2.3.1 High-Grain Diets Increase the Risk for Ruminal Acidosis

The prevailing strategy used by North American feedlot producers is to feed diets high in non-forage components, herein referred to as concentrates. Concentrates include non-forage fiber and non-structural carbohydrate sources, as well as mineral and protein supplements. The high-grain diets used by North American feedlot producers typically contain between 5-15 % forages (Owens, 1987; Vasconcelos and Galyean, 2007), which serve to reduce the risk for ruminal acidosis and increase performance (Kreikemeier et al., 1990). The degradation and fermentation of non-structural carbohydrates (pectins, starch, and sugars) occurs much more rapidly than for structural carbohydrates (cellulose, hemicellulose, and lignin). For instance, the ruminal

fermentation rate of sugars is very rapid, with estimated rates of at least 300%/h (Sniffen et al., 1983; Weisbjerg et al., 1998). Weisbjerg et al. (1998) estimated the rate of ruminal sucrose hydrolysis at approximately 1200 to 1400%/h, while lactose hydrolysis ranged from 200 to 500%/h. Furthermore, the rate of ruminal monosaccharide fermentation from dissacharide hydrolysis was found to range between 300 to 700%/h (Weisbjerg et al., 1998). Comparatively, the rate of ruminal starch digestion is intermediate and highly variable between 3 to 65%/h (Sniffen et al., 1992), whereas that for available structural carbohydrates is lower at approximately 5%/h (Smith et al., 1972; Varga and Hoover, 1983; Sniffen et al., 1992). However, there is extensive variation in the degradation and fermentation rates of both neutral detergent fiber (**NDF**) and starch (Nocek and Russell, 1988; Nocek and Tamminga, 1991).

Despite this variation, a meta-analysis of the data from 74 multi-catheterized cattle and 90 multi-catheterized sheep found dietary fermentable organic matter to correlate highly (R^2 = 0.95) with the net portal appearance of SCFA (Loncke et al., 2009). In addition, it has long been established that ruminal pH correlates positively with both dietary crude fiber (R^2 = 0.95; Kaufmann, 1976) and acid detergent fiber (**ADF**) (R^2 = 0.30; Erdman, 1988). Hence, when the dietary intake of non-structural carbohydrates is increased, the increased rate and extent of fermentation can result in an increased production of organic acids (Sutton et al., 2003). Because the accumulation of organic acids is thought to be the principal cause of ruminal acidosis (Briggs et al., 1957; Rumsey et al., 1970; Kleen and Cannizzo, 2012) an in-depth understanding of how the various physiological rumen buffering strategies are affected by high-grain feeding is paramount to the understanding and management of ruminal acidosis.

2.3.2 Gradual Dietary Transition Decreases the Risk for Ruminal Acidosis

Abruptly feeding a high-grain diet to unadapted cattle has been shown to decrease both ruminal pH and DMI (Uhart and Carroll, 1967; Fulton et al., 1979a; Brown et al., 2000). Decreased ruminal pH (Garrett et al., 1999; Penner et al., 2007; Khafipour et al., 2009c) and increased pH variability (Nocek et al., 2002; Bevans et al., 2005) when feeding high concentrate diets are thought to be prominent indicators of ruminal acidosis (Slyter, 1976; Owens et al., 1998; Nagaraja and Titgemeyer, 2007). In addition, due to the relationship between pH and DMI, the decrease in DMI observed during dietary adaptation is also indicative of risk for ruminal

acidosis (González et al., 2012) and can contribute to decreased performance (Burrin et al., 1988).

One strategy used to mitigate the negative effects of feeding a high-grain diet is to transition cattle from approximately 40 to 55% concentrate to the high-grain diet over a transition period consisting of between 14 and 28 d with numerous "step-up" diets intermediate in their level of dietary concentrate (Bevans et al., 2005; Brown et al., 2006; Klinger et al., 2007). This is the most commonly used strategy in North American feedlots (Vasconcelos and Galyean, 2007). The 2007 Texas Tech University Survey (Vasconcelos and Galyean, 2007) surveyed 29 nutritionists responsible for establishing and carrying out industry practices in feedlots across the continental United States. They found that the majority of these nutritionists (76%) recommend utilizing a transition period consisting of multiple step-up diets. Specifically, this survey suggested that the industry standard in the United States is to transition cattle from approximately 40% to 90% concentrate over a 21 d period consisting of 3 intermediate diets with 7 d of feeding each (Vasconcelos and Galyean, 2007).

The scientific literature backing the strategy for dietary transition is extensive. Bauer et al. (1995) found evidence of ruminal acidosis after both an abrupt transition from 50% to 90% concentrate and a 12 d transition from 50% to 100% concentrate, suggesting that a 12 d dietary adaptation is not adequate. Leedle et al. (1995) found that even a stepwise dietary transition with large increases in the proportion of concentrate (25%) resulted in ruminal acidosis. Burrin et al. (1988) found that cattle that were gradually adapted over 14 d from 55% to 95% concentrate experienced greater efficiency of gains over the 21 d adaptation period than those cattle that were rapidly adapted over 7 d, despite being fed the finishing diet for less time during this period. Bevans et al. (2005) reported that gradually adapted cattle experienced decreased between steer variation in the duration of time that ruminal pH was detected below 5.6. This was evident during the first few days that cattle were fed both a 65% and 90% concentrate diet (Bevans et al., 2005).

Even with a gradual transition to the high-grain diet, a traditional step-up program fed ad libitum may result in overconsumption of the transition diets (Stock et al., 1995), which can increase the risk for ruminal acidosis. Bartle and Preston (1992) found that limiting the feed available to steers during a 4-wk dietary transition period (65, 75, 85, 90%) tended to increase weight gain efficiency during both the dietary transition and the entire finishing period. Although

they could not attribute this increased performance to decreased prevalence or severity of ruminal acidosis, this work suggests that there are productive merits in limiting the amount of rapidly fermentable carbohydrates fed during the dietary adaptation period. Increased productivity has also been reported during the finishing period by restricting intakes to 85% ad libitium (Hicks et al., 1990), or by employing programmed weight gain strategies (Knoblich et al., 1997; Klinger et al., 2007). The increased productivity when limit feeding is thought to be at least partially attributable to a decrease in digestive disturbances by limiting variation in DMI among days (Hicks et al., 1990; Choat et al., 2002). Thus, by minimizing the incremental daily changes in the amount of rapidly fermentable carbohydrates consumed, restricting intake is similar in principle to imposing a dietary transition. It has even been suggested that restricting intake of the finishing diet can alone serve as the transition period, rather than utilizing a traditional dietary adaptation (Choat et al., 2002).

2.3.3 Microbial Adaptations to High-Grain Diets

Feeding high-grain diets drastically increases the number of amylolytic and lactobacillus bacteria at the cost of fibrolytic bacteria (Slyter et al., 1970; Goad et al., 1998; Tajima et al., 2001). The increase in substrate availability (Slyter et al., 1970; Kreikemeier et al., 1990) for amylolytic bacteria and decrease in protozoal predation and competition for substrate (Coleman, 1986) likely favours amylolytic bacteria to outcompete cellulolytic bacteria when cattle are fed diets rich in non-structural carbohydrates. Because of the decrease in the numbers (Slyter et al., 1970; Russell et al., 1979) and consequently activity (Kaufmannn et al., 1980; Hiltner and Dehority, 1983) of cellulolytic bacteria, roughage is poorly digested in finishing diets (DeGregorio et al., 1982; Brink and Steele, 1985; Stock et al., 1987). Furthermore, because amylolytic and lactobacillus bacteria ferment their substrates much more rapidly than cellulolytic bacteria (Sniffen et al., 1992), their increase in numbers as a result of high-grain feeding causes ruminal pH to decline (Hungate et al., 1952; Nagaraja and Titgemeyer, 2007). A decrease in ruminal pH causes cellulolytic bacterial populations to decline further (Slyter et al., 1970).

Allison et al. (1964) suggested that ruminal acidosis can be averted by inoculating the rumen with digesta from grain-adapted sheep. Microbiological adaptation to high-grain diets has since been shown to result in an increase in the populations of bacteria that metabolize lactate to

SCFA, the most important of which is *Megasphaera elsdenii* (Mackie et al., 1978; Counotte et al., 1981; Fernando et al., 2010). Goad et al. (1998) confirmed that prior to inducing acidosis those cattle that were adapted to a high-grain diet contained more lactate-utilizing bacteria. However, this microbial adaptation was insufficient in decreasing susceptibility to an induced bout of ruminal acidosis, as hay-adapted and grain-adapted cattle experienced a similar severity of sub-acute ruminal acidosis (Goad et al., 1998). Because the acidotic challenge induced in this trial did not result in elevated ruminal lactate concentrations, microbial adaptation to high-grain feed may therefore become more important in decreasing the susceptibility to a more severe, or acute bout of ruminal acidosis. However, it has recently been suggested that the prebiotic supplementation of *M. elsdenii* (strain NCIMB 41125) can reduce the susceptibility to both acute (Meissner et al., 2010) and sub-acute ruminal acidosis (Aikman et al., 2011). Therefore, the proliferation of lactate-utilizing bacteria, whether through prebiotic supplementation or dietary adaptation, may help reduce the risk for ruminal acidosis.

Ciliated protozoa are thought by some to help increase and stabilize ruminal pH (Nagaraja et al., 1986; Newbold et al., 1986; Nagaraja and Titgemeyer, 2007) through indirect mechanisms including sequestering starch (Mackie et al., 1978; Mendoza et al., 1993) and bacterial predation (Bonhomme, 1990). Conveniently, gradually increasing the amount of grain in the diet usually results in an initial increase in the number of ruminal protozoa (Abe and Iriki, 1978; Dennis et al., 1983). However, feeding high concentrate finishing diets usually results in a drastic decrease or elimination (defaunation) of ruminal protozoa (Slyter et al., 1970; Lyle et al., 1981; Franzolin and Dehority, 1996). Interestingly, significant variability in the protozoal response to high-grain feeding has been reported (Slyter et al., 1970; Lyle et al., 1981; Towne et al., 1990). Since the defaunation is thought to be largely caused by low ruminal pH (Purser and Moir, 1959; Whitelaw et al., 1984; Franzolin and Dehority, 1996), this variability in the protozoal response suggests that there is significant variability in the other high-grain adaptations that govern pH regulation. However, pH is not the sole determinant of protozoal numbers (Towne et al., 1990), as protozoa have been observed to thrive at low pH (Slyter et al., 1970) and defaunation has been observed to occur prior to a significant decrease in ruminal pH (Lyle et al., 1981). One mechanism that might influence protozoal concentrations is the digesta passage rate. As the passage rate increases the risk for defaunation increases with it (Christiansen et al., 1964; Kreikemeier et al., 1990). Mechanisms governing resistance to protozoal defaunation are not yet

fully understood but should be investigated because protozoa are believed to ameliorate ruminal pH and may be an important part of the microbial adaptation to high-grain feeding (Towne et al., 1990).

2.3.4 Influence of High-Grain Diets on Saliva Production and SCFA Passage

Because salivary composition is not greatly affected by diet (Erdman, 1988) and the buffering capacity of saliva increases with a decrease in ruminal pH (Allen, 1997; Aschenbach et al., 2011), one might anticipate an increase in the relative importance of salivary buffering during the adaptation to high-grain feed. However, because dietary inclusion of forages increases the total amount of time spent chewing (Santini et al., 1983; Mertens, 1997; Maewaka et al., 2002), it was long believed that high-grain diets greatly decrease total saliva production (Bailey and Balch, 1961; Kaufmann et al., 1980; Erdman, 1988; Allen, 1997; Owens et al., 1998). The importance of eating (Bailey, 1961; Santini et al., 1983) and rumination (Welch and Smith, 1969; Santini et al., 1983) towards saliva production has long been established. Bailey (1961) found the rate of mixed saliva secretion to be approximately 2.5 fold greater during eating than resting in dairy cattle, which was later verified by work conducted in beef cattle (Yarns et al., 1965). The rate of saliva production during eating is believed to be similar to that observed during rumination (Cassida and Stokes, 1986), and ratios of 1.2-2.2 in dairy cattle (Cassida and Stokes, 1986; Maewaka et al., 2002; Bowman et al., 2003) have since been reported for saliva produced during eating or rumination relative to resting.

In a review of the literature, Allen (1997) found forage particle size to be positively correlated with total time spent chewing and ruminal pH. This suggests that in addition to the level of forage included in the diet, the amount of physically effective NDF (**peNDF**) is also important in encouraging saliva production (Beauchemin, 1991; Allen, 1997). To date, 2 studies in dairy cattle have suggested that increasing the peNDF in the diet can improve ruminal pH by increasing chewing activity (Krause et al., 2002; Beauchemin et al., 2003). Beauchemin and Yang (2005) found that dairy cattle preferentially sort for peNDF as ruminal pH declines, which may indicate a synchronization of behavioural (sorting) and physiological (saliva production) adaptations to improve ruminal pH. Beauchemin et al. (2008) suggested that because of reduced particle size, cattle consume concentrates much more rapidly than forages which decreases

salivary ensalivation (saliva produced/g DMI) which could potentially contribute to a decrease in total saliva production (although not detected in this particular study).

However, despite the long held belief that saliva production is reduced by high-grain feeding (Bailey and Balch, 1961; Kaufmannn et al., 1980; Allen, 1997), Maekawa et al. (2002) found that increasing the amount of forage in the diet by 20% did not influence the rate of resting or total saliva production, despite increasing the amount of time spent ruminating and the total amount of time spent chewing. These results are similar to those found by Cassida and Stokes (1986), whereby increasing the level of dietary concentrate by 10% did not affect the total rate of salivation. Moreover, Yang and Beauchemin found peNDF to increase both ruminating chews (2006a), total ruminating time (2006a,b), and total chewing time (2006b) without significantly impacting ruminal pH, and other studies have also found that increasing peNDF does not increase ruminal pH (Kononoff and Heinrichs, 2003; Plaizier, 2004; Beauchemin and Yang, 2005). According to Bannink et al. (2012) a decrease in resting saliva secretion may mitigate the increased saliva secretion that has been observed during ruminating and eating while on a high-grain diet (Bailey, 1961). Because more studies have failed to find a link between peNDF and pH (Kononoff and Heinrichs, 2003; Plaizier, 2004; Beauchemin and Yang, 2005; Yang and Beauchemin 2006a, b) than those that have (Krause et al., 2002; Beauchemin et al., 2003), it is likely that other adaptations to high-grain feed play a more important role in the regulation of ruminal pH.

Despite the contention surrounding peNDF, total saliva production, and ruminal pH, Bannink et al. (2012) estimated that total ruminal bicarbonate provided by saliva decreases from 47% to 35% when dairy cattle are adapted from a high roughage to a high concentrate diet, with the remainder of total ruminal bicarbonate provided by SCFA absorption. Therefore, high-grain diets decrease the total time spent chewing and ruminating (Welch and Smith, 1969; Santini et al., 1983; Mertens, 1997), which results in either a decrease (Bailey and Balch, 1961; Kaufmann et al., 1980; Allen, 1997) or does not impact (Cassida and Stokes, 1986; Maewaka et al., 2002) total saliva production. Regardless, because saliva production does not appear to increase as a result of high-grain feeding, the relative importance of saliva production as a mechanism governing ruminal pH likely becomes less important as beef cattle are transitioned to their finishing diet.

Increasing the amount of grain in the diet has also been shown to decrease both solid (Evans et al., 1981b) and liquid (Evans, 1981a; Owens and Goetsch, 1986; Kreikemeier et al., 1990) fractional passage rates (%/h). Assuming SCFA production and absorption remains equal, the decrease in fractional liquid passage will result in decreased fractional rates of SCFA passage, increased SCFA accumulation, and decreased ruminal pH. Kreikemeier et al. (1990) found a high-grain diet to decrease ruminal dilution by 38% and that level of intake increased ruminal SCFA concentrations. As discussed previously, dilution is not expected to influence ruminal pH to a significant extent (Allen, 1997). However, the increase in SCFA concentration suggests that the improvement in digesta passage with level of intake is not sufficient in compensating for reduced passage (Owens and Goetsch, 1986; Kreikemeier et al., 1990) and increased SCFA production (Sutton et al., 2003) due to high-grain feeding. Evidentially, the lack of an adequate adaptive response in both saliva production and liquid passage necessitate an increase in SCFA absorption in order to improve pH regulation when high-grain diets are fed (Dirksen et al., 1985).

2.3.5 Epithelial Adaptation to High-Grain Diets

Half a century ago, it was discovered that the inclusion of hay and calf starter in the diets offered to Holstein calves encouraged rumen development and increased SCFA absorption (Sutton et al., 1963). Shortly thereafter, two independent studies associated damage to the rumen epithelium (parakeratosis) with decreased rates of SCFA absorption (Bull et al., 1965; Hinders and Owen, 1965). Bull et al. (1965) suggested that a negative response to the dietary inclusion of grain was responsible for the decreased absorptive capacity. Meanwhile, it was also suggested that inclusion of grain increased ruminal concentrations of SCFA (Wheaton et al., 1970), and that ruminal SCFA promoted the development of the ruminal mucosa (Flatt et al., 1958; Sander et al., 1959). Evidence for the influence of grain on rumen epithelial development was suggested by Harrison et al. (1960), then by Tamate et al. (1962), whose lab later emphasized the stimulatory effect of ruminal SCFA, namely butyrate, on epithelial proliferation (Sakata and Tamate, 1978, 1979). In addition, Thorlacius and Lodge (1973) showed that Holstein cows fed a high concentrate diet experienced greater ruminal clearance rates of SCFA. Taken together, these

early studies suggested that gradual adaptation to dietary grain during development is vital in promoting epithelial health and SCFA absorption.

It has long been speculated that increased weight and surface area of a healthy rumen epithelium improves ruminal pH regulation (Kaufmannn et al., 1980). Dirksen et al. (1985) measured changes in epithelial morphology and SCFA absorption in adult dairy cattle offered both low (approximately 30-40% crude fiber) and high (approximately 10% crude fiber) levels of concentrate. This work showed that adaptation to high-grain diets resulted in increased papillae surface area and improved ruminal SCFA absorption, and that the adaptation process may take between 6-8 weeks (Dirksen et al., 1985). Researchers are still attempting to quantify the time necessary for epithelial adaptation when dietary concentrates are offered to ruminants (Bannink et al., 2008; Etschmann et al., 2009). Bannink et al. (2008) reported that the adaptive response for increased epithelial surface area can be reduced (from 8 to 4 weeks) by increasing the proportion of concentrate in diets offered to Holstein cows in the post-partum phase of the transition period. Etschmann et al. (2009) reported that 4 wk was required to achieve the maximal increase in ruminal sodium transport following dietary inclusion of grains. Interestingly, they also found that 73% of this response occurred in the first week of dietary adaptation, suggesting that epithelial adaptations governing the increase in absorptive capacity per unit epithelia are much more rapid than increases in absorptive surface area (Etschmann et al., 2009).

Since the study of Dirksen et al. (1985), the positive effect of dietary inclusion of grain on the growth of rumen papillae has been widely supported (Gaebel et al., 1987; Reynolds et al., 2004; Shen et al., 2004). However, one study did not find such a change in papillae surface area (Andersen et al., 1999) and another found a grain challenge to reduce the number but increase the size of ruminal papillae (Odongo et al., 2006). The mechanism behind the apparent influence of grain feeding on epithelial growth is not yet known; however, the conflicting effect of butyrate in vivo (Sakata and Tamate, 1978) and in vitro (Neogrády et al., 1989; Gálfi et al., 1993; Wang and Jiang, 2010) on ruminal epithelial proliferation suggests that butyrate initiates an endocrine response (Baldwin, 1999; Steele et al., 2011a). This is thought to primarily occur through an increase in insulin-like growth factor (**IGF**)-1 (Baldwin, 1999), and a modulation in its regulatory binding proteins (Firth and Baxter, 2002). Indeed, the inclusion of dietary concentrates has been found to increase plasma IGF-1 levels and rumen epithelial proliferation

(Shen et al., 2004; Zitnan et al., 2005). Moreover, Steele et al. (2011a, 2012a) found dairy cattle offered a high-grain diet to have elevated ruminal mRNA expression of insulin-like growth factor binding protein (**IGFBP**)-5 and decreased ruminal mRNA expression of IGFBP-3. The IGFBP-5 isoform has been shown to stimulate the proliferative influence of IGF-1 (Firth and Baxter, 2002), while IGFBP-3 has been shown to have the opposite effect on IGF-1 (Albiston et al., 1992). However, future research is warranted to elucidate the mechanism behind the involvement of butyrate, IGF-1, IGFBP-5, and IGFBP-3 in inducing ruminal epithelial proliferation.

It is also now well established that SCFA absorption increases with high concentrate feeding (Thorlacius and Lodge, 1973; Gäbel et al., 1991; Sehested et al., 2000). Gäbel et al. (1991) found that sheep fed 81% concentrate and 19% hay had greater rates of acetate, propionate, butyrate, and total SCFA absorption than those that were fed 100% hay, and attributed the effect to enhanced $SCFA^-/HCO_3^-$ exchange. Previous work done by this group also showed that adaptation to high-grain feed increases papillae surface area and causes an increase in ruminal Na^+, Cl^-, and Mg^{2+} absorption (Gaebel et al., 1987). An increase in ruminal Cl^- absorption is interesting, as the currently proposed model for $SCFA^-/HCO_3^-$ exchange involves Cl^-/HCO_3^- transporters (Kramer et al., 1996; Aschenbach et al., 2009; Penner et al., 2011). In addition, increased Na^+ uptake on high-grain feed has been reported by others (Etschmann et al., 2009), and is likely caused by increased expression of Na^+/H^+ exchangers (NHE) (Penner et al., 2011; Yang et al., 2012), resulting in a reduction in intracellular H^+ and therefore contributing to intraepithelial pH homeostasis (Müller et al., 2000; Aschenbach et al., 2011; Penner et al., 2011).

Although Dirksen et al. (1985) attributed the observed increase in absorptive capacity to structural adaptations of the rumen epithelia, Sehested et al. (2000) showed that dairy cattle with elevated dietary inclusion of barley grain experienced greater rates of butyrate transport without a concomitant increase in epithelial surface area (Andersen et al., 1999). Increased SCFA absorption per unit epithelia could be influenced by adaptive processes that increase the rates of lipophillic diffusion, such as increased blood flow (Dobson, 1984; Kristensen and Harmon, 2004), decreased diffusion distance (Steele et al., 2011a; Bannink et al., 2012), or increased membrane fluidity due to alterations in cholesterol biosynthesis (Steele et al., 2011b; 2012a,b). However, because the vast majority of SCFA exist in the ionized state in the rumen at physiological pH (Aschenbach et al., 2011), it is likely that SCFA absorption per unit epithelia is

influenced to a greater extent by adaptive processes that increase the rates of facilitated diffusion, such as increased expression or activity of transporters thought to be involved in anionic $SCFA^-/HCO_3^-$ exchange (Aschenbach et al., 2009; Penner et al., 2009a; Penner et al., 2011). In fact, Connor et al. (2010) found that calves fed milk replacer followed by grain or hay to have elevated ruminal mRNA expression of downregulated-in-adenoma (DRA), a candidate $SCFA^-/HCO_3^-$ transporter (Bilk et al., 2005; Penner et al., 2011), relative to calves fed milk replacer only.

In regards to recent work conducted by the lab of Brian McBride (University of Guelph, Guelph, ON), decreased epithelial depth (Steele et al., 2011a) and altered cholesterol biosynthesis (Steele et al., 2011b; Steele et al., 2012a, b) were detected under conditions where ruminal acidosis was induced. Moreover, enzymes responsible for cholesterol biosynthesis were found to be downregulated, suggesting a possible decrease in membrane fluidity and therefore a negative impact of high-grain feeding on lipophillic permeability. However, because these processes are usually investigated during induced bouts of ruminal acidosis (Steele et al., 2011a,b; Steele et al., 2012a,b), we can only speculate how they are affected by gradual adaptation to high-grain feeding (Bannink et al., 2012).

Because propionate and butyrate are extensively metabolized by the rumen epithelia (Britton and Krehbiel, 1993; Kristensen and Harmon, 2004), it is intuitive that epithelial adaptation to high-grain feed may also involve the upregulation of enzymes involved in SCFA metabolism. High-grain feeding has been shown to increase the activity of propionyl-CoA synthetase (Nocek et al., 1980), an enzyme responsible for propionate activation. However, Harmon et al. (1991) reported that high dietary grain inclusion decreased the activity of butyryl-CoA synthetase, and Penner et al. (2009b) found no changes in the mRNA expression of acyl-CoA synthetases. Because butyrate is most extensively metabolized (Britton and Krehbiel, 1993; Kristensen and Harmon, 2004) and much of it undergoes ketogenesis (Weigand et al., 1975), it is also likely that enzymes and transcriptional factors that stimulate ketogenesis are upregulated during high-grain feeding (Meertens et al., 1998; Hegardt, 1999). However, despite increasing concentrations of circulating BHBA, high-grain feeding has not yet been shown to increase the mRNA expression of ketogenic enzymes (Penner et al., 2009b; Steele et al., 2011b; Steele et al., 2012a,b). Thus, it is possible that increased circulating BHBA is driven by increased available substrate only (Steele et al., 2011b). It is also possible that high-grain feeding influences post-

transcriptional, or post-translational modifications of ketogenic and/or oxidative enzymes or that enzyme abundance and activity do not limit metabolism.

The work discussed above outlining the current state of knowledge on rumen epithelial adaptation was almost entirely conducted with either dairy cattle or sheep. There is a paucity of data regarding epithelial adaptation in beef cattle. This is surprising as dietary concentrates are included in the finishing diet of beef cattle at a much higher proportion than high-energy lactating diets fed to dairy cattle. As a result, beef cattle are likely at much greater risk for ruminal acidosis (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011) than dairy cattle.

2.4 Summary and Conclusions

Ruminal acidosis is primarily defined by low ruminal pH (Kleen et al., 2003; Plaizier et al., 2008; Aschenbach et al., 2011), and has been shown to drastically alter the rumen microbiome (Mackie and Gilchrist, 1979; Goad et al., 1998; Khafipour et al., 2009a), and reduce barrier function (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012). These physiological and microbiological changes are thought to contribute to the manifestation of other feedlot disorders (Nocek, 1997; Emmanuel et al., 2007; Plaizier et al., 2012), which increases the importance of ruminal acidosis as an animal welfare concern. Feeding diets rich in rapidly fermentable non-structural carbohydrates has been associated with increased risk for ruminal acidosis (Owens et al., 1998; Krause and Oetzel 2006; Kleen and Canizzo, 2012), but can also promote epithelial adaptations that improve SCFA absorption (Dirksen et al., 1985; Bannink et al., 2008). Because SCFA absorption is the most important source of ruminal bicarbonate (Allen, 1997; Bannink et al., 2012), and elevated rates of SCFA absorption have been shown to decrease the risk for ruminal acidosis (Penner et al., 2009a), the impact that time on high-grain feed has on functional adaptation of the ruminal epithelium and susceptibility to ruminal acidosis warrants investigation in beef cattle.

2.5 Hypotheses

Increasing the amount of time that cattle are fed a high-grain diet will increase SCFA absorption and decrease the susceptibility to, and the time required to recover from, a bout of ruminal acidosis.

2.6 Objectives

This study was designed to evaluate the effect of the duration of time that cattle are fed a high-grain diet on the absorptive capacity of the reticulo-rumen and the susceptibility to, and recovery from, an induced bout of ruminal acidosis. For clarity, the resulting data have been divided into 2 chapters with Chapter 3 describing the effect of time on high-grain feed on the susceptibility to, and recovery from, ruminal acidosis, and Chapter 4 describing the effect of time on high-grain feed on the absorption of SCFA and lactate.

3. DURATION OF TIME FED A HIGH-GRAIN DIET AFFECTS THE RECOVERY FROM A BOUT OF RUMINAL ACIDOSIS

3.1 Introduction

Ruminal acidosis is thought to be the most prevalent digestive disorder in feedlot cattle (Nagaraja and Titgemeyer, 2007) and is associated with an increase in ruminal short-chain fatty acid (SCFA) production (Sutton et al., 2003; Loncke et al., 2009) and concentration (Penner et al., 2009a,b), and a decrease in ruminal pH (Penner et al., 2007). These symptoms have been associated with decreased energy intake (Fulton et al., 1979a,b; Brown et al., 2000) and growth, and liver abscesses (Brent, 1976; Nagaraja and Chengappa, 1998). Additionally, exposure of the ruminal epithelium to low ruminal pH (< 5.5) has been shown to decrease absorptive capacity (Gaebel et al., 1989; Wilson et al., 2012) and barrier function (Aschenbach and Gäbel., 2000; Penner et al., 2010; Wilson et al., 2012).

Gradually transitioning cattle to high-grain diets may reduce the risk for ruminal acidosis (Bevans et al., 2005) by providing sufficient time for behavioural, microbial, and epithelial adaptation. Adaptations that increase SCFA absorption should correspond to lower risk for ruminal acidosis (Gäbel et al., 2002; Penner et al., 2009a); however, few studies have evaluated adaptation of the ruminal epithelium in beef cattle. Reported timelines for ruminal epithelial adaptation range between 7 d for initial increases in functional activity (Etschmann et al., 2009) to 42 d for measurable increases in surface area (Dirksen et al., 1985). This range suggests that even with a gradual dietary transition, the ruminal adaptive process may not be complete and that ruminal adaptation may proceed with advancing days on feed. Thus, it was hypothesized that increasing the amount of time that cattle are fed a high-grain diet would decrease the susceptibility to, and the time required to recover from, a bout of ruminal acidosis. The objective of the study was to determine if the duration of time that cattle are fed a high-grain diet influences their susceptibility to, and recovery from, a bout of ruminal acidosis.

3.2 Materials and methods

This manuscript evaluates whether the duration of time cattle are fed high-grain diets improves the resistance to, and recovery from, an induced bout of ruminal acidosis with a focus on DMI, ruminal SCFA concentrations, and ruminal pH. A companion paper (Chapter 4) reports the results obtained for SCFA and lactate absorption, saliva production, and blood metabolites. The procedures and heifers used in this study were pre-approved by the Animal Care Committee of the Agriculture and Agri-Food Canada Lethbridge Research Centre (Lethbridge, AB, Canada) and the study was conducted according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada). Unless otherwise stated, all analyses were conducted in triplicate and reanalyzed when the CV was > 3.0%.

3.2.1 Animals, Diets, and Experimental Design

Sixteen ruminally cannulated Angus heifers were used in this study. Each heifer was fitted with a 10-cm ruminal cannula (model 9C; Bar Diamond, Parma, ID) at approximately 9 mo of age and provided at least 5 wk of recovery before the start of the study. Heifers were housed in tie-stalls and were provided exercise daily. At the time of surgery, the mean BW ± SEM was 261 ± 6.1 kg. Prior to the start of the study, heifers were adapted from a high-forage diet (forage-to-concentrate ratio (F:C) of 95:5) to a diet with a F:C of 60:40; hereafter designated as the backgrounding diet (Table 3.1). The dietary transition from the high-forage diet to the backgrounding diet occurred over 7 d using 3 intermediate diets that all contained 10% supplement (DM basis). During the first 2 d heifers were fed 30% barley silage, 50% grass hay, 10% barley grain, and 10% supplement; for the following 3 d heifers were fed 40% barley silage, 30% grass hay, 20% barley grain, and 10% supplement; and during the last 2 d heifers were fed 55% barley silage, 10% grass hay, 25% barley grain, and 10% supplement (DM basis).

Based on recovery from surgery, heifers were assigned to 1 of 4 blocks and, within block, assigned to 1 of 2 treatments designated as long adapted (LA) and short adapted (SA). Within block, average BW across treatments was balanced. Heifers on the LA treatment were fed the backgrounding diet for 7 d prior to being transitioned to a barley-based finishing diet; whereas, SA heifers were fed the backgrounding diet for 33 d before being exposed to the same dietary transition protocol (Table 3.1). Differences in the duration of the backgrounding period allowed a delay in the start of the dietary transition for the SA animals so that the LA and SA heifers

were fed the barley-based finishing diet for 34 and 8 d, respectively, prior to the induction of ruminal acidosis. The transition from the backgrounding diet to the finishing diet was accomplished over a period of 20 d using 5 intermediate diets with each diet fed for 4 d. Throughout the study, feed was offered once daily at 0900 h allowing for ad libitum intake, except for the day of, and the day preceding, the acidosis challenge.

The study design consisted of 4 distinct measurement periods including 8 d for baseline measurements (BASE), the day of the acidosis challenge (CHAL), and 2 consecutive 8-d recovery periods (REC1 and REC2). The ruminal acidosis induction protocol was similar to that of Dohme et al. (2008) with the following modifications. Within each block, the proportion of feed intake relative to BW over 31 consecutive d (d 3 to d 33 on the finishing diet) for the LA heifers was used to calculate the feed restriction and challenge dose. Body weight was measured once a week during this time, and a linear growth rate was used to estimate daily intake as a proportion of BW. The challenge severity was normalized across dietary treatments by applying the same challenge dose relative to BW for LA and SA heifers. On the day prior to the challenge, feed intake for both LA and SA was restricted to 50% of DMI as a proportion of BW. Data from the d of feed restriction was not used in the statistical analysis. On the day of the CHAL, heifers (excluding those in block 1) were provided with an intraruminal infusion of ground barley grain (ground to pass through a 4.5-mm sieve) at 0900 h equating to 10% of DMI as a proportion of BW measured prior to feed restriction. Heifers in block 1 were infused with the same lot of ground barley, but at 20% of DMI as a proportion of BW; however, because of the severity of the resulting acidosis, the amount of grain infused was subsequently reduced for the cattle in the remaining blocks. Heifers were then given their full diet allocation 1 h after the intraruminal infusion (1000 h). Beginning at the time of challenge, the pH of strained ruminal fluid from the ventral sac was measured every 2 h for the first 12 h and then every 4 h for the next 12 h using a portable pH meter (Accumet 25; Fisher Scientific, Ottawa, ON). When ruminal pH was ≤ 4.2 an additional pH measurement was made 1 h later. If ruminal pH remained ≤ 4.2, heifers were provided an intraruminal dose of 250 g of sodium bicarbonate. This intervention was necessary for 2 of the 4 heifers exposed to the ruminal acidosis induction protocol in block 1 (both LA), and for this reason, subsequent intraruminal barley infusions were decreased from 20% to 10% of DMI as a proportion of BW, as indicated previously. Data from these heifers were used in the data analysis. Interventions were not required for cattle offered the modified challenge dose.

Table 3.1. Dietary transition protocol, ingredient inclusion rates, and the chemical composition of diets fed to heifers provided a long- (LA) or short-adaption (SA) to the high-grain diet prior to the acidosis induction protocol.

	Experimental Diet						
Item	BG[1]	Step 1	Step 2	Step 3	Step 4	Step 5	HG[2]
Time on each diet, d							
LA	7	4	4	4	4	4	34
SA	33	4	4	4	4	4	8
Ingredients, g/kg DM							
Barley silage	600	500	400	300	200	130	90
Barley grain[3]	300	400	500	600	700	770	810
Supplement[4]	100	100	100	100	100	100	100
Chemical composition[5], g/kg ± SEM							
n	8	4	4	4	4	4	16
DM	468 ± 7.4	486 ± 11.7	538 ± 11.5	597 ± 8.4	660 ± 7.9	732 ± 4.3	777 ± 4.3
CP	138 ± 1.0	134 ± 1.0	133 ± 1.5	135 ± 2.8	130 ± 2.8	131 ± 3.1	134 ± 1.8
NDF	377 ± 8.3	363 ± 7.4	330 ± 3.5	301 ± 3.5	273 ± 9.1	251 ± 7.9	248 ± 8.6
ADF	208 ± 4.3	192 ± 4.6	166 ± 2.5	140 ± 2.4	116 ± 1.4	107 ± 3.3	91 ± 1.0
Starch	320 ± 9.0	357 ± 3.0	389 ± 8.6	437 ± 12.5	478 ± 5.2	501 ± 13.3	510 ± 5.2

[1]BG = Backgrounding diet

[2]HG = High-grain finishing diet

[3]Barley grain processing index (volume weight after processing expressed as a percentage of volume weight before processing, DM basis) was 82.8 ± 0.25%.

[4]Supplement prepared as a mash and contained (DM basis): 50.0% beet pulp, 33.0% canola meal, 12.0% calcium carbonate, 2.5% urea, 1.6% salt, 0.5% vitamin and mineral mix, 0.3% melengestrol acetate (200 mg/kg), and 0.1% molasses. The concentration of minerals and vitamins (DM basis) were: zinc sulphate monohydrate (55.7 mg/kg), copper sulphate pentahydrate (14.2 mg/kg), manganese sulphate monohydrate (25.6 mg/kg), EDDI (0.6 mg/kg), selenium (0.3 mg/kg), vitamin A (9281 IU/kg), vitamin D_3 (464 IU/kg), and vitamin E (13 IU/kg).

[5]All analysis except for DM are reported on a DM basis.

3.2.2 Data and Sample Collection

The amount of feed offered and refused was recorded daily throughout the study. Once the cattle were fed the high-grain diet, feed offered and refused were analyzed daily for DM content to determine DMI. In addition, TMR samples of the backgrounding and finishing diets were collected twice weekly, and samples of each transition diet were collected and stored at -20°C until analysis. Prior to analysis, TMR samples were allowed to thaw and the following feeding periods were composited by block to yield 4 samples each: Steps 1-5, extended high-grain feeding (LA only), BASE, CHAL and REC1, and REC2. This resulted in a total of 16 samples used for chemical analysis of the high-grain diet, and 4 samples for each of the transition diets (Table 3.1). In addition, the backgrounding diet was composited by treatment and block to yield 8 samples. Ingredients were also collected for DM content and chemical analysis. Barley silage was also collected twice weekly, grass hay and barley grain were collected weekly, and samples of the supplement were collected monthly as the same production lot was used for the entire study. All samples were composited to yield a monthly sample and were stored at -20°C for subsequent analysis (Table 3.2).

Ingredient and TMR samples were dried at 55°C for 48 h and ground to pass through a 1-mm sieve (SM100, Retsch; Hann, Germany). The analytical DM content was determined by drying samples at 135°C for 2 h (AOAC, 1995) and was used to calculate nutrient composition on a DM basis. Crude protein was estimated from the N concentration (CP = N × 6.25), which was determined using flash combustion (Carlo Erba Instruments; Milan, Italy). Neutral detergent fiber and ADF were determined using an Ankom Fiber Analyzer (Ankom Technology Corporation; Fairport, NY) using separate runs. Heat-stable α-amylase and sodium sulfite were used in the NDF procedure (Van Soest et al., 1991). Starch content was determined using enzymatic hydrolysis of α-linked glucose polymers as described by Rode et al. (1999) with minor modifications. Briefly, 100 to 500 mg of sample was diluted in 25 mL 0.1 N Na-acetate buffer and 200 µL of α-amylase (Termamyl, Novo Nordisk; Bagsvaerd, Denmark) was added. Tubes were vortexed immediately and at 10, 20 and 30 min while incubating at 95°C in a constantly shaking water bath. Incubation at 95°C continued for an additional 0.5 h. Subsequently, the incubation temperature was lowered to 65°C and 200 µL amyloglucosidase (208-469; Boehringer Mannheim, Laval, QC, Canada) was added to the tubes. Tubes were

vortexed immediately and after 30 and 60 min of incubation at 65°C. Incubation at 65°C continued for an additional 1 h followed by cooling for 5 min. Tubes were then centrifuged (29,000 × g for 15 min at 4°C) and diluted 1:20 using double distilled water. Fifty µL of sample was added to a microplate and 300 µL glucose trinder reagent (315-100; Sigma-Aldrich, St. Louis, MO) was added to all wells. Following a 20-min incubation at 39°C, glucose was determined by reading the absorbance at 508 nm (Appliscan Multiplate Reader, Thermo Electron Company; Waltham, MA). Means from four glucose determinations were compared in duplicate and results were confirmed when the CV < 5.0 %.

Table 3.2. Chemical composition of dietary ingredients used throughout the study.

Item	Grass hay	Supplement [1]	Barley silage	Barley grain
n	3	5	5	5
Chemical composition[2], g/kg ± SEM				
DM	872 ± 19.4	931 ± 3.0	340 ± 9.1	878 ± 4.1
CP	76 ± 1.4	258 ± 3.7	120 ± 2.2	112 ± 2.5
NDF	709 ± 10.5	290 ± 4.5	506 ± 8.6	209 ± 7.9
ADF	420 ± 4.9	190 ± 2.1	292 ± 3.0	62 ± 1.8
Starch	ND[3]	ND	199 ± 5.4	593 ± 9.7

[1]Supplement prepared as a mash and contained (DM basis): 50.0% beet pulp, 33.0% canola meal, 12.0% calcium carbonate, 2.5% urea, 1.6% salt, 0.5% vitamin and mineral mix, 0.3% MGA (200 mg/kg), and 0.1% molasses. The concentration of minerals and vitamins (DM basis) were zinc sulphate monohydrate (55.7 mg/kg), copper sulphate pentahydrate (14.2 mg/kg), manganese sulphate monohydrate (25.6 mg/kg), EDDI (0.6 mg/kg), selenium (0.3 mg/kg), vitamin A (9281 IU/kg), vitamin D_3 (464 IU/kg), and vitamin E (13 IU/kg).
[2]All analysis except for DM are reported on a DM basis.
[3]ND = Not determined.

3.2.2.1 Ruminal pH Measurement

Ruminal pH data were recorded every 1 min using an indwelling pH measurement system (LRCpH Data Logger system; Dascor, Escondido, CA) positioned at the bottom of the cranial-ventral sac as described by Penner et al. (2006). Measurement started on d 1 of BASE, and persisted through to the final day of experimentation resulting in 26 d of measurements. Ruminal pH systems were standardized before ruminal incubation and were re-standardized in pH buffers 7 and 4 at 39°C before and after cleaning in Terg-A-Zyme (Alconox; White Plains, NY) solution for 10 min upon weekly removal from the rumen. The resulting data were transformed from mV recordings to pH based on the pre- and post-incubation standardizations with the pre-cleaning measurement used for the post-incubation standardization values. A linear drift correction over time was used to correct pH data (Penner et al., 2006). The daily minimum, mean, and maximum pH, as well as duration (min) and area (min × pH) that pH was < 5.5 were calculated. The day of the dietary restriction prior to CHAL was discarded from analysis, as were pH data from days when the temporarily isolated and washed reticulo-rumen procedure was conducted (d 5 of BASE, d 2 of REC1, and d 1 of REC2; see Schwaiger et al., submitted). After these deletions, 22 d of pH data remained for each cow (7 d for BASE, 1 d for CHAL, and 7 d each for REC1 and REC2). Additionally, pH was monitored during the first 7 d that the LA and SA cattle were fed the high-grain diet to test whether the duration of time on the backgrounding diet affected the response.

3.2.2.2 Ruminal Fluid Sampling and Analyses

Ruminal digesta was sampled on d 3 of BASE, on the d of CHAL, and on d 7 of REC1. Starting at the time of feeding (0900 h), samples were collected every 2 h for a total of 7 samples over 12 h. Due to an observed continued response to the challenge during block 1, the sampling protocol was extended to cover a 24 h duration on the challenge day for blocks 2 to 4. This extended sampling was used to collect 3 additional ruminal fluid samples with a 4-h interval between consecutive samples.

Digesta was collected from 3 locations in the rumen (cranial, central-ventral, and caudal sacs) with an equal volume from each location used to prepare a composite (250 mL each for

750 mL total). Composited digesta was strained through PECAP polyester monofilament (pore size 355 µm; part 7-355/47; B & SH Thompson, Ville Mont-Royal, QC, Canada) and aliquots were stored at -20°C for determination of SCFA (1.5 mL), lactate (1.5 mL) and osmolality (2 mL). For SCFA and lactate determination, 1.5 mL aliquots were preserved with 0.3 mL frozen 25% (wt/vol) metaphosphoric acid.

Ruminal fluid osmolality (mOsm/kg) was determined in duplicate by freezing-point depression. Samples were centrifuged at 16,100 × g for 30 min, and 250 µL of supernatant was loaded into the osmometer (Advanced Instruments 3250; Norwood, MA). For quality control, double distilled H_2O and standards (290 and 500 mOsm/kg) were analyzed at the beginning and end of each d.

Ruminal fluid SCFA and lactate concentrations were determined by gas chromatography (GC) using a flame ionization detector. The gas chromatograph (Hewlett Packard 5890; Santa Clara, CA) was fitted with a Zebron capillary column (ZB-FFAP; 30 M × 0.32 mm i.d. × 1.0 µm phase thickness; Phenomenex, Torrance, CA), and crotonic acid (trans-2-butenoic acid) and malonic acid (propanedioic acid) were used as internal standards for SCFA and lactate, respectively. In both cases, helium was used as carrier gas (28.5 cm/s). For SCFA determination, 1 µL was injected using a split ratio of 50:1. The injector temperature was set at 225°C and the column temperature was held at 150°C for 1 min followed by a 5°C/min increase in temperature until reaching 195°C, after which temperature was held for 5 min. The detector temperature was held constant at 250°C. For lactate determination, lactate methyl esters were prepared using BF_3-MeOH and a 1-µL splitless injection was used. The injector temperature was set at 225°C and the column temperature was held at 45°C for 1 min followed by a 30°C/min increase in temperature until reaching 160°C then a 5°C/min increase in temperature until reaching 195°C, after which temperature was held for 5 min. The detector temperature was held constant at 250°C. Lactate concentrations below the lowest standard (0.21 m*M*) were assumed to be 0 m*M*.

3.2.3 Statistical Analyses

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). Block was considered a fixed effect, and was left in the model except when performing analysis of covariance as described

below. Significance was declared when $P \leq 0.050$ and tendencies are discussed when $0.050 < P \leq 0.100$. Mean separation was conducted with use of the LSMEAN procedure of SAS, Tukey's post-hoc mean separation test, and the SAS pdmix800 macro (Saxton, 1998). The PROC UNIVARIATE procedure of SAS was used to determine if residual data were normally, identically, and independently distributed (NIID). If necessary, outliers were removed in a stepwise fashion until the normal probability plot indicated that residual data were NIID.

Ruminal pH and DMI data were summarized by day and cow. The fixed effect of treatment was investigated over the first 7 d on the high-grain diet. Then, to compare daily means across periods with a different number of d, a split-plot design was used. In this manner the fixed effects of treatment, period, treatment × period, period × day, and treatment × period × day were investigated with the random effects of cow × block and cow × period × treatment × block. The effects of period × day and treatment × period × day were left in the model, but not reported due to their lack of physiological importance. The effects of treatment and treatment × period were also not reported, as each sampling period was considered biologically distinct.

The fixed effects of treatment, day, and treatment × day were then investigated within BASE using day as a repeated measure for the subject cow × treatment × block. The fixed effect of treatment was then investigated within CHAL and analysis of covariance was conducted for each variable with respect to day using daily means from BASE and CHAL to determine if there were significant linear, quadratic or cubic effects of day. In order to accomplish this, the fixed effects of treatment, day, treatment × day, day^2, treatment × day^2, day^3, and treatment × day^3 were tested with cow × treatment as the subject. Starting with the highest order term, insignificant ($P > 0.050$) terms were removed from the model in a stepwise fashion until only significant ($P \leq 0.050$) terms remained in the model. When an interaction was significant, the lower order term was removed.

The fixed effects of treatment, day, and treatment × day were then investigated within REC1 using day as a repeated measure for the subject cow × treatment × block. Analysis of covariance was then conducted for each variable with respect to day within REC1 to determine if there were significant linear, quadratic, or cubic effects of day. This analysis was conducted in the same manner as previously described for BASE and CHAL.

The fixed effects of treatment, period, and treatment × period were investigated for ruminal fluid fermentation products by summarizing the data by cow and period. Only the first

12 h of challenge sampling was used for ruminal fluid fermentation products (extended sampling on CHAL was ignored). Period was used as a repeated measure for the subject cow × treatment × block. Then, within each sampling period the fixed effects of treatment, time, and treatment × time were investigated for ruminal fluid fermentation products by summarizing the data by cow, period and time. Time was used as a repeated measure for the subject cow × treatment × block.

One SA heifer was removed from the study prior to the acidosis induction due to low intake, frothy ruminal contents, and keratinized epithelia; however, baseline data from this animal were used in statistical analysis. A failed pH electrode resulted in the loss of 6 d of pH data from one cow (SA; BASE d 6 to d 8, CHAL, REC1 d 1 to d 2). Dry matter intake data were missing for one heifer (SA) on d 5 of REC2.

3.3 Results

3.3.1 Ruminal pH

As the aim of this study was to evaluate whether duration of time that heifers were fed a high-grain diet affects the susceptibility to, and recovery from, an induced bout of ruminal acidosis, we first needed to test whether the duration of time on the backgrounding diet affected the response to dietary transition. Thus, comparisons were made for BW, DMI and ruminal pH during the first 7 d that heifers from each treatment were fed the high-grain diet (Table 3.3). The 26 additional days that the SA cattle were fed the backgrounding diet increased BW ($P < 0.001$), and tended to increase DMI ($P = 0.094$) during the first 7 d on the high-grain diet; however, there was no effect of treatment on DMI when reported as a percentage of BW ($P = 0.70$). There were also no differences between treatments for minimum and mean ruminal pH, nor were there differences for the duration or area that pH < 5.5. Overall, we interpret these data to suggest that the duration of time that heifers were fed the backgrounding diet did not have a significant impact on DMI or ruminal fermentation, and thus responses observed for the subsequent tables can be attributed to the duration of time that heifers were fed the high-grain diet.

Table 3.3. Mean daily BW, DMI, and ruminal pH for the first 7 d that long-adapted (LA) and short-adapted (SA) heifers were fed the high-grain diet[1]. Long-adapted heifers were fed the high-grain diet for 34 d compared to 8 d for the SA heifers.

	Treatment			
Item	LA	SA	SEM	*P* value
BW[2], kg	314	342	2.4	< 0.001
DMI, kg	8.7	9.3	0.25	0.094
DMI, % BW	2.8	2.7	0.07	0.70
Minimum pH[3]	5.04	5.06	0.032	0.65
Mean pH	5.79	5.72	0.042	0.24
Maximum pH	6.68	6.49	0.037	0.001
pH < 5.5				
Duration, min/d	475	522	50.3	0.52
Area, (min × pH)/d	153	166	22.5	0.67

[1]Forage:concentrate ratio = 9:91
[2]Mean BW was estimated assuming linear growth between measurements separated by 20 d (LA) and 13 d (SA).
[3]Data for 7 d of indwelling ruminal pH measurement.

To illustrate the effects of the induced challenge on ruminal pH, mean pH at 10-min intervals was plotted for the 2 treatments over a 5-d duration surrounding the induced bout of ruminal acidosis (Figure 3.1). From the rapid drop in pH, and the extent to which pH dropped, it is clear that a bout of ruminal acidosis was successfully induced using the acidosis challenge protocol.

Comparisons by period were conducted to evaluate whether the acidosis protocol affected DMI and ruminal pH (Table 3.4). Dry matter intake tended ($P = 0.072$) to be affected by period, whereby DMI in CHAL tended to be greater than during the other periods. The acidosis challenge reduced minimum ($P < 0.001$) and mean pH ($P < 0.001$) relative to BASE with the effect reversed during REC1 and REC2, such that minimum and mean pH were higher during REC than during BASE and CHAL. Maximum pH increased on the day of CHAL due to the elevated pH caused by the dietary restriction ($P < 0.001$; Figure 3.1). This elevated pH persisted for approximately 1 h following the induced challenge. The duration ($P < 0.001$) and area ($P < 0.001$) that pH was < 5.5 was greater during CHAL relative to BASE. Specifically, the CHAL nearly doubled the duration that pH was < 5.5 relative to BASE (1020 vs. 531 min/d) (Table 3.4). The increases in duration and area during CHAL were both reversible with lower duration observed during REC1 and REC2 than during BASE, and the area that pH was < 5.5 during REC1 and REC2 were not different than during BASE but lower than CHAL. Treatment ($P > 0.100$) or treatment × period interactions ($P > 0.100$) were not detected.

To evaluate the effect of treatment and day on DMI and pH prior to the CHAL, the data were investigated within BASE (Table 3.5). There was no effect of treatment on DMI or pH variables during BASE ($P > 0.050$). Dry matter intake was greatest on the first d of BASE ($P = 0.038$); however, minimum ($P = 0.010$), mean ($P = 0.030$), and maximum ($P = 0.027$) pH all reached the lowest values on d 6 of BASE, during which time both duration (min/d; $P = 0.064$) and area (pH × min/d; $P = 0.075$) that pH remained below 5.5 tended to be greatest. A tendency for a treatment × day interaction for maximum pH ($P = 0.063$) indicated that the LA heifers tended to experience an increase in maximum pH between d 1 and 4 during BASE, while the SA heifers tended to experience a decrease in maximum pH during this time (data not shown).

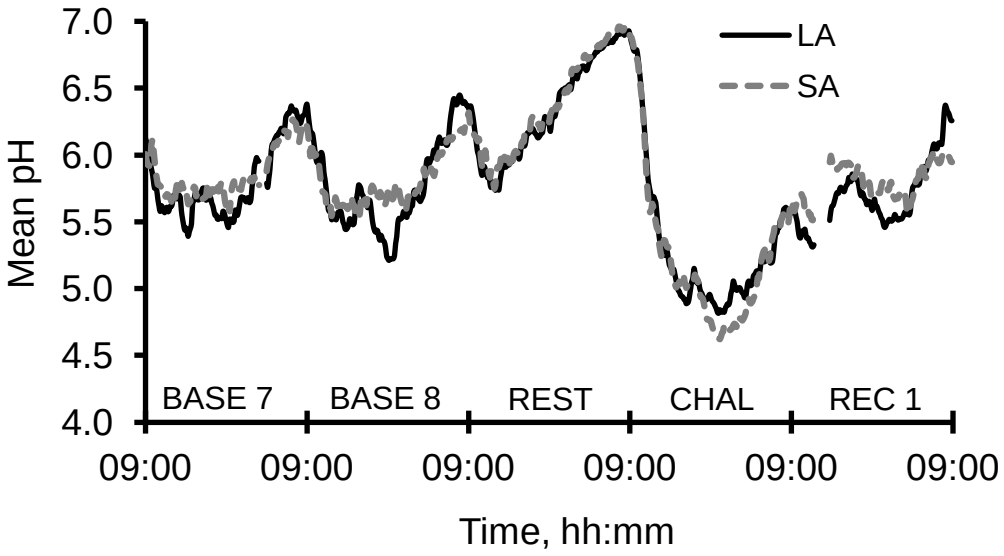

Figure 3.1. Mean ruminal pH for the 5 d surrounding the induced challenge. Data were summarized by min for each heifer, and means were calculated for every 10 min for long-adapted (LA) and short-adapted (SA) heifers during the last 2 d of baseline (BASE 7 and 8), during the dietary restriction (REST), during the day of the induced challenge (CHAL), and during the first day of recovery from the challenge (REC 1). All (n=8) LA heifers were used for all means shown, while 7 (n=7) SA heifers were used during BASE, and 6 (n=6) SA heifers were used during REST, CHAL, and REC 1. One SA heifer was removed from the study after BASE, and another had a failed pH electrode.

Table 3.4. Mean daily DMI and ruminal pH across measurement periods when heifers were fed the high-grain diet[1]. Data are reported by biologically distinct sampling periods with data from treatments pooled for analysis as there were no treatment and treatment × period interactions ($P > 0.100$)[2].

	Period[2]					
Item	BASE	CHAL	REC1	REC2	SEM	*P* value
Number of days, d	7	1	7	7		
DMI, kg	9.3	10.5	9.2	9.6	0.46	0.072
Minimum pH	5.03[b]	4.57[c]	5.20[a]	5.26[a]	0.030	< 0.001
Mean pH	5.73[b]	5.27[c]	5.86[a]	5.93[a]	0.043	< 0.001
Maximum pH	6.54[b]	6.96[a]	6.52[b]	6.60[b]	0.038	< 0.001
pH < 5.5						
Duration, min/d	531[b]	1020[a]	294[c]	259[c]	55.7	< 0.001
Area, (min × pH)/d	176[b]	595[a]	72[b]	58[b]	36.0	< 0.001

[abc]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc mean separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]BASE = Baseline measurement period that consisted of 8 d on the final diet prior to dietary restriction (50% DMI/BW). CHAL = Challenge day that occurred 1 d after dietary restriction and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation. REC = Recovery period that started 24 h after the challenge and is separated into two consecutive 8 d periods (REC1 and REC2). Data from the WRR days during BASE, REC1, and REC2 have been omitted.

Table 3.5. Mean daily DMI and pH variables for the 8 days prior to the induced acidosis challenge (BASE). Long-adapted (LA) heifers were fed the high-grain diet[1] for 26 additional days prior to this measurement period; short-adapted (SA) heifers received the high-grain diet on d 1 of the BASE period.

	Treatment			Day of baseline period (BASE)[2]								*P* value[3]		
Item	LA	SA	SEM	1	2	3	4	6	7	8	SEM	T	D	T × D
DMI, kg	9.3	9.3	0.64	10.5	9.5	9.6	8.3	8.8	8.5	9.6	0.58	1.00	0.038	0.97
Minimum pH	4.99	5.06	0.037	5.11^{ab}	5.02^{ab}	4.93^{ab}	5.04^{ab}	4.88^{b}	5.12^{a}	5.09^{ab}	0.058	0.20	0.010	0.69
Mean pH	5.73	5.72	0.056	5.78	5.66	5.63	5.82	5.58	5.82	5.78	0.078	0.93	0.030	0.56
Maximum pH	6.59	6.48	0.043	6.65^{a}	6.56^{ab}	6.52^{ab}	6.55^{ab}	6.45^{b}	6.46^{ab}	6.56^{ab}	0.060	0.088	0.027	0.063
Ruminal pH < 5.5														
Duration, min/d	547	516	72.6	474	581	639	485	680	397	465	94.3	0.77	0.064	0.29
Area, (min × pH)/d	190	167	31.2	138	210	223	147	261	127	141	44.2	0.60	0.075	0.53

[ab]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc mean separation test was used for mean separation. For DMI and mean pH, mean separation was not achieved despite a significant effect of day.

[1]Forage:concentrate ratio = 9:91

[2]BASE = The baseline measurement period consisted of 8 d on the final diet prior to dietary restriction and the acidosis challenge. Day 5 was the day of the temporarily isolated and washed reticulo-rumen technique. Due to manipulation of the rumen DMI and pH data were not included.

[3]T = treatment, D = day of baseline period.

The impact of the duration of dietary adaptation on the susceptibility to an induced bout of acidosis was investigated solely using data within CHAL (Table 3.6). There was no effect of treatment on DMI or pH variables on CHAL ($P > 0.100$). Although treatment differences were not detected within BASE or CHAL, analysis of covariance was used to comprehensively evaluate the effect of treatment on the between-day responses for the duration of time that pH was below 5.5 (min/d) prior to and leading into the CHAL. From Figure 3.2, it is evident that over the 9 d BASE and CHAL, SA heifers had greater rates of change in duration that pH was < 5.5 over time. The fitted data indicated significant treatment differences between y-intercepts ($P = 0.047$), linear slopes ($P = 0.007$), quadratic coefficients ($P = 0.003$), and cubic coefficients ($P = 0.001$) with trend line equations of $y = 567.4 + 48.7\ d - 23.1\ d^2 + 2.2\ d^3$ for LA, and $y = -173.6 + 653.1\ d - 150.5\ d^2 + 9.8\ d^3$ for SA. In support of this observed increase in between-day variability in pH, SA heifers also tended to have a greater SD in the duration of time that pH was < 5.5 over the 8 d considered in this analysis when compared to LA heifers (data not shown; $P = 0.096$).

Table 3.6. Mean DMI and ruminal pH variables on the challenge day[1]. Long-adapted (LA) heifers were fed the high-grain diet[2] for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			
Item	LA	SA	SEM	*P* value
DMI, kg	11.0	10.1	0.82	0.47
Minimum pH	4.60	4.58	0.134	0.90
Mean pH	5.30	5.31	0.095	0.94
Maximum pH	6.95	6.96	0.066	0.93
Ruminal pH < 5.5				
Duration, min/d	951	1013	58.6	0.51
Area, (min × pH)/d	583	545	123.1	0.85

[1]Challenge day occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.
[2]Forage:concentrate ratio = 9:91

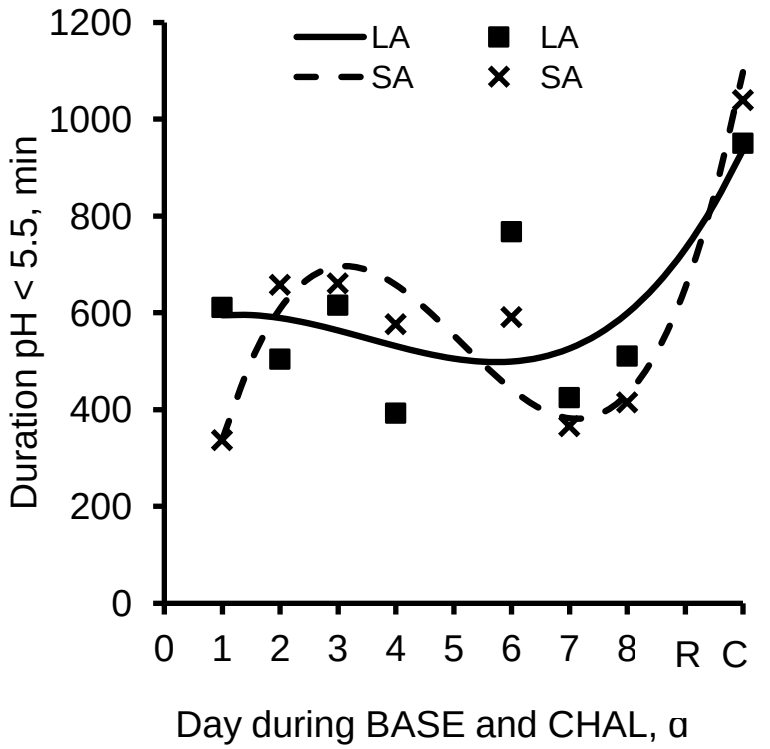

Figure 3.2. Change in the duration of time that pH < 5.5 over the days leading up to and including the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet for 26 days prior to this measurement period; short-adapted (SA) heifers received the high-grain diet on day 1 of the baseline period. Trend lines were constructed using analysis of covariance. Daily means were used from the 7 d during baseline (BASE; d 5 = WRR) and the challenge day (CHAL, C; d 10). Restriction (R; d 9) was not included in the analysis. Significant treatment differences were found between y-intercepts (P = 0.047), linear slopes (P = 0.007), quadratic coefficients (P = 0.003) and cubic coefficients (P = 0.001). The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y = 567.4 + 48.7\ d - 23.1\ d^2 + 2.2\ d^3$ (LA), and $y = -173.6 + 653.1\ d - 150.5\ d^2 + 9.8\ d^3$ (SA).

The effect of treatment and day on DMI and pH after the CHAL was investigated within REC1 (Table 3.7). There was no effect of treatment on DMI or pH variables during REC1 ($P >$ 0.100). Dry matter intake increased linearly ($P < 0.001$; not shown) from d 1 to d 8, culminating in the greatest value on d 8 ($P = 0.001$). A tendency for a treatment × day interaction for minimum pH ($P = 0.088$) indicated that minimum pH tended to increase to a greater extent between d 1 and d 3 for LA cattle, but reached a greater value for SA cattle on d 6 and 7 (data not shown). Although there was no effect of day for maximum pH, mean pH reached its greatest values on d 5 and 6 ($P = 0.012$), while minimum values for duration ($P = 0.019$) and area ($P =$ 0.018) were observed on d 7 and 5, respectively. A tendency for a treatment × day interaction for the duration of time that pH was < 5.5 ($P = 0.085$) indicated that the duration (min/d) that pH was < 5.5 tended to decrease between d 1 and d 3 for LA cattle, and instead increased from d 1 to d 4 for SA cattle (data not shown).

The tendencies for treatment × day for minimum pH ($P = 0.088$) and duration pH < 5.5 ($P = 0.085$) both can be interpreted to suggest that the LA heifers recovered more quickly from the CHAL. Because analysis of covariance is a more appropriate method of detecting a change in a variable over time than mean separation, it was utilized to examine the effect of treatment on the between-day responses for these variables during REC1. During REC1 (Figure 3.3) the LA cattle experienced a greater linear reduction in the duration of time that pH was < 5.5 over the first few days after the challenge (Figure 3.3; - 588 vs. 369 min/d; $P = 0.019$).

Table 3.7. Mean daily DMI and ruminal pH during recovery (REC1) after an induced acidosis challenge. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			Day of recovery (REC1)[2]								*P* value[3]		
Item	LA	SA	SEM	1	3	4	5	6	7	8	SEM	T	D	T × D
DMI, kg	9.1	9.5	0.56	8.0^{cd}	8.6^{d}	8.8^{bcd}	9.9^{abc}	9.5^{abcd}	10.0^{ab}	10.2^{a}	0.48	0.69	0.001	0.38
Minimum pH	5.16	5.25	0.043	5.07	5.26	5.18	5.26	5.28	5.2	5.19	0.064	0.17	0.21	0.088
Mean pH	5.87	5.84	0.039	5.75	5.86	5.78	5.94	5.94	5.91	5.81	0.058	0.64	0.012	0.31
Maximum pH	6.56	6.49	0.053	6.44	6.51	6.52	6.60	6.56	6.53	6.54	0.051	0.39	0.54	1.00
pH < 5.5														
Duration, min/d	297	274	56.0	458	281	407	192	167	167	326	75.8	0.78	0.019	0.085
Area, (min × pH)/d	83	71	23.2	178^{ab}	61^{ab}	106^{a}	42^{b}	32^{ab}	44^{ab}	76^{ab}	28.6	0.70	0.018	0.37

^{abcd}Means within a dependent variable differ ($P \leq 0.050$). Tukey was used for mean separation. For mean pH and duration of pH < 5.5, mean separation was not achieved despite a significant effect of day.

[1]Forage:concentrate ratio = 9:91

[2]REC1 = Recovery 1 measurement period that started 24 h after the acidosis challenge and consisted of 8 d. Day 2 was the day of the REC1 WRR. Due to manipulation of the rumen DMI and pH data were not included.

[3]T = treatment, D = day of recovery.

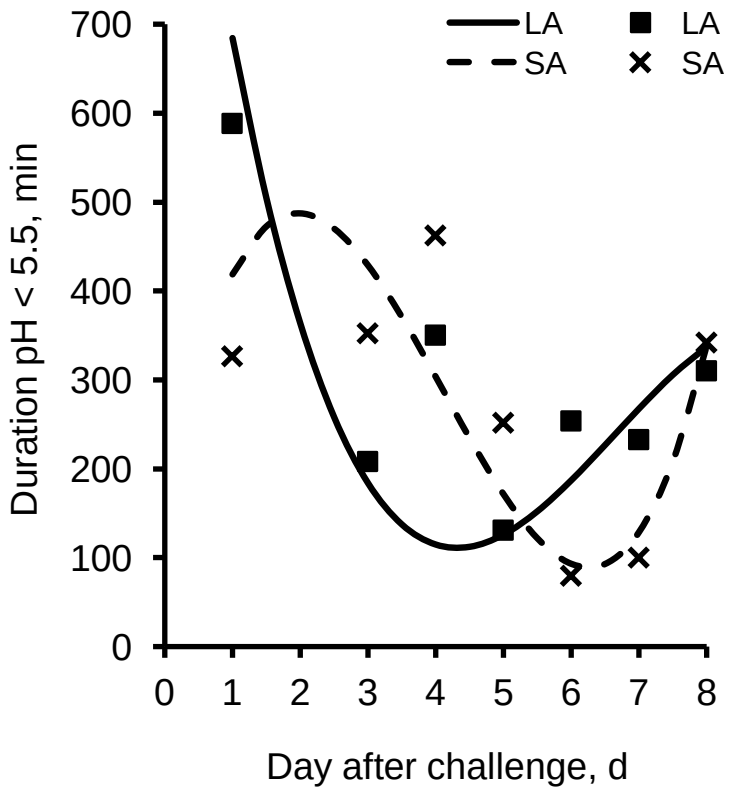

Figure 3.3. Change in the duration of time that pH < 5.5 over the days following the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet for 34 d compared to 8 d for the short-adapted (SA) heifers. Trend lines were constructed using analysis of covariance. Daily means were used from the first 7 d following the induced challenge (REC1; d 2 = WRR). Significant treatment differences were found between y-intercepts (P = 0.048), linear slopes (P = 0.019), quadratic coefficients (P = 0.026) and cubic coefficients (P = 0.037). The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y = 1176.4 - 587.8\ d + 100.9d^2 - 5.06\ d^3$ (LA), and $y = 162.8 + 369.4\ d - 123.7\ d^2 + 10.0\ d^3$ (SA).

3.3.2 Ruminal Short-Chain Fatty Acids, Lactate, and Osmolality

There were no significant effects of treatment, or interactions between treatment and period for mean or maximum ruminal fluid SCFA, lactate, or osmolality when compared across periods (Table 3.8). Maximum acetate concentrations tended ($P = 0.080$) to increase during CHAL and decrease during REC1. Maximum propionate concentrations were greatest during BASE and lowest during REC1 ($P = 0.018$), while both maximum total SCFA (165.7 vs. 143.2 m*M*; $P = 0.011$) and maximum ruminal fluid osmolality (432 vs. 407 mOsm/kg; $P = 0.030$) were greatest during the CHAL, and lowest during REC1.

Mean ($P = 0.013$) and maximum ($P = 0.008$) ruminal fluid lactate concentrations increased during the CHAL relative to BASE but BASE and REC1 were not different (Table 3.8). Mean and maximum daily ruminal lactate concentrations ranged from 0.3 to 30.5 m*M*, and 1.0 to 76.4 m*M*, respectively during CHAL. Ruminal lactate concentration > 5.0 m*M* was observed in 12 of the 15 heifers during CHAL. In contrast, none of the heifers experienced a maximal daily ruminal lactate concentration ≥ 5.0 m*M* during either BASE or REC1 (data not shown). Although there was significant between heifer variability in response to the challenge, we were able to successfully induce an average bout of acute ruminal acidosis when data from all heifers were considered (Table 3.4 and Table 3.8). By implementing our challenge model, we observed a significant increase in ruminal fluid lactate concentration (Tables 3.8 and 3.10) with a peak mean lactate concentration of 19.0 m*M* observed 8 h after providing the challenge dose. The daily mean lactate concentration of 11.4 m*M*, and daily maximum lactate concentration of 29.3 m*M* are both above the suggested threshold of 5.0 m*M* to indicate acute ruminal lactic acidosis (Aschenbach et al., 2011).

On the challenge day (Tables 3.9, 3.10), mean pH reached a minimum ($P < 0.001$), and the duration that ruminal pH was < 5.5 reached a maximum ($P < 0.001$) between 10 and 12 h post-challenge, while the area that pH was < 5.5 peaked between 14 and 16 h after the barley infusion ($P < 0.001$). Ruminal acetate concentrations remained elevated between 6 and 20 h after challenge induction (Table 10, $P < 0.001$). Ruminal propionate ($P < 0.001$), butyrate ($P < 0.001$), total SCFA ($P < 0.001$), and lactate concentrations ($P = 0.027$) all peaked at 8 h after challenge induction. Ruminal fluid osmolality reached a maximum at hours 6, 8, and 16 following the barley infusion ($P < 0.001$).

Table 3.8. Rumen fluid short-chain fatty acids (SCFA), lactate, and osmolality as affected by treatment and sampling period. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Treatment			Period[2]				*P* value[3]		
Item	LA	SA	SEM	BASE	CHAL	REC1	SEM	T	P	T × P
Total SCFA, m*M*										
Mean	129.7	125.9	5.02	128.2	133.0	122.0	4.32	0.61	0.14	0.26
Maximum	158.9	152.1	4.09	157.7^{ab}	165.7^{a}	143.2^{b}	5.00	0.25	0.011	0.16
Acetate, m*M*										
Mean	64.7	64.4	1.05	62.2	67.8	63.6	1.62	0.85	0.22	0.65
Maximum	77.2	75.6	1.62	75.5	80.0	73.6	1.98	0.49	0.080	0.25
Propionate, m*M*										
Mean	49.6	45.8	4.47	50.4	50.0	42.8	3.88	0.56	0.12	0.53
Maximum	61.7	56.8	4.74	65.0^{a}	61.7^{ab}	51.0^{b}	4.35	0.48	0.018	0.43
Butyrate, m*M*										
Mean	15.7	16.4	0.97	15.2	17.9	15.0	1.55	0.62	0.23	0.78
Maximum	21.5	21.1	1.72	20.1	24.5	19.4	2.10	0.88	0.20	0.42
Lactate, m*M*										
Mean	4.4	3.4	1.45	0.3^{b}	11.4^{a}	0.1^{b}	1.04	0.63	0.013	0.36
Maximum	11.1	9.6	3.44	1.3^{b}	29.3^{a}	0.5^{b}	2.55	0.76	0.008	0.59
Osmolality, mOsm/kg										
Mean	371	367	4.3	369	369	369	5.3	0.52	0.99	0.12
Maximum	420	414	5.6	412^{ab}	432^{a}	407^{b}	6.9	0.51	0.030	0.19

[abc]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]BASE = Baseline sampling occurred 7 d prior to challenge. CHAL = Challenge sampling occurred on day of induced challenge. REC1 = Recovery sampling occurred 7 d after challenge. Daily mean and maximum values were determined from samples collected at the time of feeding and every 2 hours for 12 h after feeding.

[3]T = treatment, P = period.

Table 3.9. Rumen fluid pH, short-chain fatty acids (SCFA), lactate, and osmolality as affected by treatment during the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Treatment			*P* value[2]	
Item	LA	SA	SEM	T	T × t
Mean pH	5.54	5.50	0.106	0.79	0.94
Ruminal pH < 5.5[3]					
Duration	85	94	9.3	0.52	0.83
Area	49	45	13.6	0.84	0.82
Total SCFA, m*M*	121.6	122.7	6.69	0.91	0.34
Acetate, m*M*	61.6	63.1	2.65	0.70	0.39
Propionate, mM	45.7	43.4	4.76	0.74	0.17
Butyrate, m*M*	14.2	16.3	1.81	0.44	0.41
Lactate, m*M*	12.7	11.9	5.20	0.92	0.53
Osmolality, mOsm/kg	376	376	5.9	0.98	0.075

[1]Forage:concentrate ratio = 9:91

[2]T = Treatment, t = time after feed. Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed. Extended sampling occurred on challenge day at 16 h, 20 h and 24 h after feed. To correspond with other variables reported, pH variables were calculated over 2 h prior to each sample.

[3]Duration pH < 5.5 measured in min/120 min, and area pH < 5.5 measured in (min × pH)/120 min.

Table 3.10. Rumen fluid pH, short-chain fatty acids (SCFA), lactate, and osmolality as affected by time during the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Time After Feed (h)[2]											
Item	0	2	4	6	8	10	12	16	20	24	SEM	*P* value
Mean pH	6.93^{a}	6.70^{b}	5.78^{c}	5.33^{df}	5.06^{eg}	5.02^{defg}	4.92^{g}	4.83^{fg}	5.09^{fg}	5.54^{cde}	0.105	< 0.001
pH < 5.5[3]												
Duration	ND[4]	ND	39^{c}	89^{ab}	111^{a}	117^{a}	119^{a}	106^{a}	87^{ab}	47^{bc}	8.8	< 0.001
Area	ND	ND	7^{e}	31^{cd}	52^{ab}	55^{abc}	66^{ab}	82^{a}	56^{ac}	25^{bde}	12.3	< 0.001
Total SCFA[3]	47.8^{e}	88.3^{d}	123.5^{c}	149.9^{ab}	156.3^{a}	136.7^{bc}	136.5^{abc}	135.7^{abc}	130.4^{abc}	116.2^{bcd}	7.35	< 0.001
Acetate	28.4^{g}	51.7^{f}	66.2^{cde}	74.8^{ab}	74.8^{ac}	66.0^{bde}	67.8^{abcd}	69.0^{abcd}	66.0^{abcdf}	59.0^{df}	3.21	< 0.001
Propionate	12.8^{e}	25.5^{d}	42.3^{c}	54.1^{ab}	59.1^{a}	51.7^{bc}	51.1^{abc}	52.1^{abc}	51.4^{abc}	45.3^{abc}	3.87	< 0.001
Butyrate	6.4^{d}	11.1^{c}	14.8^{bc}	20.8^{ab}	22.3^{a}	18.8^{ab}	17.6^{abc}	14.8^{bc}	13.6^{bcd}	12.2^{bc}	1.75	< 0.001
Lactate[3]	ND[4]	5.3^{bc}	3.6^{bc}	10.0^{b}	19.0^{a}	20.5^{abc}	21.4^{abc}	22.6^{abc}	8.2^{abc}	0.7^{c}	4.25	0.027
Osmolality[3]	285^{d}	325^{c}	367^{b}	404^{a}	414^{a}	386^{ab}	388^{ab}	416^{a}	398^{ab}	375^{ab}	9.6	< 0.001

^{abcdefgh}Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed. Extended sampling occurred on challenge day at 16 h, 20 h and 24 h after feed. To correspond with other variables reported, pH variables were calculated over 2 h prior to each sample. For example, t=0 corresponds to pH measured between 07:01 and 09:00.

[3]Duration pH < 5.5 measured in min/120 min, and area pH < 5.5 measured in (min × pH)/120 min. Total and individual SCFA, as well as lactate, measured in m*M*. Osmolality measured in mOsm/kg.

[4]ND = No data. Times with means of 0 were removed from analysis due to causing negative LSMeans.

3.4 Discussion

In North American feedlots, beef cattle are typically fed diets with a high proportion of grain to maximize energy intake, and improve performance and feed efficiency (Owens et al., 1997). However, feeding highly fermentable diets increases the risk for ruminal acidosis (Aschenbach et al., 2011) as rates of acid production in the rumen may exceed rates of acid removal (Penner et al., 2009a). While prevalence rates for ruminal acidosis in feedlot cattle are not currently available, several studies utilizing continuous ruminal pH measurement in feedlot cattle indicate that based on a threshold pH value of 5.5, the risk for ruminal acidosis and likelihood of cattle experiencing ruminal acidosis is high (Wierenga et al., 2010; Li et al., 2011; Moya et al., 2011). In addition, acute ruminal acidosis is primarily characterized by an increase in ruminal lactate concentration (Nocek, 1997; Najaraga and Titgemeyer, 2007; Aschenbach et al., 2011). For these reasons, we used the duration and area that pH < 5.5 to detect ruminal acidosis (both sub-acute and acute) and ruminal fluid lactate concentrations to detect acute ruminal acidosis.

A number of studies have investigated changes in ruminal pH, SCFA, and lactate concentrations that occur in beef cattle subjected to an induced bout of ruminal acidosis (Hibbard et al., 1995; Goad et al., 1998; Brown et al., 2000). However, none of those studies have examined the duration (min) and area (min × pH) of ruminal pH depression in beef cattle following feed restriction and a grain challenge using a continuous indwelling ruminal pH measurement system (Dado and Allen, 1993; Penner et al., 2006; Penner et al., 2009c). Continuous measurement of pH has been widely used in dairy cattle (Krause and Oetzel, 2005; Dohme et al., 2008; Khafipour et al., 2009) and has facilitated the growing knowledge gap between the characterization of ruminal acidosis in dairy cattle (Nocek, 1997; Krause and Oetzel, 2006; Kleen and Cannizzo, 2012) and beef cattle (Huber, 1976; Owens et al., 1998; Nagaraja and Titgemeyer, 2007). As the dietary inclusion rates of cereal grains are much higher in finishing diets for beef cattle relative to diets fed to lactating dairy cattle, it is important that comprehensive measurement approaches are used to improve our understanding of ruminal acidosis in general, and to improve our knowledge regarding the severity and prevalence of ruminal acidosis in beef cattle.

Our challenge model was successful in decreasing mean and minimum ruminal pH, and increasing both the duration and area that pH was < 5.5. However, the extent of ruminal pH depression and lactate accumulation was more severe than most previous studies implementing a grain challenge to induce ruminal acidosis in dairy (Krause and Oetzel, 2005; Dohme et al., 2008; Khafipour et al., 2009b) and beef (Burrin and Britton, 1986; Goad et al., 1998) cattle. In fact, the challenge in the current study was more in line with a challenge model using ruminal glucose infusion (Harmon et al., 1985) or consecutive challenges (Nagaraja et al., 1985; Coe et al., 1999). The severe challenge induced in the current study was likely the result of low ruminal pH for heifers when fed the basal diet. In fact, the mean duration that ruminal pH was < 5.5 was 531 min/d during the baseline period. This prolonged duration of pH depression during BASE was greater than the severity of SARA induced in dairy cattle by Keunen et al. (2002), Obsorne et al. (2004), and Gozho et al. (2007) and was similar in severity to the grain challenge imposed on hay-adapted and grain-adapted steers by Goad et al. (1998), as well as the most severe challenge induced by Dohme et al. (2008) in lactating dairy cattle (9.5 h/d that pH was < 5.5). Therefore, it is clear that the heifers in our study were already coping with a substantial acidotic challenge prior to the induced bout of acidosis.

The severity of ruminal acidosis observed during BASE was caused primarily by the high fermentability of the high-grain diet, and was similar to the severity of acidosis observed in other studies feeding similar high-grain barley-based finishing diets to beef cattle (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011). Because these diets and dietary transitions closely represent feedlot dietary conditions in western Canada, the BASE ruminal pH data suggest that the normal prevalence and severity of acidosis in feedlot cattle may be alarmingly high. This severely depressed pH can greatly increase the risk of an acute bout of ruminal acidosis, as is evident by the severity of our challenge and studies that have investigated multiple challenges as part of an acidosis induction protocol in cattle (Nagaraja et al., 1985; Coe et al., 1999; Dohme et al., 2008). The current findings in combination with past studies underline the importance of continued work with indwelling pH measurement systems and beef cattle fed finishing feedlot diets.

It is evident that we induced a severe, or acute, bout of lactic acidosis. The nearly doubling of the duration that pH was < 5.5 during CHAL relative to that during BASE, and the observed minimum pH of 4.57, are supported by the high ruminal fluid lactate concentrations.

Because the observed minimum pH was below the pKa of SCFA (4.8 to 4.9; Aschenbach et al., 2011), the SCFA would have actually been acting as bases by stabilizing pH to approximately 4.8. However, the observed concomitant increase in lactate concentration (pKa = 3.9; Aschenbach et al., 2011) ensured that pH continued to plummet for hours after maximum SCFA and lactate concentrations were observed. When both SCFA and lactate concentrations peaked at 8 h after the challenge, SCFA concentrations were 8-fold greater than lactate concentrations. Over the next 8 h, a shift in organic acid accumulation, and presumably production, resulted in a decrease in SCFA concentrations while lactate concentration remained elevated. However, the concentration of SCFA in the ruminal fluid remained 6-fold greater than lactate at 16 h after the challenge. While for sub-acute ruminal acidosis, high production rates and dissociation of SCFA is clearly the driving factor for low ruminal pH, the observed response is an example of when ruminal lactate becomes a more effective organic acid at reducing ruminal pH than the more abundant SCFA.

One major challenge with feeding cattle high-grain diets is the variation in the ruminal pH response among cattle, even when fed the same diet (Brown et al., 2000; Bevans et al., 2005; Nagaraja and Titgemeyer, 2007). Leading up to and including the challenge, we observed that SA heifers had greater between-day variability in the duration pH was < 5.5, suggesting that the short duration on the high-grain diet may have increased the between-day variability in ruminal pH buffering. Greater day-to-day variation in ruminal pH for the SA cattle was observed despite a lack of a treatment effect on the between-day rate of change for DMI. Bevans et al. (2005) reported that dramatic differences in the variation in ruminal pH occurred in feedlot cattle during a dietary transition protocol to a high-grain diet. They also demonstrated that accelerating the rate of dietary transition to a high-grain finishing diet increased the variability in the ruminal pH response without affecting daily DMI.

The decreased pH variability for LA than SA heifers observed in our study and that of Bevans et al (2005) for cattle that provided a gradual dietary adaptation suggests that extending the time on feed may improve the ability to resist dramatic changes in ruminal pH that are inherent when consuming a high-grain diet. However, the day-to-day variation in pH does not appear to be linked to similar variation in DMI indicating that ruminal acid production or buffering strategies may differ for cattle exposed to SA and LA. This finding also presents a new

opportunity to assess the between day variability in ruminal pH as an appropriate indicator for cattle experiencing ruminal acidosis.

While numerous studies have investigated ruminal acidosis (Dohme et al., 2008; Khafipour et al., 2009b; Penner et al., 2009a), few have evaluated the recovery following an episode of ruminal acidosis (Gaebel and Martens, 1988; Krehbiel et al., 1995; Zhang et al., 2013). Based on the recovery pattern following the induced bout of acidosis, LA cattle experienced their lowest daily duration that ruminal pH was < 5.5 after approximately 4 d, while it took the SA cattle approximately 6 d to experience their lowest daily duration. The mean daily duration that ruminal pH was < 5.5 for REC1 was 294 min/d, when averaged over both treatment groups; however, it took the LA heifers 3 d to reduce the duration that pH was < 5.5 below 294 min/d, while SA heifers required 5 d. The shorter amount of time required for the LA heifers to reach mean and minimum values for duration of time that pH < 5.5 during the first week of recovery from the induced challenge can be appropriately quantified by their greater linear decrease in pH duration during this time (Figure 3.3). This is the first study, known to the authors, to suggest that time on high-grain feed may decrease the time necessary for beef cattle to recover from an induced bout of acute ruminal acidosis.

Epithelial adaptation to high-grain diets (Thorlacius and Lodge, 1973; Gäbel et al., 1991; Sehested et al., 2000) has been suggested to increase the buffering capacity of the rumen. Thus, increased rates of SCFA absorption with advancing days on the high-grain diet offers one potential explanation for the observed reduced recovery time in LA cattle, and is addressed in our companion study (Chapter 4). In addition, it is also possible that advancing days on high-grain feed may have stabilized the microbial communities (Mackie et al., 1978; Mackie and Gilchrist, 1979; Counotte et al., 1981). For example, the populations of lactate-utilizing bacteria may have increased to help prevent lactate accumulation during the days following CHAL (Goad et al., 1998); however, we did not evaluate whether microbial composition differed among treatments and over time and therefore cannot confirm or refute this mechanism.

It was recently reported by Zhang et al. (2013b) that the severity of feed restriction influences the rate of recovery for DMI and ruminal pH when beef cattle resume ad libitum consumption of a diet containing 40% concentrate. In that study, heifers that were subjected to an imposed feed restriction experienced elevated duration and area that pH < 5.5 when ad libitum feeding resumed. The recovery, with respect to DMI and ruminal pH, from the feed restriction

challenge imposed by Zhang et al. (2013b) was complete by the second wk of ad libitum feeding, and was greatest for those cattle who were subjected to the greatest severity of imposed feed restriction. The challenge induced in the current study was much more severe than that of Zhang et al. (2013b), as was the time necessary for pH to stabilize. Taken together with the results of the current study, it can be hypothesized that the severity of the ruminal acidosis bout may positively correlate to the number of days required for a recovery in ruminal pH, and that cattle provided more time for adaptation to the diet may have a greater ability to recover following a bout of ruminal acidosis.

3.5 Conclusions

Although the severity of acidosis was equivalent across treatments, heifers that spent more time on a high concentrate diet before the bout of imposed ruminal acidosis exhibited decreased between day variation in ruminal pH without corresponding variation in DMI. However, the observed increased stability in ruminal pH leading up to the challenge did not influence the susceptibility to a bout of induced ruminal acidosis. Rather, the benefits of high-grain adaptation were delayed until after the induced challenge such that heifers offered a high concentrate diet for an additional 26 days prior to the induced challenge required less time to recover from the induced bout of acute ruminal acidosis. These results are interpreted to suggest that time on high-grain feed stabilizes ruminal pH both prior to and following a bout of ruminal acidosis, although adaptation time does not affect the risk for ruminal acidosis.

4. DURATION OF TIME FED A HIGH-GRAIN DIET AFFECTS SHORT-CHAIN FATTY ACID AND LACTATE ABSORPTION

4.1 Introduction

Finishing beef cattle are fed high-grain diets to meet the energy requirement for rapid growth (Vasconcelos and Galyean, 2007), but this feeding practice predisposes cattle to ruminal acidosis. Ruminal acidosis has been shown to decrease DMI (Fulton et al., 1979a,b; Brown et al., 2000) and short-chain fatty acid (**SCFA**) absorption (Gaebel et al., 1989; Wilson et al., 2012), and therefore likely limits the productivity of affected animals (Burrin et al., 1988). Improving the understanding of the physiological mechanisms used by cattle to resist ruminal acidosis, and how these mechanisms respond under high-grain feeding systems, may improve the ability to achieve rapid rates of growth while mitigating potential detrimental effects caused by ruminal acidosis.

The ruminal acid load is cleared through neutralization with salivary and ruminal epithelial secreted bicarbonate, absorption of SCFA, and passage of protons out of the rumen (Allen, 1997; Aschenbach et al., 2011). Ruminal SCFA absorption accounts for the greatest proportion of acid removal with every mol of SCFA absorbed equating to approximately 0.5 mol of acid removed (Gäbel et al., 1991, 2002). Gäbel et al. (1991) reported that feeding a greater proportion of concentrate increases SCFA absorption, which should improve ruminal buffering (Penner et al., 2009a). However, dietary adaptation to a high-grain diet requires 4 to 8 wk to fully increase the ruminal papillae surface area (Dirksen et al., 1985; Bannink et al., 2008, 2012). It was therefore hypothesized that increasing the amount of time that beef cattle are fed a high-grain diet would increase SCFA absorption thereby decreasing the susceptibility to, and increasing the rate of recovery from, an induced bout of acidosis.

The objective of this study was to determine if the duration of time that heifers are fed a high-grain diet affects the absorption of SCFA and lactate, saliva production, and the response to an induced bout of ruminal acidosis.

4.2 Materials and methods

This manuscript evaluates whether the duration of time cattle are fed high-grain diets improves the resistance to, and recovery from, an induced bout of ruminal acidosis with a focus on SCFA and lactate absorption, saliva production, and blood metabolites. A companion paper (Chapter 3) reports responses of DMI, ruminal SCFA concentrations, and ruminal pH. The procedures and heifers used in this study were pre-approved by the Animal Care Committee of the Agriculture and Agri-Food Canada Lethbridge Research Centre (Lethbridge, AB, Canada) and the study was conducted according to the guidelines of the Canadian Council on Animal Care (Ottawa, ON, Canada). Unless otherwise stated, all analyses were conducted in triplicate and were repeated if the CV was > 3.0%.

4.2.1 Animals, Diets, and Experimental Design

A detailed description of the heifers, dietary treatments, and experimental design has been reported (Chapter 3). Briefly, heifers (n = 16, BW ± SEM = 261 ± 6.1) were assigned to 1 of 4 blocks and within block, randomly assigned to 1 of 2 treatments designated as long adapted (**LA**) and short adapted (**SA**). Long adapted and SA heifers differed in the number of days that they were fed the finishing diet (34 and 8 d, respectively) prior to the induction of ruminal acidosis. The study design consisted of 4 distinct measurement periods including 8 d for baseline (prior to feed restriction; **BASE**), the day of the acidosis challenge (**CHAL**) and 2 consecutive 8-d recovery periods (**REC1** and **REC2**).

4.2.2 Data and Sample Collection

4.2.2.1 Short-Chain Fatty Acid and Lactate Absorption

Absorption of SCFA and lactate was measured on d 5 of BASE, d 2 of REC1, and d 1 of REC2 using the isolated and washed reticulo-rumen technique (**WRR**), as originally described by Care et al. (1984) but adapted for cattle by Zhang et al. (2013a). The reticulo-rumen was evacuated and washed twice with 5 L of tap water at 39°C, followed by 3 washes using 5 L of pre-heated wash buffer (39°C). The wash buffer contained 105 m*M* NaCl, 25 m*M* $NaHCO_3$, 20 m*M* Na-propionate, and 10 m*M* acetic acid, and was adjusted to pH 6.2. Throughout the

procedure, digesta was stored in an insulated and sealed container. Rumen fluid, wash water, wash buffer, and incubation buffer were all removed from the reticulo-rumen with use of a wet/dry vacuum with care not to allow the vacuum to damage the rumen epithelium. After cleaning the reticulo-rumen, a 75-mL Foley catheter (Bard Canada; Oakville, ON) was inserted into the omasum to block fluid passage out of the reticulo-rumen, and a saliva-collecting device was inserted into the esophagus. The saliva-collecting device had an occluding cuff that was inflated to prevent saliva from entering the reticulo-rumen. This device was then attached to a vacuum pump (UN86KT.45P; KNF Neuberger Inc., Trenton, NJ) to prevent saliva accumulation in the esophagus.

After successful placement and inflation of the occluding devices, the reticulo-rumen was washed a final time with 5 L of wash buffer, and incubated for 50 min with 15 L of incubation buffer (Table 4.1). During incubation, CO_2 was continuously bubbled into the ventral sac to promote mixing, and saliva was collected into a 10-L flask and weighed to measure saliva production during the WRR. A modified ruminal cannula plug was used to prevent spilling of the buffer, and to facilitate gassing and sampling without opening the cannula. A quality control sample was taken before the incubation buffer was poured into the rumen. After infusion, samples of incubation buffer were collected from the ventral sac at 5 and 50 min. During the incubation, the evacuated digesta was weighed and sampled. Sampled digesta was dried at 55°C for 48 h and re-weighed to determine DM content. In addition, BW was estimated for these days by conducting 3 BW measurements separated by 13 d (BASE) and 20 d (REC1 and REC2) and assuming linear growth.

Samples of incubation buffer were used for SCFA, lactate, and Co determination. For SCFA and lactate analysis, 1.5 mL samples were added to 0.3 mL of frozen 25% (wt/vol) metaphosphoric acid and allowed to mix before being stored at -20°C. Analysis using gas chromatography was the same as described for rumen fluid SCFA and lactate analysis in our companion study (Schwaiger et al., submitted). For Co determination, 25 mL samples were stored at 4°C for up to 5 h, treated with 125 µL nitric acid (HNO_3) and stored at -20°C. Cobalt concentration was determined using inductively coupled plasma optical emission spectrometry at emission line 228.631 (Spectro Ciros ICP-OES with Smart Analyzer Vision software; Spectro Analytical Instruments, Kleve, Germany).

Table 4.1. Chemical composition of WRR incubation buffer.

Item	Formulated	Observed[1]
n	42	42
Chemical composition[2], m*M* ± SEM		
Acetate	65	65 ± 0.3
Propionate	35	34 ± 0.2
Butyrate	15	14 ± 0.2
L-lactate[3]	5	5 ± 0.3
Cobalt	2	2 ± 0.0
EDTA	2	ND[4]
Sodium	103	ND
Potassium	40	40 ± 0.2
Chloride	18	ND
Bicarbonate	25	ND
Magnesium	2	2 ± 0.1
Calcium	2	2 ± 0.1
Osmolarity (mOsm/L)	314	ND
pH	6.2	

[1]All data are from quality control samples.
[2]Ingredients used to achieve chemical composition: 30 m*M* Na-acetate, 35 m*M* K-acetate, 35 m*M* Na-propionate, 8 m*M* Na-butyrate, 7 m*M* butyric acid, 5 m*M* L-lactate, 2 m*M* Co-EDTA, 5 m*M* NaCl, 5 m*M* KCl, 25 m*M* $NaHCO_3$, 2 m*M* $MgCl_2$, 2 m*M* $CaCl_2 \times 2H_2O$.
[3]L-lactate was added to buffer and total (L+D) lactate was measured.
[4]ND = No data.

Samples were diluted 20-fold in 1% HNO_3, and Co was determined in duplicate by the intensity of emission at a wavelength of 286 nm. In the same run K, Ca, and Mg were determined for quality control samples only. Cobalt was used as a liquid marker in order to determine changes in volume of the incubation buffer during incubation. To calculate absorption, the concentrations of SCFA and lactate were multiplied by corrected liquid volume at each sampling time. The absolute (mmol/h) and fractional (%/h) disappearance rates from the incubation buffer were calculated from the 45-min incubation (50 min - 5 min). Disappearance of SCFA and lactate was assumed to equate to absorption, therefore positive values indicate net absorption (disappearance) and negative values indicate net secretion (appearance).

4.2.2.2 Blood Sampling and Analyses

Blood was sampled on d 3 of BASE, on the d of the acidosis challenge, and on d 7 of REC1. Starting at the time of feeding (0900 h), samples were collected every 2 h for a total of 7 samples over 12 h. Due to an observed delayed response to the challenge, this was extended to 24 h sampling on the challenge day for blocks 2 to 4. This extended sampling was used to collect one additional blood sample at 24 h during CHAL. Rumen fluid sampling occurred concurrently with blood sampling and is reported elsewhere (section 3.2.2.2).

Blood was collected from the jugular vein via a catheter (V-CATH Peripherally Inserted Central Catheter; NeoMedical Inc., Fremont, CA) fitted with a peel away introducer over the needle (Mila International Inc.; Erlanger, Kentucky). Blood was collected into one 6-mL tube containing 10.8 mg K_2-EDTA (Beton Dickinson; Franklin Lakes, NJ), one 10-mL tube containing 158 USP units Li-heparin (Beton Dickinson; Franklin Lakes, NJ) and one 10-mL tube with no preservative (Beton Dickinson; Franklin Lakes, NJ). The latter was incubated at room temperature for 20 minutes, while K_2-EDTA and Li-heparin tubes were immediately placed on ice. Between sampling, catheters were filled with heparinized (20 IU/ mL) 0.9% saline to prevent clotting.

Tubes containing Li-heparin were centrifuged at 3,000 × g at 4°C for 20 min and 1 mL aliquots of plasma were transferred and stored (-20°C) for osmolality, glucose, and insulin determination. For serum, the tubes lacking preservative were centrifuged at 3,000 × g at 4°C for 20 min and 1-mL aliquots of serum were transferred and stored (-20°C) for β-hydroxybutyrate

(BHBA) and lactate determination. Tubes containing K_2-EDTA were used for packed cell volume (**PCV**). Packed cell volume was determined in duplicate by centrifuging (Autocrit Ultra3; Beton Dickinson; Franklin Lakes, NJ) whole blood contained in Fisherbrand Microhematocrit Capillary Tubes (Waltham, MA) for 6 min.

Plasma osmolality (mOsm/kg) was determined by freezing-point depression in duplicate (Advanced Instruments 3250; Norwood, MA). For quality control, double distilled H_2O and standards (290 and 500 mOsm/kg) were analyzed at the beginning and end of each day. Plasma glucose was determined by enzymatic oxidation of glucose to gluconic acid and the subsequent enzymatic oxidation of o-dianisidine, as described by Penner et al. (2009c). Oxidized o-dianisidine was detected by absorption at 450 nm (SPECTRAmax PLUS384; Molecular Devices Corporation, Sunnyvale, CA). Plasma insulin was determined in duplicate using a commercial kit (Bovine Insulin ELISA; Mercodia AB, Uppsala, Sweden). Serum BHBA was determined by enzymatic oxidation of BHBA to acetoacetate and subsequent reduction of NAD, as described by Penner et al. (2009c). The increase in NADH caused by incubation of 3-hydroxybutyrate dehydrogenase (Roche Applied Science, Laval, QC) was detected by absorption at 340 nm (SPECTRAmax PLUS384; Molecular Devices Corporation, Sunnyvale, CA).

Serum L- and D-lactate were both determined by enzymatic conversion to pyruvate and subsequent reduction of NAD as described by Engel and Jones (1978) and Brandt et al. (1980), respectively, with the following modifications. For both L- and D-lactate determination, serum samples were not deproteinized, and 2 mg/mL NAD (N7004; Sigma-Aldrich, St. Louis, MO) was mixed with alkaline hydrazine-glycine buffer (pH = 9.5). The increase in NADH caused by incubation of L-lactate dehydrogenase (L2500; Sigma-Aldrich, St. Louis, MO) at 37°C for 60 min was detected by absorption at 340 nm (SPECTRAmax PLUS384; Molecular Devices Corporation, Sunnyvale, CA). The standard curve was extended to include 0.4 to 5.0 m*M* L-lactate, and linearity was confirmed in this range. The increase in NADH caused by incubation of D-lactate dehydrogenase (L2011; Sigma-Aldrich, St. Louis, MO) at room temperature for 60 min was detected by absorption at 340 nm (SPECTRAmax PLUS384; Molecular Devices Corporation, Sunnyvale, CA). The standard curve was extended to include 0.2 to 6.0 m*M* D-lactate, and linearity was confirmed in this range.

4.2.3 Statistical Analyses

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC). Block was considered a fixed effect, and was left in the model except when performing analysis of covariance as described below. Significance was declared when $P \leq 0.050$ and tendencies are discussed when $0.050 < P \leq 0.100$. Mean separation was conducted with use of the LSMEAN procedure of SAS, Tukey's post-hoc mean separation test, and the SAS pdmix800 macro (Saxton, 1998). The PROC UNIVARIATE procedure of SAS was used to determine if residual data were normally, identically, and independently distributed (NIID). If necessary, outliers were removed in a stepwise fashion until the normal probability plot indicated that residual data were NIID. Correlation analysis was conducted using the PROC CORR procedure of SAS (version 9.2; SAS Institute Inc., Cary, NC).

The fixed effects of treatment, period, and treatment × period were investigated for SCFA and lactate absorption, saliva production, and blood variables by summarizing the data by cow and period. Only the first 12 h of challenge sampling was used for blood variables (extended sampling on CHAL was ignored). Period was used as a repeated measure for the subject cow × treatment × block. Then, within each sampling period the fixed effects of treatment, time and treatment × time were investigated for blood variables by summarizing the data by cow, period and time. Time was used as a repeated measure for the subject cow × treatment × block.

Correlation analysis was conducted to determine the relationships between the various blood and rumen fluid variables (n = 333), which were collected every 2 h for 12 h during BASE (n = 112) and REC1 (n = 105), with an additional 24 h sample included during CHAL (n = 116). The rumen fluid variables used are described in Chapter 3 and included rumen fluid osmolality and concentrations of lactate, acetate, butyrate, propionate, and total SCFA.

One SA heifer was removed from the study prior to the acidosis induction due to low intake, frothy rumen contents, and keratinized epithelia; however, baseline data from this heifer were used in statistical analysis.

Six WRR were omitted from the data set (of 48 total). Two were due to removal of heifer (SA REC1 and REC2), 2 due to excessive loss of incubation buffer during the procedure (2 LA REC1), and 2 due to observed net appearance of SCFA (SA: 1 BASE, and 1 REC2). All data for

the day of the WRR reflect these removed data points (n = 42). In addition, total digesta DM pool and liquid pool are missing an additional 11 data points (n = 31), as digesta DM was not collected for the first block.

4.3 Results

4.3.1 SCFA Absorption and Saliva Production

There was no effect of treatment on BW, although BW did increase throughout the study ($P < 0.001$; Table 4.2). Total digesta weight, digesta DM, and liquid digesta weight were not affected by treatment or period. There was also no effect of treatment on saliva production when reported as kg/h or when saliva production was corrected for BW (g saliva/(kg BW × h). Long adapted heifers tended to have greater absolute rates of butyrate absorption (94 vs. 79 mmol/h; $P = 0.087$), although absolute absorption rates for total SCFA, acetate, propionate, and lactate did not differ between LA and SA. The fractional rate of total SCFA absorption (37 vs. 32%/h; $P = 0.100$) tended to be higher for LA than SA. In addition, LA heifers had greater fractional absorption rates of propionate (42 vs. 34 %/h; $P = 0.045$) and butyrate (45 vs. 36 %/h; $P = 0.019$).

The absolute (mmol/h) and fractional (%/h) absorption of total SCFA and all individual SCFA (acetate, propionate, and butyrate) increased from REC1 to REC2, with intermediate absorption occurring during BASE ($P \leq 0.050$; Table 4.2). The increase in absolute SCFA absorption ranged from 47 to 51% from REC1 to REC2, while the numerical decrease in absolute SCFA absorption from BASE to REC1 equated to a 20 to 23% reduction.

There were no effects of period for either absolute or fractional lactate absorption ($P > 0.100$). However, a tendency for a treatment × period interaction for absolute lactate absorption ($P = 0.072$; data not shown), and a treatment × period interaction for the fractional rate of lactate absorption ($P = 0.024$) were detected (Figure 4.1). Lactate absorption was greatest for the LA heifers during REC1 (Figure 4.1) when compared to SA but lactate absorption during BASE and REC2 were not different between treatments. The fractional rate of lactate absorption during REC1 for LA was 43%/h and was the only measured time point where lactate absorption differed from 0 (data not shown).

4.3.2 Blood Packed Cell Volume, Insulin, Metabolites, and Osmolality

The duration of time that heifers were fed a high-grain diet did not affect mean or maximum PCV, plasma insulin, plasma glucose, serum BHBA, serum L-lactate and plasma osmolality (Table 4.3). However, LA heifers had a greater mean serum D-lactate concentration than SA heifers during CHAL and all heifers during REC1 (Figure 2, $P = 0.003$).

There was no effect of period on mean or maximum PCV (Table 4.3). Mean ($P = 0.001$) and maximum ($P = 0.014$) plasma insulin concentrations were greater during REC1 than BASE, with intermediate concentrations observed during CHAL. Following a similar pattern to insulin, mean plasma glucose concentration tended to increase during REC1 relative to BASE and CHAL ($P = 0.098$). Mean serum BHBA concentration tended to increase during CHAL ($P = 0.058$). Mean total serum lactate concentration increased from BASE to CHAL (1.56 to 1.83 m*M*), then decreased during REC1 (1.35 m*M*) to a value below that of BASE ($P < 0.001$). Maximum total serum lactate also decreased during REC1 relative to BASE and CHAL ($P < 0.001$), as did mean ($P < 0.001$) and maximum ($P < 0.001$) serum L-lactate concentrations. The mean ($P = 0.004$) and maximum ($P < 0.001$) serum D-lactate concentrations (Table 4.3) were lowest during REC2 compared with BASE and CHAL. Mean (279 to 276 mOsm/kg; $P = 0.010$) and maximum (285 to 280 mOsm/kg; $P = 0.001$) plasma osmolality significantly decreased from CHAL to REC1 with intermediate values for BASE.

Table 4.2. Absorptive capacity, saliva production, BW, and digesta weight as affected by treatment and sampling period. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Treatment			Period2				*P* value[3]		
Item	LA	SA	SEM	BASE	REC1	REC2	SEM	T	P	T × P
BW, kg	367	353	9.4	349^{c}	359^{b}	372^{a}	6.7	0.30	< 0.001	0.72
Digesta, kg										
Total	34.3	33.0	1.92	31.2	35.8	34.1	1.71	0.64	0.13	0.21
DM pool	5.1	4.9	0.45	4.6	5.2	5.3	0.37	0.84	0.26	0.74
Liquid pool	27.6	27.2	2.11	26.5	27.9	27.8	1.6	0.91	0.50	0.89
Saliva production										
Total, kg/h	4.57	4.75	0.298	4.15^{b}	5.14^{a}	4.70^{ab}	0.320	0.67	0.018	0.99
Mass specific[4]	12.4	13.4	0.81	11.8^{b}	14.3^{a}	12.7^{ab}	0.86	0.40	0.019	0.98
Absolute absorption, mmol/h[5]										
Total SCFA	613	544	43.1	595^{ab}	461^{b}	679^{a}	52.6	0.27	0.024	0.53
Acetate	310	284	24.2	310^{ab}	235^{b}	347^{a}	29.4	0.46	0.038	0.56
Propionate	209	181	14.0	199^{ab}	158^{b}	228^{a}	17.1	0.18	0.026	0.53
Butyrate	94	79	6.3	86^{ab}	69^{b}	104^{a}	7.8	0.087	0.012	0.52
Lactate	18	5	12.3	1	18	16	11.0	0.49	0.30	0.072
Fractional absorption, %/h[5]										
Total SCFA	37	32	2.3	35^{ab}	29^{b}	40^{a}	2.8	0.100	0.031	0.66
Acetate	33	29	2.3	32^{ab}	25^{b}	36^{a}	2.9	0.24	0.046	0.68
Propionate	42	34	2.4	38^{ab}	32^{b}	44^{a}	2.9	0.045	0.033	0.67
Butyrate	45	36	2.5	40^{ab}	34^{b}	47^{a}	3.1	0.019	0.022	0.60
Lactate	11	2	14.5	-11	17	12	13.2	0.68	0.35	0.024

[abc]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]BASE = Baseline sampling occurred 5 d prior to challenge. REC1 = Recovery 1 sampling occurred 2 d after challenge. REC2 = Recovery 2 sampling occurred 9 d after challenge. All measurements were made on WRR day, except for BW that was estimated based on linear growth from measurements separated by 13 d (BASE) and 20 d (REC1 and REC2).

[3]T = Treatment, P = Period.

[4]Mass specific saliva production measured in g/(kg BW × h).

[5]Positive values indicate net absorption, while negative values indicate net secretion.

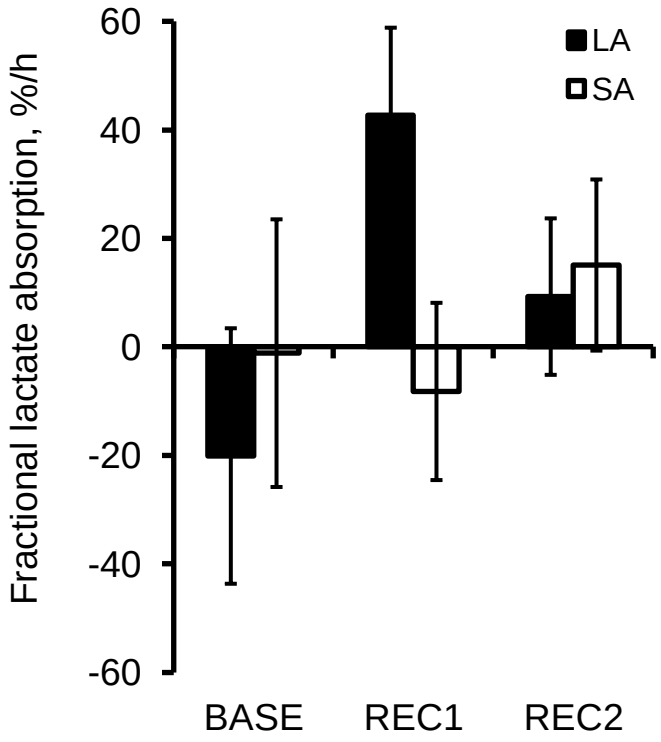

Figure 4.1. Interaction between time on high-grain feed and measurement period on fractional lactate absorption (P = 0.024). Long-adapted (LA) heifers were fed the high-grain diet for 34 d compared to 8 d for the short-adapted (SA) heifers. Baseline (BASE) occurred 7 d prior to the challenge, recovery 1 (REC1) occurred 2 d after the induced challenge, and recovery 2 (REC2) occurred 9 d after the challenge. During the isolated and washed reticulo-rumen technique, L-lactate (5 m*M*) was included in the incubation buffer, and total lactate was determined from samples separated by a 45 min incubation. Hourly fractional lactate absorption was calculated by dividing the positive disappearance of lactate by the initial value, multiplying by 100%, and dividing by (45 min / 60 min). Therefore, positive values indicate net absorption (disappearance) and negative values indicate net secretion (appearance). Mean separation was not achieved using the Tukey post-hoc separation test.

Table 4.3. Daily blood composition as affected by treatment and sampling period. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Treatment			Period[2]				*P* value[3]		
Item	LA	SA	SEM	BASE	CHAL	REC1	SEM	T	P	T × P
PCV, %										
Mean	31	30	0.8	31	31	30	0.6	0.46	0.49	0.97
Maximum	33	32	0.7	32	32	32	0.6	0.50	0.83	0.89
Plasma insulin, μg/L										
Mean	2.6	2.5	0.29	2.0^{b}	2.4^{ab}	3.1^{a}	0.32	0.83	0.001	0.91
Maximum	4.2	4.4	0.51	3.4^{b}	4.7^{ab}	4.7^{a}	0.55	0.87	0.014	0.87
Plasma glucose, mg/dL										
Mean	84.4	84.6	1.36	83.2	82.7	87.5	1.66	0.92	0.098	0.94
Maximum	90.8	91.1	1.38	89.4	89.8	93.6	1.70	0.90	0.17	0.78
Serum BHBA, mg/dL										
Mean	8.2	8.1	0.35	7.8	9.1	7.7	0.43	0.77	0.058	0.45
Maximum	10.2	10.0	0.56	9.4	11.2	9.6	0.69	0.80	0.16	0.36
Total serum lactate, m*M*										
Mean	1.64	1.53	0.069	1.56^{b}	1.83^{a}	1.35^{c}	0.064	0.29	< 0.001	0.26
Maximum	1.81	1.79	0.054	1.85^{a}	2.00^{a}	1.55^{b}	0.066	0.77	< 0.001	0.78
Serum L-lactate, m*M*										
Mean	1.02	0.97	0.037	1.01^{a}	1.10^{a}	0.87^{b}	0.036	0.35	< 0.001	0.105
Maximum	1.20	1.18	0.048	1.21^{a}	1.35^{a}	1.01^{b}	0.048	0.79	< 0.001	0.55
Serum D-Lactate, m*M*										
Mean	0.56	0.50	0.014	0.56^{a}	0.55^{a}	0.49^{b}	0.017	0.003	0.004	0.003
Maximum	0.65	0.64	0.027	0.64^{a}	0.75^{a}	0.54^{b}	0.028	0.82	0.001	0.74
Plasma osmolality, mOsm/kg										
Mean	279	278	0.9	280^{ab}	279^{a}	276^{b}	1.3	0.22	0.010	0.86
Maximum	284	282	1.3	284^{ab}	285^{a}	280^{b}	1.5	0.41	0.001	0.94

^{abc}Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]BASE = baseline sampling occurred 7 d prior to challenge. CHAL = challenge sampling occurred on day of induced challenge. REC1 = recovery sampling occurred 7 d after challenge. Daily mean and maximum values were determined from samples collected at the time of feed and every 2 hours for 12 h after feed.

[3]T = treatment, P = period.

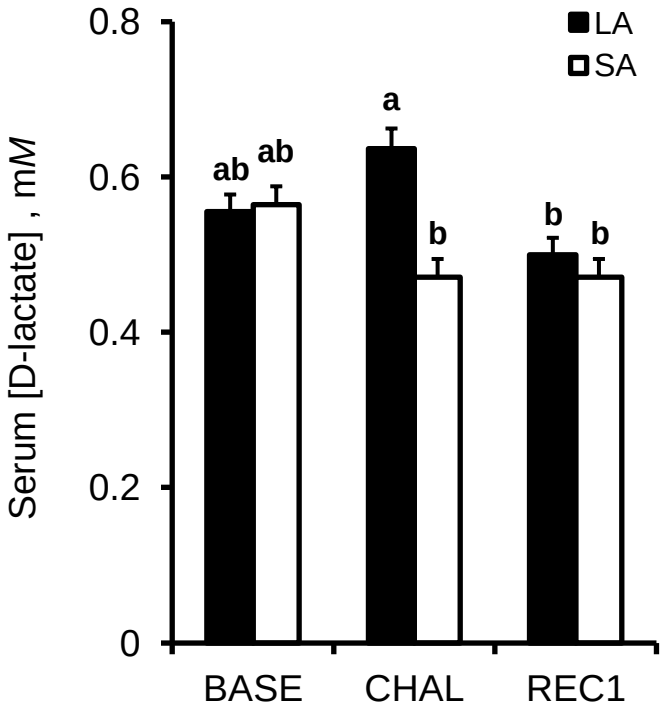

Figure 4.2. The interaction between treatment and period on mean daily serum D-lactate concentrations. Long-adapted (LA) heifers were fed the high-grain diet for 34 d compared to 8 d for the short-adapted (SA) heifers. Baseline sampling (BASE) occurred 7 d prior to the challenge. Challenge sampling (CHAL) occurred on the day of the induced challenge. Recovery sampling (REC1) occurred 7 d after the challenge. Mean daily serum D-lactate concentrations were determined from samples collected at the time of feed and every 2 hours for 12 h after feed. Means with a different letter differ (P = 0.003). The Tukey post-hoc separation test was used for mean separation.

On the day of CHAL (Table 4.4) there was no effect of treatment on PCV, plasma insulin concentration, plasma glucose concentration, serum BHBA concentration, or plasma osmolality. However, LA heifers tended to have greater total serum lactate concentrations (1.85 vs. 1.62 m*M*; $P = 0.054$) than SA heifers (Table 4.4). The LA heifers also had greater serum L-lactate concentrations (1.15 vs. 1.02 m*M*; $P = 0.042$), and tended to have greater serum D-lactate concentrations (0.67 vs. 0.58 m*M*; $P = 0.075$) than SA heifers.

During CHAL, all measured blood variables changed significantly during the day ($P \leq 0.050$; Table 4.5), which was not the case during BASE (Appendix Table 8.3) and REC1 (Appendix Table 8.4). During CHAL, PCV reached a minimal value 4 h after the challenge dose ($P = 0.017$), and maximal plasma insulin concentrations were observed between 6 and 8 h after the challenge dose ($P = 0.003$). Maximal plasma glucose concentrations were observed at the time of the challenge dose ($P = 0.006$). Serum BHBA reached maximal values between 6 and 10 h after challenge dose during CHAL ($P < 0.001$), and total lactate and L- and D-lactate reached maximum concentrations in serum at approximately 8 h after the challenge dose (Table 4.4). Plasma osmolality reached maximal values between 4 and 8 h after the challenge dose.

Table 4.4. Blood composition as affected by treatment over 24 h during the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Treatment			*P* value[2]	
Item	LA	SA	SEM	T	T × t
PCV, %	31	31	0.8	0.84	0.052
Plasma insulin, µg/L	2.6	2.1	0.44	0.44	0.24
Plasma glucose, mg/dL	82.6	83.2	1.64	0.78	0.59
Serum BHBA, mg/dL	9.1	8.4	0.50	0.36	0.19
Serum lactate, m*M*	1.85	1.62	0.078	0.054	0.96
Serum L-lactate, m*M*	1.15	1.02	0.040	0.042	0.98
Serum D-lactate, m*M*	0.67	0.58	0.031	0.075	0.83
Plasma osmolality, mOsm/kg	280	277	1.2	0.15	0.59

[1]Forage:concentrate ratio = 9:91

[2]T = Treatment, t = time after feed. Samples collected immediately before feed (t=0) and every 2 hours for 12 h after feed. Extended sampling occurred on challenge day at 24 h after feed.

Table 4.5. Blood composition as affected by time over 24 h during the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers. The induced challenge occurred 1 d after dietary restriction (50% DMI/BW) and consisted of an intraruminal infusion of ground barley grain (10% DMI/BW), followed by full diet allocation.

	Time relative to feeding[2]									
Item	0 h	2 h	4 h	6 h	8 h	10 h	12 h	24 h	SEM	*P* value
PCV, %	32^{ab}	32^{a}	30^{b}	31^{ab}	31^{ab}	31^{ab}	31^{ab}	33^{ab}	0.7	0.017
Plasma insulin, µg/L	0.7^{d}	1.3^{cd}	1.7^{bcd}	2.9^{a}	3.8^{a}	3.3^{ab}	2.6^{abc}	2.1^{abcd}	0.44	0.003
Plasma glucose, mg/dL	86.0^{a}	80.6^{b}	81.4^{ab}	84.2^{ab}	84.0^{ab}	80.7^{ab}	82.7^{ab}	83.4^{ab}	1.88	0.006
Serum BHBA, mg/dL	5.9^{d}	7.6^{b}	9.2^{ac}	10.1^{a}	10.1^{a}	10.2^{a}	9.3^{ab}	7.3^{bcd}	0.52	< 0.001
Serum lactate, m*M*	1.59^{ab}	1.49^{b}	1.81^{ac}	1.80^{ab}	1.89^{a}	1.96^{ab}	1.90^{ab}	1.43^{b}	0.094	0.010
Serum L-lactate, m*M*	1.03^{ab}	0.96^{b}	1.19^{a}	1.17^{a}	1.21^{a}	1.14^{ab}	1.08^{ab}	0.91^{b}	0.053	< 0.001
Serum D-lactate, m*M*	0.56^{ab}	0.54^{b}	0.63^{ac}	0.64^{ac}	0.69^{a}	0.69^{ab}	0.71^{ab}	0.50^{bc}	0.033	0.035
Plasma Osmolality, mOsm/kg	275^{c}	281^{ab}	283^{a}	282^{a}	281^{a}	278^{abc}	276^{bc}	273^{c}	1.3	< 0.001

[abcd]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (t=0) and every 2 hours for 12 h after feed. Extended sampling occurred on challenge day at 24 h after feed.

4.3.3 Correlation Analysis: Rumen Fluid and Blood Variables

Within BASE there was a negative correlation between plasma insulin and glucose concentration (r = -0.282, $P = 0.003$). However, a positive correlation was evident within both CHAL (r = 0.382, $P < 0.001$), and REC1 (r = 0.344, $P < 0.001$). When the data were pooled across periods, there was an overall positive correlation between plasma insulin and glucose concentration (r = 0.255, $P < 0.001$).

Serum BHBA concentration was positively correlated with rumen fluid butyrate concentration during BASE (r = 0.424, $P < 0.001$), CHAL (r = 0.734, $P < 0.001$), REC1 (r = 0.790, $P < 0.001$), and overall (r = 0.667, $P < 0.001$). The correlation between serum BHBA concentration and rumen fluid propionate concentration was not significant overall ($P > 0.050$) or during BASE ($P > 0.050$), but was positive during CHAL (r = 0.214, $P = 0.021$), and negative during REC1 (r = -0.486, $P < 0.001$).

During CHAL, serum L-lactate (r = 0.244, $P = 0.009$), D-lactate (r = 0.513, $P < 0.001$), and total lactate concentrations (r = 0.534, $P < 0.001$) were all positively correlated with rumen fluid lactate concentrations. In addition, during CHAL serum L-lactate concentrations were positively correlated with both rumen fluid propionate (r = 0.235, $P = 0.011$) and total SCFA concentrations (r = 0.233, $P = 0.012$).

4.4 Discussion

High-grain feeding has been shown to increase both SCFA absorption, and the appearance of bicarbonate in the rumen of sheep, relative to sheep fed high-forage diets (Gäbel et al., 1991). In addition, increased SCFA absorption has been shown to decrease the susceptibility to ruminal acidosis (Penner et al., 2009a), suggesting that epithelial adaptation may impart a protective response to high-grain feeding. Etschmann et al. (2009) reported that the ruminal epithelium can increase absorptive capacity within 1 wk of dietary transition, and that this functional response likely precedes increases in papillae surface area, which can take up to 4 to 8 wk (Dirksen et al., 1985; Bannink et al., 2008). We therefore offered cattle a high-grain diet for either 8 or 34 d and hypothesized that additional time on high-grain feed may increase SCFA absorption and reduce susceptibility to ruminal acidosis in beef cattle. In a companion paper

(Chapter 3), we have shown that increasing the number of days that heifers were fed a high-grain diet decreases their between-day variation in ruminal pH without influencing their susceptibility to an acute bout of ruminal acidosis. Furthermore, the heifers adapted to the high-grain diet for the additional 26 d required less time to recover from the induced bout of acute ruminal acidosis.

In the current study, we found that increasing the number of days that heifers were fed a high-grain diet increases the fractional rates (%/h) of butyrate and propionate absorption, and the absolute absorption rate of butyrate (mmol/h). While these findings are the first to report SCFA absorption as affected by time on a high-grain diet in beef cattle, they are in alignment with the increase in absorptive capacity reported over 14 wk of feeding an energy rich diet to dairy cattle (Dirksen et al., 1985). Despite the increase in the fractional rates of absorption of butyrate and propionate, we observed that the fractional rate of acetate absorption was not affected. We speculate that a partial explanation for the lack of treatment differences for ruminal pH during CHAL reported in Chapter 3 was because increasing the duration of time that heifers were fed the high-grain diet did not affect acetate absorption. The vast majority of SCFA are dissociated at ruminal pH 6.2 (the pH of the experimental buffer) thereby posing a constraint for lipophilic diffusion. Of the ruminal SCFA, acetate is the most abundant (Bergman, 1990), least lipophillic (Walter and Gutknecht, 1986), and, relative to propionate and butyrate, relies on bicarbonate-dependent transport to the greatest extent (Aschenbach et al., 2009, 2011). Thus, lack of treatment effect on acetate absorption would indicate that the ruminal bicarbonate supply provided by bicarbonate-dependent SCFA absorption was similar for SA and LA heifers.

The increase observed for butyrate but not acetate absorption for LA compared to SA heifers suggests that the increase is likely not due to an increase in absorptive surface area alone as we would expect an increase in ruminal surface area to increase the absorption rates for all SCFA (Dirksen et al., 1985). Although we did not attempt to measure papillae density or surface area and therefore cannot confirm this suggestion, results from Sehested et al. (2000) and Andersen et al. (1999) collectively support our suggestion, as dietary inclusion of barley grain was reported to increase ruminal butyrate transport (Sehested et al., 2000) without a concomitant increase in rumen epithelial surface area (Andersen et al., 1999). The increase observed for butyrate absorption in LA compared to SA heifers could be, in part, caused by an increase in butyrate metabolism (Penner et al., 2009c). Increased intraepithelial metabolism of butyrate, whether through increased oxidation or ketogenesis (Krehbiel et al., 1992; Britton and Krehbiel,

1993), increases the concentration gradient thereby promoting lipophilic and facilitative uptake. Because the extent of intraepithelial SCFA metabolism mirrors lipophilic permeability (Britton and Krehbiel, 1993; Kristensen and Harmon, 2004), an increase in SCFA metabolism may help explain the apparent increased fractional absorption rates of butyrate and propionate, but not acetate. High-grain feeding has been shown to increase the activity of propionyl-CoA synthetase in the rumen epithelium (Nocek et al., 1980). In contrast, Harmon et al. (1991) found dietary grain inclusion to decrease the activity of butyryl-CoA synthetase, and others have found no changes in the mRNA expression of acyl-CoA synthetases (Penner et al., 2009b) or enzymes involved in ketogenesis (Penner et al., 2009b; Steele et al., 2011b, 2012a). However, it is also possible that high-grain feeding influences post-transcriptional, or post-translational modifications of ketogenic and/or oxidative enzymes (Gygi et al., 1999; Bondzio et al., 2011). While increased metabolism appears to be a plausible mechanism, we did not detect changes in serum BHBA, a potential indicator of ruminal ketogenesis in a fed state, and did not attempt to quantify the activity of enzymes involved in SCFA metabolism. Therefore, the results of this study would be complimented by further investigation into the effects of advancing days on high-grain feed on epithelial surface area and thickness, blood flow, and the expression and localization of the proteins involved in intraepithelial metabolism.

In addition to the possibility of increased butyrate metabolism with adaptation to high-grain feeding, increased butyrate absorption could also be facilitated by increased blood flow. Blood flow to the rumen epithelium has been shown to increase following a meal (Dobson, 1984), and portal blood flow has been shown to increase with dietary inclusion of concentrates (Huntington et al., 1981), likely in response to increased concentrations of SCFA (Kristensen et al., 2000; Kristensen and Harmon, 2004; Storm et al., 2011). Increased blood flow to and from the rumen epithelium increases the concentration gradients for both lipophillic and facilitated SCFA transport, and has been shown to increase SCFA absorption (Storm et al., 2011). Therefore, increased blood flow in the LA cattle cannot be discounted as a possibility.

In addition to an apparent increase of butyrate and propionate absorption, we have found evidence to suggest that time on high-grain feed may increase lactate absorption during the recovery from an acute bout of ruminal acidosis. Monocarboxylate transporters (MCT) are responsible for the co-transport of lactate and a proton (Müller et al., 2002), and have been localized to both the apical (MCT4; Kirat et al., 2007) and basolateral (MCT1; Müller et al.,

2002) membranes of the ruminal epithelium. However, it has not yet been established whether time on high-grain feed influences the expression or localization of transporters involved in lactate absorption or clearance from the ruminal epithelium in beef cattle. The potential impact that an increase in lactate absorption might have on ruminal pH also merits further investigation. Because the treatment difference in the recovery of ruminal pH during the week after the induced challenge occurred most prominently between d 1 and d 3 after CHAL (Chapter 3), one cannot discount the observed increase in lactate absorption for LA heifers measured on d 2 as a potential contributing factor.

Although we did not observe a strong effect of dietary adaptation to high-grain feed on the absorption of acetate, we did observe a numerical decrease, followed by a remarkable and unanimous increase in absolute absorptive capacity of all SCFA during recovery from the induced bout of acute ruminal acidosis. Gaebel and Martens (1988) also observed a decrease in absorptive function for 2 d when the rumen of sheep was exposed to a buffer containing a pH of 4.8 for 1 h. Our induced challenge was at least this severe, as the average minimum ruminal pH reached 4.57 on the challenge day (Chapter 3). In their study, absorptive function returned after 5 d of recovery (Gaebel and Martens, 1988), which is in line with the time observed in our study for rumen pH to recover to baseline readings after the challenge. Similarly, Krehbiel et al. (1995) found that lambs exposed to an acute bout of ruminal acidosis experienced a decrease in fractional SCFA absorption that persisted for up to 3 mo after the challenge.

The current study is the sole effort to date that shows an apparent reversible decrease in both absolute and fractional rates of all 3 SCFA investigated and total SCFA following an induced bout of acute ruminal acidosis. Although we cannot deduce the mechanism responsible from this study alone, it is likely that decreased SCFA absorption rates in response to an acidosis challenge enables the animal to maintain intraepithelial pH homeostasis. More work is required to elaborate this mechanism in relation to other confounding factors that occur during an induced bout of acute ruminal acidosis (for example, reduced rumen motility; Ash, 1959; Gregory, 1987; Kezar and Church, 1979).

Saliva production increased shortly after the induced challenge, indicating it may also be an adaptive response to acidosis. It should be acknowledged that saliva flow rates measured in this study could be affected by the technique used for collection, although relative differences between treatments and between periods are not expected to be affected by the technique. The

opposite response of SCFA absorption and saliva production suggests that cattle may possess the ability to increase saliva production in order to compensate for a decrease in SCFA absorption. In fact, assuming that the saliva produced was equal in composition between treatments, and contained approximately 126 m*M* of bicarbonate (Bailey and Balch, 1961), the extra 0.99 kg/h of saliva produced during REC1 supplied approximately 125 mmol/h of ruminal bicarbonate, which is remarkably close to the 134 mmol/h decrease in total SCFA absorption. Therefore, the increase in saliva production appears to compensate for the decrease in acid removal (due to decreased lipophilic diffusion of H-SCFA) and neutralization (decreased bicarbonate secretion resulting from the $SCFA^-/HCO_3^-$ exchange) by the ruminal epithelia. These data suggest that heifers have the ability to modulate the ruminal buffering mechanisms when exposed to an induced bout of ruminal acidosis with the relative importance of saliva increasing immediately after the bout of ruminal acidosis.

The observed plasma glucose concentrations for heifers in the current study were in range with values previously reported for beef cattle fed high-grain diets (Arai et al., 2003; Yang et al., 2010; McGilchrist et al., 2011). However, the observed insulin concentrations were high, but in range with those reported by Mir et al. (2000, 2002). The dramatic increase in plasma insulin concentrations across periods, tendency for plasma glucose concentrations to increase during recovery, and overall positive correlation between plasma glucose and insulin during both CHAL and REC1 suggests that the heifers may have become decreasingly responsive to insulin.

The ability of ruminal butyrate concentrations to explain the majority of the variation in circulating BHBA concentrations during both CHAL (54%) and REC1 (63%) suggest an increase in the relative importance of ketogenesis during an acute bout of ruminal acidosis, and after a week of recovery. However, the loss of the negative correlation between plasma glucose and serum BHBA concentrations during CHAL suggests that the normal antagonistic relationship between intraepithelial SCFA metabolism and glucose utilization (Beck, 1984; Weise, B.I., P. Gorka, T. Mutsvangwa, E. Okine, and G.B. Penner, unpublished) is compromised during a severe bout of ruminal acidosis. The apparent disregulation between epithelial ketogenesis and glycolysis may have been driven by the apparent decrease in absorptive capacity caused by the acute bout of ruminal acidosis. However, circulating concentrations of metabolites and hormones can merely inspire a suspicion of metabolite disregulation. A comprehensive study utilizing the multi-catheterized model (Huntington et al., 1989; Kristensen, 2005) is required to

further investigate the relationships between plasma insulin, plasma glucose, and ketogenesis during and following an acute bout of ruminal acidosis.

4.5 Conclusions

Heifers that were fed a high-grain diet for 34 d had greater rates of SCFA absorption than those that were fed the same diet for only 8 d. Moreover, increased time of feeding the high-grain diet appeared to enhance lactate absorption shortly after an acute bout of ruminal acidosis. Regardless of treatment, the acute bout of ruminal acidosis appeared to temporarily decrease the absorption of acetate, propionate, and butyrate, and increase saliva production. The inverse response of saliva production and SCFA absorption following an acute bout of ruminal acidosis suggests that beef cattle may be able to increase saliva production in order to compensate for decreased absorptive capacity resulting from a bout of acidosis.

5. GENERAL DISCUSSION

The paucity of research investigating ruminal acidosis, epithelial adaptation, SCFA absorption, and saliva production in beef cattle was stated in the literature review (Chapter 2), and many important implications of the findings of this research were discussed in Chapters 3 and 4. Thus, the following section will elaborate on the importance of continued work towards investigating the factors that influence the severity and prevalence of, susceptibility to, and recovery from ruminal acidosis in feelot beef cattle. This will be followed by a suggestion of a few future directions that may improve our knowledge of ruminal buffering strategies.

5.1 Determining the Severity of Ruminal Acidosis

The severity of sub-acute ruminal acidosis (SARA) is typically determined by the extent of ruminal pH depression, without a concominant increase in ruminal lactate concentration (Kleen et al., 2003; Plaizier et al., 2008; Aschenbach et al., 2011). Several studies, including the current study, that characterize the severity of ruminal acidosis in beef cattle fed barley based finishing diets suggest that SARA may be severe and highly variable in North American feedlots (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011). However, due to the constraints surrounding the measurement, detection, and classification of ruminal acidosis (section 1.2) there have not yet been estimates made of the prevalence or severity of ruminal acidosis in feedlots. Therefore, future research is warranted to assess both the prevalence and severity of ruminal acidosis among beef cattle on finishing diets in feedlots.

In addition to low ruminal pH and increased ruminal lactate concentrations, elevated concentrations of lactate in the blood have previously been used as an indicator for acute ruminal acidosis (Harmon et al., 1985; Krehbiel et al., 1995; Nagaraja and Titgemeyer, 2007). In the current study, we found that time on high-grain feed increased circulating concentrations of lactate during an acute bout of ruminal acidosis. However, the equal concentrations of ruminal SCFA and lactate, as well as equal ruminal pH and osmolality observed between treatments suggests that circulating lactate concentrations should not be used alone to quantify the severity of acute ruminal acidosis. Rather, as this study has suggested, elevated circulating lactate may indicate increased absorptive capacity for ruminal lactate or increased lactate production and release by gastrointestinal tissues. Although sub-acute ruminal acidosis is undoubetly much more

prevalent in both dairy (Stone, 2004) and beef cattle (Nagaraja and Titgemeyer, 2007) than acute ruminal acidosis, the results of this study suggest that the risk for acute ruminal acidosis in beef cattle fed a finishing feedlot diet is high. Thus, the determination of ruminal lactate concentrations may be warranted in future studies that examine the severity and prevalence of ruminal acidosis in feedlot beef cattle.

5.2 Determining the Prevalence of Ruminal Acidosis

Although the severity and prevalence of ruminal acidosis has not yet been determined in feedlot beef cattle, numerous studies have investigated the prevalence of ruminal acidosis in dairy herds. Kleen and Canizzo (2012) define prevalence of ruminal acidosis as the number of cows at any given time that are experiencing ruminal acidosis, and incidence of ruminal acidosis as the number of cows that experience ruminal acidosis in any given day. Garrett et al. (1999) found a pH threshold of 5.5 to maximize the probability that sampled dairy cattle were representative of the whole herd (compared to the pH thresholds 5.4, 5.6, and 5.7), when sampled using rumenocentesis 3 to 4 h after feed was offered (20 to 25% NDF on a DM basis, offered as TMR). These authors went on to suggest that a detection of ruminal pH < 5.5 in at least 3 of 12 sampled dairy cattle (25%) is the most efficient method of detecting sub-acute ruminal acidosis (SARA) in over 30% of the cattle within any given herd, whereas if 2 or fewer of the 12 cattle are observed to have ruminal pH < 5.5, then it is likely that the prevalence rate for that herd is 15% or less (Garrett et al., 1999).

Studies have since used these criteria to determine the prevalence of SARA in Italian (Morgante et al., 2007), Irish (O'Grady et al., 2008), Dutch (Kleen et al., 2009), and Iranian (Tajik et al., 2009) dairy herds. Morgante et al. (2007) found 3 of 10 Italian dairy herds to have prevalence rates > 33%, when sampling 4 to 8 h after feeding (30 to 40% NDF on a DM basis, offered as TMR). Similarly, O'Grady et al. (2008) found 3 of 12 Irish dairy herds to have prevalence rates of at least 25%, with an overall prevalence rate of 11% across the 12 herds, when sampling 6 h after pasture feeding. Kleen et al. (2009) surveyed 18 Dutch dairy herds and found an overall prevalence rate of 14%, when sampling 3 h after feed (dietary information inadequate). Of those 18 herds, 11 were sampled according to Garrett et al. (1999), of which 2 were found to have prevalence rates greater than 25% (Kleen et al., 2009). Tajik et al. (2009)

found a much greater overall prevalence of SARA of 28% across 10 Iranian dairy herds, when sampling between 4 to 6 h after TMR feeding (inadequate dietary information). Of these 10 herds, 7 of them were found to have prevalence rates of at least 25% (Tajik et al., 2009).

In light of the large variability in ruminal pH (Nocek et al., 2002; Bevans et al., 2005; Aschenbach et al., 2011) and the emergence of continuous pH measuring systems that can effectively capture this variability (Dado and Allen, 1993; Penner et al., 2006; Penner et al., 2009b), future studies should use continuous pH measurements to determine the daily prevalence (or incidence as described by Kleen and Canizzo, 2012) for feedlot beef cattle. Based on the suggested diagnosis of SARA of Gozho et al. (2005) for dairy cattle, whereby ruminal pH must remain below 5.6 for at least 3 h/d, and the support for the use of a pH threshold of 5.5 (Fulton et al., 1979a; Gaebel et al., 1989; Garrett et al., 1999), a duration of at least 3 h/d below pH 5.5 could be used as a valid threshold to detect the upper limit of ruminal acidosis in beef cattle.

Applying this threshold to the current study reveals a prevalence rate of SARA of 78% among the 112 daily observations recorded during BASE (7 d period with 16 heifers). The calculation of a weekly prevalence rate for each heifer during BASE increases the prevalence to 88%, as only 2 of the 16 heifers experienced an average daily duration of less than 3 h/d below pH 5.5. In addition, we found that the heifers fed our high-grain diet experienced moderate to severe SARA during this period, as ruminal pH was below 5.5 for an average of over 8 h/d. Our results during BASE coupled with other studies conducted with beef cattle (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011) suggest that ruminal acidosis may be a much more alarming problem for beef cattle in North American feedlots than for the dairy cattle from which most studies investigating ruminal acidosis have been conducted (Dirksen et al., 1985; Bannink et al., 2008; Kleen and Cannizzo, 2012). Therefore, studies investigating the severity and prevalence of ruminal acidosis in feedlot beef cattle are required to make the scientific community, industry, and the public aware of the extent of this problem.

5.3 Determining the Susceptibility to Ruminal Acidosis

In order to determine susceptibility to ruminal acidosis, a controlled bout of ruminal acidosis must be induced (Nagaraja and Titgemeyer, 2007; Dohme et al., 2008; Khafipour et al., 2009b). Although some may believe that a controlled bout of ruminal acidosis may generate a

bout of ruminal acidosis that is more severe than observed in the feedlot, the severity of acidosis observed during BASE and in other studies that have utilized similar barley-based finishing diets (Bevans et al., 2005; Wierenga et al., 2010; Moya et al., 2011) are on par with the severity of acidosis observed after an induced bout of acidosis in beef cattle fed a corn-based finishing diet (Goad et al., 1998). The continued use of an acidosis challenge model will allow us to increase our knowledge of the factors that influence susceptibility to ruminal acidosis, so that we can attempt to mitigate the elevated risk for ruminal acidosis in beef cattle. In addition, very few studies have examined the susceptibility to multiple consecutive induced bouts of ruminal acidosis (Nagaraja et al., 1985; Coe et al., 1999; Dohme et al., 2008). As ruminal acidosis is often a recurring digestive disorder (Owens et al., 1998; Kleen et al., 2003), future research inducing multiple bouts of ruminal acidosis in both beef and dairy cattle is warranted.

Ruminal acidosis can be induced by promoting ruminal fermentation using an abrupt dietary transition to a diet containing a greater proportion of dietary concentrates (Keunen et al., 2002; Gozho et al., 2005; Khafipour et al., 2009b), by imposing a dietary restriction followed by either ad libitium feeding of the baseline ration (Albornoz et al., 2013; Zhang et al., 2013b), an intraruminal dose of a challenge ration (Brown et al., 2000), ad libitium feeding of a challenge ration (Krause and Oetzel, 2005) and baseline ration (Dohme et al., 2008), or an intraruminal dose of a challenge ration, followed by ad libitium feeding of the baseline ration. Alternatively, ruminal fermentation can be promoted by dosing the rumen with a glucose solution (Harmon et al., 1985). An acidosis challenge that doses the rumen directly with rapidly fermentable carbohydrates will effectively control for behavioural responses that may minimize the severity of acidosis (Fulton et al., 1979a; Domhe et al., 2008).

In the current study, the option of a short-term moderate feed restriction (1 d at 50% of DMI) followed by an intraruminal challenge and provision of the normal diet was applied. This approach in inducing ruminal acidosis is practical, as it simulates the involuntary feed restriction that has been shown to occur during transportation (Cole et al., 1988; Loerch and Fluharty, 1999), and voluntary feed restriction upon arrival to the feedlot (Hutcheson and Cole, 1986; Fluharty et al., 1994). The intraruminal dose of barley grain provides a controlled simulation of the overfeeding that can occur during realimentation from a mild feed restriction (Drouillard et al., 1991). In addition, heat stress can also cause voluntary feed restriction (Albright and Alliston, 1971; Knapp and Grummer, 1991; Holter et al., 1996) that may increase the risk for

overfeeding and subsequently, ruminal acidosis. However, heat stress does not only increase the risk for ruminal acidosis through feed restriction, as hyperventilation results is a loss of circulating CO_2, which is compensated for by increased urinary secretion of circulating HCO_3^- (Kadzere et al., 2002), effectively decreasing the most important source of rumen HCO_3^- (Aschenbach et al., 2009).

Based on the results of the current study, the challenge model used was successful in inducing, on average, an acute bout of ruminal acidosis. Zhang et al. (2013a,b) recently suggested that the severity of the imposed dietary restriction can influence the severity of the response to the challenge. In that study, imposed feed restriction tended to decrease SCFA absoption, regardless of the severity of feed restriction (75%, 50%, 25%; Zhang et al., 2013a). However, the most severe feed restriction (25%) was found to delay the recovery in SCFA absorption and DMI when ad libitium feeding resumed (Zhang et al., 2013b). Although the induced bout of acidosis was much more severe in the current study (Chapter 3), both studies observed a reduction in SCFA absorption either during (Zhang et al., 2013b) or after (Chapter 4) the imposed feed restriction. In addition, the extent of the week-long recovery of SCFA absorption was remarkably similar between the 50% feed restriction employed by Zhang et al. (2013b) and the challenge induced in the current study (Chapter 4). Taken together, these findings suggest that the severity of feed restriction is responsible for the recovery of SCFA absorption, and is therefore likely responsible for the recovery of ruminal pH following a bout of ruminal acidosis. However, because we induced an acute bout of ruminal acidosis, the increased absorption of lactate during the recovery period can not be discounted as a potential factor influencing recovery.

5.4 Determining the Pattern of Recovery from Ruminal Acidosis

Although many studies have investigated the susceptibility to ruminal acidosis using an acidosis induction model in dairy cattle (Keunen et al., 2002; Krause and Oetzel, 2005; Dohme et al., 2008), very few studies have examined the daily pattern of recovery with continuously monitored ruminal pH following an induced bout of ruminal acidosis (Albornoz et al., 2013; Zhang et al., 2013b). Recently, Albornoz et al. (2013) suggested that dietary inclusion of concentrates offered prior and during a 5 d feed restriction decreased the susceptibility to SARA

when ad libitium feeding resumed, despite a lack of an effect on SCFA absorption. The current study found that the time necessary to recover from an induced bout of acute ruminal acidosis is decreased for cattle that spent an additional 26 d on high-grain feed prior to the acidotic insult, despite the equal susceptibility to ruminal acidosis observed between the treatments. Taken together, these studies suggest that the inclusion of dietary concentrates prior to an induced bout of ruminal acidosis may decrease the susceptibility to a bout of SARA (Albornoz et al., 2013), and feeding high-grain diets for a greater duration may reduce the time necessary to recover from an acute bout of ruminal acidosis (Chapter 3). Because the current study found that time on high-grain feed appeared to increase both SCFA absorption (across all periods), and lactate absorption during REC1, it is not yet clear which mechanism of organic acid removal played a larger role in the improved recovery response. Therefore, future work must be conducted in order to determine the relative importance of the observed elevated rates of SCFA absorption and lactate absorption in improving the recovery time from an induced bout of acute ruminal acidosis.

Due to high fermentability of the diets that are fed to feedlot beef cattle (Vasconcelos and Gaylean, 2007), ruminal acidosis cannot be avoided altogether. In fact, as previously discussed, ruminal acidosis caused by feed restriction (Hutcheson and Cole, 1986; Loerch and Fluharty, 1999) or heat stress (Kadzere et al., 2002) can often be predicted. Therefore, continued investigation into the factors that improve the pattern of recovery from a bout of ruminal acidosis may arguably be just as important as the factors that influence susceptibility to ruminal acidosis. The results of the current study suggest that epithelial adaptation to high-grain feeding may influence the recovery response. Therefore, future research dedicated to investigating the adaptive response in beef cattle fed high-grain diets, should also consider the influence on recovery from a bout of ruminal acidosis.

5.5 Future Directions for Investigating Ruminal Buffering Strategies

There is a paucity of data describing the absorptive physiology and saliva production in beef cattle. For example, studies showing that inclusion of dietary concentrates can result in increased rates of ruminal SCFA absorption have primarily been conducted in dairy cattle (Thorlacius and Lodge, 1973; Dirksen et al., 1985; Sehested et al., 2000) and sheep (Gäbel et al., 1991). While the current study has shown that time on high-grain feed appears to increase SCFA

absorption in beef cattle, only one study to date has shown that increasing the level of dietary concentrates increases SCFA absorption in beef cattle (Amat et al., 2012). In addition, the vast majority of recent research investigating saliva production has been conducted in dairy cattle (Krause et al., 2002; Maekawa et al., 2002; Beauchemin et al., 2003). Although it is generally assumed that the physiological mechanisms governing ruminal acid removal are the same for sheep, dairy cattle, and beef cattle, the gap in our knowledge for beef cattle fed low-forage diets represents an opportunity for researchers to validate this assumption. The use of multi-catheterization (Huntington et al., 1989; Kristensen, 2005) would allow for researchers to determine daily rates of SCFA and lactate absorption, in order to improve the resolution of the recovery response in absorptive capacity following a bout of acute ruminal acidosis observed in the current study.

The physiological plasticity displayed by the cattle in the current study, by increasing saliva production when SCFA absorption was compromised, raises many interesting questions that warrant future research. For example, do cattle fed an all forage diet respond in a similar manner when absorptive capacity is compromised, and what signalling mechanisms are responsible for relaying information regarding the cessation of SCFA absorption at the rumen epithelia to the salivary glands? However, there is a need to develop more precise methodologies in order to better estimate or directly measure total daily saliva production (Erdman, 1988; Allen, 1997). In particular, a focus on developing technologies to improve our ability to measure, rather than estimate, total daily saliva production and composition would provide invaluable information regarding the adaptation of saliva production with advancing days on high-grain feed, and in response to a bout of ruminal acidosis.

6. CONCLUDING REMARKS

This study demonstrated that advancing time on high-grain feed results in an increase in both SCFA and lactate absorption, which does not decrease the susceptibility to ruminal acidosis, but may reduce the time necessary to recover from an acute bout of ruminal acidosis. In addition, this study suggested that beef cattle may possess the ability to compensate for decreased SCFA absorption shortly after an acute bout of ruminal acidosis by increasing saliva production. Further investigation into the mechanisms governing these observations would help us better understand the factors that influence the risk for ruminal acidosis. With an improved understanding of the risk for and response to ruminal acidosis, we may be able to manipulate the natural microbiological and physiological mechanisms that regulate ruminal pH in order to decrease the prevalence and severity of ruminal acidosis in beef cattle, and therefore improve animal welfare and productivity.

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8. DATA APPENDIX

8.1 Effect of Time on Ruminal Fermentation during BASE and REC1

Similarly to CHAL (Table 3.10), during BASE (Table 8.1) and REC1 (Table 8.2), mean pH reached a minimum ($P < 0.001$) 12 h after feed. The duration ($P < 0.001$) and area ($P = 0.011$) that ruminal pH was < 5.5 reached a maximum at 12 h during BASE. However, during REC1 there was no effect of time on the duration or area pH < 5.5 ($P > 0.05$). Also similar to CHAL (Table 3.10), ruminal acetate ($P < 0.001$), propionate ($P < 0.001$), butyrate ($P < 0.001$), and total SCFA ($P < 0.001$) concentrations, as well as ruminal osmolality ($P < 0.001$), all peaked between 6 and 12 h after feed during BASE (Table 8.1). However, during REC1 (Table 8.2) ruminal acetate ($P < 0.001$), propionate ($P < 0.001$), butyrate ($P < 0.001$), total SCFA ($P < 0.001$) and osmolality ($P < 0.001$) all reached maximal values later, at 12 h after feeding. Despite drastically different severities of ruminal acidosis (CHAL>BASE>REC1; Table 3.4) the similar daily patterns in ruminal fermentation observed between the three sampling periods suggests that the challenge induction model employed during CHAL induces an increase in ruminal fermentation that follows a similar (although much more severe) pattern over time. Interestingly, during BASE (Table 8.1) the LA heifers tended to have greater ruminal concentrations of propionate ($P < 0.001$) and total SCFA ($P < 0.001$), and exhibited greater ruminal osmolality ($P < 0.001$). Because LA heifers had elevated rates of fractional propionate absorption (Table 4.2), this may suggest that they also experienced increased ruminal fermentation during BASE. However, there were no effects of treatment on ruminal pH (Table 8.1).

Table 8.1. Rumen fluid pH, short-chain fatty acids (SCFA), lactate, and osmolality as affected by treatment and time during the baseline period (BASE). Long-adapted (LA) heifers were fed the high-grain diet1 for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			Time After Feed (h)[2]								*P* value[3]		
Item	LA	SA	SEM	0	2	4	6	8	10	12	SEM	T	t	T × t
Mean pH	5.71	5.76	0.096	6.21^{a}	6.18^{a}	5.90^{b}	5.58^{c}	5.53^{cd}	5.45^{cd}	5.29^{d}	0.089	0.73	< 0.001	0.14
pH < 5.5[4]														
Duration	49	43	13.2	10^{ef}	15^{def}	22^{cf}	56^{abde}	65^{abc}	63^{bcd}	91^{a}	11.6	0.77	0.011	0.35
Area	16	13	3.5	3^{b}	4^{b}	4^{b}	13^{ab}	22^{ab}	21^{ab}	35^{a}	4.1	0.56	0.004	0.47
Total SCFA[4]	133.5	122.1	4.12	94.7^{c}	109.1^{bc}	125.8^{ab}	139.9^{a}	144.8^{a}	133.7^{a}	146.8^{a}	5.44	0.065	< 0.001	0.59
Acetate	63.7	60.8	1.58	47.6^{c}	56.5^{b}	62.5^{ab}	68.0^{a}	68.9^{a}	63.4^{ab}	68.9^{a}	2.31	0.23	< 0.001	0.70
Propionate	54.1	46.6	2.97	35.2^{c}	39.7^{c}	48.1^{b}	55.5^{ab}	58.6^{a}	54.6^{ab}	60.9^{a}	3.08	0.093	< 0.001	0.52
Butyrate	15.8	14.7	1.89	11.9^{ab}	13.0^{ab}	15.2^{b}	16.5^{ab}	17.4^{a}	15.8^{ab}	17.1^{ab}	1.61	0.71	0.015	0.53
Osmolality[4]	380	358	6.6	321^{c}	350^{b}	369^{ab}	384^{a}	390^{a}	376^{ab}	391^{a}	7.7	0.038	<0.001	0.42

^{abcdef}Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed. To correspond with other variables reported, pH variables were calculated over 2 h prior to each sample. For example, t=0 corresponds to pH measured between 07:01 and 09:00.

[3]T = Treatment, t = time after feed.

[4]Duration pH < 5.5 measured in min/120 min, and area pH < 5.5 measured in (min × pH)/120 min. Total and individual SCFA, as well as lactate, measured in m*M*. Osmolality measured in mOsm/kg.

Table 8.2. Rumen fluid pH, short-chain fatty acids (SCFA), lactate, and osmolality as affected by treatment and time during the recovery period (REC 1). Long-adapted (LA) heifers were fed the high-grain diet1 for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			Time After Feed (h)[2]								*P* value[3]		
Item	LA	SA	SEM	0	2	4	6	8	10	12	SEM	T	t	T × t
Mean pH	6.03	5.94	0.071	6.27^{a}	6.15^{ab}	6.03^{bc}	5.90^{cd}	5.93^{bcd}	5.88^{cd}	5.72^{d}	0.07	0.39	< 0.001	0.38
pH < 5.5[4]														
Duration	11	11	5.9	0	3	8	14	10	16	28	5.6	0.95	0.39	0.29
Area	2	1	1.2	0	0	1	3	1	2	5	1.1	0.97	0.60	0.19
Total SCFA[4]	120.8	124.6	3.14	104.2^{c}	117.7^{b}	126.0^{ab}	130.5^{ab}	122.6^{b}	120.6^{b}	137.2^{a}	3.80	0.41	< 0.001	0.63
Acetate	64.0	63.0	1.30	54.7^{c}	62.7^{ab}	65.5^{ab}	67.3^{ab}	62.6^{ab}	62.2^{bc}	69.3^{a}	1.77	0.58	< 0.001	0.65
Propionate	42.4	45.9	5.16	37.5^{d}	41.2^{bde}	45.7^{ac}	47.0^{abc}	44.8^{abcd}	42.9^{cde}	49.9^{ab}	3.95	0.64	< 0.001	0.35
Butyrate	14.4	15.9	2.21	12.0^{bd}	13.9^{abc}	14.9^{abc}	16.5^{ac}	15.4^{abc}	15.6^{cd}	18.0^{ab}	1.77	0.64	< 0.001	0.97
Osmolality[4]	368	372	7.3	344^{b}	368^{ab}	373^{ab}	383^{a}	367^{ab}	369^{ab}	387^{a}	8.4	0.73	0.002	0.68

^{abcde}Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed. To correspond with other variables reported, pH variables were calculated over 2 h prior to each sample. For example, t=0 corresponds to pH measured between 07:01 and 09:00.

[3]T = Treatment, t = time after feed.

[4]Duration pH < 5.5 measured in min/120 min, and area pH < 5.5 measured in (min × pH)/120 min. Total and individual SCFA, as well as lactate, measured in m*M*. Osmolality measured in mOsm/kg.

8.2 Effect of Treatment on the Dissappearance of Ruminal Propionate and Appearance of Serum BHBA During CHAL

An analysis of covariance for serum BHBA concentration with respect to time indicated that the LA heifers had a greater cubic appearance of serum BHBA during CHAL (Figure 8.1B, $P < 0.001$). The increased appearance of serum BHBA for LA heifers mirrors their greater quadratic disappearance of rumen fluid propionate on the challenge day (Figure 8.1A, $P < 0.001$). Similarly, LA heifers also exhibited a numerically greater quadratic rate of rumen fluid butyrate disappearance on the challenge day ($P = 0.12$, not shown).

We did not measure SCFA absorption on the challenge day. However, the increased rate of disappearance of rumen fluid propionate and increased rate of serum BHBA appearance for LA cattle suggests that LA cattle may have had greater ratea of SCFA absorption and metabolism on the challenge day. It has been shown that both butyrate and propionate undergo first-pass metabolism by the rumen epithelia (Kristensen et al., 2000; Kristensen and Harmon, 2004) and are in part converted into BHBA and lactate, respectively (Krehbiel et al., 1992; Weigand et al., 1975; Kristensen et al., 2005). Therefore, increased rates of disappearance of ruminal butyrate and propionate in addition to increased appearance of serum BHBA and lactate (Table 4.4) may indicate increased rates of intraepithelial SCFA metabolism. However, this hypothesis is speculative, as a change in ruminal fluid SCFA conentration can also be attributable to a change in fermentation, and metabolite concentrations can change for a variety of reasons.

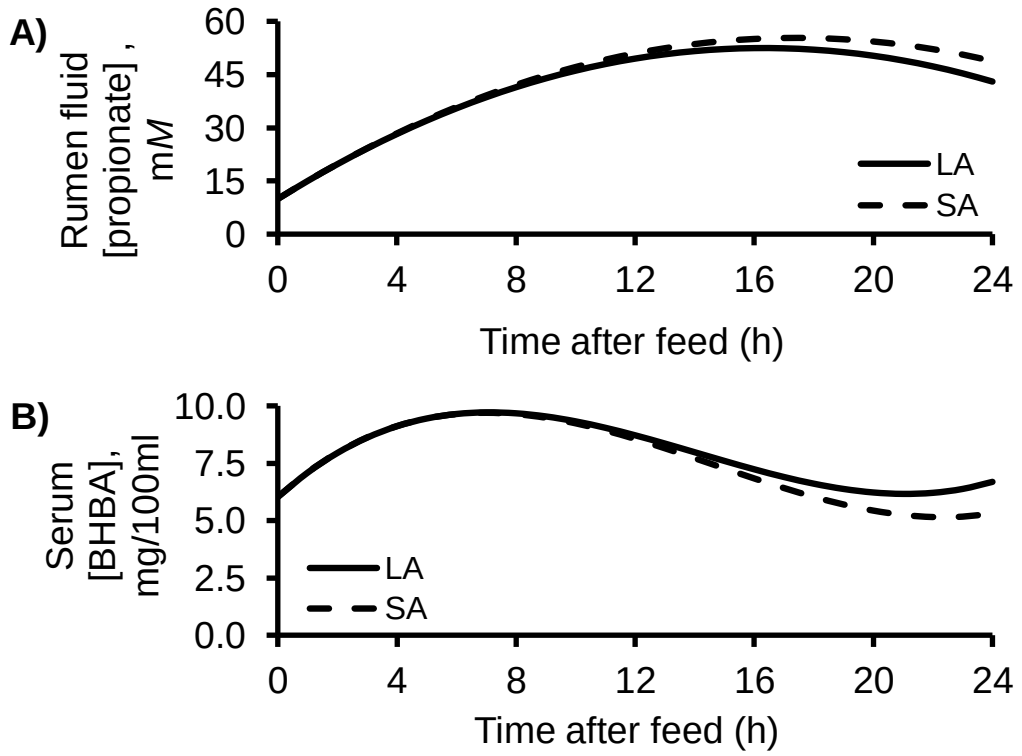

Figure 8.1. Change in rumen fluid propionate and serum β-hydroxybutyrate (BHBA) concentrations throughout the day of the induced challenge. Long-adapted (LA) heifers were fed the high-grain diet for 34 d compared to 8 d for the short-adapted (SA) heifers. Samples were collected immediately before feed and every 2 hours for 12 h after feed. Extended rumen fluid sampling occurred at 16, 20, and 24 h after feed, and extended blood sampling occurred at 24 h after feed. Trendlines were constructed using analysis of covariance. Rumen fluid propionate (A) showed a significant linear ($P < 0.001$) effect over time as well as a significant quadratic ($P < 0.001$) treatment effect. The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y = 9.99 + 5.22\ t - 0.16\ t^2$ (LA), and $y = 9.99 + 5.22\ t - 0.15\ t^2$ (SA). Serum BHBA (B) showed a significant linear ($P < 0.001$) and quadratic ($P < 0.001$) effect over time, as well as a significant cubic ($P < 0.001$) treatment effect. The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y= 6.04 + 1.17\ t - 0.11\ t^2 + 0.0026\ t^3$ (LA), and $y=6.04 + 1.17t - 0.11\ t^2 + 0.0025\ t^3$ (SA).

8.3 Effect of Time on Blood Composition During BASE and REC1

The extent of the severity of the daily fluctuation in blood composition appears to be positively influenced by the severity of ruminal acidosis (CHAL>BASE>REC1; Table 3.4). During CHAL, all 8 blood variables changed significantly during the day ($P \leq 0.050$; Table 4.5), while 7 of the 8 variables changed during BASE ($P \leq 0.050$; Table 8.3) and 3 of the 8 changed during REC1 ($P \leq 0.050$; Table 8.4). During CHAL, PCV reached a minimal value 4 h after the challenge dose (Table 4.5; $P = 0.017$), and it did not change over 12 h during both BASE (Table 8.3) and REC1 (Table 8.4). Maximal plasma insulin concentrations were observed between 6 and 8 h after the challenge dose during CHAL ($P = 0.003$), and accumulated much faster than the maximal insulin concentrations observed at 12 h after feed during BASE ($P = 0.010$) and REC1 ($P < 0.001$). Maximal plasma glucose concentrations were observed at the time of feeding and challenge dose during BASE ($P = 0.047$) and CHAL, respectively, (Table 4.5; $P = 0.006$), and at 4 and 8 hours after feed during REC1 ($P = 0.042$). Serum BHBA reached maximal values between 6 and 10 h after challenge dose during CHAL ($P < 0.001$), but remained elevated between 4 to 12 h after feeding during BASE ($P = 0.001$) and did not change during REC1 ($P = 0.103$). Total lactate and L- and D-lactate reached maximum concentrations in serum at approximately 8 h after the challenge dose during CHAL (Table 4.5), and after feeding during BASE (Table 8.3). However, serum lactate did not change over the 12 h of monitoring during REC1 (Table 8.4). Plasma osmolality reached maximal values between 4 and 8 h after the challenge dose during CHAL (Table 4.5; $P < 0.001$), at 6 h after feeding during BASE ($P < 0.001$), and at 2 h after feeding during REC1 ($P = 0.022$).

Table 8.3. Blood composition as affected by treatment and time during the baseline period (BASE). Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			Time After Feed (h)[2]								*P* value[3]		
Item	LA	SA	SEM	0	2	4	6	8	10	12	SEM	T	t	T × t
PCV, %	32	31	0.7	31	31	31	31	31	31	31	0.5	0.34	0.60	0.43
Insulin, µg/L	1.9	1.8	0.37	1.2^{c}	1.5^{bc}	1.6^{abc}	1.8^{abc}	2.4^{abc}	2.3^{ab}	2.3^{a}	0.30	0.80	0.010	0.56
Glucose, mg/100 ml	82.6	83.7	1.52	86.6^{a}	84.3^{ab}	84.3^{ab}	82.5^{ab}	82.5^{ab}	80.5^{b}	81.1^{ab}	1.49	0.61	0.047	0.74
BHBA, mg/100 ml	7.4	8.1	0.35	6.3^{b}	7.4^{ab}	8.0^{a}	8.1^{a}	8.3^{a}	8.5^{a}	8.0^{a}	0.38	0.17	0.001	0.77
Total Lactate, m*M*	1.55	1.61	0.074	1.52^{ab}	1.49^{b}	1.56^{ab}	1.59^{ab}	1.68^{a}	1.66^{ab}	1.54^{ab}	0.065	0.56	0.035	0.59
L-lactate, m*M*	0.99	1.04	0.054	0.98^{ab}	0.95^{b}	1.00^{ab}	1.03^{ab}	1.08^{a}	1.08^{ab}	0.99^{ab}	0.048	0.51	0.035	0.51
D-Lactate, m*M*	0.56	0.57	0.021	0.54^{ab}	0.54^{b}	0.56^{ab}	0.57^{ab}	0.59^{a}	0.58^{ab}	0.55^{ab}	0.019	0.73	0.029	0.73
Osmolality[4]	280	279	1.3	278^{d}	281^{abc}	282^{ab}	282^{a}	280^{bcd}	278^{bcd}	277^{cd}	1.2	0.61	< 0.001	0.39

[abcd]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed.

[3]T = Treatment, t = time after feed.

[4]Osmolality measured in mOsm/kg.

Table 8.4. Blood composition as affected by treatment and time during the recovery period (REC1). Long-adapted (LA) heifers were fed the high-grain diet[1] for 34 d compared to 8 d for the short-adapted (SA) heifers.

	Treatment			Time After Feed (h)[2]								*P* value[3]		
Item	LA	SA	SEM	0	2	4	6	8	10	12	SEM	T	t	T × t
PCV, %	31	31	1.2	31	31	31	31	31	30	31	0.9	0.90	0.28	0.17
Insulin, µg/L	3.1	3.4	0.65	2.5^{c}	2.7^{bc}	3.0^{abc}	3.6^{ab}	3.6^{ab}	3.2^{abc}	3.9^{a}	0.50	0.78	< 0.001	0.80
Glucose, mg/100 ml	87.7	87.0	3.35	89.6^{ab}	87.7^{ab}	88.8^{a}	88.1^{ab}	88.0^{a}	85.6^{ab}	83.7^{b}	2.56	0.90	0.042	0.50
BHBA, mg/100 ml	8	7.2	0.81	6.9	6.9	7.6	8.1	7.8	7.7	8.5	0.69	0.50	0.103	0.44
Total Lactate, m*M*	1.39	1.32	0.055	1.42	1.32	1.38	1.33	1.35	1.38	1.33	0.048	0.38	0.26	0.20
L-lactate, m*M*	0.89	0.85	0.039	0.91	0.84	0.89	0.86	0.87	0.88	0.85	0.034	0.44	0.35	0.20
D-Lactate, m*M*	0.50	0.47	0.019	0.51	0.47	0.49	0.48	0.48	0.50	0.48	0.016	0.35	0.13	0.28
Osmolality, mOsm/kg	277	275	1.0	278^{ab}	278^{a}	277^{ab}	277^{ab}	276^{ab}	274^{b}	274^{b}	0.9	0.29	0.022	0.43

[abc]Means within a dependent variable differ ($P \leq 0.050$). The Tukey post-hoc separation test was used for mean separation.

[1]Forage:concentrate ratio = 9:91

[2]Samples collected immediately before feed (09:00, t=0) and every 2 hours for 12 h after feed.

[3]T = Treatment, t = time after feed.

8.4 Decrease in Insulin Sensitivity During an Acute Bout of Ruminal Acidosis

Analysis of covariance was able to detect a decrease in plasma glucose and an increase in plasma insulin throughout the day during both BASE (Figure 8.2A) and REC1 (Figure 8.2C). However, during CHAL analysis of covariance was unable to detect a change in plasma glucose with respect to time (Figure 8.2B). This data supports the increasing concentrations of insulin and glucose across periods (Table 4.3), and the loss of the negative correlation between insulin and glucose observed during BASE (Chapter 4.3.3) in suggesting that there may be a loss in insulin sensitivity during and after an acute bout of ruminal acidosis.

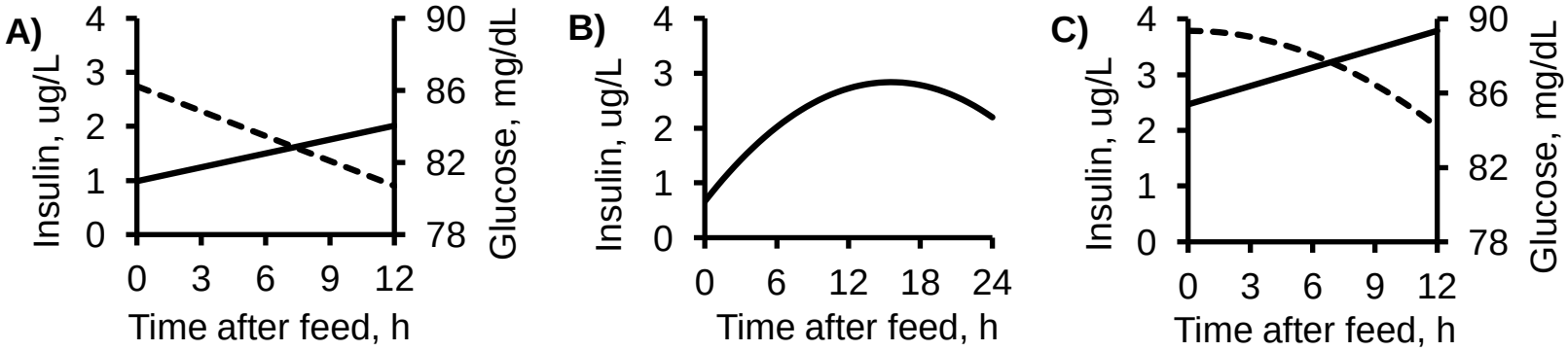

Figure 8.2. Change in plasma insulin and glucose concentrations during baseline, challenge, and recovery. Long-adapted (LA) heifers were fed the high-grain diet for 34 d compared to 8 d for the short-adapted (SA) heifers. Samples were collected immediately before feed and every 2 hours for 12 h after feed. During the challenge, extended rumen fluid sampling occurred at 24 h after feed. Trendlines were constructed using analysis of covariance. During baseline (A) plasma insulin increased ($P = 0.001$) and plasma glucose decreased ($P = 0.008$) linearly. The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y = 0.99 + 0.085\ t$ (insulin), and $y = 86.22 - 0.46\ t$ (glucose). During the challenge (B) plasma insulin increased linearly ($P < 0.001$) and decreased quadratically ($P = 0.002$), while a change in glucose with respect to time was not detected with use of analysis of covariance (mean glucose was 82.9 mg/dL). Therefore, a significant amount of variation in insulin can be represented by the following equation: $y = 0.66 + 0.28\ t - 0.009\ t^2$. During recovery (C) plasma insulin increased linearly ($P < 0.001$) and plasma glucose decreased quadratically ($P = 0.003$). The resulting equations, therefore, account for a significant amount of variation in the dependant variable and were used in constructing the trend lines: $y = 2.47 + 0.11\ t$ (insulin), and $y = 89.37 - 0.036\ t^2$ (glucose)