

# TEMPORAL EFFECTS OF RUMINAL BUFFERS ON RUMINAL FLUID FROM DAIRY COWS

P. Le Ruyet<sup>1</sup> and W.B. Tucker<sup>2</sup>

## Story in Brief

In vitro characteristics of several buffers and alkalinizing agents were evaluated. Ruminal fluid was collected from five cows consuming a diet containing concentrate and sorghum silage in a 68:32 ratio (DM basis). This fluid was incubated with either  $\text{NaHCO}_3$ , a natural sodium sesquicarbonate, a multielement buffer or  $\text{MgO}$  (7.1 g/L rumen fluid) or no buffer for 48 h; flasks were removed and analyzed for pH, buffering capacity and buffer value index every 12 h during the 48-h incubation. The buffer value index separated these buffering compounds into two categories;  $\text{NaHCO}_3$  and sodium sesquicarbonate exhibited similar buffer value indexes; both were markedly higher than those for the multielement buffer and  $\text{MgO}$ . Whereas  $\text{NaHCO}_3$  and sodium sesquicarbonate each increased ruminal fluid pH and buffering capacity sharply, the multielement buffer only increased pH and buffering capacity moderately. The increase in buffer value index for  $\text{MgO}$  was due primarily to an increase in pH. Both  $\text{NaHCO}_3$  and sodium sesquicarbonate were fully active within the first 12 h of incubation; activity of multielement buffer and  $\text{MgO}$  plateaued at 24 h. Compared to the multielement buffer and  $\text{MgO}$ ,  $\text{NaHCO}_3$  and sodium sesquicarbonate should be more beneficial in preventing short-term postprandial increases in ruminal fluid hydrogen ion concentration; because of their slower release rates, the multielement buffer and  $\text{MgO}$  should help stabilize ruminal acid-base status but efficacy might be reduced due to passage out of the rumen.

(Key Words: Buffers, Ruminal Fluid, Dairy Cows.)

## Introduction

Dietary buffers are used to lessen the ruminal acid load resulting from dietary acids and nutrient fermentation. Buffers that are highly soluble in ruminal fluid have an immediate impact on ruminal acid-base status; however,

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<sup>1</sup>Visiting Scholar <sup>2</sup>Assistant Professor

these effects may be transient. Ideally, buffers should either be released during the interval of most severe acid production in the rumen, or they should provide a continuous release to prevent fermentation-related increases in free proton ( $H^+$ ) concentration from becoming detrimental to fiber digestion. Hence, our objective was to characterize the buffering capacity of several buffers during a 48 h incubation period in ruminal fluid.

## Materials and Methods

Five ruminally-cannulated Holstein cows in a separate 5x5 Latin square study with 3-wk experimental periods were utilized to provide ruminal fluid to evaluate the *in vitro* release pattern of 4 different buffers. Cows were fed a total mixed diet (Table 1) consisting of concentrate plus sorghum silage (68:32 ratio, DM basis) twice daily. Mean daily dry matter intake was 19.2 (SE = 2.4) kg.

Three days before the end of each experimental period in the separate study, ruminal fluid was collected from a single cow receiving the diet in Table 1. Ruminal fluid (1.95 L) was collected from the ventral sac of the

**Table 1. Ingredient and nutrient composition of experimental diet (DM basis).**

Ingredient	%	Nutrient	%
Forage sorghum, silage	32.04	DM	45.10
Ground shelled corn	43.96	CP	15.80
Soybean meal, 44% CP	20.64	NE <sub>L</sub> , Mcal/kg	1.59
Megalac <sup>a</sup>	.52	ADF	21.00
Limestone	1.07	NDF	31.10
Dicalcium phosphate	.87	Ca	.81
Dynamate	.35	P	.44
Trace mineralized salt	.54	Mg	.35
Vitamin A premix <sup>c</sup>	.01	K	1.22
Vitamin E premix <sup>d</sup>	.01	S	.31
		Na	.26

<sup>a</sup> Calcium salts of fatty acids.

<sup>b</sup> Double sulfate of potassium and magnesium.

<sup>c</sup> Supplied 30,000,000 IU Vitamin A per kg of premix.

<sup>d</sup> Supplied 500,000 IU Vitamin E per kg of premix.



rumen with an electric vacuum pump at 2 h postfeeding. Seventy milliliters of the fluid were dispensed into each of 25 (5 sampling times for 5 buffers from one of 5 cows in 5 different periods), 125-ml flasks; each flask received either no buffer (Control), .5 g  $\text{NaHCO}_3$  (Bic), .5 g of a natural sodium sesquicarbonate (NSSC), .5 g of a multielement buffer (MEB) or .5 g  $\text{MgO}$  and was incubated in a shaking water bath at 39° C for 0, 12, 24, 36 or 48 h. Upon removal from the water bath, ruminal fluid was analyzed for pH, buffering capacity (BC) and buffer value index (BVI).

## Results and Discussion

Buffers provided the largest source of variation in our statistical model for  $\text{H}^+$ , BC and BVI (Table 2). The buffer-by-sample time interaction was significant for each indicator of acid-base status; hence, buffer effects are presented for each sample time (Figures 1 and 2).

Ruminal fluid pH (Figure 1) appeared to be increased most sharply by NSSC and  $\text{NaHCO}_3$  prior to 24 h of incubation; after 24 h,  $\text{MgO}$  increased pH to that of NSSC and  $\text{NaHCO}_3$ , and was higher than for MEB addition. Magnesium oxide appeared to have the greatest effect on ruminal fluid pH after 24 h of incubation.

Ruminal fluid  $\text{H}^+$  (Figure 2A) was decreased by addition of any buffer but increased for the Control at 12 h of incubation. Because  $\text{H}^+$  was measured immediately after addition of buffers to the ruminal fluid at 0 h, the reduction in  $\text{H}^+$  at 12 h likely was related to dissolving of the buffering compounds. The increase in  $\text{H}^+$  for the Control at 12 h probably resulted from fermentation-related acid production. After 12 h, there was no change (NS) in ruminal fluid  $\text{H}^+$  with added buffers, whereas in Control flasks,  $\text{H}^+$  tended to decrease ( $P = .06$ ) from 24 to 48 h.

As compared to Control, ruminal fluid  $\text{H}^+$  was reduced most markedly by  $\text{NaHCO}_3$  and NSSC at 0 h, whereas all buffers reduced  $\text{H}^+$  after 12 h of incubation. No differences in  $\text{H}^+$  were observed among buffers at any sampling time. By contrast, Tucker et al. (1992) reported that  $\text{NaHCO}_3$  and NSSC were more effective in reducing  $\text{H}^+$  than MEB when ruminal fluid was incubated for only 5 h; in that study, each of the buffers reduced  $\text{H}^+$  compared to an unbuffered control. It appears that MEB requires more than 5 h to fully release its acid neutralizing potential.

Ruminal fluid BC (Figure 2B) for  $\text{NaHCO}_3$  and NSSC increased from 0 to 12 h, but remained constant for the remainder of the incubation interval. Buffering capacity peaked and stabilized at 24 h for MEB and  $\text{MgO}$ , whereas the Control increased throughout the 48-h interval. Compared to the rapid reduction in ruminal fluid  $\text{H}^+$  with MEB, BC appeared to increase more gradually for MEB; response patterns for these two variables appeared to be

Table 2. Mean squares for indicators of ruminal acid-base status<sup>a</sup>.

Source	df	H <sup>+</sup>		BC		BVI	
		MS	P	MS	P	MS	P
Cow	4	2,832,943	.001	3155	.001	54.3	.033
Buffer	4	11,848,858	.001	8107	.001	2249.6	.001
Cow-by-Buffer	16	1,006,654	.001	218	.005	104.2	.001
Sample time	4	1,198,704	.001	3966	.001	550.3	.001
Sample time-by-Buffer	16	420,232	.004	225	.004	68.0	.001
Residual	80	170,283		90		19.7	

<sup>a</sup>H<sup>+</sup> = ruminal fluid hydrogen ion concentration; BC = ruminal fluid buffering capacity; BVI = ruminal fluid buffer value index.



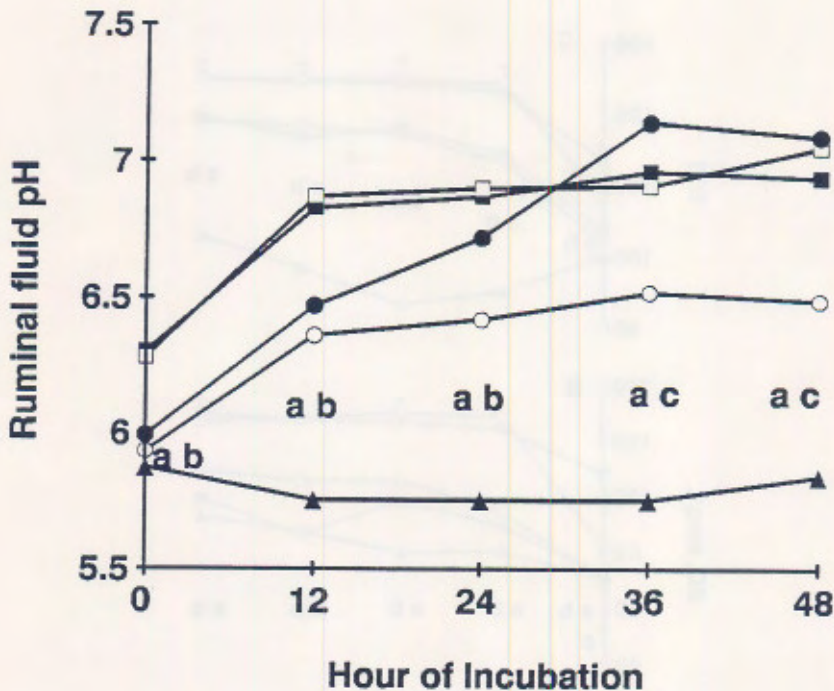


Figure 1. Ruminant fluid pH throughout 48 h of incubation with 7.1 g buffer added per liter. Solid triangle = no buffer (Control); solid square =  $\text{NaHCO}_3$ ; open square = natural sodium sesquicarbonate (NSSC); open circle = multielement buffer (MEB); solid circle =  $\text{MgO}$ ; a, b, c = significant effects ( $P < .05$ ): within each sampling time, a = Control vs. buffers, b = sodium buffers ( $\text{NaHCO}_3$  and NSSC) vs. other buffers (MEB and  $\text{MgO}$ ), c = MEB vs.  $\text{MgO}$ .

more closely and inversely related for the remaining buffers. The increase in BC over time for MEB likely was related to more gradual dissolution of this buffering compound; the increase over time for the Control may have resulted from increased production of VFA, which would buffer the ruminal fluid close to pH 5. Ruminant fluid BC appeared to increase over time for  $\text{MgO}$ , although a sharp drop was evident at 36 h.

Ruminant fluid BC (Figure 2B) was higher for  $\text{NaHCO}_3$  than for Control at each sample time, was higher for NSSC and MEB than for Control after 12 h, and was higher for  $\text{MgO}$  than for Control only at 24 h. In addition, BC was higher for sodium buffers ( $\text{NaHCO}_3$  and NSSC) than for MEB and  $\text{MgO}$ , and

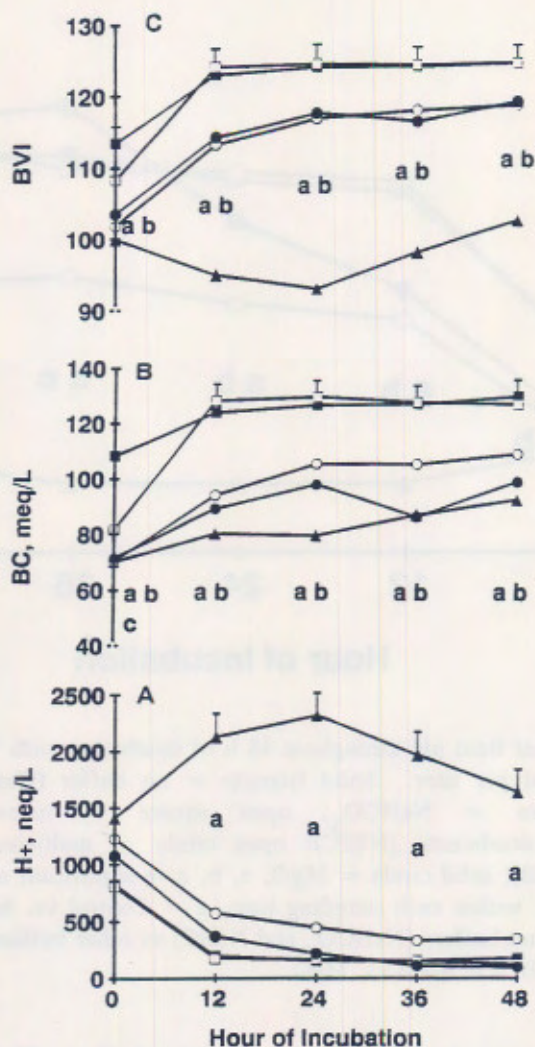


Figure 2. Ruminal fluid hydrogen ion concentration (panel A), buffering capacity (BC, panel B) and buffer value index (BVI, panel C) throughout 48 h of incubation with 7.1 g buffer added per liter. Solid triangle = no buffer (Control); solid square =  $\text{NaHCO}_3$ ; open square = natural sodium sesquicarbonate (NSSC); open circle = multielement buffer (MEB); solid circle =  $\text{MgO}$ ; a, b, c = significant effects ( $P < .05$ ): within each sampling time, a = Control vs. buffers, b = sodium buffers ( $\text{NaHCO}_3$  and NSSC) vs. other buffers (MEB and  $\text{MgO}$ ), c =  $\text{NaHCO}_3$  vs. NSSC.



was higher for  $\text{NaHCO}_3$  than NSSC at 0 h; after 12 h,  $\text{NaHCO}_3$  and NSSC had similar BC and both were superior to MEB and MgO. Buffering capacity for MEB and MgO were similar except for the 36-h sample, when the BC of MgO decreased inexplicably.

Throughout the 48-h incubation interval, BVI was higher for sodium buffers than for MEB and MgO (Figure 2C). The BVI for the four buffers increased until 12 h of incubation but subsequently was constant for the remaining 36 h. The BVI for the Control tended to decrease ( $P = .10$ ) between 0 and 24 h, but then it increased until 48 h. The sharp increase in BVI for the buffers at 12 h was a result of both a reduction in  $\text{H}^+$  and an increase in BC; BC increased more slowly for MEB and MgO than for  $\text{NaHCO}_3$  and NSSC. Again, these responses likely were attributable to more complete dissolution of the buffers at 12 h. The reduction in BVI for Control during the first 24 h of incubation appears to be related to fermentation-induced increases in  $\text{H}^+$ ; the subsequent increase in BVI, due to a reduction in  $\text{H}^+$ , has no available explanation.

At 0 h, only  $\text{NaHCO}_3$  and NSSC had higher BVI than Control; however, by 12 h, BVI was higher for all buffers than for Control. Ruminal fluid  $\text{H}^+$  was somewhat higher for MEB than MgO during the last 36 h of the incubation interval; however, BC also was higher for MEB. As a result, BVI for MEB and MgO were similar throughout the study. The BVI accounts for buffer-related alterations in both  $\text{H}^+$  and BC; hence, it provided a more accurate evaluation of the apparent ( $\text{H}^+$ ) and potential (BC) benefits of dietary buffers to the rumen (Tucker et al., 1992). In a short term (5 h) incubation comparing  $\text{NaHCO}_3$ , NSSC and MEB, Tucker et al. (1992) reported that BVI was higher for  $\text{NaHCO}_3$  than for NSSC or MEB. Although  $\text{NaHCO}_3$  and NSSC yielded similar initial pH values in that study, NSSC had a lower BC and, hence, a lower BVI. Ruminal fluid BC was similar for  $\text{NaHCO}_3$  and NSSC in the present study after 12 h of incubation, yet it was higher for  $\text{NaHCO}_3$  after only 5 h of incubation (Tucker et al., 1992); hence, NSSC appeared to release its buffering potential more slowly than  $\text{NaHCO}_3$  did. Of the four buffers that we tested,  $\text{NaHCO}_3$  had the fastest release rate, followed by NSSC, MEB and MgO.

Compared to the unbuffered control, all buffering compounds increased ruminal fluid BVI. However, BVI separated buffering compounds into two categories;  $\text{NaHCO}_3$  and NSSC at 7.1 g/L exhibited similar BVI which were markedly higher than the BVI for the second category of compounds, MEB and MgO. Whereas  $\text{NaHCO}_3$  and NSSC each sharply increased both ruminal fluid pH and BC, the BVI for MEB increased due to more moderate increases in pH and BC. The increase in BVI for MgO was due primarily to an increase in pH. Both  $\text{NaHCO}_3$  and NSSC released their full BC and acid neutralizing effects within 12 h of incubation; MEB and MgO reacted more slowly, plateauing at 24 h. Compared to MEB and MgO,  $\text{NaHCO}_3$  and NSSC rapidly

buffered ruminal fluid and should be beneficial in preventing postprandial increases in ruminal fluid  $H^+$ . Because of their slower release rates, MEB and MgO should stabilize ruminal acid-base status later but might be less efficacious due to passage out of the rumen.

### Literature Cited

- Tucker, W.B. et al. 1992. A buffer value index to evaluate effects of buffers on ruminal milieu in cows fed high or low concentrate, silage or hay diets. *J. Dairy Sci.* (in press)