BATCH CULTURE PROCEDURES FOR EVALUATING RUMINAL BUFFERS

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Story in Brief

Characteristics of several buffer and substrate combinations were evaluated. Ruminal fluid was collected from 3 cows fed three different diets. These diets contained concentrate and sorghum silage in three ratios: 70:30, 60:40, and 50:50 (dry matter basis). Ruminal fluid was incubated in a shaking water bath with either purified corn starch, the grain mix consumed by the cow providing the ruminal fluid for incubation, or the total mixed diet consumed by the cow providing the ruminal fluid for incubation. In addition, incubation flasks (75 ml of ruminal fluid) received either .5 g of a 2:1 mixture of $NaHCO_3$ and MgO, or no buffer. A flask representing each substrate and buffer combination was removed every hour for 5 hours and analyzed for pH, buffering capacity, and buffer value index. Based upon alterations in ruminal fluid acid-base status during incubation, a mixture of 75 ml of ruminal fluid from a cow consuming a 60:40 grain to forage diet, .5 g of the total mixed diet being fed to the cow serving as the source of ruminal fluid, and .5 g of the buffer being tested provided the most acceptable model for evaluating rapidly the release rates of ruminal buffers.

(Key Words: In Vitro, Buffers, Ruminal Fluid, Substrate.)

Introduction

Several laboratory procedures have been used to imitate the changes that occur in the rumen after a meal. These include, in order of decreasing complexity, continuous culture, semicontinuous culture and batch culture approaches. In batch culture, fermentation endproducts are not removed; hence, it does not imitate precisely the dynamics of digestion and absorption that occur in the rumen. However, this approach does imitate the increased

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acidity and accumulation of fermentation endproducts that occur postfeeding in the rumen; hence, the batch culture procedure should provide a rapid, reliable procedure for evaluating release rates of ruminal buffers. Our objective was to determine the optimum combination of ruminal fluid, substrate and test buffer to be used in batch culture.

Materials and Methods

Three ruminally-cannulated Holstein cows were used in a 3x3 Latin square study with 3-wk experimental periods to provide ruminal fluid to evaluate in vitro systems designed to screen rapidly the release rates of ruminal buffers. Three different total mixed diets (Table 1) were fed to the donor cows. These contained concentrate plus sorghum silage (70:30, 60:40, or 50:50 ratio, dry matter basis) and were fed twice daily (0550 and 1750).

Beginning three days before the end of each experimental period, ruminal fluid was collected from one cow each day. Fermentation treatments were added in a 2x4 factorial arrangement. These factors were the amount of buffer added (0 or .5 g of a 2:1 mixture of $NaHCO_3$ and MgO) and the type of substrate added to the fluid (no substrate, .5 g purified corn starch, .5 g of the grain mix consumed by the cow from which ruminal fluid was collected, or .5 g of the total mixed diet consumed by the cow from which ruminal fluid was collected).

Seventy-five milliliters of ruminal fluid was dispensed into each of six, 125-ml erlenmeyer flasks; each of these flasks contained the same substrate and buffer combination. One of the six flasks was analyzed immediately for pH and buffering capacity; the remaining flasks were incubated in a shaking water bath at 39 °C. An incubation was begun every 7.5 min until each of the eight buffer and substrate combinations was added to the water baths. A flask representing each substrate and buffer combination was removed each 60 min for 5 h after initiation of incubation to measure pH, buffering capacity (BC) and buffer value index (BVI).

Results and Discussion

Although the difference between diets fed to the cow when averaged across the incubation interval was nonsignificant, the diet by incubation interval interaction was an important source of variation for ruminal fluid hydrogen ion (H^+) and buffer value index (Table 2). Acid content of the ruminal fluid increased during incubation, presumably in response to the fermentation of substrate. Ruminal fluid acidity increased sharply for fluid from cows consuming a 70:30 grain to forage ratio; temporal changes for the

Table 1. Ingredient and nutrient composition (%) of experimental diets containing 50:50, 60:40 or 70:30 grain to forage ratios (DM basis).

Ingredient	Diet 1 50:50	Diet 2 60:40	Diet 3 70:30
Forage sorghum, silage	50.01	40.03	30.02
Shelled corn, ground	23.44	34.45	44.66
Soybean meal, 44% CP	23.95	22.87	22.58
Limestone	.75	.91	1.06
Dicalcium phosphate	.91	.88	.82
Dynamate	.39	.36	.41
Trace mineralized salt	.53	.49	.43
Vitamin A premix	.01	.01	.01
Vitamin E premix	.01	.01	.01
Nutrient			
DM	38.8	40.9	48.0
CP	14.6	16.0	16.5
NE_L, Mcal/Kg	1.43	1.58	1.65
Ca	.59	.73	.75
P	.32	.46	.47
S	.26	.28	.30
K	1.31	1.27	1.22
Na	.20	.20	.20

Table 2. Mean squares for indicators of ruminal fluid acid-base status.

Source[a]		pH		H^+, neq/L		BC, meq/L		BVI	
Main plots	df	MS[b]	*P*	MS	*P*	MS	*P*	MS	*P*
Cow	2	3.770	.214	15,647,487	.399	3,747	.462	1,097	.368
Period	2	.678	.603	5,689,399	.646	1,156	.736	507	.557
Diet	2	3.392	.233	23,217,292	.309	183	.946	2,359	.213
Cow by diet	2	1.028		10,401,435		3,217		638	
Subplots									
Buffer	1	66.550	<.001	238,370,713	<.001	96,128	<.001	46,380	<.001
Substrate	3	.622	<.001	2,849,087	<.001	205	.014	278	<.001
Incubation interval	5	.684	<.001	6,760,557	<.001	73	.273	601	<.001
Buffer by substrate	3	.014	.333	1,465,463	<.001	108	.130	106	<.001
Buffer by incubation interval	5	.211	<.001	4,504,726	<.001	265	<.001	333	<.001
Substrate by incubation interval	15	.038	<.001	168,895	.577	40	.787	19	.391
Diet by substrate	6	.038	.005	238,085	.278	20	.909	25	.222
Diet by incubation interval	10	.017	.159	389,430	.028	64	.343	44	.008
Diet by buffer	2	.119	<.001	13,075,054	<.001	652	<.001	996	<.001
Residual	373	.012		189,982		57		18	

[a]Main plot variables (cow, period and diet) were tested against cow by diet interaction; all other variables were tested against residual error.
[b]Type I mean squares.

50:50 and 60:40 diets were more moderate. The diet by incubation interval interaction was not significant (NS) for ruminal fluid BC (Table 2). Because buffering capacity was similar for fluid from the three diets, changes in ruminal fluid buffer value index (Table 2) were dictated primarily by changes in ruminal fluid H^+; buffer value index dropped more sharply with incubation for the 70:30 diet than for fluid from the 60:40 and 50:50 diets.

Our intent was to characterize the rapidity of fermentation-induced changes in ruminal fluid acid-base status occurring in batch culture for ruminal fluid from low, intermediate and high-grain diets. This information is essential to the selection of appropriate proportions of grain and forage to feed to cows used as sources of ruminal fluid for in vitro evaluation of the release rates of buffers. Dietary buffers have been more beneficial for cows consuming diets with high than with low grain content (Erdman, 1988); ideally, the acidity and buffering capacity of incubated fluid should follow patterns occurring in cows consuming high grain diets. However, the fluid also should have a low enough gas content to allow accurate measurement of fluid volume in the laboratory. In the present study, ruminal fluid from the 70:30 diet clearly yielded the sharpest increase in ruminal fluid acidity upon incubation; however, the high gas content of this fluid prior to incubation caused excessive foaming. When using a graduated cylinder to measure 75 ml of fluid for filling the batch culture flask, this foam interfered with accurate measurement of the fluid. This problem did not occur for fluid from the 60:40 or 50:50 diets. Although not apparent in our study, fluid from 60:40 diets should yield a higher acidity than that for 50:50 diets. Hence, of the diets tested in our study, the 60:40 grain to forage ratio should provide the most acceptable combination of handling and fermentation characteristics for evaluating release rates of buffers in batch culture.

Addition of .5 g of buffer to our batch culture flasks dramatically affected all measures of ruminal fluid acid-base status (Table 2). However, several buffer interactions were evident. Buffer by incubation interval interaction was identifiable for all measures of acid-base status. Ruminal fluid acidity increased sharply during incubation for unbuffered flasks, but was lower and fairly stable when buffer was added. Ruminal fluid acidity increased consistently from 0 to 5 h of incubation in the unbuffered flasks. Ruminal fluid buffering capacity was quite stable for buffered flasks, but it tended to increase during incubation for unbuffered flasks. Ruminal fluid buffer value index decreased by 13 units for unbuffered flasks during incubation, but changed by only 2 units for buffer addition. All indicators of acid-base status were remarkably constant during incubation for buffered flasks.

Acidity after 5 h of incubation was highest for ruminal fluid incubated with total mixed diet, followed by grain, starch and no substrate. Ruminal fluid buffering capacity was approximately 2.5 meq/L lower for starch than for the other substrates. No explanation for this is apparent, but the numerical

difference was so small that ruminal fluid buffer value index was dictated primarily by differences in H^+ for the four substrates; buffer value index was highest for no substrate, followed by starch, grain and total mixed diet.

Although substrate was a significant source of variation for each measure of acid-base status, the buffer by substrate interaction also was significant for H^+ and buffer value index (Table 2). For the unbuffered flasks, ruminal fluid acidity decreased as the similarity of the substrate to the source diet decreased; i.e., acidity was highest for total mixed diet, followed by grain, starch and no substrate.

In summary, .5 g of purified corn starch, .5 g of the grain mix consumed by the cow providing ruminal fluid for incubation, or .5 g of the total mixed diet each yielded temporal alterations in ruminal fluid acid-base status that were similar to those observed previously in vivo. However, because ruminal fluid acidity tended to develop more rapidly when the total mixed diet was used as a substrate, we recommend that this substrate should be used in batch culture systems to evaluate release rates of ruminal buffers. Based upon the combination of handling characteristics and temporal acid generation, we suggest that donor cows should be fed a 60:40 grain to forage ratio to provide ruminal fluid for batch culture evaluations of release rates of ruminal buffers.

Literature Cited

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