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Determination of Henry's constant, the dissociation constant, and the buffer capacity of the bicarbonate system in ruminal fluid

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ABSTRACT

Despite the clinical importance of ruminal acidosis, ruminal buffering continues to be poorly understood. In particular, the constants for the dissociation of H_2CO_3 and the solubility of CO_2 (Henry's constant) have never been stringently determined for ruminal fluid. The pH was measured in parallel directly in the rumen and the reticulum in vivo, and in samples obtained via aspiration from 10 fistulated cows on hay- or concentrate-based diets. The equilibrium constants of the bicarbonate system were measured at 38°C both using the Astrup technique and a newly developed method with titration at 2 levels of partial pressure of CO_2 (pCO_2; 4.75 and 94.98 kPa), yielding mean values of 0.234 ± 0.005 mmol·L^{-1}·kPa^{-1} and 6.11 ± 0.02 for Henry's constant and the dissociation constant, respectively (n/n = 31/10). Both reticular pH and the pH of samples measured after removal were more alkalic than those measured in vivo in the rumen (by ΔpH $= 0.87 \pm 0.04$ and 0.26 ± 0.04). The amount of acid or base required to shift the pH of ruminal samples to 6.4 or 5.8 (base excess) differed between the 2 feeding groups. Experimental results are compared with the mathematical predictions of an open 2-buffer Henderson–Hasselbalch equilibrium model. Because pCO_2 has pronounced effects on ruminal pH and can decrease rapidly in samples removed from the rumen, introduction of a generally accepted protocol for determining the acid-base status of ruminal fluid with standard levels of pCO_2 and measurement of base excess in addition to pH should be considered.

Key words: rumen, bicarbonate, dissociation constant, Henry's constant

INTRODUCTION

Acute and subacute ruminal acidosis is a fermentational disorder afflicting ruminants with symptoms can negatively affect milk fat content, feed intake, and milk yield. The condition affects some 10 to 40% of dairy cows at least once in a lifetime and is associated with a low ruminal pH, inflammation of the ruminal epithelium, and loss of ruminal barrier function (Enemark, 2008; Plaizier et al., 2012). Efflux of pathogens and their toxins into the portal circulation follows (Plaizier et al., 2008). Symptoms include systemic immunological complications with lameness, liver abscesses, and dehydration due to ruminal bloating that can be lethal (Kleen et al., 2003; Plaizier et al., 2008).

Early diagnosis is crucial because animals can recover after shifting dietary balance away from starches and toward physically effective neutral detergent fiber to stimulate chewing activity and the production of salivary buffers such as $NaHCO_3$ (Allen, 1997; Mertens, 1997; Yang and Beauchemin, 2006; Zebeli et al., 2012; Maulfair et al., 2013). Although correlations exist between SARA and parameters such as a drop in milk fat or a rise renal net acid excretion, these criteria have yet to replace ruminal pH as the leading diagnostic tool (Enemark, 2008). Investigators have suggested various pH thresholds ranging from pH <5 to pH <5.2 for the acute form of the disease, whereas a range of pH <5.5 to pH <6.0 or even higher has been suggested for SARA (Duffield et al., 2004; Krause and Oetzel, 2006; Plaizier et al., 2008). Depending on the study, these thresholds can refer to measurements at a certain time point, the daily means, or the nadir of a continuous measurement. Even if the procedure is identical, the pH ranges given are clearly too large to satisfy the clinician in the field who has to make a decision on whether dietary intervention is necessary or not (Li et al., 2012). Accordingly, various other parameters for identifying animals or herds at risk are being discussed, which include the concentrations of ruminal acetic, propionic,

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isobutyric, butyric, and caproic acids, lactate, and ammonia (Krause and Oetzel, 2005; Bramley et al., 2008).

A parameter that is curiously missing from this rising list is the partial pressure of CO_2 (**pCO_2**) in the rumen, which is known to vary between 36.7 and 66.5 kPa (Turner and Hodgetts, 1955; McArthur and Miltimore, 1961; Kölling, 1974; Counotte et al., 1979) and can be expected to drop sharply after removal of a sample, in particular when pressure drops during aspiration. Spurious changes of this nature can thus be expected to add to the poor outcomes of attempts at identifying precise thresholds for the onset of ruminal acidosis.

When trying to assess the magnitude of a variation in pCO_2 on ruminal pH, another omission emerges: the only available value for the dissociation constant (**pK_{app}**) of H_2CO_3 in ruminal fluid was determined decades ago at an inappropriate temperature (25°C; Turner and Hodgetts, 1955) and using a value for the solubility of CO_2 (α) that was obtained in 1917 in aqueous solution (Van Slyke and Cullen, 1917). In one of the very few more recent detailed studies of the issue (Counotte et al., 1979), the authors concluded that "bicarbonate will not play an important role as a base, until the pH in the rumen is 6.25 or lower." Made in the context of an otherwise exemplary study, this statement suggests that the authors are erroneously treating the rumen as a closed buffer system in which buffer capacity is maximal around the pK value of the bicarbonate system.

Several studies since have clarified that due to absorption of CO_2 from the rumen and regular eructation, the bicarbonate system of the rumen has to be regarded as an open buffer system comparable to that of the blood (Allen, 1997; Kohn and Dunlap, 1998). Protons react with HCO_3^- to form H_2O and CO_2 ($H^+ + HCO_3^- \Leftrightarrow H_2CO_3 \Leftrightarrow H_2O + CO_2$). Because CO_2 does not accumulate in the system but escapes, the bicarbonate system buffers strongly at neutral pH and beyond. A further problem that emerges when trying to understand ruminal buffering is that equations solving the underlying mixed SCFA-HCO_3^- buffer problem are lacking in the literature. These have been now been derived and are given in the Appendix of this study.

A major question that remains is whether the values for Henry's constant (α) and the dissociation constant (pK) that were determined for blood plasma (Andersen, 1962; Segel, 1976) can be applied to ruminal fluid. Both α and the pK of the bicarbonate system are known to vary not only with the osmolarity and the temperature, but also with protein content and various other parameters (Heisler, 1986). Given that the effect of short-chain fatty acids (**SCFA**) on both constants has never been studied, a rigorous determination of both constants for fluid from the rumen appears overdue. In the current study of bovine ruminal solution, Henry's constant α and the pK of H_2CO_3 are determined using a classical variation of the Astrup technique. These results are confirmed by those obtained via a novel titration method developed in the course of this study. Finally, the concept of base excess was adapted to allow a better evaluation of the acid-base status of ruminal fluid.

MATERIALS AND METHODS

Care and Use of Animals

All experiments were performed in accordance with German laws for the protection of animals [permits L0016/09 (Berlin) and 33.12–42502–04–14/1501 (Braunschweig)].

Animals and Sampling

A first set of samples was obtained from 2 fistulated cows (**h1** and **h2**) that were kept in tethered housing on straw at the faculty of veterinary medicine in Berlin. Initially, the animals received a hay-only diet at 0730 and 1500 h and water ad libitum. These animals will be referred to as hay/silage-fed in what follows because the animals received small amounts of grass silage in addition to hay toward the end of the experimental period.

The 2 hay/silage-fed cows were sampled twice weekly for a period of 3 wk. On experimental days, samples were taken immediately before the morning meal and approximately 4 h afterward (0700 and 1100 h) so that, in total, 12 samples were obtained from each of the 2 cows.

Before sampling, a 3-way stopcock was fitted to the fistula plug, allowing the measurement of intraruminal pressure using a differential pressure measurement device (Testo 510, Testo, Letzkirch, Germany). Negative basal values of -0.71 ± 0.06 kPa ($n = 24$) inside the rumen were observed versus barometric pressure outside, which alternated with short pressure bouts above the barometric level reflecting eructation.

After opening the fistula, intraruminal pH was measured approximately 10 to 15 cm below liquid level using a portable pH meter (pH-Meter 1140, Mettler Toledo, Gießen, Germany) calibrated at 37°C with standard buffer (pH 4 and 7) immediately before use. Subsequently, approximately 200 mL of ruminal fluid were obtained after insertion of an oro-ruminal sampling device into the rumen through the ruminal fistula. The pH of this sample was also recorded. Fluid aliquots were immediately frozen in liquid nitrogen to stop fermentation processes and stored at −20°C.