

## Repeated Ruminal Acidosis Challenges in Lactating Dairy Cows at High and Low Risk for Developing Acidosis: Ruminal pH<sup>1</sup>

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### ABSTRACT

The primary objective of this experiment was to determine whether lactating dairy cows that are at high (HR) or low (LR) risk for experiencing ruminal acidosis, because of their diet and stage of lactation, differ in their response to an acidosis challenge. A secondary objective was to determine whether the severity of acidosis changes with repeated challenges. The experiment was a completely randomized design with 2 groups (risk scenarios, HR vs. LR) and 3 periods corresponding to 3 repeated acidosis challenges. Eight lactating ruminally cannulated cows were assigned to 1 of 2 groups: HR, early lactation cows fed a 45% forage diet, or LR, midlactation cows fed a 60% forage diet. Cows were exposed to 3 acidosis challenges, each separated by 14 d. The challenge consisted of restricting total mixed rations to 50% of ad libitum intake for 24 h, followed by a 1-h meal of 4 kg of ground barley-wheat before allocating the total mixed rations. Ruminal pH was measured continuously for 9 of the 14 d each period using an indwelling system. Subacute acidosis (SARA) was described at 2 thresholds: pH <5.8 and pH <5.5. As expected, HR cows had lower ruminal pH profiles (curves) compared with LR cows: mean pH (5.81 vs. 6.21) and nadir pH (5.13 vs. 5.53). The HR cows also experienced SARA to a greater extent than LR cows during the experiment (pH <5.8, 10.6 vs. 3.5 h/d; pH <5.5, 5.9 vs. 1.6 h/d). The pH profiles of cows in both

risk categories decreased with each challenge period; mean pH was 6.13, 6.03, 5.77, and nadir pH was 5.52, 5.34, and 5.14 in periods 1, 2, and 3, respectively. The challenges caused a similar decrease in pH for cows in both risk categories, but because the HR cows had a lower baseline pH, they experienced more severe SARA with each subsequent challenge. Feed restriction the day before administering the acidosis challenge caused ruminal pH to gradually increase. On the challenge day, the entire grain allotment was consumed by all cows in period 1, six cows in period 2, and only 3 cows in period 3. The pH plummeted immediately after each grain challenge. Ruminal pH remained very low during the first day after the challenge for all cows, but LR cows began their recovery more quickly than HR cows. Regardless of risk category, with each successive challenge, the pH decrease on the challenge day was more severe: nadir pH on the challenge day was 5.19, 5.07, and 4.90 and duration of SARA (pH <5.8) was 12.2, 13.4, and 15.8 h/d in periods 1, 2, and 3. This study indicates that cows become more prone to acidosis over time even though they decrease intake of the challenge grain to avoid acidosis. The severity of each subsequent bout of acidosis increases, especially for cows fed diets low in physically effective fiber and at high acidosis risk. Therefore, a bout of acidosis that occurs due to improper feed delivery or poor diet formulation can have long-term consequences on cow health and productivity.

**Key words:** acidosis, subacute acidosis, ruminal pH, ruminal health

### INTRODUCTION

Dairy cows fed for maximum milk production are at risk of experiencing ruminal acidosis, as recently reviewed by Krause and Oetzel (2006). In high-producing dairy cows, ruminal pH fluctuates over the course of the day as the processes of eating, rumination, ruminal digestion, and VFA absorption occur. If ruminal pH decreases below 5.2 for several hours, the ruminal acidosis is characterized as acute (Owens et al., 1998), because it can lead to metabolic or systemic acidosis

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requiring intervention. Ruminal acidosis is characterized as subacute (**SARA**) when ruminal pH decreases into a zone that is suboptimal for ruminal function (pH 5.2 to 6.0; Plaizier et al., 2008). Unlike acute metabolic acidosis, SARA occurs in bouts with pH recovering without intervention to baseline values within several minutes or hours. Blood pH is typically not affected by SARA (Krause and Oetzel, 2006).

The occurrence of acute acidosis in commercial dairy cows is low, whereas the prevalence of SARA is widespread. For example, surveys indicate that in Wisconsin, 19 to 26% of lactating cows fed TMR diets experienced SARA (Garrett et al., 1997; Oetzel et al., 1999), and in Ireland, almost 50% of grazing cows from 12 herds experienced moderate to severe SARA (O'Grady et al., 2008). Subacute ruminal acidosis causes the dairy industry significant financial losses associated with lameness, treatment of sick animals, and decreased milk production (Krause and Oetzel, 2006).

Several risk factors predispose dairy cows to SARA. As discussed by Stone (2004), the main nutritional factors include the amount of OM fermented in the rumen, rate and extent of starch digestion in the rumen, concentration of NDF and forage NDF in the diet, and particle size of the TMR. Management and environmental factors that contribute to SARA include heat stress, overcrowding, component feeding, and inconsistent feed delivery (Stone, 2004). Susceptibility of cows to ruminal acidosis also depends on stage of lactation; cows in early lactation have a high incidence of SARA (Fairfield et al., 2007; Penner et al., 2007).

Several challenge models have been used to experimentally induce ruminal acidosis in cattle (Nagaraja and Titgemeyer, 2007). Most challenge models involve measuring changes in ruminal pH after feeding or intraruminally dosing animals with rapidly fermentable carbohydrates. These models facilitate the study of ruminal and systemic changes associated with acidosis while closely monitoring ruminal pH such that the experiment can be terminated should the acidosis become acute. Even though challenge models have been used successfully to induce SARA in lactating dairy cows (Krause and Oetzel, 2005; Gozho et al., 2007), it is not known whether diet and other risk factors affect the response of such cows to a ruminal acidosis challenge.

Furthermore, the effect of repeated acidosis challenges is unknown. Ruminal acidosis can decrease the absorptive capacity of the ruminal epithelium by causing abnormalities of ruminal papillae and rumenitis (McGavin and Morrill, 1976; McManus et al., 1977). Decreased absorptive capacity of the rumen as a result of a ruminal acidosis challenge may, thus, increase the severity of subsequent challenges. There is also the possibility that cows once subjected to a ruminal

acidosis challenge may alter their intake behavior to avoid further episodes of SARA. Much evidence exists to suggest that ruminants may alter their feeding behavior in response to a toxicosis (Provenza, 1995). Examples of this include cows tending to sort in favor of long forage particles when they experienced a nadir ruminal pH near 5 (Beauchemin and Yang, 2005). Similarly, Phy and Provenza (1998a,b) observed that when lambs were given diets high in barley and wheat, they preferred pellets with sodium bicarbonate to pellets with sodium chloride and increased their intake of sodium bicarbonate, respectively. Phy and Provenza (1998a) also observed a decreased preference of lambs for barley when barley was consumed too frequently or in excess.

The objective of our experiment was to determine whether lactating dairy cows that are at high (**HR**) or low (**LR**) risk for experiencing ruminal acidosis differ in their response to an acidosis challenge and whether this response changes with repeated acidosis challenges. We hypothesized that those cows at HR for ruminal acidosis would have a lower ruminal pH profile (i.e., pH curve) during baseline measurements and thus would experience a more severe bout of SARA when exposed to an acidosis challenge. To test whether the severity of SARA changes over time, the acidosis challenge was repeated during 3 periods. We hypothesized that both HR and LR cows would be increasingly reluctant to consume the grain challenge with repeated exposure and that this reluctance would minimize the severity of SARA invoked with each challenge.

## MATERIALS AND METHODS

### *Animals, Diet, and Experimental Design*

The experimental protocol was approved by the Animal Care Committee at the Lethbridge Research Centre (Alberta, Canada) before beginning the study. For the duration of the study, the dairy cows were cared for according to the guidelines of the Canadian Council on Animal Care (1993). For a minimum of 1 mo before starting the experiment, all cows received the standard lactation diet offered at the Dairy Unit.

The experiment was a completely randomized design with 2 groups (risk scenarios, HR vs. LR) and 3 periods corresponding to 3 repeated acidosis challenges. The acidosis risk scenarios related to the stage of lactation of the cows and their diet. The HR scenario consisted of dairy cows in early lactation fed a low-forage diet, and the LR scenario consisted of cows in midlactation fed a greater-forage diet. An acidosis challenge model was used to induce acidosis during each period.

**Table 1.** Ingredient composition of the experimental diets fed to lactating dairy cows at high and low risk for experiencing ruminal acidosis (DM basis)

Ingredient, % DM	Treatment	
	High risk	Low risk
Barley silage <sup>1</sup>	39.6	52.7
Chopped grass-legume hay <sup>2</sup>	5.5	7.4
Corn grain, dry-rolled	8.0	2.4
Barley grain, steam-rolled	19.6	10.6
Canola oil	1.4	2.6
Pelleted supplement	25.9	24.3
Ground barley	3.67	3.35
Ground corn	0.13	0.12
Canola meal, heat-treated (Alberta Gold) <sup>3</sup>	5.38	5.06
Treated soybean meal (SoyPass) <sup>4</sup>	5.35	5.02
Beet pulp	3.09	2.90
Corn gluten meal	4.42	4.15
Molasses, beet	1.67	1.57
Limestone	0.45	0.43
Dicalcium phosphate	0.70	0.66
Sodium bicarbonate	0.39	0.39
Flavor (Anise 422 Powder) <sup>5</sup>	0.01	0.01
Trace mineralized salt <sup>6</sup>	0.64	0.64

<sup>1</sup>Chemical composition of barley silage (DM basis) was  $49.0 \pm 1.28\%$  NDF,  $32.9 \pm 0.50$  ADF, and  $12.7 \pm 0.26\%$  CP.

<sup>2</sup>Chemical composition of hay (DM basis) was  $52.2 \pm 2.43\%$  NDF,  $37.9 \pm 2.24$  ADF, and  $15.8 \pm 0.60\%$  CP.

<sup>3</sup>Canbra Foods Ltd., Lethbridge, Alberta, Canada.

<sup>4</sup>LignoTech USA Inc., Rothschild, Wisconsin.

<sup>5</sup>Canadian Bio-Systems Inc., Calgary, Alberta, Canada.

<sup>6</sup>Contained 58.8% NaCl, 16.0% Dynamate (Pitman Moore Inc., Mundelein, IL; 18% K, 11% Mg, 22% S, 1,000 mg of Fe/kg), 2% ZnSO<sub>4</sub>, 2.4% MnSO<sub>4</sub>, 0.01% CoSO<sub>4</sub>, 0.009% Na<sub>2</sub>SeO<sub>3</sub>, 0.012% ethylenediamine dihydroiodide, 0.8% CuSO<sub>4</sub>, 2,000,000 IU/kg of vitamin A, 200,000 IU/kg of vitamin D, and 2,000 IU/kg of vitamin E.

Eight multiparous Holstein cows (mean BW  $\pm$  SD,  $688 \pm 55.3$  kg), that had been previously ruminally cannulated, were assigned to 2 groups (HR vs. LR) based on their milk production and DIM at the start of the study. The HR cows were on average  $60 \pm 19.4$  DIM producing  $40 \pm 3.1$  kg/d of milk. These cows were assigned to a high-energy, low-forage diet. The LR cows were on average  $105 \pm 85.7$  DIM producing  $34 \pm 1.6$  kg/d and were assigned to a high-forage diet. Thus, diets were intentionally confounded with DIM (and milk production) to represent 2 different ruminal acidosis risk scenarios.

The forage-to-concentrate ratio (DM basis) of the diet fed to HR cows was 45:55, and the ratio of the diet fed to LR cows was 60:40 (Table 1). Consequently, the diet fed to the HR cows was greater in NFC and lower in fiber (NDF and ADF) than the diet fed to the LR cows (Table 2). Both experimental diets were formulated using the Cornell-Penn-Miner System (CPM Dairy, Version 3.0.4a; University of Pennsylvania, Kennett Square, PA, Cornell University, Ithaca, NY, and William H. Miner Agricultural Research Institute, Chazy,

**Table 2.** Nutrient composition and particle size distribution of the experimental diets fed to lactating dairy cows at high and low risk of experiencing ruminal acidosis (means  $\pm$  SD)

Item	Treatment	
	High risk	Low risk
Chemical composition		
DM, %	$59.8 \pm 0.43$	$53.7 \pm 0.72$
CP, % of DM	$18.3 \pm 0.58$	$18.7 \pm 0.89$
NDF, % of DM	$34.1 \pm 1.87$	$40.1 \pm 0.39$
Forage NDF, % of NDF	67	80
ADF, % of DM	$22.2 \pm 1.69$	$26.3 \pm 0.56$
Starch, %	$29.7 \pm 1.29$	$21.4 \pm 0.96$
NFC, %	40.0	32.0
Predicted NE <sub>L</sub> , Mcal/kg of DM	1.60	1.49
Particle size distribution, % of DM		
>19 mm	8.7	14.3
8 to 19 mm	35.6	37.6
1.18 to 8 mm	44.4	39.7
<1.18 mm	11.3	8.5
pef <sub>8.0</sub>	0.443	0.519
pef <sub>1.18</sub>	0.887	0.916
peNDF <sub>8.0</sub> , <sup>1</sup> % of DM	10.6	19.4
peNDF <sub>1.18</sub> , <sup>1</sup> % of DM	21.1	34.3

<sup>1</sup>pef<sub>8.0</sub> = total proportion retained on the >19-mm and 8- to 19-mm sieves; pef<sub>1.18</sub> = the total proportion retained on the >19-mm, 8- to 19-mm, and 1.18- to 8-mm sieves; peNDF<sub>8.0</sub> and peNDF<sub>1.18</sub> = physically effective NDF determined as NDF content of TMR multiplied by pef<sub>8.0</sub> and pef<sub>1.18</sub>, respectively.

NY). The TMR was offered ad libitum 3 times daily at 0600, 1500, and 1800 h. Cows also had free access to water. The cows were housed in tie-stalls on rubber mats bedded with wood shavings and were milked twice daily at 0630 and 1600 h in their stalls.

### The Acidosis Challenge Model

After 2 wk of adaptation to their respective TMR, each cow was used in 3 consecutive 14-d experimental periods. Each period started with 3 baseline days (d -4, -3, -2 prechallenge) in which the cows had ad libitum access to the TMR. The day before the challenge (d -1, restricted feeding day), feed was restricted to 50% of the ad libitum intake measured the 3 previous days and offered in 2 meals at 0600 and 1500 h. In the morning of the challenge day, SARA was induced by feeding 4 kg (as-fed basis) of ground barley and wheat (1:1) at 0600 h for 1 h followed by TMR ad libitum. Any grain that was not consumed within the hour was removed and weighed before distributing the TMR. During the remaining days, cows once again received TMR ad libitum.

### Feed Intake and Composition

The weight of feed offered and refused was recorded daily throughout the experiment. Samples of both TMR



were collected on 5 d during each experimental period. One subsample was taken to measure the particle size distribution using the Penn State Particle Size Separator (Kononoff et al., 2003). The other subsample was stored at  $-20^{\circ}\text{C}$ , composited by period, and used later for chemical analysis.

The feed samples were dried at  $55^{\circ}\text{C}$  for 48 h and then ground to pass a 1-mm screen (Wiley standard model 4, Arthur H. Thomas Co., Philadelphia, PA). Analytical DM content of the samples was by drying at  $135^{\circ}\text{C}$  for 3 h (AOAC, 1995). Acid detergent fiber and NDF were analyzed using an Ankom 200/220 Fiber Analyzer (Ankom Technology Corporation, Fairport, NY). Heat-stable  $\alpha$ -amylase and sodium sulfite were used in the NDF procedure. The N content for CP calculation ( $\text{CP} = \text{N} \times 6.25$ ) was determined by flash combustion (Carlo Erba Instruments, Milan, Italy). Starch was determined enzymatically after gelatinization as described by Rode et al. (1999). Daily ort samples were collected and composited (volume basis) by cow and period and analyzed for DM.

### Ruminal pH

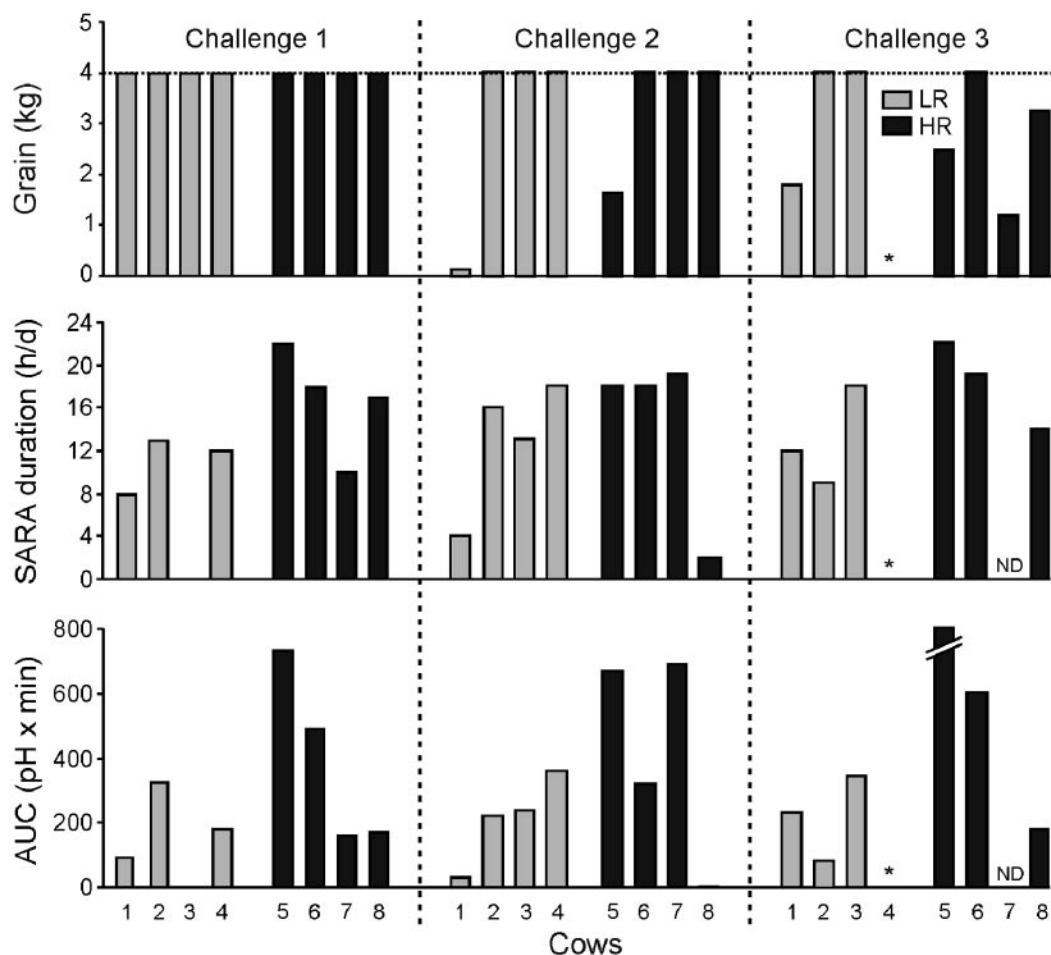
Ruminal pH was continuously measured for 9 of the 14 d during each experimental period using the Lethbridge Research Centre Ruminal pH Measurement System (Dascor, Escondido, CA) as described in detail by Penner et al. (2006). Ruminal pH readings were taken every 30 s. The standardization of the electrodes and the transfer of the data were carried out every 3 d as recommended by Penner et al. (2007). The ruminal pH data were averaged each minute and summarized daily as nadir pH, maximum pH, pH range (difference between nadir and maximum pH), and mean pH. The occurrence of SARA was determined using 2 thresholds: 5.8 for total SARA and 5.5 for severe SARA. These threshold values were used because  $\text{pH} < 5.8$  is harmful to ruminal cellulolytic bacteria (Russell and Wilson, 1996), whereas  $\text{pH} < 5.5$  is detrimental to the ruminal epithelium and VFA absorption (Gäbel et al., 2002). The duration (h/d) and total area ( $\text{pH} \times \text{h}$ , AUC) that pH was below each SARA threshold were calculated as a measure of the severity of ruminal acidosis. The AUC was calculated by adding the absolute value of negative deviations in pH from 5.5 or 5.8 for each 30-s interval. Furthermore, the frequency of daily bouts (no./d) and duration of those bouts (min/d) of total and severe SARA were calculated. A bout was defined to begin when ruminal pH was below the predefined threshold and ended when ruminal pH met or exceeded the threshold.

### Statistical Analysis

The ruminal pH data, summarized by day within period for each cow, were analyzed using the mixed model procedure of SAS (Release 9.1, SAS Institute Inc., Cary, NC). The restricted maximum likelihood method was used for estimating the variance components, and degrees of freedom were adjusted using the Kenward-Roger's option. The model included the fixed effects of the acidosis risk category of the cow (LR, HR), the challenge period (1, 2, 3), and the day of pH measurement (1 to 9), all 2-way interactions, and the 3-way interaction. Cow within risk category was considered a random effect, and challenge period and day were considered repeated measures. The variance-covariance error structure was unstructured by first-order autoregressive or unstructured by compound symmetry depending upon which model gave the lowest Akaike information criterion fit statistic.

Effects were considered different when  $P < 0.05$  and trends were discussed at  $P < 0.15$ . Period means were compared using an LSD test, and when risk  $\times$  period interactions ( $P < 0.05$ ) occurred, the comparisons were made within risk category. There was an effect of day ( $P < 0.001$ ) for all pH variables, and day  $\times$  treatment ( $P < 0.05$ ) and day  $\times$  challenge period ( $P < 0.05$ ) interactions occurred for most pH variables, with the exception of bout frequency and duration, which had no day  $\times$  challenge period ( $P > 0.05$ ) interactions. Further, there were no day  $\times$  treatment  $\times$  challenge period interactions for any of the pH variables ( $P > 0.05$ ). Thus, the effect of day was examined by risk category and by period (with the exception of the bout frequency and duration data) by removing the risk or period effects from the model. The effects of days within challenge period or within risk category were examined using contrast statements; baseline pH measurements (d  $-4$  to  $-2$ ) were compared with d 1 postchallenge (0 to 24 h postchallenge), d 2 postchallenge (24 to 48 h postchallenge), and to the recovery phase (d 3 to 5 postchallenge).

To test whether HR cows suffered from a greater proportion of long ruminal acidosis bouts, we used the univariate procedure of SAS to output the 0, 5, 10, 25, 50, 75, 90, 95, 99, and 100% quantiles of the bout duration data, summarized by period for each cow. A base-10 logarithm transformation was used to normalize these data, and then the data were analyzed using the mixed model procedure of SAS (Release 9.1, SAS Institute Inc.). The model included the fixed effects of risk category, challenge period and quantile, all 2-way interactions, and the 3-way interaction of risk  $\times$  challenge period  $\times$  quantile. Cow within period



**Figure 1.** Intake of the grain challenge and the corresponding subacute acidosis (SARA; pH <5.8) variables on the challenge day for individual cows (AUC = area between pH 5.8 and the pH profile of the cow). ND = no data because of logger failure; LR = low risk; HR = high risk. \* = cow was removed from the study due to poor health.

was considered a random effect, and challenge period and quantile were considered repeated measures. The variance-covariance error structure was unstructured by first-order autoregressive based upon the lowest Akaike information criterion fit statistic.

Means are presented for diet composition variables (Tables 1 and 2), and least squares means are presented for all other results. For several pH variables, the complex model failed to converge, and a simplified model was used. Use of a simplified model is indicated in footnotes in the tables.

## RESULTS

One of the LR cows was diagnosed with hemorrhagic bowel syndrome during period 3 and was removed from the study. Thus, only the data from periods 1 and 2 for that cow were included in the analysis. In addition, a

pH logger failed during period 3 for one of the HR cows, resulting in missing data in period 3.

The grain challenge was consumed in its entirety within 1 h by all cows during the first challenge (Figure 1). However, only 6 of the 8 cows consumed the entire grain challenge in period 2, and only 3 of the 7 cows consumed the entire grain challenge in period 3. All but one of the LR cows in one period (period 1) experienced SARA immediately after the acidosis challenge.

Dry matter intake averaged 21.2 kg/d during the study and was not affected by acidosis risk or period (Table 3). However, DMI was affected by day within challenge period, but the effect of day was not consistent among periods (day  $\times$  period,  $P = 0.009$ ). During the first period, DMI was similar among days, with only a trend ( $P = 0.08$ ) for lower DMI during recovery compared with baseline days (Table 4). During the second period, DMI was actually greater on d 2 postchal-

**Table 3.** Effects of repeated acidosis challenge periods (1, 2, or 3) on ruminal pH measured in lactating dairy cows at high and low risk for experiencing ruminal acidosis<sup>1</sup>

Item	High risk			Low risk			SEM <sup>3</sup>	<i>P</i> -value <sup>2</sup>		
	1	2	3	1	2	3		R	P	R × P
DMI, kg/d	21.2	22.4	21.3	21.1	21.8	19.4	1.94	0.50	0.21	0.81
Mean pH	5.94 <sup>a</sup>	5.85 <sup>a</sup>	5.63 <sup>b</sup>	6.32 <sup>a</sup>	6.20 <sup>a</sup>	6.10 <sup>b</sup>	0.109	0.02	0.01	0.68
Nadir pH	5.34 <sup>a</sup>	5.17 <sup>b</sup>	4.88 <sup>b</sup>	5.69 <sup>a</sup>	5.51 <sup>b</sup>	5.40 <sup>b</sup>	0.146	0.04	0.01	0.63
Maximum pH	6.59 <sup>a</sup>	6.50 <sup>ab</sup>	6.39 <sup>b</sup>	6.90 <sup>a</sup>	6.82 <sup>ab</sup>	6.76 <sup>b</sup>	0.093	0.02	0.046	0.81
Range	1.25 <sup>a</sup>	1.33 <sup>a</sup>	1.53 <sup>b</sup>	1.21 <sup>a</sup>	1.31 <sup>a</sup>	1.38 <sup>b</sup>	0.077	0.47	0.001	0.39
SARA <sup>4</sup> <5.8, h/d	7.9 <sup>a</sup>	10.1 <sup>ab</sup>	13.9 <sup>b</sup>	2.4 <sup>a</sup>	3.4 <sup>ab</sup>	4.7 <sup>b</sup>	2.08	0.03	0.02	0.37
SARA <5.5, h/d	2.9 <sup>a</sup>	5.4 <sup>a</sup>	9.5 <sup>b</sup>	0.8 <sup>a</sup>	1.2 <sup>a</sup>	1.8 <sup>b</sup>	1.74	0.02	0.04	0.14
AUC <sup>5</sup> <5.8, pH units × min/d	136 <sup>a</sup>	231 <sup>a</sup>	475 <sup>b</sup>	37 <sup>a</sup>	50 <sup>a</sup>	93 <sup>a</sup>	88.3	0.003	0.02	0.11
AUC <sup>6</sup> <5.5, pH units × min/d	42 <sup>a</sup>	91 <sup>a</sup>	291 <sup>b</sup>	8	10	23	—	—	—	—

<sup>a,b</sup>Within a row and risk category, means for challenge periods without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Ruminal pH was measured for 9 d in each challenge period.

<sup>2</sup>Effect of day ( $P < 0.001$ ) for all variables except AUC. Day × treatment ( $P < 0.05$ ) and challenge period × day ( $P < 0.05$ ) for most variables. No day × treatment × challenge period interactions for any variable ( $P > 0.05$ ). R = acidosis risk; P = challenge period.

<sup>3</sup>Greatest SEM is shown.

<sup>4</sup>SARA = subacute ruminal acidosis measured as duration below the pH threshold (5.5 or 5.8).

<sup>5</sup>AUC = area between the pH threshold (5.5 or 5.8) and the pH profile of the cow.

<sup>6</sup>The complex model failed to converge. The least squares means are for a simplified model that examined the effect of day and period within acidosis risk. Period effects for ( $P = 0.08$ ) high-risk cows and day effects ( $P = 0.01$ ) for low-risk cows, with no other effects ( $P > 0.15$ ). SEM for high-risk cows = 100.0; SEM for low-risk cows = 11.6.

lence and during recovery compared with the baseline days. During the third period, DMI was lower during recovery compared with baseline. Furthermore, DMI was more variable for cows in both risk categories during the third challenge compared with the other 2 challenges.

The average pH profiles for LR and HR cows in each challenge period are shown in Figure 2 with pH variables summarized by risk category and challenge period in Table 3. As intended, HR cows had lower ruminal pH profiles compared with LR cows (Figure 2). Specifically, mean pH (5.81 vs. 6.21), nadir pH (5.13 vs. 5.53), and maximum pH (6.49 vs. 6.83) were all lower for HR cows (Table 3). The HR cows also experienced a greater degree (duration and AUC) of SARA during the experiment. Despite the lower profiles of HR cows, the pH range was similar for both risk categories (HR vs. LR: 1.37 vs. 1.30 pH units), indicating that the ruminal

pH for both groups fluctuated each day by a similar amount.

The pH profiles of cows in both risk categories decreased with each subsequent acidosis challenge period (Table 3). There were no risk category × challenge period effects indicating that the extent of the decline in mean, nadir, and maximum pH with each challenge was similar for both risk categories. Because HR cows had lower pH during challenge period 1, they experienced more severe SARA (duration and AUC) than LR cows with each subsequent challenge. By challenge period 3, severe SARA (pH <5.5) occurred in HR cows for 9.5 h/d when averaged over the entire period, compared with 1.8 h/d for LR cows. The AUC markedly increased with repeated challenges for HR cows, whereas there tended to be less change for LR cows (risk category × challenge period effects,  $P = 0.11$ ). Thus, LR cows were less susceptible to SARA during the study.

**Table 4.** Effect of day relative to acidosis challenge on DMI of lactating dairy cows during 3 challenge periods<sup>1</sup>

Challenge period	Day relative to acidosis challenge <sup>2</sup>				SEM <sup>3</sup>	Contrast		
	Baseline (B)	d 1 postchallenge (D1)	d 2 postchallenge (D2)	Recovery (R)		B vs. D1	B vs. D2	B vs. R
1	21.8	22.3	20.9	20.9	0.88	0.43	0.21	0.08
2	21.9	20.8	23.4	23.1	0.74	0.12	0.05	0.03
3	22.2	20.0	19.3	19.7	2.26	0.22	0.18	0.048

<sup>1</sup>Least squares means averaged over the high- and low-risk acidosis scenarios.

<sup>2</sup>Baseline days = mean of d -4, -3, and -2 before acidosis challenge; d 1 postchallenge = 0 to 24 h after acidosis challenge; d 2 postchallenge = 24 to 48 h after acidosis challenge; recovery days = mean of d 3, 4, and 5 after acidosis challenge.

<sup>3</sup>Greatest SEM is shown.

**Table 5.** Effects of repeated acidosis challenge periods (1, 2, or 3) on frequency and duration of acidosis bouts measured in lactating dairy cows at high and low risk of experiencing ruminal acidosis<sup>1</sup>

Ruminal acidosis	High risk			Low risk			SEM <sup>3</sup>	P-value <sup>2</sup>		
	1	2	3	1	2	3		R	P	R × P
Bout frequency (no./d)										
pH <5.8	15.4	11.8	10.7	4.2 <sup>a</sup>	5.4 <sup>ab</sup>	10.3 <sup>b</sup>	3.22	0.005	0.52	0.036
pH <5.5	6.8	10.8	9.1	1.9	2.4	4.7	3.37	0.02	0.15	0.38
Bout duration (min/bout)										
pH <5.8	42.6	176.1	209.1	19.5	34.9	27.1	82.4	0.026	0.12	0.18
pH <5.5 <sup>4</sup>	17.3 <sup>a</sup>	26.4 <sup>a</sup>	116.5 <sup>b</sup>	11.4	17.3	12.9	23.6	0.14	0.01	0.01

<sup>a,b</sup>Within a row and risk category, means for challenge periods without a common superscript differ ( $P < 0.05$ ).

<sup>1</sup>Ruminal pH was measured for 9 d in each challenge period.

<sup>2</sup>Effect of day ( $P < 0.15$ ) for all variables. Day × treatment effect ( $P < 0.1$ ) for bout frequencies at pH <5.8 and pH <5.5. No day × treatment × challenge period interactions for any variable ( $P > 0.05$ ). No challenge period × day interactions for any variable. Day × treatment × challenge period effect ( $P = 0.05$ ) only for bout frequency at pH <5.5. R = acidosis risk; P = challenge period.

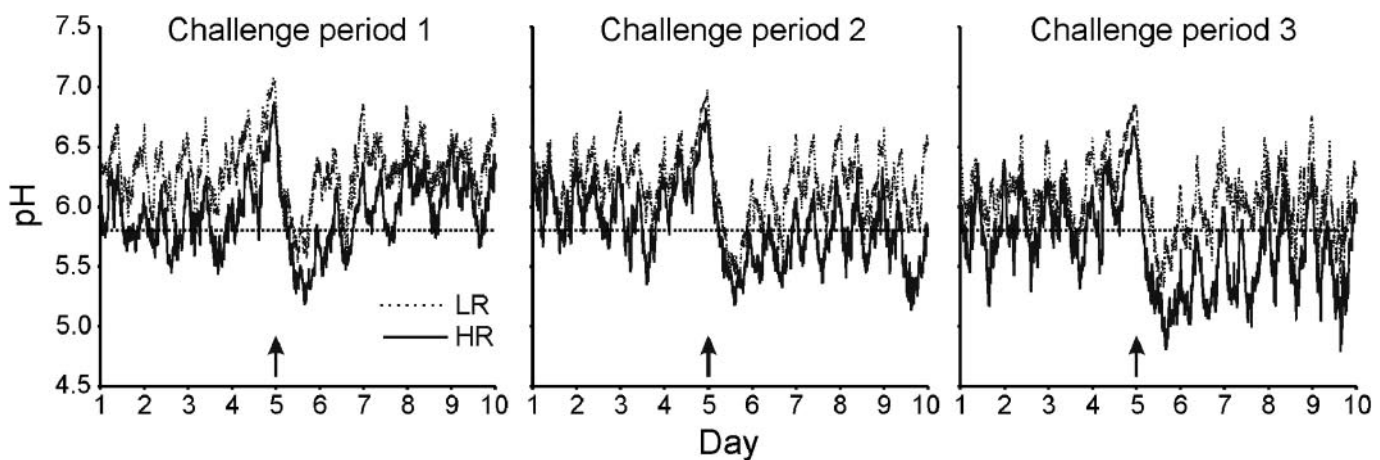
<sup>3</sup>Greatest SEM is shown.

<sup>4</sup>The complex model failed to converge. The least squares means were produced using a simplified model that examined the effect of acidosis risk and period, without the effect of day in the model.

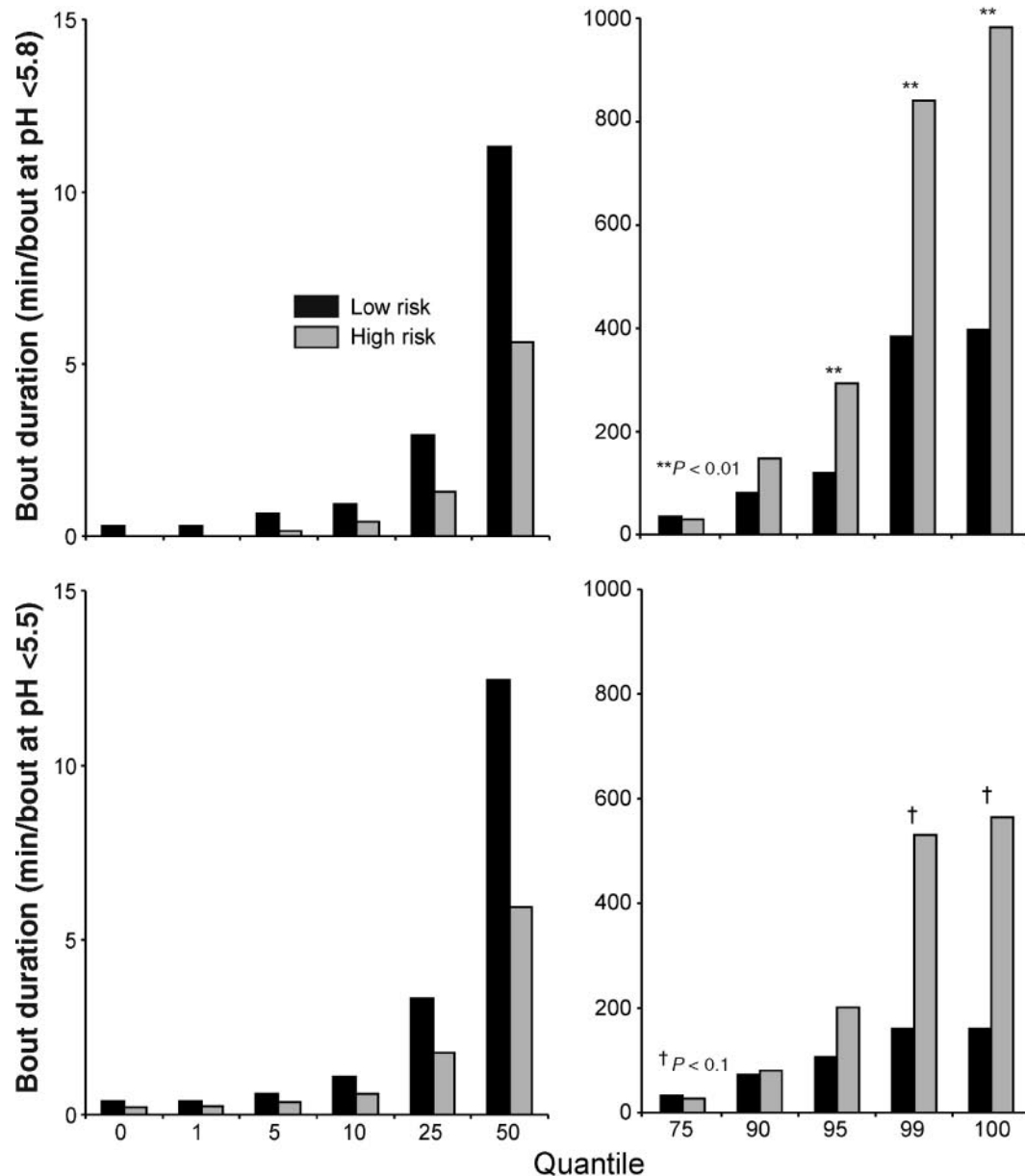
Differences in ruminal pH between the HR and LR cows were moderated by changes in the frequency and duration of SARA bouts (Table 5). During the experiment, HR cows experienced more frequent bouts of SARA at pH <5.8 (12.6 vs. 6.6 bouts/d) and pH <5.5 (8.9 vs. 3.0 bouts/d) compared with LR cows. These bouts were also much longer for HR cows (142.6 vs. 27.2 min/bout at pH <5.8; 52.6 vs. 12.9 min/bout at pH <5.5). The SARA bouts (pH <5.8) tended ( $P = 0.12$ ) to become longer with each subsequent acidosis challenge. The quantile plots of SARA bout duration at pH <5.8 and 5.5 (Figure 3) indicate that differences in bout duration between HR and LR cows were caused mainly by the very long bouts, which represented only a small

proportion of the total bouts. Indeed, 90% of the bouts at pH <5.8 were similar in length, with only the top 5% being extremely longer for the HR cows. Similarly, at pH <5.5, only the top 1% of bouts were longer for the HR cows.

Day of measurement affected all pH variables ( $P < 0.001$ ). For most pH variables, the effects of day depended on the risk category of the cows (day × risk;  $P < 0.05$ ) and challenge period (challenge period × day;  $P < 0.05$ ). However, there were no day × risk × challenge period interactions for nearly all variables measured ( $P > 0.05$ ). Thus, the means are presented by day and risk in Tables 6 and 7 and by day and challenge period in Table 8.



**Figure 2.** Ruminal pH in high- and low-risk cows subjected to 3 acidosis challenge periods. Each challenge period consisted of 3 baseline days (d 1, 2, and 3 of the period corresponding to d -4, -3, and -2 relative to the challenge), a feed restriction day (d 4 of the period corresponding to d -1 relative to the challenge), d 1 postchallenge (d 5, 0 to 24 h postchallenge), d 2 postchallenge (d 6, 24 to 48 h postchallenge), and 3 recovery days (d 7, 8, 9 of the period corresponding to d 3, 4, and 5 postchallenge). The arrow indicates the grain challenge. LR = low risk; HR = high risk.



**Figure 3.** Quantile plots for duration of acidosis bouts below thresholds of pH <5.8 (SEM = 88.8) and <5.5 (SEM = 83.2) for high and low acidosis risk cows.

Feed restriction the day before administering the acidosis challenge caused ruminal pH to gradually increase (Figure 2). The pH then plummeted immediately after the grain challenge was offered. Ruminal pH remained very low on d 1 postchallenge for all cows, but LR cows began their recovery from the acidosis challenge more quickly than HR cows (Table 6). There was evidence that recovery had begun on d 2 postchallenge for LR cows, but that was not the case for HR cows. For example, for LR cows, the duration of SARA at pH <5.5 was restored to baseline levels on d 2 postchallenge un-

like for HR cows. As expected, the duration of acidosis bouts increased immediately after the grain challenge for both HR and LR cows (Table 7). The frequency of acidosis bouts also increased, except bouts under pH <5.8 for HR cows. These cows experienced fewer, but longer, bouts under pH <5.8 in the days after the challenge.

The effect of administering an acidosis challenge on the decline in ruminal pH and subsequent recovery depended upon the challenge period (Table 8). With each successive challenge, the decrease in pH on the chal-



**Table 6.** Ruminal pH before, during, and after acidosis challenges in lactating dairy cows at high and low risk of experiencing ruminal acidosis<sup>1</sup>

Item	Day relative to acidosis challenge <sup>2</sup>				SEM	Contrast		
	Baseline (B)	d 1 postchallenge (D1)	d 2 postchallenge (D2)	Recovery (R)		B vs. D1	B vs. D2	B vs. R
High acidosis risk								
Mean pH	5.88	5.56	5.59	5.77	0.118	0.006	0.02	0.34
Nadir pH	5.21	4.89	5.01	5.07	0.143	0.04	0.21	0.37
Maximum pH	6.47	6.67	6.24	6.42	0.104	0.03	0.02	0.61
SARA <sup>3</sup> <5.8, h/d	9.2	16.1	16.2	10.7	2.87	0.001	0.003	0.45
SARA <5.5, h/d	3.3	10.9	11.0	6.9	2.22	0.003	0.006	0.16
AUC <sup>4</sup> <5.8, pH units × min/d	23	193	60	47	21.0	<0.001	0.15	0.29
AUC <5.5, pH units × min/d	5	57	15	12.5	9.1	<0.001	0.35	0.43
Low acidosis risk								
Mean pH	6.25	5.88	6.08	6.23	0.075	<0.001	0.04	0.77
Nadir pH	5.60	5.20	5.33	5.55	0.122	0.004	0.07	0.72
Maximum pH	6.80	6.95	6.78	6.78	0.064	0.03	0.83	0.84
SARA <5.8, h/d	1.9	11.3	4.8	2.9	1.0	<0.001	0.02	0.33
SARA <5.5, h/d	0.5	4.5	1.2	1.1	0.51	<0.001	0.22	0.25
AUC <5.8, pH units × min/d	155	516	442	303	142.0	0.003	0.03	0.26
AUC <5.5, pH units × min/d	45	300	211	205	104.7	0.06	0.21	0.15

<sup>1</sup>Least squares means are averaged over the 3 acidosis challenge periods. Period × day interactions ( $P > 0.05$ ) for the variables presented.

<sup>2</sup>Baseline days = mean of d -4, -3, and -2 before acidosis challenge; d 1 postchallenge = 0 to 24 h after acidosis challenge; d 2 postchallenge = 24 to 48 h after acidosis challenge; recovery days = mean of d 3, 4, and 5 after acidosis challenge.

<sup>3</sup>SARA = subacute ruminal acidosis measured as duration below the pH threshold (5.5 or 5.8).

<sup>4</sup>AUC = area between the pH threshold (5.5 or 5.8) and the pH profile of the cow.

challenge day was more severe: pH decreased to a lower nadir and the duration of SARA (at both thresholds) increased. During the first challenge period, the pH increased on the day postchallenge such that mean,

maximum, and nadir pH were actually greater, with less SARA, during the recovery phase. In challenge periods 2 and 3, these pH variables were not restored to baseline values by the recovery phase.

**Table 7.** Frequency and duration of acidosis bouts before, during, and after acidosis challenges in lactating dairy cows at high and low risk of experiencing ruminal acidosis<sup>1</sup>

	Day relative to acidosis challenge <sup>2</sup>					Contrast		
Item	Baseline (B)	d 1 postchallenge (D1)	d 2 postchallenge (D2)	Recovery (R)	SEM	B vs. D1	B vs. D2	B vs. R
High acidosis risk								
Bout frequency (no./d)								
pH <5.8	17.3	8.6	7.4	12.4	3.13	0.027	0.016	0.15
pH <5.5	7.4	14.5	12.7	9.1	2.74	0.018	0.07	0.45
Bout duration (min/bout)								
pH <5.8 <sup>3</sup>	39.9	276.3	313.4	155.3	85.7	0.01	0.003	0.07
pH <5.5 <sup>3</sup>	21.7	155.5	51.4	45.5	45.8	0.007	0.54	0.49
Low acidosis risk								
Bout frequency (no./d)								
pH <5.8	4.3	12.7	11.5	7.3	2.47	0.007	0.019	0.19
pH <5.5	1.1	9.9	3.1	3.5	1.72	<0.001	0.35	0.18
Bout duration (min/bout)								
pH <5.8	22.5	56.8	32.0	19.7	8.31	<0.001	0.22	0.6
pH <5.5	11.3	25.1	21.6	14.8	6.11	0.01	0.047	0.32

<sup>1</sup>Least squares means are averaged over the 3 acidosis challenges periods. No period × day interactions ( $P > 0.05$ ) for the variables presented.

<sup>2</sup>Baseline days = mean of d -4, -3, and -2 before acidosis challenge; d 1 postchallenge = 0 to 24 h after acidosis challenge; d 2 postchallenge = 24 to 48 h after acidosis challenge; recovery days = mean of d 3, 4, and 5 after acidosis challenge.

<sup>3</sup>The complex model failed to converge. The least squares means were produced using a simplified model that examined the effect of sampling day within acidosis risk scenario, without the effect of period in the model.

**Table 8.** Challenge period  $\times$  day of sampling interaction for ruminal pH variables measured before, during, and after an acidosis challenge in lactating dairy cows<sup>1</sup>

Item	Day relative to acidosis challenge <sup>2</sup>					Contrast		
	Baseline (B)	d 1 postchallenge (D1)	d 2 postchallenge (D2)	Recovery (R)	SEM <sup>3</sup>	B vs. D1	B vs. D2	B vs. R
Challenge period 1								
Mean pH	6.07	5.82	6.01	6.24	0.079	<0.001	0.42	<0.001
Nadir pH	5.42	5.19	5.30	5.72	0.110	0.03	0.23	<0.001
Maximum pH	6.62	6.86	6.72	6.74	0.062	<0.001	0.08	0.004
SARA <sup>4</sup> <5.8, h/d	5.8	12.2	8.0	2.4	1.40	<0.001	0.13	0.002
SARA <5.5, h/d	1.9	6.2	3.1	0.51	1.05	<0.001	0.28	0.09
AUC <sup>5</sup> <5.8, pH units × min/d	94	269	124	60	42.8	<0.001	0.49	0.03
AUC <5.5, pH units × min/d	30	100	26	3	21.1	0.007	0.87	0.12
Challenge period 2								
Mean pH	6.11	5.75	5.84	5.98	0.090	<0.001	<0.001	0.01
Nadir pH	5.47	5.07	5.15	5.25	0.115	<0.001	0.002	0.002
Maximum pH	6.67	6.81	6.46	6.58	0.067	0.01	<0.001	0.02
SARA <5.8, h/d	4.5	13.4	10.7	7.4	1.92	<0.001	<0.001	<0.001
SARA <5.5, h/d	1.6	7.4	6.6	3.5	1.48	<0.001	<0.001	0.046
AUC <5.8, pH units × min/d	66	315	252	162	69.8	<0.001	0.007	0.047
AUC <5.5, pH units × min/d	15	123	94	63	44.1	0.02	0.09	0.15
Challenge period 3								
Mean pH	6.04	5.59	5.69	5.81	0.115	<0.001	<0.001	<0.001
Nadir pH	5.33	4.90	5.09	4.99	0.180	<0.001	0.04	<0.001
Maximum pH	6.64	6.78	6.41	6.54	0.077	0.02	<0.001	0.03
SARA <5.8, h/d	5.9	15.8	12.8	12.3	2.50	<0.001	<0.001	<0.001
SARA <5.5, h/d	2.4	9.0	7.7	6.6	2.40	<0.001	0.003	0.002
AUC <5.8, pH units × min/d	107	470	364	302	119.3	<0.001	0.008	0.005
AUC <5.5, pH units × min/d	34	247	183	145	82.1	0.006	0.048	0.03

<sup>1</sup>Least squares means are averaged over high- and low-risk scenarios.

<sup>2</sup>Baseline days = mean of d -4, -3, and -2 before acidosis challenge; d 1 postchallenge = 0 to 24 h after acidosis challenge; d 2 postchallenge = 24 to 48 h after acidosis challenge; recovery days = mean of d 3, 4, and 5 after acidosis challenge.

<sup>3</sup>Greatest SEM is shown.

<sup>4</sup>SARA = subacute ruminal acidosis measured as duration below the pH threshold (5.5 or 5.8).

<sup>5</sup>AUC = area between the pH threshold (5.5 or 5.8) and the pH profile of the cow.

## DISCUSSION

The HR and LR cows responded in a similar manner to an acidosis challenge, with ruminal pH plummeting immediately after administration of the challenge. The extent to which pH decreased was similar for both risk categories, but because HR cows had a lower baseline pH profile before the challenge, the severity of the SARA arising from the challenge (measured as duration and AUC under pH thresholds of 5.8 and 5.5) was greater for HR cows than for LR cows. Thus, cows at a high risk for ruminal acidosis because of stage of lactation or diet, or both, are more likely to experience severe SARA as a result of feeding mismanagement.

We also demonstrated that the severity of SARA resulting from a challenge worsens with repeated challenges even though some cows in both risk categories became increasingly reluctant to consume the grain challenge. Contrary to our expectations, avoidance did not minimize the severity of SARA invoked with each challenge. To our knowledge, this is the first report of cows participating in multiple acidosis challenges in

which cows were permitted to recover between challenges. Krause and Oetzel (2005) subjected cows to 2 acidosis challenges, but these were on 2 consecutive days. Keunen et al. (2002) imposed 2 consecutive challenges separated by 1 wk, but these challenges failed to induce SARA as we have defined it.

### The Acidosis Challenge Model

The acidosis challenge model succeeded in causing an extended period of SARA in both HR and LR cows. This extended period of SARA was characterized by a nadir pH of 4.89 in HR cows and 5.20 in LR cows, when averaged across the 3 challenges. Because the nadir pH was lower for the HR cows, the bouts of ruminal acidosis after the challenge lasted longer for HR cows. Ruminal acidosis increasingly worsened with each successive challenge.

Several challenge models have been used previously in research to induce both acute and SARA in cattle, as reviewed by Nagaraja and Titgemeyer (2007). It can be difficult to cause SARA without incurring acute meta-

bolic acidosis requiring intervention. Most challenge models consist of a fasting period followed by carbohydrate loading either by feeding a source of rapidly fermentable carbohydrate or by dosing it intraruminally. Fasting before offering grain helps ensure that consumption of the rapidly fermentable carbohydrate source occurs. The results from our study, however, indicate that the fast is also implicated in the pH decrease that occurs after the grain challenge. For example, some cows (e.g., cow 5, Figure 1) avoided consuming the challenge grain allocation, yet SARA still occurred. Regardless of grain intake, all cows rapidly consumed the TMR offered after the challenge. Feed restriction may have destabilized the ruminal microbial flora due to starvation of some bacteria (Van Kessel and Russell, 1997). Destabilization, combined with rapid TMR intake, may have contributed to the decrease in pH on the challenge day even when cows avoided consuming the challenge grain.

The acidosis challenge model used in our study was based on that used with dairy cows by Krause and Oetzel (2005), although the grain was mixed into the TMR in that study. Feeding the grain separately from the TMR allowed us to determine whether the intake behavior of the cow changed with repeated acidosis challenges. Although all cows consumed the entire grain allotment during the first challenge period, the number of cows that refused some portion of the grain progressively increased in subsequent periods (Figure 1), indicating that avoidance increased with repeated exposure. This observation corroborates the study by Phy and Provenza (1998a), in which lambs fed barley too frequently or in excess decreased their preference for barley, and the study by Keunen et al. (2002), in which dairy cows experiencing SARA preferred long hay rather than pelleted forage. Furthermore, avoidance of the grain challenge supports the theory that ruminants will alter their feed intake to correct imbalances in the ruminal environment (Provenza, 1995). Evidence for this theory includes studies that report sheep preferred a diet supplemented with sodium bicarbonate when offered diets considered likely to cause SARA (Cooper et al., 1996; Phy and Provenza, 1998b). Further evidence is given by studies that show that dairy cows will selectively sort through a TMR and consume long particles when fed diets that lower ruminal pH (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006). However, others (Keunen et al., 2003; Paton et al., 2006) have reported that cattle experiencing ruminal acidosis did not consume sodium bicarbonate, when offered free choice, in quantities that would elevate rumen pH. The results from our study lend much support to the theory that animals will alter their feed consumption to correct ruminal imbalances, but our study also shows that

this change in behavior does not necessarily attenuate the effects of SARA.

### **Acidosis Risk Scenarios**

The HR acidosis scenario was created by feeding a low-forage diet (45% of DM) to cows in early lactation, whereas the LR acidosis scenario consisted of a high-forage diet (60% of DM) fed to cows in midlactation. The diets provided a range in forage to concentrate ratio typical of rations fed to lactating dairy cows in North America. The lower pH profiles of the HR cows were expected based on the earlier stage of lactation of these cows (Fairfield et al., 2007; Penner et al., 2007) and the lower fiber, greater NFC contents of the diet (Zebeli et al., 2008). The greater acidosis risk of cows in early lactation is thought to be related to the relatively short adaptation time that these cows have had to the change in diet composition before and after parturition (Penner et al., 2007).

Fairfield et al. (2007) reported that SARA increased dramatically after parturition in dairy cows fed a lactation diet consisting of 54% forage DM (34% NDF and 39% NFC, DM basis). During the first week after calving, mean pH averaged 6.19 and time below pH 6.0 and pH 5.6 were 7.3 and 1.9 h/d, respectively. During the sixth week after calving, these were 6.36, 3.4, and 0.9 h/d, respectively. Penner et al. (2007) also reported that the incidence of SARA was high in early lactation cows. In that study, first-lactation cows were fed a lactation diet containing 47% forage DM (29% NDF, DM basis), and mean pH averaged 5.98, nadir pH averaged 5.37, and pH remained below 5.8 for 7.7 h/d the first 8 wk postpartum.

The occurrence of SARA is also highly correlated to intake of dietary physically effective NDF (Yang and Beauchemin, 2006). Physically effective NDF (**peNDF**) is a measure that combines the physical characteristics of fiber (e.g., particle length) and NDF content. Increasing the peNDF intake of cows by increasing forage proportion in the diet and the chop length of forages are often the major strategies used to decrease the risk of SARA in dairy cows. In our study, cows in the 2 risk scenarios received diets that varied substantially in peNDF content (Table 2). The peNDF intake was increased by increasing the proportion of forage in the diet, which increased NDF content and decreased NFC content. We did not vary particle size of the forage, but particle size of the TMR varied as a result of changing the proportion of forage in the TMR. Thus, the HR scenario diet supplied less peNDF, which would have contributed to the lower pH profiles of the HR cows. Similarly, Yang and Beauchemin (2007) reported that lactating dairy cows experienced about 11.5 h/d of pH

<5.8 when fed a diet (35% forage, 30.5% NDF, 16% forage-NDF, DM basis) containing <10% peNDF (calculated using 2 sieves as was done in the present study and reported in Table 2).

Our study indicates that regardless of stage of lactation or diet, dairy cows are susceptible to SARA when challenged. A challenge model was used in our study to induce SARA, but in commercial feeding operations, a similar effect could arise as a result of feeding mismanagement such as the overprocessing of feeds, errors in TMR mixing, or fluctuating the amount and timing of feed delivery (Stone, 2004; Krause and Oetzel, 2006). Our study demonstrates that the episode of SARA that is likely to occur as a result of feeding management will be more severe if cows have a lower baseline pH because of risk factors such as their diet or stage of lactation.

### ***Effects of Repeated Challenges***

The increasing severity of SARA with repeated challenges may have been related to the relatively short recovery phase provided to the cows between challenge periods. After the first challenge, the ruminal pH recovered and actually exceeded baseline values, indicating that the recovery phase was adequate for a single challenge. Full recovery did not, however, occur within the allotted time after the second and third challenges. Increased severity of SARA and failure to fully recover may have resulted in part from instability of the ruminal microflora (Nagaraja and Titgemeyer, 2007). Instability in the ruminal microbial populations would have occurred as a result of the low ruminal pH, the pH fluctuations, and the inconsistent delivery of nutrients arising from the challenge model (e.g., feed restriction followed by excessive supply of fermentable carbohydrates). Substrate deprivation causes bacteria to die (Wells and Russell, 1996), whereas low pH prevents growth of cellulolytic bacteria and favors the growth of acid-resistant species (Russell and Wilson, 1996).

Potentially decreased absorptive capacity of the ruminal epithelium (Harmon et al., 1985) may also have contributed to an accumulation of VFA in the rumen. Krehbiel et al. (1995) showed that a short-term, severe insult of acute acidosis decreased ruminal VFA absorption for an extended period of time, presumably caused by damage to the ruminal wall (McGavin and Morrill, 1976; McManus et al., 1977). Decreased absorptive capacity of the rumen as a result of an acidosis challenge may, thus, increase the severity of subsequent acidosis challenges. This may explain why the severity increased more for the HR cows who experienced dramatically longer bouts of ruminal acidosis as compared with the LR cows (Figure 3). In contrast, when given

a chance to adapt through slowly increasing the levels of concentrate offered, the absorption capacity of the rumen can actually be increased by up to 4-fold (Gäbel et al., 2002), reducing the accumulation of VFA in the rumen and subsequent risk of ruminal acidosis.

### ***Variability Among Cows***

The cows in this study varied considerably both in terms of their intake of the grain challenge and the extent of SARA that resulted (Figure 1). Variability in grain challenge consumption is the reason that many researchers have opted to dose it intraruminally (Nagaraja and Titgemeyer, 2007). Dosing intraruminally would allow for testing the effect of the substrate on ruminal acidosis, but it does not account for the behavioral response of the cow. For example, cow 5 in the HR category demonstrated a particularly interesting response to the challenge. In challenge period 1, it consumed the entire allotment and then experienced the longest and most severe bout of SARA of all the cows. In challenge period 2, the cow consumed just less than half the grain offered, yet the resulting episode of SARA was as severe, or more severe, as that experienced by her contemporaries. In challenge period 3, the cow consumed slightly more grain than in challenge period 2, and the resulting SARA was even more severe than that experienced in challenge period 1. Thus, a change in intake behavior did not eliminate the resulting ruminal acidosis. A similar conclusion can be made for cow 1 in the LR category. Another interesting response was cow 3 in challenge period 1, who did not experience SARA, even though it consumed the entire grain allotment. Such individuality in cow susceptibility may explain why, despite efforts to transition the rumen during the close-up dry period, many cows still experience SARA in early lactation (Penner et al., 2007).

Individual variation among cows clearly demonstrates that animals markedly vary in their ability to cope with the dietary factors that predispose them to acidosis (Schwartzkopf-Genswein et al., 2003). The source of such variability may include age of the cow, its genetics, the inherent ruminal microbial population, and previous exposure to acidosis. It is clear that this variability makes it difficult to study the effects of imposed treatments on ruminal acidosis and highlights the need to use sufficient animal numbers within a study. Studies that examine treatment effects within cow, such as Latin square designs, may not be appropriate experimental designs for conducting acidosis research because of the potential long-term carryover effects of acidosis.

Ruminal pH reflects the complex balance between the production of fermentation acids during feed diges-



tion and their neutralization or removal, or both, from the rumen (Allen, 1997). Thus, several factors may contribute to why some cows experience ruminal acidosis whereas others are metabolically capable of coping with a challenge. As shown in this study, individual cows differ in their intake behavior in response to ruminal acidosis. They may also differ with respect to ruminal microbial populations (Weimer et al., 1999), salivary secretion rates (Bailey, 1961), absorptive capacity from the ruminal epithelium (Gäbel et al., 2002), diet selection behavior (Leonardi and Armentano, 2003), and digestion kinetics or passage rates of feed from the rumen (K. A. Beauchemin, unpublished data).

## CONCLUSIONS

Early lactation cows fed a high-concentrate diet had lower pH profiles than midlactation cows fed a high-forage diet. Both groups of cows experienced SARA after an acidosis challenge, but the bouts of SARA were more severe in HR cows. The severity of ruminal acidosis worsened with repeated challenges even though cows in both risk categories became increasingly reluctant to consume the grain offered each challenge. Thus, avoidance of grain intake did not minimize the severity of ruminal acidosis. We conclude that reducing the risk factors that contribute to ruminal acidosis, such as increasing dietary physically effective fiber, confers a degree of protection to cows against SARA should they be subjected to feeding mismanagement. Once cows experience a bout of SARA, subsequent bouts of SARA become increasingly severe. Therefore, a bout of SARA that occurs due to improper feed delivery or poor diet formulation can have long-term consequences on cow health and productivity. It is not known, however, how long of a recovery time after a bout of SARA is needed to minimize the severity of subsequent bouts.

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