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Understanding Variation in the Susceptibility to Ruminal Acidosis

by

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DEDICATION

I dedicate this thesis to my loving wife Charla and daughter Carly. Throughout this degree they have undoubtedly made sacrifices which have enabled me to successfully complete my coursework and research projects. I am truly grateful for their encouragement, support, and patience!

ABSTRACT

Ruminal acidosis is a persisting digestive disorder in modern ruminant production; however, the susceptibility of cows to ruminal acidosis differs among cows fed a common diet. The overall objective of this research was to evaluate factors affecting the susceptibility of cows to ruminal acidosis. This research demonstrated that feeding sucrose in replacement for corn grain to Holstein cows in early lactation does not increase the risk for ruminal acidosis and may actually increase ruminal pH. However, regardless of dietary treatment, cows in early lactation were at risk for ruminal acidosis. In Study 2, diets differing in the forage-to-concentrate ratio were fed to Holstein cows to evaluate changes in the in vivo rate of short-chain fatty acid absorption (SCFA) and the expression of genes coding for transporters and enzymes involved in the absorption and metabolism of SCFA in ruminal tissue. Contrary to the hypothesis, the fractional rate of absorption and expression of genes involved in SCFA absorption and metabolism were not affected by the forage-to-concentrate ratio. Considerable variation among individual cows for the severity of ruminal acidosis was detected for cows on the diet containing the low forage-to-concentrate ratio. To determine the cause of this variation, a ruminal pH measurement system was developed to accurately and precisely measure ruminal pH in non-cannulated small ruminants. Sheep were then subjected to a ruminal acidosis challenge model in vivo, and the absorption of acetate and butyrate across the isolated ruminal epithelia was measured in vitro in Ussing chambers. The results of this study demonstrated that differences in the severity of ruminal pH depression among animals could largely

be accounted for by differences in the absorptive capability of the ruminal epithelium. In summary, although ruminal acidosis is a common digestive disorder in dairy production systems, variation in the susceptibility to ruminal acidosis is common. The cause for much of this variation is due to differences in the absorptive capacity of the ruminal epithelia.

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α-ketoglutarate-dehydrogenase	Oxoglutarate alpha- ketoglutarate dehydrogenase lipoamide
Acetyl-CoA synthetase	Acetyl-CoA synthetase 2
Acyl-carnitine	Solute carrier family 25, carnitine/acylcarnitine translocase, member 20
Acyl-CoA synthetase	Acyl-CoA synthetase short-chain family member 1
AV	Acidogenic value
BCS	Body condition score
BDH2	3-hydroxybutyrate dehydrogenase, type 2
BHBA	β-hydroxybutyric acid
BHD1	3-hydroxybutyrate dehydrogenase, type 1
Butyrl-CoA synthetase	Acyl-CoA synthetase medium-chain family member 1
CP	Crude protein
DGGE	Denaturing gradient gel electrophoresis
DMI	Dry matter intake
HMGL	3-hydroxymethyl-3-methylglutaryl-CoA lyase hydroxymethylglutaricaciduria
HMGS	3-hydroxy-3-methylglutaryl-CoA synthase 1
HS	High sugar diet containing 8.8% sugar on a dry matter basis
H-SCFA	Undissociated short-chain fatty acid
IDH	Isocitrate dehydrogenase 1 NADP+ soluble
LDH	lactate dehydrogenase
LRCpH	Lethbridge Research Centre Ruminal pH Measurement System

LS	Low sugar diet containing 4.5% sugar on a dry matter basis
MCT	Monocarboxylate transporter
MCT4	Solute carrier family 16 member 3 monocarboxylic acid transporter 4
Na/H antiporter	Solute carrier family 9 sodium/hydrogen exchanger member 1 antiporter $Na+/H^+$ amiloride sensitive
NA/K ATPase	ATPase Na+/K+ transporting alpha 1 polypeptide
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NHE	Sodium/hydrogen exchanger
NR	Non-responders
OM	Organic matter
PC	Pyruvate carboxylase
PDH	Pyruvate dehydrogenase lipoamide alpha 1
peNDF	Physically effective neutral detergent fiber
PUN	Plasma urea nitrogen
RES	Responders
RPLP0	Ribosomal protein, large P0
SARA	Sub-acute ruminal acidosis
$SCFA^-$	Dissociated short-chain fatty acid
SCFA	Short-chain fatty acids, refers to both the dissociate and undissociated states
SRS	Small Ruminant pH Measurement System
VFA	Volatile fatty acids

1.0 INTRODUCTION

1.1 General Overview of Ruminal Acidosis

Ruminal acidosis is a persisting digestive disorder that negatively affects animal health (Nocek, 1997), fibre digestibility (Calsamiglia et al., 2002), and animal productivity (Stone, 2004). Conventional feeding practices focus on maximizing energy intake through the use of fermentable carbohydrates to achieve high levels of milk yield or a rapid rate of growth in dairy and beef animals, respectively. However, feeding highly fermentable diets can lead to ruminal acidosis. For the dairy industry, ruminal acidosis is arguably one of the most prominent digestive disorders with prevalence rates, diagnosed by ruminocentesis using a threshold pH of 5.5, in Wisconsin dairy herds estimated to range between 12 to 30% during lactation (Krause and Oetzel, 2006). Although prevalence rates were not reported in Penner et al. (2007), they reported that on average primiparous cows spent 7.3, 9.0, 8.3, and 6.1 h/d with a ruminal pH below 5.8 during the first 5 d of lactation, and on d 17, 37, and 58, respectively. Thus, it is apparent that ruminal acidosis is a common digestive disorder in lactating Holstein cows and that research directed to reduce the prevalence and severity of ruminal acidosis is warranted.

Ruminal acidosis is generally characterized as acute or sub-acute in severity. Acute ruminal acidosis is caused by an abrupt increase in the intake of rapidly fermentable carbohydrate. This increases fermentation acid production decreasing pH, and ultimately increasing lactic acid production and accumulation leading to a further decrease in rumen pH (Owens et al., 1998). Acute ruminal

acidosis results in overt clinical signs including anorexia, diarrhea, and possibly death (Owens et al., 1998). In contrast, the decrease in pH associated with sub-acute ruminal acidosis (SARA) is driven by the accumulation of volatile fatty acids without a marked increase in lactic acid concentration (Krause and Oetzel, 2006), and animals experiencing SARA often do not present clinical signs (Nocek, 1997).

While the severity of ruminal acidosis varies on a continuum from sub-acute to acute, the pH thresholds used to define such events, especially SARA, differ among researchers. Commonly used pH thresholds for SARA range between 5.5 and 5.8 (Krause and Oetzel, 2006; Penner et al., 2007), and in some instances also include the time below the threshold in the criteria (Gozho et al., 2005). Differences in the threshold values used among groups can likely be attributed to their priority on the outcomes of low ruminal pH, such as decreased fiber digestion (Calsamiglia et al., 2002; Beauchemin et al., 2003), decreased ruminal epithelial barrier and absorptive functions (Nocek, 1997; Penner et al., 2007), and the acute phase protein response (Gozho et al., 2005). To develop feeding and management strategies that reduce the extent and severity of ruminal acidosis, a comprehensive understanding of ruminal acid production and removal is required.

Excellent reviews describing the etiology (Owens et al., 1998; Krause and Oetzel, 2006; Plaizier et al., 2009), physiological consequences (Nocek, 1997; Plaizier et al., 2009), and strategies for the treatment and prevention of ruminal acidosis (Enemark, 2009) have previously been published. To avoid repetition

and provide new insight into ruminal acidosis, this review will focus on factors affecting ruminal pH and the risk factors for ruminal acidosis.

1.2 Factors Affecting Ruminal pH

Ruminal pH is a balance between acid production and acid removal from the rumen through neutralization, absorption, and passage. Thus, ruminal acidosis occurs when the rate of acid production exceeds the rate of acid removal. To better understand ruminal acidosis, a thorough understanding of factors influencing both the production and removal of acid from the rumen is essential. A previous review by Allen (1997) greatly improved the current understanding on the relative contribution of acid removal pathways involved in ruminal pH stabilization. In that review, the importance of acid absorption was emphasized and it was further suggested that salivary buffer supply had previously been over-emphasized with regard to acid neutralization in the rumen. The current understanding of ruminal acid production and removal has greatly increased thereby exposing some limitations of the model proposed by Allen (1997).

1.2.1 Ruminal Acid Production

Anaerobic fermentation of organic matter in the rumen provides a source of energy for ruminal bacteria to carry out maintenance and growth, and yields by-products of fermentation including short-chain fatty acids (SCFA), carbon dioxide, and hydrogen gas (Russell and Hespell, 1981). The primary SCFA

arising from microbial fermentation are acetate, propionate, and butyrate; however, other minor acids including isobutyrate, valerate, isovalerate and lactate are also produced. Although the relationship between the concentration of SCFA in ruminal fluid and ruminal pH is weak (r^2 = 0.13, P < 0.001; Allen, 1997), production of 1 mol of SFCA is associated with the production of 1 mol of H^+. The reason that SCFA concentration only explains a small proportion of variation in ruminal pH is due to dissociation of SCFA and the removal of H^+ from the rumen. The logarithmic dissociation constants measured at 25°C for acetate, propionate, and butyrate are 4.76, 4.87, and 4.81, respectively. Therefore, according to the Henderson-Hasselbalch equation, more than 95% of the acetate, propionate, and butyrate would be in the dissociated state at a ruminal pH of 6.0. Thus, neutralization of H^+ through formation of CO_2 and H_2O via the carbonic anhydrase reaction or through incorporation into dihydrogen phosphate (pKa = 7.20; Allen, 1997) can occur independent of SCFA removal.

As mentioned previously, each individual SFCA can dissociate to yield 1 H^+. However, the type of SCFA produced through fermentation may result in a different quantity of acid produced per unit of hexose fermented. For example, 1 mol of glucose can be fermented to yield 2 mol of acetic, 2 mol of propionic, or 1 mol of butyric acid. Thus, fermentation promoting butyric acid production produces less acid compared to acetic and propionic. However, simply measuring SCFA concentration is not an adequate approach for measuring acid production in the rumen because of extensive carbon inter-conversion between acetic and butyric acid and to a lesser extent propionic acid within the rumen (Sutton et al.,

2003). Further, the absorption rates of acetate, propionate, and butyrate have been shown to differ (Dijkstra et al., 1993).

Numerous other factors may also affect the production of acid in the rumen including carbohydrate fermentability, rate of carbohydrate digestion, rate of passage from the rumen, and microbial efficiency (yield/kg OM). The contribution of these factors towards ruminal acid production has previously been extensively discussed by Allen (1997). In particular, the efficiency of microbial synthesis may have a large impact on the production of SCFA. In a summary by Martin et al. (2001), rates of SCFA production for sheep fed various diets ranged from 7.5 to 19.8 mol SCFA/kg digestible OM intake further confirming variation in the output of SCFA per unit of digestible OM. Assuming a digestible organic matter intake, of 1.5 kg for sheep, increasing fermentation efficiency from 7.5 to 19.8 mol SCFA/kg digestible OM could result in SCFA production rates differing by 18.5 mol/d.

Rates of SCFA production are most accurately measured using isotope dilution (Martin et al., 2001; Sutton et al., 2003). Rates of SCFA production in sheep fed at high and low intake levels (90% and 45% ad libitum intake), estimated using [1-^{13}C]propionate, range between 8.8 and 14.8 mol/d (Martin et al., 2001). For dairy cows fed diets with concentrate-to-hay ratios of 60:40 and 90:10, total net SCFA production rates ranged between 79.8 and 90.0 mol/d (Sutton et al., 2003). Other studies have estimated absorption based on portal appearance, but these values are highly affected by extensive intraepithelial metabolism of SCFA and differential intraepithelial metabolism among individual

SCFA. Approximately 70, 50, and 30% of the butyrate, propionate, and acetate are metabolized by the rumen epithelia, respectively (Bergman, 1990). As such, portal appearance of SCFA is not a good estimator of SCFA production.

1.2.2 Absorption of Acid from the Rumen

Short chain fatty acids may exist in the protonated (H-SCFA) or dissociated ($SFCA^-$) forms in ruminal contents. However, under normal ruminal pH conditions (mean pH = 5.8) approximately 90% of the SCFA are dissociated. Protonated SFCA are lipophilic and may cross the apical cell membrane via passive diffusion (Walter and Gutknecht, 1986; Gäbel et al., 2002). Accordingly, absorption of H-SCFA across the rumen wall should remove acid from the rumen and thereby stabilize ruminal pH. Supporting this view, Allen (1997) calculated that the largest proportion of H^+ removal (approximately 53%) would occur through the absorption of SCFA across the rumen wall. The model of Allen (1997); however, does not consider rapid intracellular dissociation of H-SCFA and the potential for apically directed H^+ secretion by the rumen epithelia (Müller et al., 2000; Graham et al., 2007). Furthermore, the model of Allen (1997) does not consider differences in transport processes and regulation for the different SCFA (see Figure 1.1).

As mentioned previously, H-SCFA can cross the apical membrane of the ruminal epithelia via passive diffusion (Gäbel et al., 2002). However, if the absorption of H-SCFA is to be a permanent contribution towards the stabilization of ruminal pH, we need to assume that absorbed H^+ are not recycled back into the

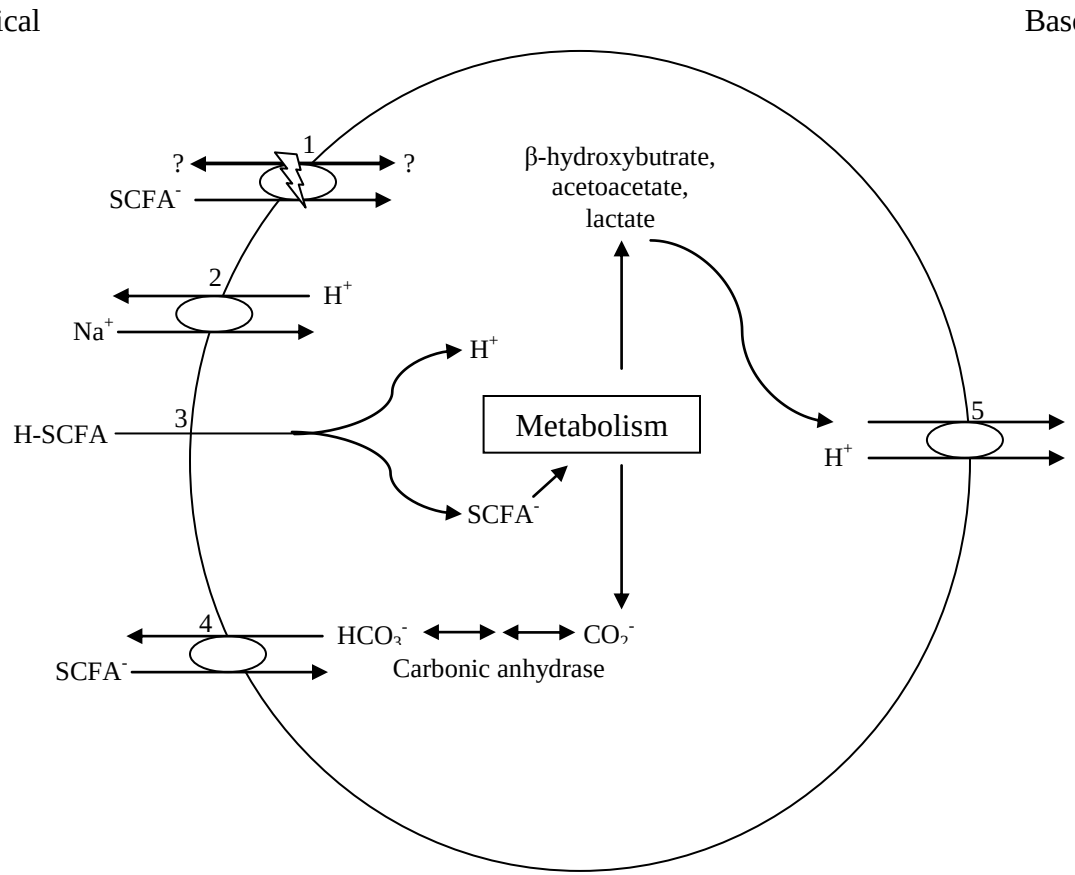

Figure 1.1 Pathways involved in the absorption of short chain fatty acids (SCFA) and H^+ recycling in the rumen epithelia: 1 = bicarbonate-independent nitrate-sensitive transport, 2 = $Na^{+/}H^+$ antiporter, 3 = passive diffusion of SCFA, 4 = bicarbonate-dependent transport, and 5 = monocarboxylate transporter. Although 1 cell is depicted, it should be acknowledged that a number of cells across different strata are involved. Adapted from Gäbel and Aschenbach (2006) and Aschenbach et al. (2009).

rumen, which may not be true as protonated SCFA absorbed across the apical membrane would rapidly dissociate due to an intracellular pH of 7.4. In fact, according to the Henderson-Hasselbalch equation, at an intracellular pH of 7.4 more than 99.7% of SCFA are in the dissociated state. Thus, mechanisms for intracellular pH homeostasis are required. Graham et al. (2007) demonstrated the apical localization of NHE1 (Na^+/H^+ exchanger) using immunohistochemistry and suggested that the apically directed secretion of H^+ helps to maintain an acidic microclimate on the luminal side of the stratum corneum. This microclimate would promote further protonation of SCFA enhancing passive diffusion.

The relative proportion of H^+ secreted back into the rumen for those originally absorbed as H-SCFA is not currently known. Furthermore, the proportion of H^+ secreted to the rumen likely changes based on rumen pH, intracellular acid load, and type of SCFA. For example, numerous studies have demonstrated that grain feeding and increased SCFA concentration result in an increased net flux rate of Na^+ across the rumen epithelia (Sehested et al., 2000; Uppal et al., 2003). The increase in Na^+ flux is correlated to increased H^+ secretion into the rumen (Sehested et al., 2000; Gäbel and Aschenbach, 2006; Graham et al., 2008). In contrast, Gaebel et al. (1989) exposed isolated ovine ruminal epithelia to pH 7.0, 6.5, 6.0, or 5.5 in Ussing chambers and reported that decreasing pH from 6.0 to 5.5 markedly decreased the net Na^+ flux. The decrease in net flux in that study was due to both decreased mucosal-to-serosal flux and increased serosal-to-mucosal flux. Decreased mucosal to serosal flux may indicate difficulty in maintaining the Na^+ concentration gradient across the apical

cell membrane due to limited activity of Na^+/K^+ ATPase whereas increased serosal-to-mucosal flux may be a result of altered barrier function thus allowing for passive leaking of Na^+. Together, these data suggest that the low pH impaired tissue barrier function and possibly cellular ATP production.

Recycling of H^+ may also differ based on the type of SCFA absorbed. For example, acetate absorption is primarily mediated through transport proteins (Aschenbach et al., 2009) whereas butyrate is mainly absorbed via passive diffusion (Sehested et al., 1999; Gabel et al., 2002). In addition, extensive intraepithelial metabolism of butyrate (Sehested et al., 1999; Kristensen, 2005) may help limit H^+ recycling. Metabolism of butyrate results in the production of ketone bodies such as β-hydroxy butyric acid (BHBA) (Britton and Krehbiel, 1993) which can be exported via the monocarboxylate$^-$/H^+ co-transporter (MCT1) located on the basolateral side (Müller et al., 2002; Graham et al., 2008). Thus, the basolaterally directed co-transport of a ketone and H^+ results in the permanent removal of a H^+ from the rumen. In contrast to butyrate, acetate is not extensively metabolized in the rumen epithelia (Bergman, 1990; Britton and Krehbiel, 1993), and Stumpff et al. (2009) recently proposed the existence of a large-conductance anion channel located on the basolateral side of ruminal epithelial cells which can function as an important exit pathway for acetate anions and possibly propionate anions. Acetate anions transported via this channel would leave behind a H^+ in the cytosol thereby requiring additional transporters to remove intracellular H^+. Such transporters may be located either apically or basolaterally. Thus, it seems

likely that H^+ recycling back into the rumen may differ between acetate, propionate, and butyrate.

The model of Allen (1997) assumes that SCFA are exclusively absorbed across the rumen in the protonated form, however it should be acknowledged that the absorption of $SCFA^-$ has been extensively documented for ovine (Gäbel et al., 1991; Gäbel et al. 2002) and bovine epithelia (Sehested et al., 1999; Sehested et al., 2000). In fact, protein mediated transport of acetate in the dissociated state accounts for more than 50% of the total acetate transport (Aschenbach et al., 2009). The assumption of exclusive H-SCFA absorption should not be held as a cudgel against the model of Allen (1997) as absorption of H-SCFA and the secretion of HCO_3^- associated with $SCFA^-$ absorption both contribute to the removal of 1 H^+ from the rumen, providing absorbed H^+ are not recycled back into the rumen as discussed above. However, the current understanding of $SCFA^-$ absorption suggests counter transport of $SCFA^-$ and HCO_3^- (Bilk et al., 2005). The ratio of SCFA absorption to HCO_3^- secretion ranges from 1 SCFA to 0.50 to 0.55 HCO_3^- (Ash and Dobson, 1963; Gäbel et al., 1991). As such, absorption of $SCFA^-$ would contribute to the removal of a H^+ due to HCO_3^- secretion and the subsequent neutralization of a H^+ in the rumen.

In conclusion, H-SCFA absorption contributes to the stabilization of pH; however, the contribution has likely been overestimated by Allen (1997) due to H^+ recycling associated with the absorption of H-SCFA (Gäbel et al., 2002; Gäbel and Aschenbach, 2006) and the relative importance of $SCFA^-$ transport (Gäbel and Aschenbach, 2006; Aschenbach et al., 2009). Further research is required to

quantify the relative contribution of H-SCFA and $SCFA^-$ absorption towards the stabilization of ruminal pH, and how H^+ secretion by the ruminal epithelia changes in response to ruminal pH, intracellular pH, and the type of SCFA absorbed.

1.2.3 Passage of Acid from the Rumen

Short chain fatty acids and H^+ are soluble in water and are primarily located in the liquid phase of the rumen (Walter and Gutknecht, 1986). As such, liquid passage from the rumen should account for the majority of acid passage from the rumen. However, some acid may also pass from the rumen with feed particles (Allen, 1997).

Allen (1997) suggested that only a small proportion of H^+ would be removed from the rumen through passage but that passage accounted for approximately 13.4% of the total acid removal from the rumen which is supported by other studies. For example, Rezende Júnior et al. (2006) reported a liquid clearance rate of 15.1 %/h in lactating dairy cattle averaging 17.7 kg DMI; however, slower rates of liquid clearance (11-12%/h) have been reported (Maekawa et al., 2002a,b).

At a mean ruminal pH of 6.0, over 94% of the SCFA would be dissociated. Thus, quantitatively, passage of H^+ from the rumen as H-SCFA should not have a large impact on ruminal pH. In addition, it is unlikely that ruminal pH would accurately reflect the pH of the cranial region of the rumen due to the proximity of saliva inflow. Duffield et al. (2004) evaluated the pH of various regions in the

rumen and found that the ventral cranial region (6.42) had higher pH than the central rumen (6.10). Thus, the free H^+ concentration in fluid passing from the rumen is likely even lower than the free H^+ concentration in ruminal fluid.

The calculated proportion of SCFA in the undissociated state is expected to be < 10% at physiologically normal pH ranges. As such, assuming a pH of 6.4 in the cranial region, a liquid pool size of 80 L, total SCFA concentration of 120 m*M*, passage rate of 15 %/h, and average SCFA pKa of 4.8, one could predict that a total of 1.32 mol of H-SCFA would leave the rumen via passage/d. With estimated SCFA production rates ranging between 79.8 and 90.0 mol/d for dairy cows fed diets containing ratios of concentrate to hay of 60:40 and 90:10, respectively (Sutton et al., 2003), 1.32 mol of acid removed from the rumen by passage of H-SCFA would only account for 1.6% and 1.4% of the H^+ removed, respectively. Allen et al. (1997) proposed values approximately double to those calculated above.

1.2.4 Neutralization of Acid in the Rumen

1.2.4.1 Epithelial Secretion of Bicarbonate

An early study by Ash and Dobson (1963) clearly demonstrated that SCFA absorption across the rumen was associated with bicarbonate secretion. In that study anaesthetized sheep were used to prepare an isolated reticulo-rumen. Buffer solutions containing different concentrations of HCO_3^- and acetate were placed in the rumen. In that study, acetate disappearance corresponded to a linear

appearance HCO_3^-, such that for every mol of acetate absorbed, 0.5 mol of HCO_3^- appeared in the ruminal contents. Although Ash and Dobson (1963) documented some of the first work demonstrating HCO_3^--dependent-absorption, these researchers did not realize that HCO_3^- was secreted by the rumen epithelia and suggested that HCO_3^- was produced in the rumen through the carbonic anhydrase reaction where CO_2 and H_2O were converted to HCO_3^- and H^+.

Subsequently, Gäbel et al. (1991) examined the relationship between HCO_3^- secretion and SCFA absorption in the washed reticulo-rumen of sheep fed 100% hay (1400 g/d), or 81% concentrate and 19% hay (1300 g concentrate + 300 g hay). In that study, the relative contribution per mol of acetate absorption was 1 mol/0.55 mol HCO_3^-. They further proposed the existence of an anion exchanger capable of transporting $SCFA^-$ and possibly Cl^- in exchange for HCO_3^-. Further in vitro studies have confirmed the findings of Gäbel et al. (1991) in bovine (Sehested et al., 1999; 2000), and ovine epithelia (Bilk et al., 2005; Aschenbach et al., 2009) demonstrating the dependence of SCFA absorption on bicarbonate secretion by ruminal epithelial cells.

According to the results of Ash and Dobson (1963) and Gäbel et al. (1991), a mean of 0.53 mol of HCO_3^- would be secreted by the epithelium per mol of VFA absorbed. Assuming a SCFA production rate of 74 mol/d (Sutton et al., 2003), it could be assumed that 39.2 mol of HCO_3^- would be secreted by the epithelia. The pKa of HCO_3^- in a closed system at 25°C has been determined to be 6.1, but since CO_2 can be removed from the rumen through eructation the dissociation constant of HCO_3^- is likely higher than reported (Allen, 1997). Allen

(1997) suggested that the buffering capacity of HCO_3^- ranges from approximately 50% at a neutral pH to 100% at pH 5.5. Thus, assuming a mean ruminal pH of 6.0, the HCO_3^- buffering capacity may approximate 70% equating to a potential for the removal of 37% of the H^+ produced/d (total SCFA production 74 mol/d).

From past research it is clear that the rumen epithelium is a potent source of intra-ruminal HCO_3^-. However, data from Aschenbach et al. (2009) suggest that the importance of HCO_3^--dependent acetate transport increases with increased acetate concentration and at lower pH. In fact, maximal HCO_3^--dependent acetate uptake was 3 times higher than maximal HCO_3^--independent uptake. Apparently, the requirement for HCO_3^- is not met through the intra-cellular carbonic anhydrase activity as Sehested et al. (1999) reported that acetazolamide, an inhibitor of carbonic anhydrase, did not reduce the net flux of butyrate across the bovine rumen epithelia in Ussing chambers. However, butyrate seems to have a lower reliance on HCO_3^- than acetate and propionate (Sehested et al., 1999; Aschenbach et al., 2009). These data suggest that in addition to the apical co-transport of $SCFA^-$ and HCO_3^-, there must be a basolateral HCO_3^- transporter involved. Future studies are required to determine the net contribution of $SCFA^-$ absorption to the HCO_3^- secretion by ruminal epithelia and factors affecting HCO_3^- secretion.

1.2.4.2 Salivary Buffer Supply

A considerable emphasis has been placed on the role of salivary buffer supply towards the stabilization of ruminal pH. Since the bicarbonate and

phosphate concentrations do not differ due to rate of secretion (when the rate is greater than 30 mL/min) or diet (Bailey and Balch, 1961); estimates of salivary secretion during eating, ruminating, and resting can provide reliable estimates of the salivary buffer supply to ruminal contents. Bailey and Balch (1961) reported a concentration of 125 mEq of HCO_3^- and 26 mEq of phosphate/mL of saliva.

Rates of saliva secretion during resting, and eating have been estimated in a number of publications (Baily and Balch, 1961; Cassida and Stokes, 1986; Maekawa et al., 2002a,b; Beauchemin et al., 2003; Bowman et al, 2003; Beauchemin et al., 2008). From these studies, salivary secretion rates during eating range from 166 to 253 mL/min (Cassida and Stokes, 1986 and Maekawa et al., 2002a) and rates during resting range from 88 to 173 mL/min (Cassida and Stokes, 1986 and Maekawa et al., 2002b). Although the rate of salivary secretion does not differ among diets (Cassida and Stokes, 1986; Maekawa et al., 2002a,b; Bowman et al., 2003; Beauchemin et al., 2008), eating time may be affected by feed type (forage-to-concentrate ratio and type of forage) thereby altering the total salivary buffer supply (Beauchemin et al., 2008). For example, Beauchemin et al. (2008) compared the saliva secretion from cows fed diets containing different types of forage (51% barley silage, 53% alfalfa silage, 52% alfalfa hay, or 16% barley straw). Although forage type did not affect the rate of saliva secretion with an average of 212.6 mL/min across treatments, saliva secretion per unit DM and per unit NDF differed among the forages tested with barley straw yielding increased amounts of saliva/g DM. This increase in salivary production for barley straw was primarily attributed to increased meal duration and NDF content.

In Cassida and Stokes (1986), Maekawa et al. (2002a,b), and Bowman et al. (2003) total saliva output was estimated to range between 201-304 L/d. The total saliva output was calculated assuming that saliva production during rumination was equal to saliva production during eating. Based on the published literature including 3 studies with a total of 10 treatments, a mean salivary secretion of 254.8 L/d could be expected. Assuming a HCO_3^- concentration of 125 m*M*, saliva could contribute to a total of 31.9 mol of HCO_3^-/d. Thus, theoretically, salivary HCO_3^- could provide buffer for approximately 43% of H^+/d, but this assumes that HCO_3^- has 100% buffering capacity. As described above, the pKa of HCO_3^- in a closed system has been determined to be 6.1, but since CO_2 can be removed from the rumen through eructation, the dissociation constant of HCO_3^- is likely higher than reported (Allen, 1997). Therefore, assuming total SCFA production of 74 mol/d and a HCO_3^- buffering capacity of 70%, HCO_3^- could potentially remove approximately 30% of the H^+ produced/d.

1.2.4.3 Other Minor Buffers

Other potential buffers to the rumen environment include, HPO_4^{2-}, NH_3, and the endogenous buffering capacity of feeds (Allen, 1997). As mentioned above, saliva also contains HPO_4^{2-} (Bailey and Balch, 1961), which contributes to the overall buffering capacity of saliva. The pKa of HPO_4^{2-} is 7.2 indicating more potent buffering capacity at an alkaline ruminal pH. Based on the estimated saliva flow described above, HPO_4^{2-} could be expected to supply 13.2 mol of HPO_4^{2-} with approximately 5% in the acid form ($H_2PO_4^-$) at pH 6.0. Thus, HPO_4^{2-}

could be expected to buffer approximately 6.6 mol of H^+/d (7.4% of the H^+ produced/d).

The concentration of NH_3 in the rumen is diet dependent, but often ranges between 3 and 11 m*M* (Beauchemin et al., 2003; Penner et al., 2009). However, NH_3 provides little buffering capacity to the rumen since at a pH of 6.2 (pKa = 9.25) more than 99% of the ammonia would occur as NH_4^+. Assuming a concentration of 8 mmol/L for NH_3 and NH_4^+ and a rumen volume of 80 L, the calculated NH_3-N pool size would equate to approximately 64 m*M*. Thus, the buffering potential of NH_3 is of marginal impact to ruminal pH as previously described by Allen (1997).

Feed ingredients may also provide some inherent buffering capacity. While the buffering capacity is difficult to measure, the acidogenic value (AV) has been proposed as a measure to characterize the potential for acid production from a feedstuff (Wadhwa et al., 2001). The AV is determined in vitro and is based upon the dissolution of Ca^{2+} from insoluble $CaCO_3$ after a 24-h incubation period (Wadhwa et al., 2001). To validate the technique Wadhwa et al. (2001) evaluated 28 feed ingredients varying in starch, protein, and neutral detergent fiber (NDF) concentration. Generally, the AV was highest for starch feeds, intermediate for forages, and lowest for protein supplements (Wadhwa et al., 2001; Rustomo et al., 2006a). Others (Rustomo et al., 2006a,b,c) have also used the AV to predict the effect of feed ingredients and found that changes in in vitro ruminal fluid pH were better predicted by the AV value than starch content. Rustomo et al. (2006b) further demonstrated that a low AV diet can help maintain higher rumen pH than

high AV diet; however, the diet differed in source of starch (wheat and barley for high AV vs. oats and wheat bran for low AV). Future studies should further investigate the use of the AV to reduce the occurrence of ruminal acidosis.

1.3 Risk Factors Predisposing Dairy Cattle to Ruminal Acidosis

1.3.1 Increased Risk During the Transition Period

The transition period in dairy cows has been defined as the last 3 wk of gestation through the first 3 wk of lactation (Grummer, 1995). The pre-partum phase of the transition period is characterized by up to a 50% reduction in dry matter intake (DMI) with the majority of the decrease occurring in the last week prior to parturition (Hayirli et al., 2002). Following parturition, cows are often fed a diet containing increased dietary energy density relative to the pre-partum diets occurring during a time when DMI increases dramatically. Penner et al. (2007) recently reported that primiparous Holstein cows increased DMI by nearly 200% from the wk prior to parturition to wk 4 of lactation. Changes in DMI and dietary composition that occur during the transition period may cause increased risk for ruminal acidosis.

In a large epidemiological study including more than 60,000 cows, the prevalence of ruminal acidosis was greatest in the months that most calvings occurred (Grohn and Bruss, 1990). Others (Fairfield et al., 2007; Penner et al., 2007) have reported that ruminal acidosis is highly prevalent and severe during early lactation. In multiparous cows, Fairfield et al. (2007) reported that cows

spent over 3 h/d with a ruminal pH < 6 during the 1st wk following calving. Similarly, Penner et al. (2007) reported that primiparous cows spent more than 7 h/d with a ruminal pH < 5.8. These studies indicate that ruminal acidosis is a common digestive disorder during the transition period in dairy production and demonstrates the necessity for the development of programs to reduce the risk.

A classical study by Dirksen et al. (1985) suggested that high prevalence of ruminal acidosis following calving was due to inadequate surface area and functional characteristics of the ruminal papillae. However, Penner et al. (2007) fed primiparous heifers according to NRC (2001) recommendations or a pre-partum dietary adaptation protocol containing diets with a lower forage-to-concentrate ratio than the NRC recommendations (i.e. increased dietary energy density). The latter was designed to adapt the ruminal epithelia and microflora to the post-partum diet prior to calving. The results of that study demonstrated that the pre-partum dietary adaptation protocol did not affect post-partum ruminal pH. Furthermore, Penner et al. (2006) reported that ruminal papillae surface area reached the maximal size 14-d prior to calving, which is contrary to the work of Dirksen et al. (1985). Thus, papillae surface area is likely not a limiting factor for SCFA absorption under North American feeding conditions. However, the metabolic adaptation of ruminal epithelial cells requires further investigation.

Past reviews have suggested that high DMI is a predisposing factor for the occurrence of ruminal acidosis (Krause and Oetzel, 2006; Zebeli et al., 2008). While this may be a correct general assumption for cows fed highly fermentable diets, high DMI did not correspond to the greatest occurrence rates of ruminal

acidosis during early lactation in a recent study (Figure 1.1; Penner et al., 2007). Reasons for the high occurrence of ruminal acidosis in early lactation may be due to instability in the ruminal microflora (Nocek, 1997) and decreased absorptive capacity of the ruminal epithelia (Gäbel et al., 1993). As described previously, as cows approach parturition DMI decreases drastically. Gäbel et al. (1993) and Gäbel and Aschenbach (2002) have demonstrated that withholding feed for 2 d results in drastic decreases in SCFA, electrolyte, and glucose absorption by the ruminal epithelia. As such, the depression in intake that occurs around parturition may be the primary factor predisposing cows to ruminal acidosis post-partum.

1.3.2 Previous Exposure to Ruminal Acidosis

Ruminal acidosis is a digestive disorder that may directly affect both the host animal and the microbial inhabitants predisposing the host to future episodes of ruminal acidosis. With respect to the host animal, decreased ruminal pH in vivo (pH 4.79; Gaebel et al., 1987) and in vitro (pH 5.5; Gaebel et al., 1989) has been reported to cause decreased capacity for the transport of Na and Cl across the ovine epithelia. Altered electrolyte absorption with low pH (5.5) was accompanied by increased mitochondrial diameter and alterations of the mitochondrial cristae (Gaebel et al., 1989). Although SCFA absorption was not measured in that study, Na^+ transport is positively correlated to SCFA transport in ruminal epithelia (Diernæs et al., 1994; Sehested et al., 1999) suggesting that

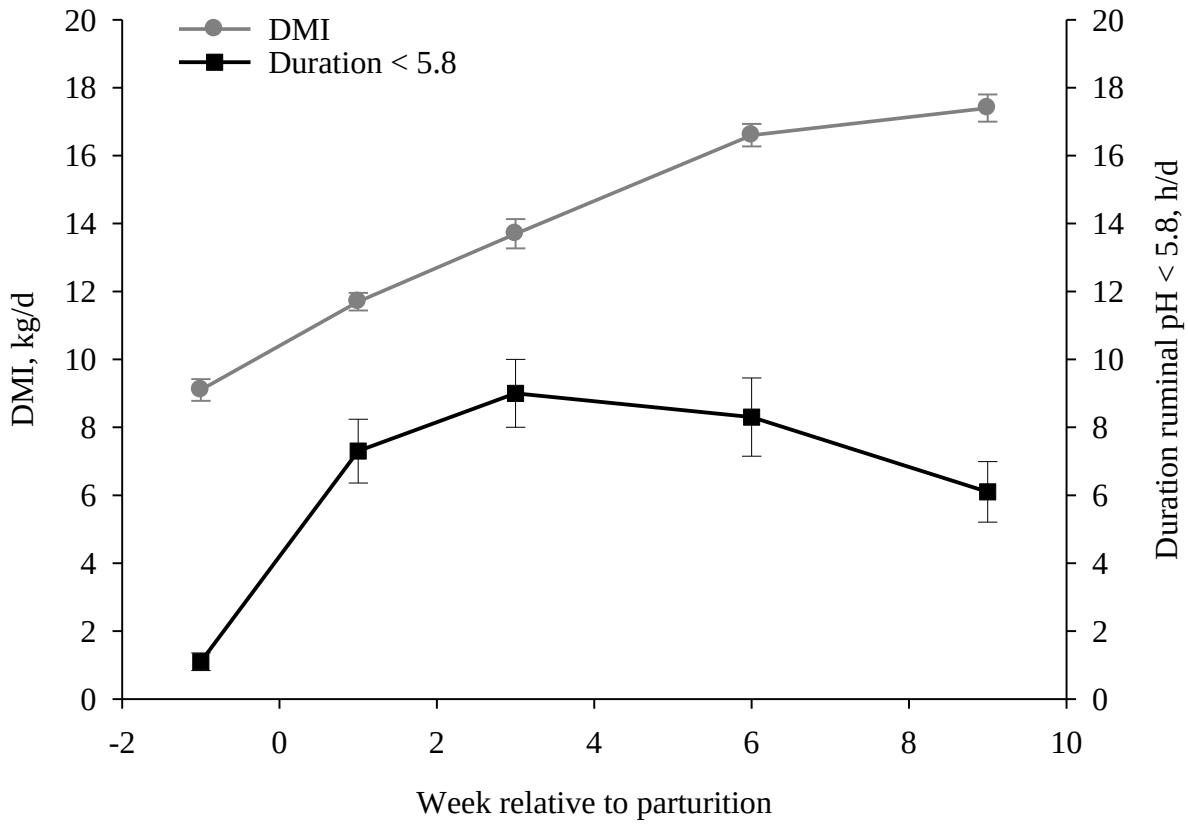

Figure 1.2 Changes in DMI (linear *P* < 0.001, quadratic = 0.042) and the duration that ruminal pH was below 5.8 (quadratic *P* < 0.001) during the periparturient period in primiparous Holstein cows (n = 14). Adapted from Penner et al. (2007).

SCFA absorption may have also decreased. In a subsequent study, Krehbiel et al. (1995) induced ruminal acidosis in sheep by supplying 0, 6, 12, or 18 g glucose/kg body weight in an effort to determined the effects of ruminal acidosis on the absorption SCFA using the washed reticulo-rumen model 10 d, 3 mo, and 6 mo after the induction of acidosis. Results from this study demonstrated that ruminal acidosis did not affect the fractional rates of SCFA absorption 10 d after induction. However, lambs that were exposed to 18 g glucose/kg BW, and experienced the most severe ruminal acidosis, tended to have lower propionate absorption 3 mo after the acidosis challenge compared to lambs on the control treatment (0 g glucose/kg BW).

More recently, Dohme et al. (2008) exposed cows fed a low forage (45.1% forage) or high forage (60.1% forage) diet to an acidosis induction model. In that study, feed was restricted to 50% of the DMI 1 d prior to the challenge and then cows were offered 4 kg of a ground barley:ground wheat mixture, which was followed by their TMR diet ad libitum. Cows were exposed to this protocol in 3 consecutive periods such that each challenge was separated by 14 d. Although more cows selectively refused a portion of the challenge with subsequent inductions, the severity of ruminal acidosis increased from the 1st challenge to the 3rd challenge. The authors were not able to precisely determine the cause for increase severity with subsequent challenges but suggested that damage to the rumen epithelium or alterations in the composition and activity of microflora may be involved. These data support the work of Gaebel et al. (1987, 1989) and

further suggest that ruminal acidosis may alter ruminal epithelial function and predispose animals to subsequent episodes of ruminal acidosis.

As acknowledged by Dohme et al. (2008), the onset of ruminal acidosis induces changes in the microbial population including decreases in the number of gram negative bacteria and increases in the number of gram positive bacteria (Nagaraja and Titgemeyer, 2007). Goad et al. (1998) induced ruminal acidosis in beef steers, adapted to a grain-based (80% concentrate) or hay-based (20% concentrate) diet, by withholding feed for 24 h followed by the provision of a diet primarily containing cracked corn over the next 3 d. In that study, induction of ruminal acidosis resulted in increased total viable anaerobic bacteria, increased amylolytic bacteria, increased lactate producers and utilisers, and decreased protozoal populations. Although microbial changes occur, the effect of microbial composition on subsequent exposure to ruminal acidosis is currently unknown. Further research is required to determine the mechanisms by which previous exposure to ruminal acidosis increase the susceptibility of ruminants for subsequent episodes.

1.3.3 Effect of Dietary Composition

1.3.3.1 Physically Effective NDF

Dietary neutral detergent fibre (NDF) can be easily measured with chemical analysis, however, as proposed by Mertens (1997), chemical characterization of

fiber does not describe physical properties of fiber and the resulting effect on the stimulation of rumination and the maintenance of milk fat concentration. As such, Mertens (1997) proposed to use physically effective NDF (peNDF) to account for both the physical and chemical characteristics of fiber that influence chewing activity and the maintenance of rumen stratification. Because particle size is a major determinant of peNDF (Mertens, 1997), Lammers et al. (1996) and Kononoff et al. (2003) proposed the use of the Penn State Particle Size Separator to measure the particle size distribution of forages and rations. There are a number of methods to determine the peNDF content including multiplying the NDF concentration by the proportion retained on a 1.18-mm sieve (Mertens et al., 1997), multiplying the NDF concentration by the sum of the proportions retained on 19- and 8-mm sieves (Lammers et al., 1996), or multiplying the NDF concentration by the sum of the proportions retained on the 19-, 8-, and 1.18-mm sieves (Kononnoff et al., 2003).

The recommended peNDF content (sum of the proportions retained on the 1.18-mm sieve multiplied by the NDF concentration) of diets for lactating dairy cattle suggest that approximately 19% peNDF is required to maintain ruminal pH around 6.0 (Zebeli et al., 2006). However, more recently, recommendations as high as 30 to 33% of dietary DM or alternatively the ratio of peNDF to ruminally degradable starch content of the dietary concentrate should be maintained around 1.45:1 (Zebeli et al., 2008) to optimize fat corrected milk yield and minimize the risk for ruminal acidosis. However, peNDF contents above 21% tended to

decrease DMI and diets exceeding a peNDF content of 32% resulted in a more drastic decrease in DMI (Zebeli et al., 2008).

The concept of peNDF originally focussed on promoting rumination and it was thought that the additional ruminating activity increases the supply of salivary buffer to the rumen (Mertens, 1997). Although ruminal pH increased with increased dietary peNDF in a curve-linear manner (Mertens 1997), according to the equation of Mertens (1997), increasing the peNDF by 10 percentage units (20% to 30% DM) content would increase ruminal pH by 0.24 pH units. The assumption that peNDF only impacts salivary buffer supply is incorrect as greater ruminal motility is expected to expose more SCFA to the rumen wall increasing SCFA absorption. Further, increases in the peNDF concentration likely also decrease the rate of fermentation acid production. Thus, the importance of peNDF likely extends beyond effects on ruminating activity such as decreased fermentation acid production including increases in the rate of SCFA absorption, epithelial secretion of bicarbonate, and decreased fermentation acid production.

1.3.3.2 Carbohydrate Source

Combined with the inclusion level of dietary concentrate, ruminal fermentability of the grain source can have a large impact on ruminal pH. The type of grain (McCarthy et al., 1989) and variety of grain (Silveira et al., 2007a,b) can have marked impacts on the rumen degradable organic matter and the rate of fermentation. Past reviews have extensively discussed the effects of grain type, variety of grain, and processing of grain on digestion (for reviews see Allen,

1997; Owens et al., 1997; Allen et al., 2000; Dehghan-Banadaky et al., 2008). However, no reviews known to the author have compared starch and sugar with respect to ruminal fermentation and effects on ruminal pH.

Sugars are often considered to be rapidly fermented in the rumen and past research investigating the in vitro rate of sucrose hydrolysis has reported rates ranging between 1200 and 1400%/h with fermentation of the comprising monosaccharides ranging between 400 and 620%/h (Weisbjerg et al., 1998). Despite evidence for rapid fermentation, feeding sugar has been reported to have either no effect or a positive effect on ruminal pH in vivo (Heldt et al., 1999; Broderick and Radloff, 2004; DeFrain et al., 2004; Penner et al., 2009) and in vitro (Vallimont et al., 2004).

The type of sugar may also have an effect on the depression in ruminal pH. Weisbjerg et al. (1998) reported that introducing 1 kg of sucrose into the rumen decreased ruminal pH to a greater extent than the same dose of glucose or lactose; however, in that study ruminal pH was only monitored for 180-min following the sugar challenge. Heldt et al. (1999) reported no negative effect of sugar on ruminal pH but found that the pattern of pH depression was more rapid for steers fed sugar than for those fed starch; however, the recovery of ruminal pH was also more rapid for sugar than starch. For studies reporting a decrease in ruminal pH with the provision of sugar, the decrease can likely be attributed to increased dietary NSC content (Krehbiel et al., 1995; Lee et al., 2003; Hall and Weimer, 2007) rather than to the effect of sugar itself. As such, it seems that replacement of starch with sugar may not decrease rumen pH.

Data describing the mechanisms by which sugar does not decrease ruminal pH is limiting. One possibility is that the disappearance of sugar from the rumen may not necessarily result in fermentation acid production. For example, Hall and Weimer (2007) demonstrated, in vitro, that the addition of 65, 130, or 195 mg sucrose to 130 mg of isolated NDF resulted in increased glycogen concentration at 0 and 4 h but found that glycogen concentration decreased thereafter. It is therefore possible that bacteria convert dietary sugars to glycogen as a short-term storage of energy that is utilized later, thereby temporarily reducing fermentation acid production.

In addition, it is possible that the replacement of starch for sugar may improve the efficiency of microbial N production. In continuous culture, Ribeiro et al. (2005) showed that the efficiency of microbial N production per kg of OM digested in vitro increased linearly from 36.7 to 56.4% when sucrose concentration increased from 0 to 8% of the diet. Hall and Herejk (2001) reported higher trichloroacetic acid precipitated CP for in vitro incubations containing starch compared to pectin and sucrose. If sugar supplementation increases the efficiency of microbial cell yield, ruminally degraded OM available for fermentation acid production may consequently be reduced (Allen, 1997).

Furthermore, sugar has been shown to increase the concentration of butyrate in the rumen (Nombekela et al., 1994; DeFrain et al., 2006). As described above, fermentation leading to butyrate production may decrease H^+ production relative to acetate and propionate production. However, this is a theoretical speculation assuming that butyrate concentration in the rumen arises

from direct production and not from SCFA carbon inter-conversion (Sutton et al., 2003).

Alternatively, monosaccharide transport across the rumen epithelia may also explain the higher ruminal pH for cows fed sugar compared to starch. Past studies have demonstrated the molecular and functional evidence of the sodium-glucose linked co-transporter 1 (Aschenbach et al., 2000) and facilitated glucose transporters such as GLUT 2, 5, 8, and 10 (Aschenbach et al., 2005). These proteins are capable of transporting glucose and possibly fructose and thereby may reduce the amount of monosaccharides fermented in the rumen. The quantitative significance of monosaccharide transport across the rumen is not known. Hall and Weimer (2007) added 65, 130, or 195 mg sucrose to 130 mg of isolated NDF in vitro and reported glucose concentrations (12 h after the initiation of fermentation) which were 3 to 10 times higher than previous values reported in vivo (Lynd et al., 2002); albeit, glucose concentrations were low (< 1m*M*). Differences between concentrations in vitro and in vivo may be related to absorption of glucose across the rumen epithelia. Whether absorption across the rumen epithelia can remove enough monosaccharides to yield improvements in rumen pH requires further study.

1.4 Individual Variation in the Susceptibility to Ruminal Acidosis

Arguably, the most challenging and perplexing aspect of ruminal acidosis is the considerable variation in the susceptibility among individual animals fed a common diet. Such variation has received considerable attention in a recent study

investigating dietary adaptation in beef cattle (Bevans et al., 2005) and in recent reviews focusing on dietary adaptation for feedlot cattle (Brown et al., 2000; Schwartzkopf-Genswein et al., 2003). Despite the documented evidence for variation in the susceptibility in beef cattle, few studies in dairy cattle have identified such variation although it is clearly present (Penner et al., 2007).

The variation among animals for the susceptibility to ruminal acidosis is a concern in dairy production since it is desirable to reduce the occurrence and severity without decreasing performance. Penner et al. (2007) conducted a study investigating the impact of pre-partum dietary adaptation on post-partum ruminal acidosis and reported substantial variation in the susceptibility of heifers regardless of dietary treatment. In fact, mean ruminal pH during the first 5 d of lactation ranged between 5.37 and 6.25, and the duration that pH was below 5.8 ranged between 0 and 17.7 h/d. A physiological understanding of why animals differ in their tolerance to SARA may provide new strategies to reduce SARA.

1.5 Summary

Ruminal acidosis persists as a prevalent digestive disorder in intensive dairy production due to the desire to optimize energy intake to achieve high levels of productivity. Factors influencing the potential for ruminal acidosis include the quantity and rate of acid production in the rumen and the quantity and rate of acid removal through neutralization, absorption, and passage. Regarding acid production, future research is warranted to investigate the effect of carbohydrate

source (starch vs. sugar) on ruminal pH at times when cows are at elevated risk for ruminal acidosis.

As described by Allen (1997) and in the current review, absorptive processes have a dominant role in acid removal from the rumen. Since ruminal pH depression occurs when acid production exceeds acid removal, studies should be conducted to determine the effect of nutritional management on acid removal from the rumen (i.e. absorption and epithelial bicarbonate secretion) and the mechanisms regulating it. Further studies should examine differences in the absorptive capacity of animals differing in their susceptibility to ruminal acidosis.

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2.0 INCREASING DIETARY SUGAR CONCENTRATION MAY IMPROVE DRY MATTER INTAKE, RUMINAL FERMENTATION, AND PRODUCTIVITY OF DAIRY COWS IN THE POST-PARTUM PHASE OF THE TRANSITION PERIOD[1]

2.1 Introduction

The onset of lactation imposes a considerable metabolic challenge to dairy cows as energy intake lags behind the energy expenditure required to achieve and sustain high levels of milk production (NRC, 2001). As a result, cows show a homeorhetic response in which extensive mobilization of peripheral tissues including muscle and adipose tissue occurs (Chibisa et al., 2008) in order to support lactation (Bauman and Currie, 1980). While numerous studies have been conducted to evaluate the carry-over effects of pre-partum diet on post-partum performance (Dann et al., 1999; Rabelo et al., 2003; Penner et al., 2007), there is a paucity of data on nutritional strategies during the post-partum phase of the transition period to improve nutrient intake and lactation performance.

Conventional strategies to improve nutrient intake and performance focus on increasing the dietary energy density. For example, Rabelo et al. (2003) increased the energy density of diets (1.63 and 1.57 Mcal/kg NE_L) by substituting

[1]A version of this chapter has been published. Penner, G.B. and M. Oba. 2009. Increasing dietary sugar concentration may improve dry matter intake, ruminal fermentation, and productivity of dairy cows in the post-partum phase of the transition period. J. Dairy Sci. 92: 3341-3353.

the proportion of ground corn and soybean meal in diets for alfalfa and corn silage. They found that feeding the higher energy diet did not improve milk yield or energy balance, but decreased ruminal pH during a time when cows are already at risk for ruminal acidosis (Penner et al., 2007). Dann et al. (1999) increased energy density of diets by replacing cracked corn grain with steam-flaked corn, and reported increased milk yield for cows fed steam-flaked corn during the first 63 d of lactation without a change in energy balance. However, it is not clear whether the responses observed by Dann et al. (1999) were due to increased dietary energy density or the proportion of ruminally fermentable carbohydrate.

The replacement of starch with sugar has been shown to increase DMI and milk yield for Holstein cows in mid lactation (Broderick and Radloff, 2004). Moreover, Vallimont et al. (2004) observed a quadratic increase in NDF digestibility when sucrose replaced corn starch in continuous culture, and Ribeiro et al. (2005) showed that bacterial OM production in continuous culture increased linearly from 12.3 to 14.4 g/d as the concentration of sucrose increased from 0 to 8%. Potential greater risk for ruminal acidosis must be considered with inclusion of sugar in the diets for post-partum transition cows as they are susceptible to ruminal acidosis (Penner et al., 2007). However, a recent study by Penner et al. (2009a) demonstrated that replacement of cracked corn with sucrose did not decrease ruminal pH. As such, the replacement of starch with sucrose may have the potential to improve nutrient supply and digestibility to transition cows without negatively affecting ruminal fermentation.

The current study was undertaken to investigate the effect of feeding diets containing low or high sugar content to post-partum transition cows on productivity, ruminal fermentation and nutrient digestibility. We hypothesized that the high sugar diet would increase DMI and improve lactation performance. The secondary objective was to characterize changes in sorting behavior, ruminal fermentation, and diet digestibility over the first 4 weeks of lactation.

2.2 Materials and Methods

This experiment was conducted from November 2006 to August 2007 at the University of Alberta Dairy and Research Technology Centre. All experimental procedures were pre-approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and were performed in accordance to the guidelines of the Canadian Council of Animal Care (Ottawa, ON, Canada).

2.2.1 Experimental Design

Fifty-two Holstein cows (28 primiparous and 24 multiparous cows), free of clinical metabolic disorders, were used in this study starting on d 1 of lactation. Twelve additional cows were originally assigned to treatments but were removed from the study due to displaced abomasum (n = 6), mastitis (n = 1), metritis (n = 2), and low feed intake (< 3 kg/d as fed basis, n = 3). For cows on the high sugar (**HS**) treatment, reason for removal from the study were displaced abomasum (n = 2), mastitis (n = 1), and low feed intake (n = 2). Cows on the low sugar (**LS**)

treatment were removed due to displaced abomasum (n = 4), metritis (n = 2), and low feed intake (n = 1). Seven of these cows were primiparous (HS, n = 4; LS, n = 3) and the remaining 5 were multiparous (HS, n = 2; LS, n=3). Ten of the 24 multiparous cows were fitted with a ruminal cannula during a previous lactation such that each treatment consisted of 5 ruminally cannulated cows. Throughout the study, cows were housed in individual tie stalls and were released into an exercise lot for 2 h/d. Cows were fed once daily at 0900 h and the amount of TMR offered was given to yield feed refusals between 5 and 10% of the total feed offered on an as fed basis. Cows were milked twice daily at 0400 and 1500 h.

Based on expected calving date and pre-partum diet, cows were randomly assigned to one of two treatments differing in dietary sugar concentration. Pre-partum diets consisted of either timothy hay based diets with a forage to concentrate ratio of 63:37 (Penner et al., 2008) or the standard pre-partum diet for the University of Alberta Dairy Research and Technology Centre that contained a forage to concentrate ratio of 73: 27 (DM basis). Cows were fed their respective experimental diet on the day of calving if they calved before 1100 h or the following day if cows calved after 1100 h. Dietary treatments in this study were designated as LS (n = 27) or HS (n = 25), and contained 4.5 or 8.8% sugar on a DM basis (Table 1). To increase the dietary sugar concentration without changing the dietary CP and EE content, cracked corn was replaced by sucrose (Wetaskawin Co-op, Wetaskawin, AB, Canada), urea, and canola oil for the HS diet relative to the LS diet. The LS diet did not contain any additional sucrose.

Table 2.1 Ingredient composition, nutrient composition, and particle size distribution of low sugar (LS) and high sugar (HS) diets fed to transition cows during the first four weeks of lactation.

	Treatment	
Ingredient composition, % DM	LS	HS
Barley silage	39.6	39.4
Alfalfa hay	10.1	10.1
Basal concentrate mix[1]	17.5	17.4
rolled barley grain	7.9	7.9
rolled corn grain	7	6.9
Cracked corn grain	4.8	. . .
Sucrose	. . .	4.7
Soy plus	4.2	4.3
Canola meal	2.9	3
Corn gluten meal	2.1	2.2
Rolled peas	1.4	1.4
Sodium bicarbonate	1.3	1.3
Canola oil	0.7	0.9
Calcium carbonate	0.3	0.3
Urea	0.1	0.3
Nutrient composition, % DM		
DM	51.2	51.4
OM	90.8	90.9
CP	20.5	20.2
NDF	34.2	33.4
EE	2.7	2.7
Starch	20.6	18.5
Total ESC[2]	4.5	8.7
NFC[3]	33.4	34.5
Particle Size distribution, %		
>18 mm	31.7	28.9
9 to 18 mm	41.3	37.2
1.18 to 9 mm	18.4	20.1
Pan	8.7	13.8

[1]Contained 39.0% canola meal, 14.0% high fat product (ADM Alliance Nutrition, Lethbridge, AB, Canada), 11.0% AminoPlus (Ag Processing Inc., Omaha, NE), 8.25% ground wheat, 5.75% soybean meal, 4.15% Enertia (ADM Alliance Nutrition, Lethbridge, AB, Canada), 4.00% ground barley, 3.25% Mill-run, 2.13% micromineral and vitamin mix, 2.00% linseed meal, 1.62% limestone, 1.50% sodium bicarbonate, 1.50% canola oil, 1.05% calcium phosphate, and 0.80% salt.

[2]Total ethanol soluble carbohydrates determined according to Hall et al., 1999 using sucrose as a standard.

[3]Nonfiber carbohydrates = 100 - (%NDF + %CP + %EE + %Ash)

Diets were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0.8; University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY, and William H. Miner Agricultural Research Institute, Chazy, NY) to supply adequate NE_L and metabolizable protein for a 650 kg cow producing 35 kg of milk with a fat concentration of 3.5%, and both diets were formulated to contain similar CP and forage NDF concentrations.

2.2.2 Data and Sample Collection

Data and samples were collected on 3 consecutive days during wk 1, 2, 3, and 4 of lactation. The actual initial days of sampling were (average ± SE) d 5.2 ± 0.3, 12.2 ± 0.3, 19.2 ± 0.3, and 26.1 ± 0.3 relative to parturition for wk 1, 2, 3, and 4, respectively.

During the collection periods, the weight of feed offered and refused was recorded daily. A representative sample of the total daily refusal (12.5%) was collected and composited over the 3-d collection period. Feed ingredient samples were collected once weekly except forage samples (barley silage and alfalfa hay) which were collected twice weekly. Individual feed ingredient samples and weekly refusal composites were analyzed for particle size distribution using the modified Penn State Particle Size Separator (Kononoff et al., 2003). All feed ingredient samples were dried in a forced-air oven at 55°C for 48 h to determine the DM content. Diets were adjusted for DM content weekly, if required, to maintain the desired forage-to-concentrate ratio on a DM basis.

Body weight and BCS (5 point scale; Wildman et al., 1982) were measured at the beginning of each collection period. Milk yield was recorded, and milk samples were collected for 6 consecutive milkings during the collection period. Furthermore, blood and fecal samples were collected every 9 h over a 72-h duration. Blood was sampled from the coccygeal vessel into a vacutainer tube containing Na heparin (Fisher Scientific Company, Nepean, Ontario, Canada) and was immediately placed on ice until centrifugation at 2,500 × *g* for 20 min. Harvested plasma was then thawed and composited by cow and week, and stored at -20°C. Fecal samples were also composited by cow and week, stored at -20°C, and the composite was dried at 55°C.

For the 10 ruminally cannulated cows, ruminal pH was measured every 30 s over a 48-h duration in each collection period using the Lethbridge Research Center Ruminal pH Measurement system (Dascor, Escondido, CA) as described by Penner et al. (2006a). Daily nadir, mean, and maximum pH as well as the duration and area that ruminal pH was below 5.8 were determined as described by Penner et al. (2007). Additionally, ruminal digesta was collected from the cranial, ventral, and caudal dorsal regions, and strained through a perforated material (Peetex, pore size = 355 µm; Sefar Canada Inc., Scarborough, Ontario, Canada) immediately after collection. Samples were collected at the same time as blood and feces collection. Ruminal fluid samples were immediately placed on ice until being stored at -20°C. Samples were then thawed and composited to yield one sample per cow per period for analysis.

2.2.2 Sample Analysis

Dried feed ingredient and fecal samples were ground to pass through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA). Fecal samples, weekly forage composites, and monthly concentrate composites were analyzed for concentrations of DM, OM, starch, NDF, and indigestible NDF. All feed ingredient samples were then composited by month, and additionally analyzed for the concentrations of CP, EE, and total ethanol soluble carbohydrates.

Dry matter concentration was determined after drying samples at 135°C for 2 h (AOAC, 2002; method 930.15). Organic matter concentration was calculated as the difference between the DM content and ash content. Ash content was determined after placing samples in a muffle furnace for 5 h at 550°C (AOAC 2002; method 942.05). Starch was measured by an enzymatic method (Karkalas, 1985) after samples were gelatinized with sodium hydroxide and the glucose concentration was measured using a glucose oxidase/peroxidase enzyme (Sigma No. P7119) and dianisidine dihydrochloride (Sigma No. F5803). Absorbance was determined with a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). Neutral detergent fiber concentration was determined using amylase and sodium sulfite (Van Soest et al., 1991). Indigestible NDF was determined after incubating feed ingredient and fecal samples in the rumen of a lactating cow for 120 h, and used as an internal marker to determine the apparent total tract nutrient digestibility (Cochran et al., 1986). Crude protein concentration was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; Rhee et al.,

2005). Ether extract (EE) was determined using a Goldfisch extraction apparatus (Labconco, Kansas City, MO; Rhee, 2005). Total ethanol soluble carbohydrates were determined according to Hall et al. (1999) and Dubois et al. (1956).

Individual milk samples were analyzed for concentrations of fat, CP, lactose, and MUN by infrared spectroscopy (MilkOScan 605, Foss Electric, Hillerod, Denmark; AOAC, 1996) at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada). Milk samples were composited by cow and period based on the yield of milk fat from each milking, and analyzed to determine milk fatty acid profile. Milk fatty acids were extracted and esterified using methanolic acid, and fatty acid profiles were determined using gas chromatography (Khorasani et al., 1991).

Plasma samples were analyzed for glucose, insulin, BHBA, NEFA, and plasma urea nitrogen (PUN) concentrations. Plasma glucose concentration was measured using a glucose oxidase/peroxidase enzyme (No. P7119, Sigma, St. Louis, MO) and dianisidine dihydrochloride (No. F5803, Sigma). Absorbance was determined with a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). A commercial kit was used to determine the plasma concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Plasma BHBA concentration was determined using the enzymatic oxidation of BHBA to acetoacetate using 3-hydroxybutrate dehydrogenase (No. H6501; Roche, Mississauga, ON, Canada), and the concomitant reduction of NAD to NADH was determined using a plate reader at the wavelength of 340 nm. A commercial kit was used to determine the concentration of NEFA in plasma

(NEFA HR 2, Wako Diagnostics, Richmond, VA). The concentration of PUN was determined according to Fawcett and Scott (1960) with modifications for use of a plate reader.

Ruminal fluid samples were centrifuged at 26,000 × *g* for 15 min, and supernatants were collected. The centrifuged supernatant was analyzed for VFA by gas chromatography according to the method described by Khorasani et al. (1996). Rumen NH_3-N concentration was determined colorimetrically as described by Fawcett and Scott (1960).

2.2.3 Calculations and Statistical Analysis

Sorting behavior was evaluated by calculating the sorting index for particles retained on each sieve of the Penn State Particle Size Separator (NASCO, Modesto, CA). The sorting index was calculated by expressing the actual intake of each fraction as a percentage of the theoretical intake of the corresponding fraction (Leonardi and Armentano, 1993). Values <100% and >100% indicate selective refusal and selective consumption, respectively.

The NE_L intake was calculated from apparent total tract DM digestibility according to NRC (2001) with the following modifications. The TDN from EE was calculated assuming that fatty acid content is EE – 1(Allen, 2000) and that fatty acids are completely digestible (NRC, 2001). Thus the equation used to estimate dietary TDN was;

TDN = digested DM + (digested fatty acids × 1.25).

The TDN was then used to calculate dietary NE_L as described in NRC (2001). The energy required for maintenance was calculated as NE_M = 0.080 Mcal/kg $BW^{0.75}$ and NE_L (Mcal/d) was calculated according to NRC (2001) with the observed milk yield and concentrations of milk fat, milk CP, and milk lactose.

Data were analyzed using the PROC MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) accounting for repeated measures. The model included the fixed effects of treatment, parity (primiparous vs. multiparous), week of lactation, pre-partum management, and the two- and three-way interactions between treatment, parity, and week of lactation. The effect of pre-partum management (n = 3) was included in the model because this study was conducted over a 9-mo period and cows received different pre-partum management. The BCS measured in wk 1 was used as a covariate and week of lactation was included as a repeated measure. The treatment × parity × week interaction was not significant for dependent variables of primary interest and therefore was removed from the final statistical model. Treatment means and interaction terms were separated using the Bonferonni test. The SEM presented is a weighted average of the SEM as treatments contained a different number of cows (LS, n = 27; HS, n = 25). Significance was declared when $P < 0.05$ and tendencies are discussed when $P < 0.10$. For ruminally cannulated cows the same statistical model was used; however, the model did not include parity as only multiparous cows were used.

Data were also analyzed using the PROC MIXED procedure of SAS (Version 9.1.3; SAS Institute Inc., Cary, NC) with week of lactation as a

regression variable to further characterize the response over time. Linear and quadratic effects of week were tested.

2.3 Results

2.3.1 Dry Matter Intake and Sorting Behavior

Feeding HS increased DMI (P = 0.035) during the first 4 wk of lactation by 1.1 kg/d when compared to cows fed LS (Table 2). Further, feeding HS altered the sorting behavior of cows including decreased (P = 0.031) sorting for particles retained on the 8-mm sieve and increased (P = 0.006) sorting for particles retained on the pan compared to cows fed LS. Interactions between treatment and week of lactation were detected for the sorting of particles retained on the 8-mm sieves (P = 0.035; data not shown). Cows fed LS sorted for long particles in wk 1, 2, and 4 compared to cows fed HS. Additionally, a treatment × parity interaction was observed for particles retained on the 1.18-mm sieve (P = 0.033) where primiparous cows fed LS sorted against particles retained on the 1.18-mm sieve but no differences were observed for multiparous cows.

Dry matter intake increased linearly over the first 4 wk of lactation (P < 0.001) with average intakes of 14.9, 17.4, 19.0, and 19.8 kg/d for wk 1, 2, 3, and 4 of lactation, respectively. Sorting behavior was also affected by week of lactation. For particles retained on the 19-mm sieve, a quadratic response over time was observed (P = 0.010) as cows tended to increase the selective consumption for long particles from wk 1 to wk 2 but selectively refused

Table 2.2 Dry matter intake and sorting behaviour for cows fed low sugar (LS; n = 27) or high sugar (HS; n = 25) diet during the first four weeks of lactation

	Treatment				Week					*P* value	
Variable	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
DMI, kg/d	17.2	18.3	0.5	0.035	14.9	17.4	19.0	19.8	0.6	< 0.001	0.055
Sorting index[2]											
19 mm sieve	95.7	98.1	2.2	0.335	95.6	101.5	97.8	92.6	2.6	0.187	0.010
8 mm sieve[3]	102.4	99.6	1.1	0.031	101.7	99.9	99.5	103.1	1.3	0.350	0.009
1.18 mm sieve[4]	94.9	97.5	1.7	0.215	92.8	94.4	99.2	98.4	1.9	0.019	0.414
Pan	99.6	108.4	2.9	0.006	110.7	99.9	102.3	103.3	3.4	0.143	0.022

[1]SEM is presented as weighted SEM.

[2]The sorting index was calculated by expressing the actual intake of each fraction as a percentage of the theoretical intake of the corresponding fraction (Leonardi and Armentano, 1993). Values <100% and >100% equate to selective refusal and selective consumption, respectively. Feed refusals and individual feed ingredients were separated using the modified Penn State Particle Size Separator as described by Kononoff et al. (2003).

[3]Treatment × week interaction ($P = 0.035$).

[4]Treatment × parity interaction ($P = 0.033$).

thereafter. A quadratic (P = 0.009) effect of week of lactation was detected for particles retained on the 8-mm sieve where cows increased their selective consumption of this fraction in wk 4 relative to wk 2 and 3. Although cows sorted against particles retained on the 1.18-mm sieve, the selective refusal decreased linearly (P = 0.019) from wk 1 to wk 4 of lactation. Further, a quadratic response (P = 0.022) was observed for sorting for particles retained on the pan; cows decreased the selective consumption of fine particles from wk 1 to wk 2, but the selective consumption did not change after wk 2.

2.3.2 Ruminal Fermentation

For the ruminally cannulated cows used in this study, BW and DMI did not differ between treatments averaging 631 kg and 17.3 kg/d, respectively (Table 3). Feeding HS tended ($P \leq 0.092$) to increase nadir, mean, and maximum ruminal pH by 0.20, 0.15, and 0.18 pH units, respectively. The number of episodes that ruminal pH was below 5.8 was not different between treatments. Furthermore, the duration (min/d and min/kg DMI) and area (pH × min/d) that ruminal pH was below 5.8 were not affected by dietary treatment. Dietary treatment did not affect ruminal VFA concentration, the molar proportions of individual VFA, or concentration of NH_3-N. However, treatment × week interactions ($P \leq 0.019$) were detected for the molar proportions of iso-butryate and iso-valerate where the concentrations increased from wk 1 to wk 4 for cows fed LS but not for cows fed HS.

Table 2.3 Body weight, DMI, and ruminal fermentation for ruminally cannulated cows fed low sugar (LS) or high sugar (HS) diets during the first four weeks of lactation (n = 5 for each treatment)

	Treatment				Week					*P* value	
	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
BW, kg	600	662	24	0.100	634	628	617	647	20	0.657	0.207
DMI, kg/d	16.7	17.9	1.1	0.421	14	17.4	18.3	19.5	1.0	< 0.001	0.128
Ruminal pH											
Nadir	5.42	5.62	0.07	0.092	5.44	5.55	5.54	5.56	0.08	0.227	0.505
Mean	6.06	6.21	0.05	0.076	6.02	6.17	6.18	6.15	0.07	0.113	0.168
Maximum	6.65	6.83	0.06	0.074	6.58	6.82	6.79	6.79	0.06	0.070	0.056
Ruminal pH < 5.8											
Bouts, episodes/d	10.1	6.0	1.9	0.174	9.3	8.2	8.1	6.4	2.2	0.240	0.850
Duration, min/d	322	174	62	0.133	352	209	164	268	100	0.396	0.257
Duration, min/kg DMI	25.7	13.1	5.6	0.153	41.0	12.2	10.2	14.2	8.3	0.051	0.066
Area, pH × min/d	79.4	38.3	19.9	0.184	102.6	46.7	26	60.1	31.8	0.285	0.187
Ruminal VFA and NH_3											
Total VFA, m*M*	120	117	5	0.735	117	101	125	131	5	0.006	0.035
Molar proportions, %											
Acetate	58.9	58.6	1.4	0.868	57.8	57.7	60.6	58.8	1.1	0.08	0.248
Propionate	24.2	24.1	1.1	0.948	25.6	23.7	23.1	24.1	1.0	0.173	0.082
Butyrate	12.2	12.8	0.4	0.256	12.2	13.5	11.9	12.2	0.5	0.522	0.337
Iso-butryate[2]	0.98	0.93	0.06	0.507	0.86	0.99	0.92	1.05	0.05	0.004	0.993
Valerate	1.78	1.69	0.09	0.494	1.74	1.87	1.57	1.76	0.09	0.499	0.690
Iso-valerate[3]	1.53	1.43	0.19	0.736	1.33	1.64	1.45	1.49	0.14	0.303	0.035
Caproate	0.44	0.53	0.05	0.288	0.45	0.52	0.45	0.53	0.05	0.328	0.921
Acetate: propionate	2.46	2.5	0.16	0.874	2.34	2.47	2.66	2.47	0.14	0.144	0.086
NH_3-N, mg/dL	18.8	19.1	2.2	0.927	17.7	16.5	20.4	21.3	1.8	0.005	0.347

[1]SEM is presented as weighted SEM.

[2]Treatment × week interaction (*P* = 0.014).

[3]Treatment × week interaction (*P* = 0.019).

Although week of lactation did not affect BW for the ruminally cannulated cows, DMI increased linearly from wk 1 to wk 4. Nadir, and mean ruminal pH did not change during the first 4-wk of lactation, but the tendency (P = 0.056) for a quadratic effect of week was observed for maximum pH where it increased by 0.24 pH units from wk 1 to wk 2, but did not change after wk 2. The number of episodes, and the duration and area that ruminal pH was below 5.8 were not affected by week of lactation. When the duration that ruminal pH was below 5.8 was normalized for DMI (Penner et al., 2009b), tendency for a quadratic (P = 0.066) effect of week of lactation was detected where the duration/kg DMI was decreased from wk 1 to wk 2, but did not change thereafter.

The concentration of ruminal VFA increased quadratically (P = 0.035) during the first 4 wk of lactation with a numerical reduction in the concentration in wk 2 compared to wk 1, but increased concentration in wk 3 and 4 relative to wk 2 (Table 3). Molar proportions of the majority of individual VFA were not affected by week of lactation with the exception of the branch-chain VFA iso-butyrate and iso-valerate. The molar proportion of iso-butyrate increased linearly (P = 0.004) from wk 1 to wk 4 and the molar proportion of iso-valerate changed quadratically (P = 0.035) with an increase in wk 2 compared to wk 1 but no differences were observed between wk 2, 3, and 4. Ruminal NH_3-N concentration increased linearly (P = 0.005) during the first 4-wk of lactation.

Table 2.4 Apparent total tract digestibility for cows fed low sugar (LS; n = 27) or high sugar (HS; n = 25) diet during the first four weeks of lactation

	Treatment				Week					*P* value	
Variable	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
Digestibility, %											
DM	62.7	64.0	0.8	0.123	64.0	62.4	63.7	63.2	0.9	0.804	0.436
OM	64.7	65.8	0.7	0.169	65.9	64.3	65.5	65.1	0.9	0.747	0.434
NDF[2]	43.0	43.4	1.2	0.761	44.1	41.4	43.6	43.6	1.5	0.842	0.252
Starch[3]	93.3	93.8	0.4	0.331	94.6	93.6	92.9	93.0	0.5	0.007	0.218

[1]SEM is presented as weighted SEM.

[2]Treatment × parity interaction ($P = 0.022$).

[3]Treatment × parity interaction ($P = 0.014$).

2.3.3 Apparent Total Tract Digestibility

The apparent total tract digestibility of DM, OM, NDF, and starch was not affected by dietary treatment averaging 63.3, 65.2, 43.2, and 93.5%, respectively (Table 4). Treatment × parity interactions ($P \leq 0.022$) were detected for the digestibility of NDF and starch (data not shown). The interaction for NDF digestibility was a result of a greater difference in the observed digestibility between primiparous and multiparous cows fed LS (48.5 vs. 37.5 %) than that between primiparous and multiparous cows fed HS (45.9 vs. 40.8 %). Starch digestibility was not different between primiparous and multiparous cows fed HS (93.8%), but multiparous cows fed HS had lower starch digestibility than primiparous cows fed LS (91.5 vs. 95.1 %). Further, multiparous cows fed LS had lower starch digestibility than primiparous and multiparous cows fed HS. Week of lactation did not affect the digestibility of DM, OM, or NDF, but starch digestibility decreased linearly ($P = 0.007$) during the first 4 wk of lactation.

2.3.4 Plasma Metabolites and Hormones

Plasma glucose concentration was lower ($P = 0.006$) for cows fed HS compared to cows fed LS (51.3 vs. 54.0 mg/dL; Table 5); however, plasma insulin concentration was not affected by treatment averaging 4.7 µIU/mL across treatments. Feeding HS increased the concentrations of plasma BHBA (17.5 vs. 10.5 mg/dL; $P = 0.001$) and NEFA (344 vs. 280 µEq/L; $P = 0.013$). Treatment × parity interactions were detected for the concentrations of BHBA and NEFA

Table 2.5 Plasma metabolites and hormones for cows fed low sugar (LS; n = 27) or high sugar (HS; n = 25) diet during the first four weeks of lactation

	Treatment				Week					*P* value	
Variable	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
Glucose, mg/dL	54.0	51.3	0.9	0.006	52.2	51.7	52.6	54.2	1.1	0.082	0.200
Insulin, µIU/mL	4.22	4.11	0.37	0.799	4.12	3.55	4.48	4.53	0.46	0.181	0.477
BHBA[2], mg/dL	10.5	17.5	1.8	0.001	12.3	14.8	14.5	14.5	2.3	0.470	0.515
NEFA[3], µEq/L	280	344	23	0.013	398	349	276	225	28	< 0.001	0.970
PUN, mg/dL	8.01	8.95	0.52	0.098	8.16	7.81	8.84	9.12	0.63	0.079	0.500

[1]SEM is presented as weighted SEM.

[2]Treatment × parity interaction ($P < 0.001$).

[3]Treatment × parity interaction ($P = 0.003$).

($P \leq 0.003$) in plasma where multiparous cows fed HS had higher plasma concentrations of BHBA and NEFA than all other cows. Plasma urea nitrogen concentration tended to be higher ($P = 0.098$) for cows fed HS than cows fed LS. Between wk 1 and 4 of lactation, plasma glucose tended ($P = 0.082$) to increase linearly but plasma insulin concentration was not affected. The concentration of BHBA was not affected by week of lactation, but NEFA concentration decreased linearly ($P < 0.001$) from wk 1 to wk 4. Plasma urea nitrogen concentration tended to increase ($P = 0.079$) during the first 4 wk of lactation.

2.3.5 Lactation Performance

Dietary treatment did not affect BW or milk yield (Table 6). However, the yield of milk fat tended ($P = 0.096$) to be increased by feeding HS compared to LS. Additionally, a treatment × parity interaction was detected for milk fat yield where multiparous cows fed HS had greater milk fat yield than all other cows, and primiparous cows fed HS had the lowest milk fat yield. No differences in milk fat yield were detected between primiparous and multiparous cows fed LS. Milk composition was not affected by treatment but interactions between treatment × parity were detected for milk fat concentration ($P = 0.024$). The interaction for milk fat concentration was a result of multiparous cows fed LS having greater milk fat concentration than primiparous and multiparous cows fed HS but primiparous cows fed LS had lower milk fat concentration than primiparous and multiparous cows fed HS. Concentration of MUN was not affected by dietary treatment.

Table 2.6 Body weight and lactation performance for cows fed low sugar (LS; n = 27) or high sugar (HS; n = 25) diet during the first four weeks of lactation

	Treatment				Week					*P* value	
Variable	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
BW, kg	573	581	7	0.338	598	577	565	567	9	0.001	0.13
Yield, kg/d											
Milk	33	34.4	1.0	0.185	28.8	33.2	35.5	37.3	1.2	< 0.001	0.196
Fat[2]	1.35	1.44	0.05	0.096	1.38	1.39	1.39	1.41	0.06	0.685	0.886
CP	1.05	1.09	0.03	0.226	1.04	1.05	1.07	1.10	0.04	0.147	0.744
Lactose	1.47	1.53	0.05	0.225	1.21	1.47	1.61	1.70	0.06	< 0.001	0.059
Milk composition, %											
Fat[3]	4.21	4.27	0.12	0.668	4.87	4.23	4.01	3.86	0.15	< 0.001	0.047
CP	3.26	3.23	0.05	0.682	3.68	3.21	3.08	3.00	0.06	< 0.001	< 0.001
Lactose	4.43	4.43	0.04	0.994	4.21	4.43	4.54	4.54	0.05	< 0.001	0.013
MUN, mg/dL	14.2	14.2	0.4	0.908	13.6	14	14.6	14.7	0.4	0.017	0.557

[1]SEM is presented as weighted SEM.

[2]Treatment × parity interaction ($P = 0.024$).

[3]Treatment × parity interaction ($P < 0.001$).

Body weight decreased linearly from wk 1 to wk 4 resulting in a net loss of 31 kg. Over the same time, milk yield and milk lactose yield increased linearly ($P < 0.001$). The increase in milk yield between wk 1 and wk 4 was 8.5 kg/d, and lactose yield increased by nearly 0.5 kg/d. No changes in the milk fat or CP yield were detected over the first 4 wk of lactation, but the concentrations of milk fat ($P = 0.047$) and CP ($P < 0.001$) decreased quadratically from wk 1 to wk 4 of lactation with greater reductions at the earlier stage of lactation. Milk lactose concentration increased quadratically ($P = 0.013$) with greater increases at the earlier stage of lactation. Milk urea nitrogen concentration increased linearly ($P = 0.017$).

2.3.6 Milk Fatty Acid Composition

Feeding HS had a minor effect on milk fatty acid composition as the majority of measured fatty acids were not affected by treatment. However, cows fed HS had lower concentrations of $C_{4:0}$ ($P = 0.013$) and $C_{18:1trans}$ ($P = 0.042$) fatty acids in milk fat (Table 7). A treatment × week interaction ($P = 0.010$) was detected for the concentration of $C_{16:0}$ where cows fed LS had a numerically higher concentration than cows fed HS in wk 1.

Week of lactation had strong effects on the concentration of fatty acids with differences being detected in nearly all measured individual fatty acids ($P \leq 0.010$). In general, the concentrations of short and mid-chain fatty acids increased from wk 1 to wk 4 with the exception of butyric acid. Long-chain fatty acids $C_{16:0}$, $C_{17:1}$, $C_{18:2}$, and $C_{20:0}$ were not affected by week of lactation but the

Table 2.7 Milk fatty acid composition (% of total fat) for ruminally cannulated multiparous cows fed low sugar (LS) or high sugar (HS) diet during the first four weeks of lactation (n = 5 for each treatment).

	Treatment				Week					*P* value	
Milk fatty acid composition, %	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
$C_{4:0}$	1.78	1.51	0.06	0.013	1.68	1.7	1.54	1.65	0.1	0.581	0.939
$C_{6:0}$	1.33	1.15	0.09	0.179	1.12	1.23	1.28	1.33	0.08	0.009	0.564
$C_{8:0}$	0.91	0.8	0.07	0.273	0.69	0.8	0.91	1.02	0.06	< 0.001	0.991
$C_{10:0}$	2.36	2.03	0.24	0.370	1.75	1.96	2.37	2.69	0.2	< 0.001	0.699
$C_{12:0}$	2.73	2.38	0.29	0.427	2.12	2.25	2.75	3.11	0.25	< 0.001	0.469
$C_{14:0}$	9.63	8.84	0.78	0.498	8.23	8.47	9.71	10.52	0.66	< 0.001	0.467
$C_{14:1}$	0.62	0.73	0.05	0.158	0.58	0.6	0.72	0.79	0.05	< 0.001	0.489
$C_{15:0}$	0.95	0.86	0.08	0.442	0.79	0.83	0.96	1.04	0.07	< 0.001	0.551
$C_{16:0}$[2]	27.3	26.6	1.1	0.650	27.7	26.6	26.8	26.7	0.9	0.138	0.164
$C_{16:1}$	2.19	2.32	0.14	0.532	2.45	2.44	2.14	2	0.13	0.001	0.548
$C_{17:0}$	0.65	0.66	0.03	0.882	0.73	0.68	0.61	0.59	0.03	< 0.001	0.654
$C_{17:1}$	0.44	0.36	0.14	0.698	0.52	0.27	0.54	0.26	0.17	0.460	0.873
$C_{18:0}$	11.4	11.4	0.4	0.959	11.8	12.1	11.1	10.7	0.4	0.011	0.316
$C_{18:1\ trans}$	3.14	2.72	0.12	0.042	2.71	2.84	3.11	3.07	0.13	0.010	0.458
$C_{18:1\ cis}$	28.9	31.4	1.6	0.309	31.4	31.4	29.3	28.5	1.4	0.018	0.655
$C_{18:2}$	2.66	2.68	0.18	0.922	2.71	2.66	2.71	2.6	0.15	0.440	0.731
$C_{20:0}$	0.51	0.67	0.07	0.141	0.61	0.58	0.59	0.58	0.05	0.366	0.548
$C_{20:1}$	0.39	0.39	0.04	0.978	0.34	0.36	0.43	0.43	0.03	< 0.001	0.547
C_4 to C_1	19.4	17.5	1.4	0.378	16.2	17.1	19.4	21.1	1.2	< 0.001	0.630
C_{18} to C_{20}	49.4	52	2.1	0.424	52.2	52.3	49.9	48.3	1.8	0.010	0.437

[1]SEM is presented as weighted SEM.

[2]Treatment × week interaction ($P = 0.010$).

concentrations of $C_{16:1}$, $C_{17:0}$, $C_{18:0}$, $C_{18:1\ trans}$, $C_{18:1\ cis}$, and $C_{20:1}$ were affected by the week of lactation ($P \leq 0.018$). Generally, the concentration of long chain fatty acids decreased during the first 4 wk of lactation, but the concentrations of $C_{18:1\ trans}$ and $C_{20:1}$ increased linearly.

2.3.7 Energy Balance

Energy intake was increased ($P = 0.018$) by feeding HS compared to LS (Table 8). The energy expended for lactation tended ($P = 0.094$) to be higher for cows fed HS than cows fed LS, but no differences were observed for the net energy required for maintenance. The calculated total energy output for cows fed HS tended ($P = 0.084$) to be greater than that for cows fed LS, and consequently the calculated energy balance was not different between treatments averaging -7.92 Mcal/d over the first 4 wk of lactation.

A linear ($P < 0.001$) increase in energy intake was observed between wk 1 and 4 of lactation. However, this increase in energy intake corresponded to a linear increase ($P = 0.008$) in the energy expended in milk and a linear decrease ($P = 0.001$) in the energy required for maintenance. Overall energy output increased linearly ($P = 0.041$) from wk 1 to wk 4. These changes resulted in a linear increase ($P < 0.001$) in the energy balance from wk 1 to wk 4, but cows were still in a negative energy balance in the 4^{th} wk of lactation.

Table 2.8 Calculated energy intake, expenditure, and energy balance for cows fed low sugar (LS; n = 27) or high sugar (HS; n = 25) diet during the first four weeks of lactation

	Treatment				Week					*P* value	
Variable	LS	HS	SEM[1]	*P* value	1	2	3	4	SEM[1]	Linear	Quadratic
NE_L intake, Mcal/d	25.2	27.2	0.7	0.018	22.3	25.3	28.1	29.2	0.9	< 0.001	0.227
NE_L output[2], Mcal/d	24.1	25.4	0.7	0.094	23.4	24.5	25.2	25.9	0.8	0.008	0.739
NE_M, output Mcal/d	9.35	9.44	0.09	0.369	9.66	9.4	9.25	9.27	0.11	0.001	0.134
Total net energy output, Mcal/d	33.5	34.9	0.7	0.084	33	34	34.5	35.2	0.9	0.041	0.852
Net energy balance, Mcal/d	-8.31	-7.52	0.86	0.393	-10.81	-8.62	-6.33	-5.91	1.04	< 0.001	0.317

[1]SEM is presented as weighted SEM.

[2]Treatment × parity interaction ($P = 0.041$).

2.4 Discussion

2.4.1 Effects of Dietary Sucrose Concentration

During the postpartum phase of the transition period, cows undergo marked changes including rapid increases in both DMI and milk yield (NRC, 2001). These changes are accompanied by an increase in hepatic gluconeogenesis and adipose tissue mobilization to support the energy demand for lactation (Ingvartsen and Andersen, 2000; Drackley et al., 2001). Despite the well documented changes during the transition period (Reynolds et al., 2003; Reynolds et al., 2004) and the number of review papers dedicated to this unique stage of production (Drackley, 1999; Ingvartsen and Andersen, 2000; Overton and Waldron, 2004), few studies have focused on dietary strategies to optimize performance during the postpartum phase of the transition period.

Past studies have reported increases in DMI (Nombekela et al., 1994; Broderick and Radloff, 2004) or total organic matter intake (Heldt et al., 1999) when diets contained sucrose. For example, Broderick and Radloff (2004) fed dried or liquid molasses as a source of sugar in replacement for high moisture corn grain, and found increased DMI with additional sugar. In a more recent study, Broderick et al. (2008) reported a linear increase in DMI as the proportion of sugar increased from 0 to 7.5% in increments of 2.5%. Dietary modifications that increase DMI during the postpartum phase of the transition period should also increase energy intake and may improve lactation performance.

The results of the current study confirm previous findings (Broderick and Radloff, 2004; Broderick et al., 2008) that replacement of corn with sucrose

increases DMI, and demonstrate a potential nutritional strategy for increasing DMI during early lactation. Although we hypothesized that feeding HS would increase DMI and lactation performance, feeding HS did not increase milk yield even though it increased energy intake. However, milk fat yield and milk energy output tended to be higher for cows fed HS compared to cows fed LS. Because energy intake was increased and energy output tended to be higher for cows fed HS, the overall energy balance was not different between treatments. The data from the current study demonstrate that increasing the energy supply to cows in early lactation may not result in improvements in energy balance.

Based on the data collected in the current study it is difficult to define mechanisms responsible for the greater DMI observed for cows fed HS over LS. In a past study, Nombekela et al. (1994) evaluated the palatability of diets with a sweet, sour, salty, or bitter taste by comparing the voluntary DMI and preferential eating order of each respective diet. They found that diets containing sucrose were preferentially eaten 59% of the time, and that sucrose inclusion (1.5% DM basis) increased DMI by 12% compared to the control diet. In a subsequent study, Nombekela and Murphy (1995) fed diets with (1.5% of dietary DM) or without sucrose to cows during the first 12 wk of lactation. In that study they found that the inclusion of sucrose did not affect DMI, and as such, small improvements in palatability likely have a marginal impact on DMI during early lactation. Past studies have reported increased NDF passage to the omasum (Broderick et al., 2008) with increased dietary sucrose concentration while others have reported increases in the solid or liquid passage rates with sucrose (Rooke et

al., 1987; Sutoh et al., 1996). As such, it may be possible that in the current study sucrose increased digesta passage rate leading to the observed increase in DMI. Further studies are warranted to determine the mechanisms responsible for increased DMI when sucrose replaces corn grain.

In the literature, the effect of sucrose on milk yield is variable with some studies reporting increases in milk yield (Broderick and Radloff, 2004) and others reporting no effect of sugar supplementation on milk yield (Nombekela and Murphy, 1995; Cherney et al., 2003; Broderick et al., 2008). In the current study, sucrose did not affect milk yield. The response may be partially due to the amount of sugar included; Broderick and Radloff (2004) found quadratic effects of dietary sugar inclusion on milk yield where low dietary sugar concentration (up to 7%) increased milk yield but diets exceeding 7% sugar decreased milk yield. They further concluded that the optimal dietary sugar concentration was approximately 5% (DM basis). In our study, dietary sugar concentration as determined using total ethanol soluble carbohydrates were 4.5 and 8.7% for LS and HS, respectively. As such, our LS treatment was close to the optimal level defined by Broderick and Radloff (2004) and the HS treatment was above the range defined as optimal, which may explain the lack of treatment effect on milk yield.

In the current study, we found that milk fat yield tended to be higher for cows fed HS compared with LS. Our results are supported by past research demonstrating a linear increase in milk fat yield with increasing dietary sucrose concentration (Broderick et al., 2008). In contrast, Nombekela and Murphy

(1995) utilized cows during the first 12-wk lactation and found no effect of sucrose on milk fat yield and further reported a decrease in milk CP yield for cows fed 1.5% sucrose. Differences between our study and Nombekela and Murphy's study (1995) may be due to the higher sucrose inclusion rate used in our study. In addition, Nombekela and Murphy (1995) investigated the first 12 wk of lactation whereas we investigated the first 4-wk of lactation only. The increase in milk fat yield in our study may be attributed to increased mobilization of adipose tissue for cows fed HS compared to those fed LS as milk fat yield was positively related to the concentrations of plasma NEFA ($r = 0.384$, $P < 0.001$; data not shown). In addition, in our study, milk fat yield was positively related to plasma BHBA concentration ($r = 0.308$, $P < 0.001$; data not shown). Kessel et al. (2008) also reported that cows with elevated levels of BHBA in plasma had greater milk fat yield. Further, the increase in milk fat yield observed in our study is consistent with the improved ruminal pH status for cows fed HS compared to those fed LS.

During the postpartum phase of the transition period, cows are at risk for ruminal acidosis (Penner et al., 2007) and increases in the ruminally degradable carbohydrate may exacerbate this condition. Contrary to our pre-trial hypothesis, we observed tendencies for increased nadir, mean, and maximum ruminal pH for cows fed HS compared to those fed LS. Past research has shown that the in vitro rate of sucrose hydrolysis ranges between 1200 and 1400%/h with fermentation of the comprising monosaccharides ranging between 400 and 620%/h (Weisbjerg et al., 1998). As such, the observed tendencies for an increase in nadir ruminal pH

and mean pH, and an increase in maximum pH for cows fed HS compared to those fed LS is surprising. In fact, cows fed HS had higher rumen pH during wk 1 and 4 of lactation than cows fed LS. Previous in vitro (Vallimont et al., 2004) and vivo studies (Heldt et al., 1999; Broderick and Radloff, 2004; Broderick et al., 2008) have reported no effect of sucrose on rumen pH and more recently, Penner et al. (2009a) reported that sucrose tended to improve ruminal pH which is consistent with our results.

There are several possible explanations for why the replacement of starch for sucrose improved ruminal pH status in the current study. One possibility is that a portion of the dietary sucrose was respired prior to consumption by the cows; however, the proportion of sucrose aerobically oxidized is expected to be insignificant as diets were made fresh daily (Owens et al., 2008). Secondly, disappearance of carbohydrates from the rumen may not necessarily result in fermentation acid production if organic matter is converted to microbial N (Allen, 1997), or stored as glycogen (Hall and Weimer, 2007). Although in vitro, sucrose has previously been shown to increase the efficiency of microbial N production per kg OM (Ribeiro et al., 2005), ruminal NH_3-N was not affected by treatment questioning whether possible increases in microbial N production in the current study could result in the improved pH status observed. Further, we did not measure bacterial glycogen content and are unable to speculate on its contribution to improved pH status in the current study. The results of the current study demonstrate that the replacement of corn starch with sucrose does not increase the

risk of ruminal acidosis, and therefore may be a viable nutritional strategy during early lactation.

Data in the current study were covariate adjusted using BCS in the first week of lactation. Therefore, it is not expected that differences in the concentrations of plasma BHBA and NEFA between treatments were due to differences in BCS at the time of parturition. We observed that cows fed HS had 23 and 67% increases in the plasma concentrations of NEFA and BHBA, respectively, compared to cows fed LS. The increase in BHBA and NEFA concentrations may be cause for concern for ketosis and fatty liver disease. Duffield et al., (2009) recently suggested that plasma BHBA concentrations exceeding 14.4 mg/dL during the first 2-wk of lactation place cows at greater risk of experiencing hyperketonemia. This indicates that cows fed HS may be at greater risk for hyperketonemia than cows fed LS; however, cows fed HS had greater DMI than cows fed LS suggesting that the elevated concentrations of BHBA did not negatively impact animal performance.

Hepatic NEFA uptake is related to blood flow and the plasma concentration of NEFA (Grummer, 2008). Although we did not measure liver triglyceride content, it is possible that cows fed HS had increased liver triglyceride which may have decreased hepatic gluconeogenesis. This is supported by the observation of lower plasma glucose concentration for cows fed HS compared to those fed LS. However, the calculated energy balance was not different between treatments. As such, the elevated concentrations of BHBA and

NEFA for cows fed HS may indicate impaired hepatic function, but further research is required to confirm this speculation.

2.4.2 Effects of Week of Lactation

Dry matter increased linearly over the first 4-wk of lactation, which is consistent with past studies (Reynolds et al., 2003; Reynolds et al., 2004). To the authors' knowledge this is the first study that investigated the sorting behavior of cows during early lactation. During the first 4 wk of lactation, we observed marked changes in the sorting behavior regardless of the dietary treatment. In general, cows decreased sorting for fine particles from wk 1 to wk 2, but increased sorting for fine particles from wk 2 to wk 4. It is unclear why cows altered their sorting behavior but past studies using cows in mid-lactation have suggested that changes in sorting behavior may occur in response to ruminal pH status (Yang and Beauchemin, 2007; DeVries et al., 2008).

Few studies have comprehensively examined changes in ruminal pH during early lactation; however, the results of Fairfield et al. (2007), Penner et al. (2007), and the current study all suggest that cows in early lactation experience severe ruminal acidosis. For example, we observed that cows spent on average 352, 210, 164, and 268 min/d with ruminal pH below 5.8 during wk 1, 2, 3, and 4 of lactation,. Similarly, Penner et al. (2007) reported that the severity of ruminal acidosis, as indicated by the duration that ruminal pH was below 5.8, did not differ between the first 5-d of lactation and on d 17, and d 37 of lactation, but they did report a reduction in the severity of ruminal acidosis by d 58 of lactation.

Although DMI was lowest during the first week of lactation, the time that ruminal pH was below 5.8 per kg of DMI was greatest. The intake of fermentable organic matter is one of the factors influencing ruminal pH (Nocek, 1997; Allen, 1997), yet clearly during early lactation other factors are also playing a role. A classical study by Dirksen et al. (1985) suggested that the absorptive surface area of the ruminal papillae limits VFA absorption and they further recommended feeding additional fermentable carbohydrate pre-partum to increase ruminal papillae surface area and speculated that it would prevent ruminal pH depression post-partum. However, subsequent studies utilizing diets that are typically fed in North America (Andersen et al., 1999; Penner et al., 2006b) have not supported Dirksen et al. (1985). Alternatively, the activity of the ruminal epithelia may be decreased by short-term feed restriction (Gäbel and Aschenbach, 2002) as cows approaching parturition reduce DMI by approximately 30% (Hayirli et al., 2002). Thus, the combined effect of reduced epithelia function and increased diet fermentability following parturition may partially explain the increased severity of ruminal acidosis. However, other factors such as un-adapted microflora (Nagaraja and Titgemeyer, 2007) during diet transition may lead to increased lactate production (Owens et al., 1998) although lactate accumulation in dairy cows is rare (Nocek et al., 1997). A recent study did not detect ruminal lactate concentrations above 5m*M* when ruminally cannulated transition cows were fed pre-partum diets differing in the forage-to-concentrate ratio (Penner et al., 2007).

Visceral tissue mass increases in early lactation in response to increased DMI (Reynolds et al., 2004), and therefore we hypothesized that nutrient

digestibility would also increase as lactation progresses due to hypertrophy of the digestive tract. However, the results of the current study do not support our hypothesis. Apparent total tract digestibility of DM, OM, and NDF did not change over the first 4 wk of lactation and the digestibility of starch decreased over time. Past studies have either not investigated the change in digestibility over time (measured at 23 to 26 DIM; Dann et al., 1999) or have evaluated changes later in lactation (wk 6 and 14; Aikman et al., 2008). It is unclear why starch digestibility decreased over time as the ruminal microflora would be expected to adapt to the diet during the first 4 wk of lactation increasing starch digestion (Tajima et al., 2000) and increasing the supply of ruminal fermentable carbohydrate has been shown to increase ruminal starch digestion (Oba and Allen, 2003). It may be possible that the increase in DMI observed during the first 4 wk of lactation is associated with an increase in passage rate thereby decreasing starch digestion if hydrolysis of starch via pancreatic amylase in the small intestine is limited (Richards et al., 2003; Harmon et al., 2004). However, increased passage rate should have also affected the digestibility of other nutrients such as NDF, which did not occur in the current study. As such, we are unable to speculate why starch digestibility decreased over time and further studies are warranted to verify this finding.

2.5 Conclusions

The results of the present study demonstrate that the replacement of cracked corn grain with sucrose may increase DMI and increase milk fat yield for

post-partum transition cows although it may decrease plasma glucose concentration and increase adipose tissue mobilization. Further, the replacement of corn with sucrose may reduce the severity of ruminal acidosis but the mechanisms behind this response require further investigation. Collectively, the results of the current study indicate that replacement of cracked corn grain with sucrose may provide a viable strategy to improve the productivity of post-partum transition cows.

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3.0 EFFECT OF DIETARY FORAGE TO CONCENTRATE RATIO ON VOLATILE FATTY ACID ABSORPTION AND THE EXPRESSION OF GENES RELATED TO VFA ABSORPTION AND METABOLISM IN RUMINAL TISSUE[2]

3.1 Introduction

Ruminal acidosis is one of the most prominent digestive disorders in commercial dairy production with prevalence, diagnosed using ruminocentesis, ranging between 12 and 30% throughout lactation (Krause and Oetzel, 2006). The majority of past research examining ruminal acidosis has focused on dietary factors including ration particle size distribution, diet fermentability, and microbial factors (Nagaraja and Titgemeyer, 2007; Enemark, 2008). In contrast, the absorption and clearance of acid from the rumen has received little attention. Allen (1997) calculated that approximately 53% of H^+s produced during anaerobic fermentation were removed from the rumen through the absorption of VFA across the ruminal wall implying that absorptive metabolism may have a strong impact on the regulation of ruminal pH.

[2]A version of this chapter has been published. Penner, G.B., M. Taniguchi, L.L. Guan, K.A. Beauchemin, and M. Oba. 2009. The dietary forage to concentrate ratio does not affect the rate of volatile fatty acid absorption but alters the expression of genes regulating energy metabolism in rumen tissue. J. Dairy Sci. 92: 2767-2781.

Past research has demonstrated that diet can affect the absorptive metabolism in ruminal tissue. Diets containing a higher proportion of concentrate have previously been shown to increase VFA disappearance from the rumen by absorption (Gäbel et al., 1991) and the net absorption rate measured in Ussing chambers (Uppal et al., 2003). Further, feeding high concentrate diets increased the oxidation of acetate and butyrate in steers per unit of tissue (Harmon et al., 1991), and the activity of Na^+/K^+ ATPase/mg protein in rumen tissue from lambs (McLeod and Baldwin, 2000) relative to those fed low concentrate diets suggesting that the regulation of VFA transport likely differs between animals fed diets and the extent to which animals are predisposed to low ruminal pH and a high ruminal VFA concentration. Gäbel and Aschenbach (2006) reviewed the literature and identified numerous regulatory factors involved in the absorption of VFA including regulation of intracellular pH and catabolism of VFA. However, the majority of reviewed studies were conducted using sheep epithelia justifying further investigation into regulatory mechanisms in cattle.

We hypothesized that feeding more concentrate would increase the fractional rate of VFA absorption in vivo, and would up-regulate the relative expression of genes involved in the regulation of VFA absorption and metabolism. As such, the objective of this study was to investigate the in vivo rate of VFA absorption, and the expression of genes encoding for transporters and enzymes involved in VFA absorption and energy metabolism in ruminal tissue when cattle were fed high or low concentrate diets.

3.2 Materials and Methods

3.2.1 Animals and Dietary Treatments

Twelve non-pregnant, non-lactating, ruminally cannulated Holstein cows from the Lethbridge Research Centre were used for this study in accordance to the guidelines of the Canadian Council on Animal Care (Ottawa, ON). All procedures were pre-approved by the Lethbridge Research Centre Animal Care Committee. Cows were housed in tie-stalls bedded with wood shavings and were allowed to exercise daily.

Cows were randomly assigned to one of two dietary treatments after being blocked by age and BW. The treatments were designated as LC and HC, and contained concentrate at 8 and 64% of the dietary DM (Table 1), respectively. Diets were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0.8.01; University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY, and William H. Miner Agricultural Research Institute, Chazy, NY) to supply adequate metabolizable protein and energy for a dry cow weighing 650 kg. The nutrient composition of the HC and LC diets is presented in Table 1. Increasing the proportion of barley grain in replacement for barley silage numerically increased the dietary DM from 48.5% to 67.8%, and reduced the concentration of dietary NDF by nearly 16%. The concentration of CP was similar across treatments averaging 12.7%. The LC and HC diets had forage to concentrate ratios of 92:8 and 36:64, respectively. Adaptation onto the HC diet

Table 3.1 Ingredient and nutrient composition of the low (LC) and high concentrate (HC) diets

	Treatment	
	LC	HC
Ingredient, % DM		
Alfalfa hay	14.5	5.7
Rolled barley grain	4.1	44.3
Barley Silage	77.2	30.1
Ground barley grain	2.0	16.9
Canola meal	1.2	1.3
Mineral and vitamin mix[1]	1.0	1.7
Canola oil	0.02	0.09
Nutrient Composition		
DM	48.5	67.8
Ash, % DM	9.8	6.7
CP, % DM	12.5	12.9
NDF, % DM	44.2	28.3
Forage NDF, % NDF	97.0	59.0
Forage NDF, % DM	42.9	16.7
% Forage	91.7	35.8

[1]Contained 36.3% Na, 55.7% Cl, 9151 ppm Zn, 2294 ppm Cu, 3545 ppm Mn, 33.1 ppm Co, 82.4 ppm I, 761.1 KIU Vitamin A, 190.3 KIU Vitamin D, and 4507.0 KIU Vitamin E.

was carried out over 10 d, and cows received their respective treatment for at least 28 d before slaughter. Cows were fed for ad libitum intake in two equal portions at 0800 and 1300 h daily, and had free access to water.

3.2.2 Experimental Measurements and Procedures

Each blocked pair of cows was subjected to a 5-d collection period after a 28-d adaptation period. Collection periods were staggered for each pair. Body weight and BCS (5-point system; Wildman et al., 1982) were measured on the first day of collection. The amounts of feed offered and refused were measured daily during the entire collection period, and samples of the feed refusals were composited over the collection period and dried for DM determination at 55°C. Feed ingredients were collected daily and composited by week. Dried dietary ingredients were ground to pass through a 1-mm screen using a Wiley mill (Thomas-Wiley, Philadelphia, PA), and analyzed for concentrations of DM, ash, CP, and NDF. Dry matter concentration was determined after drying samples at 135°C for 2 h (AOAC, 1990; method 930.15), and the ash concentration was determined after placing the samples in a muffle furnace for 5 h at 550°C (AOAC 1990; method 942.05). Crude protein concentration was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy; Rhee et al., 2005). Neutral detergent fiber concentration was determined using amylase and sodium sulphite (Van Soest et al., 1991).

3.2.2.1 Ruminal pH Measurement.

Ruminal pH was measured every 30 s, and data were averaged over 1-min intervals for the first 72 h of each collection period using the Lethbridge Research Centre Ruminal pH Measurement System (**LRCpH**; Dascor, Escondido, CA) as previously described by Penner et al. (2006a). Daily minimum, mean, and maximum pH values were averaged for each cow. The occurrence and extent of ruminal pH depression was summarized as the number of episodes, duration, and area below the pH threshold of 5.8 (Penner et al., 2007). An acidosis index was calculated by dividing the area that ruminal pH was less than 5.8 by DMI to evaluate the severity of ruminal acidosis normalized for DMI. The use of acidosis index allows us to evaluate whether the severity of ruminal acidosis is related to differences in absorptive metabolism of VFA by removing confounding effects of DMI on ruminal pH.

3.2.2.2 Ruminal Fluid and Blood Collection and Analysis.

Ruminal fluid and blood were collected every 9 h over a 72-h duration starting on d 1 of each collection period to account for diurnal variation. Sub-samples of ruminal fluid were collected in equal proportions from the cranial dorsal, cranial ventral, central rumen, caudal dorsal, and caudal ventral regions (250 mL from each region), and combined to form one sample. Ruminal fluid was strained through a perforated material (Peetex, pore size = 355 µm; Sefar Canada Inc., Scarborough, Ontario, Canada), and the 10-mL sample was added to 2 mL of 25%-metaphosphoric

acid. Samples were stored at -20°C and a composite sample for each cow was used for analysis.

Ruminal VFA were separated and quantified by gas chromatography. Samples were injected by an auto sampler (model 8200, Varian, Walnut Creek, CA) into a Stabilwax®- DA column (30 m × 0.53 mm I.D. × 0.5 µm DF) on a Varian GC (model 3400, Varian). The samples were run at a split ratio of 20:1 with a column temperature of 90 to 170°C with an increase of 10°C/min followed by a 2-min hold. The injector and detector temperatures were 170 and 190°C, respectively. Peak integration was performed using Galaxie Software (Varian). All ruminal fluid samples were assayed in duplicate. Ruminal NH_3-N concentration was determined colorimetrically as described by Fawcett and Scott (1960).

Blood was collected from the coccygeal vessel into evacuated tubes containing Na-heparin (Fisher Scientific Company, Nepean, Ontario, Canada). Plasma was harvested by centrifuging the blood samples at 3000 × *g* for 25 min immediately after collection. A composite sample of plasma was prepared from the 8 individual sampling times, and all samples were stored at -20°C until analysis.

Plasma samples were analyzed for glucose, insulin, BHBA, and plasma urea nitrogen (**PUN**) concentrations. Plasma glucose concentration was measured using a glucose oxidase/peroxidase enzyme (No. P7119, Sigma, St. Louis, MO) and dianisidine dihydrochloride (No. F5803, Sigma). Absorbance was determined with a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA). A commercial kit was used to determine the plasma

concentration of insulin (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Plasma BHBA concentration was determined using the enzymatic oxidation of BHBA to acetoacetate using 3-hydroxybutrate dehydrogenase (No. H6501; Roche, Mississauga, ON, Canada), and the concomitant reduction of NAD to NADH was determined using a plate reader at a wavelength of 340 nm. The concentration of PUN was determined according to Fawcett and Scott (1960), but modified to include urease at the beginning of the procedure.

3.2.2.3 Ruminal VFA Clearance and Volume.

Ruminal VFA clearance rate was estimated on d 4 of each collection period using Co-EDTA (Uden et al., 1980) as a fluid passage marker, and n-valeric acid as an indicator of VFA clearance from the rumen (Allen et al., 2000; Resende Júnior et al., 2006). The n-valeric acid was used as a marker for VFA absorption as Resende Júnior et al. (2006) found that the fractional rate of VFA absorption and passage obtained using the Co-EDTA and n-valeric acid method did not differ from results obtained using individual VFA labelled with ^{13}C. To minimize fluctuations in baseline valeric acid concentration, cows were provided their feed in three equal proportions at 1300, 2100, and 0500 starting the day before the marker dose. Each cow received a 2.5-L solution containing 300-g n-valeric acid (VWR International, Edmonton, AB, Canada), and 125-g Co-EDTA buffered to pH 6.2 with NaOH. The Co-EDTA/n-valeric acid solution was thoroughly mixed by hand into the ruminal contents. An initial sample was collected immediately before the marker dose (time 0), and subsequent ruminal fluid samples were

collected at 30, 60, 120, 180, 360, 720, 1080, and 1440 min after the dose. Individual samples of ruminal fluid were analyzed for valeric acid concentration as previously described, and for Co concentration using atomic absorption (model AA240FS, Varian, Palo Alto, CA).

Baseline concentrations (time 0) of Co and n-valeric acid were used to correct concentrations measured at each individual time point (i.e. 30, 60, 120, 180, 360, 720, 1080, and 1440 min relative to dosing). The exponential rate of decay for Co and n-valeric acid were calculated as described by Resende Júnior et al. (2006) using the PROC NLIN procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) using the equation $R_t = R_0 \times e^{-k \times t}$.

Where:

R_t = concentration at a given time

R_0 = concentration at time 0

k= fractional rate of clearance

t = time, h

To calculate the fractional rate of VFA absorption, the rate of Co clearance was subtracted from the rate of n-valeric acid clearance.

Ruminal content volume was determined by completely evacuating the rumen 1500 min after the marker dose. The ruminal digesta was thoroughly mixed and duplicate samples were taken to determine ruminal content DM (55°C) and the solid and liquid pool sizes.

3.2.2.4 Cow Transportation and Slaughter.

One to 2 d following the collection period, cows were transported to a licensed abattoir, and euthanized by captive bolt stunning and exsanguination. Whole ruminal tissue samples including the mucosal and submucosal layers were collected from the ventral sac, rinsed in 90% saline, and snap-frozen in liquid nitrogen. Tissues were stored at -80°C until further analysis.

3.2.2.5 RNA Extraction and Reverse Transcription.

Ruminal tissue was ground using a mortar and pestle under liquid nitrogen. Total RNA was extracted from ground ruminal tissue using Trizol (Invitrogen, Burlington, ON, Canada) as described by the manufacturer. The RNA concentration was determined by measuring the absorbance at 260 and 280 nm using NanoDrop (ND-1000; NanoDrop Technologies, Wilmington, DE). All samples had an absorbance ratio (260:280) between 1.91 and 2.29 indicating high RNA purity. Samples were then diluted to contain 100 ng RNA/µL. All RNA samples were treated with DNase I (Invitrogen) to remove potential genomic DNA contamination. The Superscript II kit (Invitrogen, Burlington, ON, Canada) was used to synthesize single strand complementary DNA (cDNA).

3.2.2.6 Quantitative Real-Time PCR (qRT-PCR).

Bovine specific primers and Taqman probes used in this study were designed by Primer Express software (Applied Biosystems, Foster City, CA), and it was

confirmed that they included the intron/exon boundary. The target genes of interest and their respective Genbank accession number and primer sequences are listed in Table 2. Quantitative real-time PCR was conducted using a 7900HT Fast Real-Time PCR System (Applied BioSystems) with a 10 min pre-incubation at 95°C followed by 40 cycles composed of 15 s at 95°C and 60 s at 60°C. All samples were analyzed in triplicate.

The point at which the florescent signal of the PCR product crossed the threshold was deemed to be the threshold crossing value (C_T). The average C_T for each cow and gene was compared to each cow's respective average C_T for the housekeeping gene ribosomal protein, large P0 (**RPLP0)** by subtracting C_T of RPLP0 from the C_T of the target gene to calculate ΔC_T. To compare the expression of genes between treatments, the cow with the lowest acidosis index (pH < 5.8 × min/d ÷ DMI, kg/d) was used as the calibrator. The cow with the lowest acidosis index was on the LC treatment and had an acidosis index value of 0.00. The ΔC_T of this cow was then used as a reference value to calculate $\Delta\Delta C_T$ which yielded a $\Delta\Delta C_T$ value of 0 for the cow used as the calibrator. Furthermore, the fold change in gene expression was calculated as $2^{-\Delta\Delta CT}$ (Livak et al., 2001; Ontsouka et al., 2004).

3.2.3 Statistical Analysis

Data were analyzed as a randomized complete block design using the PROC MIXED procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC) with the fixed effects of block (6 blocks were used to pair cows balanced for age and

Table 3.2 Gene name, NCBI accession number, and primer and probe sequences for quantitative real-time PCR analysis

Gene name (abbreviation)	Accession number	Primer and Probe Sequences	Functional Pathway
Ribosomal protein, large, P0 (RPLP0)	NM_001012682	Forward: AGGGCGTCCGCAATGTT Reverse: CGACGGTTGGGTAACCAATC Probe: CCAGCGTGTGCCTG	Endogenous control
Acyl-CoA synthetase short-chain family member 1 (acyl-CoA synthetase)	BC114698.1	Forward: CCGATCAGGTCCTGGTAGTGA Reverse: GCCTCCGCATGACTTTTCC Probe: CGTCTTCCAAAAACT	SCFA activation
Acetyl-CoA synthetase 2 (acetyl-CoA synthetase)	DQ489534	Forward: GCTCTCACTGAGGAGCTCAAGAA Reverse: AATCCGGTGTGGCAATGG Probe: AGATTAGAGAAAAGATTGGC	SCFA activation
Acyl-CoA synthetase medium-chain family member 1 (butyrl-CoA synthetase)	BC109602	Forward: ACCCTTTGACATTCAGATCATTGAT Reverse: CCAATGTTTCCTTCCGTGTTG Probe: CAAGGGCAATATCCAGC	SCFA activation
Solute carrier family 25, carnitine/acylcarnitine translocase, member 20 (acyl-carnitine)	NM_001077936	Forward: CTCAAGTCCCGCTTCCAGACT Reverse: CCTCAGCACATCTTTGAAACCA Probe: CTCCTGGGAAATATCCTA	Fatty acid transport
Acetyl-CoA acyltransferase 1 (acetyl-CoA acyltransferase)	BC102927	Forward: CACTGGCTTCCCAGCAAAA Reverse: ACGATCTCGGCCTGGAAAC Probe: AGCCAGAGCCCAGAGA	Ketogenesis
3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGS)	AY581197	Forward: AGGATACTCATCACTTGGCCAACT Reverse: CATGTTCCTTCGAAGAGGGAAT Probe: CATTCCCCAGAGTTCCA	Ketogenesis
3-hydroxymethyl-3-methylglutaryl-CoA lyase, hydroxymethylglutaricaciduria (HMGL)	NM_001075132	Forward: TGCAGATGGGAGTGAGTGTCA Reverse: GACGCCCCCTGTGCATAG Probe: TGGCAGGACTGGGAG	Ketogenesis

Table 3.2 Continued. Gene name, NCBI accession number, and primer and probe sequences for quantitative real-time PCR analysis

Gene name (abbreviation)	Accession number	Primer and Probe Sequences	Functional Pathway
3-hydroxybutyrate dehydrogenase, type 1 (BDH1)	NM_001034600	Forward: GACTGCCACCACTCCCTACAC Reverse: TCCGCAGCCACCAGTAGTAGT Probe: CGCTACCATCCCATG	Ketogenesis
3-hydroxybutyrate dehydrogenase, type 2 (BDH2)	NM_001034488	Forward: CTGTGGCTTCCAGCATCAAA Reverse: CGCCTTGGTTGTGCTGTACA Probe: CGTTGTGAACAGGTGC	Ketogenesis
Propionyl Coenzyme A carboxylase, alpha polypeptide (Propionyl-CoA carboxylase)	BC123876	Forward: AGAATGGAAGATGCCCTGGAT Reverse: CCTCTCGAAGCAATGCGATAT Probe: TTATGTTATTCGAGGTGTTACAC	Propionate metabolism
Lactate dehydrogenase isoform A (LDHa)	BC146210	Forward: GGCAAAGACTATAATGTGACAGCAA Reverse: ACGTGCCCCAGCTGTGA Probe: CTCCAGGCTGGTTATT	Pyruvate metabolism
Lactate dehydrogenase isoform B (LDHb)	BC142006	Forward: CCAACCCAGTGGATATTCTCACA Reverse: TCACACGGTGCTTGGGTAATC Probe: TGTTACCTGGAAACTAAG	Pyruvate metabolism
Pyruvate carboxylase (PC)	NM_177946	Forward: CTCCCACCATCTGTCCTTTCC Reverse: TTTATTTGGCAGGAGATGAATACG Probe: TGCGGTCGACAGTCA	Pyruvate metabolism
Pyruvate dehydrogenase, lipoamide, alpha 1 (PDH)	NM_001101046	Forward: CAGTTTGCTACTGCTGATCCTGAA Reverse: AGGTGGATCGTTGCAGTAAATGT Probe: ACCTTTGGAAGAACTCGGCTA	Pyruvate metabolism

Table 3.2 Continued. Gene name, NCBI accession number, and primer and probe sequences for quantitative real-time PCR analysis

Gene name (abbreviation)	Accession number	Primer and Probe Sequences	Functional Pathway
Isocitrate dehydrogenase 1, NADP+, soluble (IDH)	BC103368	Forward: CTGGACCTGCACAGCTACGAT Reverse: TGGTGACCTGGTCGTTGGT Probe: TAGGCATAGAGAATCGCGA	Tricarboxylic acid cycle
Oxoglutarate, alpha-ketoglutarate dehydrogenase, lipoamide (α-ketogluturate dehydrogenase)	NM_001076030	Forward: CTGTCCACGTGGTTGTCAACA Reverse: GCCATTCGCGGGTCTGT Probe: CCAGATCGGCTTCAC	Tricarboxylic acid cycle
Solute carrier family 25, mitochondrial carrier; adenine nucleotide translocator, member 4 (ADP/ATP translocator 4)	NM_174658.1	Forward: TTTGTACTTAAGCACTATCCTCTCTTTTG Reverse: AAATGCCCGACGGAGAAAC Probe: CAGAGCCGCCTACTT	Oxidative phosphorylation
Solute carrier family 25, mitochondrial carrier; adenine nucleotide translocator, member 5 (ADP/ATP translocator 5)	NM_174659.1	Forward: AGGGCGCAAAGGAACTGATA Reverse: GCAATCTTCCTCCAGCAGTCA Probe: CATGTACACAGGCACGC	Oxidative phosphorylation
Solute carrier family 16, member 3, monocarboxylic acid transporter 4, (MCT4)	NM_001109980	Forward: ATCTACGCGGGATTCTTTGGAT Reverse: AAGGTCCATCAGCGTTTCAAAC Probe: TGCTTTTGGGTGGCTC	Intracellular pH regulation
ATPase, Na+/K+ transporting, alpha 1 polypeptide (Na/K ATPase)	NM_001076798	Forward: CATCTTCCTCATCGGCATCA Reverse: ACGGTGGCCAGCAAACC Probe: TGTAGCCAACGTGCCAG	Intracellular pH regulation
Solute carrier family 9 sodium/hydrogen exchanger, member 1 antiporter, $Na+/H^+$, amiloride sensitive (Na/H antiporter)	U49432	Forward: GAAAGACAAGCTCAACCGGTTT Reverse: GGAGCGCTCACCGGCTAT Probe: AAGTACGTGAAGAAGTGTCT	Intracellular pH regulation

BW) and treatment. Upon analysis, block was removed from the model as it was not significant for variables of primary interest. Therefore, data were re-analyzed as a completely randomized design with the fixed effect of treatment. Significance was declared when $P < 0.05$ and trends are discussed when $P < 0.10$.

3.3 Results

3.3.1 Dry Matter Intake, Ruminal Fermentation, Ruminal Volume, and VFA Absorption

Dietary treatment did not affect DMI (Table 3) however, large variation was observed in both treatments. For example, DMI ranged from 10.6 to 20.2 kg/d and from 11.3 to 20.4 kg/d for the HC and LC treatments, respectively. Body weight and BCS were not affected by treatment with mean values of 816 kg, and 3.85 BCS across treatments, respectively.

Cows fed HC had greater total VFA concentration in ruminal fluid than cows fed LC (138 vs. 114 m*M*; $P < 0.01$). Furthermore, feeding HC reduced ($P < 0.01$) the molar proportion of acetate by 20%, and increased ($P < 0.05$) the molar proportions of propionate, butyrate, valerate, and isovalerate by 28, 32, 39, and 53% respectively. The acetate to propionate ratio was lower ($P < 0.01$) for cows fed the HC treatment than cows fed the LC treatment (2.36 vs. 2.98).

Minimum and mean pH were nearly 0.6 and 0.5 pH units lower ($P < 0.01$), respectively, for cows fed HC than those fed LC. Maximum pH was not different

Table 3.3 Effect of feeding low (LC) or high concentrate (HC) diet on DMI and rumen fermentation

	Treatment			
Variable	LC	HC	SEM	*P* value
DMI, kg/d	14.0	15.8	1.4	0.387
BW, kg	813	820	46	0.914
BCS	3.83	3.88	0.24	0.904
Rumen VFA				
Total VFA, m*M*	113.7	138.0	4.8	0.005
Acetate, %	68.6	54.8	1.4	< 0.001
Propionate, %	17.4	24.2	1.4	0.006
Butyrate, %	10.3	15.2	1.1	0.009
Isobutyrate, %	1.0	0.9	0.0	0.155
Valerate, %	1.2	1.9	0.2	0.025
Isovalerate, %	1.2	2.5	0.4	0.030
Caproate, %	0.4	0.5	0.2	0.797
Acetate:propionate	3.98	2.36	0.22	< 0.001
Rumen pH				
Minimum	5.90	5.31	0.06	< 0.001
Mean	6.48	6.03	0.04	< 0.001
Maximum	6.85	6.72	0.05	0.110
Standard deviation	0.20	0.34	0.02	0.002
Ruminal pH < 5.8				
Episodes, no/d	0.3	13.5	1.1	< 0.001
Duration, min/d	10	376	43	< 0.001
Area, pH × min/d	0.8	88.7	12.9	< 0.001

between treatments. Feeding the HC diet effectively increased the severity of ruminal acidosis as indicated by increased episodes (13.5 vs. 0.3 /d; $P < 0.01$), duration (376 vs. 10 min/d; $P < 0.01$), and area (88.7 vs. 0.8 pH × min/d; $P < 0.01$) that pH was below 5.8.

3.3.2 Ruminal Volume and VFA Absorption

Ruminal digesta weight and DM concentration were not different between treatments, averaging 87.5 kg and 13.5%, respectively (Table 4). Dietary treatment did not affect the fractional rate of VFA absorption or passage from the rumen, averaging 23.4 and 9.6 %/h, respectively. In addition, diet did not affect the estimated rates of total VFA or acetate absorption, which averaged 2.22 and 1.16 mol/h, respectively. However, the estimated rates of propionate and butyrate absorption were 0.23 and 0.14 mol/h greater ($P < 0.05$) for cows fed HC compared to those fed LC, respectively. The estimated passage rate of VFA (mol/h) was not different between treatments.

3.3.3 Plasma Hormones and Metabolites

Plasma glucose concentration was not affected by dietary treatment, averaging 64.3 mg/dL, but plasma insulin concentration was three times higher for cows fed HC compared to LC ($P = 0.02$; Table 5). In addition, cows fed the HC treatment had higher plasma BHBA concentration than cows fed the LC

Table 3.4 Effect of feeding low (LC) or high concentrate (HC) diet on rumen volume, and VFA absorption and passage

	Treatment			
Variable	LC	HC	SEM	*P* value
Rumen volume				
Rumen contents, kg	90.0	85.0	5.5	0.530
Rumen DM, %	13.3	13.7	0.6	0.593
Rumen DM, kg	11.9	11.6	0.7	0.831
Liquid fraction, kg	78.2	73.4	5.0	0.513
VFA absorption and passage				
Total VFA absorption[1], %/h	22.7	24.2	1.9	0.591
Total VFA passage[2], %/h	9.6	9.7	1.0	0.944
Total VFA absorption[3], mol/h	1.97	2.47	0.23	0.190
Acetate[4], mol/h	1.18	1.15	0.13	0.880
Propionate[5], mol/h	0.35	0.58	0.06	0.046
Butyrate[6], mol/h	0.18	0.32	0.03	0.024
Passage[7], mol/h	0.83	1.00	0.09	0.228

[1]Total VFA absorption was estimated as the fractional rate of n-valeric acid clearance - the fractional rate of Co-EDTA clearance (Allen et al. 2000; Resende Júnior et al., 2006).

[2]Total VFA passage was assumed to be equal to the fractional clearance rate of Co-EDTA (Allen et al. 2000; Resende Júnior et al., 2006).

[3]VFA concentration (mol) × rumen liquid volume (kg) × fractional rate of VFA absorption (%/h).

[4]Estimated according to Resende Júnior et al. (2006) where the fractional rate of acetate absorption = 5.19 × fractional rate of n-valeric acid + 0.7427.

[5]Estimated according to Resende Júnior et al. (2006) where the fractional rate of propionate absorption = 19.2 × fractional rate of n-valeric acid + 0.4073.

[6]Estimated according to Resende Júnior et al. (2006) where the fractional rate of butyrate absorption = 7.21 × fractional rate of n-valeric acid + 0.7000.

[7]VFA concentration (mol) × rumen liquid volume (kg) × fractional rate of VFA passage (%/h).

Table 3.5 Effect of feeding low (LC) or high concentrate (HC) diets on plasma glucose, insulin, BHBA, and urea N

	Treatment			
Variable	LC	HC	SEM	*P* value
Glucose, mg/dL	64.2	66.4	1.78	0.400
Insulin, μIU/mL	9.5	28.5	4.6	0.015
BHBA, mg/dL	12.2	13.5	0.4	0.053
Urea N, μg/mL	14.1	13.9	1.2	0.929

treatment (13.5 vs. 12.2 mg/dL; P = 0.05). The concentration of plasma urea N was not affected by dietary treatment.

3.3.4 Gene Expression

The majority of the genes evaluated in ruminal tissue were not affected by dietary treatment (Table 6). Gene expression for three enzymes involved in the activation of VFA prior to metabolism, namely acyl-CoA synthetase short-chain family member 1 **(acyl-CoA synthetase)**, acetyl-CoA synthetase 2 **(acetyl-CoA synthetase)**, and acyl-CoA synthetase medium-chain family member 1 **(butyrl-CoA synthetase)** were not affected by treatment. Further, the gene expression for the solute carrier family 25, carnitine/acylcarnitine translocase, member 20 **(acyl-carnitine)** was not affected by dietary treatment. We selected five genes to evaluate in the pathway of ketogenesis including acetyl-CoA acyltransferase 1 **(acetyl-CoA acyltransferase)**, 3-hydroxy-3-methylglutaryl-CoA synthase 1 **(HMGS)**, 3-hydroxymethyl-3-methylglutaryl-CoA lyase hydroxymethylglutaricaciduria **(HMGL)**, and two isoforms of 3-hydroxybutyrate dehydrogenase: 3-hydroxybutyrate dehydrogenase, type 1 (**BDH1**) and type 2 **(BDH2).** There was no effect of feeding HC on the expression of any of the genes evaluated for the ketogenesis pathway.

Pyruvate is a key intermediate that can enter numerous pathways. As such, we evaluated the expression of genes coding for pyruvate carboxylase (**PC**), pyruvate dehydrogenase lipoamide alpha 1 **(PDH)**, and two isoforms of lactate dehydrogenase (**LDHa** and **LDHb**). Although the expression for PC and both

Table 3.6 Fold change in the expression of genes encoding for enzymes and transporters involved in the absorption and metabolism of VFA in rumen tissues for cows fed high concentrate (HC) or low concentrate (LC). Data are presented as fold change relative to the cow with the lowest acidosis index (DMI ÷ area pH < 5.8, pH × min).

		Relative FC[1]			
Gene (abbreviation)	Functional Pathway	LC	HC	SEM	*P* value
Acetyl-CoA synthetase 2 (acetyl-CoA synthetase)	SCFA metabolism	1.38	1.47	0.41	0.878
Acyl-CoA synthetase short-chain family member 1 (acyl-CoA synthetase)	SCFA metabolism	2.65	4.05	0.73	0.214
Acyl-CoA synthetase medium-chain family member 1 (butyrl-CoA synthetase)	SCFA metabolism	5.42	3.06	2.49	0.523
Solute carrier family 25, carnitine/acylcarnitine translocase, member 20 (acyl-carnitine)	SCFA transport	0.87	0.87	0.09	0.993
Acetyl-CoA acyltransferase 1 (acetyl-CoA acyltransferase)	SCFA metabolism	2.89	1.99	1.12	0.590
3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGS)	SCFA metabolism	1.29	0.94	0.25	0.345
3-hydroxymethyl-3-methylglutaryl-CoA lyase, hydroxymethylglutaricaciduria (HMGL)	SCFA metabolism	1.42	1.33	0.27	0.804
3-hydroxybutyrate dehydrogenase, type 1 (BDH1)	SCFA metabolism	2.48	2.05	0.33	0.384
3-hydroxybutyrate dehydrogenase, type 2 (BDH2)	SCFA metabolism	0.89	1.1	0.13	0.270
Propionyl Coenzyme A carboxylase, alpha polypeptide (Propionyl-CoA carboxylase)	SCFA metabolism	1.49	1.46	0.18	0.896

Table 3.6 Continued. Fold change in the expression of genes encoding for enzymes and transporters involved in the absorption and metabolism of VFA in rumen tissues for cows fed high concentrate (HC) or low concentrate (LC). Data are presented as fold change relative to the cow with the lowest acidosis index (DMI ÷ area pH < 5.8, pH × min).

		Relative FC[1]			
Gene (abbreviation)	Functional Pathway	LC	HC	SEM	*P* value
Lactate dehydrogenase isoform A (LDHa)	Pyruvate metabolism	0.96	0.99	0.06	0.753
Lactate dehydrogenase isoform B (LDHb)	Pyruvate metabolism	1.55	1.47	0.37	0.877
Pyruvate carboxylase (PC)	Pyruvate metabolism	1.38	3.48	1.85	0.445
Pyruvate dehydrogenase, lipoamide, alpha 1 (PDH)	Pyruvate metabolism	0.76	0.53	0.08	0.063
Isocitrate dehydrogenase 1, NADP+, soluble (IDH)	Tricarboxylic acid cycle	1.05	0.79	0.13	0.185
Oxoglutarate, alpha-ketoglutarate dehydrogenase, lipoamide (α-ketogluturate dehydrogenase)	Tricarboxylic acid cycle	1.03	0.95	0.22	0.795
Solute carrier family 25, mitochondrial carrier; adenine nucleotide translocator, member 4 (ADP/ATP translocator 4)	Oxidative phosphorylation	0.75	0.85	0.13	0.614
Solute carrier family 25, mitochondrial carrier; adenine nucleotide translocator, member 5 (ADP/ATP translocator 5)	Oxidative phosphorylation	0.67	0.66	0.22	0.970
Solute carrier family 16, member 3, monocarboxylic acid transporter 4, (MCT4)	SCFA transport	1.42	1.34	0.2	0.775
ATPase, Na+/K+ transporting, alpha 1 polypeptide (Na/K ATPase)	SCFA transport	1.41	1.16	0.42	0.685
Solute carrier family 9 sodium/hydrogen exchanger, member 1antiporter, $Na+/H^+$, amiloride sensitive (Na/H antiporter)	SCFA transport	1.57	1.11	0.36	0.396

[1]Relative FC was calculated based on the cow with the lowest acidosis index

isoforms of LDH were not affected by dietary treatment, cows fed HC tended to have 1.4 times lower expression (0.53 vs. 0.76; $P = 0.06$) of PDH than cows fed LC. Isocitrate dehydrogenase 1 NADP+ soluble **(IDH)** and oxoglutarate alpha-ketoglutarate dehydrogenase lipoamide **(α-ketogluturate dehydrogenase)** are two rate-limiting enzymes in the citric acid cycle; however, dietary treatment did not affect the expression of genes encoding for either enzyme. In addition, we evaluated the expression of genes encoding for three transporters involved in intracellular pH regulation, and found that dietary treatment did not affect the expression of the ATPase Na+/K+ transporting alpha 1 polypeptide **(Na/K ATPase)**, solute carrier family 9 sodium/hydrogen exchanger member 1 antiporter $Na+/H^+$ amiloride sensitive **(Na/H antiporter)** and solute carrier family 16 member 3 monocarboxylic acid transporter 4 **(MCT4)**.

3.3.5 Observed Variation within the HC Treatment

There was substantial variation detected among the 6 animals fed the HC diet for many response variables (data not shown). Daily minimum, mean, and maximum pH ranged from 5.1 to 5.6, 5.9 to 6.2, and 6.5 to 6.9, respectively. The duration that ruminal pH was below 5.8 ranged between 127 and 557 min/d with an average value of 376 min/d. Similarly, the area that ruminal pH was below 5.8 ranged from 14.9 to 154 pH × min/d. The SEM for the area that ruminal pH was below 5.8 was greater than the area for the cow with the lowest value (18.2 vs. 14.9 pH × min/d respectively) indicating substantial variation. The acidosis index

was calculated to normalize the response in ruminal pH for DMI and ranged between 6.4 and 17.2 pH × min/kg DMI with a mean of 11.9 pH × min/kg DMI.

Among cows fed the HC diet, no significant relationships were detected between the rate (%/h and mol/h) of in vivo VFA absorption and the expression of genes related to VFA absorption and metabolism in ruminal tissue (data not shown). However, PDH tended to be negatively correlated to the fractional rate of VFA absorption ($P = 0.09$, r = -0.75; data not shown).

Furthermore, among cows fed the HC diet, the acidosis index was negatively or tended to be negatively related with the expression of genes involved in the activation of butyrate and enzymes in the pathway of ketogenesis, namely butyrl-CoA synthetase and HMGL ($P = 0.01$ and 0.08, respectively; Figure 1). In both cases, one cow was detected as an outlier (DFFITS coefficient > 1.26), and therefore the data are presented with and without the outlier. In the case of HMGL, removal of the outlier improved the correlation and resulted in a significant relationship. Furthermore, the expression of LDHb and PC were negatively related to the acidosis index ($P = 0.03$ and 0.02, respectively; Figure 2), but the negative relationships for these genes were not observed when an outlier was removed from the dataset. The gene expression for MCT4 tended ($P = 0.07$) to be positively related to the acidosis index (r = 0.77; data not shown).

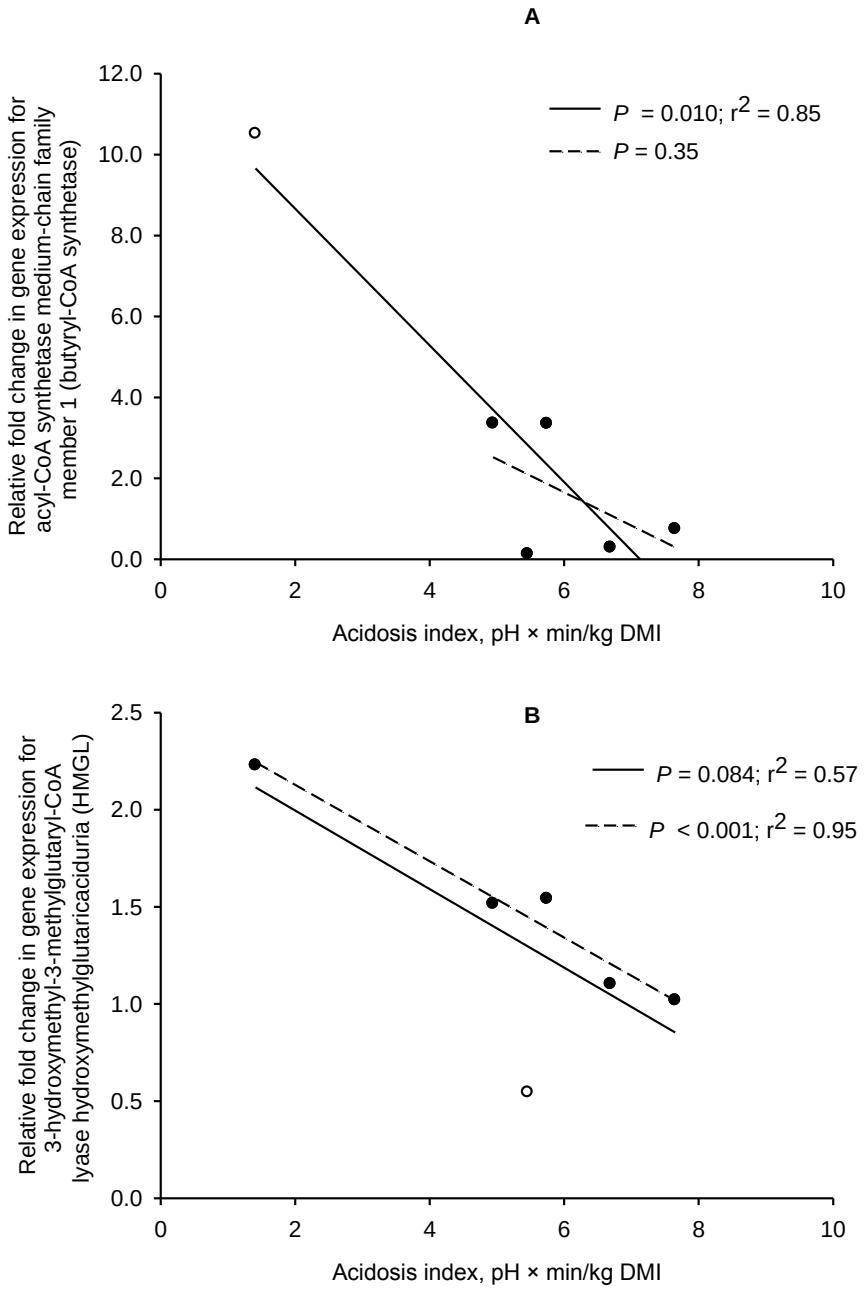

Figure 3.1 Relationship between the acidosis index and the relative fold change in gene expression for acyl-CoA synthetase medium-chain family member 1 (butyrl-CoA synthetase) (A: all data pair; *P* = 0.01, r^2 = 0.85, without outlier; *P* = 0.35) and 3-hydroxymethyl-3-methylglutaryl-CoA lyase hydroxymethylglutaricaciduria (HMGL) (B: all data pair; *P* = 0.08, r^2 = 0.57, without outlier; *P* < 0.01, r^2 = 0.95). The solid line indicates the regression including all data points and the dashed line indicates the regression with the outlier removed. Solid black filled place markers indicate data points used in both regression lines and the white filled place marker indicates the outlier. Outliers were assessed using a DFFITS coefficient of 1.26.

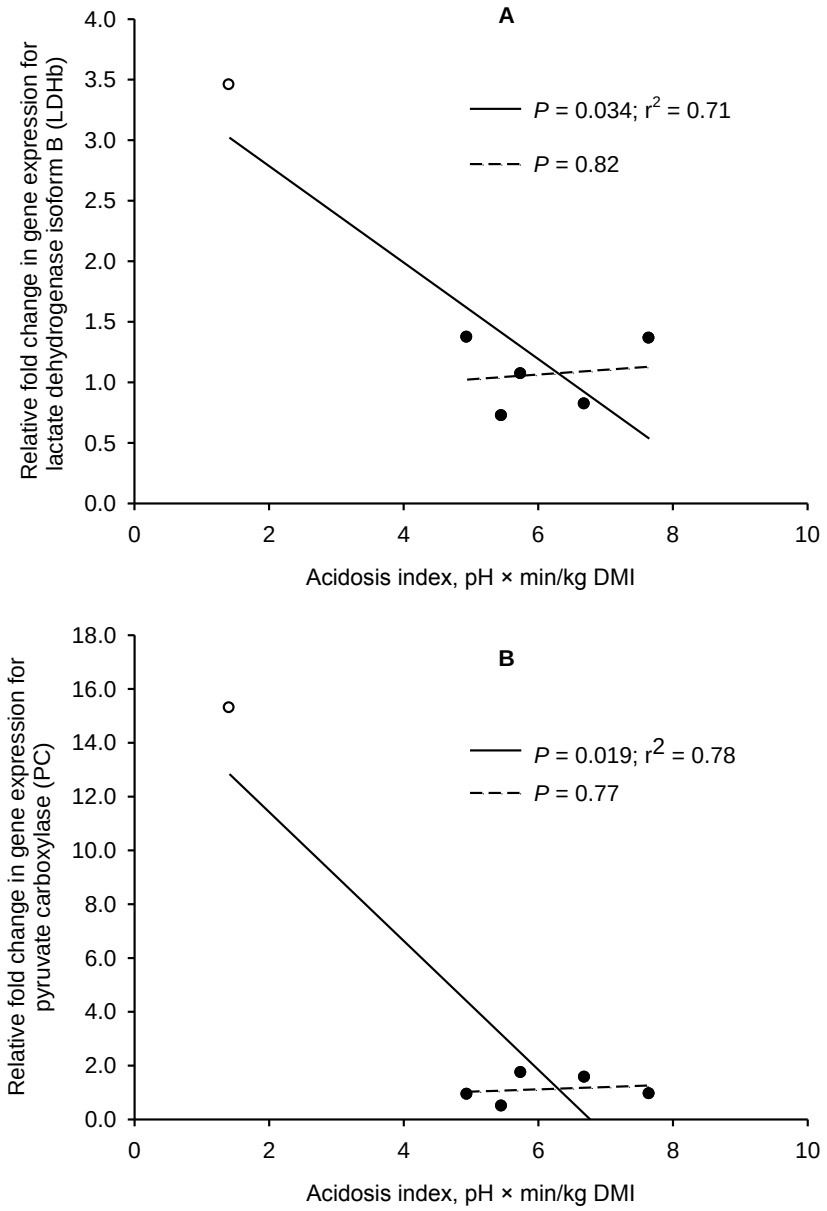

Figure 3.2 Relationship between the acidosis index and the relative fold change in gene expression for lactate dehydrogenase isoform B (LDHb) (A: all data pair; *P* = 0.03, r^2 = 0.71, without outlier; *P* = 0.82) and pyruvate carboxylase (PC) (B: all data pair; *P* = 0.02, r^2 = 0.78, without outlier; *P* = 0.77), and. The solid line indicates the regression including all data points and the dashed line indicates the regression with the outlier removed. Solid black filled place markers indicate data points used in both regression lines and the white filled place marker indicates the outlier. Outliers were assessed using a DFFITS coefficient of 1.26.

3.4 DISCUSSION

3.4.1 VFA Absorption

The treatments used in this study successfully induced large differences in ruminal fermentation as indicated by differences in the total VFA concentration, molar proportions of individual VFA, and ruminal pH variables including mean pH, and the duration and area that pH was below 5.8. In fact, cows on the LC treatment experienced a depression in ruminal pH below 5.8 for only 10 min/d; whereas, cows on the HC treatment had ruminal pH below 5.8 for nearly 6.5 h/d. These data indicate that cows fed the HC diet experienced ruminal acidosis as defined by Penner et al. (2007) although no clinical signs were noted. The results reported in this study for increased total VFA concentration, decreased molar proportion of acetate, and decreased ruminal pH in response to feeding the HC treatment are in agreement with previous studies that used diets with differing forage to concentrate ratios (Harmon et al., 1991, Baldwin and McLeod, 2000).

We observed marked differences in the ruminal fermentation measurements including VFA profiles and ruminal pH, but we did not detect differences in the fractional (%/h) or the estimated (mol/h) rate of total VFA absorption or acetate absorption (mol/h) and their passage from the rumen between cows fed HC and LC diets. However, when the absorption rates of individual VFA were estimated according to the calculation described by Resende Júnior et al. (2006), we found that the rates of propionate and butyrate absorption (mol/h) were higher for cows fed HC compared to LC. This was primarily due to the greater ruminal pool sizes of propionate and butyrate for HC cows compared

to LC cows as fractional rate of VFA absorption was not affected by treatment. Although the estimated absorption rates of propionate and butyrate (mol/h) were greater for cows fed HC compared with those fed LC, we did not observe differences in the mRNA transcript abundance for the majority of genes investigated nor did we detect correlations between the fold change in gene expression and the estimated absorption rate for propionate or butyrate. As such, the current study does not support the idea that differences observed in the estimated rate of propionate and butyrate absorption are caused by nor result in greater absorptive metabolism of propionate and butyrate.

We had hypothesized that the fractional rate of VFA absorption would be greater for cows fed HC compared to LC as previous studies have consistently demonstrated that lower ruminal pH and feeding high grain diets increase the rate of VFA absorption. For example, Dijkstra et al. (1993) used the washed reticulo-rumen technique to measure the fractional rate of VFA absorption as influenced by ruminal infusates with different pH values. They reported that as the pH of the infusate decreased from 7.2 to 4.5, the disappearance rate of propionate and butyrate increased, but that of acetate was not affected. Furthermore, Sehested et al. (2000) reported increases in the net flux rate of butyrate (μmol/cm^2/h) across ruminal epithelia for cows fed grain once daily compared to those not fed grain.

There are several possible explanations for our observation that the overall fractional rate of VFA absorption did not differ between cows fed HC vs. LC. Firstly, it may be possible that diets and ruminal fermentation do not affect fractional rate of VFA absorption for non-lactating non-pregnant cows that have

low energy demand. Supporting this theory, Seal et al. (1992) reported that the rate of VFA absorption, as detected in the portal vein, was not different for steers fed an all forage pellet or a diet containing 50% of the forage pellet and 50% flaked-maize. Other studies examining VFA absorption have been conducted in vitro and have measured VFA absorption per area of epithelia exposed (Sehested et al., 2000) or have measured the fractional rate of VFA disappearance under artificial conditions in vivo (washed reticulo-rumen technique; Dijkstra et al., 1993), and therefore should be interpreted with caution. For example, with the washed reticulo-rumen technique, the ratio of the ruminal content volume to ruminal surface area is lower than that under physiological conditions. Decreasing the ratio of ruminal content volume to ruminal surface area would likely increase the efficiency of ruminal contractions to mix ruminal contents thereby exposing more substrate to the ruminal epithelia, and possibly over-estimating the fractional rate of VFA absorption. Supporting this speculation, Dijkstra et al. (1993) reported a 25% reduction in the fractional rate of butyrate disappearance from the rumen when ruminal content volume was increased by 20 L.

The second possibility is that the treatments imposed in this study did not elicit large changes in the absorptive surface area of the ruminal epithelia, and consequently did not affect the fractional rate of absorption. Dirksen et al. (1985) suggested that maximum papillae growth is achieved after 6-8 weeks of feeding a more fermentable diet, but Penner et al. (2006b) suggested that less time is required under conventional feeding systems in North America where higher

energy diets are fed. Further, the assumption that papillae surface area limits absorption has been questioned based on in vitro studies (Andersen et al., 1999; Sehested et al., 2000).

A third possibility is that the low ruminal pH observed for the HC treatment may have caused additional keratinization of the ruminal epithelia thereby reducing the fractional absorption rate. We did not conduct measurements on ruminal papillae gross morphology or histology in this study, and therefore are unable to speculate whether changes in surface area or keratinisation affected our results for VFA absorption.

Finally, it is also possible that the model used to measure the fractional rate of VFA absorption is not sensitive enough to detect differences. The lack of sensitivity may be due to inherent limitations of the method. For example, in the current study, following the ruminal dose of the marker, we observed large increases in ruminal osmolarity from a baseline of 323 mOsm before dosing to a peak osmolarity of 357 mOsm at 30 min after the dose (data not shown). The temporal increase in osmolarity may have decreased the absorption rate (Owens et al., 1998) for both treatments masking treatment effects. Furthermore, the Co-EDTA/n-valeric acid method relies on three assumptions: 1) n-valeric acid is not metabolized extensively by the ruminal microflora, 2) the rate of passage from the rumen is equal for Co-EDTA and n-valeric acid, and 3) rate of n-valeric acid absorption is similar to the rates of absorption for other VFA (Allen et al., 2000). The later assumption likely causes a decrease in accuracy for this method due to the interactions between VFA chain length and ruminal pH for the fractional rate

of absorption (Dijkstra et al., 1993; Sehested et al., 1999). More recently, Resende Júnior et al. (2006) compared absorption rates using ^{13}C labelled VFA and the absorption rates obtained using the Co-EDTA/n-valeric acid method. Their results demonstrated that VFA chain length did not affect in vivo clearance rates and that the Co-EDTA/n-valeric acid method produced similar results to the stable isotope method; however, they did not account for the intraruminal conversion of ^{13}C among VFA as demonstrated by Sutton et al. (2003), which may have affected their results. Future research should be directed towards an improved understanding of factors affecting the absorption of VFA across the rumen under physiological conditions.

3.4.2 Ketogenesis

Harmon et al. (1991) fed diets containing 90% forage or 90% grain, and found that the activity of butyrl-CoA synthetase was increased for cows fed 90% grain but no differences were observed for the activities of acetyl-CoA synthetase and propionyl-CoA synthetase. In the current study we evaluated the gene expression of acyl-CoA synthetase, acetyl-CoA synthetase, and butyrl-CoA synthetase but did not detect any differences between treatments. It must be acknowledged that changes in gene expression do not necessarily indicate differences in protein abundance or activity due to post-translational regulation. Furthermore, the relationship between butyrate activation and butyrate absorption is questionable as Harmon et al. (1991) found increases in the activity of butyrl-CoA synthetase, but no differences in the mucosal uptake of butyrate/mg tissue.

Extensive metabolism of butyrate occurs across the ruminal epithelia. Sehested et al. (1999) reported that approximately 95% of the butyrate absorbed from the isolated bovine mucosa was metabolized, and that 94% of the metabolized butyrate was converted to non-CO_2 metabolites. As such, we hypothesized that gene expression for enzymes involved in ketogenesis would increase when cows were fed the HC diet. Genes evaluated included acetyl-CoA acyltransferase, which is the enzyme catalyzing the first reaction where two acetyl-CoA molecules are converted into one acetoacetyl-CoA molecule. We also investigated the relative abundance of mRNA transcripts for HMGS, HMGL and two isoforms of BDH. Gene expression between HC and LC treatments was not different for ketogenic enzymes, which was unexpected as cows fed the HC treatment had higher plasma concentrations of BHBA. Because the cows were non-lactating and fed diets to meet their ME requirements, BHBA was not likely produced from incomplete oxidation of fatty acids in the liver, but rather from ketogenesis in the ruminal epithelia. This is consistent with higher butyrate concentration in ruminal fluid for HC cows compared to LC cows. Although the expected ruminal butyrate supply and plasma BHBA concentration were greater for cows fed HC, no differences were observed for the expression of genes encoding for ketogenic enzymes in the current study. Harmon et al. (1991) reported no differences in the net production of acetoacetate and BHBA from acetate and butyrate by ruminal papillae slices collected from cattle fed a 90%-concentrate or 90%-forage diet; however, increased oxidation of both acetate and butyrate was detected in papillae slices when cattle were fed 90% concentrate

compared to 90% forage. These data lead to the speculation that the dietary forage to concentrate ratio may affect the partitioning of acetate and butyrate metabolism in ruminal tissue.

3.4.3 Pyruvate Metabolism

Pyruvate is a central intermediate for energy metabolism, and partitioning of pyruvate metabolism can greatly affect energy supply to the cell. No previous studies known by the authors have investigated the expression of genes related to pyruvate metabolism in bovine ruminal tissue. We found that cows fed HC had reduced expression of PDH, the enzyme that catalyzes the conversion of pyruvate to acetyl-CoA. In ruminal tissue, pyruvate can be produced via glycolysis or via oxaloacetate as catalyzed by phosphoenolpyruvate carboxykinase. However, if feeding HC increased the oxidation of butyrate and acetate as discussed previously, the reliance on PDH to supply acetyl-CoA may be reduced. Although cows fed HC and LC had similar fractional rates of VFA absorption, increases in the acetyl-CoA pool size could be expected in the current study as cows fed HC had higher VFA concentration in the rumen. Therefore, as the estimated rate of butyrate absorption (mol/h) was higher for cows fed HC than LC, increased butyrate supply could lead to a greater acetyl-CoA pool size due to metabolism of butyrate. Propionate supply from the rumen is also expected to be greater for cows fed HC compared with those fed LC, but we are unable to speculate on the contribution of propionate to the pyruvate pool through phosphoenolpyruvate carboxykinase. Although we used 3 different primer sequences for

phosphoenolpyruvate carboxykinase, the tested sequences were not successful in producing a single amplicon or producing an amplicon in sufficient quality. Future studies should utilize stable isotopes to investigate the partitioning of VFA metabolism in ruminal tissue for cows fed various dietary regimens and to determine if the expression of genes coding for regulating enzymes correlates with the production of their intermediates.

3.4.4 Observed Variation

In the present study, considerable variation existed in the response of cows fed the HC diet. Although six cows on the HC treatment were fed a common diet, one cow had markedly higher mean pH (6.19) and a lower acidosis index (6.4) than the other cows. Other studies have also noted large variation in ruminal pH, and the severity of acidosis within a treatment. For example, Brown et al. (2000) challenged 5 steers by ruminally dosing steam flaked corn at 3% BW after 1-d of feed restriction. The response to the challenge in that study ranged from euthanization due to acute ruminal acidosis to undetectable ruminal acidosis in another steer. Bevans et al. (2005) subjected beef heifers to a rapid adaptation (40% concentrate diet to a 90% concentrate diet in 4 d with one intermediate diet) or gradual adaptation protocol (40% concentrate diet to a 90% concentrate diet over 16 d with 5 intermediate diets). Bevans et al. (2005) showed that some heifers on both treatments were able to tolerate their adaptation protocol with minimal disruption to fermentation (normal diurnal variation in ruminal pH and maintenance of DMI), whereas others responded poorly (extended periods of low

ruminal pH and variation in DMI). They also noted that some cows were able to tolerate low ruminal pH without noticeable decreases in animal performance. However, we are not aware of any studies investigating the causes for the variation.

Negative relationships were observed between the acidosis index and the expression level of butyrl-CoA synthetase, PC, and LDHb genes, and a tendency for a negative relationship between the acidosis index and HMGL. These observations indicate that cows experiencing less severe ruminal acidosis had greater gene expression. Sensitivity analysis (DFFITS) of the regressions revealed that one cow was an outlier, and upon removal of that cow no relationships were observed. The exception was the negative relationship between the acidosis index and HMGL, which was improved with the removal of the outlier. However, in this case the outlier was a different cow than for the other variables. These data are collectively interpreted to suggest that as cows experienced more severe ruminal acidosis (increased acidosis index), the expression of genes related to VFA metabolism were decreased. It must be noted that this regression approach does not imply a cause-and-effect relationship, and the data should be interpreted with caution as only 6 animals were used to derive the relationships. Further research is warranted to determine the relationship between ruminal VFA absorption and metabolism and the severity of ruminal acidosis.

We expected that differences in the gene expression for enzymes and proteins related to the absorption and metabolism of VFA would be positively

associated with the estimated rates of VFA absorption (mol/h). However, we did not detect these relationships in the current study. The VFA absorption via passive diffusion is thought to be mediated by concentration gradients between the ruminal contents, the cytosol, and portal circulation and by the lipophilic nature of individual VFA (Dijkstra et al. 1994; Rémond et al. 1996). As such, it is unclear why cows fed HC did not have an increased fractional rate of VFA absorption as ruminal pH was lower. The concentration of undissociated VFA in ruminal fluid should be greater for cows fed HC than LC. Alternatively, it is possible that the metabolism of VFA have a regulatory role in VFA absorption. For example, metabolism would help maintain a concentration gradient between the ruminal contents and the cytosol and increased VFA metabolism should thereby increase VFA absorption. However, our data do not support this speculation, but the lack of relationships may be due, at least in part, to limited sensitivity of the CoEDTA/n-valeric acid method to determine VFA absorption as described previously.

3.5 Conclusion

Feeding a diet containing a greater proportion of concentrate reduced ruminal pH and increased VFA concentration; however, the rate of total VFA absorption in vivo was not affected but the estimated rates of propionate and butyrate absorption were increased. Further research is needed to determine factors affecting the absorption of total VFA and individual VFA in vivo. Further, we found negative correlations between the severity of ruminal acidosis,

as indicated by the acidosis index, and the expression of genes related to VFA activation, ketogenesis, and pyruvate metabolism. A greater understanding of mechanisms involved in VFA absorption and metabolism will increase our knowledge of energy metabolism by ruminal tissue, and may lead to the development of strategies to reduce the occurrence and severity of ruminal acidosis.

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4.0 TECHNICAL NOTE: EVALUATION OF A CONTINUOUS RUMINAL PH MEASUREMENT SYSTEM FOR USE IN NON-CANNULATED SMALL RUMINANTS[3]

4.1 Introduction

Continuous acquisition of ruminal pH data has greatly improved the current understanding of the interactions among diet fermentability, intake, and ruminal pH. Dado and Allen (1993) documented an early system capable of continuous pH measurement but this system required animals to be tethered and thus application was limited to animals housed in tie stalls. More recently, Penner et al. (2006) evaluated a system capable of measuring ruminal pH in ruminally cannulated cattle without restraining them, thereby, increasing the versatility of ruminal pH measurement.

Although continuous measurement of ruminal pH in unrestrained cattle is possible, the physical size of current pH measurement systems precludes their use in small ruminants such as sheep, goats, and young calves. Furthermore, all existing systems to measure ruminal pH require ruminal cannulation, limiting their application due to concerns with surgery and the maintenance of a cannula in

[3]A version of this chapter has been published. Penner, G.B., J.R. Aschenbach, G. Gäbel, and M. Oba. 2009. Technical note: evaluation of a continuous ruminal pH measurement system for use in non-cannulated small ruminants. J. Anim. Sci. 87: 2363-2366.

small or growing animals. The objective of this study was to evaluate the precision and accuracy of an indwelling ruminal pH measurement system that could be used in small ruminants without requiring ruminal cannulation relative to those obtained using a portable pH meter.

4.2 Materials and Methods

All experiments in this study were conducted at the Universität Leipzig, Germany, and all procedures were pre-approved by the Regierungspräsidium Leipzig (TVV 06/08).

4.2.1 Small Ruminant Ruminal pH Measurement System (SRS)

The SRS was manufactured by Dascor (Escondido, CA) and consisted of a surgical stainless steel body housing a data logger equipped with a temperature sensor (model SRL-T6, Dascor) and a pH sensor (model 971819, Dascor; see Figure 4.1). The SRS had a diameter of 20.6 mm, a length of 138 mm, and a fully assembled unit had a mass of approximately 245 g which included the 6-V battery used to power the pH and temperature sensors, and the data logger. The latter had a memory capacity of 32.8 KB allowing 25 h of measurement (pH and temperature, and battery voltage) with a sampling interval of 30 s or 7 d of measurement with a sampling interval of 2 min. Battery voltage was monitored to be able to retrospectively detect battery failure should it occur during measurements. The initialization of the data logger and the transfer of data were

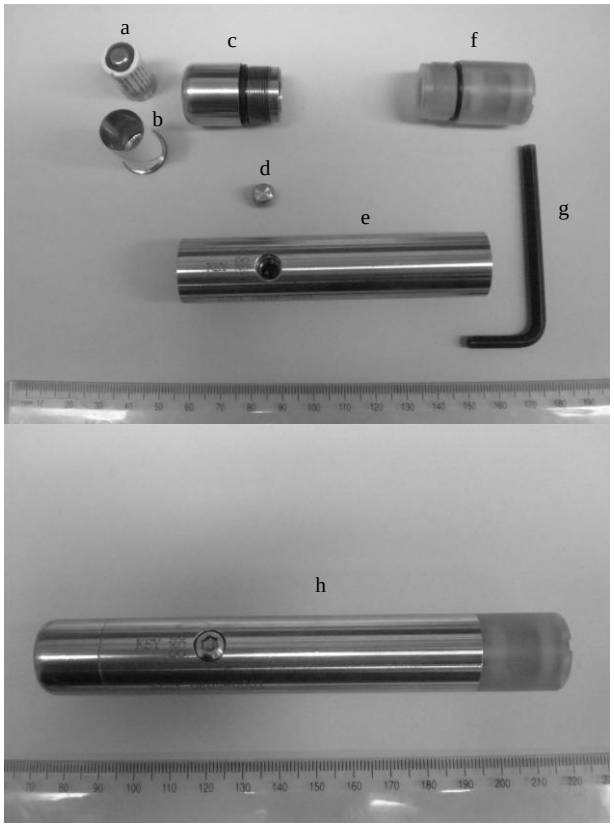

Figure 4.1 Illustration of the Small Ruminant Ruminal pH Measurement System (SRS) in unassembled and assembled forms: a = 6-volt battery, b = battery holder, c = threaded battery cap, d = computer access port plug, e = stainless steel housing with data logger, f = pH electrode, g = hex key for computer access port plug, and h = assembled SRS.

performed by a Microsoft Windows®-compatible software package supplied by Dascor (M1b version 6.1.2h).

4.2.2 Experiment 1

One Friesian wether sheep (94.1 kg BW) previously fitted with a ruminal cannula was used to compare in vivo pH measurements between a portable pH meter and the SRS. Prior to the experiment the sheep was fed a hay diet ad libitum and had free access to water and a mineral block. A composite sample of the hay was analyzed for nutrient content at a commercial laboratory (Landwirtschaftliche Kommunikations- und Servicegesellschaft; LKS-GmbH, Niederwiesa, Germany) and contained (DM basis) 13.1% CP and 8.1 MJ/kg ME. The experiment lasted 230 min during which the sheep was housed in a stanchion and did not have access to feed or water.

The first 45 min of the experiment were utilized to obtain pre-challenge pH readings in vivo. Subsequently, 1,176 mL of a 40% (wt/vol; 2.2 *M*) glucose solution was administered into the rumen to provide 5 g of glucose/kg BW. The glucose challenge was designed to provide data in the relevant range of ruminal pH values in vivo without causing acute ruminal acidosis. Ruminal pH was monitored for 185 min following the glucose challenge. Throughout the duration of the experiment, ruminal pH was measured simultaneously using two different pH measurement devices as described below.

4.2.2.1 Measurement of ruminal pH using a portable pH meter.

Ruminal pH was measured using a portable pH meter (model WTW multi 350i; WTW, Weilheim, Germany) connected to a pH electrode (Sentix 41-3, WTW), and the data was recorded using MultiLab Pilot (version 4.31, WTW). Prior to pH measurement, the pH meter was calibrated with buffers at 7.0 and 4.0 (Sensorex, Garden Grove, CA) at 39 ºC. Ruminal pH was measured every 30 s, and 1-min averages were calculated. The pH electrode was placed in the ventral sac of the rumen with the cable passing through the ruminal cannula stopper. The ruminal cannula was closed during pH measurement except during the administration of glucose.

4.2.2.2 Measurement of ruminal pH using the SRS.

Ruminal pH was measured using the SRS following a similar procedure previously described by Penner et al. (2006). Briefly, pre-measurement standardization was conducted by recording baseline mV readings in buffers 7.0 and 4.0 (Sensorex, Garden Grove, CA) at 39 ºC. Standardization was conducted using the same buffers as for the portable pH meter. The SRS was then set to collect mV readings every 30 s and placed in the ventral sac of the rumen through the ruminal cannula. Final placement of the SRS was within close proximity to the sensor attached to the portable pH meter. Upon removal from the rumen, via the ruminal cannula, post-measurement standardization was conducted by recording the baseline mV readings in buffers 7.0 and 4.0 at 39 ºC. The mV

values obtained in buffers 7.0 and 4.0 during the pre- and post-measurement standardizations were used to derive two linear regression equations (starting and ending) that were used to convert mV values obtained during in vivo measurement to ruminal pH values. A linear offset was used to account for changes in the linear equations between the pre- and post-measurement standardizations such that the post-measurement standardization equation had increased weighting as the measurement period progressed. Changes in baseline readings from the pre- and post-measurement standardizations were minimal (< 25 mV equivalent to 0.04 pH units). Ruminal pH data from the SRS were summarized as 1-min averages.

4.2.3 Experiment 2

In Experiment 2, a total of 30 mature female German Merino sheep were used to evaluate the suitability of the SRS to measure ruminal pH in non-cannulated sheep. All sheep were fed the same diet as described in Experiment 1. Sheep (72.3 ± 10.1 kg of BW; mean ± SD) were randomly assigned to either the control (n = 8) or glucose drench treatment (n = 22). For the glucose drench treatment, sheep received a ruminal infusion of a 40% (wt/vol, 2.2*M*) glucose solution via an orogastric tube (standard model for foals, outer diameter 12 mm, 150 cm long; Heiland Vet GmbH, Hamburg, Germany) to supply 5 g of glucose/kg BW as described above. Sheep on the control treatment received a ruminal infusion of water in equal volume as infused for sheep in the glucose drench treatment (12.5 mL/kg BW). All sheep were subjected to their respective

treatment at 0600 h and killed by captive bolt stunning followed by exsanguination at 0900 h.

Ruminal pH was measured every 2 min for a minimum of 48 h prior to the oral drench and for 3 h after the drench. Prior to administration of the SRS, mV readings in standard buffers 7.0 and 4.0 were recorded at 39ºC as described above. To orally administer the SRS, a sheep was positioned to sit on its rump. The SRS was wetted with water for lubrication, and placed in a bolus gun for oral administration. The sheep's head was gently elevated to provide a straight pathway for the SRS from the oral cavity down the esophagus. The bolus gun was placed in the mouth of the sheep and the plunger was gently depressed until the sheep swallowed the SRS. The abdomen of the sheep was then massaged for 1 min to facilitate movement of the SRS into the caudal region. While the sheep remained on its rump, the gross anatomical position of the SRS in the rumen was verified to be located near in the posterior area of the abdomen using a metal detector. The sheep was then helped back to a standing position and restrained to allow the SRS to move into the ventral ruminal sac and its position was verified again with a metal detector.

Following euthanization, the ventral ruminal sac was exposed and opened and the location of the SRS was identified. Ruminal pH was measured manually using a portable pH meter as described previously. The time of manual pH measurement was recorded to compare the manual reading to the corresponding pH measurement obtained from the SRS. Thereafter, the SRS was recovered for post-measurement standardization and data processing as described above.

4.2.4 Statistical Analysis

The extent of agreement between pH recordings from the SRS and the portable pH meter was evaluated using the Pearson correlation coefficient using the PROC REG procedure of SAS (version 9.13, SAS Institute Inc., Cary, NC) and the concordance correlation coefficient (Lin et al., 1989, 1992). In Experiment 2, the location of the SRS was found to be in the reticulum on three separate occasions. Data from these observations were included in the analysis as DFFITS analysis did not identify them as outliers.

4.3 Results

4.3.1 Experiment 1

Measurements from the portable pH meter and the SRS had a high degree of agreement with a Pearson correlation and a concordance correlation of 0.97 and 0.96 respectively (Table 4.1 and Figure 4.2). Although there was a high degree of agreement between the two ruminal pH measurement methods as indicated by a location shift value of zero, a scale shift of 1.28 indicates that ruminal pH was lower for the SRS than the portable pH meter at a lower pH range and higher for the SRS than the portable pH meter at a higher range.

Table 4.1 Accuracy and precision of the Small Ruminant Ruminal pH Measurement System (SRS) relative to a portable pH meter

Variable	Experiment 1	Experiment 2
N	226	26
Pearson correlation coefficient	0.97	0.96
Concordance correlation coefficient	0.96	0.95
Location shift	0.00	0.04
Scale shift	1.28	1.16

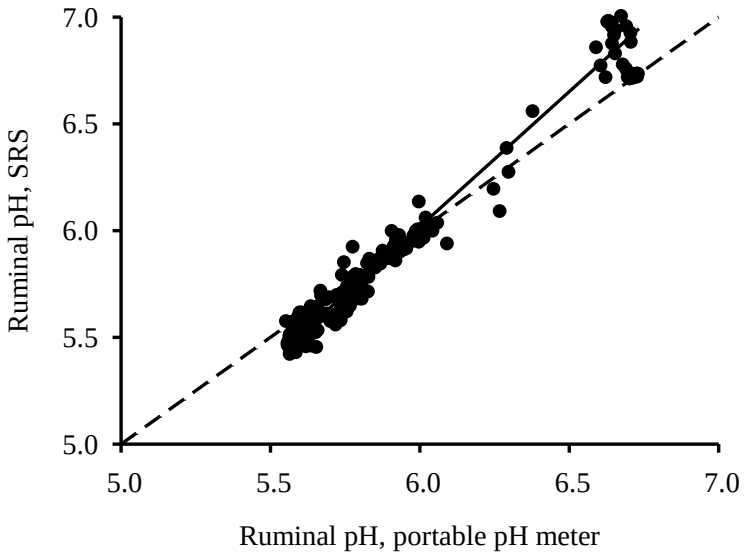

Figure 4.2 Relationship between measurements conducted using the Small Ruminant Ruminal pH Measurement System (SRS; y-axis) and a portable pH meter (x-axis) in a sheep ruminally dosed with a 40% glucose solution (wt/vol; 2.2 *M*) to supply 5 g of glucose/kg BW (Experiment 1). The solid line depicts the regression line of the data pairs and the dashed line depicts the line where y=x.

4.3.2 Experiment 2

The SRS was successfully administered without any problems in all 30 sheep. At the time of euthanization, the SRS was located in the ventral sac for 27 animals, and in the reticulum for the remaining 3 animals. Four devices failed to measure ruminal pH for the entire measurement period. Reasons for failure were due to pH sensor failure (n = 3) and failure to maintain a water-tight barrier (human error; n = 1).

The relationship between measurements obtained using the SRS and the portable pH meter had a high degree of agreement with a Pearson correlation coefficient and concordance correlation coefficient of 0.96 and 0.95, respectively (Table 4.1 and Figure 4.2). The location shift approached 0, again indicating a high degree of concordance. The scale shift was 1.16, indicating that relative to the portable pH meter the SRS recorded lower values for the low pH range and higher values for the high pH range.

4.4 Discussion

Continuous ruminal pH measurement in non-cannulated animals poses several challenges including the oral administration of a ruminal pH measurement device and the positioning of the device within the rumen. Most previous devices to measure ruminal pH have required ruminal cannulation (Dado and Allen, 1993; Penner et al., 2006) thereby preventing the continuous acquisition of ruminal pH in situations where ruminal cannulation is limited due to concerns with surgery

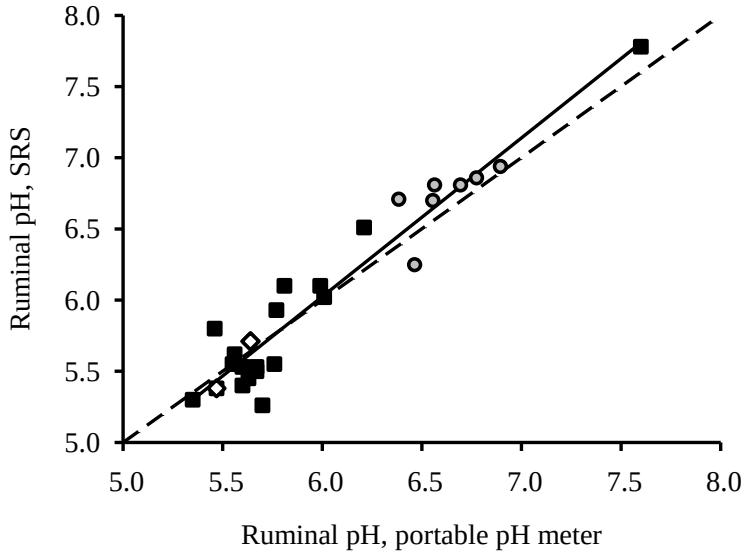

Figure 4.3 Relationship between measurements of ruminal pH conducted using the Small Ruminant Ruminal pH Measurement System (SRS; y-axis) and a portable pH meter (x-axis) in 26 non-cannulated sheep (Experiment 2) immediately after euthanization. Data points marked with a grey circle, black square, or open diamond symbol are from sheep receiving the control treatment (water), glucose treatment, or sheep in which the SRS was located in the reticulum, respectively. The solid line depicts the regression line of the data pairs and the dashed line depicts the line where y=x.

and the maintenance of a cannula in small or growing animals such as calves, lambs, or kids. Enemark et al. (2003) developed a device which could be orally dosed in dairy cows, but the device consistently measured reticular pH rather than ruminal pH. Furthermore, all previous devices to continuously measure ruminal pH were too large for use in small ruminants such as sheep, goats, and calves. To the best of our knowledge, we have demonstrated the first device capable of continuous ruminal pH measurement in small ruminants without the requirement of ruminal cannulation. Because currently available systems for telemetric data transfer were too bulky to be used with the SRS, recovery of the device from the rumen was required to download the data, and correct the data for post-measurement standardization. Thus, although surgery is not required for the measurement of ruminal pH data, post-measurement recovery of the data does require either surgery or euthanization. As such, the SRS may be most beneficial in experiments using terminal animals as it may remove the requirement of ruminal cannulation for the measurement of ruminal pH. This system could also have application in experiments utilizing cannulated small ruminants as continuous acquisition of ruminal pH data provides much more comprehensive data than manual pH measurement (Penner et al., 2006).

Pearson correlation and concordance coefficient values for both experiments in the current study show that measurements obtained from the SRS were in good agreement with measurements conducted using a portable pH meter. In addition, the correlations observed in this study are similar to those previously reported by Penner et al. (2006) for an indwelling device designed for ruminally

cannulated cattle. Furthermore, the precision and accuracy were similar between Experiments 1 and 2, supporting the suitability of the SRS to measure ruminal pH over both short and extended periods of time.

A scale shift was noted, especially in Experiment 1, suggesting that relative to the portable pH meter the SRS recorded lower pH values at the low pH range and recorded higher pH values at the high pH range. There are several possible causes for the scale shift observed. Firstly, it is possible that the resulting scale shift was a result of drift in the SRS pH electrode between pre- and post-measurement standardizations. However, the SRS was standardized before and after in vivo measurement and we noted that the drift could only account for 0.04 pH units. Alternatively, it is possible that the scale shift was a result of drift in readings for the portable pH meter electrode. The pH electrode used for the SRS is designed for submersible application in sewage environments and is likely to withstand the ruminal environment better than a standard pH electrode. Finally, in Experiment 1 both pH electrodes were in the ventral sac of rumen at the same time, however, it is possible that the pH electrodes were exposed to different microenvironments in the ruminal contents leading to the scale shift observed.

The present study thus describes the first device capable of ruminal pH measurement in small ruminants without requiring ruminal cannulation. Relative to measurements taken using a portable pH meter, the SRS-recorded values were in high agreement. As such, the SRS provides the opportunity to measure ruminal pH in cannulated or non-cannulated small ruminants under research and field

situations, and can be used to further understand the ruminal pH response in small ruminants under different dietary settings.

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5.0 EPITHELIAL CAPACITY FOR APICAL UPTAKE OF SHORT CHAIN FATTY ACIDS IS A KEY DETERMINANT FOR INTRARUMINAL PH AND THE SUSCEPTIBILITY TO SUB-ACUTE RUMINAL ACIDOSIS IN SHEEP[4]

5.1. Introduction

Ruminants like cattle and sheep have phylogenetically adapted to use fiber carbohydrates such as cellulose and hemicellulose as a main source of metabolic energy (Bergman, 1990). Since fiber is not amenable to hydrolysis by mammalian enzymes, ruminants have a forestomach system in which microorganisms convert ingested carbohydrates to short-chain fatty acids (SCFA; mainly acetate, propionate and butyrate; Russell and Hespell, 1981). The rumen is the largest part of this forestomach system and its epithelial lining additionally mediates efficient absorption of SCFA (Rupp et al., 1994), which contributes to at least 65-75% of the total metabolizable energy supply (Bergman, 1990).

In contrast to the phylogenetic adaptation, current feeding practices in dairy and beef industries use highly fermentable diets to maximize energy intake in order to achieve high levels of milk yield or rapid growth rates. But, highly fermentable diets increase the rate of fermentation acid production in the rumen.

[4]A version of this chapter has been published. Penner GB, J.R. Aschenbach, G. Gäbel, R. Rackwitz, and M. Oba. 2009. Epithelial capacity for apical uptake of short chain fatty acids is a key determinant for intraruminal pH and the susceptibility to sub-acute ruminal acidosis in sheep. J. Nutr. 139:1714-1720.

Accumulation of acid can cause ruminal pH to drop; negatively impacting microbial fermentation, ruminal epithelial function, and finally animal health and productivity (Nocek, 1997; Müller et al., 2002). While the severe state of acute ruminal acidosis results in obvious clinical illness (Nocek, 1997), less severe states called sub-acute ruminal acidosis (SARA) are often hard to detect. Nonetheless, SARA is highly prevalent in intensive production systems (Krause and Oetzel, 2006) and has economic consequences associated with decreased animal performance (Stone, 2004). One perplexing aspect of ruminal acidosis is the considerable variation in the susceptibility among individual animals, even when fed a common diet (Brown et al., 2000; Bevans et al., 2006; Penner et al., 2007). The variation among animals is a concern because diets are often formulated for the average animal, thus, predisposing some animals to SARA. A physiological understanding of why animals differ in their tolerance to SARA may provide new strategies to reduce this nutritional disorder.

Ruminal pH is a balance between acid production and the removal of acid from the rumen through absorption, neutralization, and passage. Hence, variation in the susceptibility to SARA could be due to variation in any single or any combination of these four factors. Allen (1997) previously suggested that SCFA absorption accounts for the largest proportion of proton removal (~ 53%) from the rumen of lactating dairy cows using a simplified model assuming nearly exclusive passive diffusion of protonated SCFA. However, absorption pathways are complex (Aschenbach et al., 2009). Gäbel et al. (1991) demonstrated that for every 1 mol of SCFA absorbed, approximately 0.5 mol of bicarbonate buffer was

secreted into the contents in vivo. Bicarbonate dependence was later verified in isolated ruminal epithelia for butyrate transport in cattle (Sehested et al., 1999b) and for acetate transport in sheep (Aschenbach et al., 2009). In addition, acetate transport is partially mediated by a bicarbonate-independent transport protein that is sensitive to inhibition by nitrate (Aschenbach et al., 2009). Either of these absorption pathways could be limiting for ruminal pH regulation during SARA. Thus, the aim of this study was to determine if the severity of SARA could be linked to inefficiency of certain pathways for SCFA absorption. To assess those pathways, apical uptakes of acetate and butyrate were characterised in vitro in Ussing chambers following an episode of SARA in vivo.

5.2 Materials and Methods

This study was conducted between April and August 2008 at the Universität Leipzig, Germany, and all procedures were pre-approved by the Regierungspräsidium Leipzig (TVV 06/08). In addition, procedures were approved by the Faculty Animal Policy and Welfare Committee at the University of Alberta and were in accordance with the guidelines of the Canadian Council of Animal Care.

5.2.1 Animals and experimental design

Twenty-four female German Merino sheep (2-5 yr old) sourced from two locations were used in this study. Sheep had ad libitum access to hay ad libitum

(DM basis; 13.1% CP and 8.1 MJ/kg ME) and had free access to a mineral block and water for at least 21 d prior to the experiment.

Sheep were randomly assigned to either the control (SHAM; n = 7) or glucose treatment (n = 17) that respectively consisted of a single orally administered drench of water (12 mL/kg BW) or a 2.2 mol/L glucose solution to supply 5 g of glucose/kg BW. The oral drench was administered using an oro-gastric tube (outer diameter 12 mm, 150 cm long; Heiland Vet GmbH, Hamburg, Germany). Only 1 sheep was handled each day and the orally administered drench was provided at 0600 h. The glucose drench was designed to induce SARA. Sheep that received the glucose drench were further classified according to the area that pH was below the pH threshold 5.8 (pH × min; for calculation see Penner et al., 2007). The area below pH 5.8 accounts for both the duration and extent of pH depression and is thought to be an appropriate indicator of the severity of ruminal acidosis (Penner et al., 2009b). The seven sheep with the smallest area below pH 5.8 (range, 0.0 - 12.3 min) were classified as non-responders (NR) to SARA induction, while the seven sheep with the largest area below pH 5.8 (range, 29.2 - 84.0 min) were classified as responders (RES).

5.2.2 Data and sample collection

Sheep were weighed 3 d prior to oral drench, and placed in an individual pen bedded with wood shavings. Following body weight measurement, sheep were orally dosed with a Small Ruminant Ruminal pH Measurement System (**SRS**; Dascor, Escondido, CA) as described in detail by Penner et al. (2009a).

The SRS was standardized in buffers at pH 7.0 and 4.0 (Sensorex, Garden Grove, CA) at 39°C prior to insertion and was initialized to collect a reading every 2 min.

At 0900 h (i.e. 3 h after the oral drench), blood was collected from the jugular vein. Tubes containing either 16 IU lithium heparin/mL blood or a clotting factor (both from Sarstedt AG and Co., Nümbrecht, Germany) were used to harvest plasma and serum, respectively. Blood used to harvest plasma was placed on ice immediately and centrifuged at 3,000 × *g* at 4°C for 10 min within 20 min of collection. Blood used for serum collection was stored at room temperature for 30 min and then centrifuged at 3,000 × *g* at 4°C for 10 min.

Immediately following blood collection, sheep were killed by captive bolt stunning followed by exsanguination. The abdominal cavity was opened and ruminal epithelial tissue (~300 cm^2) was collected from the ventral sac of the rumen and processed for Ussing chamber studies as previously described (Aschenbach et al., 2002). The ruminal content was weighed and a representative sample of ruminal fluid was collected by straining through a cotton cloth. Ruminal fluid was preserved in 85%-orthophosphoric acid (4 mL ruminal fluid: 1 mL 85%-orthophosphoric acid) and stored at -20°C.

The SRS was removed from the digesta and post-measurement standardizations were conducted in standard pH buffers 7.0 and 4.0 (Sensorex) at 39°C. Ruminal pH data collected during the 48 h preceding the oral drench and 3 h following the oral drench were summarized separately. Data collected prior to the oral drench were summarized for each 24-h period and the mean of the two 24-h periods was used for statistical analysis. For each sheep and data set (pre-

drench and post-drench) the nadir, mean, maximum pH values, and the duration (min) and area that ruminal pH was below pH 5.8 (pH × min) were determined (Penner et al., 2007).

5.2.3 Ussing Chamber Technique

5.2.3.1 Buffer Solutions for Ruminal Epithelia

The buffer used for washing and transport of ruminal epithelium contained (mmol/L) 15.6 NaCl, 5.5 KCl, 1.3 $MgCl_2$, 0.6 NaH_2PO_4, 2.4 Na_2HPO_4, 1.0 L-glutamine, 10.0 HEPES free acid, 24.0 $NaHCO_3$, 5.0 Na-D/L-lactate, 10.0 Na-acetate, 10.0 Na-propionate, 10.0 butyric acid, 120.0 mannitol, and 10 NaOH. The pH of the transport buffer was adjusted to 7.4 using NaOH (1 mol/L) or gluconic acid (3 mol/L), and the osmolarity was 293 ± 5.1 mOsm/L (mean ± SD). The chemical composition of the experimental buffer solutions is presented in Table 5.1. The use of buffer solutions that contained bicarbonate, excluded bicarbonate, and excluded bicarbonate but contained nitrate were used to determine the components of uptake previously characterized by Aschenbach et al. (2009); i.e. total, bicarbonate-dependent, bicarbonate-independent nitrate-sensitive, and bicarbonate-independent nitrate-insensitive uptake. Buffer solutions used for incubation of the mucosal (i.e. luminal) and serosal (i.e. bood) side were prepared to a final pH of 6.1 and 7.4, respectively. The use of mucosal and serosal buffer solutions differing in pH was intended to provide physiologically representative pH values of in vivo conditions. Buffers

containing bicarbonate were gassed with 95%O_2/5%CO_2, and those free of bicarbonate were gassed with 100% O_2.

5.2.3.2 Determination of Acetate and Butyrate uptake

Apical uptakes of acetate and butyrate were measured simultaneously based on the Ussing chamber technique previously described by Aschenbach et al. (2002). Briefly, ruminal epithelia were mounted in Ussing chambers where mucosal and sersoal buffers solutions were thermostated to 37°C and circulated gas lift. Epithelia were clamped to a transepithelial potential difference of 0 mV (short-circuit conditions) using a computer-controlled voltage-clamp device (Ing. Büro für Mess- und Datentechnik, Aachen, Germany). A solution containing acetate and butyrate to give a final concentration of 10 mmol/L each, spiked with [2-^{3}H]-acetate (100 kBq) and [1-^{14}C]-butyrate (74 kBq; both radiochemicals from Hartmann Analytic GmbH, Braunschweig, Germany), was added to the mucosal side for 1 min. Thereafter, epithelia were washed three times with ice-cold transport buffer and dismounted. Epithelial lysate was harvested in a pre-cooled lysing device with 4 mL ice-cold 100 mmol/L NaOH, and cleared by centrifugation (3000 × *g* at 4°C for 15 min). For each sheep, two pieces of ruminal epithelia were exposed to each in vitro treatment and the average of the two measurements was considered as one observation.

The uptake of acetate and butyrate in the buffer containing bicarbonate was considered as total uptake. Bicarbonate-dependent uptake was calculated as the difference between total uptake and the uptake in the bicarbonate-free buffer.

Further, bicarbonate-independent uptake was characterized as nitrate-insensitive and nitrate-sensitive uptake.

5.2.4 Analytical Methods

Plasma glucose concentration was measured as described by Penner et al. (2009b) and insulin concentration was measured using a commercial kit (Coat-A-Count, Diagnostic Products Corporation, Los Angeles, CA). Plasma BHBA concentration was determined according to Williamson et al. (1962). Serum was deproteinized using 0.6 mol/L perchloric acid and was used for the determination of L-lactic acid (Gutmann and Wahlefeld, 1974). Concentrations of SCFA in ruminal fluid were quantified by gas chromatography as previously described (Penner et al., 2009b). Concentration of L-lactate was determined in deproteinized ruminal fluid as described above for serum.

The [2-^{3}H]-acetate and [1-^{14}C]-butyrate radioactivity was measured in samples from the incubation buffer and the lysate to determine apical uptakes of acetate and butyrate. Liquid scintillation fluid (Aquasafe 300 Plus; Zinsser Analytic, Maidenhead, UK) was added to all samples before they were analyzed in duplicate using a scintillation counter (WALLAC 1409 LSC; Berthold, Bad Wilbach, Germany). The software (DSA version 2.4) automatically analyzed the scintillation spectrum to count ^{3}H and ^{14}C in parallel in the same sample. Lysate protein concentration was determined in triplicate according to Smith et al. (1985).

Table 5.1 Chemical composition of the mucosal and serosal buffer used to determine the total, bicarbonate-dependent, bicarbonate-independent nitrate-sensitive, and bicarbonate-independent nitrate-insensitive uptakes of acetate and butyrate.

	Buffer solution				
	Bicarbonate-containing		Bicarbonate-free		Bicarbonate-free with nitrate
Substance, *mmol/L*	Serosal	Mucosal	Serosal	Mucosal	Mucosal
Na-gluconate	50.6	50.6	69.6	69.6	34.6
K-gluconate	5.5	5.5	5.5	5.5	5.5
Ca-gluconate	1.0	1.0	1.0	1.0	1.0
Mg-gluconate	1.3	1.3	1.3	1.3	1.3
NaH_2PO_4	0.6	0.6	0.6	0.6	0.6
Na_2HPO_4	2.4	2.4	2.4	2.4	2.4
L-Glutamine	1.0	1.0	1.0	1.0	1.0
HEPES-free acid	10.0	10.0	10.0	10.0	10.0
$NaHCO_3$	24.0	24.0	0.0	0.0	0.0
Glucose	10.0	10.0	10.0	10.0	10.0
Na-nitrate	0.0	0.0	0.0	0.0	40.0
Acetazoleamide	0.0	0.0	0.1	0.1	0.1
Mannitol	110.0	90.0	115.0	109.0	109.0
Gluconic acid	0.0	20.0	0.0	6.0	1.0
NaOH	0.0	0.0	5.0	5.0	0.0
pH	7.4	6.1	7.4	6.1	6.1
Osmolarity, *mOsm/L*[1]	290 ± 3.0	290 ± 4.0	295 ± 4.2	296 ± 3.9	298 ± 3.3

[1]Values are presented as means ± SD

5.2.5 Chemicals.

If no supplier is mentioned in the text, chemicals were purchased from Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany) or Sigma-Aldrich (Deisenhofen, Germany).

5.2.6 Statistical Analysis

Data were analyzed as a randomized complete block design using the Proc Mixed procedure of SAS (version 9.1; SAS Institute Inc., Cary, NC). The model included the fixed effects of grouping and block. Data are presented as least square means ± standard error of the mean. Differences between treatments were considered significant when $P < 0.05$ and tendencies are discussed when $P < 0.10$. When the F-test was significant, the Bonferroni mean separation test was used to determine if means differed. There was no measureable bicarbonate-independent nitrate-sensitive butyrate uptake and as such these data were removed. Regression analysis was conducted using the Proc Reg procedure of SAS to determine if there were possible relationships between the mean pH prior to the oral drench and the apical uptake of acetate and butyrate in vitro.

5.3 Results

5.3.1 In Vivo Treatment Characterization and Ruminal pH

Sheep body weight and ruminal content mass did not differ among the three groups of sheep (Table 5.2). Furthermore, the effective dose of glucose (i.e. the amount of glucose per kg ruminal content) did not differ between the NR and RES sheep, indicating that differences in the ruminal pH response were not biased by different glucose availability for ruminal microbial fermentation.

Although grouping sheep into the NR and RES categories was based on the area (pH × min) that pH was < 5.8 after the oral drench, corresponding differences in the ruminal pH response prior to the oral drench were also evident. The NR sheep had higher mean pH, and tended to have higher nadir and maximum pH values than RES sheep. However, the nadir, mean, and maximum pH prior to the oral drench did not differ between SHAM and RES sheep.

The oral glucose drench induced a continuous drop in ruminal pH in both NR and RES sheep reaching a nadir value after approximately 2.5 h, followed by a slight recovery (data not shown). Accordingly, sheep provided glucose (NR and RES) had lower mean and nadir ruminal pH values and greater duration that pH was < 5.8 compared to SHAM sheep (Table 5.2). As a reflection of the grouping criteria, NR sheep had a shorter duration and smaller area that pH was < 5.8 than RES sheep. In addition, the nadir, mean, and maximum pH values were all higher for NR than RES sheep

.

Table 5.2 Body weight and ruminal pH for sheep receiving 12 mL water/kg BW (SHAM) or sheep classified as non-responders (NR) or responders (RES) based on their ruminal pH response following an oral drench of a 2.2 mol/L glucose solution to supply 5 g glucose/kg BW[1]

	In vivo classification			
Variable	SHAM	NR	RES	*P* value
BW, *kg*	75.1 ± 2.7	69.8 ± 3.1	73.2 ± 3.7	0.505
Rumen content mass, *kg*	10.12 ± 0.65	8.89 ± 0.86	9.44 ± 1.01	0.609
Effective dose, *g glucose/kg rumen mass*	0.00 ± 0.00[b]	48.8 ± 9.9[a]	44.3 ± 6.7[a]	<0.001
Pre-challenge ruminal pH				
Maximum	6.93 ±0.08	7.17 ±0.07	6.89 ±0.11	0.073
Mean	6.47 ± 0.10[b]	6.72 ± 0.05[a]	6.37 ± 0.08[b]	0.016
Nadir	5.98 ± 0.15	6.33 ± 0.06	5.90 ± 0.14	0.054
Duration < 5.8, *min/d*	73.8 ± 47.2	0.00 ± 0.00	72.7 ± 61.6	0.38
Area < 5.8, *pH × min/d*	6.85 ± 4.16	0.00 ± 0.00	7.94 ± 7.06	0.42
Post-challenge ruminal pH				
Maximum	6.94 ± 0.08[a]	6.73 ± 0.08[a]	6.29 ± 0.07[b]	<0.001
Mean	6.67 ± 0.08[a]	5.97 ± 0.06[b]	5.57 ± 0.05[c]	<0.001
Nadir	6.47 ± 0.09[a]	5.61 ± 0.05[b]	5.36 ± 0.05[c]	<0.001
Duration < 5.8, *min*	1.1 ± 0.0[c]	67.8 ± 19.2[b]	153.1 ± 10.2[a]	<0.001
Area < 5.8, pH × *min*	0.26 ± 0.00[b]	6.80 ± 1.96[b]	47.84 ± 7.53[a]	<0.001

[abc]Means within a row without common superscripts differ significantly ($P < 0.05$).

[1]Data are presented as means ± SEM, $n = 7$.

5.3.2 Ruminal SCFA Concentration

Total SCFA concentration was higher for sheep provided with glucose (NR and RES) than for SHAM sheep (Table 5.3), but did not differ between NR and RES sheep. The higher total SCFA concentration for sheep provided with glucose compared to SHAM sheep was due to higher concentration of the three major SCFA (acetate, propionate, and butyrate); while isobutyrate and isovalerate were lower for sheep provided with glucose. Furthermore, sheep that received glucose had a higher concentration of L-lactic acid in ruminal fluid compared to SHAM sheep.

5.3.3 Plasma and Serum Metabolites and Hormones

Plasma glucose concentration at slaughter was not different among the groups of sheep (Table 5.4). However, sheep receiving glucose (NR and RES) had nearly three times higher plasma insulin concentration than SHAM sheep, but no differences were observed between NR and RES sheep. Although the concentration of plasma BHBA was not different between SHAM and RES sheep, NR sheep had more than 400 μmol/L higher plasma BHBA concentration than RES sheep. Serum L-lactic acid concentration did not differ among treatments.

Table 5.3 Ruminal short chain fatty acid concentration at the time of slaughter for SHAM, NR, and RES sheep[1]

	In vivo classification			
Variable	SHAM	NR	RES	*P* value
Ruminal short chain fatty acids, *mmol/L*				
Total SCFA	81.9 ± 7.1[b]	110.4 ± 4.1[a]	121.6 ± 2.7[a]	<0.001
Acetate	58.9 ± 4.9[b]	69.9 ± 2.8[ab]	80.9 ± 4.2[a]	0.005
Propionate	14.3 ± 1.9[b]	26.2 ± 1.9[a]	26.4 ± 2.0[a]	0.004
Isobutyrate	0.79 ± 0.02[a]	0.42 ± 0.04[b]	0.42 ± 0.03[b]	<0.001
Butyrate	6.39 ± 0.99[b]	12.5 ± 0.70[a]	12.5 ± 0.79[a]	<0.001
Isovalerate	0.95 ± 0.04[a]	0.49 ± 0.07[b]	0.53 ± 0.05[b]	<0.001
Valerate	0.51 ± 0.05[b]	0.66 ± 0.03[a]	0.55 ± 0.05[ab]	0.007
L-lactate	0.09 ± 0.00[b]	0.49 ± 0.12[a]	0.75 ± 0.20[a]	0.009

[abc]Means within a row without common superscripts differ significantly ($P < 0.05$).

[1]Data are presented as means ± SEM, $n = 7$.

Table 5.4 Plasma and serum metabolites and hormone concentrations at the time of slaughter for SHAM, NR, and RES sheep[1]

	In vivo classification			
Variable	SHAM	NR	RES	*P* value
Plasma glucose, *mmol/L*	3.76 ± 0.13	4.33 ± 0.47	4.33 ± 0.53	0.452
Plasma insulin, *pmol/L*	35.8 ± 2.7[b]	94.7 ± 14.4[a]	97.2 ± 12.4[a]	<0.001
Plasma BHBA, *µmol/L*	848 ± 143[b]	1231 ± 75[a]	819 ± 70[b]	0.018
Serum L-lactic acid, *mmol/L*	0.61 ± 0.14	0.67 ± 0.12	0.46 ± 0.09	0.482

[abc]Means within a row without common superscripts differ significantly ($P < 0.05$).

[1]Data are presented as means ± SEM, $n = 7$.

5.3.4 Apical Uptake of Acetate and Butyrate

Total acetate uptake by the isolated ruminal epithelia was not different between SHAM and RES, but was markedly higher for NR sheep than SHAM and RES (Table 5.5). Compared to RES and SHAM, the apical uptake of acetate was 234 and 158% greater for NR sheep. Numerically, the largest contribution for the higher total acetate uptake was due to a tendency for greater bicarbonate-dependent uptake (P = 0.071) for NR compared to SHAM and RES sheep. Additionally, NR had higher bicarbonate-independent nitrate-sensitive acetate uptake than RES sheep. SHAM sheep had an intermediate level of bicarbonate-independent nitrate-sensitive acetate uptake which did not differ from NR and RES sheep. The bicarbonate-independent nitrate-insensitive acetate uptake did not differ among groups.

Total butyrate uptake did not differ between SHAM and RES sheep, but was greater for NR sheep than SHAM and RES. Bicarbonate-dependent uptake did not differ among SHAM, NR, and RES sheep. Thus, the higher total butyrate uptake for NR sheep was a response of greater bicarbonate-independent uptake compared to SHAM and RES. No differences were detected for the bicarbonate-independent butyrate uptake between SHAM and RES.

Table 5.5 Apical uptakes of acetate and butyrate by the isolated ovine ruminal epithelia harvested from SHAM, NR, and RES sheep[1].

	In vivo classification			
Variable	SHAM	NR	RES	*P* value
Acetate uptake, *nmol · mg protein^{-1} · min^{-1}*				
Total	4.69 ± 0.60^{b}	7.40 ± 0.95^{a}	3.16 ± 0.75^{b}	0.005
Bicarbonate-dependent	2.41 ± 0.52	3.82 ± 0.88	1.35 ± 0.65	0.071
Bicarbonate-independent nitrate-sensitive	0.68 ± 0.32^{ab}	1.42 ± 0.35^{a}	-0.08 ± 0.12^{b}	0.007
Bicarbonate-independent nitrate-insensitive	1.6 ± 0.23	2.14 ± 0.15	1.89 ± 0.19	0.164
Butyrate uptake, *nmol · mg protein^{-1} · min^{-1}*				
Total	8.86 ± 0.92^{b}	13.71 ± 1.10^{a}	8.77 ± 1.03^{b}	0.004
Bicarbonate-dependent	2.67 ± 0.81	3.95 ± 1.36	2.22 ± 0.62	0.458
Bicarbonate-independent	6.19 ± 0.71^{b}	9.76 ± 1.09^{a}	6.55 ± 0.84^{b}	0.022

[abc]Means within a row without common superscripts differ significantly ($P < 0.05$).

[1]Data are presented as means ± SEM, $n = 7$.

5.3.5 Relationship Between the Severity of SARA and the Uptake of Acetate and Butyrate

For all sheep included in this study, regression analysis revealed a positive relationship between the mean ruminal pH prior to the oral drench and the total uptakes in vitro of both acetate and butyrate (Figure 5.1).

5.4 Discussion

Ruminal acidosis is a persisting digestive disorder in ruminant production that negatively affects animal health, fiber digestibility, and animal productivity (Nocek, 1997). Sub-acute ruminal acidosis is characterized by ruminal pH values ranging between 5.2 and 5.8 for any extended period of time (Penner et al., 2007). The depression in ruminal pH is largely caused by an increase in SCFA concentration without a concomitant increase in lactate concentration that is typical for acute ruminal acidosis (Stone, 2004; Krause and Oetzel, 2006). In the current study, we intended to induce SARA through the provision of an orally supplied glucose drench. The oral drench of glucose successfully decreased ruminal pH and increased the intraruminal SCFA concentration compared to the SHAM treatment. It also slightly increased intraruminal lactate concentration relative to SHAM sheep, but the lactate concentration remained below the threshold (5 mmol/L) previously used to distinguish SARA from acute forms of ruminal acidosis (Nocek, 1997). Thus, the present experimental approach fulfilled the criteria of an appropriate SARA induction model.

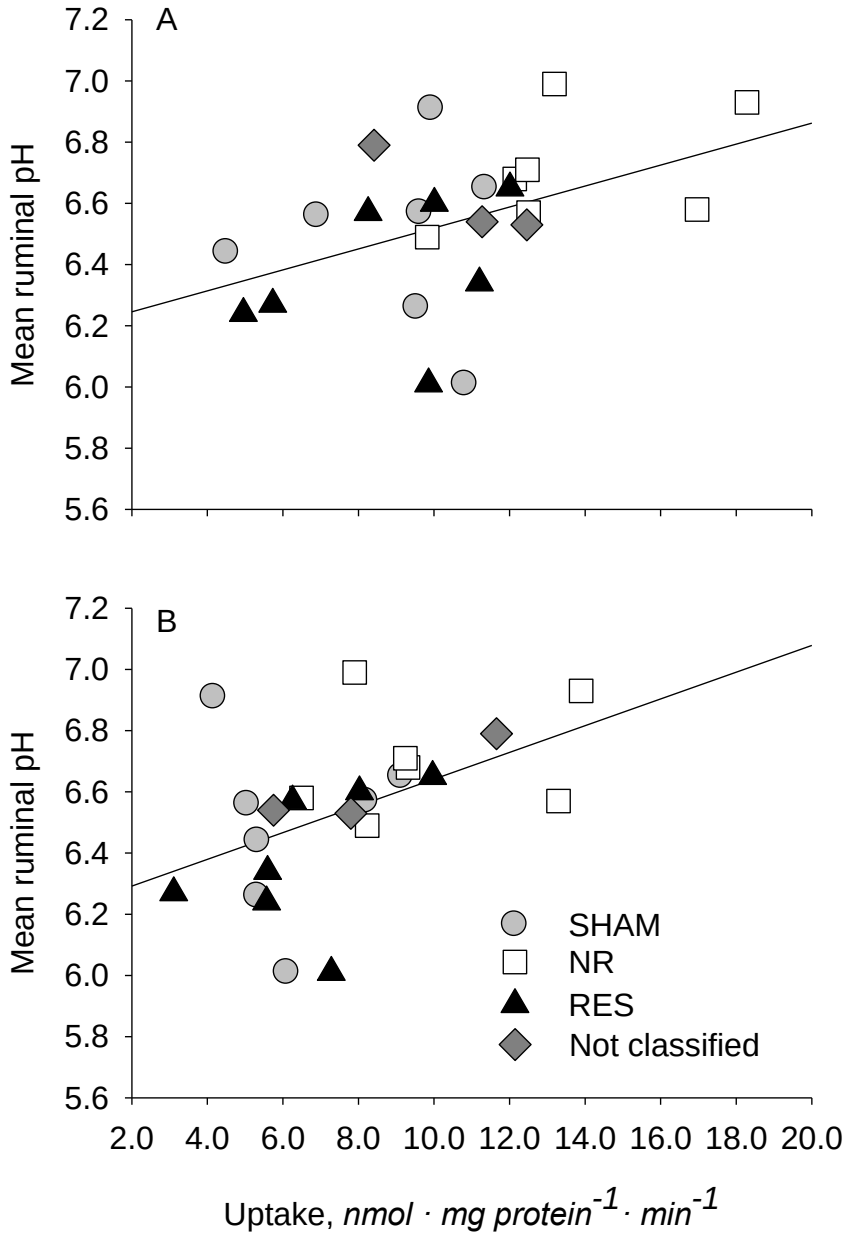

Figure 5.1 Relationship between the total uptakes of acetate (A) or butyrate (B) and the 48-h mean pH of each individual sheep prior to the oral drench. Regressions were (A) mean ruminal pH = 0.0460 × total acetate uptake, nmol · mg protein^{-1} · min^{-1} + 6.31 (n = 24, *P* = 0.024, r^2 = 0.212) and (B) mean ruminal pH = 0.0343 × total butyrate uptake, nmol · mg protein^{-1} · min^{-1} + 6.18 (n = 24, *P* = 0.033, r^2 = 0.191). The figure includes data for SHAM (n = 7), NR (n = 7) and RES sheep (n = 7), as well as for glucose-drenched sheep not classified into NR or RES (n = 3).

It was obvious, however, that not all sheep responded to the glucose drench with an episode of SARA. In fact, NR sheep had a negligible area below the threshold pH of 5.8 and had a nadir ruminal pH value of 5.61. In contrast, RES sheep had nearly seven times greater area that ruminal pH was below 5.8 than NR sheep, and the nadir pH for RES was as low as 5.36. The separation of glucose-drenched sheep into NR and RES allowed for the comparisons between groups providing new insight into the causes for variation in susceptibility among individual animals. Extreme examples of this variation were presented in a past study by Brown et al. (2000), in which five steers were provided steam flaked corn after having feed restricted for one day. The responses to the challenge in that study ranged from euthanization due to acute ruminal acidosis to ruminal acidosis being undetectable. The present study was not intended to investigate such extremes and, therefore, avoided the severe disturbances in ruminal fermentation (Owens et al., 1998) and compromises in ruminal epithelial function (Gäbel and Aschenbach, 2002) induced by feed restriction.

The main finding was that ruminal epithelia from NR sheep had a greater capacity for the uptake of acetate and butyrate in vitro. This suggests that higher ruminal pH following the glucose drench was due to greater absorptive capacity for SCFA in NR sheep than RES sheep as both groups were subjected to the same treatment. Further support for higher ruminal pH with greater absorptive capacity is provided by the positive correlations between total acetate and butyrate uptake and the mean ruminal pH before the oral drench for all sheep enrolled in the

study. Collectively, these results emphasize the importance of absorption for the regulation of ruminal pH during times of slow and rapid fermentation.

For butyrate, greater uptake in NR sheep was primarily due to increased bicarbonate-independent uptake, which should represent passive diffusion of the undissociated (protonated) SCFA. Passive diffusion of undissociated butyrate has previously been accepted as the major pathway for butyrate uptake into ruminal epithelial cells of sheep (Gäbel and Aschenbach, 2006) and is based on the higher lipophilicity of butyrate compared to acetate (Bergman, 1990). Since only the undissociated butyric acid is diffusible (Walter and Gutknecht, 1986), one mole of proton is taken up into the cells with each mole of butyrate absorbed. To make this a permanent contribution to proton removal from the rumen, it is required that absorbed protons are not recycled back into the rumen via the apical Na^+/H^+ exchanger (Müller et al., 2000). One pathway that helps limit proton recycling is the extensive intraepithelial metabolism of butyrate observed previously in vivo (Kristensen, 2006) and in vitro (Sehested et al., 1999a). Metabolism converts a significant proportion of butyric acid to BHBA (Sehested et al., 1999a; Kristensen, 2006) which can be exported via the monocarboxylate/H^+ co-transporter (MCT1) located on the basolateral side (Müller et al., 2000; Graham et al., 2007). Thus, the basolatorally directed co-transport of a ketone and proton results in the permanent removal of a proton from the system. Consistent with this concept, NR sheep had increased plasma BHBA concentration compared to RES sheep suggesting greater ketone and proton transport from the rumen. Since metabolism of butyrate helps to promote passive diffusion by maintaining the

concentration gradient between the intracellular and ruminal environments (Gäbel and Aschenbach, 2006), enhanced butyrate metabolism in NR sheep would also explain the greater bicarbonate-independent uptake rate for NR compared to RES sheep. In contrast, the minor amount of acetate metabolism in ruminal epithelia (Kristensen, 2006) may partially explain why bicarbonate-independent uptake of acetate uptake did not differ between NR and RES sheep.

Aschenbach et al. (2009) recently demonstrated that acetate uptake predominantly occurred via protein-mediated pathways rather than via lipophilic diffusion. They further demonstrated two distinct protein-mediated transport principles; 1) a bicarbonate-dependent mechanism that should operate as an HCO_3^-/acetate exchanger and 2) a novel bicarbonate-independent mechanism that can be inhibited by nitrate (Aschenbach et al., 2009). In the current study, the bicarbonate-dependent and bicarbonate-independent nitrate-sensitive pathways accounted for 51.6 and 19.2% of the total acetate uptake in NR sheep compared to 42.7% for bicarbonate-dependent and a negligible amount for bicarbonate-dependent nitrate-sensitive uptake in RES sheep. Additionally, the tendency for greater bicarbonate-dependent uptake of acetate for NR compared to RES sheep implies that greater activity of anion exchangers may be involved in the apical uptake of dissociated SCFA (acetate) in exchange for bicarbonate (Aschenbach et al., 2009; Bilk et al., 2005). Previous studies using the washed reticulorumen technique determined that for every 1 mol of SCFA uptake, approximately 0.5 mol of bicarbonate was secreted back into the ruminal contents (Ash and Dobson, 1963; Gäbel et al., 1991), but our data demonstrated that this proportion can vary

among animals. Absorption of acetate in the dissociated state, which is associated with the secretion of bicarbonate from the ruminal epithelia (Gäbel and Aschenbach, 2006; Aschenbach et., 2009) and the subsequent buffering of the ruminal contents likely contribute to net removal of protons to a greater extent than removal of protons with acetate via passive diffusion.

Collectively, while not ignoring the complexity and the involvement of other factors (e.g. fermentation and salivary secretion; Allen, 1997; Maekawa et al., 2002; Beauchemin et al., 2005) in ruminal pH regulation, the present study clearly underlines the importance of SCFA absorption for the removal of acid from the rumen. Greater capacity for the apical uptake of acetate and butyrate for NR compared to RES sheep provides a plausible explanation for the higher ruminal pH in this group after the oral glucose drench. Correspondingly higher rates of acid extraction from NR sheep rumen in vivo can be concluded from increased plasma BHBA concentrations in the case of butyrate and numerically lower intraruminal acetate concentrations in the case of acetate. Mechanisms on the apical ruminal membrane with proposed relevance for this variation in acid extraction and, consequently, the severity of SARA are bicarbonate-dependent uptake of acetate, bicarbonate-independent nitrate-sensitive uptake of acetate, and bicarbonate-independent uptake of butyrate. A better understanding of the molecular determinants of those pathways would provide the opportunity to characterize the absorptive capacity through genotype testing and may lead to new strategies to limit the occurrence of SARA, e.g., by means of breeding or genotype-adjusted feeding regimens.

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6.0 GENERAL DISCUSSION

6.1 Effect of Carbohydrate Source on Ruminal Acidosis in Early Lactation

The susceptibility of dairy cows to ruminal acidosis is dependent on a number of factors including stage of lactation and diet fermentability (Penner et al., 2007; Dohme et al., 2008). In addition, past studies have clearly demonstrated that there is considerable variation in the fermentable organic matter tolerance among animals fed a common diet (Brown et al., 2000; Bevans et al., 2005; Penner et al., 2007). To address the above mentioned points, 3 studies were conducted. Study 1 evaluated the source of carbohydrate on ruminal fermentation in early lactation by feeding sucrose in place of starch. In Study 2, changes in indicators for rumen epithelial function were determined for animals fed high forage and low forage diets by examining the rate of in vivo SCFA absorption and the expression of selected genes involved in the transport and metabolism of SCFA in ruminal tissue. Study 3 was conducted as a pre-requisite to Study 4 in order to continuously measure ruminal pH. The aim of Study 4 was to determine if the capacity for the apical uptake of acetate and butyrate by ruminal epithelia explains the variation in the susceptibility of individual animals to ruminal acidosis.

In the rumen, organic matter is fermented to provide energy for maintenance and growth functions by ruminal bacteria and yields by-products of fermentation including SCFA and CO_2 (Russell and Hespell, 1981). Although sugars are expected to have a greater rate of ruminal fermentation (Weisbjerg et al., 1998),

and consequently should reduce ruminal pH to a greater extent, our study demonstrated that partial replacement of corn grain for sucrose tended to increase rumen pH. Others have also reported that replacement of corn grain with sucrose tends to increase rumen pH (Penner et al., 2009) or does not reduce ruminal pH (Broderick et al., 2008). Based on the data collected in our study and the studies of others (Penner et al., 2007; Broderick et al., 2008) we are unable to determine the mechanism responsible for the lack of reduction in ruminal pH when sucrose is fed in replacement of starch. However, it is likely that there are multiple mechanisms working in concert. For example, replacing starch with sucrose may result in increased glycogen synthesis by ruminal bacteria (Hall and Weimer, 2007) or improvements in microbial OM efficiency (Ribiero et al., 2005) which would both decrease fermentation acid production.

Alternatively, Aschenbach et al. (2000) demonstrated that glucose is absorbed across ruminal tissue. As such, if feeding sucrose increased the concentration of free-monosaccharides in ruminal fluid, there may also be increased monosaccharide transport across the ruminal epithelia via SGLT-1 (Aschenbach et al., 2000) or GLUT transport proteins (Aschenbach et al., 2005). Future studies are warranted to evaluate the mechanisms involved for the tendency of increased ruminal pH when sugar is fed as a partial replacement of corn grain. Furthermore, studies should be conducted to determine the quantitative significance of monosaccharide transport across the ruminal epithelia which should include the measurement of glucose concentration in ruminal fluid under different feeding regimens.

Regardless of the mechanisms involved, we demonstrated that partial replacement of corn grain with sucrose may effectively increase ruminal pH during early lactation; a period when cows are at high risk for ruminal acidosis. This response is expected to be more pronounced if sucrose was fed in replacement for a starch source that is more rapidly degraded in the rumen than corn grain (e.g. barley grain). Future studies should be conducted to evaluate the potential to use disaccharides as a source of fermentable carbohydrate during early lactation in an effort to reduce the occurrence and severity of ruminal acidosis.

6.2 Regulation of Ruminal Acidosis during Early Lactation

As reported by Fairfield et al. (2007) and Penner et al. (2007), cows in early lactation experience severe ruminal acidosis. Consistent with those studies, we observed that when data were pooled across treatments, cows spent on average 352, 209, 164, and 268 min/d with a ruminal pH < 5.8 during wk 1, 2, 3, and 4, respectively. These data further support that cows in early lactation experience severe ruminal acidosis based on previously defined thresholds for SARA (Penner et al., 2007). To further characterize the ruminal pH response, we calculated the duration of time that ruminal pH was < 5.8, min/kg of DMI. The duration that pH was < 5.8, min/kg DMI, tended to decrease linearly (P = 0.051) and quadratically (P = 0.066) from wk 1 to wk 4 of lactation. From these data, it is clear that the increase in DMI is not the primary cause for SARA in early lactation.

It is plausible that changes in the rumen microflora are involved in the decreased acid load during wk 1 to wk 4. However, samples of ruminal fluid

from our study were collected 3h post-feeding and microbial diversity was evaluated using denaturing gradient gel electrophoresis (DGGE; Guan et al., unpublished). The results of the DGGE analysis showed that bacterial diversity did not differ between diet or week of lactation suggesting that factors other than microbial adaptation may mediate the response in rumen pH.

In addition to microbial adaptation, the rumen mucosa has been shown to adapt during the transition period as indicated by increased papillae surface area and increased capacity for SCFA absorption when cows are fed more fermentable diets (Dirksen et al., 1985). Functional changes in the rumen epithelia may result in increased ruminal pH as $SCFA^-$ are absorbed in exchange for HCO_3^- (Ash and Dobson, 1969; Gäbel et al., 1991) and because absorption of H-SCFA may result in the removal of a H^+ from the rumen. Thus, a subsequent study was conducted to evaluate differences in the absorption of SCFA and indicators of SCFA metabolism for animals fed diets differing in the forage-to-concentrate ratio.

6.3 Effect of Dietary Forage-to-Concentrate Ratio on the Rate of SCFA Absorption and Energy Metabolism by Ruminal Tissue

Past studies have demonstrated that animals adapted to diets with a lower forage-to-concentrate ratio have increased rates of SCFA absorption in vivo (Gäbel et al., 1991) and in vitro (Uppal et al., 2003), and increased oxidation of acetate and butyrate (Harmon et al., 1991) and Na/K ATPase transporter activity (McLeod and Baldwin, 2000) in ruminal tissue. Assuming that absorption of SCFA contributes to the stabilization of ruminal pH, diets containing a lower

forage-to-concentrate ratio should cause an increase in the rates of SCFA absorption and should increase the expression of key enzymes involved in energy metabolism in ruminal tissue.

Contrary to our hypothesis we did not observe differences in the fractional rate of SCFA absorption suggesting that under the conditions imposed, the forage-to-concentrate ratio does not affect the fractional rate of SCFA absorption (%/h). However, the estimated rates of absorption of propionic and butyric acid (mol/d) were greater for cows fed low concentrate (LC) than high concentrate (HC) diets. Further, cows fed HC had lower expression of pyruvate dehydrogenase lipoamide alpha 1 (PDH), the enzyme involved in the conversion of pyruvate to acetyl-CoA. The lower expression of PDH may be due to a decreased reliance on pyruvate for synthesis of acetyl-CoA as the increase in butyrate and propionate may have provided acetyl-CoA and oxaloacetate, respectively. Although we did detect some differences in the relative mRNA abundance, the lack of difference observed between LC and HC cows may be in part related to the management of the cows prior to collection of the tissue. For example, cows in the current study did not have access to feed for 12 h prior to euthanization. Past studies measuring transport of SCFA, Na, and Cl (Gäbel et al., 1993) or a glucose analogue (3-O-methyl-α-D-glucose; Gäbel and Aschenbach, 2002) have demonstrated that withholding feed for 48 h decreases the transport capacity of the ruminal epithelia. Reductions in transport capacity may be mediated through transcription and translation and therefore, the short-term feed restriction in the current study may

have impacted our results for the expression of genes coding for transporters and enzymes involved in SCFA absorption and metabolism.

However, collectively these data suggest that the forage-to-concentrate ratio does not affect the fractional rate of SCFA absorption but may alter energy metabolism in ruminal tissue. Future studies are needed to characterize SCFA metabolism in ruminal tissue and to determine the effects of metabolism on SCFA absorption and energy utilization by ruminal epithelia.

6.4 Variation in Ruminal pH Depression

One common theme observed in Studies 1 and 2 was the marked variation observed in the severity of ruminal pH depression for cows fed a common diet. For example, in Study 1 the duration that pH was < 5.8 ranged between 0 to 1305, 30 to 390, 63 to 277, and 55 to 897 min/d for the 5 ruminally cannulated cows fed the LS diet during wk 1, 2, 3, and 4 of lactation, respectively. Similarly, in Study 2, a large variation in the ruminal pH response was observed with the duration that ruminal pH was < 5.8 ranging between 127 and 557 min/d among 6 cows fed the HC diet. Other past studies have demonstrated substantial variation in the severity of ruminal pH depression under common feeding management protocols (Brown et al., 2000; Bevans et al., 2005; Penner et al., 2007). To understand the potential causes for such variation, regression analysis was used to compare the expression of genes related to the absorption and metabolism of SCFA to the duration that ruminal pH was < 5.8, min/kg DMI (Study 2). Although these comparisons were made with a limited data set (n=6), significant relationships

were observed between the expression of mRNA for 2 enzymes involved in the metabolism of SCFA and the duration that ruminal pH was < 5.8, min/kg DMI.

To further examine the relationship between the absorptive capacity of the ruminal epithelia and the severity of ruminal pH depression, we used an in vivo challenge model followed by the in vitro measurement of acetate and butyrate absorption across the isolated ruminal epithelia (Study 4). Consistent with the previous studies (Studies 1 and 2), we observed substantial variation in rumen pH among the group of sheep receiving the oral drench of glucose. This variation allowed for the separation of two distinct populations of sheep which were classified as responders (RES) or non-responders (NR) based on the area that ruminal pH was < 5.8 following the glucose drench. From this study, it is evident that a mild exposure to low ruminal pH does not affect the apical uptake of acetate or butyrate as the apical uptake of acetate and butyrate did not differ between SHAM sheep and RES. However, NR had markedly higher total acetate and butyrate uptake than RES. Thus, since NR and RES received the same dose of glucose, differences in the ruminal pH response can be attributed to differences in the absorptive capacity of the ruminal epithelia.

From Study 4, it is also evident that the type of SCFA and pathway of absorption may have an impact on the stabilization of ruminal pH. For example, if absorption of H-SCFA is to remain a permanent contribution toward the stabilization of ruminal pH, it is required that H^+s are not recycled back to the rumen. However, this is an incorrect assumption as H-SCFA would rapidly dissociate with an intracellular pH of 7.4. Furthermore, past studies have

demonstrated that there are NHE located on the apical membrane that are capable of secreting H^+ back into the rumen contents (Müller et al., 2000; Graham et al., 2007). With respect to acetate, NR tended to have greater bicarbonate dependent transport than RES suggesting that the secretion of bicarbonate from ruminal epithelia may help to stabilize ruminal pH.

In contrast, for butyrate, NR had greater bicarbonate independent uptake (passive diffusion) suggesting that for butyrate, H^+s absorbed across the apical membrane are also likely transported across the basolateral membrane. One pathway that may help to limit H^+ recycling is the extensive intraepithelial metabolism of butyrate observed both in vivo (Kristensen et al., 2005) and in vitro (Sehested et al., 1999). Metabolism in the ruminal epithelial cells converts a significant proportion of butyric acid to BHBA (Britton and Krehbiel, 1993) which can be exported via the monocarboxylate/H^+ co-transporter (MCT1) located on the basolateral side (Müller et al., 2002; Gäbel and Aschenbach, 2006). Thus, the basolaterally directed co-transport of a ketone and a H^+ results in the permanent removal of a H^+ from the rumen. Consistent with this concept, NR sheep had higher plasma BHBA concentration compared to RES. Further proof of the concept that absorption of SCFA stabilizes ruminal pH is that ruminal pH prior to the oral drench was positively related to the rates of apical uptake of acetate and butyrate. To my knowledge, this is the first study to document that sheep with increased absorptive capability have higher ruminal pH both prior to and following SARA induction.

6.5 Measurement of Ruminal pH in Small Ruminants

Continuous measurement of ruminal pH has previously been limited to cattle fitted with a ruminal cannula (Penner et al., 2006). However, comprehensive measurement of ruminal pH is desirable in small ruminants as it can provide a great deal of information regarding the nutritional management of small ruminants, and provide more detailed information of ruminal pH when small ruminants are used as a model for large ruminants. Previous limitations for the measurement of ruminal pH in small ruminants included difficulty in maintaining a ruminal cannula and the physical size of current systems (Graf et al., 2006; Penner et al., 2007). In Study 3, we overcame these limitations by developing the Small Ruminant pH Measurement System (SRS) that could be dosed orally into the rumen of small ruminants such as sheep. Although the SRS requires either euthanization or surgery to remove it from the rumen for the collection of data and post-measurement standardization, it provides the ability to measure ruminal pH in situations that were not previously possible. Further, the concordance correlation coefficient between manual pH measurement and measurement using the SRS indicated that pH can be measured both accurately and precisely.

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7.0 CONCLUSIONS

Although ruminal acidosis is a persisting digestive disorder in ruminant production, various strategies can be applied to reduce the prevalence and severity of ruminal acidosis. Such strategies may include the partial replacement of dietary starch with sucrose or increasing the forage-to-concentrate ratio. However, for cows in early lactation, increasing the forage-to-concentrate ratio may also decrease energy intake and thereby predispose cows to other metabolic disorders and may decrease performance. In contrast, the partial replacement of starch with sucrose does not compromise energy intake and may decrease ruminal acidosis.

In addition to dietary modifications, these are the first data to demonstrate that differences in the absorptive and metabolic capacity of the ruminal epithelia account for a large proportion of the variation among animals for the susceptibility to ruminal acidosis. As such, it is clear that animals themselves have inherent differences with regard to the susceptibility to ruminal acidosis. Future work is required to determine the molecular regulation for the capacity of the ruminal epithelia with respect to the absorption and metabolism of SCFA. This could lead to selective breeding programs and the identification of animals that are susceptible and resistant to ruminal acidosis. Feeding strategies can then be designed to optimize animal performance while minimizing the prevalence and severity of ruminal acidosis.