Subacute ruminal acidosis and total mixed ration preference in lactating dairy cows¹

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ABSTRACT

Subacute ruminal acidosis (SARA) is a condition where the pH of the rumen becomes abnormally acidic because of increased and altered production of volatile fatty acids. The objective of this experiment was to determine how a SARA challenge affects total mixed ration selection in dairy cows. In this study, 8 multiparous, lactating, ruminally cannulated Holstein cows were given a choice between a long-forage-particle-size diet with slow-fermenting starch (LC) and a short-forage-particle-size diet with fast-fermenting starch in a crossover design. Cows were allowed to adapt to this feeding scheme and were then subjected to a rumen challenge to induce a bout of SARA. The rumen challenge successfully decreased rumen pH and altered rumen volatile fatty acid profiles. Daily average rumen pH decreased from 6.02 to 5.77, and average minimum rumen pH decreased from 5.59 to 5.28. In addition, following the rumen challenge, concentrations of acetate, butyrate, and valerate, and acetate-to-propionate ratio increased. In response to the rumen challenge, intake of LC increased from the baseline level of 18.1% of total daily dry matter intake to 38.3% for that day. During the first recovery day after the rumen challenge, LC intake moderated to 28.0% of total daily dry matter intake. On the second recovery day, LC intake returned to baseline levels at 18.6%. These results indicate that cows are able to alter their diet preference for higher physically effective fiber and slower starch fermentability during a bout of SARA and that they can effectively fully recover from this type of SARA within 72 h when appropriate diets are available.

Key words: acidosis, diet selection, particle size, sorting

INTRODUCTION

Subacute ruminal acidosis is a major concern in the modern high-producing dairy cow. It is defined as a moderately depressed rumen pH in the range of 5.5 to 5.0 (Nocek, 1997; Krause and Oetzel, 2006). Krause and Oetzel (2006) suggested that 3 major causes of SARA exist in dairy herds: excessive intake of rapidly fermentable carbohydrates, inadequate ruminal adaptation to a highly fermentable diet, and inadequate ruminal buffering caused by inadequate dietary fiber or inadequate physical fiber. The negative effects of SARA are vast and varied; ranging from decreased DMI (Britton and Stock, 1987; Nocek, 1997) and reduced feed efficiency (Huntington, 1993; Nocek, 1997) to decreased milk fat yield (Nocek, 1997) and contributing to lameness (Nocek, 1997; NRC, 2001; Stone, 2004). A study that evaluated 154 cows in 14 Wisconsin dairy herds determined that 20.1% of lactating cows had SARA when tested using rumenocentesis (Oetzel et al., 1999). In a case study of a 500-cow dairy in central New York State, Stone (1999) estimated that SARA could cost up to \$475/cow per year in lost milk production and components only. Clearly, SARA warrants extensive research and management.

Several studies have examined diet and feed selection changes when sheep or lambs were subjected to acidotic rumen conditions. For example, in a study by Phy and Provenza (1998b), lambs were fed a meal of rolled barley and then offered a choice of flavored (onion or oregano) rabbit pellets that either contained NaHCO₃ and lasalocid or NaCl. The authors determined that after a grain meal, lambs preferred rabbit pellets that contained NaHCO₃ and lasalocid over pellets that contained NaCl. Another study by Phy and Provenza (1998a) examined the effect that eating a meal of rapidly fermentable feed had on the preference for rapidly fermentable feed later in the day. Lambs fed a lower amount (400 g) of rolled barley for a meal exhibited equal preference for rolled barley and alfalfa pellets (52 and 48% of total intake respectively) during the next 4 h. However, when a higher amount (1,200 g) of rolled barley was fed, the lambs increased their preference for alfalfa pellets over rolled barley (71 and 29% of total

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intake, respectively) during this same time (Phy and Provenza, 1998a). All of these results show that lambs prefer feeds that attenuate acidosis after a grain meal to maintain ruminal health.

In addition, studies have examined the influence of SARA in dairy cows on eating behavior. Keunen et al. (2002) conducted an experiment where 25% of DMI of cows being fed a TMR was replaced by wheat and barley pellets to induce SARA. The choice of 2 feeds (long alfalfa hay and alfalfa pellets) was then offered 2 times per day for 30 min each. Cows with SARA increased their consumption of long alfalfa hay over alfalfa pellets compared with their consumption without SARA; 85 and 60% of test feeds were consumed as long alfalfa hay for SARA and non-SARA cows, respectively (Keunen et al., 2002). DeVries et al. (2008) used a rumen challenge model to induce SARA in early and mid-lactation Holstein cows. The rumen challenge consisted of restricting feed to 50% of ad libitum DMI for 1 d and then feeding 4 kg of barley and wheat, after which cows had ad libitum access to TMR. Changes in eating behavior were measured by determining the particle size distribution of offered feed and refusals and calculating a selection index for each particle fraction. After the rumen challenge, cows in both groups changed their sorting behavior. DeVries et al. (2008) determined that early lactation cows generally increased their sorting for medium particles and against short and fine particles and exhibited no change in sorting long particles. Mid-lactation cows exhibited variable responses with sorting activity changing with day and period. DeVries et al. (2008) suggested that both early and mid-lactation cows altered their sorting behavior to consume a diet that would help attenuate their bout of SARA.

Despite evidence of dairy cattle altering their eating behavior or diet choice based on their rumen environment, no research has been published where cattle had access to 2 distinct diets to observe the influence of SARA on diet preference and eating behavior. Therefore, the objective of this experiment was to induce a bout of SARA in lactating dairy cows that had ad libitum access to 2 distinct diets that varied in forage particle size and starch fermentability and to determine how SARA affects TMR selection in dairy cows.

MATERIALS AND METHODS

Diets, Cows, and Experimental Design

Cows used in this research were cared for and maintained according to a protocol approved by The Pennsylvania State University Institutional Animal Care and Use Committee (University Park). Eight

lactating, multiparous, ruminally cannulated, Holstein cows averaging 219 ± 61 DIM and 44 ± 7 kg of milk production/d, weighing 702 ± 56 kg, and with parity of 3.13 ± 0.99 (mean $\pm$ SD) were studied. The trial consisted of two 7-d adaptation periods, followed by 8-d collection periods in a crossover design.

For the duration of the study, cows were fed 2 different diets simultaneously: a long-particle-size-diet with slowly fermentable starch (**LC**) and a short-particle-size-diet with fast starch fermentability (**SF**). Diets were offered to cows in tie-stall feed bunks divided into halves via a plywood panel that eliminated cross-contamination of TMR. The side of the feed bunk that the diets were offered was alternated each day to limit the possibility for bias of bunk location or relationship to water bowls. The 2 rations fed contained identical ingredients and proportions, but varied in the particle length of corn silage and the particle size of dry ground corn. The LC diet included long corn silage (**LCSLG**) and dry cracked corn (**CC**) and the SF diet included short corn silage (**SCSLG**) and dry fine ground corn (**FC**). Ingredients and their percentage of ration DM were corn silage (42.6), dry ground corn (22.2), alfalfa haylage (15.4), canola meal (9.4), roasted split soybeans (7.1), mineral/vitamin mix (2.5), salt (0.4), and Optigen (Alltech Inc., Nicholasville, KY; 0.4).

The corn silage hybrid was Pioneer 34M78 (Pioneer Hi-Bred International Inc., Johnston, IA) that was planted on April 19, 2010, and harvested on August 30, 2010. Corn silage was harvested with a John Deere 6750 forage harvester (John Deere, Moline, IL) equipped with a kernel processor set at approximately 6.35 mm. The cutterhead of the harvester used 16 knives (maximum capacity is 48 knives) with the length-of-cut transmission at its highest setting to produce a theoretical length of cut of 47.1 mm. After harvesting, corn silage was ensiled in an Ag-Bag (Ag-Bag, St. Nazianz, WI) and allowed to ferment for 62 d before beginning the study. Corn silage that was removed from the Ag-Bag and mixed into TMR without further processing was considered LCSLG. A cut-and-throw type, single-row forage harvester that was modified to operate on a trailer and be fed manually with a 25 horsepower V-Twin small gas engine was used to reduce the particle size of corn silage to produce SCSLG. Corn silage was rechopped twice through the custom forage chopper on a daily basis to minimize the chemical variance between LCSLG and SCSLG. Dry corn was ground through a Roskamp roller mill (California Pellet Mill Co., Crawfordsville, IN) to produce the CC used in this study. This corn was then ground further with a Case International 1250 grinder-mixer (Case IH, Racine, WI) using a 3.18-mm screen to produce FC. Diets were mixed

separately using an I. H. Rissler model 1050 TMR mixer (E. Rissler Mfg. LLC, New Enterprise, PA).

The 4 consecutive days immediately following the adaptation period (d 8 to 11) were designated the baseline for feed preference and rumen conditions. On d 12, feed intakes for each diet were restricted to 75% of baseline intake. Following feed restriction on d 13 at 0745 h, 4 kg (as fed) of fine ground wheat was thoroughly mixed into the rumen digesta of each cow via the rumen cannulas to provide a rumen challenge by initiating SARA. Each cow was then allowed ad libitum access to both diets at 0800 h, the amount of TMR offered allowed for approximately 115% of total daily baseline intake to be consumed from either diet offered. Ad libitum TMR feeding continued on d 14 and 15 to monitor recovery from the rumen challenge.

Animals were housed in individual stalls, milked twice per day at 0500 and 1700 h, and fed once per day at approximately 0800 h for ad libitum consumption. Cows were fed for a 10% refusal rate except when either treatment diet intake was below 6 kg of DM/d, which was set as the minimum amount of feed to be offered to always allow for an opportunity to choose either diet. Feed was pushed up 3 times/d at 1230, 1730, and 2400 h. Rations were balanced to meet or exceed NRC (2001) requirements for cows producing 52.2 kg of milk/d containing 3.75% fat and 3.07% true protein, assuming a DMI of 29.5 kg/d, and water was available for ad libitum consumption.

Rumen Sampling

On d 11 of the study, ruminal contents were collected from dorsal, ventral, cranial, caudal, and medial areas of the rumen at 0.0, 1.5, 3.5, 5.5, 8.5, 11.5, 14.5, 18.0, 21.5, and 24.5 h after feeding (Kononoff et al., 2003b) to determine baseline rumen conditions. Rumen sampling also occurred on d 12 (feed restriction) at 11.5, 14.5, 18.0, and 21.5 h after feeding; d 13 (rumen challenge) at 0.0, 1.5, 3.5, 5.5, 8.5, 11.5, 14.5, 18.0, 21.5, and 24.5 h after feeding; and d 14 (recovery) at 3.5, 8.5, 14.5, and 21.5 h after feeding. At each rumen sampling, collected digesta was mixed thoroughly, sampled, and filtered through 2 layers of cheesecloth. Rumen liquid pH was immediately determined using a hand-held pH meter (HI 98121; Hanna Instruments Inc., Woonsocket, RI). Approximately 15 mL of filtered liquid was placed into bottles containing 3 mL of 25% metaphosphoric acid and 3 mL of 0.6% 2-ethylbutyric acid (internal standard) and stored at approximately 2°C. Within 24 h after collection, samples were centrifuged 3 times at $4000 \times g$ for 30 min at 4°C to obtain a clear supernatant and were analyzed for VFA concentration using gas chromatography (Yang and Varga, 1989).

Feed, Refusal, and Particle Size Analysis

Feed bunk contents for each animal were weighed and sampled on d 8 to 15 at 0 and 24 h after feeding to determine particle size distribution and DM content of the remaining feed. Additionally, feed bunk contents were weighed on d 8 to 11 and d 13 at 2, 4, 8, and 16 h after feeding. All samples were sieved in duplicate in the American Society of Agriculture and Biological Engineers (ASABE; St. Joseph, MI) forage particle separator, which can determine 6 particle fractions (>26.9 , >18.0 , >8.98 , >5.61 , >1.65 , and <1.65 mm; screen diagonal; ASABE, 2007). Whole samples were then placed in a forced-air oven at 65°C for 48 h to determine DM content. Samples of forages, ground corn, and TMR were taken on d 11 and 13 and analyzed by Cumberland Valley Analytical Services Inc. (Hagerstown, MD) for CP (AOAC International, 2000), ADF (AOAC International, 2000), NDF (Van Soest et al., 1991), ash (AOAC International, 2000), NFC (Van Soest et al., 1991), and NE_L (NRC, 2001). Starch contents of forages, ground corn, and TMR were determined by grinding (0.5-mm screen; Wiley mill; Arthur H. Thomas Co. Inc., Swedesboro, NJ) dried samples and then using the starch procedure reported by Zanton and Heinrichs (2009). Particle size distributions of forages and TMR were determined via sieving with the ASABE forage particle separator (ASABE, 2007). To determine particle size distributions of ground corn, samples were placed on a series of stacked sieves (sizes 0.15, 0.425, 0.60, 0.85, 1.18, 1.70, 2.36, 3.35, 4.75, and 6.7 mm; VWR, Arlington Heights, IL) contained in a Retsch AS 200 Control sieve shaker (Retsch GmbH, Haan, Germany) and were sieved for 10 min at 2.5-mm amplitude. There was approximately a 41% increase between each sieve screen size, except between the 0.15- and 0.425-mm sieves. Particles retained on each sieve were then weighed to determine their proportion of total sample DM. Two procedures were used to calculate physically effective NDF (peNDF) concentration: $peNDF_{8.0} = \% \text{ of particles } >8.98 \text{ mm} \times \text{NDF concentration of whole sample}$ [similar to top 2 sieves of the Penn State Particle Separator (The Pennsylvania State University)] and $peNDF_{1.18} = \% \text{ of particles } >1.65 \text{ mm} \times \text{NDF concentration of whole sample}$ (similar to top 3 sieves of the Penn State Particle Separator; Kononoff et al., 2003a). Corn grain fermentability was determined via in situ bags incubated in quadruplicate in the rumen of 2 lactating cows (each cow incubated 2 bags of each sample for each time point) for 0.5, 1, 2, 4, 6, 8, 12, 16, 24, and 48 h. After removal from the rumen, bags were rinsed in cold water by hand until the water was almost clear. Bags were then dried in a forced-air oven

at 65°C for 48 h and then weighed to determine remaining DM.

Statistical Analyses

Statistical analysis was conducted using PROC MIXED of SAS (version 9.2; SAS Institute Inc., Cary, NC). Dependent variables were analyzed as a cross-over design. All denominator degrees of freedom for F-tests were calculated according to Kenward and Roger (1997) and repeated measurements for ruminal pH, ruminal VFA concentrations, and ground corn DM disappearance were analyzed using the first-order autoregressive covariance structure (Littell et al., 1998) as well as terms for time and interaction of treatment by time. Because of unequally spaced rumen sampling, the weighted mean daily pH and VFA concentrations were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972). Area under the curve for the SARA thresholds of 5.8 and 5.5 were also calculated using the trapezoidal rule (Shipley and Clark, 1972). For each cow, the 4 baseline d (8, 9, 10, and 11) were averaged before analysis to provide equal number of observations between baseline and rumen challenge day. A selection index based on refusals was calculated for each of the 6 particle size fractions. This index was calculated as the actual intake of each fraction (Y_i to pan), expressed as a percentage of the expected intake. Expected intake of Y_i equals intake multiplied by the fraction of Y_i in the fed TMR (Leonardi and Armentano, 2003). Values >1.0 indicate that cows were sorting for the particle fraction and values <1.0 indicate that cows were sorting against the particle fraction. The 95% confidence limits were used to determine if a selection index was significantly different from 1.0. All data are presented as least squares means and treatment effects are considered significant at $P < 0.05$ and a trend at $P < 0.10$.

RESULTS AND DISCUSSION

Chemical Composition and Particle Size Distribution of Diets

Particle size distributions and chemical compositions of forages used in this study are shown in Table 1. A large difference was observed in particle size distribution between LCSLG and SCSLG. When separated with the ASABE particle separator, LCSLG had many more particles retained on 26.9- and 18.0-mm screens, equal particles on the 8.98-mm screen, and many fewer particles on 5.61- and 1.65-mm screens and the pan than SCSLG. The approximate equivalency of Penn State Particle Separator fractions to the ASABE screens

are top (26.9 + 18.0 mm), middle (8.98 mm), lower (5.61 + 1.65 mm), and pan (pan). The particle size distribution of alfalfa haylage was similar to SCSLG. Chemical compositions of the corn silages were similar and not practically different despite some statistically significant differences for DM, ADF, NDF, NFC, and NE_L. Sampling error may be responsible for the small differences seen between LCSLG and SCSLG, as they were taken from the same bag each day as a single batch, with part being rechopped as the only difference. Rechopping of corn silage could conceivably increase DM content through increased drying rate. The peNDF measures were, as expected, very different between corn silages, but a much greater difference was observed for peNDF_{8.0} than for peNDF_{1.18}. The LCSLG was 1.81 and 1.15 times greater than SCSLG for peNDF_{8.0} and peNDF_{1.18}, respectively.

The particle size distributions, chemical compositions, and rates of disappearance for corn grains used in this study are shown in Table 2. The particle size distributions of CC and FC were different at all 11 particle fractions. The greatest differences occurred at screen sizes 2.36 mm and larger, where CC had 67.4% and FC had 5.6% of particles retained, and at screen sizes 1.18 mm and smaller, where CC had 18.4% and FC had 78.2% of particles retained. The chemical compositions of CC and FC were similar and not practically different despite being statistically different in DM and CP content. The rates of disappearance of CC and FC were different at every time point except 48 h ($P = 0.15$). The greatest differences between CC and FC were in the first 2 h of incubation, where FC had about 2.1 times more DM disappearance than CC. The disappearance of FC continued to be greater than CC at each time point (except 48 h), but the differences between them decreased with increasing incubation time. These data should be interpreted with caution, as the effect of eating and rumination on ground corn was not a factor in this analysis and it is reasonable to assume that chewing would have a larger effect on CC because of its greater potential for further particle size reduction.

The particle size distributions and chemical compositions of the treatment TMR are shown in Table 3. Each particle fraction was different between LC and SF; the 4 largest particle fractions (>26.9 , >18.0 , >8.98 , and >5.61 mm) were greater for LC, whereas the 2 smallest particle fractions (>1.65 mm and pan) were greater for SF. The chemical compositions of the TMR were similar and not practically different. The CP, NDF, forage NDF, and starch content of the TMR were approximately 16.3, 31.8, 21.5, and 30.1% of DM, respectively. The peNDF measures were very different between LC and SF diets, with the greatest difference occurring

Table 1. Chemical compositions and particle size distributions determined with the American Society of Agriculture and Biological Engineers (ASABE; St. Joseph, MI) particle separator for alfalfa haylage and long and short corn silage

Item	Alfalfa haylage	Corn silage			
		Long	Short	SEM	<i>P</i> -value
Particle size, ¹ as-fed % retained					
26.9 mm	1.9	12.6	0.9	0.65	<0.01
18.0 mm	6.0	31.3	13.2	0.54	<0.01
8.98 mm	26.3	28.7	28.7	1.02	1.00
5.61 mm	24.0	13.7	21.7	1.95	0.04
1.65 mm	31.8	11.9	26.8	1.32	<0.01
Pan	10.0	1.8	8.8	1.83	0.03
Composition, % of DM					
DM	46.0	39.2	40.7	0.92	<0.01
CP	18.5	8.6	8.5	0.06	0.15
ADF	36.0	20.7	19.1	0.47	0.04
NDF	46.4	34.9	32.6	0.67	0.05
peNDF _{8.0} ²	15.9	25.4	14.0	0.66	<0.01
peNDF _{1.18} ³	41.8	34.3	29.7	0.97	<0.01
Ash	10.4	3.1	3.2	0.11	0.49
NFC	23.3	50.2	52.8	0.72	0.05
Starch	0.77	39.7	41.4	1.61	0.49
NE _L , ⁴ Mcal/kg	1.36	1.73	1.78	0.01	0.05

¹Approximate equivalency to Penn State Particle Separator (The Pennsylvania State University, University Park): top sieve (26.9 + 18.0 mm), middle sieve (8.98 mm), lower sieve (5.61 + 1.65 mm), and pan (pan).

²Physically effective NDF_{8.0} (peNDF_{8.0}) = % of particles >8.98 mm × NDF concentration of whole sample (similar to top 2 sieves of Penn State Particle Separator; Kononoff et al., 2003a).

³Physically effective NDF_{1.18} = % of particles >1.65 mm × NDF concentration of whole sample (similar to top 3 sieves of Penn State Particle Separator; Kononoff et al., 2003a).

⁴NRC (2001).

with peNDF_{8.0}, where LC was 2.12 times higher than SF (13.8 vs. 6.5%). The LC diet was only 1.30 times higher than SF for peNDF_{1.18} (27.7 vs. 21.3%).

Rumen Characteristics

The effect of the rumen challenge model on rumen pH is shown in Figure 1. On the baseline day, rumen pH gradually decreased after feeding to a low of 5.61 at 11.5 h after feeding. Rumen pH then gradually increased to preprandial levels by 24 h after feeding. The following day (feed restriction day), rumen pH was measured starting at 11.5 h after feeding and rumen pH was not different from baseline levels at 11.5 and 14.5 h after feeding. Rumen pH then increased faster and remained higher than baseline levels for the remainder of the day. The following day (rumen challenge day), ground wheat was mixed into the rumen via the cannulas of all cows 15 min before feeding. Rumen pH, which began at a higher level than baseline, then dropped sharply after feeding until 3.5 h after feeding and remained constant for 18 h after feeding. The rumen challenge day rumen pH had a 3.8-fold larger decrease from feeding to 1.5 h after feeding and a 6.1-fold larger decrease from feeding to 3.5 h after feeding compared with the baseline day rumen pH. Also, the rumen challenge day rumen pH

was lower than the baseline day rumen pH at 3.5, 5.5, 8.5, and 18.0 h after feeding. By 21.5 h after feeding on the rumen challenge day, the rumen pH had returned to baseline levels and stayed at baseline levels for the remainder of the rumen challenge day and the following recovery day, except at 21.5 h after feeding on the recovery day; the cause of this difference is not apparent.

Ruminal pH daily weighted average was lower and the area under ruminal pH 5.8 and 5.5 was greater during the rumen challenge day compared with baseline (Table 4). This indicates that the rumen challenge was successful in inducing SARA and the decrease in average rumen pH (0.25-unit decrease) was comparable to other studies attempting to induce SARA in dairy cattle, such as Keunen et al. (2002) and Dohme et al. (2008), with 0.14- and 0.35-unit decreases, respectively. The area under ruminal pH of 5.8 was increased by 5 fold (50.9 to 254.8 pH units × min/d) on the rumen challenge day and the area under ruminal pH of 5.5 was essentially 0.0 during baseline but increased to 37.3 pH units × min/d on the rumen challenge day. Dohme et al. (2008) showed similar areas under the curve for their early-lactation cows subjected to rumen challenges, where areas under 5.8 ruminal pH were 136, 231, and 475 pH units × min/d and under 5.5 ruminal pH were 42, 91, and 291 pH units × min/d

Table 2. Chemical compositions, particle size distributions, and rates of disappearance determined via in situ incubation for dry cracked corn, dry fine ground corn, and ground wheat

Item	Ground wheat	Ground corn			
		Cracked	Fine	SEM	<i>P</i> -value ¹
Particle size, as-fed % retained					
6.70 mm	0.0	2.2	0.0	0.18	<0.01
4.75 mm	0.0	10.0	0.1	1.38	<0.01
3.35 mm	0.3	29.7	0.3	1.51	<0.01
2.36 mm	9.3	25.5	5.2	1.29	<0.01
1.70 mm	25.9	14.2	16.2	1.22	0.05
1.18 mm	22.9	6.5	17.7	0.54	<0.01
0.85 mm	12.7	3.2	13.3	0.29	<0.01
0.60 mm	7.9	2.7	11.0	0.33	<0.01
0.425 mm	5.5	2.0	11.4	0.80	<0.01
0.15 mm	8.9	3.0	22.3	0.78	<0.01
Pan	6.7	1.0	2.5	0.22	0.01
Composition, % of DM					
DM	86.4	90.3	88.3	0.36	0.03
CP	11.7	8.8	9.4	0.16	0.04
ADF	4.4	5.1	4.4	0.38	0.27
NDF	14.1	11.3	11.1	0.52	0.77
Ash	1.5	1.4	1.6	0.12	0.31
NFC	71.0	75.1	74.7	0.94	0.75
NE _L , Mcal/kg	2.27	1.96	1.96	0.00	1.00
Rate of disappearance, ² %					
0.5 h	—	17.7	36.1	2.96	<0.01
1.0 h	—	18.4	38.3	2.96	<0.01
2.0 h	—	19.0	42.0	2.96	<0.01
4.0 h	—	27.6	48.8	2.96	<0.01
6.0 h	—	34.2	58.2	2.96	<0.01
8.0 h	—	41.9	65.8	2.96	<0.01
12.0 h	—	56.1	76.9	2.96	<0.01
16.0 h	—	59.1	83.8	2.96	<0.01
24.0 h	—	76.4	92.0	2.96	<0.01
48.0 h	—	90.7	96.2	2.96	0.15

¹*P*-values express differences between cracked corn and ground corn.

²Nylon bags were incubated in quadruplicate in the rumen of 2 lactating cows (each cow incubated 2 bags of each sample for each time point).

(during the first, second, and third rumen challenge, respectively). In addition, more variation was observed in rumen pH on the rumen challenge day, as it had a lower minimum (5.28 vs. 5.59) and a higher maximum (6.95 vs. 6.69) over 24 h. Rumen VFA concentrations were also determined to be different between these 2 d. The daily weighted average concentration for acetate, butyrate, valerate, and isobutyrate, as well as acetate-to-propionate ratio increased on the rumen challenge day. Propionate and isovalerate concentrations were not affected by the rumen challenge, which was unexpected and is unexplained. Khafipour et al. (2009a), using a similar grain-based SARA challenge, had similar shifts in rumen pH and a decrease in acetate and an increase in propionate and other VFA, as would be expected. In a similar study using alfalfa pellets to induce SARA (Khafipour et al. (2009b), the authors found that all VFA increased again, with similar decreases in rumen pH over the experiment. In their study, the diet change was more related to forage particle size (replacing alfalfa

hay with alfalfa pellets) and also a result of increasing DMI as more pellets were added to the diets.

TMR Preference, DMI, and Refusals

The TMR preference was measured as the amount of LC diet DM consumed divided by total daily DMI and expressed as a percentage. The average LC consumption for all cows over the 4 baseline days was 18.1% of total daily DMI (Figure 2). This ratio remained the same for the feed restriction day, as both diets were restricted to 75% of baseline intake and there were virtually no ration refusals for either diet (Table 5). These results are in agreement with the results of Castle et al. (1979), where 3 grass silages of different particle lengths were fed simultaneously to 3 pregnant Ayrshire heifers. The heifers consumed 15.9, 31.9, and 52.2% of total DMI as long, medium, and short silages, respectively. After the rumen challenge, LC intake increased dramatically to 38.3%, followed by 28.0% on the first recovery day.

Figure 1. Effect of rumen challenge while offering 2 free-choice TMR containing long forage and slowly fermentable starch or short forage and rapidly fermentable starch on rumen pH over time for baseline, feed restriction, rumen challenge, and recovery days.

Table 3. Chemical composition and particle size distributions determined with the American Society of Agriculture and Biological Engineers (ASABE; St. Joseph, MI) particle separator for TMR containing long forage and slowly fermentable starch (LC) or short forage and rapidly fermentable starch (SF)

Item	TMR		SEM	P-value
	LC	SF		
Particle size, ¹ as-fed % retained				
26.9 mm	3.2	0.7	0.10	<0.01
18.0 mm	15.0	4.3	0.26	<0.01
8.98 mm	19.9	17.3	0.32	<0.01
5.61 mm	22.0	18.0	1.08	0.04
1.65 mm	24.2	32.6	0.64	<0.01
Pan	15.6	27.2	1.53	0.01
Composition, % of DM				
DM, %	50.1	50.7	0.49	0.26
CP	16.4	16.1	0.36	0.54
ADF	20.8	19.1	0.42	0.04
NDF	32.8	30.7	0.64	0.06
Forage NDF	21.8	21.1	0.42	0.19
peNDF _{8.0} ²	13.8	6.5	0.88	<0.01
peNDF _{1.18} ³	27.7	21.3	0.76	<0.01
Ash	6.6	6.1	0.18	0.03
NFC	41.0	44.2	0.32	<0.01
Starch	28.9	31.3	0.89	0.04
NE _L ⁴ Mcal/kg	1.68	1.70	0.01	0.10

¹Approximate equivalency to Penn State Particle Separator (The Pennsylvania State University, University Park): top sieve (26.9 + 18.0 mm), middle sieve (8.98 mm), lower sieve (5.61 + 1.65 mm), and pan (pan).

²Physically effective NDF_{8.0} (peNDF_{8.0}) = % of particles >8.98 mm × NDF concentration of whole sample (similar to top 2 sieves of Penn State Particle Separator; Kononoff et al., 2003a).

³Physically effective NDF_{1.18} = % of particles >1.65 mm × NDF concentration of whole sample (similar to top 3 sieves of Penn State Particle Separator; Kononoff et al., 2003a).

⁴NRC (2001).

Table 4. Effect of rumen challenge while offering 2 free-choice TMR containing long forage and slowly fermentable starch or short forage and rapidly fermentable starch on rumen pH and VFA for the baseline and rumen challenge day

Item	Baseline	Challenge	SEM	P-value
Rumen pH				
Weighted average ¹	6.02	5.77	0.04	<0.01
Minimum	5.59	5.28	0.06	<0.01
Maximum	6.69	6.95	0.10	<0.01
AUC ² <5.8, pH units × min/d	50.85	254.83	24.31	<0.01
AUC <5.5, pH units × min/d	0.11	37.22	7.81	<0.01
VFA weighted average, µmol/mL				
Total	143.45	150.35	3.89	0.07
Acetate	81.78	86.50	1.42	<0.01
Propionate	38.39	36.97	3.24	0.39
Butyrate	15.00	17.90	0.57	<0.01
Valerate	2.69	2.90	0.38	0.01
Isovalerate	2.25	2.30	0.11	0.53
Isobutyrate	1.05	1.20	0.04	0.01
Acetate:propionate	2.29	2.58	0.25	0.03

¹Weighted averages determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972).

²AUC = area under curve [area below pH threshold (5.5 or 5.8) and above pH profiles of cows].

On the second recovery day after rumen challenge, LC intake returned to baseline levels at 18.6% of total daily DMI. Their results clearly show that the cows very consistently (small SE values) changed their TMR preference in response to a rumen challenge and also they appeared to fully recover from this rumen challenge within 72 h.

The DMI and refusals for each diet for the baseline, feed restriction, rumen challenge, and recovery days are shown in Table 5. The average daily DMI during the baseline period was 30.7 kg/d (5.3 and 25.4 kg/d for

LC and SF, respectively). The following day, feed was restricted to 75.9% of baseline intake at 23.3 kg/d (4.0 and 19.3 kg/d for LC and SF, respectively). Rumen challenge day DMI increased from the baseline for LC by 136% and decreased for SF by 20% for a total daily intake of 32.7 kg/d (excluding ground wheat). Intake of LC recovered to baseline levels by recovery d 1 and SF DMI recovered to baseline levels by recovery d 2. The amount of TMR delivered to the cows was adjusted daily to maintain a refusal rate of 10%, with the exception of the restricted intake day before the rumen challenge. However, as the minimum amount of feed offered per diet per day was set at 6 kg of DM and most cows consumed less than this amount of the LC diet, LC refusals were much higher than SF refusals during the baseline period (31.8 vs. 9.6%). On the rumen challenge day, a drastic increase in refusals occurred for both diets because cows were offered approximately 115% of total daily baseline intake for each diet (230% of total daily baseline intake combined), so they had the ability to consume their entire daily intake from only one diet if they preferred. Therefore, LC refusals were 63.0% and SF refusals were 45.5% on the rumen challenge day. Refusal rates remained elevated during the 2 recovery days because larger amounts of feed continued to be offered to allow cows to return to their baseline LC:SF intake ratios without the influence of low diet refusals. Whether TMR was delivered on the left or right side of the feed bunk or whether TMR was delivered to the side of the feed bunk that was adjacent to a water bowl did not affect the percentage of LC consumed as a percentage of total daily DMI ($P = 0.68$ and 0.63 , respectively) during the baseline period.

Figure 2. Effect of rumen challenge while offering 2 free-choice TMR containing long forage and slowly fermentable starch or short forage and rapidly fermentable starch on preference for TMR with long forage (expressed as a percentage of total daily intake). Means with different letters (a–c) differ ($P \leq 0.05$).

Table 5. Effect of rumen challenge while offering 2 free-choice TMR containing long forage and slowly fermentable starch (LC) or short forage and rapidly fermentable starch (SF) on DMI and refusals for baseline, feed restriction, rumen challenge, and recovery days

Day ¹	DMI, kg		Refusal, %	
	LC	SF	LC	SF
Baseline	5.3 ^{bc}	25.4 ^a	31.8 ^c	9.6 ^d
Feed restriction	4.0 ^c	19.3 ^c	4.8 ^d	0.5 ^e
Rumen challenge	12.5 ^a	20.2 ^c	63.0 ^a	45.5 ^a
Recovery 1	7.8 ^b	21.6 ^{bc}	42.7 ^{bc}	27.1 ^b
Recovery 2	5.0 ^c	23.5 ^{ab}	49.5 ^b	18.4 ^c
SEM	1.4	2.1	5.7	3.1
<i>P</i> -value	<0.01	<0.01	<0.01	<0.01

^{a-c}Means within a column with different superscripts differ ($P \leq 0.05$).

¹No differences in sorting indices were found among the baseline, rumen challenge, and recovery days (all $P > 0.10$).

The cumulative percentages of daily intakes for each diet for the baseline and rumen challenge day are shown in Figure 3. The cows consumed approximately 21.9% of their total daily intake by 2 h after feeding and no differences were observed in the cumulative percentages of daily intakes among the diets or days. At 4 h after feeding the cows consumed an average of 32.1% of their daily diet intakes; however, some small differences were observed among the diets and days. The SF intake was lower on the baseline day compared with LC and SF intake on the rumen challenge day. By 8 and 16 h after feeding, the cows had consumed approximately 51.7 and 81.5%, respectively, of daily diet intakes and no differences existed among diets and days. These results show that the cows consumed the diets simultaneously and in the same ratio throughout the day, independent of which day it was. In other words, the cows did not consume a larger proportion of one diet at certain times of the day and a larger proportion of the second diet at another time of day. These data also show how heavily a cows' daily DMI is skewed toward immediately after feeding when only 1 meal is fed per day in an individual stall housing system, even though ample feed was available before feeding based on consistently having high levels of refusal.

Ration Sorting

Ration sorting was measured via selection indices calculated by comparing TMR particle size distributions at time of feeding to 24 h after feeding. The selection index uses the actual consumption of a particle fraction divided by the estimated consumption of the particle fraction if no sorting occurred to produce the index value. Index values >1.0 indicate sorting for a particle fraction and values <1.0 indicate sorting against. No differences were found in sorting indices among the

baseline, rumen challenge, and recovery d (all $P > 0.10$); therefore, all days were averaged (Tables 5 and 6). Based on these selection indices, sorting occurred in all 6 particle fractions when cows were fed the LC diet. They sorted against particles retained on the 3 largest screens (26.9, 18.0, and 8.98 mm) and for particles in the 3 smallest particle fractions (>5.61 , >1.65 mm, and pan). All of the LC sorting indices were different from 1.0 based on their 95% confidence limits, even though they did not have very large numerical differences. Much less ration sorting occurred on the SF diet, as 4 of the 6 particle fractions had no significant sorting occurring. The cows fed the SF diet did sort against particles retained on the 18.0- and 8.98-mm screens. In addition, the sorting indices for each particle fraction were different between the diets except for the 18.0-mm screen. It is unlikely that the minimal amount of sorting described in this study influenced cow performance and rumen fermentation, because in a previous study by Maulfair et al. (2010), much greater ration sorting activity was found to have no effects on milk production or rumen fermentation patterns. Evidence exists, however (Penner et al., 2010), that a mild episode of SARA may be sufficient to induce rumen epithelial permeability and create a situation to begin acute ruminal acidosis.

CONCLUSIONS

Lactating cows were given the choice between an LC diet and an SF diet. Cows were allowed to adapt to this 2-diet feeding scheme until the intake ratio of LC:SF remained constant. Cows were then given a rumen challenge to induce a bout of SARA. Dairy cattle can significantly alter their TMR preference, when faced with SARA, to a diet with increased peNDF and slower starch fermentability that may help alleviate

Table 6. Effect of offering 2 free-choice TMR containing long forage and slowly fermentable starch (LC) or short forage and rapidly fermentable starch (SF) on mean selection indices¹ of baseline, rumen challenge, and recovery days (4 d)

Screen, ² mm	TMR		SEM	P-value
	LC	SF		
26.9	0.78	0.98 ³	0.05	<0.01
18.0	0.85	0.90	0.03	0.21
8.98	0.91	0.96	0.01	<0.01
5.61	1.03	1.00 ³	0.01	0.02
1.65	1.06	1.01 ³	0.01	<0.01
Pan	1.15	1.02 ³	0.02	<0.01

¹Values = 1.00 indicate no sorting, values <1.00 indicate sorting against, and values >1.00 indicate sorting for.

²Approximate equivalency to Penn State Particle Separator (The Pennsylvania State University, University Park): top sieve (26.9 + 18.0 mm), middle sieve (8.98 mm), lower sieve (5.61 + 1.65 mm), and pan (pan).

³Sorting index is not significantly different from 1.00 based on a 95% confidence limit.

their acidotic condition. In addition, this study showed that dairy cattle with this severity of a single bout of SARA can fully recover within 72 h after onset. As cattle were only fed once per day and only subjected to 1 bout of SARA in this study, further research is warranted to evaluate effects of multiple SARA bouts and different feeding times and feeding systems on diet selection.

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Figure 3. Effect of rumen challenge while offering 2 free-choice TMR containing long forage and slowly fermentable starch (LC) or short forage and rapidly fermentable starch (SF) on cumulative percentage of diet daily intake at various times after feeding for the baseline and rumen challenge day. Means within a time point with different letters (a and b) differ ($P \leq 0.5$).

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