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Effect of a low-moisture buffer block on ruminal pH in lactating dairy cattle induced with subacute ruminal acidosis

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ABSTRACT

The objective of this study was to evaluate the effect of a low-moisture buffer block on ruminal pH and milk production in cows induced with subacute ruminal acidosis (SARA). Sixteen ruminally cannulated cows were randomly assigned to treatment (access to buffer blocks) or control (no buffer blocks). Ruminal pH was recorded each minute; dry matter intake (DMI), milk yield, and milk composition were measured daily. The experiment lasted 12 d and consisted of a 3-d pre-SARA period (without access to buffer blocks; d 1 to 3), after which 8 cows were given access to buffer blocks and 8 cows continued without access to buffer blocks. The next 4 d (d 4 to 7) were for evaluating the response to buffer blocks. On d 8, cows were restricted to 50% of previous DMI, and on d 9 SARA was induced (addition of 4 kg of wheat/barley pellet to pre-SARA total mixed ration (TMR). Cows were then monitored for a 3-d recovery period (d 10 to 12). The SARA challenge was successful in decreasing mean ruminal pH and time and area below pH 5.6. Intake of buffer blocks averaged 0.33 kg of DM/cow per day and was greatest on d 4 and d 8. Total DMI (TMR plus buffer block) and yields of milk and milk components were not affected by treatment. Although there was no overall effect of treatment on any of the ruminal pH variables measured, there were significant treatment by period interactions for several ruminal pH variables. Cows on the control treatment tended to experience a greater decrease in mean ruminal pH when induced with SARA than cows with access to buffer blocks (−0.55 vs. −0.20 pH units). Cows on the control treatment also experienced a greater increase in time (9.7 vs. 4.1 h/d) and area (249 vs. 83 min × pH units/d) below pH 5.6 compared with cows with access to buffer blocks. Ruminal volatile fatty acids, lactate, ethanol, and succinate concentrations during the SARA challenge did not differ between treatments. Eating behavior was not affected by treatment. Size of the first meal of the day was greater on the SARA challenge day than during the pre-SARA period (11.0 vs. 5.7 kg, as fed). Giving cows access to a buffer-containing molasses block may reduce the duration and the severity of a 1-d SARA challenge.
Key words: buffer block, subacute ruminal acidosis

INTRODUCTION

Subacute ruminal acidosis (**SARA**) is defined as periods of moderately depressed ruminal pH (between 5.2 and 5.6) that are between acute and chronic in duration (Cooper and Klopfenstein, 1996). The most consistent and immediate clinical sign of SARA is depressed feed intake, evidently because excess organic acids disrupt rumen function (Cooper et al., 1995, 1996) and cause malaise (Provenza et al., 1994). Periods of low ruminal pH impair the function of the fibrolytic microflora (Shi and Weimer, 1992) and decrease ruminal fiber digestion in vivo (Krajcarski-Hunt et al., 2002). These factors combine to reduce milk yield and hence, profitability for dairy producers.

Subacute ruminal acidosis may be caused by feeding excess NFC, a rapid increase in dietary content of NFC, or insufficient ruminal buffering (NRC, 2001). Dietary buffers, particularly sodium bicarbonate (**SB**), have been added to dairy cattle diets in an attempt to meet this shortage in ruminal buffers and decrease the incidence of SARA. Buffers can either be force-fed to cattle (i.e., added directly to the cow's mixed ration or grain mix) or offered free choice. Force feeding of buffers may result in greater expense than necessary, because not all cows require the same extent of dietary buffering. Free-choice feeding of buffers could be economically efficient, because in theory it allows cows to consume buffers only as needed. This requires that the cow has the "nutritional wisdom" to consume buffers in proportion to her need for dietary buffering. However, Cottee et al. (2004) reported that cows, when subjected to SARA, showed no difference in preference of an SB-supplemented water source to unsupplemented water.

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During this SARA challenge, cows experienced a significant decrease in mean ruminal pH of 0.28 pH units and significant increases in time and area below ruminal pH 5.6. Similarly, Keunen et al. (2003) found no preference for free-choice SB in cows induced with SARA. These studies indicate that cows do not attempt to correct an imbalance in rumen environment by increasing their intake of SB. Because undiluted SB may be unpalatable, cows with low ruminal pH may not consume enough free-choice SB to elicit a positive reinforcement. When SB was included in a pelleted, high-energy density feed, Cooper et al. (1996) found that feed intake of sheep increased. In addition, Phy and Provenza (1998) found that after feeding rolled barley, lambs preferred pellets with SB (2% as-fed basis) to pellets with NaCl. These findings suggest that incorporating SB into a highly palatable free-choice supplement might increase intake of SB during a bout of SARA.

Sodium bicarbonate can be incorporated into a low-moisture molasses block. Mixing SB with molasses has the advantage of masking the flavor of bicarbonate and encouraging free-choice consumption. Furthermore, intake of a low-moisture block is only possible by licking the surface area, which will stimulate saliva production and thereby the production of endogenous buffers. The objective of this experiment was to evaluate the effect of a free-choice, low-moisture buffer block on ruminal pH and milk production in cows challenged with a bout of SARA.

MATERIALS AND METHODS

Cows and Diets

Sixteen ruminally cannulated cows in 2 groups of 8 cows each were utilized in this study. The first group of cows were all in their first lactation and averaged 171 ± 68 DIM (mean ± SD) and 538 ± 48 kg of BW (mean ± SD) at the start of the experiment (June 2002). The second group (April 2004) were all multiparous cows. Three cows were in their second lactation, 3 in their third, and the remaining 2 were in their fourth and fifth lactations, respectively. These cows averaged 249 ± 113 DIM (mean ± SD) at the start of the experiment in 2004 and weighed 665 ± 18 kg of BW (mean ± SD). Cows were paired by stage of lactation (≤150 DIM and >150 DIM) within group. Treatments were assigned randomly within pair. Cows assigned to the control treatment averaged 195 ± 92 DIM (mean ± SD) and 602 ± 77 kg of BW (mean ± SD). Cows assigned to the buffer block treatment averaged 208 ± 107 DIM (mean ± SD) and 600 ± 77 kg of BW (mean ± SD). The study was conducted at the US Dairy Forage Research Center (Prairie du Sac, WI). It was approved and overseen by the University of Wisconsin-Madison Research Animal Resource Center and School of Veterinary Medicine Animal Care and Use Committee. Cows were housed in individual tie stalls and were managed according to standard protocols at the US Dairy Forage Research Center. Cows past the ninth week of lactation were injected once every 14 d with recombinant bST (Posilac, Monsanto, St. Louis, MO). Cows in the first group were fed their TMR once daily at approximately 0730 h and milked twice daily in a milking parlor at approximately 0530 and 1730 h. Cows in the second group were fed at 0600 h and milked at 0430 and 1530 h.

Cows were fed the experimental diets for a minimum of 1 wk before the start of the experiment. The schedule for each group consisted of a 3-d initial pre-SARA period (d 1 to 3, without buffer blocks available), a 4-d period to evaluate the response to the buffer blocks (Buffer-lyx, Ridley Block Operations, Mankato, MN; d 4 to 7, with buffer blocks available to the cows assigned to the buffer block treatment for the remainder of the experiment), 1 d of 50% feed restriction (d 8), 1 d of induced SARA (d 9), and a 3-d recovery period (d 10 to 12). On d 8 for each group, intake was reduced to 50% of each cow's average DMI for d 1 through 7. The SARA induction diet (basal TMR plus 3.5 kg of wheat/barley pellet on a DM basis) was then offered ad libitum on d 9. The wheat/barley pellet was mixed with the TMR by hand before feeding. This SARA induction protocol has proven to successfully induce SARA in lactating dairy cows (Krause and Oetzel, 2005).

Physical exams (rectal temperature, respiratory and heart rate, general appearance, and visual fecal scores) were conducted daily and every 4 h during the challenge day. Temperature was considered elevated if >39.4°C, heart rate elevated if >100 beats per minute, respiratory rate abnormal if >40 breaths per minute, and fecal score abnormal if <2 (Ireland-Perry and Stallings, 1993).

Dry matter contents of corn silage, alfalfa silage, and high-moisture corn were determined 3 times weekly by drying in a forced air oven at 60°C for 48 h, and diets were adjusted accordingly. Samples of TMR were also collected 3 times weekly and frozen for later analysis. Cows were fed for ad libitum intake (110%). Composition of the basal TMR for each group is presented in Table 1.

According to the manufacturer, the buffer blocks used in this study were a proprietary formulation comprising approximately 55% molasses, 40% sodium bicarbonate and alkalinizers, 5% hydrolyzed vegetable oil (DM basis), and low levels of minerals and vitamins. The buffer blocks had a DM content of 96%. Buffer blocks were manufactured to the same specifications for both groups of cows (in 2002 and 2004).

Table 1. Feed ingredients and nutrient composition of the basal TMR in first-lactation (group 1) and second- or greater lactation (group 2) cows

Ingredient or nutrient, % of DM	Basal TMR	
	Group1	Group 2
Corn silage	27.0	25.6
Alfalfa silage	22.1	28.2
High-moisture shelled corn	26.3	23.6
SoyPLUS[1]	10.1	9.2
Whole cottonseed (with lint)	8.3	7.5
Wheat/barley pellet	3.2	3.0
Limestone	1.4	1.3
Energy Booster 100[2]	0.56	0.55
Salt, plain white	0.38	0.37
Bloodmeal	0.33	0.34
Dynamate[3]	0.16	0.16
Vitamin/trace mineral mix	0.09	0.09
Magnesium oxide	0.09	0.09
DM, % as fed	57.2	55.7
NE_L, Mcal/kg of DM	1.72	1.68
NDF, % of DM	29.1	29.1
ADF, % of DM	19.0	22.1
NFC,[4] % of DM	40.9	43.7
CP, % of DM	17.5	17.0
Ether extract, % of DM	5.3	5.3
Ash, % of DM	7.21	4.9

[1]SoyPLUS, West Central Cooperative, Ralston, IA.

[2]Energy Booster 100, MSC Specialty Nutrition, Dundee, IL.

[3]Dynamate, The Mosaic Co., Plymouth, MN.

[4]NFC were calculated as 100% − (CP % + ether extract % + NDF % + ash %), all on a DM basis.

Milk weights were recorded and milk samples were collected daily at each milking. Milk components were determined by AgSource (Menomonie, WI) using a near infrared reflectance spectroscopy analyzer (MilkoScan 605, Foss Electric, Hillerød, Denmark).

Feed Analysis

Composite samples of the basal TMR were dried for 48 h at 60°C to determine DM content and then ground to pass a 2-mm screen (Wiley mill, Arthur H. Thomas, Philadelphia, PA). Samples were analyzed for nutrient content by Cumberland Valley Analytical Services (Maugansville, MD). Organic matter was determined according to AOAC (2000), but modified to use a sample weight of 0.5 g and a furnace temperature of 535°C. Neutral detergent fiber was measured using the procedure of Goering and Van Soest (1970), whereas ADF was analyzed according to AOAC (2000), with both procedures modified to use Whatman 934-AH glass microfiber filters with 1.5-μm particle retention during the filtering process. Crude protein was determined using a nitrogen combustion analyzer (Leco FP-528, Leco, St. Joseph, MI) as described by AOAC (2000). Ether extract was determined using a Tecator Soxtec System HT 1043 Extraction unit (Tecator, Foss, Eden Prairie, MN) as described by AOAC (1990), but with the following modifications: samples were extracted in petroleum ether, boiled 20 min, and rinsed 20 min.

Ruminal pH and VFA Concentrations

Ruminal pH was measured continuously during the experiment using an indwelling pH electrode (Epoxy body sealed combination pH electrode, no. 970061, Sensorex, Garden Grove, CA) placed in the ventral sac of the rumen. A 1-kg weight was attached to the electrode to prevent it from shifting in the rumen. Ruminal pH was measured continuously and recorded as 1-min averages and downloaded to a computer using the program LabTech Notebook 7.5 (LabTech, Andover, MA). Data acquisition was interrupted twice daily for approximately 1 h at the time of milking. Electrodes were calibrated twice weekly using pH 4 and 7 standard buffers. Ruminal pH data were summarized by calculating average pH, time below pH 5.6, and area below pH 5.6 for each 24-h period. Daily nadir pH and time to nadir after feeding was identified using −15 min/+15 min rolling averages of ruminal pH values to eliminate false nadirs caused by very-short-duration irregularities in ruminal pH data.

During the SARA induction period (d 9), ruminal fluid was collected every 30 min by aspiration through a stainless steel strainer located next to the ruminal pH electrode. Ruminal fluid samples were then strained through 2 layers of cheesecloth and acidified to 1.0% sulfuric acid (0.2 mL of 50% sulfuric acid added to 10 mL of strained fluid) before freezing at −20°C. Ruminal fluid was analyzed for acetate, propionate, butyrate, 2-3 butanediol, lactate (D plus L isomers), succinate, formate, and ethanol concentrations by HPLC (Shimadzu class-VP, version 5.03, Shimadzu Scientific Instruments Inc., Columbia, MD) as described by Siegried and Stumpf (1984).

Feed and Buffer Block Intake

Wooden feed mangers designed to hold a full day's amount of TMR were suspended in front of each cow. Hanging electronic load cells were used to measure daily TMR intake (S-beam hanging load cells, model number LC101-500, Omega Engineering, Stamford, CT). Feed manger weights were transmitted from load cells to a second data acquisition system separate from the ruminal pH data acquisition system (LabTech Notebook 7.5, LabTech). Feed manger weights were measured continuously and recorded as 1-min averages. Load cells were calibrated at the start of each group using 3 known weights representing the range of expected TMR weights. For cows on the buffer block treatment,

reported daily DMI consists of buffer block and TMR intakes.

The buffer blocks provided by the manufacturer were broken into pieces using a hammer and melted at 60°C into rectangular metal pans 15 cm wide, 23 cm long, and 6 cm deep. The blocks were allowed to reharden after removal from the oven. The metal pans containing the blocks were mounted on platform load cells (single-point load cells, model number LCAE-15KG, Omega Engineering). Weights from the load cells were sent to the computers with the same data acquisition system as for the feed manger weight data. The entire buffer block assembly was protected inside a 3-sided wooden box placed next to the feed manger in front of cows assigned to the buffer block treatment. Platform load cells were calibrated at the start of each group using 3 known weights representing the range of expected buffer block weights. Buffer blocks were replaced when between one-half and two-thirds of the block was consumed.

Dry matter intake was evaluated by tracking the weight change of hanging mangers. Daily weight changes of the mangers were plotted and short-duration weight changes or weight increases were removed. It was assumed that eating activity took place when manger weights were decreasing. A meal was defined as a decrease of at least 0.5 kg in the weight of the manger occurring after at least 5 min without any change in manger weight. Number of daily meals and length of meals were recorded.

Weights of the buffer blocks consumed by the cows assigned to the buffer block treatment were also measured continuously and recorded once per minute using the second data acquisition system. Buffer blocks were cleaned once daily before weighing to remove straw, grain, and excessive moisture. Buffer blocks were consumed gradually throughout the day and no meals of the buffer blocks could be discerned. The recorded weight changes of the buffer blocks were only used to calculate daily intake (start weight minus end weight).

Before the start of the study, all cows were placed on the experimental diet and given access to the buffer blocks for a minimum of 3 d to avoid first-time exposure to the buffer blocks during the actual experiment. Buffer block intakes were recorded and analyzed after cows were randomly assigned to either the control or buffer block group. Block intakes during the pretrial period were not significantly different between treatment groups following treatment assignments at the start of the trial (data not shown).

Statistical Analysis

Response variables measured daily were evaluated using the mixed model procedure of SAS (SAS Institute, 1999). Fixed effects included in the model were group, treatment, period, and their 2-way interactions. Three-way interactions were not included, as *P*-values for these were consistently >0.80. Day of experiment was used as a repeated measurement with first-order autoregressive covariance structure. The covariance structures were chosen based on the best fit according to the Schwarz Bayesian criterion. Random effects included in the model were pair, pair by treatment, pair by period, and pair by period by treatment. The effect of period on DMI was analyzed without the restricted period in the model because DMI was intentionally restricted on this day. The effect of period on DMI was evaluated using the moving average covariance structure for repeated measures. Comparisons of outcomes for ruminal pH variables were made using the estimate statement using Bonferroni adjustment.

Ruminal pH on the SARA challenge day and ruminal VFA and organic acid concentrations were analyzed as repeated measures (first-order autoregressive covariance structure) using the mixed model procedure of SAS. Ruminal pH values were averaged across hourly intervals for ease of analysis. Fixed effects included treatment, time postfeeding, and treatment by time postfeeding. Group was initially included in the model, but was not close to significance ($P > 0.65$) and was therefore removed. Random effects included in the model were pair and its associated interactions.

Significance was declared at $P \leq 0.05$. A trend was considered to exist if $0.05 < P \leq 0.10$. All reported values are least squares means unless otherwise stated.

RESULTS AND DISCUSSION

Days in milk, daily milk yield, mature-equivalent milk production, daily DMI, and buffer block intake were not different between treatment groups at the start of the trial (data not shown).

One cow on the control treatment in group 1 was diagnosed with clinical mastitis on her SARA challenge day (d 9). Her data showed a sharp drop in DMI starting on d 5, so only data collected before d 5 for this cow were included in the analysis. Three cows in group 1 had ruminal pH coil assembly problems: 1 cow on the buffer block treatment had no valid pH data until the afternoon of d 9, whereas 2 other cows had no data on d 1 and 2 of the experiment; their pre-SARA period ruminal pH averages were determined from d 3 alone. One cow in group 2 experienced health problems and was replaced after the first day of the trial; therefore, her replacement had no data collected on d 1. Otherwise, no data were lost for group 2, except for a few hours of ruminal pH values because of broken electrodes or electrical connections. Besides the problems mentioned

Table 2. Effect of access to buffer blocks and period on DMI, milk yield, and milk components

Variable	Period[1]					SEM[2]
	Pre-SARA (d 1–3)	Block (d 4–7)	Restricted (d 8)	SARA challenge (d 9)	Recovery (d 10–12)	
Control treatment						
DMI, kg/d	18.5[b]	19.5[b]	10.5[3]	23.4[a]	17.8[b]	1.1
Milk yield, kg/d	30.9[a]	29.9[ab]	28.5[b]	24.2[c]	25.7[c]	2.4
Milk fat, %	3.60[c]	3.77[bc]	4.07[ab]	4.40[a]	3.84[bc]	0.23
Milk protein, %	3.04	3.06	3.06	3.18	3.14	0.14
Fat yield, kg/d	1.09[ab]	1.12[a]	1.16[a]	1.05[ab]	0.95[b]	0.20
Protein yield, kg/d	0.93[a]	0.92[a]	0.88[a]	0.77[b]	0.79[b]	0.11
Buffer block treatment						
DMI, kg/d	18.2[b]	19.5[b]	10.1[3]	23.1[a]	19.6[b]	1.1
Buffer block intake, g/d	—	304[b]	519[a]	235[b]	324[b]	44
Milk yield, kg/d	28.5[a]	28.4[a]	26.2[b]	23.6[c]	26.9[ab]	2.4
Milk fat, %	3.82[b]	3.70[b]	3.93[ab]	4.29[a]	3.67[b]	0.22
Milk protein, %	3.06	3.06	3.01	3.02	3.00	0.13
Fat yield, kg/d	1.07	1.02	1.02	0.99	0.98	0.19
Protein yield, kg/d	0.86[a]	0.85[a]	0.77[bc]	0.71[c]	0.79[ab]	0.11

[a–c]Means within a row with different superscripts differ ($P < 0.05$).

[1]There was no effect of treatment or any treatment by period interaction on any of the variables presented in this table.

[2]Greatest standard error of the mean is shown.

[3]Arithmetic mean for DMI only.

above, there were no other physical examination abnormalities recorded during the trial.

Intake of the buffer blocks averaged 335 g of DM/cow per day for the days they were available. Block intakes were significantly different ($P < 0.001$) for the 2 groups, with intakes being greater for group 1 compared with group 2 (475 vs. 216 g of DM/d, respectively; data not shown). Buffer block composition did not differ between groups and cannot explain this difference in block intake. No other data are available to support a greater intake of buffers or molasses by first-lactation vs. second- or greater lactation cows. Daily block intakes ranged from 59 to 875 g of DM. Block intake was significantly ($P < 0.05$) greater on d 4 than on d 5, 6, and 9 (data not shown). Block intake was also significantly ($P < 0.05$) greater on d 8 than on all other days (Table 2). These results indicate that cows consumed more of the buffer blocks on the day the blocks were first introduced (d 4) and on the day of restricted feed intake (d 8). Cows did not consume more buffer block when their ruminal pH was low on the SARA challenge day. This observation agrees with the study by Cottee et al. (2004), who found that cows did not change their preference for SB-supplemented water when subjected to SARA.

Total DMI (TMR plus buffer block) and milk production outcome variables were not affected by treatment (Table 2). Dry matter intake was greater ($P < 0.05$) on the SARA challenge day (when an additional 4 kg of pellets was fed) than during any of the other periods. Dry matter intake on the feed-restricted day averaged 10.5 and 10.1 kg for control cows and cows with access to buffer blocks, respectively. Cows receiving the buffer blocks consumed numerically more DM than control cows on the first day of recovery after the SARA challenge (17.7 kg vs. 14.6 kg; data not shown); however, there was no significant treatment effect on DMI in any of the defined periods.

Milk yield responses for the 2 treatments during the different experimental periods are presented in Table 2. There was no effect of treatment on daily milk yield. Milk yield was similar for the pre-SARA and buffer access periods, but decreased significantly on the day of restricted feeding, and decreased further on the day of the SARA challenge. Although milk yield increased again during the recovery period, it did not return to the levels observed during the pre-SARA and buffer access periods, and remained approximately 3 kg less for the control cows and 1.5 kg for the cows with access to buffer blocks.

There was no effect of treatment on milk fat percentage (Table 2), but milk fat percentage was greater during the SARA challenge than during any of the other periods. This response is similar to that in an earlier study in which cows were subjected to the same SARA induction protocol (Krause and Oetzel, 2005). Yield of milk fat was also unaffected by treatment and was very similar for all periods. Decreased milk fat percentage has often been associated with SARA (Nocek, 1997), and Allen (1997) found a positive relationship between milk fat percentage and ruminal pH ($P < 0.0001$; $r^2 = 0.39$). Decreased milk fat percentage probably oc-

curs following repeated bouts of SARA, but not during induction of a single bout.

Milk protein percentage was not affected by treatment and period (Table 2), but milk protein yield was numerically reduced on the restricted feeding day and further reduced on the SARA challenge day, just as milk yield was. Although milk protein yield increased after the SARA challenge, it did not reach pre-SARA level.

The effects on intake and production when adding SB to diets for lactating dairy cows has been extensively investigated. Hu and Murphy (2005) evaluated the addition of SB to diets fed to early- and mid-lactation cows by a statistical analysis of 27 published studies. The authors reported no benefit of feeding buffer on DMI or milk production when the main forages fed were other than corn silage. Cows fed corn silage and supplemented with SB at between 0.7 and 1.0% of diet DM consumed 1.24 kg/d more DM than unsupplemented cows. This increase in DMI did not result in an increase in milk yield, but milk fat percentage and yield increased when cows were supplemented with SB. The authors also reported an increase in ruminal pH of 0.13 units. Average intake of buffer block in the current study was 335 g of DM/d, which would result in an intake of approximately 132 g of $NaHCO_3$. This level of bicarbonate intake is substantially lower than when SB is included in the TMR, as in the studies evaluated by Hu and Murphy (2005). The low level of buffer intake and the limited number of cows used in the current study probably explain the lack of response in DMI and milk production to buffer supplementation.

According to Kohn and Dunlap (1998) intake of 132 g of $NaHCO_3$ should increase ruminal pH from 6.00 (mean pH during pre-SARA period for cows with access to buffer blocks) to 6.53, assuming a rumen fluid volume of 50 L; however, no increase in ruminal pH was observed when cows were given access to buffer blocks. The above calculation ignores saliva flow and ruminal passage rates, along with other factors, but still indicates that this amount of buffer can affect ruminal pH significantly. Although there was no main effect of treatment on any of the pH variables measured, there was a significant treatment by period interaction for both mean ruminal pH and hours and area below ruminal pH 5.6 (Table 3). Cows were assigned to treatments randomly; however, cows on the buffer block treatment had numerically lower mean ruminal pH during the pre-SARA and block adaptation periods than did the control cows. Both treatment groups of cows had significantly increased ruminal pH on the restricted feeding day to be followed by a significant decrease on the SARA challenge day. Nevertheless, control cows experienced a decrease in mean pH from 6.50 to 5.69, and the cows with access to buffer blocks experienced a decrease from pH 6.31 to 5.87. In both treatment groups of cows ruminal pH increased again during the recovery period. Whereas control cows returned to a pH similar to the pre-SARA and buffer block adaptation period level, buffer block treatment cows returned to a ruminal pH significantly higher than their pre-SARA level and numerically higher than the level during the buffer block adaptation period.

Average buffer block intake was numerically greater during the recovery period compared with the buffer block adaptation period (324 vs. 304 g/d), especially in group 2 (185 vs. 232 g/d; data not shown). It seems unlikely that this small difference in buffer block intake can explain the numerical increase in mean ruminal pH from the buffer block adaptation period to the recovery period for cows with access to buffer blocks. As noted above, control cows had higher ruminal pH than buffer cows at the beginning of the trial before the introduction of the buffer blocks. However, when the pH values collected during the pre-SARA period were used as a covariate in the analysis of treatment effect on ruminal pH, this covariate was not significant (data not shown). This difference in pre-SARA ruminal pH between treatment groups could indicate that some cows were not adapted to the experimental diet or that the cows were recovering from a previous SARA challenge. However, all cows were allowed at least 1 wk of adaptation to the experimental diet. Moreover, the experimental diet was not drastically different from the normal herd ration that all cows were fed before the experiment. We have no explanation, therefore, for the lower and more variable mean ruminal pH observed in cows assigned to the buffer treatment. In hindsight, it might have been beneficial to use baseline ruminal pH when assigning cows to treatments.

Because there is a large variation in the response of individual animals to an acidosis challenge (Dougherty et al., 1975; Brown et al., 2000) and because the number of experimental units per treatment was relatively low in this experiment, we looked at changes in ruminal pH within each cow, using each cow as her own control. Changes in mean ruminal pH from the buffer block adaptation period to the SARA challenge, and from the buffer access period to the recovery period are presented in Table 4. Cows on the control treatment tended ($P = 0.06$) to experience a greater decrease in mean ruminal pH when induced with SARA compared with cows with access to buffer blocks (−0.55 vs. −0.20 pH units). The cows with access to buffer blocks also tended to recover from the SARA incident better than the control cows ($P = 0.06$), and their ruminal pH actually increased (0.15 pH units) during recovery compared with the period before the SARA challenge. In contrast, control

Table 3. Effect of access to buffer blocks and period on ruminal pH variables

Outcome variable	Period[1]					SEM[2]
	Pre-SARA (d 1 to 3)	Block (d 4 to 7)	Restricted (d 8)	SARA challenge (d 9)	Recovery (d 10 to 12)	
Control treatment						
Mean pH	6.13[b]	6.24[b]	6.50[a]	5.69[c]	6.07[b]	0.10
Hours <5.6, h/d	1.9[b]	1.1[b]	1.0[b]	10.9[a]	2.6[b]	1.3
Area <5.6, pH × min/d	17[b]	15[b]	12[b]	264[a]	36[b]	35
Nadir pH[3]	5.62[a]	5.66[a]	5.84[a]	4.89[b]	5.55[b]	0.14
Time of nadir postfeeding,[3] h	11.0[ab]	11.8[a]	6.8[b]	14.6[a]	8.0[b]	2.1
Buffer block treatment						
Mean pH	6.00[cd]	6.07[bc]	6.31[a]	5.87[d]	6.26[ab]	0.10
Hours <5.6, h/d	5.9[ab]	3.7[b]	2.8[bc]	7.7[a]	0.7[c]	1.3
Area <5.6, pH × min/d	123[ab]	76[bc]	49[c]	158[a]	6[c]	35
Nadir pH	5.40[b]	5.42[b]	5.54[ab]	4.95[c]	5.81[a]	0.14
Time of nadir postfeeding, h	11.6[a]	11.0[a]	3.9[b]	12.7[a]	11.2[a]	2.1

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]SARA = subacute ruminal acidosis.

[2]Greatest standard error of the mean is shown.

[3]There was no significant effect of treatment or any treatment by period interaction on these variables

cows had lower ruminal pH during recovery than in the period before the SARA challenge (−0.16 pH units). Cows with access to buffer blocks also had numerically greater DMI during the recovery period compared with control cows (19.7 vs. 17.0 kg); these findings are consistent with the higher ruminal pH values.

It is important to consider not only mean ruminal pH, but also time and area spent below a critical pH value. Area below pH 5.6 considers the duration and extent of the deviation from pH 5.6. As for mean ruminal pH, cows with access to buffer blocks experienced numerically more hours below pH 5.6 during the pre-SARA and buffer block period than did the control cows (Table 3), despite the fact that cows were assigned to treatments randomly. Although not statistically significant, cows on the buffer block treatment had decreased hours and area below pH 5.6 when given access to the buffer block (5.9 vs. 3.7 h below pH 5.6/d, and 123 vs. 76 min × pH units/d), suggesting that voluntary intake of the buffer block might have improved ruminal pH.

Cows on the control treatment experienced between 1 and 2 h/d below pH 5.6 before the SARA challenge (Table 3), which increased dramatically to 10.9 h/d on the SARA challenge day. However, cows returned to pre-SARA levels during the recovery period. The same was true for area below pH 5.6, where cows had a significantly greater area below pH 5.6 on the SARA challenge day than during any of the other periods. These changes in hours and area below pH 5.6 combined with the decrease in mean ruminal pH demonstrate that our model for inducing SARA in dairy cows was successful. Cows with access to buffer blocks also experienced more hours and had greater area below pH 5.6 on the SARA challenge day compared with the buffer block period; however, these values were similar to the pre-SARA values. During the recovery period, cows had signifi-

Table 4. Effect of access to buffer blocks on change of selected ruminal pH variables across experimental periods

Variable	Treatment		SEM	*P*-value
	Control	Buffer block		
Change from buffer access period to SARA[1] challenge				
Mean pH	−0.55	−0.20	0.10	0.06
Hours <5.6, h/d	9.7	4.1	1.4	0.02
Area <5.6, pH × min/d	249	83	39	0.01
Nadir pH	−0.77	−0.47	0.15	0.34
Change from buffer access period to recovery				
Mean pH	−0.16	0.18	0.10	0.06
Hours <5.6, h/d	1.5	−3.0	1.4	0.06
Area <5.6, pH × min/d	21	−69	39	0.20
Nadir pH	−0.11	0.39	0.15	0.06

[1]SARA = subacute ruminal acidosis.

cantly less time below pH 5.6 than during pre-SARA periods and numerically less area below pH 5.6.

Control cows increased time below pH 5.6 by 9.7 h/d from the buffer block period to the SARA challenge, whereas cows with access to buffer blocks only increased 4.1 h/d ($P = 0.02$; Table 4). Area below pH 5.6 increased 249 min × pH units/d for control cows, whereas cows with access to buffer blocks only increased 83 min × pH units/d ($P = 0.01$; Table 4). These results indicate that cows with access to buffer blocks were able to handle the SARA challenge better than the control cows. When comparing the buffer access period to the recovery period, cows with access to buffer blocks tended ($P = 0.06$) to decrease hours below pH 5.6 slightly, whereas control cows increased hours below pH 5.6 slightly. There was no difference in the change of area below pH 5.6 ($P = 0.20$) between the 2 treatments.

Daily minimum ruminal pH (nadir) did not differ between treatments, but was significantly lower on the SARA challenge day than during any of the other periods (Table 3). Although the decrease in daily nadir pH was greater for control cows than for cows with access to buffer blocks when comparing pre-SARA to the SARA challenge day, this was not significant (−0.77 and −0.47, respectively; Table 4). Treatment tended ($P = 0.06$; Table 4) to affect change in nadir from the buffer block period to the recovery period, with a negative change in nadir for the control cows (−0.11) and a positive change for cows with access to buffer blocks (0.39).

Time of nadir occurred approximately 11 h postfeeding during the pre-SARA and buffer block adaptation periods (Table 3), but occurred significantly earlier (6.8 and 3.9 h postfeeding for control and buffer block cows, respectively) during the day of feed restriction. Nadir occurred numerically later (14.6 and 12.7 h for control and buffer block cows, respectively) on the SARA challenge day, which could be caused by a significantly larger first meal of the day and total DMI compared with other periods (see Table 5). Meal size and total DMI could be factors affecting the time after feeding of nadir pH. A larger meal could result in the production of fermentation acids for an extended period, thereby delaying the time of ruminal pH nadir.

Hourly ruminal pH averages on the SARA challenge day were analyzed for treatment effects. Hourly mean ruminal pH on the SARA challenge day was not different for the 2 treatments ($P = 0.16$) and averaged 5.70 and 5.94 for the control cows and the buffer block cows, respectively (Figure 1). There was no interaction between treatment and hours postfeeding, indicating that the postprandial pattern in ruminal pH was similar for the control and buffer block cows. Figure 1 also illustrates that although there was no overall treatment effect on ruminal pH for those 24 h, cows with access to buffer blocks had numerically higher ruminal pH than control cows from 10 h postfeeding until next feeding.

Ruminal fluid was collected only during the SARA challenge and therefore, cannot be compared with pre-SARA or recovery values. Ruminal VFA and metabolite concentrations did not differ between treatments (P

Table 5. Effect of experimental group (first-lactation cows in group 1; second- and greater lactation cows in group 2) and period on eating behavior

	Period[1]					
Variable	Pre-SARA (d 1 to 3)	Block (d 4 to 7)	Restricted (d 8)	SARA challenge (d 9)	Recovery (d 10 to 12)	SEM[2]
Group 1						
Meal size, kg	2.5	2.5	2.4	2.1	2.3	0.6
No. of meals per d	12.9^{b}	13.7^{b}	10.1^{c}	17.4^{a}	13.1^{b}	1.2
Length of meal, min	14.8	15.9	15.7	14.4	14.7	1.5
Time spent eating, min/d[2]	182^{c}	206^{b}	140^{d}	243^{a}	187^{bc}	16
First meal of the day						
Size, kg as fed	4.7^{b}	7.8^{a}	6.1^{ab}	8.1^{a}	4.9^{b}	2.8
Length, min	25.6^{b}	43.3^{a}	28.6^{b}	46.6^{a}	27.1^{b}	6.8
Group 2						
Meal size, kg	2.8^{bc}	3.3^{bc}	2.5^{c}	4.4^{a}	3.8^{ab}	0.8
No. of meals per d	13.9^{a}	12.8^{ab}	7.3^{c}	10.1^{bc}	9.7^{c}	1.7
Length of meal, min	14.0^{b}	17.4^{b}	15.5^{b}	24.4^{a}	22.5^{a}	2.2
Time spent eating,[3] min/d	184^{b}	206^{b}	103^{c}	244^{a}	215^{ab}	22
First meal of the day						
Size, kg as fed	6.7^{b}	7.0^{b}	7.5^{b}	14.0^{a}	5.7^{b}	3.9
Length, min	32.0^{b}	22.9^{b}	35.1^{b}	65.6^{a}	26.7^{b}	9.6

[a–d]Means within a row with different superscripts differ ($P < 0.05$).

[1]SARA = subacute ruminal acidosis.

[2]Greatest standard error of the mean is shown.

[3]There was no significant effect of group or period by group interaction for this variable

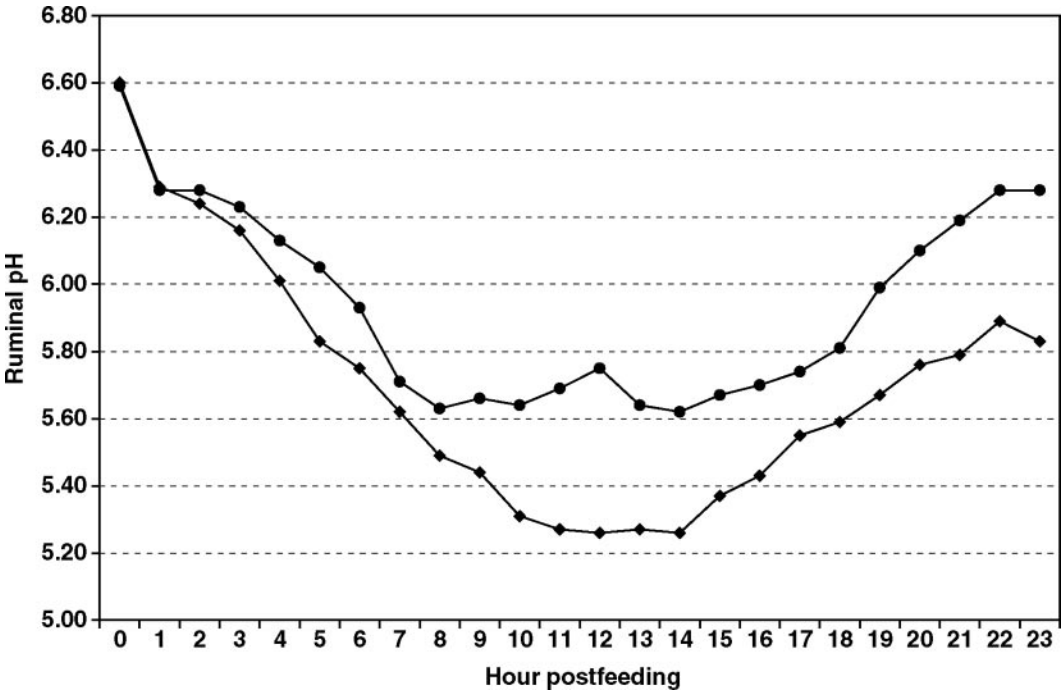

Figure 1. Hourly mean ruminal pH on subacute ruminal acidosis (SARA) challenge day for control cows and cows with access to buffer blocks. Control: ◆; buffer block treatment: ●. SEM = 0.16 pH units.

> 0.10) or between groups ($P > 0.10$). All concentrations were significantly affected ($P < 0.05$) by time of sampling except for succinate ($P = 0.09$) and formate ($P = 0.40$). Half-hourly least squares mean concentrations of acetate, propionate, and butyrate are presented graphically in Figure 2. Of the 3 major VFA, acetate increased to the greatest extent during the SARA challenge day. Least squares mean concentrations of acetate for the SARA challenge day were 64.8 and 61.3 m*M* (SEM = 2.2) for the control and buffer block treatments, respectively. Propionate concentration averaged 19.4 and 19.3 m*M* (SEM = 1.2) and butyrate 14.1 and 14.1 m*M* (SEM = 1.3) for the control and buffer block treatments, respectively. The acetate to propionate ratio stayed above 2 at all times. A ratio <2 has been associated with SARA and decreased milk fat production (Sauvant and Mertens, 1998). However, as mentioned earlier, repeated bouts of SARA might lead to changes in ruminal microbial populations and different effects on ruminal VFA than we observed in the current study.

Lactate concentration averaged 5.47 and 5.37 m*M* (SEM = 2.49) for the control and buffer block treatments, respectively. Three of the 16 cows had peak ruminal lactate concentrations >40 m*M*, which are similar to ruminal lactate concentrations found in acute ruminal acidosis (Owens et al., 1998). The other 13 cows' ruminal lactate concentrations peaked below or around 10 m*M* (individual cow data not shown). Half-hourly least squares mean concentrations of lactate and ethanol are shown in Figure 3. The lactate peaks observed in the current study were brief (see Figure 3). Ruminal lactate concentration peaked between 9 and 15 h postfeeding and showed a biphasic pattern with a smaller peak a few hours postfeeding followed by a return to pre-SARA levels and then a much larger peak. The fact that most cows had low (<10 m*M*) ruminal lactate concentrations during the SARA challenge suggests that lactate was not the primary cause for the decreased ruminal pH in these cows. Similarly, Oetzel et al. (1999) found that the majority of cows diagnosed with SARA by rumenocentesis had normal (<5 m*M*) ruminal lactate concentrations, indicating that elevated total VFA concentration was the main cause of low ruminal pH.

Ethanol concentration averaged 1.48 and 1.04 m*M* (SEM = 0.28) for the control and buffer block treatments, respectively. Average concentration of ethanol increased from around 0 m*M* to >2 m*M* during the SARA challenge day. Peak concentration occurred at approximately the same time as ruminal lactate peaked, which also coincided with the ruminal pH nadir. Ethanol and lactate are produced by heterofermentative lactobacilli. A substantial increase in the population

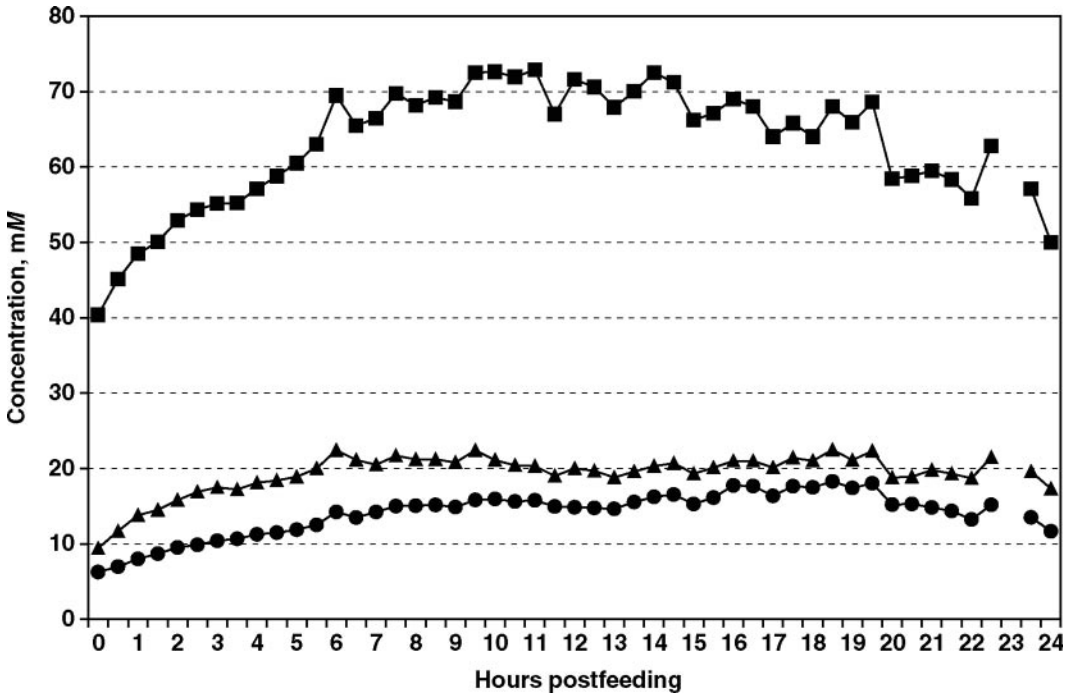

Figure 2. Ruminal acetate, propionate, and butyrate concentrations on subacute ruminal acidosis (SARA) challenge day for control cows and cows with access to buffer blocks. Acetate: ■, SEM = 3.27 m*M*; propionate: ▲, SEM = 1.40 m*M*; butyrate: ●, SEM = 1.39 m*M*.

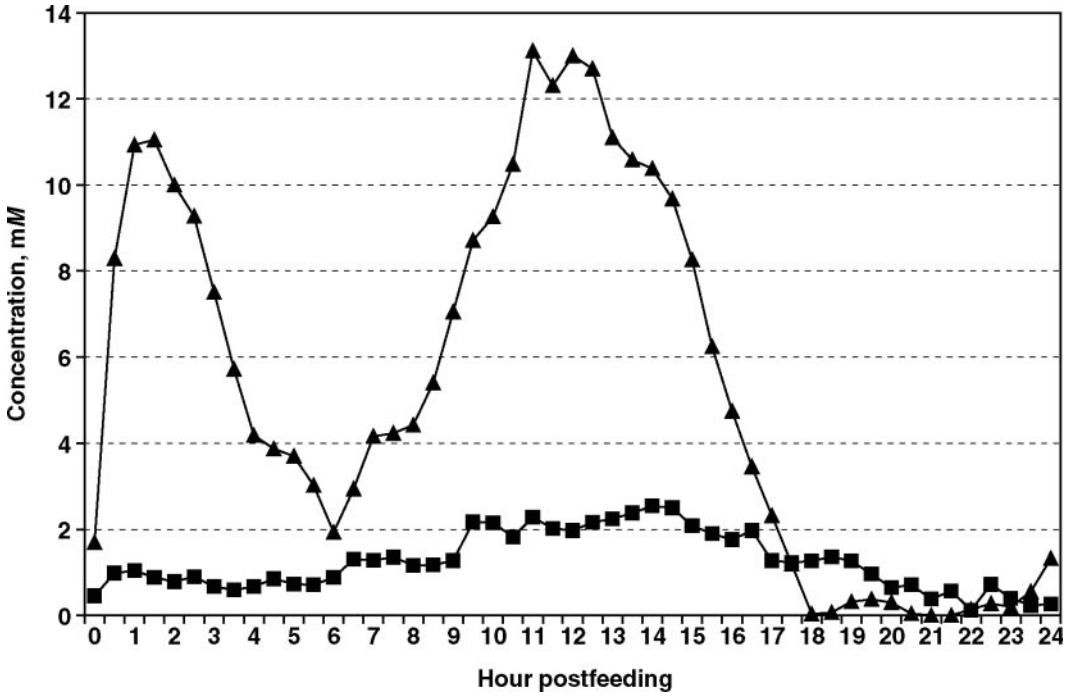

Figure 3. Ruminal lactate and ethanol concentrations on subacute ruminal acidosis (SARA) challenge day for control cows and cows with access to buffer blocks. Lactate: ▲, SEM = 2.50 m*M*; ethanol: ■, SEM = 0.36 m*M*.

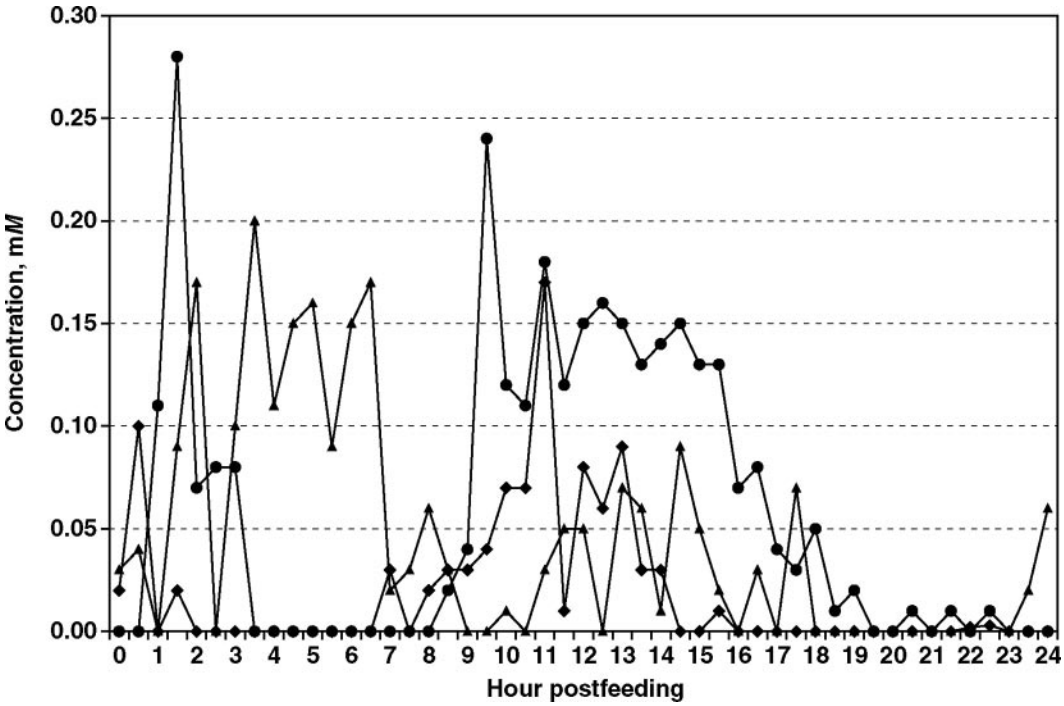

Figure 4. Ruminal succinate, formate, and 2,3-butanediol concentrations on subacute ruminal acidosis (SARA) challenge day for control cows and cows with access to buffer blocks. Succinate: ◆, SEM = 0.04 m*M*; formate: ▲, SEM = 0.06 m*M*; 2,3-butanediol: ●, SEM = 0.05 m*M*.

of ruminal lactobacilli is often seen during acute and subacute ruminal acidosis (Slyter, 1976; Nagaraja and Miller, 1989).

Half-hourly least squares mean concentrations of ruminal succinate, formate, and 2,3-butanediol are shown in Figure 4. Formate concentration averaged 0.05 and 0.04 m*M* (SEM = 0.03) for the control and buffer block treatments, respectively. Although ruminal formate concentration was not affected by time of sampling, the numerically greatest concentrations occurred 1 to 7 h after feeding. Diol 2,3-butanediol peaked 8 to 16 h after feeding, as did lactate. Average diol 2,3-butanediol was numerically greater for the control cows compared with cows with access to buffer blocks (0.10 vs. 0.02 m*M*; SEM = 0.03; $P = 0.11$). Succinate concentrations remained low throughout the sampling day and averaged 0.003 and 0.03 m*M* (SEM = 0.02) for the control and buffer block treatments, respectively. Ruminal succinate concentrations tended to be affected by time of sampling ($P = 0.09$), and the greatest concentrations of this metabolite coincided with the peak in ruminal lactate concentrations. Succinate is produced by several microorganisms associated with both starch and fiber digestion, but does not usually accumulate in the rumen due to decarboxylation to propionate by *Selenomonas ruminantium* (Hespell et al., 1997). It is not possible to define the microorganisms accounting for the fermentation products measured in this study, but the increases in these somewhat atypical metabolites suggest disruption of normal fermentation at low ruminal pH.

Eating behavior data are presented in Table 5. There was no effect of treatment on eating behavior, but meal size, number of meals per day, and length of meals differed between groups 1 and 2. A significant group by period interaction was detected; therefore, results are presented individually for each group. Total time spent eating did not differ between groups, despite a difference in DMI (18.5 vs. 21.5 kg/d, respectively). However, meal sizes were larger, meal times longer, and number of daily meals smaller in group 2 than in group 1. In addition, size and length of the first meal did not differ between groups. As mentioned earlier, only first-lactation cows were utilized in the first group, whereas all cows in group 2 were second- or greater lactation cows. This difference in parity could explain the difference in eating behavior between groups, as primiparous cows spend more time eating and perhaps have a slower eating rate than multiparous cows, as observed both by Campling and Morgan (1981) and Beauchemin and Rode (1994).

In group 1, meal size and length of meal were similar for all periods, whereas number of daily meals decreased on the feed-restricted day and was greatest on the SARA challenge day (Table 5). In contrast to

group 1, group 2 meal size was greatest on the SARA challenge day and the length of meals was greater during the SARA challenge and the recovery period than during the pre-SARA periods. For both groups, time spent eating was greatest on the SARA challenge day (which is not surprising given the greater DMI on this day) and lowest on the feed-restricted day. Also, size of the first meal of the day was significantly ($P < 0.0001$) greater on the SARA challenge day compared with pre-SARA (8.1 vs. 4.7 kg, as fed and 14.0 vs. 6.7 kg, as fed for groups 1 and 2, respectively), demonstrating that cows might overeat when fed ad libitum after 1 d of restricted feeding. Whether it was the size of the first meal, the increased proportion of easily fermentable carbohydrates in the TMR, or a combination of both factors that caused the cows in this experiment to experience SARA is unknown. Even limited fluctuations in feed delivery (±10% of ad libitum intake) tend to decrease mean ruminal pH and increase time below pH 5.8 and 5.5 in feedlot cattle fed high-grain diets (Schwartzkopf-Genswein et al., 2004).

CONCLUSIONS

Cows with access to a low-moisture buffer block consumed, on average, 0.33 kg DM of the block per day. The SARA challenge used in this trial was very effective in lowering ruminal pH. It also caused significant losses in milk yield and DMI after the challenge. Cows with access to the buffer blocks tended to experience a smaller decrease in mean ruminal pH during the SARA challenge and tended to recover better when comparing change in mean ruminal pH from before the SARA challenge to after the challenge. The effect of the buffer block intake on ruminal pH was especially pronounced with regard to hours spent below pH 5.6 and area below pH 5.6; when challenged with SARA, cows with access to buffer blocks experienced a lesser increase in both time and extent of ruminal pH below 5.6 compared with control cows. These data show that giving cows access to buffer-containing, low-moisture molasses blocks reduces the duration and the severity of a SARA challenge and tends to assist cows in returning to pre-SARA levels of ruminal pH.

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