

Short chain fatty acid absorption and regulation of ruminal pH¹

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Introduction

Ruminants have a unique ability to derive energy from complex carbohydrates as microbes ferment the carbohydrates yielding short-chain fatty acids (**SCFA**). Short-chain fatty acids have been estimated to provide up to 75% of the total metabolizable energy (Bergman, 1990) for cattle. Thus, it is not surprising that diets that are readily fermentable promote greater production of SCFA also drive productivity outcomes (e.g. milk production) to a greater extent than less fermentable diets (Kolver and de Veth, 2002; Oba and Allen, 2003a,b). However, as weak acids, SCFA will dissociate in the rumen releasing a proton thereby decreasing ruminal pH under most circumstances. This highlights the double-edged sword where promoting SCFA production leads to greater energy supply, but too much SCFA or a rate of SCFA production that exceeds the ability to neutralize the protons (acid) reduces ruminal pH and can lead to ruminal acidosis. Understanding SCFA absorption in relation to ruminal pH and factors that can stimulate SCFA absorption to increase energy supply are key to enhancing production responses.

Mechanisms of SCFA Absorption and the Linkage to Ruminal pH

The ruminal contents are highly stratified due to the compartmentalization of the reticulo-rumen and the nature of the digesta within. Short-chain fatty acid production occurs primarily at the rumen-fluid rumen-mat interface and studies have consistently identified the rumen-fluid rumen mat interface as the region that has the lowest pH (Lui et al., 2009; Storm and Kristensen, 2010) and greatest SCFA concentrations (Storm and Kristensen, 2010). While this is logical, it presents a challenge in terms of SCFA absorption: for SCFA to be absorbed, they must be exposed to the ruminal epithelium. Thus, the stratification of the rumen provides a diffusional gradient that must be overcome (Storm and Kristensen, 2010). The primary mechanism to ensure SCFA are exposed to the ruminal epithelium is through rumen motility. However, few studies have considered rumen motility as a factor promoting SCFA absorption (Storm and Kristensen, 2010).

SCFA Absorption

Once SCFA are exposed to the ruminal epithelium, they are capable of being absorbed. The ruminal epithelium is a complex tissue consisting of 4 cell strata with many cell layers. The structural complexity creates a physical barrier from the ruminal environment and metabolically active layers that can absorb and metabolize SCFA. To better understand SCFA absorption, it is important to consider the histological

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arrangement of the ruminal epithelium (Steven and Marshall, 1980). The outer-most layer of the ruminal epithelium is the stratum corneum. The cells within the stratum corneum are highly keratinized and are not metabolically active. These protect the underlying strata from physical abrasion, but likely contribute very little as a barrier or promoting factor for SCFA absorption. The next layer is the stratum granulosum. This stratum is characterized by cells that are increasingly keratinized and have few intracellular organelles. However, the stratum granulosum is the primary site for tight-cell junctions and thus acts as a physical barrier to ensure absorption of SCFA occurs while preventing passage non-desired molecules. The next layer is the stratum spinosum. The cells within the stratum spinosum are metabolically active and have gap junctions that serve to facilitate cell-to-cell communication and exchange of ions. Finally, the most inner layer is the stratum basale. The stratum basale are highly active cells and are the layer where cell division occurs. The cell division is necessary to provide new cells that mature and differentiate as cells migrate toward the stratum corneum.

Given the structural complexity of the ruminal epithelium, 2 barriers on the ruminal epithelium can be highlighted. Firstly, SCFA must cross the apical side of the stratum granulosum to be absorbed within the ruminal epithelium and secondly, SCFA and their metabolites must be exported out of the ruminal epithelium into portal blood so that they can contribute to the systemic energy supply of the host. Fortunately, the ruminal epithelium is highly vascularized promoting blood flow and the movement of SCFA and SCFA-metabolites from the epithelium into portal blood flow.

While mechanisms of SCFA have been investigated since the 1940's (Danielli et al., 1945; Masson and Phillipson, 1951; Ash and Dobson, 1963), it was largely argued that SCFA absorption occurred via passive diffusion. The suggestion for passive diffusion was based on the inability to achieve saturation of SCFA absorption with increasing SCFA concentration and that reducing pH increased SCFA absorption (Dijkstra et al., 1993; López et al., 2003; Graham et al., 2007). It is important to recognize that as pH declines, the proportion of SCFA in the undissociated state increases and that only undissociated SCFA are permeable to cross the lipid bilayer of cells (Walter and Gutknecht, 1986; Gäbel et al., 2002). Thus, a reduction in pH would increase the proportion of undissociated SCFA that could then freely diffuse across the rumen epithelium.

There are numerous theoretical constraints for a model solely relying on passive diffusion. Firstly, the proportion of SCFA in the undissociated state ($pK_a = 4.8$) is low under normal pH conditions in the rumen. Even with pH values of 5.8, more than 90% of the SCFA would be in the dissociated state. Previous researchers had suggested that there was an acidic pH microclimate on the luminal side of the ruminal epithelia (Graham and Simmons, 2005) allowing for a greater proportion of SCFA in the undissociated state immediately adjacent to the epithelium. However, basic apical pH values have been reported (7.47 to 7.68) depending on the incubation conditions (Leonhard-Marek et al., 2006). Lipophilicity constants also suggest that butyric acid should be absorbed about 14 times more rapidly than acetic acid (Walter and Gutknecht, 1986). However, similar fractional absorption rates have been reported among SCFA in vitro (Aschenbach et al., 2009) and when differences are found (Dijkstra et al., 1993; López et al., 2003), they are

not consistent with the increase predicted based on lipophilicity. Moreover, a recent study showed that although the concentration of SCFA increased from 10 to 50 mM, the rates of acetate and butyrate absorption only increased by 2.1 and 2.4 times for acetate and butyrate, respectively (Schurmann et al., 2014). The model of passive diffusion also does not consider how SCFA are transported across the basolateral (blood facing) side of the epithelium.

A simplified model showing the current understanding of the mechanisms involved in SCFA absorption and how the absorption of SCFA contributes to the stabilization of ruminal pH is depicted in Figure 1 (Aschenbach et al., 2011). The predominant mechanisms include: 1) SCFA⁻/HCO₃⁻ anion exchange; 2) passive diffusion; 3) nitrate-sensitive SCFA absorption; 4) proton-coupled SCFA⁻ transport; and 5) electrogenic SCFA transport. While these are the major absorption mechanisms, other processes such as Na⁺/H⁺ exchange, and bicarbonate import into the cells are required to enable the maintenance of intracellular pH and to promote SCFA absorption. These pathways have been reviewed extensively in Aschenbach et al. (2011).

Much of the SCFA absorption occurs through anion exchange where SCFA⁻ are absorbed in exchange for release of HCO₃⁻ into the rumen and further the SCFA⁻ can cross the basolateral membrane in exchange for HCO₃⁻ import into the cell (Bilk et al., 2005; Aschenbach et al., 2009; Penner et al., 2009b). Based on the available data, approximately 42 to 57%, 0 to 14%, and 29 to 59% of the acetate transport relies on bicarbonate-dependent, nitrate-sensitive, and passive diffusion, respectively (Penner et al., 2009a; Schurmann, 2013). For butyrate, the proportion accounted for by bicarbonate-dependent transport, nitrate-sensitive transport, and passive diffusion are 24 to 46, 0 to 4, and 25 to 76%, respectively (Penner et al., 2009a; Schurmann, 2013).

In the rumen, the vast majority of the SCFA will be in the dissociated state (**SCFA⁻**). Absorption of SCFA⁻ occurs in exchange for HCO₃⁻ in an electro-neutral process that is mediated by a number of anion exchangers (Bilk et al., 2005; Aschenbach et al., 2009; Penner et al., 2009b). This mechanism provides a source of bicarbonate to the ruminal environment where it can neutralize a proton via the carbonic anhydrase reaction producing carbon dioxide and water. Driving forces for bicarbonate-dependent transport include the concentration of ruminal SCFA and ruminal pH. In fact, the bicarbonate-dependent SCFA absorption increases with increasing luminal SCFA concentration and with decreasing ruminal pH (Aschenbach et al., 2009). The bicarbonate facilitating this transport does not seem to occur in the cytosol, but rather is transported from arterial circulation into the cell (Sehested et al., 1999; Aschenbach et al., 2009). There are several bicarbonate transporters including anion exchangers on the basolateral (blood-facing) side that may also help to export SCFA⁻ out of the cell and into arterial blood. Thus, it appears that this transport process is crucial in terms of helping to regulate ruminal pH (Penner et al., 2009a) and exporting SCFA to be metabolized by other tissues.

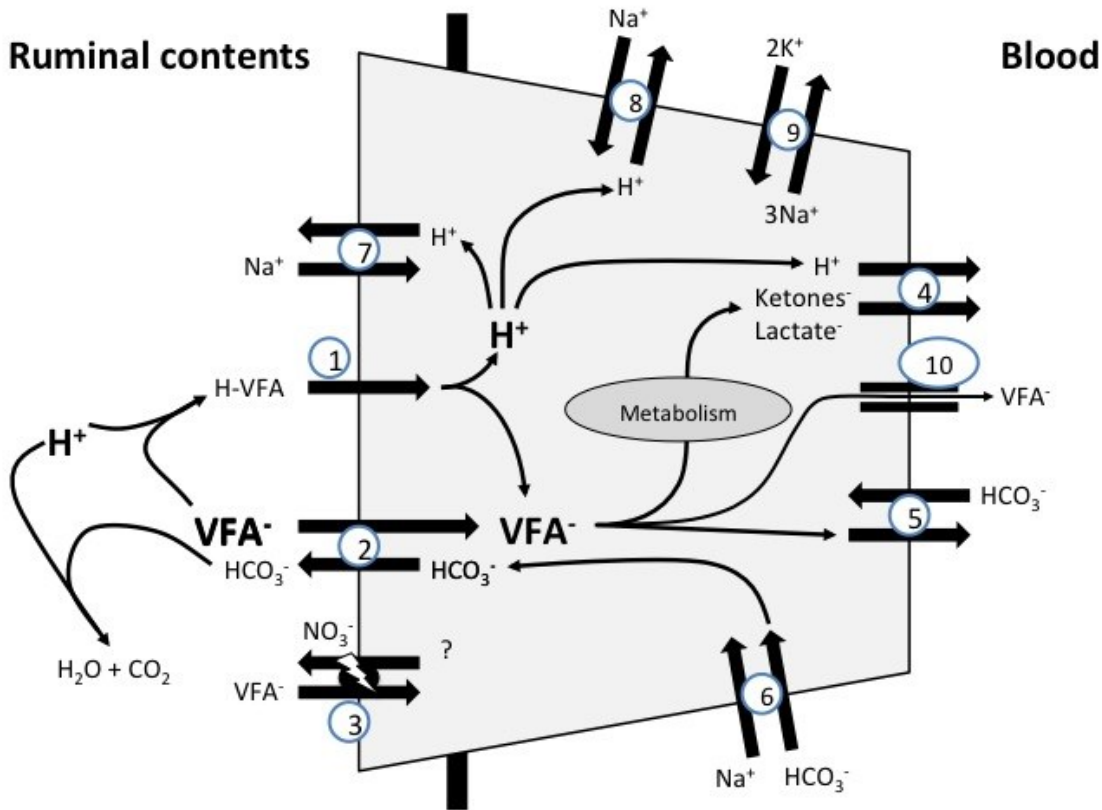


Figure 1. Partial model depicting the current understanding for SCFA absorption in relation to the stabilization of ruminal pH. 1) Diffusional absorption of SCFA facilitates the removal of a proton associated with the SCFA. This proton will rapidly dissociate in the cytosol where it can be exported by sodium/hydrogen exchanges (7, 8) or coupled with metabolites of SCFA (e.g. ketone bodies and lactate) via the monocarboxylate transporter (4). Dissociated SCFA can be absorbed in an anion exchange mechanism thereby providing a source of bicarbonate to the ruminal contents (2). This bicarbonate can then neutralize a proton through the carbonic anhydrase reaction thereby stabilizing ruminal pH. The bicarbonate supply to the epithelia is derived from blood (5, 6). The SCFA can also be absorbed via a nitrate sensitive pathway (3) and can be exported into blood via a voltage-gated channel (10). Note, the model does not show the structural complexity of the ruminal epithelia including the number of strata and cells within strata. Adapted from Aschenbach et al. (2011).

When H-SCFA are absorbed via passive diffusion, 1 proton is removed from the ruminal digesta; however, H-SCFA will dissociate in the cytosol releasing SCFA⁻ and H⁺. The proton (H⁺) released must be removed from the cell or neutralized in order to maintain intracellular pH and tissue integrity. Transporters involved in the regulation of intracellular pH include the sodium/hydrogen exchangers (**NHE**) that export protons back to the lumen

or into extra-cellular spaces. In addition to NHE, the monocarboxylate transporter (**MCT**) has been shown to be localized on the basolateral membrane (blood facing; Graham and Simmons, 2007) and can facilitate the removal of a proton along with metabolic end-products of SCFA metabolism such as ketone bodies and lactate (Müller et al., 2002; Kirat et al., 2006). Thus, the direction of proton export has major implications for whether passive diffusion contributes to the stabilization of ruminal pH. For example, if the proton is exported back into the rumen contents as a strategy to maintain intracellular pH, there would be no net proton removal from the rumen and therefore ruminal pH would not be affected. Interestingly, the expression and activity of NHE in ruminal epithelia increase when highly fermentable diets are fed (Etschmann et al., 2009; Yang et al., 2009; Schurmann, 2013). However, due to the complexity of the transport mechanisms involved and the regulation of their activity, it is very difficult to quantify or predict the proportion of protons recycled back to the lumen relative to those that account for permanent removal from the ruminal contents. That said, it is clear that under some circumstances passive diffusion does contribute to the removal of protons from the rumen (Penner et al., 2009a).

In addition, it is now known that there is a nitrate-sensitive transport pathway for SCFA. This process occurs both in the presence and absence of bicarbonate (Aschenbach et al., 2009), but currently the transporters involved are not known. Recent unpublished work (K. Wood, J.R. Aschenbach, F. Stumpff, and G.B. Penner) has shown a clear inhibitory effect with increasing concentrations of nitrate for acetate but no effect for butyrate. Future studies are required to improve our understanding of this transport mechanism and its regulation. Finally, electrogenic SCFA⁻ transport has been documented (Stumpff et al., 2009; Georgi et al., 2013). This transport process is thought to be mediated by maxi-anion channels but the total contribution to SCFA transport is not currently known.

Evidence Linking SCFA Absorption to the Stabilization of Ruminal pH

Early studies (Masson and Phillipson, 1951; Dobson and Ash, 1963; Gäbel et al., 1991) had suggested that SCFA absorption could be one mechanism for the stabilization of rumen pH. However, the first evidence supporting the pH stabilizing effect of SCFA absorption was provided by Resende Júnior et al. (2006). In that study moderate ($r^2 = 0.43$) positive correlations between the fractional rate of SCFA clearance and ruminal pH were observed suggesting that greater rates of SCFA clearance corresponded to improved ruminal pH. Resende Júnior et al. (2006) further evaluated whether the effect on pH was due to absorption of SCFA across the rumen wall or the passage of SCFA out of the rumen finding that both mechanisms were positively related to ruminal pH. In another study Penner et al. (2009b), reported negative associations between the expression of a number of genes involved in SCFA metabolism and the severity of ruminal acidosis for dairy cows fed a diet containing 64% concentrate. While these studies (Resende Júnior et al., 2006; Penner et al., 2009b; Schlau et al., 2012) showed relationships between ruminal pH or the severity of ruminal acidosis and the absorption of SCFA or indicators for intra-epithelial metabolism of SCFA, they cannot prove that SCFA absorption improves ruminal pH nor can they elucidate how the pathway of SCFA and type of SCFA affect ruminal pH.

Penner et al. (2009a) conducted a study to determine the relationship between the uptake of SCFA and the severity of ruminal acidosis. In that study, ruminal acidosis was induced in 17 lambs using an oral glucose drench (5 g glucose/kg BW). Based on the ruminal pH response over 3 hours after the drench, lambs were assigned to 1 of 2 classifications; non-responders (**NR**; the 7 lambs that had the least ruminal pH reduction) or responders (**RES**; the 7 lambs that had the greatest reduction in ruminal pH following the challenge). To evaluate the relationship between ruminal pH and SCFA absorption, the rumen epithelium was collected and the uptake of acetate and butyrate was measured *ex vivo*. Results from the NR and RES lambs were compared to a group that was not exposed to an acidotic challenge (**SHAM**). Ruminal pH differed between sheep classified as NR (67.8 min), RES (153 min) and SHAM (1.1 min) as did the uptake of acetate and butyrate. It is important to note that we assumed that the acidotic challenge imposed did not compromise the ruminal epithelium as acetate and butyrate uptake did not differ between the RES and SHAM treatments. Interestingly, we found that epithelia from NR sheep had a greater rate of total acetate and butyrate uptake than RES indicating that the improved ruminal pH response could be attributed to greater capability for SCFA uptake. In addition, retrospective correlation analysis showed that acetate and butyrate uptake was also positively related to the mean pH prior to the acidotic challenge. This is the only study (Penner et al., 2009a) that has provided comprehensive data demonstrating that the rate of acetate and butyrate uptake has a substantial effect on ruminal pH homeostasis.

As mentioned above, the pathway of SCFA absorption may play a role in the stabilization of ruminal pH. In addition to total uptake, Penner et al. (2009a) also reported that the main mechanisms facilitating acetate and butyrate uptake were different between NR and RES. For acetate, the bicarbonate-dependent and bicarbonate-independent nitrate-sensitive transport was greater for NR than RES. As mentioned above, with the bicarbonate-dependent transport, bicarbonate secretion and acetate absorption are coupled. Interestingly, for butyrate, bicarbonate-independent (passive diffusion) uptake was higher for NR than RES. Collectively these data indicate that the pathway of SCFA absorption may differ based upon the type of SCFA and thus the relative contribution towards the stabilization of ruminal pH may also differ. For example, acetate is not as lipophilic as butyrate and thus protein-mediated pathways contribute substantially towards its uptake. This is important as the bicarbonate-dependent pathway would also provide bicarbonate to buffer the rumen contents (Aschenbach et al., 2009). In contrast, butyrate has a greater potential for diffusional uptake (Walter and Gutknecht, 1986). Thus, factors promoting a concentration gradient between the rumen, cytosol, and blood should promote absorption (Gäbel et al., 2002). The suggestion that intracellular metabolism enhances butyrate absorption is in alignment with Gäbel et al. (2001) and previously reported negative correlations between the expression of genes involved in butyrate metabolism and the severity of ruminal acidosis (Penner et al., 2009b). Furthermore, we found that NR sheep had greater serum β -hydroxybutyric acid (**BHBA**; a metabolite of butyrate metabolism) than RES sheep after the 180 min acidotic challenge (Penner et al., 2009a). The increase in serum BHBA may also indicate that for butyrate, metabolism to ketone bodies and export from the cell via MCT may help to regulate ruminal pH.

Nutritional Modulation of SCFA Transport

Given the importance of SCFA transport towards meeting the energy requirement and stabilization of ruminal pH, several studies have investigated whether dietary or feeding management can modulate the response. Interestingly, past studies have clearly demonstrated that SCFA can be manipulated through management and dietary interventions.

Low Feed Intake and Feed Deprivation Decrease SCFA Absorption

The vast majority of current research has focused on rumen epithelial adaptation from an anabolic perspective, however, in times of scarcity or in response to a nutritional insult, the adaptive response certainly includes regression. In fact, the long-term changes induced by a low plane of nutrition have been shown to decrease gut mass and reduce O₂ consumption by visceral tissue, and reduce SCFA absorption (Doreau et al., 1997). Understanding how the ruminal epithelium responds to reductions in SCFA exposure due to a transient low feed intake and, more importantly, the timeline required for the epithelium to return to the pre-restriction function is needed to develop feeding strategies and mitigate disorders associated with digestive upset.

Albeit unintentional and generally short in duration, beef and dairy cattle are exposed to periods of feed restriction or complete feed deprivation. Examples include during weaning, transportation, prior to and immediately after parturition, immediately following digestive upset, while experiencing heat stress, and in association with metabolic disorders and infection. Gäbel et al. (1993) demonstrated that 48-h of complete feed reduced SCFA, Na⁺, Ca²⁺, and Mg²⁺ absorption by approximately 40 to 60%. It is important to note that these changes were likely due to a reduction in the functional capacity and blood flow rather than changes induced by epithelial surface area. More recently, the effect of the severity of short-term feed restriction, rather than complete feed deprivation, has been investigated (Zhang et al., 2013a). In this study, 18 heifers were fed ad libitum and then allocated feed equating to 75, 50, or 25% of their ad libitum DMI for a period of 5 d. A 5-d feed restriction period, regardless of the severity, tended ($P = 0.09$) to decrease total SCFA absorption and decreased acetate absorption. Additionally, heifers restricted to 50 and 25% of ad libitum intake tended ($P = 0.07$) to have lower rates for total SCFA and acetate absorption compared to those restricted to 75% of ad libitum intake. It does not appear that shifting the dietary forage-to-concentrate ratio will mitigate this effect despite expected changes in fermentability and ruminal retention time (Albornoz et al., 2013a). For example, when cattle were restricted to 25% of their ad libitum intake for 5 d, the total SCFA absorption rate decreased by 120 mmol/h relative to baseline measurements and did not differ between heifers fed a diet consisting of 92% forage vs. those fed 60% forage (Albornoz et al., 2013a). Thus, it appears that reductions in ruminal epithelial function occur rapidly in response to lower energy intake.

A rapid reduction in ruminal epithelial function may be a compensatory mechanism to reduce energy expenditure by ruminal tissue (Zhang et al., 2013a) during periods of low energy intake. However, given the transient nature of low feed intake under

conventional feeding systems, a rapid increase in epithelial function corresponding to increased energy intake would be desirable. Zhang et al. (2013b) provided heifers ad libitum access to feed, without changes in the diet composition, after a 5-d period of feed restriction. That study reported two important findings: 1) return to ad libitum feeding without dietary change induced ruminal acidosis, and 2) that time to recover absorptive function increased with increasing severity of feed restriction. In fact, heifers restricted to 25% of their ad libitum intake required 3 weeks for SCFA absorption rates to recover, whereas those restricted to 75% of their ad libitum intake recovered within 1 wk. The delayed recovery response suggests that at least a portion of the response is mediated by the epithelia and not solely due to changes in blood flow. Interestingly, the recovery response appears to be hastened when cattle are fed greater proportions of concentrate prior to dietary restriction and greater proportions of forage after feed restriction (Albornoz et al., 2013b).

Ruminal Acidosis Compromises SCFA Absorption

Providing adequate time for dietary adaptation has been recommended as a strategy to reduce the risk for ruminal acidosis. It is evident that repeated exposure to sub-acute ruminal acidosis or a single exposure to acute ruminal acidosis may also negatively affect SCFA absorption. Dohme et al. (2008) reported that the response to repeated ruminal acidosis inductions increased in severity with each consecutive challenge despite the cows consuming less grain during consecutive challenges. While there may be a number of reasons behind this response, a decrease in SCFA absorption is highly plausible because previous studies have shown that at similar pH values (< 5.4) epithelial damage was induced (Steele et al., 2009) and ion transport was impaired (Gaebel et al., 1987, Gaebel et al., 1988; Gaebel et al., 1989). That said, it is not clear whether adaptation reduces the risk for ruminal acidosis. In a recent study, we compared whether cattle fed a high-grain diet (81% barley grain, 10% vitamin and mineral supplement, 9% barley silage) for 34 d were more resistant to ruminal acidosis than cattle fed the same diet but for only 8 d (Schwaiger et al., 2013a,b). Ruminal acidosis was induced by restricting feed intake on the d before the challenge and the challenge itself included an intraruminal infusion of ground barley grain. There were no differences observed for the risk or severity of ruminal acidosis between short-adapted and long-adapted cattle. However, we did observe that ruminal pH recovered more rapidly in long-adapted cattle than short-adapted cattle. Interestingly, long-adapted cattle also had greater lactate absorption than short-adapted cattle immediately following the challenge.

While the total SCFA absorption rate was not different between the short- and long-adapted cattle, it was very clear that induction of ruminal acidosis decreased SCFA absorption (Schwaiger et al., 2013a,b) when measured 2 d following induction of ruminal acidosis but not when measured 9 d after the induction of ruminal acidosis. Moreover, there appears to be a compensatory shift in ruminal buffering strategies such that absorption is reduced following a bout of ruminal acidosis while at the same time, saliva production increases. Thus, it appears that ruminal acidosis impairs SCFA absorption but the recovery following a bout of ruminal acidosis may be rapid and that cattle may increase salivary buffer supply to compensate for the reduction in SCFA absorption. The

negative effect of severely low ruminal pH on SCFA absorption is supported by previous work in vivo (Krehbiel et al., 1995) and in vitro (Wilson et al., 2012).

Promoting SCFA Absorption

To apply the concept of nutritional challenges within the feedlot sector, a study was conducted to evaluate strategies to accelerate recovery of gastrointestinal tract following a nutritional challenge (Penner et al., unpublished). In this study, 32 lambs were assigned to 1 of 4 treatments. The treatments consisted of a finishing ration (9% barley silage, 79% barley grain, and 12% of a barley-based mineral and vitamin supplement) throughout the study (CON) or lambs that were fed the finishing ration but exposed to a 3-d period of low feed intake at 50% of voluntary intake and then 1 of 3 recovery treatments. The recovery period was 5-d. To evaluate the recovery response after low feed intake, lambs were either fed the finishing ration (FIN), or 1 of 2 diets where the proportion of barley silage was increased to 20% at the expense of barley grain. This approach is commonly referred to as a 'storm' diet in the feedlot sector. The second 'storm' diet also included a dietary additive of rumen protected betaine (0.7% of DM), superoxide dismutase (0.01% of DM) as an antioxidant, and Na-butyrate (0.2% DM). Betaine has been reported to help support gastrointestinal tract function during coccidia challenges (Kettunen et al., 2001; Fetterer et al., 2003), and superoxide dismutase has been reported to improve gastrointestinal tract function in mice (Vouldoukis et al. 2004). Finally, butyrate has been shown to induce positive effects at low doses (Gorka et al., 2007; Kowalski et al., 2015). We observed that the CON group did not change DMI throughout the study, thereby serving as an appropriate control as they were not exposed to a low feed intake challenge. Interestingly, lambs fed the STORM or STORM plus additive diets during recovery increased DMI relative to that during low feed intake, while lambs fed the FIN diet did not increase DMI during recovery. This suggests, that increasing the proportion of forage after a period of LFI can help recovery of DMI when fed finishing diets. While all treatments, except the CON, had lower ruminal pH during recovery than during the LFI challenge, the STORM and STORM plus additive diets had numerically greater ruminal pH during the 5-d recovery than lambs provided the finishing diet. We also found that lambs fed the STORM plus additive diet tended to have greater rates of acetate absorption and had greater butyrate absorption in the recovery period than the other treatments. This study demonstrated that moderate increases in the forage proportion can help cattle recover after a period of LFI, even with finishing diets, and that provision of additives reported to accelerate gastrointestinal tract function can help the recovery response. Future research is needed to evaluate which additives are most beneficial to improve the recovery of the gastrointestinal tract.

Dietary Fatty Acid Supply and Composition Affect SCFA Absorption

Dietary fatty acids are often used to increase energy density of the diet and can modulate composition of tissues (Owens and Gardner, 2000). However, we are not aware of any studies that have evaluate whether ruminal epithelial composition can be manipulated and whether changes in composition affect SCFA absorption. Twenty-one Holstein steers (194 ± 10.7 kg) were randomly assigned to the control (**CON**; contained

2.2% ether extract) or one of two lipid treatments (contained 5% ether extract) utilizing saturated (**SAT**) or unsaturated sources (**UNSAT**) of lipid (Verdugo and Penner, unpublished). The SAT lipid sources were primarily from tallow and palmitic acid while the UNSAT was provided from flax and megalac. After 30 d, calves were killed and samples of ruminal digesta, blood, and ruminal tissue were collected for fatty acid analysis and ruminal tissues were also used for ex vivo measurement of acetate, propionate, and butyrate uptake and flux. WE observed that inclusion of lipid increased ($P < 0.01$) the concentration of fatty acids in ruminal fluid, but SAT and UNSAT did not differ. Feeding UNSAT decreased the proportion of saturated FA and increased the proportion of mono and polyunsaturated fatty acids in ruminal fluid. The changes in ruminal fluid were also reflected in plasma and ruminal tissue. The ruminal epithelial concentration of fatty acids tended ($P = 0.10$) to be greater for calves fed lipid and for calves fed UNSAT vs. SAT ($P = 0.06$). Interestingly, calves fed supplemental lipid had greater ($P = 0.03$) butyrate uptake than CON, and butyrate uptake was 44% greater for SAT than UNSAT ($P < 0.01$) suggesting that fatty acid supply and type can modulate SCFA absorption.

Summary

Short-chain fatty acid absorption clearly helps to stabilize ruminal pH by either removing protons with passive diffusion or by the secretion of bicarbonate with anion exchange mechanisms. Interestingly, the relative contribution of individual pathways of SCFA absorption differ based on the type of SCFA absorbed and the contribution of salivary bicarbonate and epithelial buffering towards stabilization of ruminal pH appear to be affected by ruminal pH itself. A number of factors such as feed restriction and ruminal acidosis negatively affect SCFA absorption. More recent research has also highlighted that nutritional manipulation can enhance SCFA absorption providing a strategy to help support gastrointestinal function and potentially increase productivity.

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