# Calculation of the Buffering Capacity of Bicarbonate in the Rumen and In Vitro

**R. A. Kohn and T. F. Dunlap**

Department of Animal and Avian Sciences, University of Maryland, College Park 20742

**ABSTRACT:** We describe a model to calculate the buffering capacity of bicarbonate in the rumen. The addition of $NaHCO_3$ results in the release of $CO_2$ from solution and eventually from the rumen via eructation. This process directly neutralizes ruminal acidity. The degree to which the process continues depends on the partial pressure of $CO_2$ in the gas phase, the pH, and a constant (7.74), according to the Henderson-Hasselbalch equation: pH = 7.74 + log([$HCO_3^-$]/ pressure of $CO_2$ in atmospheres). The addition of $NaHCO_3$ to buffer solutions and ruminal fluid under high pressure of $CO_2$ increased pH as predicted. The buffering capacity of ruminal fluid under $CO_2$ was greater at low pH than was previously determined by titration in air. In contrast, in vitro systems in which $CO_2$ is not permitted to escape may result in reduced buffering capacity. In vitro systems in which excess $CO_2$ may escape (under $N_2$ gas pressure) may result in uncontrolled pH elevation. Dilution of ruminal fluid under constant pressure of $CO_2$ decreased ruminal pH as predicted by the model. The pH under different pressures at equilibrium and the buffering capacity are easily calculated for in vitro and in vivo systems.

Key Words: Rumen, pH, Bicarbonates, Buffers

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## Introduction

The prediction of ruminal pH has been a major concern of ruminant nutritionists for many years, and $HCO_3^-$ is thought to be an important buffer of ruminal pH (Erdman, 1988) and of most in vitro media used for fermentation studies (Goering and Van Soest, 1970). The mechanism by which $HCO_3^-$ buffers the rumen and in vitro media is often misunderstood. Because the bicarbonate system is ubiquitous in nature, physical chemists have systematically developed calculations for predicting buffering capacity as affected by the medium's pH, ionic strength, and temperature (Fogg and Gerrard, 1985). These calculations are applicable to ruminal fluid and to in vitro media used for fermentation studies.

This article describes how $HCO_3^-$ buffers the rumen, and it describes the calculation of the buffering capacity of $HCO_3^-$ in vitro and in vivo. This understanding is a prerequisite for the development of a mechanistic mathematical model to predict ruminal pH. This article addresses issues related to the function of added $NaHCO_3$ in the diet and the impact of increased salivation, which increases $NaHCO_3$ flow to the rumen. In addition, the buffering capacity is calculated for in vitro methods that use $NaHCO_3$ at different pH levels and with different pressures of $CO_2$ (i.e., continuous perfusion of $CO_2$, perfusion of $N_2$, or systems with high $CO_2$ pressures).

## Background and Equations

Buffering capacity refers to the number of moles of $H^+$ that must be added to 1 L of solution to decrease the pH by 1 unit (Segel, 1976). This value depends on the buffer system and on the pH. Weak acids and bases provide better buffering than strong acids and bases because of the establishment of equilibria between the acid and conjugate base. For example, consider the weak acid, HA, and its base, $A^-$:

$$HA \underset{\leftarrow}{\overset{\rightarrow}{}} H^+ + A^-$$

If the forward reaction is first order with respect to acid concentration, the rate is expressed as

$$\text{forward rate} = k_f[HA],$$

where $k_f$ represents the fractional rate constant and [HA] represents the concentration of acid. If the reverse reaction is first order with respect to products,

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the rate is expressed as

$$\text{reverse rate} = k_r[A^-]\ [H^+]$$

where $k_r$ represents the fractional rate constant for the reverse reaction and $[A^-]$ and $[H^+]$ represent the concentrations of $A^-$ and $H^+$ in moles per liter, respectively. If the system comes into equilibrium, the forward reaction rate equals the reverse rate,

$$k_f[HA] = k_r[A^-]\ [H^+]$$

The equilibrium constant ($k_{eq}$) for the reaction is determined as

$$k_{eq} = k_f/k_r = [A^-]\ [H^+]/[HA]$$

Because this is the constant for acid dissociation, it is also referred to as the *acid constant* ($K_a$). The negative $\log_{10}$ of the $K_a$ is referred to as the $pK_a$:

$$pK_a = -\log K_a$$
$$pK_a = -\log([A^-]\ [H^+]/[HA])$$
$$pK_a = -\log([A^-]/[HA]) - \log[H^+]$$
$$pK_a = -\log([A^-]/[HA]) + pH$$

Rearranging provides for the Henderson-Hasselbalch equation,

$$pH = pK_a + \log([A^-]/[HA])$$

The $pK_a$ is therefore the pH at which the acid is half-dissociated when in equilibrium. At > 1 unit of pH below the $pK_a$, > 90% of the buffer would be in the acid form at equilibrium, and at > 1 unit of pH above the $pK_a$, > 90% of the buffer would exist as the conjugate base at equilibrium.

Major buffers that exist in the rumen (Counotte et al., 1979) include $HCO_3^-$ ($pK_a$ = 3.80), carbonate ($pK_a$ = 10.25), phosphate ($pK_a$ = 2.12, 7.21, and 12.32), acetate ($pK_a$ = 4.76), propionate ($pK_a$ = 4.87), butyrate ($pK_a$ = 4.82), and lactate ($pK_a$ = 3.86). Most of these weak acids and bases have $pK_a$ that are outside the normal pH range of the rumen. If the ruminal pH is > 6.0, most of the VFA would be dissociated. As the pH drops to < 6.0, the rumen may be buffered by the protonization of fatty acids. Under normal conditions, these acids would provide for little buffering. The ability of phosphate in the rumen to buffer pH would decline as the pH decreases from neutrality.

### *The Bicarbonate System*

The most prevalent ruminal buffer is $HCO_3^-$ (Counotte et al., 1979; Erdman, 1988). The bicarbonate system includes two major ionic forms: $HCO_3^-$ and $CO_3^{2-}$. The $HCO_3^-$ is of primary importance to buffering the blood of animals because it may be protonated to $H_2CO_3$. The $pK_a$ for this acid is only 3.80 at 37°C and .15 *M* ionic strength (Segel, 1976). However, $H_2CO_3$ may establish an equilibrium with dissolved $CO_2$ and $H_2O$:

$$CO_2 + H_2 \underset{\leftarrow}{\overset{\rightarrow}{}} H_2CO_3 \underset{\leftarrow}{\overset{\rightarrow}{}} HCO_3^- + H^+$$

The equilibrium constant ($k_{eq}$) for the two steps of the reaction combined is the product of the individual equilibrium constants.

$$k_{eq} = k_1k_2/k_{-1}k_{-2}$$
$$= ([HCO_3^-][H^+])/([CO_2]\ [H_2O])$$

where $k_1$ and $k_2$ are fractional rate constants for each forward reaction and $k_{-1}$ and $k_{-2}$ are fractional rate constants for each reverse reaction. Considering both steps in equilibrium, the effective $pK_a$ for the system ($pK_a'$) is 6.1 for solutions of .15 *M* ionic strength at 37°C (Segel, 1976). This value is > 1 unit below blood pH. How does the system maintain blood pH? The $CO_2$ is exhaled or removed via urine, thus amounting to the removal of a proton equivalent. The breathing rate is regulated to allow blood pH to increase only to the desired neutral (pH 7.4) value.

### *The Ruminal System*

There are major similarities and differences between the blood and the ruminal environment. Both environments are similar in temperature and ionic strength, and, therefore, the equilibrium constants between the reactions are similar. However, the ruminal environment is typically lower in pH (5.0 to 6.8) than the blood environment, and the exchange between the outside air and the liquid phase of the rumen is not regulated like breathing. Whether these differences affect the buffering capacity of $NaHCO_3$ in the rumen needs to be determined.

Ruminal gases and liquids are in close contact so that an equilibrium between soluble and evolved gas may be attained. This equilibrium is in contrast to the blood buffering system in which the association of the liquid (blood) with gas (air in lungs) is regulated by breathing. The overall equilibrium constant for the ruminal system is therefore the product of the three equilibrium constants for each of the reactions in which $CO_2$ gas is converted to $HCO_3^-$. The equilibrium solubility of $CO_2$ is determined from the partial pressure in atmospheres (atm) of $CO_2$ in the gas phase ($pCO_2$) and Henry's constant (k) as

$$[CO_2]_{aq} = k(pCO_2)$$

where $[CO_2]_{aq}$ is the concentration of dissolved $CO_2$ in solution. Henry's constant (k) for $CO_2$ in solvent with .15 ionic strength (similar to blood or ruminal fluid) at 37°C is .0229 mol/atm (adapted from Segel, 1976).

The effective $pK_a$ ($pK_a'' = 7.74$) in this situation is therefore higher ($-\log(.0229) = 1.64$) than that for the two-reaction system ($pK_a' = 6.1$). The higher $pK_a''$ for the three-reaction system reflects the equilibrium between different reactants and products but does not contradict the $pK_a'$ for the two-reaction system. This high $pK_a''$ indicates that $NaHCO_3$ added to the rumen in saliva or feed will be protonated and result in $CO_2$ formation in the gas phase. A higher $pK_a'$ (6.25) was previously reported (Turner and Hodgetts, 1954) for rumen fluid at 25°C but the temperature considered here is 37°C. If we had used the higher $pK_a'$, 6.25, the $pK_a''$ would have been 7.84. The extent of the $CO_2$ release will depend on the $pCO_2$ in the rumen:

$$pH_{rumen} = 7.74 + \log([HCO_3^-]/pCO_2)$$

The final reaction to consider is the eructation of gas from the rumen. This eructation is a unidirectional process. Gas leaves the rumen when total ruminal gas pressure exceeds atmospheric pressure (Stevens and Sellers, 1960). This $CO_2$ loss is not likely to be countered by $CO_2$ intake (atmospheric $CO_2$ concentration is very low). Therefore, the ruminal gases are not in equilibrium with the outside air. For this reason, it is not necessary to consider the equilibrium constant of the process or its impact on the $pK_a''$.

### *Empirical Evaluation*

This model was tested by empirical experimentation. Distilled water or sodium acetate solutions were equilibrated with 70% $CO_2$ and 30% $N_2$ using a gas flowmeter (Cole-Parmer, Vernon Hills, IL) at 37°C and a fritted-glass bubble disperser. Sodium hydroxide was added to bring each sample to the desired initial pH (5.0, 5.5, 6.0, 6.5, and 7.0). After reaching this starting point, $NaHCO_3$ was added (.04 mol/L final volume), and equilibrium pH was recorded when it was clearly stable, which occurred within about 15 min. Each experiment was replicated three times, and the mean pH values are shown in Tables 1 and 2. Root mean square prediction error represents the mean difference of predicted vs measured values (Bibby and Toutenburg, 1977).

In most cases, the predicted pH was within .1 unit of measured pH for water samples (Table 1). A higher $pK_a''$ (7.83) would have improved the prediction of pH in distilled water ($pK_a''$ used was for .15 ionic strength). The pH of acetate solution was consistently underestimated, probably as a result of the loss of acid by volatilization. In reality, ruminal fluid VFA concentrations are lower than were used here to demonstrate the potential for a dampening of changes in pH that are due to VFA.

Ruminal fluid also was collected from a lactating cow on a high-protein, high-energy diet based on alfalfa hay and corn silage and from a nonlactating cow on a high-forage diet based on corn silage and orchardgrass hay. Whole (solids and fluid) rumen contents were strained through four layers of cheesecloth. The pH was determined after the samples were equilibrated with 70% $CO_2$ at 37°C. Sodium bicarbonate was added (.04 mol/L final volume), and the equilibrium pH was determined when it stabilized. After this determination, distilled water was added (20% of previous volume) to represent the influx of water into the rumen in response to drinking. The predicted changes in pH from both manipulations were within .02 pH unit of observed changes (Table 3).

### *Impact of Adding $NaHCO_3$*

Bicarbonate enters the rumen as part of the diet or in the saliva that is secreted during chewing (Erdman, 1988). The model described here can be used to predict the effect of added $NaHCO_3$. When eructation is considered, the total pressure of all gases in the rumen is not likely to greatly exceed 1 atm. If $CO_2$ makes up 70% of the total ruminal gases (Barry et al., 1977), the $pCO_2$ would equal .7 atm, and, if this gas is in equilibrium with the liquid phase at pH 6.5, the system can be described according to the Henderson-Hasselbalch equation as follows:

$$6.5 = 7.74 + \log([HCO_3^-]/.7)$$

or

$$[HCO_3^-] = .7 \text{ inverse } \log(6.5 - 7.74) = .040 \text{ mol/L}$$

Consider the addition of .04 mol/L (3.36 g/L of ruminal fluid or about 168 g per animal) of $NaHCO_3$. Because $[HCO_3^-]$ is much greater than $[H^+]$ for this system at equilibrium, most of the added $HCO_3^-$ remains in that form. The new pH can be calculated

Table 1. Impact on pH of adding $NaHCO_3$ (.04 mol/L) to bicarbonate media equilibrated with .7 atmosphere partial pressure of $CO_2$ at 37°C

| | Equilibrium after $NaHCO_3$ addition | | |
|---|---|---|---|
| Initial pH | Predicted $[HCO_3^-]$[b] | Predicted pH | Measured pH[c] |
| 7.00 | .167 | 7.12 | 7.05 |
| 6.50 | .080 | 6.80 | 6.85 |
| 6.00 | .053 | 6.62 | 6.64 |
| 5.50 | .044 | 6.54 | 6.52 |
| 5.00 | .041 | 6.51 | 6.58 |

[a]Predicted using $pH = pKa'' + \log([HCO_3^-]/pCO_2)$ with $pKa'' = 7.74$(estimated from literature values).
[b]Concentration of ions in moles per liter.
[c]SE = .024; n = 3; root mean square prediction error = .069.

Table 2. Impact on pH of adding $NaHCO_3$ (.04 mol/L) to acetate-bicarbonate media (.2 mol/L acetate + acetic acid) equilibrated with .7 atmosphere partial pressure of $CO_2$ at 37°C

| | Equilibrium after $NaHCO_3$ addition | | | |
|---|---|---|---|---|
| | | | Final pH | |
| Initial pH | $[HCO_3^-]$[a] | [Acetic acid][a] | Predicted[b] | Measured[c] |
| 7.00 | .167 | .001 | 7.12 | 7.22 |
| 6.50 | .079 | .002 | 6.79 | 6.93 |
| 6.00 | .045 | .003 | 6.55 | 6.68 |
| 5.50 | .020 | .006 | 6.20 | 6.38 |
| 5.00 | .003 | .035 | 5.43 | 5.63 |

[a]Concentration of ion or acid in moles per liter.
[b]Predicted using $pH = pKa'' + \log([HCO_3^-]/pCO_2)$ with $pKa'' = 7.74$, and $pH = pKa + \log([CH_3COO^-]/[CH_3COOH])$ with $pKa = 4.76$.
[c]SE = .012; n = 3; root mean square prediction error = .158.

from the equation representing the new equilibrium concentrations (excluding other buffers, for now):

$$pH = 7.74 + \log\{(.04 + .04)/.7\} = 6.80$$

The addition of $NaHCO_3$ would increase the ruminal pH from 6.5 to 6.8. This change demonstrates that added $NaHCO_3$ would increase ruminal pH. The effect would be greater at lower pH and would be reduced at higher pH (Table 1). The addition of $NaHCO_3$ would result in the dissociation of $Na^+$ and $HCO_3^-$. Some of the $HCO_3^-$ would be converted to $H_2CO_3$ and then released as $CO_2$. The amount of $H^+$ consumed in this process was calculated from the change in $H^+$ concentration ($1.6 \times 10^{-7}$ mol/L). Because most of the added $NaHCO_3$ would remain in the $HCO_3^-$ form, the initial assumption for the final concentration of $NaHCO_3$ was correct.

Some have argued (Russell and Chow, 1993) that added $NaHCO_3$ has little direct impact on ruminal fluid pH because the rumen is saturated with $CO_2$. Results and discussion presented in the current article are contrary to that argument. Added $NaHCO_3$ can directly neutralize ruminal fluid acidity even under high $pCO_2$.

### *The Buffering of Ruminal Fluid*

Eventually, the $NaHCO_3$ added in the diet or saliva is removed from the system via eructation. As more acid is produced by fermentation, the buffer is consumed to maintain the pH. Consider the addition of .01 mol/L of $H^+$ to the situation described previously at pH 6.8. This acid is adequate to reduce the pH to 2 for an unbuffered system. In the ruminal system, after equilibrium is reached, the acid releases .01 mol/L of $CO_2$. The added pressure from $CO_2$ release is relieved by eructation, so the final $pCO_2$ would still equal .7. However, .01 mol/L of $HCO_3^-$ is consumed or the final concentration of $HCO_3^-$ is .08 mol/L (shown previously) –.01 mol/L = .07 mol/L. The final pH is

$$pH = 7.74 + \log(.07/.7) = 6.74$$

which is a slight change from pH 6.8. These examples demonstrate the importance of the direct buffering capacity of the bicarbonate system on ruminal pH.

Ruminal fluid also contains other buffers that further dampen the changes in pH. If, in the first example, the pH did not adjust fully to pH 6.8 from 6.5

Table 3. Impact of adding $NaHCO_3$ (.04 mol/L) and then dilution (20.3%) on ruminal fluid pH equilibrated with .7 atmosphere partial pressure of $CO_2$ at 37°C

| | Sample one[a] | | Sample two[b] | |
|---|---|---|---|---|
| Item | Predicted[c] | Measured[d] | Predicted[c] | Measured[d] |
| Initial pH | — | 6.10 | — | 6.66 |
| pH after adding $NaHCO_3$[e] | 6.64 | 6.61 | 6.88 | 6.90 |
| pH after $NaHCO_3$ and dilution[e] | 6.55 | 6.52 | 6.79 | 6.81 |

[a]Ruminal fluid from a lactating Holstein on a high-energy, alfalfa silage-based diet 4 h after feeding.
[b]Ruminal fluid from a nonlactating Holstein on a corn silage and orchardgrass hay-based diet 16 h after feeding.
[c]Predicted using $pH = pKa'' + \log([HCO_3^-]/pCO_2)$ with $pKa'' = 7.74$ (estimated from literature values). Only the bicarbonate buffer was considered.
[d]SE = .020; n = 3.
[e]Root mean square prediction error = .033.

but rather to 6.7, how much would the other buffers be alkalized?

$$6.7 = 7.74 + \log([HCO_3^-]/.7)$$
$$[HCO_3^-] = .7 \text{ inverse log } (6.7 - 7.74) = .064 \text{ mol/L}$$

The addition of .040 mol/L of $HCO_3^-$ to the existing .040 mol/L $HCO_3^-$ results in a final concentration of only .064 mol/L. Therefore, (.040 + .040 – .064 = .016) .016 mol/L of $HCO_3^-$ is converted to $CO_2$, and an equal amount of noncarbonate buffer is regenerated. The rumen likely does not contain enough noncarbonate buffer to dampen the pH change this much at this pH. The following discussion demonstrates that the bicarbonate system is the predominant buffer of normal ruminal pH.

Consider the example of adding .04 mol/L of $NaHCO_3$ to media under .7 atm of $pCO_2$. The media contains .2 mol/L of acetic acid. Previously, we assumed that most of the added $NaHCO_3$ remained in solution, and, for unbuffered media, this assumption proved to be correct. However, in the situation in which acetic acid must be neutralized to raise the fluid pH, many more protons must be consumed. Therefore, more $NaHCO_3$ is converted to $CO_2$ and evolves from the system. In fact, the final concentration of $NaHCO_3$ is not the sum of the initial concentration and the added amount. Similarly, the amount of acetic acid is changed because of the addition of $NaHCO_3$. The pH does not increase as much as it does in the unbuffered media. The final pH can still be calculated from the Henderson-Hasselbalch equations for each buffer:

$$pH = 7.74 + \log[HCO_3^-]/.7]$$
$$= 4.76 + \log[Ac^-]/[AcH]$$

where $[Ac^-]$ and [AcH] are the concentrations (moles per liter) of acetate and acetic acid, respectively. If x is the amount of $CO_2$ gas evolved in the process of neutralizing acetic acid, the equilibrium amount of $HCO_3^-$ is the initial amount plus the added amount minus x. The equilibrium amount of AcH is the initial amount minus x. These two equations can then be solved simultaneously for x and pH. The results in Table 2 in contrast to those in Table 1 demonstrate that, at low pH, VFA dampen the effectiveness of added $NaHCO_3$ on raising ruminal fluid pH, but added $NaHCO_3$ is still very effective. The negligible impact of phosphate buffer on the effectiveness of $NaHCO_3$ to raise media pH is provided in the final section. Calculation of the effect of more than two buffers at the same time would require the solution of more than two simultaneous equations, which merely requires persistence.

These calculations show a very high buffering capacity for ruminal fluid compared to previously published results (Theodorou et al., 1994; Pitt et al., 1996). Titration curves for ruminal fluid have been generated by titration in air (Counotte et al., 1979) or under low $pCO_2$ (Theodorou et al., 1994), and therefore they only demonstrate a fraction of the full buffering capacity as pH is reduced. At any given pH, the amount of $HCO_3^-$ available for buffering is higher when the $pCO_2$ is greater.

### *Impact of Dilution Rate*

There has been much confusion about the buffering system of ruminal fluid, and researchers have sought further explanations for the maintenance of ruminal fluid pH. One such theory is that the dilution of ruminal contents with water from saliva production or water intake will raise ruminal pH (Russell and Chow, 1993) by dilution of acids and washout of starch. This theory does not seem appropriate for the rumen system. First, in vivo experiments did not show that liquid dilution rates change when $NaHCO_3$ or other buffers are included in the diet (Erdman et al., 1982). In addition, the following discussion will show that the predicted effect of dilution on ruminal pH is exactly the opposite of what was proposed. When considering the impact of $HCO_3^-$ under $CO_2$ pressure, the pH is predicted to decrease from dilution rather than increase. Dilution increases the effective volume of liquid. This additional liquid enables more $CO_2$ to be converted to $HCO_3^-$ with the release of more protons. Therefore, dilution with water would reduce pH. This demonstrates that the impact of $NaHCO_3$ on raising ruminal pH cannot be explained by dilution effects, as was suggested (Russell and Chow, 1993). However, saliva production would increase ruminal pH owing to the combined effects of added sodium and dilution.

The effect of dilution can be calculated using the model described in this paper. Consider rumen fluid at pH 6.6 under .7 atm $pCO_2$. At this pH and $pCO_2$, the $[HCO_3^-]$ = .051, as determined previously. Dilution by 20% by adding water initially reduces $[HCO_3^-]$ by 20% to .041. In order for the system to return to equilibrium, the $[HCO_3^-]$ must increase while the pH decreases. Gaseous $CO_2$ is converted to new $HCO_3^-$ while releasing one proton for every new $HCO_3^-$. Because the $[HCO_3^-]$ is much greater than $[H^+]$ at this pH, the change in $[HCO_3^-]$ is negligible. The new pH corresponding to this concentration is calculated from the Henderson-Hasselbalch equation as 6.50 = 7.74 + log(.041/.70). At a lower pH, the effect of dilution on reducing pH would be diminished as a result of the reduced solubility of $HCO_3^-$ and the buffering by VFA.

## Buffers for In Vitro Fermentation

### *Open Systems Under $CO_2$ Pressure*

Bicarbonate buffers are frequently used with the continuous perfusion of 1 atm of $CO_2$ gas for in vitro

fermentation experiments. A buffer designed by Goering and Van Soest (1970) equilibrates to pH 6.8 under 1 atm of $CO_2$. If a $NaHCO_3$ buffer at this pH is used under the more realistic .7 atm, what would be the equilibrium pH? Under 1 atm of $pCO_2$,

$$6.8 = 7.74 + \log([HCO_3^-]/1)$$
$$[HCO_3'] = \text{inverse log}(6.8 - 7.74) = .115 \text{ mol/L}$$

Now, with a reduction of $pCO_2$ to 0.7 atm,

$$pH = 7.74 + \log(.115/.7) = 6.96$$

This equation demonstrates that a buffer intended to maintain pH at 6.8 under 1 atm of $pCO_2$ would result in a higher pH if subjected to a lower $pCO_2$. The continuous perfusion of 1 atm of $pCO_2$ over the in vitro media (Goering and Van Soest, 1970) would result in a different pH (for the same buffer) than would result from allowing the pressure to be released as it accumulated from gas production (Theodorou et al., 1994). In the latter case, the $pCO_2$ would be lower because methane (if methanogens survive) and other gases would account for some of the total pressure. In both systems, the total pressure would be maintained and pH could be predicted.

### *Partially Closed Systems*

In vitro ruminal preparations are often sealed, and total pressure is allowed to increase as gas is released from the fluid (Lowe et al., 1985). In fact, this may offer the opportunity to estimate the rate of fermentation from the pressure changes (Pell and Schofield, 1993). However, are these systems adequately buffered? The pH of such systems is not easy to measure because once the samples are opened, the pressure is released and the pH may adjust to a different equilibrium. However, calculation is not difficult. Consider a system using the buffer described previously, which equilibrates to pH 6.8 under 1 atm of $pCO_2$. If the $pCO_2$ increases to 2 atm from fermentation (assuming all $CO_2$ released is from metabolic production), what is the equilibrium pH?

$$pH = 7.74 + \log(.115/2) = 6.50$$

The use of gas pressures to estimate fermentation requires a small reduction in medium pH; however, this example demonstrates that some pressure may be accumulated before the pH is driven outside the normal range for the rumen.

How much buffering capacity does $NaHCO_3$ provide in an in vitro system where pressure accumulates? Buffering capacity is a function of the number of moles of $H^+$ that may be neutralized to prevent a reduction in pH. As the $pCO_2$ increases in systems from which $CO_2$ cannot escape, $CO_2$ is forced back into solution, which results in lowering pH.

The pressure of $CO_2$ depends on the ideal gas law:

$$PV = nRT$$

where P = pressure (atmospheres), V = volume (liters), n = moles of gas, R = the gas constant (.08206 L atm/°K per mol), and T = temperature (degrees Kelvin). Under ruminal conditions, pressure remains relatively constant as moles of $CO_2$ released are eructated (volume increases). In a two-phase system where gas cannot completely escape and gas volume is held constant, the pressure increases in proportion to the moles of $CO_2$ released. If the volume is large (greater gas space in the system), the pressure increases less dramatically than if it is small. Therefore, the amount of buffering that such a semiclosed system provides depends on the volume available for the gaseous phase.

For example, what is the change in pH from the addition of .01 mol/L of $H^+$ on a semiclosed system with 100 mL of gaseous space (initially under 1 atm of $pCO_2$) and 1 L of ruminal buffer as used above (initial pH 6.8 under 1 atm of $pCO_2$)? This system was previously calculated to have .115 mol/L of $HCO_3^-$. If .01 mol of $HCO_3^-$ is lost to buffer the added $H^+$, the final concentration is .115 – .01 = .105. The change in $pCO_2$ ($\Delta P$) is proportional to the release of $CO_2$ from acidification ($\Delta n$):

$$\Delta P = \Delta nRT/V = .01 \text{ mol } (.08206 \text{ L atm per °K per mol})(310°K)/.1 = 2.54 \text{ atm}$$

or the final pressure is 3.54 atm. The final pH of this system is, therefore,

$$pH = 7.74 + \log(.105/3.54) = 6.21$$

An unbuffered system would have resulted in a pH of 2; however, if the gas were allowed to leave the system (as in the example of the rumen), the pH would have been buffered even better (6.74). If an in vitro method results in very high pressures after fermentation, the buffer may be inadequate, which would be especially true when high amounts of substrate are available, there is little gaseous volume, and other buffers are not included. Simply measuring the pH after releasing the pressure will result in an erroneous conclusion about the buffering capacity.

### *Systems Under $N_2$*

Although some in vitro fermentations may be conducted under very high $pCO_2$, other systems perfuse the media with $N_2$ gas (Hoover et al., 1976). What sort of equilibria do these systems approach? The absence of $CO_2$ in the gas above the buffer could result in the diffusion of $CO_2$ from the liquid. When $CO_2$ is removed, less $HCO_3^-$ remains in solution and the $HCO_3^-$ buffering capacity is reduced. As the $CO_2$

diffuses from the system, the $HCO_3^-$ would accept protons to form more $CO_2$. For this reason, the pH of ruminal fluid or $NaHCO_3$ buffer would increase if allowed to equilibrate with air or other gas with lower than intended $pCO_2$. Bicarbonate buffers under these conditions are only stable if the exchange of $CO_2$ with the air is controlled (as in animal respiration). The rumen and most in vitro systems are not regulated in this way. The pH seems to be maintained in these systems because the fluids are buffered by other agents besides $HCO_3^-$ and dilution rates are sufficiently high that some $HCO_3^-$ remains in solution (Hoover et al., 1976).

*Buffering by Phosphate and $NaHCO_3$*

The extent to which phosphate may buffer the rumen or the media is easily calculated. The buffer of Goering and Van Soest (1970) uses $HCO_3^-$ and $HPO_4^-$ buffers. The $HPO_4^-$ concentration in this case is approximately .02 mol/L. If the mixed buffer is equilibrated with 1 atm of $pCO_2$, the pH adjusts to 6.8. The relative concentrations of $HCO_3^-$ and phosphate ions can be calculated from the two simultaneous Henderson-Hasselbalch equations ($pK_a$ for phosphate = 7.21):

$$6.8 = 7.74 + \log([HCO_3^-]/1)$$

$$[HCO_3^-] = .115$$

$$6.8 = 7.21 + \log([HPO_4^-]/[H_2PO_4])$$

$$([HPO_4^-]/[H_2PO_4]) = .39$$

$$[HPO_4^-] + [H_2PO_4] = .02$$

$$[HPO_4^-] = .0056$$

$$[H_2PO_4] = .0144$$

Now consider the addition of .01 mol of $H^+$ to this buffer kept under 1 atm of $pCO_2$. Let x be the moles of $CO_2$ gas released, and .01 – x is, therefore, the moles of phosphate acidified. The final pH is not immediately known, but it would be the same for both systems if these were contained together,

$$pH = 7.74 + \log\{(.115 - x)/1\}$$
$$= 7.21 + \log\{(.0056 - .01 + x)/.0144\}$$
$$x = .0098$$

or 98% of the buffering comes from $HCO_3^-$ and 2% from phosphate:

$$pH = 7.74 + \log\{(.115 - .0098)/1\} = 6.78$$

and

$$pH = 7.21 + \log\{(.0056 - .01 + .0098)/.0144\}$$
$$= 6.78$$

Under these conditions, $HCO_3^-$ is the predominant buffer by far. Previous authors have come to similar conclusions based on empirical and theoretical evidence (Turner and Hodgetts, 1954; Counotte et al., 1979) but these have been forgotten by many.

## Implications

The rumen maintains a relatively constant $pCO_2$ through eructation when total gas pressures exceed atmospheric pressures. This controlled gas pressure is an essential part of the buffering system. Bicarbonate is a strong neutralizer of reductions in ruminal pH in vivo, and the buffering capacity of ruminal fluid is higher than has been determined previously by titration in air (rather than under $CO_2$). However, in vitro systems may not provide the intended buffering capacity when $HCO_3^-$ is used as the buffer because these systems may provide greater or lower $pCO_2$ than required. Buffering capacity of $HCO_3^-$ in vivo and in vitro is easily calculated from the Henderson-Hasselbalch equation and from knowledge of the $pK_a''$ (7.74) and $pCO_2$.

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