BUFFERING ABILITY OF SEVERAL COMPOUNDS IN VITRO AND THE EFFECT OF A SELECTED BUFFER COMBINATION ON RUMINAL ACID PRODUCTION IN VIVO

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ABSTRACT

The buffering ability of several compounds was tested in vitro with rumen fluid from cattle fed roughage-concentrate or all-concentrate rations. The in vitro system developed to test buffers involved incubating 100 ml rumen fluid (after flushing with carbon dioxide) with 1% buffer and 5% ground extrusion-cooked corn 6 h at 39 C. This then was titrated with acid or base for buffering capacity. With rare exception, hydroxides and oxides were poor buffers alone or in combinations because their response often was erratic and usually caused excessive pH changes immediately after addition to rumen fluid. In proper combination, carbonates and bicarbonates were the most promising anions. Occasional benefits were derived from phosphates. Some buffer salts, rated fair or poor alone, balanced out each other's defects and combined into a good buffer.

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[1]Contribution no. 958-j, Department of Dairy and Poultry Science, and no. 290-j, Department of Statistics, Kansas Agricultural Experiment Station, Manhattan 66506

[2]This research was supported in part by a grant from Church and Dwight Co., Inc., New York NY 10001

Several buffer combinations were selected by computer for both all-concentrate and concentrate-roughage rations. One such combination for the concentrate ration consisted of bentonite, monobasic potassium phosphate, magnesium carbonate, magnesium oxide, and sodium carbonate combined in a 5:22:22:35:16 ratio. This combination without bentonite was fed as a supplement (.227 kg/head per day) to diary steers consuming an all-concentrate ration. Animals fed the buffer had a slightly higher rumen fluid pH, higher rumen acetate, lower propionate, and higher lactate concentration than did the controls.

INTRODUCTION

The need for buffers in ruminant nutrition has been established (3,4,6,9,19,11). A trend towards feeding high-grain, low-roughage rations has increased the interest in buffers, and a variety of buffer compounds is available at relatively low cost. Little attention has been given to the basic actions or longevity of buffers in the rumen. Our purpose was to develop a simple in vitro system to evaluate 35 buffer compounds. After we evaluated the compounds initially, we tested several combinations of them, then selected additional combinations by computer based on each's ability to meet certain specifications for good buffering action. One of the computer selections (for an all-concentrate ration) then was tested in cattle to observe its effect on ruminal acid production.

EXPERIMENTAL PROCEDURE

Experiment 1

Rumen fluid was obtained from the ventral sac of the rumen of two rumen-fistulated adult cows just before the morning feeding. The fluid

was strained immediately through two layers of cheesecloth. One cow was fed a roughage and concentrate (1:2.5) ration and the other an all-concentrate ration in an amount to satisfy maintenance requirements. The roughage was alfalfa hay; the concentrate was 62% cracked sorghum grain, 25% cracked wheat, 10% Starea,[3] 2% dicalcium phosphate, .5% trace-mineralized salt, and .5% vitamin A and D supplement (1,000,000 units of A and D per kg of supplement). An in vitro system then was used to evaluate 35 buffer compounds in rumen fluid from the roughage-concentrate fed animal and 29 compounds in rumen fluid from the all-concentrate fed animal. Control titrations of the rumen fluids (100 ml) were over a pH range of 3 to 11 with N HCl and N NaOH. Compounds then were added in quantities of 1 g to 100 ml of rumen fluid contained in 250 ml centrifuge bottles, and the resulting change in pH was recorded. Five grams ground extrusion cooked corn grain then were added, mixed, the system flushed with CO_2, stoppered with Bunsen valves, and incubated for 6 h at 39 C. After incubation the milliliters of N HCl or N NaOH required to lower or raise the pH of the fermented mixtures to 3 or 11 were determined. All tests were in quadruplicate.

Experiment 2

The procedures were similar to those in Experiment 1 except that 21 combinations of compounds (Table 2) were tested in rumen fluid from cattle fed the roughage-concentrate and all-concentrate rations. The combinations of compounds selected were based on those used previously in ruminant nutrition (6,11).

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[3]Starea (registered trademark 860255, U.S. patent 3,642,489) is an extrusion cooked mixture of grain and urea.

Experiment 3

Six combinations of buffer compounds were tested. Selection was based on the hypothesis that results from the experiments with single buffer compounds with a given ration could be used to predetermine activities of selected combinations. These six combinations were calculated and tested in rumen fluid from the cow fed the mixed roughage-concentrate ration.

An additional series of combinations of buffer compounds was tested. Selection was by computer (1) for each of the above two rations with the following stipulations: (roughage-concentrate ration) 1)[4] 5% bentonite and the best[5] of the other buffers; 2) 5% bentonite, 35% sodium bicarbonate, and the best of the others; 3) 5% bentonite, 20% magnesium oxide, and the best of the others; (all concentrate ration) 4) 5% bentonite, and the best combination of single buffers; 5) 5% bentonite, 25% calcium carbonate monohydrate, and the best of the others; 6) 5% bentonite, 20% magnesium oxide, and the best of the others.

Also stipulated for the roughage concentrate ration was: 7) best combination excluding oxides and hydroxides; 8) 35% sodium bicarbonate and the best of the others with stipulation 7 in effect; 9) 25% calcium carbonate monohydrate, and the best of the others with stipulation 7 in effect. Stipulations similar to 7,8, and 9 were made for the all-concentrate ration numbered 10,11,12.

Experiment 4

A buffer combination of bentonite, monobasic potassium phosphate,

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[4]Number of buffer combinations shown in Table 3.

[5]Best of the other buffers refers to single buffers (Table 1) that provide maximum buffering capacity with minimum initial pH change.

magnesium carbonate, magnesium oxide, and sodium carbonate (5:22:22:35:16) appeared to be a promising buffer when tested in vitro (Table 3). The buffer was classified "fair" because it increased initial pH more than 1 pH unit. However, because the buffer showed considerable buffering capacity in the desired pH range, it was tested in vivo with 12 Holstein steers (average weight 312 kg) divided into two groups of six each. The steers were fed ad libitum twice daily the all-concentrate ration described in Experiment 1. The buffer was pelleted and crumblized, with 5% molasses as a binder, and 60% ground corn as a carrier. Due to a palatability problem, molasses replaced bentonite as a binder.

RESULTS AND DISCUSSION

Experiment 1

The buffering capacities of the 35 compounds are in Table 1. Each was evaluated as a good, fair or poor buffer. The evaluation criteria were that the immediate action of the buffer should not change drastically rumen fluid pH (plus or minus 1 pH unit) as that could affect the microbial flora and the animal's physiological state, and that the buffer should exhibit buffering capacity after incubation in the pH range of 5 to 8 (considered suitable for rumen microorganisms 8).

It appeared that incubation is needed to evaluate buffers with low solubilities like bentonite, because they require considerable time to provide buffering action in an environment similar to the rumen. It is possible that they function only after their molecular complex is broken down to release Al_2O_2 and SiO_2 and the Na ion (if present). The fair buffers (Table 1) may be valuable in mixed buffer systems. However, used alone they are of doubtful value. The poor buffers also may have value in

TABLE 1. Buffer activities of 35 compounds tested in vitro (Experiment 1).

Compound	Acid or base required (from control) after incubation to reduce or increase pH to 3 or 11 (ml/l)				pH change (from control) before incubation		pH change (from control) after incubation		Rating[d]	
	HCl Ration[a]		NaOH Ration		Ration		Ration		Ration	
	1	2	1	2	1	2	1	2	1	2
$Al(OH)_2$	-30	-32	50	22	.1	0	-1.3	-.5	F	F
NH_4 acetate	-26	-16	52	114	0	.1	-1.1	-.9	F	F
NH_4 propionate	-30	-24	48	112	-.3	-.2	-1.2	-1.0	F	F
Bentonite[b]	14	-16	38	50	0	.1	-1.1	-.9	G	F
$CaCO_3$	14	-24	50	48	-.2	.1	-1.1	-.7	G	F
$Ca(OH)_2$	216	32	-94	10	5.1	.9	4.8	.2	P	G
$Ca(H_2PO_4)_2 \cdot H_2O$	-48	-56	98	94	-1.1	-.5	-1.5	-1.0	P	F
$CaHPO_4$	-46	-46	56	76	-.2	.1	-1.4	-1.0	F	F
$Ca_{10}(OH)_2(PO_4)_6$	-34	-44	36	70	-.2	.1	-1.3	-1.0	F	F
Dolomite[b]	-44	-38	48	70	.1	.2	1.3	-.9	F	F
$MgCO_3$	104	32	0	20	.5	.7	.2	-.3	G	G
MgO	290	224	-96	-100	2.6	2.6	2.8	3.7	P	P
$MgSO_4$	-34	-62	40	106	-.3	-.2	-1.1	-1.3	F	F
$MnSO_4$	-22	--	25	--	-.7	--	-.9	--	F	-
Microlite[c]	-36	-54	42	84	-.3	-.1	1.2	-1.1	F	F
$KHCO_3$	20	0	22	64	.5	1.1	-.4	-.5	G	F
K_2CO_3	88	0	0	54	2.7	2.1	.4	-.5	F	F
KH_2PO_4	-54	-52	126	132	-.4	-.1	-1.4	-1.7	P	F
K_2HPO_4	20	-34	54	130	.6	.9	-.4	-.9	G	F
K_3PO_4	52	-14	14	98	2.6	1.3	.1	-.7	F	P
Na acetate	0	-42	40	96	-.2	.2	-1.0	-.9	G	F
$NaHCO_3$	28	0	18	34	.2	1.0	-.5	-.5	G	G
$Na_2B_4O_7 \cdot 10H_2O$	48	-12	24	72	1.7	1.9	.4	-.7	F	F
Na_2CO_3	182	94	-28	0	2.7	3.2	1.6	.7	P	F
$Na_2CO_3 \cdot H_2O$	142	52	-34	32	2.7	3.1	1.8	.1	P	F
$Na_2CO_3 \cdot 10H_2O$	154	76	-30	12	2.8	3.1	1.7	.5	P	P
Na citrate	-14	-40	44	100	-.1	.4	-1.2	-1.0	F	F
Na EDTA	-10	--	42	--	-.4	--	-.6	--	F	-
NaOH	198	--	-158	--	5.7	--	5.1	--	P	-
Na lactate	-28	-80	42	70	0	.3	1.1	-.8	F	F
NaH_2PO_4	-30	--	114	--	-.5	--	-1.3	--	F	-
Na_2HPO_4	0	-10	82	102	.4	.7	-1.2	-.9	G	F
Na_3PO_4	-64	0	102	90	2.4	1.3	-1.5	-.7	P	F
Tris	48	--	22	--	1.8	--	.3	--	F	-
$ZnSO_4$	-30	--	26	--	-.6	--	-.7	--	P	-

[a]Ration numbers: 1 - roughage-concentrate; 2 - all-concentrate.

[b]Volclay (no. 200), Western Bentonite, American Colloid Co., Skokie, IL

[c]Super Supplement, Inc., 301 W. 11th, Kansas City, MO 64103.

[d]Rating letters: G - good; F - fair; P - poor.

mixed buffer systems, especially if they are needed as a source of macro-mineral nutrients.

From the data in Table 1 it appears that although a disproportionate number of Na buffers was used, Na is the best buffer, K is better than Ca, and Ca surpasses Mg, Al, NH_4, and Mn. The Zn salt tested showed little buffering ability.

The best buffer anions appear to be acetate, bicarbonate, carbonate, and dibasic phosphate. Borate, citrate, lactate, and propionate are fair buffer anions. Oxides, hydroxides, mono- and triphosphates, and sulfates are poor buffer anions. Sodium diethylene-diaminetetraacetate and Tris were fair buffers. The two calcite rock compounds, dolomite and micro-lite, were only fair buffers while the salt-clay mineral, bentonite, was a good buffer when allowed to solubilize in rumen fluid with extended contact.

When 29 of these buffer compounds were tested in rumen fluid from the cow fed an all-concentrate ration, three compounds were classified good, 23 fair, and three poor (Table 1). With a low initial pH of rumen fluid and 6 h of incubation, one of two things occurred. First, the buffer would raise the initial pH more than 1 pH unit, eliminating it from the good classification, or second, if the buffer did not increase the initial pH considerably, the pH, after incubation, would fall below 5, again excluding it from the good classification. Thus, a high percentage of buffers are classified as fair for an all-concentrate ration. Perhaps the requirement that compounds not raise initial pH more than 1 unit is too severe a restriction for compounds required to buffer rumen fluid with a low pH in animals fed all-concentrate rations.

Experiment 2

Responses to the 21 buffer combinations tested with these rations are in Table 2. Seven combinations were rated good, nine fair, and five poor. The poor buffer combinations contained either a hydroxide or an oxide. Oxides and hydroxides apparently should not be used to buffer mixed roughage-concentrate dairy rations. However, if carefully used, they may be of value in high-concentrate rations like those fed finishing cattle where rumen pH is below ca. 5.5 The safest oxide was magnesium oxide. Combinations of bicarbonates, carbonates, and phosphates in proper portions could provide desirable buffering action for both mixed roughage-concentrate and all-concentrate rations.

Experiment 3

All the hand-calculated buffer combinations (Table 3) were rated good. To justify the calculations and to validate the basis for the combinations, two poor buffers with different responses were tested. Monobasic calcium phosphate and sodium carbonate monohydrate were each tested at a concentration of 1%, and a 1:1 mixture of the two was tested at 1%. Rumen fluid was from the animal fed a mixed ration of roughage and concentrate. The combination proved to be a good buffer (Figure 1), substantiating the validity of the basis on which the combinations were made.

Changes from initial pH immediately after the buffer was added and 6 h after incubation are in Table 3. Correlations between actual and calculated change in pH before and after incubation were .84 ($P < .0001$) and .64 ($P < .002$). Such high correlations support the use of computer system analysis for indicating the best buffer combinations. Buffer combinations selected by the computer are shown in Table 3. Two of the buffer combin-

TABLE 2. Buffer activity of 21 combinations of compounds tested in vitro (Experiment 2).

Combination of compounds	Ratio	Acid or base required (from control) after incubation to reduce or increase pH to 3 or 11 (ml/l)				pH change (from control) before incubation		pH change (from control) after incubation		Rating[b]	
		HCl Ration[a]		NaOH Ration		Ration		Ration		Ration	
		1	2	1	2	1	2	1	2	1	2
$Al(OH)_2$, MgO, $Ca(OH)_2$	1:1:1	150	168	-70	-88	3.9	3.7	3.3	3.1	P	P
$CaCO_3$, $NaHCO_3$, K_2CO_3	2:2:1	0	-32	46	165	1.1	1.0	-.9	-.8	F	F
$CaCO_3$, K_2CO_3, $MgSO_4$	1:1:1	0	-20	44	158	.9	.7	-1.1	-.7	G	F
$Ca(H_2PO_4)_2$, Ca_2HPO_4	1:1	-16	-84	50	208	.3	.9	-1.1	-.8	F	F
McDougal's mineral buffer		-38	-130	102	266	-.5	-.1	-1.3	-1.3	F	F
$Ca(OH)_2$, $NaHCO_3$	2:1	16	112	18	-44	1.7	5.3	-.7	3.5	F	P
$CaCO_3$, $NaHCO_3$, $KHCO_3$, $MgCO_3$, K_2CO_3	1:1:1:1:1	18	-16	20	122	1.5	1.4	-.5	-.6	F	P
$KHCO_3$, $NaHCO_3$, MgO	1:1:1	192	178	-52	-50	2.7	3.4	2.5	3.3	P	P
$CaCO_3$, $NaHCO_3$, K_2CO_3	2:3:1	0	-28	36	144	.9	1.1	-.9	-.7	G	F
$KHCO_3$, $NaHCO_3$	1:1	30	-20	26	135	.7	1.1	-.5	-.7	G	F
$CaCO_3$, $NaHCO_3$	1:1	22	-22	36	148	.3	.9	-.7	-.6	G	G
$NaHCO_3$, $MgCO_3$	2:1	32	0	18	132	1.0	.9	-.3	-.5	G	P
$NaHCO_3$, MgO	1:1	212	232	-82	-96	2.8	3.9	2.4	4.2	P	P
$NaHCO_3$, MgO	2:1	210	182	-60	-64	2.3	3.1	2.1	2.7	P	P
$NaHCO_3$, $CaCO_3$	2:1	0	-30	34	142	.3	.9	-.9	-.7	G	F
$NaHCO_3$, Na_2HPO_4	1:1	12	-54	48	176	.4	.9	-.7	-.9	G	F
$NaHCO_3$, Na_2HPO_4	3:1	0	-48	30	160	.7	1.1	-.7	-.8	G	F
$NaHCO_3$, $MgSO_4$, $MnSO_4$, $ZnSO_4$	200:40:1:1	34	-54	12	144	.3	1.2	-.3	-.7	G	P
$NaHCO_3$, Bentonite	1:1	-14	-64	28	148	.3	.9	-1.1	-.8	F	F
$NaHCO_3$, K_2CO_3	2:1	24	-12	34	126	1.6	.3	-.5	-.5	F	P
$NaHCO_3$, $MgCO_3$	1:1	38	10	24	118	1.0	.9	-.3	-.5	G	G

[a]Ration numbers: 1 - roughage-concentrate; 2 - all-concentrate.

[b]Rating letters: G - good; F - fair; P - poor.

TABLE 3. Buffer activities of combinations of compounds tested in vitro (Experiment 3).

Combinations of Compounds	Ratio	Acid or base required (from control) after incubation to reduce or increase pH to 3 or 11 (ml/l)				pH change (from control) before incubation		pH change (from control) after incubation		Rating[b]
		HCl Ration[a]		NaOH Ration		Ration		Ration		
		1	2	1	2	1	2	1	2	
Hand calculated selections										
Na_2CO_3, NaH_2PO_4	1:1	68		36		1.0		-.2		G
$MgCO_3$, $Na_2B_4O_7 \cdot H_2O$, Na EDTA	1:1:1	56		16		1.0		-.1		G
$MgCO_3$, K_2CO_3, $Ca(H_2PO_4)_2 \cdot H_2O$	2:1:1	72		26		.2		-.3		G
Na_2CO_3, $ZnSO_4$, Na EDTA	1:1:1	18		20		.7		-.3		G
$NaHCO_3$, $Na_2B_4O_7 \cdot 10H_2O$, Na EDTA	1:1:1	34		28		.5		-.3		G
$MgCO_3$, $Na_2B_4O_7 \cdot 10H_2O$, Na EDTA, $ZnSO_4$	1:1:1:1	10		24		.4		-.4		G
Computer selections										
1) Bentonite, $Ca(H_2PO_4)_2 \cdot H_2O$, $MgCO_3$, MgO	5:42:9:44	128		-48		1.6		2.8		P
2) Bentonite, $Ca(H_2PO_4)_2 \cdot H_2O$, KH_2PO_4, MgO, $NaHCO_3$	5:18:6:35:35	166		-42		1.7		2.5		P
3) Bentonite, KH_2PO_4, $MgCO_3$, MgO, Na_2CO_3	5:14:57:20:3	0		36		1.5		-.7		F
4) Bentonite, KH_2PO_4, $MgCO_3$, MgO, $Na_2CO_3 \cdot H_2O$	5:22:22:35:16		56		42		1.4		.1	F
5) Bentonite, $Ca(H_2PO_4)_2 \cdot H_2O$, MgO, NH_4Pr, $Na_2CO_3 \cdot H_2O$	5:20:36:14:25		38		76		2.0		-.1	F
6) Bentonite, $MgCO_3$, MgO, Na_2CO_3, NH_4Ac	5:63:20:8:4		58		0		1.4		.3	F
7) KH_2PO_4, $MgCO_3$, Na_2CO_3	4:80:16	0		52		.4		-.9		G
8) $MgCO_3$, $NaHCO_3$, Na_2CO_3	47:35:18	10		42		.5		-.8		G
9) $Ca(H_2PO_4)_2 \cdot H_2O$, $MgCO_3$, $NaCO_3 \cdot H_2O$	12:62:25	0		58		-.1		-.9		G
10) Bentonite, $MgCO_3$, Na_2CO_3	77:4:19		-20		36		.2		-.6	F
11) No solution										
12) No solution										

[a]Ration numbers: 1 - roughage-concentrate; 2 - all-concentrate.

[b]Rating letters: G - good; F - fair; P - poor.

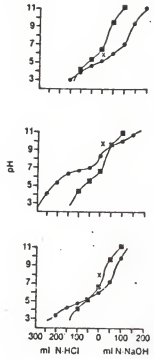

FIG. 1. Illustration of two compounds, monobasic calcium phosphate (top graph) and sodium carbonate monohydrate (middle graph) showing poor and different buffering responses when used singly but good responses (bottom graph) when used at a concentration of 1% in a 1:1 ratio (Experiment 5). No buffer, no incubation (—□—); buffer, incubated 6h (—○—); pH after addition of buffer, no incubation (X).

ations for the rumen fluid from the animal fed roughage-concentrate (numbered 1, 2, 3) rated poor and one rated fair. Combinations containing hydroxides and oxides rated low with the mixed roughage-concentrate ration, which confirms the results of Experiment 2. The buffer combinations numbered 7, 8, and 9 (roughage-concentrate rumen fluid) rated good, but they did not outperform some of the good single buffers (Table 1).

When rumen fluid with a low pH from the concentrate-fed animal was tested, buffer combinations numbered 4, 5, and 6 all rated fair, an unexpected response. Only one buffer (number 10) of the series numbered 10, 11, and 12 was selected, and this combination rated fair. Hydroxides or oxides should be included in the combinations when rumen fluid with low pH from animals fed low-concentrate is used. If hydroxides and oxides are excluded, the computer fails to compute all combinations or the buffer combinations have a low ranking. The data reveal that for all-concentrate rations, buffer combinations ranked fair would rank as good buffers if the stipulation were removed that buffers not raise initial pH more than 1 unit. An initial change of 1 pH unit probably would not be deleterious to an animal or its rumen microflora, but selected buffer combinations must be tested in cattle to determine that.

Experiment 4

The in vitro titration of the buffer supplement is in Figure 2. The buffer showed considerable buffering capacity in the desired pH range (4 to 6) for cattle fed all-concentrate rations.

When the buffer supplement was fed, feed consumption was decreased significantly ($P < .05$), and on occasion the animals went off feed completely (data not shown). The buffer-supplemented ration tended to produce less

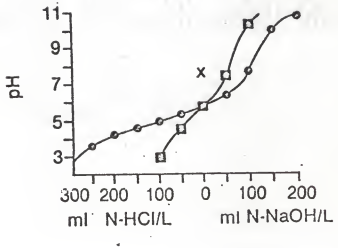

FIG. 2. Acid-base titration curves of a mixture of KH_2PO_4, $MgCO_3$, MgO, and Na_2CO_3(22:22:35:16) used to buffer fluid from a cow fed an all-concentrate ration (Experiment 3). No buffer, no incubation (—□—); buffer, incubated 6 h (—O—); pH after addition of buffer, no incubation (X).

efficient weight gain than did the control ration; however, this difference was not significant. Persistent diarrhea occurred in animals fed the buffer supplement. This was due to the large amount of magnesium in the buffer. Large amounts of magnesium oxide cause severe diarrhea (5). Bloat was in both groups. It was more difficult to obtain rumen fluid with a stomach pump from the animals consuming the buffer supplement than from those not consuming the supplement. This is probably due to a disturbance in water and electrolyte balance as evidenced by diarrhea.

The buffer supplement increased the proportion of rumen acetate ($P < .01$) and decreased the proportion of propionate ($P < .05$) (Table 4). The buffer increased rumen pH, though not significantly. The buffer appeared not to affect total VFA concentration but to increase ($P < .05$) lactic acid concentration. Apparently the buffer favored the growth of lactic acid producing but not lactic acid utilizing microorganisms.

The selection of buffers and buffer combinations by an in vitro system has merit. Much can be learned about buffer action in vitro. However, it should not be inferred that the in vitro system can predict the response in vivo. At best it can be used as an indicator of potential buffers for in vivo use. Using systems analysis to select buffer combinations also has merit; however, more information is needed on the effects of specific buffer compounds on animal well-being. Buffer actions and physiological effects need to be collated before buffer responses can be optimized. Our study has provided information on buffer action and methods for obtaining a solution to the problem.

TABLE 4. Effect of a buffer combination (KH_2PO_4, $MgCO_3$, MgO and Na_2CO_3) on ruminal acid production in Holstein steers (Experiment 4).

Period	Group	Treatment	pH	Volatile Fatty Acids						Total conc.	Lactic Acid
				Acetate	Propionate	Butyrate	Isobutyrate	Valerate	Isovalerate		
				(molar %)						µm/l	µg/l
1	1	Buffer	5.7	47.2	29.7	12.0	1.8	3.8	5.1	195.7	82.8
	2	No Buffer	5.6	41.5	34.8	12.7	1.4	5.0	4.6	275.2	48.5
2	1	No Buffer	5.5	39.3	34.6	12.9	1.9	5.4	5.7	186.3	72.8
	2	Buffer	5.6	41.8	30.8	13.1	2.2	5.0	6.9	201.1	91.8
Average of Periods 1 & 2		Buffer	5.7	44.5[c]	30.2[a]	12.6	2.0	4.4	6.0	198.4	87.3[a]
		No Buffer	5.6	40.4[d]	34.7[b]	12.8	1.6	5.2	5.2	230.8	60.6[b]

a,b Values within columns showing unlike superscripts differ significantly ($P<.05$).

c,d Values within columns showing unlike superscripts differ significantly ($P<.01$).

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BUFFERING ABILITY OF SEVERAL COMPOUNDS IN VITRO AND THE EFFECT OF A SELECTED BUFFER COMBINATION ON RUMINAL ACID PRODUCTION IN VIVO

by

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ABSTRACT

The buffering ability of several compounds was tested in vitro with rumen fluid from cattle fed roughage-concentrate or all-concentrate rations. A control titration of the rumen fluid (100 ml) over a pH range of 3 to 11 with N HCl and N NaOH was made. Compounds then were added in quantities of 1 g to 100 ml of rumen fluid contained in 250 ml centrifuge bottles. The resulting change in pH was recorded. Five grams ground extrusion cooked corn grain were added, mixed, the system flushed with CO_2, stoppered with Bunsen valves, and incubated for 6 h at 39 C. After incubation the fermented mixtures were titrated over a pH range of 3 to 11 with N NCl and N NaOH. Each compound was evaluated as a good, fair, or poor buffer. The evaluation criteria were that the immediate action of the buffer should not change drastically rumen fluid pH (plus or minus 1 pH unit), and that the buffer should exhibit buffering capacity after incubation in the pH range of 5 to 8 (considered suitable for rumen microorganisms).

With rare exception, hydroxides and oxides were poor buffers alone or in combination because their response often was erratic and usually caused excessive pH changes immediately after being added to rumen fluid. In proper combination, carbonates and bicarbonates were the most promising anions tested. Occasional benefits were derived from phosphates. Though a disproportionate number of Na buffers were used, Na appeared to be a better buffer cation than K, which in turn was better than Ca, which was better than Mg, Al, NH_4, and Mn. The Zn salt showed little buffering ability.

Two poor buffers with different buffering responses when used singly

could combine (1:1) to form a good buffer combination. This led to the hypothesis that results from the experiments with single buffer compounds with a given ration could be used to predetermine activities of selected combinations. Correlations between actual buffer responses and calculated responses supported the use of computer systems analysis for indicating the best buffer combinations. Several buffer combinations were selected by computer for both all-concentrate and concentrate-roughage rations. One such combination for the concentrate ration consisted of bentonite, monobasic potassium phosphate, magnesium carbonate, magnesium oxide and sodium carbonate combined in a 5:22:22:35:16 ratio. This combination appeared to be a promising buffer when tested in vitro and was fed as a supplement (.227 kg/head/day) to dairy steers consuming an all-concentrate ration ad libitum. Twelve Holstein steers in two groups of six were used in a cross-over design. Ruminal acid production and animal performance were observed. The general appearance of the animals consuming the buffer supplement was inferior to that of the controls. Severe diarrhea occurred in the buffered animals. This was probably due to a disturbance in the water and electrolyte balance resulting from the high magnesium content of the supplement. A significant ($P < .05$) decrease in feed intake was observed in the buffered animals. There was also a tendency for the supplemented animals to have less efficient weight gain than the controls but this was not significant. Buffered animals had a slightly higher ruminal fluid pH, significantly ($P < .01$) higher rumen acetate, significantly ($P < .05$) lower propionate and significantly ($P < .05$) higher lactate concentration than the controls.