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RUMINANT NUTRITION SYMPOSIUM: Role of fermentation acid absorption in the regulation of ruminal pH[1,2]

J. R. Aschenbach,*[3] G. B. Penner,† F. Stumpff,* and G. Gäbel‡

*Institute of Veterinary Physiology, Free University of Berlin, D-14163 Berlin, Germany;
†Animal and Poultry Science, University of Saskatchewan, Saskatoon, Saskatchewan, Canada, S7N 5A8;
and ‡Institute of Veterinary Physiology, University of Leipzig, D-04103 Leipzig, Germany

ABSTRACT: Highly fermentable diets are rapidly converted to organic acids [i.e., short-chain fatty acids (SCFA) and lactic acid] within the rumen. The resulting release of protons can constitute a challenge to the ruminal ecosystem and animal health. Health disturbances, resulting from acidogenic diets, are classified as subacute and acute acidosis based on the degree of ruminal pH depression. Although increased acid production is a nutritionally desired effect of increased concentrate feeding, the accumulation of protons in the rumen is not. Consequently, mechanisms of proton removal and their quantitative importance are of major interest. Saliva buffers (i.e., bicarbonate, phosphate) have long been identified as important mechanisms for ruminal proton removal. An even larger proportion of protons appears to be removed from the rumen by SCFA absorption across the ruminal epithelium, making efficiency of SCFA absorption a key determinant for the individual susceptibility to subacute ruminal acidosis. Proceeding initially from a model of exclusively diffusional absorption of fermentation acids, several protein-dependent mechanisms have been discovered over the last 2 decades. Although the molecular identity of these proteins is mostly uncertain, apical acetate absorption is mediated, to a major degree, via acetate-bicarbonate exchange in addition to another nitrate-sensitive, bicarbonate-independent transport mechanism and lipophilic diffusion. Propionate and butyrate also show partially bicarbonate-dependent transport modes. Basolateral efflux of SCFA and their metabolites has to be mediated primarily by proteins and probably involves the monocarboxylate transporter (MCT1) and anion channels. Although the ruminal epithelium removes a large fraction of protons from the rumen, it also recycles protons to the rumen via apical sodium-proton exchanger, NHE. The latter is stimulated by ruminal SCFA absorption and salivary Na^+ secretion and protects epithelial integrity. Finally, SCFA absorption also accelerates urea transport into the rumen, which via ammonium recycling, may remove protons from rumen to the blood. Ammonium absorption into the blood is also stimulated by luminal SCFA. It is suggested that the interacting transport processes for SCFA, urea, and ammonia represent evolutionary adaptations of ruminants to actively coordinate energy fermentation, protein assimilation, and pH regulation in the rumen.

Key words: ammonia, lactic acid, nutrient absorption, ruminal acidosis, ruminal pH, short-chain fatty acid

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INTRODUCTION

Microbial fermentation of OM induces shifts in pH that act on the microbial ecosystem, thus determining the selective growth of certain microbial species, and the types and quantities of fermentation products (Russell and Diez-Gonzalez, 1998; Mouriño et al., 2001; Russell and Rychlik, 2001; Allen et al., 2006). Fermentation of

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[3]Corresponding author: aschenbach.joerg@vetmed.fu-berlin.de

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carbohydrates releases organic acids that readily dissociate to decrease pH (Allen, 1997; Russell and Rychlik, 2001). In contrast, fermentation of protein or NPN may release excess ammonia, which readily associates with protons to increase pH (Wang and Fung, 1996). When microbial fermentation is utilized for nutrition, either as in-body fermentation (as in the rumen) or as a technology such as silage production, the purpose is mostly to facilitate acid production from carbohydrates while preserving or assimilating protein. Thus, fermentation of nutrients leads to proton release and, thereby, decreases pH. As a consequence, fermentation usually proceeds at pH <7.

The forestomach of ruminants is one of the most refined in-body fermenters utilized for nutrition (Bergman, 1990; Russell and Rychlik, 2001). The pH homeostasis within this fermenter determines not only the biodiversity of the ruminal ecosystem and the nutritional value of the end products, but also animal health because both alkaline and acidic deviations may promote health upsets. Alkaline disturbances of ruminal pH may occur during excess protein feeding; however, description of ruminal alkalosis is limited to very few reports (Sederevicius and Kantautaité, 1993; Loste et al., 2005). Alternatively, acidic disturbances are a widely acknowledged problem because current intensive production systems rely on intake of large amounts of carbohydrates to meet the energy demand of animals. Because only easily fermentable carbohydrates have sufficient energy density and passage rates to allow for greater energy intakes, intensive feeding strategies are naturally linked to an increased risk of ruminal acidosis (Plaizier et al., 2008; Lechartier and Peyraud, 2010). Fermentation acid absorption plays a key role in counteracting acidosis, and it is evident that a complex system of acid absorption pathways contributes to this outcome. This review aims to analyze the importance of epithelial short-chain fatty acid (**SCFA**) transport pathways for ruminal pH regulation in relation to the different components that contribute to proton release, proton buffering, and proton removal in the rumen.

RUMINAL pH PROFILES AND THEIR SIGNIFICANCE

Ruminal pH is not only a physicochemical measure with relevance for the fermentation in the rumen, but it is also the central diagnostic criterion to classify ruminal acidosis as a disease that affects more organs than just the rumen (Dirksen, 1970, 1985; Gozho et al., 2005; Dohme et al., 2008; Steele et al., 2009). Clinical presentation of the mild form, called subacute ruminal acidosis (**SARA**), varies and may include mild transient anorexia, intermittent diarrhea, dehydration, poor body condition, depression, decreased rumen motility, laminitis, unexplained abscesses, and decreased milk production (Dirksen, 1970, 1985; Underwood, 1992; Nordlund and Garrett, 1994; Duffield et al., 2004; Krause and Oetzel, 2006). Because the disease presentation for SARA is variable and may be evident only because of increased culling rates, the presence of an abnormal ruminal pH is used as the final decisive criterion for diagnosis (Duffield et al., 2004; Krause and Oetzel, 2006). It may appear peculiar that a single measurement item from a sequestrated "transcellular" compartment (i.e., ruminal pH) can satisfactorily serve to diagnose a complex internal disease. Indeed, the relationship between ruminal pH and a given clinical sign (e.g., going off feed) is certainly not linear (Uhart and Carroll, 1967), which supports the view that ruminal pH is a crucial risk factor rather than a unique diagnostic criterion for ruminal acidosis. Nonetheless, if ruminal pH is the final decisive criterion to diagnose and classify a multiorgan disease, it should be mandatory to measure it as representatively as possible and to standardize and improve the diagnostic interpretation.

The 2 major challenges for collecting and comparing ruminal pH data are that ruminal pH is not homogeneously distributed throughout the rumen (Duffield et al., 2004; Zebeli et al., 2008b) and that different sampling techniques will produce different results (Nordlund and Garrett, 1994; Duffield et al., 2004). The standardized sampling site for ruminal pH is the (cranial-)ventral ruminal sac because this is the place where most mixing of ruminal contents occurs (Duffield et al., 2004). The ventral ruminal sac thus provides the most integrated information on the pH status of the whole rumen (as long as motility is intact), and the term "ruminal pH" is often used synonymously for "ventral ruminal pH". Unfortunately, pH measurements in ruminal fluid samples from the ventral ruminal sac recovered by orogastric tube or ruminocentesis show only poor to moderate correlation to the ventral ruminal pH measured in situ (Duffield et al., 2004). In situ measurement of ruminal pH is thus necessary if results with greater precision are desired. Over the last several years, great efforts have been made to accurately measure ventral ruminal pH in situ over time by indwelling systems, which has already led to a greatly improved understanding of ruminal pH dynamics. Indwelling systems are available for tethered (Dado and Allen, 1993) and nontethered, ruminally cannulated cattle (Graf et al., 2005; Penner et al., 2006; AlZahal et al., 2007), as well as for noncannulated small ruminants (Penner et al., 2009b). The great advantage of these systems is a good time resolution over several days or weeks when investigating the relationship between meal size, diet fermentability, eating behavior, and ruminal pH (Maekawa et al., 2002; Krause and Combs, 2003; Penner et al., 2009b). Because the ventral indwelling sensors reside close to the ruminal epithelium, the data collected should have greater predictive value for the pH environment above the epithelium, even though the immediate surface microenvironment of the epithelium is slightly more alkaline when urea hydrolysis (Cheng and Wallace, 1979) and epithelial HCO_3^- secretion (Leonhard-Marek et al., 2006) are active.

Whereas the time resolution of ruminal pH measurement has greatly improved, the site resolution or the time × site resolution is still a matter of concern. Rumen stratification can cause extensive pH gradients within the rumen. The pH values in the rumen mat (Zebeli et al., 2008b; Storm and Kristensen, 2010) and central portion of the rumen (Duffield et al., 2004) have been reported to be 0.16 to 0.73 units less compared with those from the ventral sac. The pH conditions under which the major part of fermentation occurs should, thus, be slightly more acidic than predicted from ventral ruminal pH. Additional measurements in the ruminal mat have, thus, a potential to improve the precision when predicting fiber digestibility, fermentation patterns, and health consequences of feeding regimens based on easily fermentable carbohydrates. Sensors for continuous indwelling measurement in the dorsal ruminal area have just become commercially available (Laporte-Uribe et al., 2010).

With regard to ruminal acidosis, the definition of tolerable pH thresholds has to take into account the vulnerability of both the ruminal ecosystem and the ruminal epithelium. It is well established that starch feeding and prolonged reductions in ruminal pH less than 6.0 greatly facilitate the growth of amylolytic bacteria (Mackie and Gilchrist, 1979) while cellulolytic bacteria and NDF digestibility are concurrently inhibited (Russell and Wilson, 1996; Krajcarski-Hunt et al., 2002). Additionally, a marked proliferation of lactate-utilizing bacteria occurs at pH 5.8 (Mackie and Gilchrist, 1979). These events are linked to profound shifts in fermentation pattern with increasing proportions of propionate and sometimes butyrate (Bannink et al., 2008). Based on changes in microbial composition and activity, a first pH threshold around 5.8 is justified. This threshold coincides with the first vulnerability threshold of the ruminal epithelium because early inflammatory responses may occur when ruminal pH is <5.6 for >1 h (Gozho et al., 2005). A second threshold exists as pH decreases further toward 5.0. Ruminal protozoa die and fermentation shifts to release large amounts of lactic acid (Dirksen, 1970; Stone, 2004; Lettat et al., 2010), which appears to be mainly attributable to an overgrowth by *Streptococcus bovis* (Hungate et al., 1952; Dirksen, 1970; Gill et al., 2000). This second threshold also coincides with a second vulnerability threshold of the ruminal epithelium, where an immediate and persistent compromise of transport and barrier function occurs at approximately pH 5.1 (Gaebel et al., 1987; Gaebel and Martens, 1988; Aschenbach and Gäbel, 2000).

When applying the quoted pH values, it needs to be considered that every biological system is dynamic by nature with no clear-cut thresholds. Additionally, the pH of the bulk solution (e.g., ventral ruminal pH) is not completely identical to the pH directly at the epithelial surface (Leonhard-Marek et al., 2006), and ruminal motility may greatly affect pH gradients from the lumen to the epithelium. As a consequence, decreasing of ruminal pH into the range defined for SARA does not always cause inflammation (Khafipour et al., 2009) or result in decreased productive performance (Oba and Allen, 2003a,b). Although we do not currently understand all the different factors that can modify the effect of low pH on the ruminal ecosystem and the ruminal epithelium, it has been specifically suggested that the intake of ruminally fermentable OM may be an important accessory factor and a useful additional criterion for the diagnosis of ruminal acidosis (Penner et al., 2009c).

The dynamic nature of the effects of low pH on the ruminal ecosystem and the ruminal epithelium have led to the use of different thresholds for acidosis classification among research groups, with thresholds ranging between 5.5 and 5.8 for SARA (Kleen et al., 2003; Krause and Oetzel, 2006; Penner et al., 2007) and 5.0 and 5.2 for acute ruminal acidosis (Dirksen, 1985; Nocek, 1997; Penner et al., 2007). The smaller threshold values (5.5. and 5.0, respectively) appear to be appropriate when taking single measurements or nadir pH as the decision criterion for subacute and acute acidosis, respectively (Dirksen, 1985; Kleen et al., 2003). The greater values (5.8 and 5.2, respectively) appear more appropriate when making decisions based on the duration or area (pH × duration) spent below these thresholds (i.e., when considering that only prolonged or repeated periods below the thresholds may lead to subacute or acute health disturbances; Gozho et al., 2005; Penner et al., 2007).

Although decreases in ruminal pH may originate from the intake of acidic feed (e.g., silage), the intraruminal production of fermentation acids from concentrate has by far the largest effect on ruminal pH (Lechartier and Peyraud, 2010; see next 2 sections). As intake and fermentation vary, ruminal pH also varies markedly throughout the day and may differ substantially within an animal for consecutive days (Figure 1). Thus, only continuous measurement of ruminal pH over an extended period can serve to adequately evaluate ruminal acid–base balance. Variation within a day is largely affected by the consumption of meals, with pH decreasing after a meal and slowly recovering until the next meal (Allen, 1997). Variation in the pH response demonstrates that, at times, the rate of acid production exceeds the rate of acid removal or proton buffering. Soluble carbohydrates and starch are most effective in decreasing ruminal pH rapidly, whereas increasing the amount of physically effective fiber in the ration is the most efficient nutritional measure to alleviate the pH decline after a meal (Allen, 1997; Zebeli et al., 2008a). The reason for variation among days has not been addressed specifically but is most likely related to DMI; however, DMI is not exclusively related to ruminal pH, at least not in the long term (Penner et al., 2007; Figure 2). Variation among animals in their pH response to a common diet is also prevalent and has received considerable attention in recent years (Brown et al., 2000; Bevans et al., 2005; Penner et al., 2009b,c). The

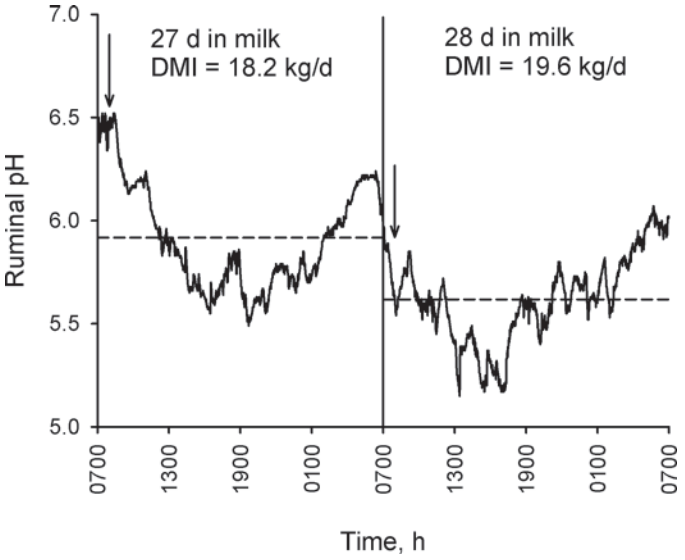

Figure 1. Variation in the ruminal pH response for 1 cow on 2 consecutive days (27 and 28 d in milk). The solid line indicates the ruminal pH value (data collected every 30 s and averaged over 1-min intervals), the dashed line indicates mean ruminal pH for each individual day (5.92 for d 27 and 5.62 for d 28), and the arrows depict the time of feeding (0800 h). The areas under the curve for pH <5.8 (pH × h/d) were 1.22 and 4.88 for d 27 and 28, respectively. Excerpt of data from Penner and Oba (2009).

latter appears to be highly correlated to differences in fermentation acid absorption (Penner et al., 2009b), as will be elaborated in subsequent sections.

ESTIMATES OF RUMINAL ACID PRODUCTION

The nutritional relevance of acid production in the rumen was first postulated by Zuntz (1879). Since then, it has been the focus of many studies, with findings demonstrating that absorption of SCFA contributes substantially to the total ME supply for ruminants (as summarized by Bergman, 1990). The major fermentation acids are the SCFA, acetic, propionic, and butyric acids, with molar proportions of each in the ruminal fluid ranging from 45 to 70%, 15 to 40%, and 5 to 20%, respectively (Bergman, 1990; Kristensen et al., 1996; Penner et al., 2009c; Udén, 2010). The 3 SCFA generally represent >95% of all fermentation acids, with a cumulative concentration of 60 to 150 mmol/L in ruminal fluid (Bergman, 1990). It has been suggested that the molar proportions of individual SCFA in the ruminal fluid are somehow indicative of their fractional production rates (Udén, 2010). However, most studies allow no or only limited conclusions on the production rates of individual SCFA.

Approaches to quantify fermentation acid production are mainly based on isotopic tracer techniques (Martin et al., 2001; Sutton et al., 2003) or the measurement of the portal appearance of SCFA and metabolites (Kristensen, 2005; Loncke et al., 2009). Although both these methods may be used to quantify acid production, they differ in the measurement outcome: measurement of total or net acid production, respectively. It should be acknowledged that both methods have major limitations. Isotopic tracer techniques are susceptible to carbon sequestration by ruminal microbes (Kristensen, 2001) and microbial carbon interconversion of SCFA as an error source (Sutton et al., 2003), whereas portal appearance studies systematically lead to an underestimation of produced acids due to differences between the rate of acid production and acid removal, as well as SCFA metabolism by the pregastric epithelia (Kristensen, 2005). Nevertheless, quantifying acid production in the rumen is of critical importance for predicting the nutrient supply and identifying the potential for pH depression.

It is clear that dietary composition and level of intake, particularly the intake of ruminally fermentable OM, affect total SCFA production and the molar proportions of individual SCFA (Bannink et al., 2008; Loncke et al., 2009). In fact, Loncke et al. (2009), based on a meta-analysis of net portal appearance data, predicted that a 5.93 mmol/(d·kg of BW) increase in SCFA production would occur for every 1 g/(d·kg of BW) increase in ruminally fermentable OM intake. Correspondingly, increasing the ruminally fermentable NDF:ruminally fermentable OM ratio increased the proportion of acetate and propionate but decreased butyrate. The models proposed by Loncke et al. (2009) covered a wide range of dietary conditions but did not include data from animals fed high-concentrate diets, such as feedlot cattle, or animals with a greater level of feed intake, such as dairy cattle in early lactation. This area requires further investigation as the amount of acid production and type of acids produced may shift (i.e., increased lactic acid production; Owens et al., 1998). The quantity of acid produced may also be affected by the extent of OM fermentation in the rumen and the efficiency of microbial fermentation (i.e., yield/kg of ruminally fermented OM; Allen, 1997). Based on

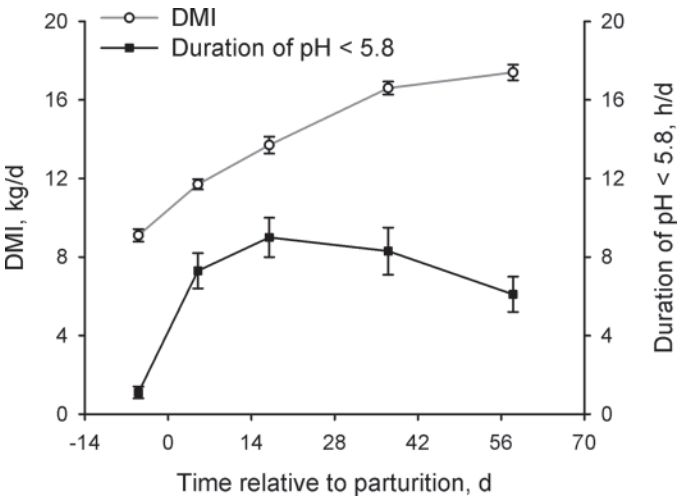

Figure 2. Changes in DMI (linear, $P < 0.001$; quadratic, $P = 0.042$) and duration that ruminal pH was <5.8 (quadratic, $P < 0.001$) during the periparturient period in primiparous Holstein cows (n = 14). Data derived from Penner et al. (2007).

the isotopic dilution method, rates of SCFA production in sheep fed at high and low intake (90 and 45% ad libitum intake, respectively) ranged between 14.8 and 8.8 mol/d (Martin et al., 2001) and for dairy cows fed diets with concentrate-to-hay ratios of 60:40 and 90:10 at moderate intake (~13 kg/d of DMI), total SCFA production rates ranged between 79.8 and 90.0 mol/d (Sutton et al., 2003).

At physiological ranges of pH, production of 1 mol of SFCA is initially associated with the release of 1 mol of H^+ (see subsequent discussion). The ratio may be somewhat less in forages based on their greater dietary cation-anion difference, because fermentation of already buffered organic anions in the plant cytosol will not release additional H^+. However, even on pasture-based diets, a negative within-study correlation has been found between the concentration of SCFA in the ruminal fluid and ruminal pH ($r^2 = 0.80$; Kolver and de Veth, 2002). Across studies with different diets, however, this relationship was rather weak ($r^2 = 0.13$; Allen, 1997). That the concentration of SCFA explains only a certain proportion of variation in ruminal pH is explained by different removal of dissociated H^+ from the rumen through neutralization, absorption, and clearance (Allen, 1997).

Although each individual SCFA can dissociate to yield 1 H^+, the type of SCFA produced through fermentation may result in a different quantity of acid produced per unit of hexose fermented. For example, fermentation of 1 mol of glucose can result in 2 mol of acetic, 2 mol of propionic, or 1 mol of butyric acid (Baldwin, 1995; Bannink et al., 2006). Thus, fermentation promoting butyric acid production may produce less acid compared with fermentation promoting acetic and propionic acids. Although substrate fermentation and the partitioning of SCFA production is accounted for in most mathematical models used to predict SCFA production (Pitt et al., 1996; Bannink et al., 2006; Loncke et al., 2009), there has been little success relating the production of SCFA to ruminal pH (Pitt et al., 1996). Thus, strategies to quantify the individual SCFA produced are needed to quantify H^+ release and further understand acid-base balance in the rumen.

IN SITU BUFFERING OF RUMINAL PROTONS

The production of large amounts of organic acids, especially SCFA, under energy-dense feeding conditions is a nutritionally desirable result of fermentation (Bergman, 1990; Allen, 1997; Gäbel and Aschenbach, 2007). However, a problem can arise from the large amounts of protons that these weak acids release when they dissociate in the ruminal environment (Gäbel and Aschenbach, 2006). The dissociation equilibrium is characterized by the pK_a value [where pK_a is $-\log(K_a)$ and K_a is the acid dissociation constant]. Considering that $pK_a = 4.8$ for SCFA (Table 1), it can be calculated from the Henderson-Hasselbalch equation that 1, 10, 50, 90, and 99% of SCFA have released their protons at pH 2.8, 3.8, 4.8, 5.8, and 6.8, respectively. As such, SCFA themselves behave like a buffer system that can release protons when pH increases and bind protons when pH decreases. From the above sample calculation according to Henderson-Hasselbalch, it is also evident that the largest part of the buffering capacity (i.e., 80%) resides in the pH range of $pK_a \pm 1$. The problem with the SCFA buffer is that it would effectively stabilize ruminal pH toward pH 4.8 (Counotte et al., 1979), which is the typical scenario when SCFA concentration increases in animals experiencing SARA (Stone, 2004; Krause and Oetzel, 2006). Short-chain fatty acid concentrations often increase to >150 mmol/L (DeFrain et al., 2002; Morgante et al., 2007) with a negative relationship between the ruminal concentration of SCFA and ruminal pH (Kolver and de Veth, 2002). The situation may become worse when rumen fermentation changes at very high amounts of concentrate feeding, when SCFA are gradually replaced by lactate. Lactate has a pK_a value of 3.86 (Table 1) and, thus, stabilizes pH about 1 unit less than an SCFA-buffered system (Stone, 2004). In contrast to SCFA, lactate is only very slowly absorbed from the rumen (Williams and Mackenzie, 1965; see subsequent discussion) and does not require increased production rates to accumulate to greater intraruminal concentrations. An accumulation of ≥5 mmol/L lactate has been suggested as the threshold to predispose a ruminant to acute ruminal acidosis (Nocek, 1997).

Because the organic acids produced during fermentation would inevitably buffer the ruminal content to low pH values, the rumen must be supplied with additional buffer bases to increase luminal pH into a range that is more compatible with microbial and host life. The repertoire of available buffer substances is listed in Table 1. In this section, we will largely focus on HCO_3^- and HPO_4^{2-} as the quantitatively most important buffer bases (Counotte et al., 1979; Allen, 1997). The role of NH_3 will be explored in a subsequent discussion toward the end of this review.

Saliva is one important source of buffer bases (Counotte et al., 1979; Erdman, 1988; Maekawa et al., 2002), especially the voluminous secretion from the parotid glands (Coats and Wright, 1957; Kay, 1960). The alkalinity of ruminant saliva has been known for almost 200 yr (Tiedemann and Gmelin, 1826) and its relevance for ruminal acid-base balance has been discussed for about 100 yr (Markoff, 1913; Scheunert and Trautmann, 1921). Ruminant saliva contains phosphate and bicarbonate in concentrations that are far greater than those in nonruminant species, making it an appropriate medium for buffering the acids produced in the rumen (McDougall, 1948; Turner and Hodgetts, 1955). Phosphate has typical concentrations in bovine mixed saliva of ~20 to 30 mmol/L (Bailey and Balch, 1961). Total phosphate secretion into the rumen may thus reach ~6 mol/d in lactating dairy cows, with peak salivary secretion rates of ~250 L/d (Cassida and Stokes, 1986; Erdman, 1988; Maekawa et al., 2002). The $HPO_4^{2-}/H_2PO_4^-$

Table 1. Buffering systems in the rumen

Buffer	pK_a	Reference
$H_2CO_3 \leftrightarrow HCO_3^- + H^+$ (closed buffer system in the absence of water)	3.80	Segel (1976)
$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow HCO_3^- + H^+$ (open buffer system in aqueous solution)[1]	p$K_a' = 6.1$	
$H_2PO_4^- \leftrightarrow HPO_4^{2-} + H^+$	7.21	Kohn and Dunlap (1998)
H-Acetate ↔ Acetate + H^+	4.75	Cistola et al. (1982); Kohn and Dunlap (1998)
H-Propionate ↔ Propionate + H^+	4.87	
H-Butyrate ↔ Butyrate + H^+	4.82	
H-Lactate ↔ Lactate + H^+	3.86	
$NH_3 + H^+ \leftrightarrow NH_4^+$	9.21	Hall (1957)
Other organic salts (e.g., pyruyate, oxaloacetate)		
Proteins, AA		
Feed ingredients		

[1]pH = 6.1 + log {[HCO_3^-]/(k_H × pCO_2)}, where k_H = 0.0229 mol/(L × atm). Kohn and Dunlap (1998) transform this equation to yield a greater effective pK_a'' = 6.1 + log(k_H^{-1}) = 7.74. Their approach describes essentially the same relationship between pH, partial pressure of CO_2 (pCO_2), and HCO_3^- as the conventional Henderson-Hasselbalch equation, provided that all items are entered in the appropriate units (i.e., mol, L, atm). Abbreviations used are concentration of HCO_3^- ([HCO_3^-]); Henry's constant (k_H); partial pressure of CO_2 (pCO_2), the negative decadal logarithm of the acid dissociation constant (pK_a), as well as effective values for pK_a that incorporate the hydration constant (pK_a') or the hydration constant and k_H (pK_a'').

buffer has a pK_a of 7.21 (Table 1), meaning that 80% of the buffering capacity resides in the pH range between 8.21 and 6.21 (i.e., pK_a ± 1). Given that typical pH in mixed saliva of a cow is 8.2 to 8.5 (Emery et al., 1960; Bailey and Balch, 1961; Cassida and Stokes, 1986) and with an assumed ruminal pH of 6.2 or less, ≥80% of buffering capacity of phosphate may be used when saliva is mixed with the ruminal content. This means that phosphate would buffer ~5 mol/d protons in the rumen of the aforementioned dairy cow. An even greater buffering capacity resides in salivary HCO_3^- with typical concentrations in bovine mixed saliva of ~120 mmol/L (Bailey and Balch, 1961; Erdman, 1988). At peak salivary secretion rates of ~250 L/d, total HCO_3^- secretion into the dairy cow rumen may reach 30 mol/d. The ruminal HCO_3^- system is more complex than the SCFA and phosphate buffering systems. The buffering capacity of HCO_3^- cannot simply be deduced from the pK_a of the pure HCO_3^-/H_2CO_3 equilibrium (pK_a = 3.8; Table 1) because this equilibrium is part of a double-open system in the rumen. The first opening to increase the efficiency of HCO_3^- buffering is that H_2CO_3 can decay to H_2O and CO_2 and is thus in equilibrium with the dissolved CO_2 in the ruminal fluid. The concentration of dissolved CO_2 ([CO_2]) can be calculated from the partial pressure of CO_2 (pCO_2) multiplied by Henry's constant [k_H = 0.0229 mol/(L × atm)]. Because only about 0.5% of the dissolved CO_2 combines with water to form H_2CO_3, the negative log of the hydration constant (−log 0.005 = 2.3) has to be added to the pK_a value of pure carbonic acid. Therefore, the resulting Hendersson-Hasselbalch equilibrium between HCO_3^- and dissolved CO_2 has an effective pK_a' of 6.1 (Table 1) and is described by pH = 6.1 + log {[HCO_3^-]/(k_H × pCO_2)} (Segel, 1976). A special feature of the rumen is that the dissolved CO_2 may escape into a gas phase and is eventually eructated or absorbed (Kohn and Dunlap, 1998). As one reaction partner is steadily diluted out of the system, the efficiency of HCO_3^- buffering increases a second time. This double-open system is a big advantage for the animal because the buffering capacity of saliva is greatly increased. A rough estimate indicates that the true percentage of HCO_3^- converted to CO_2 is in excess of 90% in the double-open system. For example, the concentration of HCO_3^- in the ruminal fluid amounts to 16 mmol/L at steady-state ruminal pH of 6.1. Assuming a salivary bicarbonate entry of 120 mmol/L and that salivary secretion rate roughly equals the liquid passage rate (Allen, 1997; Maekawa et al., 2002), 87% [i.e., (120 – 16)/120%] of salivary bicarbonate would have been used to buffer protons. Considering further that the ruminal epithelium contributes to the ruminal HCO_3^- concentration by secreting HCO_3^- in amounts that are roughly comparable with salivary HCO_3^- secretion (see subsequent discussion), the percentage of HCO_3^- converted to CO_2 increases well above 90% [e.g., (120 – 8)/120%]. For a model cow with 30 mol/d salivary bicarbonate, this means that ~27 mol/d are used to buffer ruminal protons. Salivary phosphate and bicarbonate buffers thus may bind up to ~40% of the ruminally released protons in high-yielding dairy cows (Allen, 1997).

Despite the undisputed importance of saliva in ruminal H^+ buffering, conflicting results have been obtained regarding the acidosis protection provided by saliva. In one study using 4 different energy-dense feeding conditions, increased rates of salivary secretion were paradoxically associated with increased severity of SARA (Penner and Beauchemin, 2010; Figure 3). Therefore, mechanisms other than salivary buffer secretion may be more decisive for SARA protection. These mechanisms likely reside in the ruminal epithelium. The importance of the ruminal epithelium as a buffer source for the ruminal content was acknowledged much later than the importance of saliva. The ruminal epithelium itself secretes large amounts of HCO_3^- (Gäbel et al., 1991a) and removes protonated buffer bases with a low pK_a [i.e., protonated SCFA (**HSCFA**); Gäbel and As-

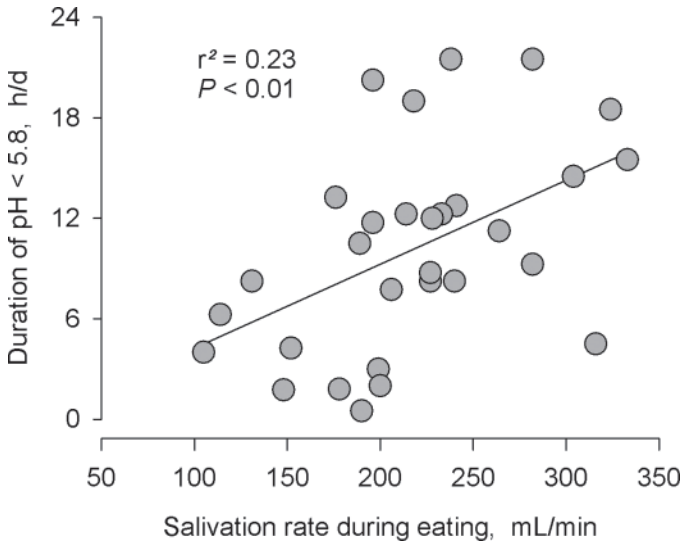

Figure 3. Relationship between the duration that ruminal pH is <5.8 and the rate of saliva production during eating in lactating dairy cows. Data are from 8 cows receiving 4 acidogenic diets containing between 40 and 60% concentrate in a double 4 × 4 Latin square design. Diet itself did not affect salivation rate during eating or the duration of pH <5.8 (for details, see Maekawa et al., 2002). However, a positive correlation existed between salivation rate during eating and the duration of pH <5.8. It indicates that the duration that pH is <5.8 cannot be ameliorated by increasing the rate of salivary secretion under acidogenic feeding conditions. Adapted from Penner and Beauchemin (2010).

chenbach, 2006, 2007]. Both processes contribute to the stabilization of ruminal pH into the physiological range. However, as these issues directly relate to the absorption of SCFA, they will be discussed subsequently.

PROTON REMOVAL BY ACID ABSORPTION

Almost exactly 100 yr after the first description of ruminal microbes (Gruby and Delafond, 1843), pioneering experiments were performed in Cambridge showing that a major part of microbially produced SCFA is directly absorbed across the forestomach wall (Phillipson and McAnally, 1942; Barcroft et al., 1944). This initiated a series of experiments extending the understanding of the role of the rumen from a mere fermentation chamber to an absorptive organ as well (Danielli et al., 1945; Masson and Phillipson, 1951; Ash and Dobson, 1963). Today, it is widely accepted that between 50 to 85% of the ruminally produced SCFA is directly absorbed across the reticuloruminal wall; only 15 to 50% passes into distal parts of the digestive system. The percentage that is not absorbed in the reticulorumen and passes into distal parts appears to be least in sheep (15%; von Engelhardt and Hauffe, 1975) and calves (15 to 20%; Edrise and Smith, 1977). Dairy cows and steers have fractional SCFA passage rates typically between 29 and 39% (Tamminga and van Vuuren, 1988; Peters et al., 1990; Penner et al., 2009c). Although energy intake clearly determines the absolute amount of SCFA available for absorption versus passage, there is no linear relationship between energy intake (Penner et al., 2009c) or SCFA production rates (Peters et al., 1990) and fractional SCFA passage into the omasum. This is because the "spillover" of the excess acids into the omasum will be counteracted by 1) increased ruminal absorption rates of SCFA at a lower ruminal pH (Dijkstra et al., 1993) and 2) decreased liquid passage rates into the omasum (Colucci et al., 1990) under energy-dense feeding conditions. However, extreme energy intakes (4 times maintenance vs. maintenance) have been found to enhance the fraction of SCFA passing unabsorbed into the omasum (Tamminga and van Vuuren, 1988). Decreased ruminal motility due to low ruminal pH could contribute to the latter phenomenon because the mixing of ruminal contents is an important factor favoring absorption (Allen et al., 2006). The greatest fractional passage rates (about 50%) have been reported in dairy cows in early lactation on fresh grass and concentrate feeding (Resende Júnior et al., 2006), which may, in part, be related to greater liquid passage rates associated with diets based on fresh forage (Reis and Combs, 2000). The fractional passage rates of SCFA, however, cannot be set equal to passage of protons into distal compartments because only a small fraction of the passing acids will carry a proton (e.g., 1% of SCFA at pH 6.8). Allen (1997) estimated the proton removal from the rumen by passage of HSCFA and other buffering substances (e.g., $H_2PO_4^-$, NH_4^+, particulate matter) to be on the order of approximately 15%. Given a salivary buffering of up to ~40% of released protons, as discussed previously, it becomes clear that the majority of protons have to be removed by absorption in most situations.

As elaborated earlier, SCFA absorption proceeds on the order of 50 to 85% of the produced acids, being equivalent to 40 to 60 mol/d in dairy cows fed a high concentrate diet (Allen, 1997; Penner et al., 2009c). Nevertheless, the importance of SCFA absorption for ruminal pH homeostasis cannot be reduced to a sole quantitative consideration of SCFA absorption rates. For the efficiency of concurrent proton removal, the mechanism of absorption deserves due consideration (Figure 4). From current perspective, it is very intriguing that the very early studies on ruminal SCFA absorption already elucidated some important characteristics of ruminal SCFA absorption; that is, the chain length (Danielli et al., 1945; Gray, 1947) and pH dependence of absorption (Gray, 1948), the partial metabolism of SCFA during absorption (butyrate > propionate > acetate; Masson and Phillipson, 1951), the contribution of SCFA absorption to ruminal pH stabilization (Masson and Phillipson, 1951; Ash and Dobson, 1963), and the appearance of bicarbonate in the rumen when SCFA are absorbed (Ash and Dobson, 1963). Research over the last 50 yr extended these early findings and provided functional explanations by using in vitro (Pennington, 1951; Hird and Weidemann, 1964; Hird et al., 1966; Stevens and Stettler, 1966a,b, 1967) and molecular techniques (Müller et al., 2002; Lane et al.,

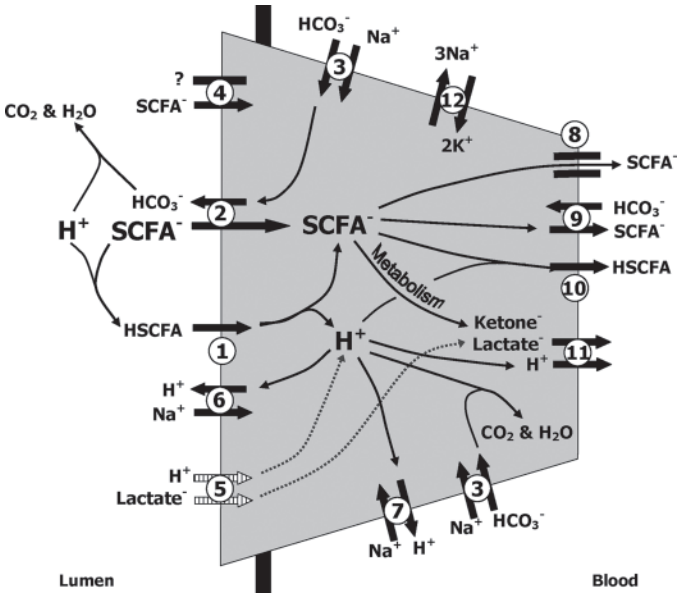

Figure 4. Model on organic acid transport in ruminal epithelial cells. On the lumen-directed apical membrane, lipophilic diffusion of undissociated short-chain fatty acids (HSCFA; 1) is an efficient way to import acids with high lipophilicity (especially butyric acid) into the cell. These acids rapidly release their protons once inside the cell. All 3 short-chain fatty acids (SCFA; i.e., acetic, propionic, and butyric acids) additionally use transport proteins that exchange their anions ($SCFA^-$) with HCO_3^- for apical uptake (2). The $SCFA^-/HCO_3^-$ exchange is especially important for acids with less lipophilicity, such as acetate, and is largely driven by HCO_3^- imported from the blood via Na^+/HCO_3^- cotransport (3). The apically exported HCO_3^- neutralizes 1 proton in the rumen; therefore, lipophilic diffusion and $SCFA^-/HCO_3^-$ exchange have a largely comparable contribution to ruminal proton removal. The acetate anion may additionally use a poorly characterized apical uptake protein with undefined role for ruminal pH homeostasis (4). Lactate anions can enter the cell in cotransport with their protons (5). However, the responsible monocarboxylate cotransporter has an extremely low functional activity in roughage-adapted sheep and its quantitative importance remains to be determined. Protons taken up with HSCFA or lactic acid can either be neutralized by HCO_3^- from basolateral Na^+/HCO_3^- cotransport (3) or expelled by Na^+/H^+ exchange across the apical (6) or basolateral membrane (7). The pathways of basolateral exit of SCFA have not been evaluated in detail but are likely to include an anion channel permeable to large anions (8), $SCFA^-/HCO_3^-$ exchange (9), and lipophilic diffusion (10). Of these, $SCFA^-/HCO_3^-$ exchange and lipophilic diffusion would contribute to net removal of protons from the epithelial cell into the blood. Butyrate is metabolized extensively to ketone bodies (i.e., β-hydroxybutyrate, acetoacetate) and propionate is metabolized partly to lactate inside the cells. These metabolic products and possibly apically imported lactate are expelled together with their protons across the basolateral membrane via monocarboxylate transporter 1 (MCT1) (11). All Na^+-driven transport mechanisms are energized by the Na^+/K^+ ATPase (12) at the basolateral membrane. The electrogenic efflux of Na^+ via this pump requires a neutralizing efflux of anions through the basolateral anion channel (8). The HCO_3^- and H^+ pool inside ruminal epithelium is partly replenished from CO_2 by carbonic anhydrase reaction (Gäbel et al., 2002), which is not shown in the current model for clarity reasons.

2002; Bilk et al., 2005). As a result, we realize today that absorption of SCFA from the rumen is not purely determined by energy input, microbial dynamics, and physicochemical factors. The ruminal epithelium itself is a dynamic key player with a major contribution to decreasing the ruminal acid and proton load.

The current model on ruminal SCFA absorption is depicted in Figure 4. Absorption of undissociated HSCFA via lipophilic diffusion has long been postulated to be the only relevant pathway of SCFA absorption (Bugaut, 1987; López et al., 2003; Graham et al., 2007). Because the permeability of lipid bilayer membranes to the electrically charged SCFA anion ($SCFA^-$) is extremely low, passive diffusion has to be attributed to the lipophilic protonated form, HSCFA (Walter and Gutknecht, 1986; Gäbel et al., 2002). Lipophilic diffusion couples the removal of $SCFA^-$ anions to the removal of protons at a ratio of 1:1, which constitutes a very efficient way of proton extraction from the rumen (Allen, 1997). However, quantitative and qualitative constraints indicate that lipophilic diffusion cannot be the only way of SCFA absorption. A quantitative constraint to lipophilic diffusion is that HSCFA (pK_a ~4.8; Table 1) constitute only a small fraction in the HSCFA–$SCFA^-$ acid–base equilibrium according to Henderson-Hasselbalch's equation; for example, only 1% at pH 6.8. Consequently, the effective luminal concentration driving apical uptake of total HSCFA may be as little as 1 m*M*. A qualitative constraint is that the acid production rates discussed previously (i.e., acetic > propionic > butyric acid) are inversely related to their lipophilic permeability (i.e., butyric > propionic > acetic acid). For example, lipid bilayer permeability decreases from butyric to acetic acid by a factor of approximately 14 (Walter and Gutknecht, 1986). A second qualitative constraint is that the rates of intracellular metabolism (i.e., butyric > propionic ≥ acetic acid; Bergman, 1990; Kristensen et al., 2000; Kristensen and Harmon, 2004) that finally enhance lipophilic diffusion by decreasing the intracellular acid concentration are also inversely related to acid production rates. Together, the 2 qualitative constraints would inevitably lead to selective sequestration of large amounts of acetate in the rumen. The latter is in sharp contrast to experimental data showing that all 3 SCFA are absorbed at rather comparable fractional rates from the ovine (Kristensen et al., 2000; Aschenbach et al., 2009) and bovine rumen at physiological ruminal pH (Dijkstra et al., 1993; Kristensen and Harmon, 2004, 2005) in vivo. Therefore, it is essential to postulate additional routes of absorption, at least, for acetic and propionic acids, that is, for acids with decreased rates of lipophilic absorption and intraepithelial metabolism compared with butyric acid.

Nondiffusional absorption of SCFA requires transport proteins and applies to the dissociated anions, $SCFA^-$. The main pathway for apical nondiffusional absorption of $SCFA^-$ has meanwhile been clearly identified as $SCFA^-/HCO_3^-$ exchange (Gäbel et al., 1991a; Kramer et al., 1996; Aschenbach et al., 2009; Figure 4). Acetate, especially, seems to utilize HCO_3^--dependent uptake for absorption (Ash and Dobson, 1963; Aschenbach et al., 2009; Penner et al., 2009a). Masson and Phillipson (1951) already had discovered that the absorption of SCFA leads to HCO_3^- enrichment in the rumen. However, this phenomenon was initially explained by carbonic acid dissociation that served to provide protons for a diffusive uptake of HSCFA, leaving HCO_3^- behind in the rumen (Ash and Dobson, 1963). Later, a

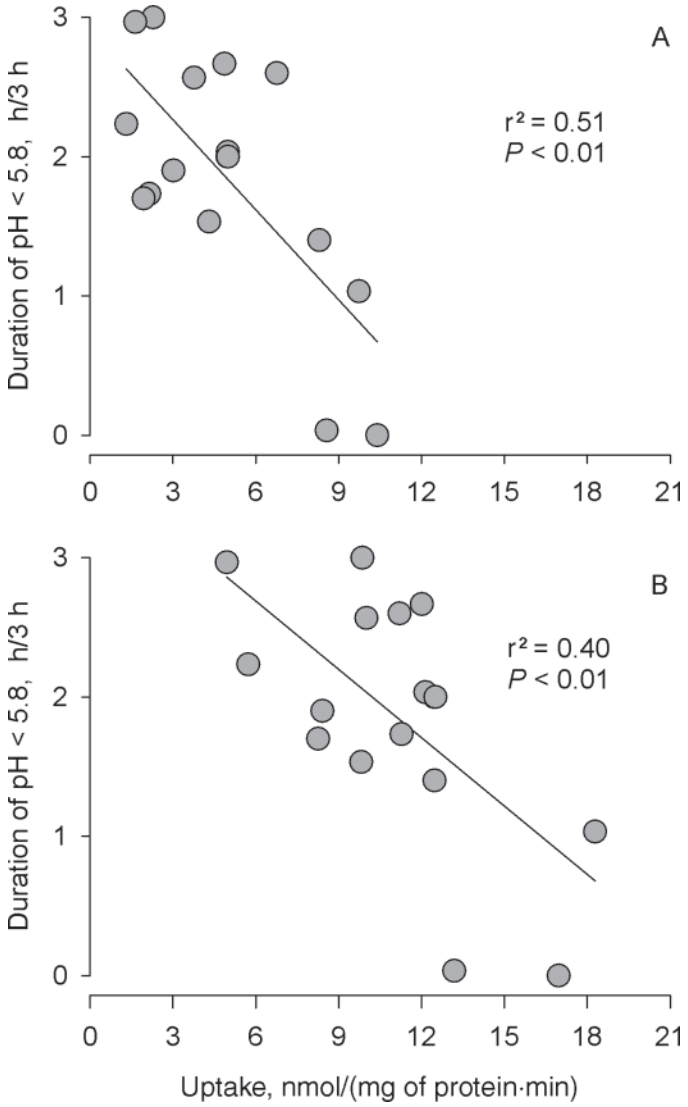

Figure 5. Relationship between acetate (panel A) and butyrate uptake (panel B) across the apical membrane of ruminal epithelial cells and the duration spent at ruminal pH <5.8. Uptake of short-chain fatty acids (SCFA) was measured in isolated ruminal epithelia at a concentration of 10 m*M* each. Donor sheep of these epithelia had been subjected to a defined induction protocol for subacute ruminal acidosis (SARA) in vivo for 3 h (oral drench of 5 g of glucose/kg of BW; for details see Penner et al., 2009a). The incidence of SARA, represented by the duration of pH <5.8, decreases markedly when ruminal epithelia are able to take up larger quantities of acetate and butyrate. An r^2 of ~0.5 indicates that ~50% of the individual susceptibility to SARA can be explained based on the absorptive capacity of the ruminal epithelium for these acids.

weak inhibitory effect of the unspecific anion transport blocker 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid on propionate fluxes in the ovine rumen (Kramer et al., 1996) and butyrate fluxes in the bovine rumen (Sehested et al., 1999) indicated the existence of some direct $SCFA^-/HCO_3^-$ exchange. The final proof for direct coupling between $SCFA^-$ absorption and HCO_3^- secretion was recently deduced from the finding that the long-known stimulatory effect of low ruminal pH on SCFA absorption is, to a major extent, attributable to a HCO_3^- gradient across the apical membrane and not to the pH gradient itself. In the absence of HCO_3^-, the stimulatory effect of pH on acetate uptake is negligible (Aschenbach et al., 2009). Furthermore, the latter studies indicated that apically exported HCO_3^- is replenished to a significant degree from extracellular sources, which indicates that HCO_3^- secretion by the ruminal epithelium involves apical $SCFA^-/HCO_3^-$ exchange and basolateral Na^+/HCO_3^- cotransport operating in series (Huhn et al., 2003; Aschenbach et al., 2009; Figure 4).

Quantitative estimates from sheep indicate that up to 50% of the SCFA can be absorbed in a HCO_3^--dependent manner (Ash and Dobson, 1963; Gäbel et al., 1991a; Penner et al., 2009a). Extrapolating these quantities to high-yielding dairy cows would mean that the ruminal epithelium supplies an amount of HCO_3^- to the ruminal content that is roughly equivalent to salivary HCO_3^- secretion (i.e., 20 to 30 mol/d). The ruminally secreted HCO_3^- enters the double-open HCO_3^- buffer system of the rumen where it combines with H^+ to form CO_2 and water (Figure 4). In this way, the exchange of 1 mol $SCFA^-$ against 1 mol HCO_3^- finally also leads to the neutralization of >0.9 mol H^+ in the ruminal content (for estimation of HCO_3^- buffering capacity; see previous section). Animals with very efficient SCFA absorption may utilize a third absorptive mechanism, which is protein-mediated but HCO_3^--independent (Penner et al., 2009a; Figure 4). Even if this third mechanism would not coeliminate ruminal protons, $SCFA^-/HCO_3^-$ exchange and lipophilic diffusion alone remove H^+ in quantities that exceed H^+ removal by saliva. Absorption of SCFA also means an extraction of low-pK_a buffers from the ruminal content. It is thus not surprising that the individual susceptibility of sheep to SARA is negatively correlated to their ruminal capacity for apical SCFA uptake (Penner et al., 2009a; Figure 5).

For ruminal pH homeostasis, 2 factors are important: how many protons are removed with $SCFA^-$ during absorption and how permanent the proton extraction is. Both the inflow of HSCFA into ruminal epithelial cells and the outflow of HCO_3^- via $SCFA^-/HCO_3^-$ exchange decrease intracellular pH (**pH_i**) in isolated ruminal epithelial cells (Müller et al., 2000; Gäbel et al., 2002), as well as intact ruminal epithelium (Abdoun et al., 2010). In principle, the ruminal epithelium can counterregulate pH_i acidification via basolateral acid–base transporters, which likely include one of the discovered Na^+/H^+ exchanger (**NHE**) isoforms (Müller et al., 2000; Gäbel and Aschenbach, 2006; Etschmann et al., 2006) as well as basolateral Na^+/HCO_3^- import (Huhn et al., 2003; Figure 4). However, a proven and significant part of pH_i regulation occurs by exporting protons back into the lumen via an apical NHE (Sehested et al., 1996; Müller et al., 2000; Gäbel et al., 2002). Both ruminal SCFA absorption (Gäbel et al., 1991b) and Na^+ supply by saliva (Sehested et al., 1996) increase the driving forces for this apical NHE and, thus, contribute to H^+ recycling. According to our current definition of SARA, based on ventral ruminal pH, we would need to regard apical NHE as a mechanism contributing to SARA because it increases the ruminal proton load to >100%

of initial proton release. In fact, however, it protects the animal from the negative consequences of SARA by securing epithelial cell function and by preventing Na^+ accumulation in the rumen, the latter counteracting the vicious cycle of rising osmolarity, rising volume, and decreasing pH seen in SARA.

The apical uptake of SCFA is one important rate-limiting step during SCFA absorption with a clear role in acidosis protection (Penner et al., 2009a; Figure 5). Less is known about the basolateral exit pathways. Only monocarboxylate transporter 1 (**MCT1**) has been unequivocally localized to the basolateral membrane (Müller et al., 2002; Kirat et al., 2006; Graham et al., 2007). It is involved in the basolateral export of ketone bodies arising from the intracellular metabolism of butyrate and for lactate arising from metabolism of propionate (Müller et al., 2002; Gäbel and Aschenbach, 2006). The velocity of butyrate metabolism to ketone bodies and the consecutive export across the basolateral membrane via MCT1 are accepted determinants for the velocity of butyrate and proton removal from the rumen (Gäbel et al., 2001; Penner et al., 2009a). Some authors also consider a direct export of SCFA via MCT1 (Kirat et al., 2006; Graham et al., 2007). Alternative pathways for basolateral export of SCFA would be lipophilic diffusion (Gäbel et al., 2002; Gäbel and Aschenbach, 2006) and $SCFA^-/HCO_3^-$ exchange (Bilk et al., 2005). All 3 mechanisms (i.e., MCT1, lipophilic diffusion, and $SCFA^-/HCO_3^-$ exchange) would coeliminate a proton from the cytosol to blood. However, a plausibility check leads to the conclusion that some SCFA have to leave the cell as a free base without a proton, because a considerable fraction of protons is buffered in the rumen by saliva. In addition, the cell-alkalizing action of apical and basolateral NHE, as well as that of the Na^+/HCO_3^- cotransporter, would not be required if apical H^+ import with $SCFA^-$ was equal to basolateral H^+ export with $SCFA^-$. The $SCFA^-$ export without a proton is most likely realized by a large-conductance anion channel in the basolateral membrane (Stumpff et al., 2009) and should be highly efficient in minimizing the SCFA concentration within the cytosol because the efflux is driven by the basolateral membrane potential.

Although the knowledge on quantitative and qualitative aspects of SCFA elimination from rumen has greatly increased over the last decades, the capacity of the ruminal epithelium to absorb lactic acid is still insufficiently assessed. The epithelium possesses an apical MCT, which is likely the MCT4 isoform (Kirat et al., 2007; Aschenbach et al., 2009). This mechanism can extract 1 proton together with each molecule of lactic acid from the rumen (Figure 4). However, the transport velocity of the apical MCT is extremely low in roughage-adapted sheep (Aschenbach et al., 2009), which conforms to the low lactate absorption rates observed in vivo (Williams and Mackenzie, 1965) and can explain the rapid accumulation of lactic acid in times of enhanced lactic acid production (i.e., during acute ruminal acidosis; McLaughlin et al., 2009; Lettat et al., 2010). However, it is an important question whether or not an adaptation of this transport system to concentrate diets occurs and whether this contributes to the acidosis resistance of concentrate-fed ruminants. This question cannot be answered at present. So far, only one study exists about a possible feed adaptation of ruminal MCT4, which could not detect any difference of ruminal MCT4 protein abundance in reindeer fed in captivity versus those on natural pasture (Koho et al., 2005).

INTERACTION OF AMMONIA AND SCFA

Ruminal ammonia ("ammonia" will be used in this section to refer to the sum of NH_3 and NH_4^+) is an essential source of nitrogen for microbial growth. Ammonia concentrations may increase within 2 h after a meal to 20 or 30 mmol/L, dependent on rumen degradable N intake (Reynolds and Kristensen, 2008), only to decrease rapidly thereafter due to utilization by ruminal bacteria (35 to 65% of the decrease), efflux to the omasum (~10% of the decrease), or absorption across the ruminal wall (McDonald, 1948; Kennedy and Milligan, 1980; Siddons et al., 1985; Obara et al., 1991). Portal flux of ammonia can reach approximately 100 mmol/kg of $BW^{0.75}$ per day (Parker et al., 1995). In the liver, ammonia is detoxified to form urea, much of which reenters the rumen via secretion across the ruminal epithelium, supported by some salivary urea secretion (Harmeyer and Martens, 1980; Marini and Van Amburgh, 2003). Upon entering the rumen, urea is reconverted by bacterial urease to release 2 mol of NH_3 per mol of urea (Figure 6). In dairy cows, absorption of ammonia can reach 25 mol/d (Delgado-Elorduy et al., 2002), whereas secretion of up to 10 mol/d of urea has been observed (Gozho et al., 2008). Unless dietary N intake is low, urea secretion into the rumen usually greatly exceeds the needs of microbial protein synthesis (Reynolds and Kristensen, 2008). However, the urea secreted in excess of microbial needs increases ruminal NH_3, which is a very potent buffer. Because of its high pK_a value (9.21; Table 1), NH_3 immediately binds H^+ in equimolar amounts to form NH_4^+ in the ruminal content. For ruminal pH homeostasis, it is decisive which form of ammonia leaves the rumen. Only efflux of NH_4^+ to the blood would finally remove a proton from the system.

Although the lipid-diffusion theory dominated the debates surrounding the absorption of SCFA for decades, nondiffusional absorption of NH_4^+ across the ruminal epithelium has long been postulated (Gärtner et al., 1961; Gärtner and von Engelhardt, 1964). The lipophilicity of uncharged NH_3 is approximately equal to that of uncharged butyric acid (Walter and Gutknecht, 1986) and at pH >7, substantial amounts of ammonia will cross the epithelium in this form. However, under the conditions found physiologically in the rumen, the

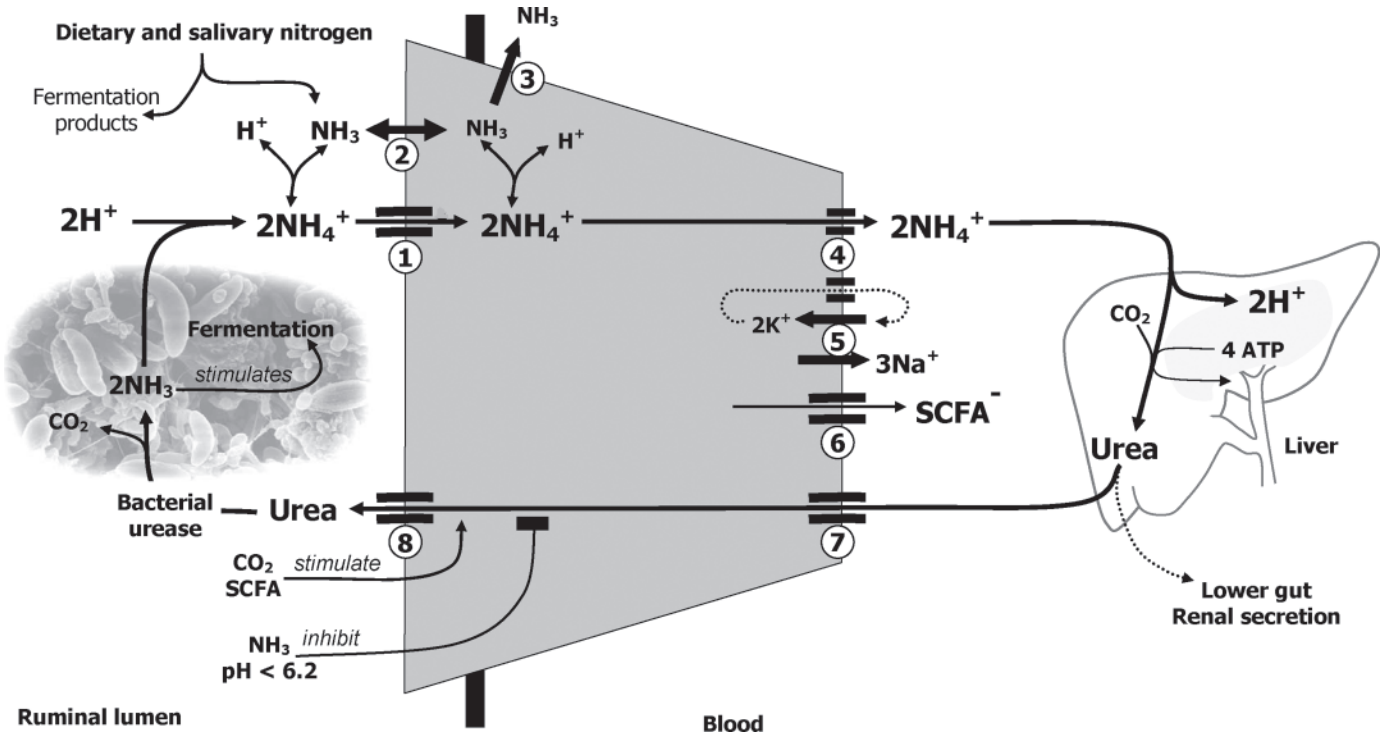

Figure 6. Model on ruminal nitrogen transport and its interaction with short-chain fatty acids (SCFA) and acid-base status. Influx of urea into the rumen and efflux of ammonia influence the amount of nitrogen available for microbial protein synthesis. Excess ammonia is absorbed as NH_4^+ through apical cation channels (1) and as NH_3 (2). At pH >7, uptake of NH_3 predominates. Within the cytosol, NH_3 is protonated to form NH_4^+, thus reducing the need for the apical extrusion of protons via Na^+/H^+ exchanger (NHE); compare (6) in Figure 4. At pH <6.8, uptake occurs primarily in the form of NH_4^+, removing a proton from the rumen directly. Increasing cytosolic concentrations of NH_3 within the neutral pH of the cytosol may result in apical recycling of ammonia (in as NH_4^+, out as NH_3), leading to a further uptake of protons that will acidify the cytosol if basolateral efflux occurred as NH_3 (3). However, in vitro evidence suggests that large quantities of protons may leave the tissue basolaterally with NH_4^+ through channels necessary for the recirculation of K^+ (4) from the Na^+/K^+ ATPase (5). Efflux of SCFA anions ($SCFA^-$) through a basolateral anion channel (6) will facilitate the basolateral efflux of NH_4^+ through K^+ channels (4) via charge-coupling. Within the liver, NH_4^+ is detoxified to form urea, thereby releasing the captured H^+. Passage back into the rumen probably occurs via serial urea transporters in the basolateral and apical membranes (7, 8) and is regulated according to the requirements of fermentation. At moderately acidic pH, urea influx into the rumen rises with the production of SCFA and CO_2, thus supplying nitrogen for microbial growth. When ruminal pH decreases too much or ammonia concentrations increase too much, the permeability of the ruminal wall to urea decreases and urea nitrogen is redirected for excretion in the lower gut or the kidney.

percentage of uncharged NH_3 in the Henderson-Hasselbalch equilibrium of NH_3/NH_4^+ ($pK_a = 9.21$) is much less than the percentage of uncharged butyric acid in the butyrate$^-$/butyric acid equilibrium ($pK_a = 4.82$; Table 1); for example, ~1/10 as much at pH 6.5 and ~1/900 as much at pH 5.5. This implies that decreasing ruminal pH should impair lipophilic diffusion of NH_3 in the same exponential manner with which it stimulates butyric acid diffusion. In contrast to this theory, epithelial uptake of ammonia remains high and may even increase slightly when ruminal pH decreases in vivo (Gärtner et al., 1961; Siddons et al., 1985; Remond et al., 1993). Electrophysiological data support the conclusion that bulk flow of ammonia proceeds via NH_4^+ at low ruminal pH when efflux of ammonia from the rumen is greatest and extraction of H^+ becomes important (Bödeker and Kemkowski, 1996; Abdoun et al., 2005). Because the atomic radii of K^+ and NH_4^+ are strikingly similar (Boron and De Weer, 1976), NH_4^+ most likely passes through the same channels that transport potassium (Bödeker and Kemkowski, 1996; Abdoun et al., 2005) and that are present in both the apical and the basolateral membranes of the ruminal epithelium (Ferreira et al., 1972; Leonhard-Marek and Martens, 1996). Apically, electrogenic NH_4^+ inflow is supported by the membrane potential that is outside positive. Basolaterally, efflux in the form of NH_4^+ (and thus removal of a proton) is facilitated by the extremely high expression of K^+ channels necessary for recirculation of K^+ entering with the Na^+/K^+-ATPase, but has to occur against an electrical gradient. Recent findings indicate that SCFA may play an important role in diminishing this electrical gradient. The outflow of $SCFA^-$ through basolateral anion channels should depolarize the basolateral membrane and thereby facilitate the basolateral outflow of NH_4^+ (Stumpff et al., 2009; Figure 6). This suggestion is in line with the classical observation that absorption of luminal ammonia is increased by the copresence of SCFA (Pilgrim et al., 1969; Bödeker et al., 1992; Remond et al., 1993).

Apart from enhancing ammonia absorption, SCFA also increase the secretion of urea (Thorlacius et al., 1971; Norton et al., 1982; Abdoun et al., 2010). Conversely, the permeability of the ruminal wall to urea rapidly declines with greater intraruminal ammonia concentration (Harmeyer and Martens, 1980) or when

ruminal pH decreases to <6.2 (Abdoun et al., 2010). A regulated protein-mediated pathway for urea has long been postulated (Gärtner et al., 1961). It was initially thought to be identical to the urea transporter-B (Ritzhaupt et al., 1998); however, urea transporter-B is at least not responsible for the large increase in ruminal urea influx capacity induced by low-protein diets, indicating that there are likely additional protein-mediated urea influx pathways (Marini et al., 2004; Muscher et al., 2010; Figure 6).

The interaction of SCFA with the secretion of urea and the absorption of ammonia appear to be important evolutionary adaptations of ruminants to actively regulate the fermentation process. When ruminants are on the low-protein diets of their natural habitats, endogenously secreted nitrogen is a pacemaker of microbial fermentation. The positive effect of SCFA on the secretion of urea allows animals to capitalize on available carbohydrates, while excess ammonia is used to buffer and transfer protons out of the rumen. Conversely, the rate of fermentation can also be reduced to prevent excessive fermentation provided that nitrogen intake is moderate. When pH declines too much and SCFA accumulate, urea influx decreases (Abdoun et al., 2010), which should decrease microbial growth and the rate of fermentation. Unfortunately, the latter regulatory pathway can be expected to be mostly annulled in high-producing cattle due to excess protein feeding. Nonetheless, the interactions between SCFA, ammonia absorption, and urea secretion are of formidable importance for understanding both the regulation of microbial dynamics and the regulation of ruminal pH.

SUMMARY AND CONCLUSIONS

Ruminants produce large quantities of organic acids in the rumen when fed on highly fermentable diets. To counteract the development and progression of acidosis, HCO_3^- inflow into the rumen is the buffer system primarily utilized. Estimates in high-yielding dairy cows indicate that HCO_3^- is introduced into the rumen to approximately equal shares via saliva and via secretion across the ruminal epithelium. The quantities of HCO_3^- introduced by saliva and the epithelium may almost be equivalent to total acid production, and it can be expected that >90% of HCO_3^- buffers a proton due to the double-open buffer system of the rumen. The major part of epithelial HCO_3^- secretion is directly coupled to the absorption of $SCFA^-$. As the ruminal epithelium additionally absorbs HSCFA via lipophilic diffusion and $SCFA^-$ via a yet unidentified mechanism, it is the key player in ruminal acid–base balance. This is compatible with the recent finding that the ruminal capacity for absorption of SCFA is a key determinant for the susceptibility of individual animals to acidosis. When estimating the proton load in the rumen, it needs to be considered that a significant fraction of already removed H^+ is recycled back into the lumen via apical NHE. The H^+ recycling is stimulated by both epithelial SCFA absorption and salivary Na^+ secretion. To better understand the acid–base status of the rumen, it will be vital to assess the quantities of recycled H^+ at different ruminal pH in future. Based on our current definition of ruminal acidosis, H^+ recycling via NHE has to be regarded as an acidosis-promoting mechanism. In fact, however, it serves to stabilize epithelial integrity and may thus be vital to protect the animal against the negative consequences of acidosis. Another important area for future research is the interaction between SCFA absorption, urea secretion, and NH_4^+ absorption. The better we understand the evolutionary strategy of ruminants to coordinate energy conversion, protein assimilation, and pH regulation in the rumen, the more successfully we can utilize these processes with due recognition of animal needs and welfare, as well as environmental concerns.

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