Inducing Subacute Ruminal Acidosis in Lactating Dairy Cows

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ABSTRACT

Data from experiments in which subacute ruminal acidosis (SARA) was induced in lactating dairy cows (days in milk = 154 ± 118) were evaluated to investigate the effectiveness of the induction protocol and its effect on production outcomes. For 13 cows in 3 trials, ruminal pH was measured continuously and recorded each minute; dry matter intake and milk yield were recorded daily. Milk composition data were obtained from 9 cows in 2 of these trials. The SARA induction protocol included 4 separate periods: 4 d of baseline [normal total mixed ration (TMR)], 1 d of 50% restricted feeding, 1 or 2 d of challenge feeding [addition of 3.5 or 4.6 kg of wheat-barley pellet (dry matter basis) to normal TMR], and 2 d of recovery measurements when feeding normal TMR. The SARA induction protocol lowered mean ruminal pH from 6.31 during the baseline period to 5.85 during the challenge period; pH remained below baseline level during the recovery period (6.16). Mean ruminal pH was highest (6.59) during the day of restricted feeding. Nadir ruminal pH decreased from baseline to challenge period (5.76 vs. 5.13). Hours below pH 5.6 increased from 1.10 to 8.26/d from baseline to challenge period and area below 5.6 ($\text{pH} \times \text{min/d}$) increased from 15.0 to 190.3. Dry matter intake was not affected by SARA induction. Milk yield dropped from 35.2 kg/d during baseline to 31.7 kg/d during the challenge period and did not return to baseline level during the recovery period (31.3 kg/d). No depression in milk fat percentage was observed when SARA was induced. Yield of fat was highest during the restricted feeding period (1.47 kg/d) and was lower during the recovery period than during the baseline period (1.12 vs. 1.31 kg/d). The protocol successfully induced SARA (low ruminal pH without signs of acute ruminal acidosis) on the challenge day. Milk yield was substantially reduced and did not recover within 2 d after the challenge.

(Key words: subacute ruminal acidosis, ruminal pH, dry matter intake, milk production)

Abbreviation key: SARA = subacute ruminal acidosis.

INTRODUCTION

Subacute ruminal acidosis (SARA) is a major concern within the dairy industry in the United States. Economic losses caused by SARA result from decreased milk production, decreased efficiency of milk production, premature culling, and increased death loss. Costs of SARA resulting from lost production alone were estimated to be \$1.12/d per cow in a herd diagnosed with SARA (Stone, 1999).

Subacute ruminal acidosis has been defined, based on research from cannulated animals with indwelling electrodes, as bouts of depressed ruminal pH between 5.2 and 5.6 (Cooper and Klopfenstein, 1996). Most research regarding SARA has been conducted on beef cattle, but Oetzel (2004) found that 20% of cows from commercial dairy herds sampled by rumenocentesis during clinical herd investigations had ruminal pH of <5.5 . Individual cow responses to low ruminal pH have not been described quantitatively. Deleterious effects of SARA in individual cows may include decreased or variable DMI, decreased efficiency of milk production, reduced milk fat test, unexplained diarrhea, and poor body condition despite adequate energy intake (Nocek, 1997). Herds with a high prevalence of SARA may have a high culling rate, increased death loss, and decreased milk production (Nocek, 1997).

To study this disease and possible solutions to it, it is desirable to be able to reliably induce it under controlled conditions. Acute ruminal acidosis has been induced in feedlot cattle by withholding feed for 12 to 24 h and then allowing cattle access to the withheld diet (Owens et al., 1998). Researchers in Canada have developed a model whereby they reduced daily mean ruminal pH 0.14 units (from 6.25 to 6.11). Time and area below pH 6.0 was also significantly increased in this model (Keunen et al., 2002). However, this model did not increase time and area below ruminal pH 5.6 (the pH value defined as the cut-off point for SARA). The objectives of the current study were to determine whether a protocol could be developed to induce SARA consistently (i.e., ruminal pH <5.6) and to evaluate quantita-

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tively the effects of SARA induction on short-term production outcomes.

MATERIALS AND METHODS

Data from 3 experiments utilizing ruminally cannulated cows were combined to evaluate the effects and repeatability of a SARA induction protocol. The first experiment utilized 4 multiparous cows (180 ± 163 DIM and 688 ± 82 kg of BW); the second experiment utilized 3 primiparous and 3 multiparous cows (149 ± 127 DIM and 629 ± 60 kg of BW); and the third experiment utilized 3 primiparous cows (170 ± 98 DIM and 608 ± 48 kg BW). No cow participated in more than one experiment. Within each experiment, cows were divided into 2 groups based on DIM: early lactation if DIM was ≤ 150 d and late lactation if DIM was >150 d. The effect of parity on the measured outcomes was confounded by experiment and was therefore not investigated.

The protocol consisted of 4 experimental periods in the following order: 1) 4 d of baseline (normal TMR fed for ad libitum intake), 2) 1 d of 50% restricted feeding, 3) 1 d (experiments 1 and 3) or 2 d (experiment 2) of challenge feeding (addition of 3.5 to 4.6 kg of DM of a wheat-barley pellet to normal TMR), and 4) 2 d of recovery measurements with normal TMR feeding. A second challenge day was introduced in experiment 2 to extend the length of time the cows would be experiencing SARA.

Physical exams (temperature, respiration and heart rate, general appearance, and visual fecal scores) were carried out daily and every 4 h during the challenge period. Temperature was considered elevated if $>39.4^{\circ}\text{C}$, heart rate was considered elevated if >100 beats/min, respiratory rate was considered abnormal if >40 breaths/min, and fecal score was considered abnormal if <2 (Ireland-Perry and Stallings, 1993).

Cows were fed a TMR for ad libitum intake once daily. Samples of forages, high moisture corn, and TMR were collected twice weekly and dried for 48 h in a 60°C forced-air oven, and diets were adjusted to account for changes in DM content. Ingredients included in the TMR differed slightly between experiments and consisted of 50 to 53% forage of which 55 to 60% was corn silage and 40 to 45% was alfalfa silage (DM basis). The corn silage and alfalfa silage were chopped at a 19- and 10-mm theoretical length of cut, respectively, at time of harvesting. The concentrate mix was based on ground high moisture shelled corn and was similar for all 3 experiments. Corn was processed by a roller mill and averaged 72% DM. All TMR contained 3.0 to 3.2% of a dry wheat-barley pellet (88.5% DM) consisting of 50% finely ground barley and 50% finely ground wheat (DM

basis). The same wheat-barley pellet was used in the SARA challenge.

Dried and ground samples of the forages and the high moisture corn were analyzed for NDF, ADF, and CP content. The NDF fraction was determined according to the procedure of Mertens (1999) adapted for Ankom²⁰⁰ Fiber Analyzer (Ankom Technology, Fairport, NY). Samples were analyzed using α -amylase and sodium sulfite and were corrected for ash content. Acid detergent fiber was determined using the procedure described by Goering and Van Soest (1970), adapted for Ankom²⁰⁰ Fiber Analyzer. Crude protein was determined by the micro-Kjeldahl method (AOAC, 1990).

Diets were formulated to meet the requirements of a 680-kg cow producing 33 kg of milk (3.5% fat and 3.0% true protein) daily (NRC, 2001). Nutrient compositions of the basal TMR for each experiment were very similar and contained between 17.5 and 18.5% CP, 19% ADF, 28.1 to 29.1% NDF, 4.8 to 5.3% ether extract, and 1.56 to 1.58 NE_L/kg (DM basis) according to wet chemistry results obtained from the forages and the high moisture corn and NRC (2001) table values for the remaining feed ingredients.

On SARA challenge days, the wheat-barley pellets were mixed with the TMR by hand at the time of feeding. The amount of wheat-barley pellets (DM basis) added to the TMR was equal to approximately 20% of average DMI during the baseline period for the group of cows, resulting in different amounts being fed for the 3 experiments: 4.6 kg of DM per cow in experiments 1 and 2 and 3.5 kg of DM per cow in experiment 3.

Ruminal pH data were collected each minute using indwelling electrodes as described by Krause et al. (2002). The 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 were identified using ± 15 -min rolling averages of ruminal pH values to eliminate false nadirs caused by electrical noise or other irregularities in the data. Dry matter intakes and milk production data were collected daily from all cows, whereas milk composition data were only obtained from cows in experiments 2 and 3 ($n = 9$). Milk components were determined by AgSource (Menomonie, WI) using a near infrared reflectance spectroscopy analyzer (MilkoScan 605; Foss Electric, Hillerød, Denmark).

Ruminal fluid was collected during the SARA challenge period for experiments 2 and 3 ($n = 9$); in experiment 2, ruminal fluid was collected during the first 24 h of the 2-d SARA challenge, and in experiment 3, ruminal fluid was collected during the entire 24-h SARA challenge period. Ruminal fluid was collected every 30 min by aspiration through a strainer located next to the pH electrode in the rumen. Samples were filtered

Table 1. Effect of period on ruminal pH in the subacute ruminal acidosis induction protocol.

Variable	LSM ¹				SED	<i>P</i>		
	Baseline	Restricted	Challenge	Recovery		Experiment	Period	Experiment × period
Mean ruminal pH	6.31 ^b	6.59 ^a	5.85 ^d	6.16 ^c	0.06	0.15	0.001	0.83
Hours <5.6, h/d	1.10 ^b	0.33 ^b	8.26 ^a	1.89 ^b	0.95	0.11	0.001	0.18
Area <5.6, pH min/d	15.0 ^b	3.4 ^b	190.3 ^a	32.5 ^b	28.5	0.06	0.001	0.15
Nadir, pH	5.76 ^b	6.06 ^a	5.13 ^c	5.56 ^b	0.10	0.10	0.001	0.68
Time to nadir postfeeding, h:min	9:44	7:42	12:25	10:22	1:41	0.78	0.15	0.84

^{a,b,c,d}Means within a row with different superscripts are different ($P \leq 0.05$).

¹Least squares means for 4 experimental periods in the following order: 4 d of baseline (normal TMR fed for ad libitum intake), 1 d of 50% restricted feeding, 1 d (experiments 1 and 3) or 2 d (experiment 2) of challenge feeding (addition of 3.5 or 4.6 kg of DM of a wheat-barley pellet to normal TMR), and 2 d of recovery measurements with normal TMR feeding.

through 2 layers of cheesecloth, and 10 mL of ruminal fluid were acidified with 0.5 mL of H₂SO₄. Acidified samples were frozen and later analyzed for acetate, propionate, butyrate, lactate (D plus L-isomers), succinate, and ethanol concentrations by HPLC (Shimadzu Class-VP, version 5.03; Shimadzu Scientific Instruments, Inc., Columbia, MD) as described by Siegfried and Stumpf (1984).

Analysis of variance of daily averages of pH data, milk yield, and milk composition for each cow was conducted using the mixed model procedure (SAS, 1999). Experiment, period (baseline, restricted, challenge, recovery), their interactions, and DIM category were included as fixed effects in the model. Day of experiment (1 to 8 or 9) was used as a repeated measurement with first-order auto regressive covariance structure. The random statement included cow. The effect of period on DMI was analyzed without the restricted period in the model and using the moving average covariance structure for the repeated measurements. Dry matter intake was intentionally restricted on this day; this would have resulted in an underestimation of the variance if this period had been included in the analysis. Values reported are least squares means with the standard error of the difference. Significance was declared at $P \leq 0.05$. All mean comparisons were by Fisher's least significant difference method after a significant period effect was detected.

Concentrations of VFA and other organic acids data collected on the SARA challenge day were analyzed using the mixed model procedure (SAS, 1999) with experiment and time postfeeding (30-min intervals) as fixed effects; cow was a random effect. Time postfeeding was used as a repeated measurement with first-order auto regressive co-variance structure.

RESULTS AND DISCUSSION

There was no effect of experiment nor any effect of an experiment × period interaction on any of the ruminal pH measures (Table 1), indicating that the effects

of this induction protocol on ruminal pH outcomes were similar in the 3 experiments. Mean ruminal pH was 6.31 during the baseline period and decreased significantly by 0.46 pH units during the challenge period (Table 1). The decrease obtained in the present experiment is greater than the 0.14 units of decrease in ruminal pH obtained using the SARA model of Keunen et al. (2002).

Ruminal pH did not return to the baseline value during the 2 d of recovery following the SARA challenge (Table 1). Changes in ruminal pH are illustrated in Figure 1, which shows hourly ruminal pH values for one individual cow during the last 6 d of experiment 3. The figure also shows that although mean ruminal pH was lower during recovery than during baseline, it appears that ruminal pH was rising during the 2 d of recovery. This pattern was typical for all cows. Mean pH across all cows on the first day of recovery was 6.07, whereas it was 6.27 on the second day of recovery.

Figure 1. Example of changes in ruminal pH in a cow during the last 6 d of experiment 3. Ruminal pH values have been averaged by hour. Gaps in graph represents missing data caused by electrode malfunction. SARA = subacute ruminal acidosis.

Hours below ruminal pH 5.6 increased from 1.10 h/d during baseline to 8.26 h/d during the challenge day (Table 1) and returned to baseline level during the recovery period. Area below pH 5.6 increased from 15.0 during the baseline period to 190.3 pH $\times$ min/d during the challenge day and returned to the pre-challenge level during the recovery period. Again, these increases in time and area below pH 5.6 were substantially greater than the changes reported by Keunen et al. (2002), who found no increase in time and area below pH 5.6 (and therefore did not induce SARA). This previous model significantly increased time and area below pH 6.0 only.

The SARA induction model in the current study induced SARA in a 1-d challenge compared with 7 d of challenge feeding in the model of Keunen et al. (2002). The current model also included a 50% feed restriction the day before the SARA challenge. It is possible that buffering capacity of the rumen fluid was decreased at the time of SARA challenge because of the prior day's low feed intake. However, we did not measure this. Rumen contents, in the form of ingested forages, have inherent buffering or acid-consuming capacities (McDonald et al., 1991). Impaired buffering capacity of the rumen content could have caused a greater decrease in ruminal pH in the current study compared with the values reported by Keunen et al. (2002).

Daily minimum pH (nadir) decreased by 0.63 pH units during the SARA challenge and returned to baseline levels during the recovery period. During the baseline period, nadir pH occurred about 10 h postfeeding. Time to nadir postfeeding was not significantly different for the 4 periods ($P = 0.15$), but did occur numerically later (12 h and 25 min postfeeding), during the challenge period. This could be related to meal size. It is possible that cows ate a relatively large amount of feed when re-fed ad libitum after 1 d of restricted feeding, which could result in production of fermentation acids for an extended period of time. When utilizing this SARA induction protocol in another experiment, where eating behavior was recorded continuously, we observed that the first meal of the day was significantly ($P < 0.05$) greater on the SARA challenge day than during baseline [11.0 vs. 5.7 kg (as-fed basis); Krause and Oetzel, unpublished data]. Ruminal pH nadirs occurred later after feeding than the 5 to 8 h suggested by Nocek (1997) and Nordlund and Garrett (1994) for testing ruminal pH in cows fed a TMR once daily near their expected nadir. However, during the baseline period, the daily ruminal pH curve was relatively flat, with nadir pH less pronounced than on the challenge day (Figure 2).

Average pH and nadir pH were highest during the restricted period, where cows were only fed 50% of the

Figure 2. Hourly mean ruminal pH for baseline (◆), restricted (■), and challenge (▲) periods (experiments 1, 2, and 3). Number of observations included in these hourly means was highest for the baseline period (4 d of observation) and lowest for the restricted period, as several hours of data were missing for this period. SEM = 0.05, 0.06, and 0.06, for baseline, restricted, and challenge period, respectively.

previous day's TMR intake. This response was expected, as lower intake should result in lower production of fermentation acids. Highest pH values collected during the trial occurred at the end of the restricted feeding day. Cows were usually out of feed by the end of the afternoon, and ruminal pH increased steadily from around this time and peaked just prior to the next feeding. Average ruminal pH at 23 h postfeeding on the restricted feeding period was 7.12 (Figure 2).

None of the cows exhibited abnormal physical examination findings during the trial. Based on the changes in ruminal pH reported previously and the lack of clinical signs of acute acidosis, the amount of wheat-barley pellet used to spike the TMR in the current protocol appeared to be appropriate to induce SARA.

The decrease in mean ruminal pH and the increase in hours and area below pH 5.6 were substantially greater than the changes observed in other SARA induction protocols (Keunen et al., 2002). The current SARA induction protocol can be used to study the short-term effects of a bout of SARA on ruminal and production outcomes. Also, the protocol can be used to evaluate new methods for alleviating SARA. The scenario of this protocol is probably close to the situations on dairy farms where cows experience day-to-day variations in intake because of feeding errors or limited access to feed. Whereas cows on a commercial dairy farm might experience this repeatedly, however, this SARA induction protocol represents only one bout of SARA. This protocol is probably not well-suited to study longer term effects of SARA, such as changes in microbial protein

Table 2. Effect of period on DMI and milk production in the subacute ruminal acidosis induction protocol.

Variable	LSM ¹				SED	P		
	Baseline	Restricted ²	Challenge	Recovery		Experiment	Period	Experiment × period
DMI, kg/d ³						0.03	0.001	0.001
Experiment 1	25.2	12.0	27.9	26.3	1.5	—	—	—
Experiment 2	23.1 ^a	11.1	17.4 ^b	21.2 ^a	1.3	—	—	—
Experiment 3	18.7 ^b	11.3	21.4 ^a	16.9 ^b	1.8	—	—	—
Milk yield, kg/d	35.2 ^{ab}	34.7 ^b	31.7 ^c	31.3 ^c	1.0	0.22	0.01	0.21
Milk fat, %	3.73 ^b	4.20 ^{ab}	4.29 ^a	3.69 ^b	0.23	0.37	0.04	0.15
Milk protein, % ³	2.86	2.87	2.95	2.82	0.08	0.14	0.44	0.04
Experiment 2	2.80	2.85	2.73	2.81	0.09	—	—	—
Experiment 3	3.00 ^b	2.98 ^b	3.25 ^a	2.93 ^b	0.12	—	—	—
Fat yield, kg/d	1.27 ^{ab}	1.43 ^a	1.22 ^b	1.08 ^c	0.09	0.04	0.02	0.62
Protein yield, kg/d	0.98 ^a	0.99 ^a	0.86 ^b	0.84 ^b	0.03	0.02	0.001	0.76

^{a,b,c}Means within a row with different superscripts are different ($P \leq 0.05$).

¹Least squares means or arithmetic means of 4 experimental periods in the following order: 4 d of baseline (normal TMR fed for ad libitum intake), 1 d of 50% restricted feeding, 1 d (experiments 1 and 3) or 2 d (experiment 2) of challenge feeding (addition of 3.5 or 4.6 kg of DM of a wheat-barley pellet to normal TMR), and 2 d of recovery measurements with normal TMR feeding.

²Arithmetic mean for DMI only.

³Least square means of period effects of individual experiments presented when an experiment × period interaction is present.

synthesis and ruminal fiber digestion or other long-term effects such as cow health.

There was a significant effect of experiment on DMI (Table 2). The DMI was higher in experiment 1 than in experiments 2 and 3. The 4 cows in experiment 1 were all multiparous, whereas the cows in experiment 2 were one-half primiparous and one-half multiparous; all cows in experiment 3 were primiparous. The effect of parity on the measured outcomes could not be tested in this study because it was confounded with experiment, but could explain the differences in DMI mentioned previously. Also, there was an interaction effect between experiment and period on DMI (Table 2). This was a result of a significantly lower DMI during the challenge period than during baseline and recovery for experiment 2 (17.4 vs. 23.1 and 21.2 kg, respectively), whereas in experiments 1 and 3, DMI was significantly higher during the challenge period than during the recovery period (Table 2). Experiment 2 differed from experiments 1 and 3 in that the SARA challenge lasted 2 d instead of 1. However, DMI in experiment 2 was lower than the baseline level on both the first and second day (18.5 and 15.3 kg, respectively) of the SARA challenge. We have no apparent explanation for the lower DMI during the SARA challenge observed in experiment 2. Despite the differences in DMI during the challenge period, there was no effect of experiment and no effect of the experiment × period interaction on any of the ruminal pH outcomes.

Milk yield dropped by 3.5 kg/d from the baseline period to the challenge period and remained lower during the recovery period (Table 2). This decrease in milk yield is close to the 2.7-kg/d decrease observed in a commercial dairy herd apparently suffering from SARA

(Stone, 1999). Dry matter intake was numerically lower (0.8 kg/d) during recovery compared with the baseline period across experiments, but this difference in DMI cannot explain fully the observed drop in milk yield.

Milk fat depression in the form of lowered milk fat percentage was not observed when cows were subjected to this SARA protocol (Table 2). Milk fat percentage was higher on the challenge day than during recovery (4.29% vs. 3.69%) but did not differ between the other periods. Milk fat yield was numerically lower during the challenge period (1.22 kg/d) compared with the baseline period (1.27 kg/d) and remained significantly depressed during the recovery period (1.08 kg/d).

Decreased milk fat percentage has often been associated with SARA (Nocek, 1997), and Allen (1997) did find a positive relationship between milk fat percentage and ruminal pH ($P < 0.0001$; $r^2 = 0.39$). Although ruminal pH was decreased significantly in the current study, we did not observe a decrease in milk fat percentage (Table 2). Decreased milk fat percentage probably occurs following repeated bouts of SARA and not during induction of a single bout. Also, the degree of milk fat depression observed during SARA will likely depend on the level of unsaturated fat in the diet (Bauman and Grinari, 2001). In the current study, cows were feed-restricted the day prior to the SARA challenge, which might have caused them to mobilize adipose tissue and incorporate a greater proportion of these fatty acids into the milk. However, we did not evaluate any measures of fat mobilization. The current SARA induction protocol was very effective in lowering ruminal pH, but is probably not well-suited for studying the effects of SARA on long-term milk production or milk fat synthesis because of its short duration.

There was no effect of period on milk protein percentage, which averaged 2.88%. However, in experiment 3, the percentage of protein was higher during the challenge day than during the restricted and recovery periods (3.21 vs. 2.94 and 2.88%, respectively), whereas there was no difference between periods in experiment 2, resulting in an experiment $\times$ period interaction (Table 2). Yield of milk protein was higher during the baseline and restricted periods than during the challenge and recovery periods (0.98 and 0.99 kg/d vs. 0.86 and 0.84 kg/d, respectively). Also, yield of protein was higher in experiment 2 than in experiment 3 (1.01 vs. 0.83 kg/d, respectively; data not shown) as was yield of fat (1.44 vs. 1.13 kg/d, respectively; data not shown). As mentioned earlier, experiment 3 utilized primiparous cows only, which could contribute to the differences in milk component yields between the 2 experiments.

As mentioned earlier, experiment 2 included a second SARA challenge day. Although the SARA challenge affected ruminal pH similarly across the 3 experiments, we observed that average daily ruminal pH for the 6 cows in experiment 2 increased from the first challenge day to the second challenge day (5.87 vs. 6.22). However, no statistical analysis of this difference was carried out. We also found that the response in DMI to the second challenge day in experiment 2 differed greatly among cows (range from -10.9 to $+3.5$ kg among the 6 cows). Because of this variation and the increase in ruminal pH observed from the first to the second challenge day, we decided to limit the SARA challenge to 1 d in the following experiment (experiment 3). So, whereas the SARA induction protocol of Keunen et al. (2002) could be and was repeated daily, the current SARA protocol is a 1-d event.

Ruminal fluid was only collected during the SARA challenge and could not be compared with baseline or recovery values. Ruminal VFA concentrations did not differ between experiments, and all VFA concentrations were significantly affected by time of sampling (results not shown). Least squares mean concentrations of acetate, propionate, and butyrate are presented graphically in Figure 3. Of the 3 major VFA, acetate increased to the greatest extent during the SARA challenge day. The acetate to propionate ratio stayed >2 at all times. A ratio <2 has been associated with SARA and decreased milk fat production (Sauvant and Mertens, 1998). However, as mentioned previously, 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.

Two of the 9 cows had peak ruminal lactate concentrations >40 mM, which are similar to ruminal lactate concentrations found in acute ruminal acidosis (Owens et al., 1998), whereas the rest peaked around 10 mM

Figure 3. Least squares mean values of ruminal acetate (■), propionate (▲), and butyrate (●) concentrations during subacute ruminal acidosis challenge day (experiments 2 and 3). SEM = 3.6, 3.2, and 1.3 mM for acetate, propionate, and butyrate, respectively.

(results not shown). The lactate peaks observed in the current study were brief (Figure 4). There was no effect of experiment on lactate, succinate, and ethanol concentrations measured during the SARA challenge. Least square mean concentrations of lactate, succinate, and ethanol are shown in Figure 4. Ruminal lactate concentration peaked between 9 and 15 h postfeeding and showed a somewhat biphasic pattern with a smaller peak a few hours postfeeding followed by a return to baseline levels and then a much larger peak. The fact that most cows had low (<10 mM) ruminal lactate concentrations during the SARA challenge suggests that

Figure 4. Least squares mean values of ruminal D- and L-lactate (■), succinate (▲), and ethanol (✚) concentrations during subacute ruminal acidosis challenge day (experiments 2 and 3). SEM = 3.7, 1.4, and 0.7 mM for lactate, succinate, and ethanol, respectively.

lactate was not the primary cause for the decreased ruminal pH in these cows. Similarly, Oetzel et al. (1999) found that the vast majority of cows diagnosed with SARA by rumenocentesis had normal (<5 mM) ruminal lactate concentrations, indicating that elevated total VFA concentration was the main cause of low ruminal pH.

The ruminal metabolites succinate and ethanol are usually only found at very low levels in the rumen, but their concentrations increased from around 0 to 3 to 4 mM during the SARA challenge day. Peak concentrations occurred at approximately the same time as ruminal lactate peaked, which also coincided with the ruminal pH nadir. Increases in the atypical metabolites suggest disruption of normal fermentation at low ruminal pH.

CONCLUSIONS

A short-term SARA induction protocol was successful in lowering ruminal pH significantly and increasing both time and area below pH 5.6 on the SARA challenge day. Mean ruminal pH did not return to pre-SARA level within 2 d of the challenge. Milk and fat yield were significantly reduced following the SARA challenge day. These results suggest that cows need several days to recover from a bout of SARA and that milk production is affected negatively following the incident. Changes in ruminal pH were similar in 3 different experiments, despite significant effects of experiment and experiment $\times$ period interactions on DMI. This SARA induction protocol may be useful to study the disease and potential methods of prevention.

REFERENCES

- Allen, M. S. 1997. Relationship between fermentation acid production in the rumen and the requirement for physically effective fiber. *J. Dairy Sci.* 80:1447–1462.
- Association of Official Analytical Chemists. 1990. Official Methods of analysis. Vol. I. 15th ed. AOAC, Arlington, VA.
- Bauman, D. E., and J. M. Griinari. 2001. Regulation and nutritional manipulation of milk fat: Low-fat milk syndrome. *Livest. Prod. Sci.* 70:15–29.
- Cooper, R., and T. Klopfenstein. 1996. Effect of Rumensin and Feed Intake Variation on Ruminal pH. Scientific Update on Rumensin/Tylan/Micotil for the Professional Feedlot Consultant. Elanco Animal Health, Greenfield, IN.
- Goering, H. K., and P. J. Van Soest. 1970. Forage Fiber Analyses (Apparatus, Reagents, Procedures, and Some Applications). Agric. Handbook No. 379. ARS-USDA, Washington, DC.
- Ireland-Perry, R. L., and C. C. Stallings. 1993. Fecal consistency as related to dietary composition in lactating Holstein cows. *J. Dairy Sci.* 76:1074–1082.
- Keunen, J. E., J. C. Plaizier, L. Kyriazakis, T. F. Duffield, T. M. Widowski, M. I. Lindinger, and B. W. McBride. 2002. Effects of a subacute ruminal acidosis model on the diet selection of dairy cows. *J. Dairy Sci.* 85:3304–3313.
- Krause, K. M., D. K. Combs, and K. A. Beauchemin. 2002. Effects of forage particle size and grain fermentability in midlactation cows. II. Ruminal pH and chewing activity. *J. Dairy Sci.* 85:1947–1967.
- McDonald, P., N. Henderson, and S. Heron. 1991. *The Biochemistry of Silage*. Chalcombe Publications, Bucks, England.
- Mertens, D. R. 1999. Variation in NDF results with modifications of the filter bag method. Pages 1–7 in Proc. National Forage Testing Association, Technical Sessions Papers and Committee Reports to the Board and Membership, June 1999, Topeka, KS. National Forage Testing Assoc., Omaha, NE.
- Nocek, J. E. 1997. Bovine acidosis: Implications on laminitis. *J. Dairy Sci.* 80:1005–1028.
- NRC. 2001. *Nutrient Requirements of Dairy Cattle*, Seventh Edition. Natl. Acad. Press, Washington, DC.
- Nordlund, K. V., and E. F. Garrett. 1994. Rumenocentesis: A technique for collecting rumen fluid for the diagnosis of subacute rumen acidosis in dairy herds. *Bovine Pract.* 28:109–112.
- Oetzel, G. R., K. V. Nordlund, and E. F. Garrett. 1999. Effect of ruminal pH and stage of lactation on ruminal lactate concentrations in dairy cows. *J. Dairy Sci.* 82(Suppl. 1):38. (Abstr.)
- Oetzel, G. R. 2004. Monitoring and testing dairy herds for metabolic disease. *Vet. Clin. Food Anim.* 20:651–674.
- Owens, F. N., D. S. Secrist, W. J. Hill, D. R. Gill. 1998. Acidosis in cattle: A review. *J. Anim. Sci.* 76:275–286.
- Siegried, R. R. H., and G. Stumpf. 1984. Method for determination of organic-acids in silage by high-performance liquid-chromatography. *Landwirtschaftliche Forschung* 37(3–4):298–304.
- SAS. 1999. *SAS User's Guide: Statistics*. SAS Inst., Inc., Cary, NC.
- Sauvant, D., and D. R. Mertens. 1998. Dietary characteristics affecting ruminal acidosis. Pages 63–65 in *Annual Research Summaries 1998*. US Dairy Forage Research Center, Madison, WI.
- Stone, W. C. 1999. The effect of subclinical rumen acidosis on milk components. Pages 40–46 in Proc. Cornell Nutr. Conf. Feed Manuf., Syracuse, NY. Cornell Univ., Ithaca, NY.