Effects of corn silage particle size, supplemental hay, and forage-to-concentrate ratio on rumen pH, feed preference, and milk fat profile of dairy cattle¹

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ABSTRACT

Two experiments (Exp.) were conducted to study effects of feeding long or short corn silage total mixed rations (TMR) on rumen pH, feed preference, and dairy cow performance and to determine the rate of recovery from grain-induced subacute ruminal acidosis (SARA). Both experiments utilized a crossover design with 12 lactating, multiparous, Holstein cows each (including 4 ruminally cannulated cows) and consisted of two 26-d periods. Each period consisted of 12 d of adaptation followed by 14 d of data collection. Each period was divided into 4 phases: adaptation, d 1 to 12; baseline, d 13 to 14; challenge, d 15 to 19; and recovery, d 20 to 26. Treatments in Exp. 1 were TMR based on corn silage with long (L) or short (ST) particle size in a 65:35 forage-to-concentrate (F:C) diet. Treatments in Exp. 2 were TMR based on corn silage with short (SH) or long (LH) particle size in a 65:35 F:C diet with 3.3% (DM basis) orchardgrass hay offered as a supplement to the diet. In both experiments, during the challenge phase cows received a 50:50 F:C diet to initiate SARA. Animals were housed individually, milked twice per day, and fed once per day for 10% refusal rate on an as-fed basis. Data were analyzed using PROC MIXED of SAS. Feeding L and LH diets increased acetate-to-propionate ratio in the rumen, which resulted in the maintenance of a ratio >2 from the start of the SARA challenge through recovery. In Exp. 1, feeding long corn silage TMR resulted in lower milk fat concentration on the third day of the challenge, whereas cows fed short corn silage TMR had lower milk fat concentration on the final day of the challenge compared with d 13. Providing supplemental hay to cows fed TMR based on long or short corn silage in Exp. 2 prevented acidosis when cows were challenged with a high-grain diet. Milk fat concentrations substantially decreased during the

challenge phase in both diets supplemented with hay, but feeding LH did not lower milk fat concentrations until d 20 compared with d 17 for cows fed SH. Under the conditions of these experiments, cows selected for shorter particles compared with longer particles, despite the rumen challenge. However, when feeding a 50:50 F:C diet, feeding long corn silage TMR or supplementing the diet with grass hay increased rumen pH, acetate-to-propionate ratio in the rumen, and rate of recovery from SARA.

Key words: subacute ruminal acidosis, ruminal pH, particle size, milk fat

INTRODUCTION

It is common practice to feed dairy cattle large quantities of concentrates and high-quality forages containing relatively low amounts of fiber to meet the high energy demands of lactation. Compared with high-forage diets, high-concentrate diets are more rapidly fermentable due to their chemical composition, contain less physically effective fiber, and may result in sub-optimal rumen conditions that may limit digestibility of some feed components. Subacute ruminal acidosis is a common and costly digestive disorder that occurs when low-forage and high-concentrate diets that contain highly fermentable carbohydrates are fed. Under the conditions of SARA, ruminal pH decreases for prolonged periods (>3 h/d) to between 5.2 and 5.6 (Kleen et al., 2003; Stone, 2004; Gozho et al., 2005).

Diets consisting of concentrates and ensiled forages, in particular, are often small in particle size. Due to their smaller particle size, cows spend less time chewing and ruminating compared with cows fed a high-forage-based diet (Santini et al., 1983). The shorter chewing time and ingestion of large quantities of carbohydrates can decrease saliva production, which may adversely affect cellulolytic activity within the reticulorumen and cause severe postprandial ruminal fluid pH depression. Consequently, diets containing both concentrates and ensiled forages may depress fiber digestion, milk production, and milk fat composition. Milk fat depression is commonly associated with SARA (Kleen et al.,

Received December 16, 2014.

Accepted February 24, 2015.

¹This research is a component of NC-2042; Management Systems to Improve the Economic and Environmental Sustainability of Dairy Enterprises.

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2003; Krause and Oetzel, 2006). High-concentrate diets are known to alter the fermentation pattern in the rumen, with possible changes in production of de novo FA (short and medium chain) by the mammary gland. Low ruminal pH may cause the biohydrogenation (BH) pathway to shift from *trans*-11 to *trans*-10 intermediates (Grinari et al., 1998). When ruminal pH was 5.6, the flow of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA decreased and the flow of *trans*-10 C18:1 and *trans*-10, *cis*-12 CLA increased when using dual-flow continuous culture (Fuentes et al., 2009). This shift in BH increases the production of bioactive FA isomers that reduce milk fat synthesis (Harvatine et al., 2009).

Long forages are necessary to help maintain ruminal function and milk fat percentage. Longer forages, in particular, promote chewing and saliva secretion, which buffers the production of VFA from feed digestion and can have a significant effect on rumen pH (Mertens, 1997). Beauchemin and Buchanan-Smith (1990) found that feeding a small amount of long hay effectively maintained optimal ruminal function in cows fed finely chopped silage. In a recent study, Kmicikewycz and Heinrichs (2014) concluded that feeding supplemental long orchardgrass hay to early and mid-lactation dairy cattle induced with SARA maintained DMI during incidents of and recovery from periods of low ruminal pH. The inclusion of small proportions of long hay, particularly into diets based on finely chopped corn silage, may be a strategy to maintain adequate physical effectiveness of the diet (Beauchemin et al., 1994).

In addition to supplementing the diet with hay, research has shown that dairy cattle may be capable of selecting feeds that have the ability to stimulate chewing in an attempt to attenuate the effects of low ruminal pH. Keunen et al. (2002) demonstrated that lactating dairy cows induced with SARA increased their preference for long alfalfa hay over pelleted alfalfa. 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 when challenged. Moreover, Maulfair et al. (2013) showed that during a bout of SARA cows were able to alter their diet preference for higher physically effective NDF (**peNDF**) and slower starch fermentability. These studies suggest that lactating dairy cattle are capable of altering their sorting behavior to consume a diet that may help attenuate their bout of SARA.

Despite evidence of the ability of dairy cattle to alter their eating behavior or diet choice based on their rumen environment, there is limited information concerning the influence of grain-induced SARA on diet preference, rumen pH, and milk fat profile of lactating dairy cows fed diets differing in forage particle size and

supplemental hay and the subsequent rate of recovery from SARA induction. Likewise, although several experiments have investigated the influence of SARA on diet preference, the potential effect of forage particle size preference under differing forage-to-concentrate ratios (**F:C**) warrants further investigation. The objectives of this study were to evaluate the effects of corn silage particle size and supplemental hay on rumen pH, feed preference, and milk fat profile of dairy cows induced with a low F:C SARA challenge and to determine the rate of recovery from this challenge. We hypothesized that providing cows with long particle size corn silage or supplemental hay with short particle size corn silage would maintain DMI and increase rumen pH during a grain-induced SARA challenge.

MATERIALS AND METHODS

Animals for Experiments 1 and 2

All experimental procedures were conducted under the approval of The Pennsylvania State University Institutional Animal Care and Use Committee (PSU IACUC #43767). Twelve lactating (4 ruminally cannulated), multiparous, Holstein cows were housed in individual tie-stalls with sawdust bedding and rubber mattresses and were used in 2 experiments (**Exp.**). Cows were fed once daily using a model 4820 mini vertical auger mixer (DS Machine Co., Loysville, PA) at approximately 0730 h for ad libitum consumption, had continuous access to fresh water, and were milked twice daily at 0500 and 1700 h. Rations were balanced to meet or exceed NRC (2001) requirements for cows producing 40.4 kg of milk/d containing 3.6% fat and 3.0% true protein assuming a DMI of 27.2 kg/d. Average parity was 2.72 ± 0.65 (mean $\pm$ SD) and average weight was 645 ± 70 kg during Exp. 1 and 694 ± 75 kg during Exp. 2. Days in milk were 74 ± 27 d at the beginning of Exp. 1 and 100 ± 27 d at the start of Exp. 2.

Experimental Design and Treatment: Experiments 1 and 2

Experiments 1 and 2 used a crossover design with 2 groups and 2 experimental periods. Experiment 1 was conducted from October to December 2013 and Exp. 2 from December 2013 to February 2014. Each experimental period lasted 26 d, with 12 d for adaptation and 14 d for data collection and sampling. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Treatments in Exp. 1 were designed to study effects of corn silage particle size at 2 F:C ratios. Treatments were TMR containing corn silage with short (**ST**) or long

(L) particle size in a 65:35 F:C diet. During the challenge phase, cows received a high-grain diet (50:50 F:C) for 5 d to initiate SARA. Cows on the L diet continued to receive long corn silage and cows on the ST diet continued to receive short corn silage during the challenge phase. Following the SARA challenge, cows were fed the baseline phase diet (65:35 F:C) from d 20 to 26 to monitor recovery from the SARA challenge. Treatments in Exp. 2 were designed to be similar to Exp. 1 and only differed with the offering of 3.3% (DM basis) unprocessed supplemental orchardgrass hay (*Dactylis glomerata* L). Treatments in Exp. 2 were TMR containing corn silage with short (SH) or long (LH) particle size in a 65:35 F:C, and both diets were supplemented with 3.3% orchardgrass hay that was offered separate from the TMR. Supplemental hay was offered at 1730 h (immediately following milking) to cows in tie-stall feed mangers divided in half by a plywood panel that reduced cross-contamination of TMR and supplemental hay. No hay was offered from 0730 to 1730 h. Similar to Exp. 1, animals received a 50:50 F:C diet for 5 d as a SARA challenge.

Ingredient composition and particle size distributions for experimental diets are shown in Table 1. Diet composition was calculated based on the formulated proportions and nutrient composition of each ingredient. Dietary DM increased and dietary NDF and peNDF (particles ≥ 4.0 mm) decreased in both treatments with decreasing F:C during the challenge phase in both experiments. Dietary starch concentration for ST diets was 27.5% (DM basis) and 26.4% for L diets in Exp. 1. The small difference in corn silages was most likely due to changes in fermentation as silages were harvested from the same field on the same day. During the challenge phase, dietary starch concentration increased to 34.4 and 34.5% for ST and L diets, respectively. In Exp. 2, dietary starch concentration increased from 26.7 and 25.7% for SH and LH diets to 33.5% during the challenge phase. The L and LH diets had a larger proportion of particles retained on the 19.0-mm sieve compared with the S and SH diets. The 65:35 F:C ST and SH diets had a higher proportion of particles retained on the 8.0- and 4.0-mm sieves compared with the 65:35 F:C L and LH diets. Subacute ruminal acidosis challenge diets composed of 50:50 F:C had greater proportions of particles retained on the 4.0-mm sieve and pan than the 65:35 F:C diets, which was expected.

The corn silage variety used in this study was Dekalb DKC 52-61 (Monsanto Company, St. Louis, MO) and was planted on May 3, 2013, and harvested on September 5, 2013. Corn silage was harvested with a John Deere 6750 self-propelled forage harvester (John Deere, Moline, IL). The cutterhead of the harvester used 12 and 48 knives (maximum capacity is 48 knives) with

the length-of-cut transmission at its highest setting to produce a theoretical length of cut of 62.7 and 5.3 mm for the long and short corn silage, respectively. After harvesting, corn was ensiled in an Ag-Bag (Ag-Bag, St. Nazianz, WI) and allowed to ferment 33 d before being fed.

Particle size distribution and chemical composition of corn silages used in this study are shown in Table 2. A large difference was observed in particle size distribution between the long and short corn silage as expected. When separated with the Penn State Particle Separator (PSPS; Nasco, Fort Atkinson, WI), long corn silage had a larger amount of particles retained on the 19.0-mm sieve ($P < 0.01$), and fewer particles retained on the 8.0- and 4.0-mm sieves compared with short corn silage. These results were expected with the treatment design. Chemical compositions of the corn silages were similar for CP and ash content; however, the long corn silage was 10.2 and 12.8% lower in DM and NFC and 17.3 and 19.1% higher in ADF and NDF content, resulting in a lower NE_L compared with short corn silage. Differences are likely a result of poorer compaction of the longer silage, thus a more prolonged fermentation that resulted in lower NFC and higher fiber levels. Corn silage was fed at the same feeding rate to test the management approach of changing the chop length. As expected, the peNDF_{4.0} measurements were different between corn silages. Long corn silage was 20.2% higher in peNDF_{4.0} than short corn silage.

Measurements and Analytical Procedures: Experiments 1 and 2

Total mixed rations for each animal were hand shoveled, mixed, and weighed before sampling to determine DMI. Approximately 1.13 kg of TMR were collected on d 13, 14, 15, 17, 19, 20, 23, and 26 of each period at 0, 8, 16, and 24 h after feeding to determine particle size distribution and DM content of the remaining feed. All samples of corn silage and TMR were sieved in the PSPS using 4 particle fractions (≥ 19.0 , ≥ 8.0 , ≥ 4.0 , and < 4.0 mm). Whole samples were then placed in a forced-air oven at 55°C for 48 h to determine DM content. Samples of all individual feed ingredients were taken on d 13, 15, and 20 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), starch (Hall, 2009), and NE_L (NRC, 2001). In Exp. 2, hay refusals for each animal were weighed on d 13 to 15, 17, 19, 20, 23, and 26 of each period 13 h after being offered to determine DMI.

Table 1. Ingredient and nutrient composition and particle size distribution of experimental diets¹

Item	Exp. 1				Exp. 2			
	65:35 F:C		50:50 F:C		65:35 F:C		50:50 F:C	
	ST	L	ST	L	SH	LH	SH	LH
Diet ingredient, %								
Corn silage	40.8	40.8	34.9	36.1	39.5	39.5	33.8	34.9
Alfalfa haylage	18.7	18.7	10.3	9.8	18.1	18.1	10.0	9.5
Orchardgrass hay	—	—	—	—	3.3	3.3	3.1	3.2
Cottonseed hulls	5.4	5.4	5.1	3.6	5.2	5.2	4.9	3.5
Ground corn	15.3	15.3	28.6	29.5	14.8	14.8	27.7	28.6
Roasted soybeans	7.3	7.3	7.9	7.4	7.1	7.1	7.7	7.1
Canola meal	9.1	9.1	9.8	10.2	8.8	8.8	9.5	9.8
Mineral and vitamin mix ²	2.9	2.9	2.7	2.8	2.8	2.8	2.6	2.7
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Urea (Optigen) ³	0.3	0.3	0.5	0.5	0.3	0.3	0.5	0.5
Composition, ⁴ % of DM								
DM	58.0	56.1	62.6	61.2	54.1	54.8	60.5	61.1
CP	15.9	16.1	16.0	16.2	16.0	16.2	16.1	16.2
ADF	22.6	24.3	18.7	19.4	23.0	24.7	19.2	19.9
NDF	32.3	34.5	28.0	29.0	33.2	35.4	29.0	30.0
peNDF _{4.0} ⁵	21.4	24.1	16.8	16.8	23.4	24.1	18.8	19.3
Ash	6.4	6.7	5.5	5.8	6.4	6.6	5.6	5.8
Starch	27.5	26.4	34.4	34.5	26.7	25.7	33.5	33.5
Particle size, DM % retained								
19.0 mm	0.9	13.6	0.2	8.6	1.1	11.0	0.5	9.2
8.0 mm	42.9	37.7	29.0	29.2	47.3	37.6	36.8	32.8
4.0 mm	22.5	18.7	30.8	20.3	22.2	19.5	27.4	22.3
Pan	33.8	30.0	40.0	41.9	29.4	31.8	35.3	35.7

¹F:C = forage-to-concentrate ratio; ST = short corn silage TMR; L = long corn silage TMR; SH = short corn silage TMR with supplemental hay; LH = long corn silage TMR with supplemental hay. Exp. = experiment.

²Contained 45.8% dried corn distillers grains with solubles; 35.8% limestone (38% Ca); 8.3% magnesium oxide (54% Mg); 6.4% salt; 1.73% vitamin ADE premix; 1.09% selenium premix (0.06% selenium); and 0.88% trace mineral mix. Composition (DM basis): 11% CP; 18% NDF; 5.2% fat; 14.9% Ca; 0.35% P; 4.58% Mg; 0.41% K; 0.31% S; 357 mg/kg of Cu; 1,085 mg/kg of Zn; 181 mg/kg of Fe; 6.67 mg/kg of Se; 125,875 IU/kg of vitamin A (retinyl acetate); 31,418 IU/kg of vitamin D (activated 7-dehydrocholesterol); and 946 IU/kg of vitamin E (DL- α tocopheryl acetate).

³Alltech, Nicholasville, Kentucky.

⁴Composition values for diets were calculated based on the actual proportions and composition of each individual ingredient.

⁵Physically effective NDF_{4.0} = % of particles ≥ 4.0 mm $\times$ NDF of whole sample.

Table 2. Particle size distribution and chemical composition of short and long corn silage and hay fed in experiment (Exp.) 1 and Exp. 2

Item	Hay	Corn silage			
		Short	Long	SEM	P-value ¹
Particle size, as-fed % retained					
19.0 mm	—	2.1	43.0	1.02	<0.01
8.0 mm	—	63.9	39.8	1.94	<0.01
4.0 mm	—	27.2	12.7	1.18	<0.01
Pan	—	6.7	4.5	0.92	0.13
Composition, % of DM					
DM	85.7	41.3	37.1	0.78	<0.01
CP	10.3	7.3	7.7	0.11	0.09
ADF	35.7	19.1	22.4	0.46	0.01
NDF	61.8	33.0	39.3	1.01	0.02
peNDF _{4.0} ²	—	17.8	21.4	0.40	<0.01
Ash	5.4	2.9	3.4	0.13	0.10
NFC	21.6	54.0	47.1	0.96	<0.01
NE _L , Mcal/kg	0.64	0.81	0.77	0.01	0.02

¹P-value reflects difference between short and long corn silage.

²Physically effective NDF_{4.0} = % of particles ≥ 4.0 mm $\times$ NDF of whole sample.

Ruminal contents were collected from dorsal, ventral, cranial, caudal, and medial areas of the rumen at 0.0, 3.5, 5.5, 8.5, 11.5, 14.5, 18, and 24.5 h after feeding (Konnoff et al., 2003) on d 13 to determine baseline rumen conditions, and on d 15, 17, and 19 (challenge phase), and d 20, 23, and 26 (recovery phase). Collected digesta was mixed thoroughly and filtered through a 0.28-mm fiberglass mesh screen (New York Wire, Mt. Wolf, PA). Rumen liquid pH was immediately determined using a handheld pH electrode (Oakton Instruments, Vernon Hills, IL). Approximately 5 mL of strained ruminal fluid was mixed with 1 mL of 25% metaphosphoric acid and 1 mL of 0.6% 2-ethylbutyric acid (internal standard) and stored at approximately -20°C . Samples were later thawed and centrifuged 3 times at $4,000 \times g$ for 30 min at 4°C to obtain a clear supernatant and were analyzed for VFA concentration. Volatile fatty acids were analyzed using gas-liquid chromatography with a column 2 m long $\times$ 2 mm i.d. packed with Carbowax 20 M on 80/100 Carbowax BPA (Supelco Inc., Bellefonte, PA; Yang and Varga, 1989). Working conditions were back inlet temperature of 220°C , front detector temperature of 225°C , oven temperature of 125°C , and N_2 used as the carrier with an overall total flow rate of 36 mL/min.

Milk production was recorded daily and 2 milk samples from each cow were taken on d 13 to 15, 17, 19, 20, 23, and 26. One sample was preserved using 2-bromo-2-nitropropane-1,3-diol and was analyzed for fat (filter B) and true protein by the Dairy One milk testing laboratory (Ithaca, NY) using infrared spectrophotometry (Foss 605B MilkoScan, Foss Electric, Hillerød, Denmark). The second milk sample (approximately 45 mL) was centrifuged at $3,500 \times g$ for 20 min at 4°C and 300 to 350 mg of fat cake were collected. Fat cakes from the morning and evening milking were composited by day according to fat yield at each milking and analyzed for FA composition as described by Rico and Harvatine (2013). In short, lipid extraction was performed according to Hara and Radin (1978) using hexane:isopropanol. Fatty acid methyl esters were prepared by base-catalyzed transmethylation according to Chouinard et al. (1999). Fatty acid methyl esters were quantified by gas chromatography using an Agilent 6890A gas chromatograph (Agilent Technologies, Palo Alto, CA) equipped with a fused-silica capillary column (SP-2560, 100 m $\times$ 0.25 mm i.d. with 0.2- μm film thickness; Supelco Inc., Bellefonte, PA), and a flame-ionization detector with hydrogen as the carrier gas. Initial oven temperature was 80°C , which was increased by $2^{\circ}\text{C}/\text{min}$ to 190°C and held for 15 min. Inlet and detector temperatures were 250°C with a 100:1 split ratio. Gas constant flows were held at hydrogen carrier

1 mL/min and detector hydrogen 25 mL/min, airflow 400 mL/min, and nitrogen plus carrier at 40 mL/min.

Fecal grab samples were collected from each cow at 0, 8, 16, and 24 h after feeding on d 13, 15, 17, 19, 20, 23, and 26 of each period. Fecal pH was measured by thoroughly mixing 10 g of fresh feces with 10 mL of distilled water (1:1 ratio) and determined using a handheld pH electrode (Oakton Instruments). Dry matter was determined by drying approximately 250 g of feces in a forced-air oven at 65°C for 48 h (Lee et al., 2011). Fecal samples were ground to pass through a 0.5-mm screen (Wiley Mill, Arthur H. Thomas Co. Inc., Swedesboro, NJ) and composited by day and time for starch analysis. Starch concentration was determined by an enzymatic method (Karkalas, 1985) after gelatinization with sodium hydroxide followed by glucose, determined using the glucose oxidase method (PGO enzyme product no. P7119, Sigma Chemical Co., St. Louis, MO; Allen and Ying, 2012).

Statistical Analyses

All data were statistically analyzed using PROC MIXED of SAS (version 9.3, SAS Institute Inc., Cary, NC). Cow was the experimental unit. Intake, sorting, and milk yield and composition data were analyzed assuming a crossover design. The following model was used:

$$Y_{ijkl} = \mu + P_i + C_j + T_k + D_l + T_k \times D_l + e_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, P_i is the random effect of period ($i = 1$ to 2), C_j is the random effect of cow ($j = 1$ to 12), T_k is the fixed effect of treatment ($k = 1$ to 2), D_l is the fixed effect of day ($l = 1$ to 7), $T_k \times D_l$ is the interaction of treatment and day, and e_{ijkl} is the residual error. Day was the repeated variable and cow by period was the subject. Repeated measurements of rumen pH, VFA, fecal pH, and fecal starch were analyzed by including a REPEATED model statement. The spatial power covariance structure SP(POW) was used on data with unequal spacing and the first order autoregressive covariance structure AR(1) was used between equal days within phase. All denominator degrees of freedom for F -tests were calculated according to Kenward and Roger (1997). Due to unequally spaced rumen sampling, the weighted mean daily ruminal pH, fecal pH, and rumen VFA concentrations were determined by calculating the area under the response curve according to the trapezoidal rule (Shipley and Clark, 1972). The area under the response curve for SARA thresholds of pH 5.8 and 5.5 were also calculated using the trapezoidal rule (Shi-

pley and Clark, 1972). A sorting index was calculated based on refusals for each of the 4 particle size fractions. This index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator (Leonardi and Armentano, 2003). Values equal to 100% indicate that cows were not sorting, values >100% indicate that cows were sorting for the particle fraction (selective consumption), and values <100% indicate that cows were sorting against the particle fraction (selective refusals). The 95% confidence limits were used to determine if a selection index was significantly different from 100%. All data are presented as least squares means, and treatment effects are considered significant at $P \leq 0.05$ and a trend at $0.05 < P \leq 0.10$. Means separation tests were conducted using the protected least significant differences (PDIF) procedure with significance at $P \leq 0.05$ and tendency at $P \leq 0.10$.

RESULTS AND DISCUSSION

Experiment 1

During the baseline phase, cows consuming L had a higher weighted average rumen pH than cows fed ST (5.86 vs. 5.69; $P = 0.02$; Table 3). The challenge diet was successful in reducing rumen pH compared with baseline and inducing SARA. Average rumen pH decreased from 5.69 to 5.42 for cows fed ST and from 5.86 to 5.54 for cows fed L. Feeding long corn silage TMR, however, tended to increase weighted average rumen pH during the challenge ($P = 0.10$) and recovery ($P = 0.10$) phases. Minimum rumen pH was similar between treatments during all 3 phases; however, a considerable decrease occurred from baseline to challenge and recovery phases for cows on both dietary treatments (treatment by day interaction not shown). During the challenge phase, minimum rumen pH for ST and L were 5.04 and 5.05, respectively, which was comparable with similar SARA induction studies reporting minimum pH values of 5.0 to 5.5 (Krehbiel et al., 1995; Goad et al., 1998). Increasing corn silage particle length increased maximum rumen pH during the challenge phase ($P = 0.05$). Treatments were similar for daily area under ruminal pH curve of 5.8 and 5.5. However, both area under the ruminal pH curve of 5.8 and 5.5 was greater during the challenge phase compared with baseline and recovery phases. An effect of day was found for ruminal pH ($P < 0.001$; Figure 1A). Cows consuming L had higher average ruminal pH on d 17 compared with SH (5.67 vs. 5.45; $P = 0.03$). After the challenge phase, ruminal pH recovered to baseline values (d 13) within the first d of the recovery phase (d 20) for both diets. However, 7 d after the start of the recovery phase, ru-

men pH for cows consuming short corn silage TMR still remained close to 5.6. Low ruminal pH after 7 d of recovery for cows consuming ST may have been a result of destabilized ruminal microflora (Nagaraja and Titgemeyer, 2007) or even damaged rumen epithelium (Harmon et al., 1985). However, this was not determined. In a series of 3 consecutive grain challenges, Dohme et al. (2008) found that after the first grain-induced SARA challenge, the ruminal pH fully recovered within 2 d, but did not fully recover after the second and third challenges. Maulfair et al. (2013) determined that dairy cattle could fully recover from a single bout of SARA within 72 h after onset, given the availability of an appropriate diet after induction. Although these results are different, the present study suggests that given a long corn silage TMR diet, cows can fully recover within 24 h after a 5-d, grain-induced SARA challenge.

An effect of day was detected for all VFA concentrations except valerate (valerate data not shown). Acetate concentration was similar between treatments ($P = 0.61$) and decreased in all treatments on d 19 and did not return to baseline concentration levels until d 23, 3 d into the recovery phase (Figure 2A). Propionate concentration increased significantly from d 13 to 15 and remained elevated until d 17 for cows fed ST ($P < 0.001$; Figure 2B). Cows consuming ST tended to have a higher concentration of propionate on d 15 ($P = 0.07$) and had a higher concentration of propionate on the third day of the challenge (d 17) compared with L ($P = 0.004$). During the recovery phase, cows consuming ST tended to have higher propionate concentration on d 20 and 23 compared with L. Propionate concentrations returned to near baseline values on the second day of the recovery (d 20). Butyrate concentration remained similar from d 13 to 17 in both diets (Figure 2C). Feeding short corn silage TMR yielded higher concentrations of butyrate in the rumen on d 13 (21.6 vs. 17.8 $\mu\text{mol/mL}$; $P = 0.04$) and 17 (23.7 vs. 19.8 $\mu\text{mol/mL}$; $P = 0.03$) compared with cows fed long corn silage. Kononoff et al. (2003) and Beauchemin and Yang (2005) found an increase in the concentration of total VFA in the rumen when corn silage particle size was reduced. Decreasing corn silage particle size may increase ruminal rate of digestion and VFA production due to an increase in the surface area for microbial attachment (Krause et al., 2002). Reducing forage particle length can also decrease saliva production and liquid passage rate, resulting in an increase of VFA concentrations in the rumen (Krause et al., 2002). The acetate:propionate (A:P) ratio decreased substantially during the challenge phase (d 15 to 19) and subsequently increased during the recovery phase (d 20 to 23; Figure 2D). Subacute ruminal acidosis and reduced milk fat production have been associated with A:P ratios <2 (Sauvant and

Table 3. Rumen pH of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in Exp. 2 during baseline, challenge, and recovery phases

Rumen pH	Exp. 1				Exp. 2			
	ST	L	SEM	<i>P</i> -value	SH	LH	SEM	<i>P</i> -value
Weighted average ¹								
Baseline	5.69	5.86	0.03	0.02	5.98	6.09	0.02	0.01
Challenge	5.42	5.54	0.04	0.10	5.65	5.72	0.04	0.29
Recovery	5.66	5.80	0.05	0.10	5.84	5.92	0.05	0.37
Minimum								
Baseline	5.34	5.21	0.09	0.18	5.30	5.48	0.05	0.11
Challenge	5.04	5.05	0.03	0.61	5.16	5.21	0.04	0.43
Recovery	5.05	5.06	0.17	0.98	5.22	5.32	0.06	0.31
Maximum								
Baseline	6.55	6.87	0.16	0.26	7.34	7.09	0.08	0.13
Challenge	6.37	6.69	0.13	0.05	6.92	6.68	0.11	0.25
Recovery	6.69	6.84	0.10	0.10	6.96	6.92	0.05	0.64
AUC ² <5.8, pH units × min/d								
Baseline	257.8	237.6	68.0	0.85	174.6	89.8	22.7	0.06
Challenge	523.2	446.8	42.1	0.28	362.7	254.8	30.4	0.07
Recovery	379.4	285.8	61.2	0.37	233.9	177.2	47.0	0.46
AUC <5.5, pH units × min/d								
Baseline	100.2	65.9	36.1	0.57	31.1	2.6	13.1	0.23
Challenge	230.4	190.0	28.1	0.38	132.7	66.5	16.2	0.10
Recovery	151.2	101.3	42.5	0.41	76.5	41.2	22.2	0.38

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 (5.8 or 5.5) and above pH profiles of cows].

Mertens, 1998). Cows consuming L had a higher A:P ratio on d 17, 19, 20, and 23 than ST. Beauchemin and Yang (2005) reported that decreasing corn silage length tended to decrease the A:P ratio. In the current study, increasing corn silage particle size in the TMR increased A:P ratio and resulted in maintenance of a ratio >2 from the start of the rumen challenge through recovery.

The concentration of starch in the feces on d 13 (baseline) was below 3.0% for both ST and L (Figure 3A). Fecal starch was lowest 16 h after feeding on all days for both diets. Cows consuming L had the highest fecal starch concentration at feeding on d 17 (5.7%) and cows consuming ST had the highest fecal starch concentration at feeding on d 20 (5.9%). Feeding diets with smaller particle size can alter retention of feed particles in the rumen, resulting in increased flow of fermentable substrates to the hindgut (Van Soest, 1994).

Average daily fecal pH was not different between treatments and averaged 6.4 and 6.3 for ST and L, respectively, on d 13 of the baseline (Table 4). Fecal pH was affected by the SARA challenge. Compared with d 13, fecal pH decreased 0.20 units for ST and 0.19 units for L on the first day of the challenge (d 15). Fecal pH did not return to baseline values and remained relatively low through the end of the recovery phase. In this current study, at the end of the challenge phase on d 19, fecal pH was lower in both treatments and cows fed ST and L had fecal pH of 6.07 and 5.94, respectively. During grain-induced SARA, escape of ex-

cessive amounts of undigested feed from the rumen and small intestine results from extensive fermentation in the hindgut, which increases the acidity of the hindgut digesta, reduces fecal pH, and increases microbial protein output in the feces (Gozho et al., 2005; Eastridge, 2006; Li et al., 2012).

Ration sorting was measured using an index calculated by comparing TMR particle size distributions at feeding (0 h) and 24 h after feeding (Table 5). During the baseline phase, cows on both diets sorted against long particles (particles retained on the 19.0-mm sieve; 85.1 and 76.7% for ST and L, respectively) and for finer particles retained on the pan (104.0 and 105.9% for ST and L, respectively). No sorting occurred for particles retained on the 8.0- and 4.0-mm sieves. During the challenge phase, cows on both diets sorted against particles retained on the 19.0-mm sieve (62.4 and 79.3% for ST and L, respectively) and sorted for particles retained on the pan (104.1 and 106.7% for ST and L, respectively). Cows fed ST sorted against particles on the 19.0-mm sieve to a greater extent than cows fed L ($P = 0.02$). No sorting of particles on the 8.0- and 4.0-mm sieves or pan occurred for either diet during the recovery phase. Cows continued to select against longer particles during the recovery phase. These results are not in agreement with studies that reported sorting for longer particles in a TMR when cows were challenged with a high starch diet (Beauchemin and Yang, 2005; Yang and Beauchemin, 2006) or ground barley and wheat diet (DeVries et al., 2008).

Figure 1. Effect of rumen challenge on ruminal pH of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in Exp. 2. (A) Weighted average ruminal pH in Exp. 1; (B) weighted average ruminal pH in Exp. 2. Preplanned contrasts tested the difference between short and long corn silage TMR at each time point; ** $P < 0.01$; * $P < 0.05$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

The DMI for ST and L diets during the baseline, challenge, and recovery phases are shown in Figure 4A. Dry matter intake was higher for cows fed TMR with short compared with long corn silage during the challenge phase on d 15 and 19 (both $P < 0.05$). The daily total DMI on baseline d 13 was lower for cows fed long corn silage TMR compared with short corn silage TMR (25.8 vs. 28.1 kg/d; $P = 0.04$). This difference may be due to increased gut fill and higher

rumen retention times associated with bulk rumen fill, as has been noted previously (Kononoff and Heinrichs, 2003; Onetti et al., 2003; Leonardi et al., 2005). These results are in agreement with studies that found that increasing particle size of corn silage decreases DMI (Stockdale and Beavis, 1994; Kononoff et al., 2003). In regard to d 13, cows fed L increased DMI from d 15 to 17 but maintained a lower intake during the recovery phase.

Figure 2. Effect of rumen challenge on ruminal VFA of dairy cows fed short (ST) or long (L) corn silage TMR in experiment 1. (A) Acetate concentration, (B) propionate concentration, (C) butyrate concentration, (D) acetate:propionate ratio. Preplanned contrasts tested the difference between short and long corn silage TMR at each time point; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt × day = interaction effect between treatment and day; error bars are SEM.

Milk production was different across diets ($P = 0.01$; Figure 5A). Decreasing corn silage particle size increased milk yield on d 19 ($P = 0.01$) and 20 ($P = 0.02$; Figure 5A) compared with long corn silage diets, which follows the daily mean DMI. Returning cows to a 65:35 F:C diet after the challenge phase did not improve milk yield. No treatment difference was found for milk fat composition; however, compared with d 13 (3.64 and 3.60% for L and ST, respectively), milk fat composition decreased starting on d 17 (3.28%; $P = 0.02$) for cows fed L and d 19 (3.06%; $P < 0.01$) for cows fed ST (Figure 5B). Milk fat concentration did not recover to baseline values until d 23 for both diets. Low milk fat content is one of the most common effects of SARA in

dairy cows (Nocek, 1997). Treatment differences also occurred for milk fat yield ($P < 0.01$). Cows consuming ST had higher fat yield compared with cows fed L on d 14 (1.55 vs. 1.35 kg/d; $P = 0.02$) and 15 (1.51 vs. 1.29 kg/d; $P = 0.01$; Figure 5C). These results are in agreement with studies that found changes in forage particle size influence milk production or components (Kononoff et al., 2003; Leonardi et al., 2005). Protein concentration was similar between treatments from d 13 to 19 (Figure 5D). During the recovery phase (d 20 to 26), however, cows consuming ST had higher concentrations of protein in the milk than L. Milk protein yield increased on d 19 and 20 ($P < 0.05$) and tended to be higher on d 23 and 26 ($P < 0.10$) compared with

Figure 3. Effect of rumen challenge on fecal starch of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with supplemental hay in Exp. 2. (A) Fecal starch (% of DM) in Exp. 1, (B) fecal starch (% of DM) in Exp. 2. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26).

Table 4. Fecal pH of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in Exp. 2 during baseline, challenge, and recovery phases

Item	Exp. 1				Exp. 2			
	ST	L	SEM	<i>P</i> -value	SH	LH	SEM	<i>P</i> -value
Baseline								
d 13	6.39	6.33	0.03	0.09	6.41	6.31	0.04	0.03
Challenge								
d 15	6.19	6.14	0.06	0.49	6.31	6.15	0.04	<0.001
d 17	6.09	6.10	0.06	0.90	6.19	6.14	0.04	0.24
d 19	6.07	5.94	0.06	0.05	6.19	6.10	0.04	0.03
Recovery								
d 20	6.06	6.04	0.05	0.76	6.23	6.11	0.04	<0.001
d 23	6.26	6.17	0.05	0.11	6.41	6.32	0.04	0.01
d 26	6.25	6.23	0.05	0.66	6.40	6.33	0.04	0.07

L (Figure 5E). Increased milk protein yields have also been observed in other studies using short corn silage particle size and are thought to be a result of increased ruminal digestibility (Clark and Armentano, 1999) due to the positive correlation between milk protein and dietary energy (Grieve et al., 1986).

Diet-induced milk fat depression is associated with a change in milk fatty acid profile including a decrease in the concentration of de novo synthesized FA (FA <16 carbons) and an increase in *trans* isomers of the alternate rumen BH pathway (Harvatine et al., 2009). In addition, milk fatty acid profile changes rapidly during induction and recovery of milk fat depression (Rico and Harvatine, 2013). There were shifts in the milk FA profile between treatments during the challenge. Milk fat concentrations of *trans*-10 C18:1, an intermediate of the alternative BH pathway, was similar between treatments during the baseline phase ($P = 0.60$; Table 6), but increased by 0.83 and 1.37% units for ST and

L, respectively, on d 19, and 0.67 and 0.85% units for ST and L, respectively, on d 23 (Figure 6A). A treatment by day interaction was detected for *trans*-11 C18:1 ($P = 0.04$) and *cis*-9, *trans*-11 CLA ($P = 0.02$). On d 19, cows fed ST had higher concentrations of *trans*-11 C18:1 and *cis*-9, *trans*-11 CLA compared with L (Supplemental Figure S1A and S1B; <http://dx.doi.org/10.3168/jds.2014-9249>), indicating a slowing of the normal BH pathway. Short- and medium-chain FA (C4:0 to C14:1) are mainly derived from de novo synthesis in the mammary gland of dairy cows (Kennelly and Glimm, 1998). In this study, increasing corn silage particle size decreased concentrations of C8:0, C10:0, C12:0, C14:0, C14:1, and C15:0, and increased C20:0 (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2014-9249>). Cows consuming ST had higher concentrations of de novo FA (<16 C) throughout the baseline, challenge, and recovery phases compared with L (all $P < 0.05$). Cows consuming L on d 13 ($P = 0.001$)

Table 5. Mean selection index¹ by sieve for dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in Exp. 2 on baseline, challenge, and recovery phases

Item	Exp. 1				Exp. 2			
	ST	L	SEM	<i>P</i> -value	SH	LH	SEM	<i>P</i> -value
Baseline								
19.0 mm	85.1*	76.7*	6.39	0.29	69.3*	87.2*	5.96	<0.01
8.0 mm	96.6	99.4	2.08	0.07	97.0*	100.2	0.76	0.01
4.0 mm	99.8	103.5	1.19	0.03	102.4	102.5	0.91	0.94
Pan	104.0*	105.9*	1.91	0.37	103.5	103.7	1.18	0.89
Challenge								
19.0 mm	62.4*	79.3*	4.87	0.02	69.3*	76.0*	4.42	0.24
8.0 mm	91.4*	98.0	2.96	0.10	97.8	100.4	1.08	0.10
4.0 mm	100.9	96.0	2.41	0.16	100.4	102.9	0.75	0.02
Pan	104.1*	106.7*	1.53	0.21	102.7	104.7	1.05	0.15
Recovery								
19.0 mm	87.1*	90.3*	3.89	0.53	85.8*	84.4*	3.75	0.77
8.0 mm	99.2	100.7	0.36	<0.01	98.4*	99.2	0.53	0.32
4.0 mm	99.2	100.6	0.77	0.18	101.4	102.8	0.58	0.03
Pan	101.8	100.8	0.97	0.36	102.0	103.7	0.74	0.03

¹Values = 100% indicate no sorting, values <100% indicated sorting against, and values >100% indicate sorting for.

*Sorting index is significantly different from 100% based on a 95% confidence limit.

Figure 4. Effect of rumen challenge on total DMI and hay DMI of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with supplemental hay in Exp. 2. (A) TMR DMI in Exp. 1, (B) TMR DMI in Exp. 2, (C) hay DMI in Exp. 2. Preplanned contrasts tested the difference between short and long corn silage TMR at each time point; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

and 23 ($P = 0.02$) had a lower concentration of de novo synthesized FA compared with ST (Supplemental Figure S2A; <http://dx.doi.org/10.3168/jds.2014-9249>). As expected, baseline concentrations of preformed FA (FA $>16C$), were higher for L compared with ST (43.3 vs. 40.7% of total FA). On d 13, cows fed L had higher concentrations of preformed FA than ST ($P = 0.01$; Supplemental Figure S2C; <http://dx.doi.org/10.3168/jds.2014-9249>). Differences in de novo synthesized fatty

acids are presumed to be due to small changes in *trans* isomer between the treatments.

Experiment 2

Cows consuming LH had a higher weighted average rumen pH than cows fed SH (5.86 vs. 5.78; $P = 0.01$; Table 3). The challenge diet successfully reduced ruminal pH; however, unlike Exp. 1, it did not induce

Figure 5. Effect of rumen challenge on milk yield and milk fat concentration and yield of dairy cows fed short (ST) or long (L) corn silage TMR in experiment 1. (A) Milk yield, (B) milk fat concentration, (C) milk fat yield, (D) milk protein concentration, (E) milk protein yield. Preplanned contrasts tested the difference between the short and long corn silage TMR at each time point; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

Table 6. Main effect of treatment on concentration of milk FA by source of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in Exp. 2

FA, % of total FA	Exp. 1				Exp. 2			
	Treatment		SEM	P-value	Treatment		SEM	P-value
	ST	L			SH	LH		
<i>trans</i> -10 C18:1								
Baseline	0.61	0.57	0.10	0.60	0.77	0.82	0.33	0.88
Challenge	1.12	1.80	0.96	0.42	0.74	0.74	0.11	0.97
Recovery	0.98	0.88	0.41	0.82	0.54	0.57	0.08	0.76
<i>trans</i> -11 C18:1								
Baseline	0.97	0.97	0.06	0.97	1.22	1.19	0.23	0.91
Challenge	1.31	0.93	0.22	0.12	1.13	1.06	0.09	0.48
Recovery	1.29	1.19	0.15	0.51	1.00	1.02	0.10	0.81
<i>cis</i> -9, <i>trans</i> -11 CLA								
Baseline	0.49	0.46	0.04	0.53	0.55	0.56	0.13	0.95
Challenge	0.66	0.51	0.07	0.07	0.62	0.53	0.05	0.09
Recovery	0.62	0.56	0.07	0.38	0.47	0.48	0.05	0.76
FA <16C ¹								
Baseline	28.2	26.2	0.75	0.02	28.2	29.4	0.99	0.24
Challenge	28.4	27.3	0.44	0.03	29.5	29.2	0.60	0.63
Recovery	28.3	27.0	0.55	0.04	29.4	28.6	0.69	0.26
FA >16C ²								
Baseline	40.7	43.3	0.91	0.02	42.0	40.3	1.58	0.33
Challenge	40.2	41.6	0.95	0.16	39.4	40.8	0.85	0.14
Recovery	41.9	42.3	0.83	0.66	40.5	42.3	1.20	0.15
16C FA								
Baseline	27.3	26.6	0.46	0.17	26.7	27.1	1.13	0.74
Challenge	27.4	27.2	0.73	0.79	27.4	26.4	0.36	0.02
Recovery	26.2	27.0	0.49	0.11	26.9	26.0	0.84	0.30

¹Fatty acids <16C originate from de novo synthesis in the mammary gland.²Fatty acids >16C originate from plasma.

SARA. During the challenge phase, rumen pH was 5.65 for SH and 5.72 for LH. Treatments within phase were similar during the challenge and recovery phases; however, both minimum and maximum rumen pH values decreased from baseline to challenge phase. During baseline ($P = 0.06$) and challenge ($P = 0.07$) phase, area under the ruminal pH curve of 5.8 tended to be lower for LH than SH. In addition, the area under the ruminal pH curve of 5.5 tended to be lower for LH during the challenge phase ($P = 0.10$). In both diets, area under the ruminal pH curve of 5.8 and 5.5 was higher during the challenge phase compared with the baseline phase. Offering supplemental hay to both diets maintained ruminal pH above the threshold of SARA during the challenge phase. Supplementing diets with hay may have maintained a more stable pH, either by increasing buffering within the rumen through increased saliva production or by altering the site of digestion of grain. After the challenge phase, cows on both diets recovered to baseline ruminal pH values after 3 d (Figure 1B). Feeding supplemental hay to cows fed short corn silage TMR accelerated recovery from SARA and maintained higher ruminal pH during recovery compared with cows consuming only short corn silage TMR in Exp. 1.

Acetate concentrations between treatments showed similar patterns throughout the study (Figure 7A).

Acetate concentrations significantly decreased from d 17 to 19 from 98.2 to 69.0 $\mu\text{mol/mL}$ for SH ($P = 0.006$) and 104.2 to 75.8 $\mu\text{mol/mL}$ for LH ($P = 0.005$). Compared with d 13, decreasing corn silage particle size increased propionate concentrations on d 17 (50.0 vs. 40.4 $\mu\text{mol/mL}$ for SH and LH, respectively; $P = 0.01$) and 20 (40.3 and 33.2 $\mu\text{mol/mL}$ for SH and LH, respectively; $P = 0.05$; Figure 7B). Highest propionate concentrations occurred on the third day of the challenge phase (d 17) and were 50.0 and 40.4 $\mu\text{mol/mL}$ for SH and LH, respectively. Propionate concentration returned to baseline values on d 23. Treatments were similar for butyrate concentration ($P = 0.69$; Figure 7C). A day effect was found for butyrate concentration ($P < 0.001$). Cows on both diets increased rumen butyrate concentration from baseline phase to the first day of the challenge (d 15) and dramatically decreased during the challenge phase. Cows returned to baseline butyrate concentrations the first day of recovery (d 20). In both SH and LH, the A:P ratio decreased considerably from d 15 to 19 (Figure 7D). Feeding long corn silage TMR and supplemental hay increased the A:P ratio on d 17 ($P = 0.04$) and 20 ($P = 0.01$). The A:P ratios were 1.84 for SH and 2.17 for LH on d 19. Increasing corn silage particle size and feeding supplemental hay helped the A:P ratio remain >2 throughout the challenge phase.

Figure 6. Effect of rumen challenge on the concentration of milk *trans*-10 C18:1 FA of dairy cows fed short (ST) or long (L) corn silage TMR in experiment (Exp.) 1 and short (SH) or long (LH) corn silage TMR with supplemental hay in Exp. 2. (A) *Trans*-10 C18:1 in Exp. 1; (B) *trans*-10 C18:1 in Exp. 2. Treatments did not differ at any time point ($P > 0.10$). Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

The concentration of starch in the feces during the baseline phase was below 3.45% for L (Figure 3B). The concentration of fecal starch increased drastically, 1.40 to 6.02%, from 16 to 24 h after feeding during baseline for SH ($P = 0.04$). On d 15, a treatment effect was found on fecal starch concentration ($P = 0.02$). Cows consuming LH had a higher fecal starch concentration compared with SH (4.62 vs. 3.14%). Highest fecal starch concentrations for SH were observed 24 h after feeding on d 17 (8.27%) and 0 h after feeding on d 20 (9.85%) for LH.

The average daily fecal pH was different between treatments and averaged 6.41 and 6.31 for SH and LH,

respectively, during the baseline phase (Table 4). Fecal pH was marginally affected by the SARA challenge. Compared with d 13, fecal pH decreased 0.10 pH units for SH and 0.16 pH units for LH on d 15. Fecal pH, however, returned to baseline values on d 23 for both diets.

During all phases of the challenge model, cows sorted against long particles (particles retained on the 19.0-mm sieve; Table 5). Cows fed SH sorted to a greater degree against the longer particles than cows fed LH ($P < 0.01$) during the baseline phase. Feeding LH did not affect sorting of particles retained on the 8.0- or 4.0-mm sieves, but cows fed SH sorted against particles retained on the 8.0-mm sieve (97.0%; $P = 0.01$). Despite the 50:50 F:C challenge, cows continued to select against long particles retained on the 19.0-mm sieve during the challenge phase (69.3 and 76.0% for SH and LH, respectively) and recovery phase (85.8 and 84.4% for SH and LH, respectively).

Figure 4B shows the DMI for each diet during the baseline, rumen challenge, and recovery phase. Cows consuming LH diets increased DMI from d 13 to 15 and subsequently decreased intake after the first day of the challenge phase. On the first day of the challenge phase (d 15), cows consumed 34.9 kg/d of the LH diet, but reduced DMI throughout the challenge phase. In a study by Soita et al. (2005), feeding a higher level of concentrate (45:55 F:C) tended to increase DMI, whereas Yang et al. (2001) reported that dietary F:C ratios of 35:65 or 55:45 had no effects on DMI in cows fed barley silage-based diets. Cows consuming SH diets increased DMI substantially from d 13 to 19 and then decreased DMI from d 19 to 20. Dry matter intake was similar throughout the recovery phase for cows consuming SH diets and averaged 31.5 kg/d. Cows consuming SH had higher DMI on d 13, 17, and 19 compared with LH. Dry matter intake peaked on d 17 for cows consuming SH and averaged 37.0 kg/d. Hay DMI was affected by corn silage particle size and hay supplementation (Figure 4C). Cows fed LH consumed more supplemental hay on d 13 (0.44 kg/d) and 19 (0.43 kg/d) compared with SH (0.26 and 0.28 kg/d on d 13 and 17, respectively). In addition, on d 17, cows consuming LH had a tendency to consume more hay than cows consuming SH ($P = 0.07$).

Milk production was similar between treatments and averaged 36.8 kg/d for SH and 35.2 kg/d for LH (Figure 8A). Milk fat and protein yields and fat concentration were not affected by treatment (Figure 8B, 8C, and 8E). Cows consuming SH averaged 3.56% milk fat compared with 3.55% for LH. Milk yield was highest on d 17 for cows consuming LH (36.9 kg/d) and on d 19 for cows consuming SH (39.9 kg/d; Figure 8A). Milk fat concentrations substantially decreased during the

Figure 7. Effect of rumen challenge on ruminal VFA of dairy cows fed short (SH) or long (LH) corn silage TMR with 3.3% supplemental hay in experiment 2. (A) Acetate concentration, (B) propionate concentration, (C) butyrate concentration, (D) acetate:propionate ratio. Preplanned contrasts tested the difference between the short and long corn silage TMR at each time point; ** $P < 0.01$; * $P < 0.05$; † $P < 0.10$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

challenge phase in both diets. The lowest concentration of milk fat occurred on d 17 for SH (3.30%) and on d 20 for LH (3.29%; Figure 8B). Decreasing corn silage particle size increased milk protein concentration (Figure 8D). Protein concentrations steadily declined from d 13 to 15 for both diets and increased progressively during the challenge phase for SH. On d 20, cows consuming SH had a higher protein concentration in the milk (3.23%) compared with LH (3.03%; $P < 0.01$).

In Exp. 1, the SARA challenge resulted in a shift in BH intermediates toward the alternate BH pathway and a decrease in de novo synthesized FA indicating

diet-induced milk fat depression. However, in Exp. 2 it appears that the higher rumen pH blunted the effect of the SARA challenge. Milk FA profile was similar between treatments in all phases of Exp. 2 (Supplemental Table S1; <http://dx.doi.org/10.3168/jds.2014-9249>). The absence of treatment effects on the yields of de novo synthesized FA in milk suggests a similar amount of mammary lipogenesis. When fed a 55:45 or 45:55 F:C diet supplemented with 1 kg of flaxseed, Soita et al. (2005) reported no effects of corn silage particle size on milk fat proportions of C10:0 to C14:1. An effect of day was detected for C15:0, a FA found in the wall

Figure 8. Effect of rumen challenge on milk yield and milk fat concentration and yield of dairy cows fed short (SH) or long (LH) corn silage TMR with supplemental hay in experiment 2. (A) Milk yield, (B) milk fat concentration, (C) milk fat yield, (D) milk protein concentration, (E) milk protein yield. Preplanned contrasts tested the difference between the short and long corn silage TMR with supplemental hay at each time point; $**P < 0.01$; $\dagger P < 0.10$. Each collection period was divided into 3 phases: baseline (d 13 to 14), challenge (d 15 to 19), and recovery (d 20 to 26). Trt = treatment effect; day = effect of day within phase; trt $\times$ day = interaction effect between treatment and day; error bars are SEM.

of the lactate-utilizing bacterium *Megasphaera elsdenii* (Fievez et al., 2012). Similarly, Colman et al. (2010) reported an increase in the proportions of C15:0 with a 50% ground wheat and 50% ground barley challenge diet. In the current Exp. 2, mean concentration of C15:0 was higher on d 17 compared with d 13 (1.06 vs. 1.20 g/100 g of FA; data not shown) and during the challenge phase (d 17 and 19) compared with the recovery phase (d 23 and 26). This increase in the proportions of C15:0 during the middle of the challenge phase may be indicative of an increase in the abundance of *M. elsdenii* and *Streptococcus bovis* (Fievez et al., 2012) in the rumen microbiome; however, this was not measured. *Streptococcus bovis* is a lactate-producing bacteria that plays a critical role in the onset of acidosis (Fievez et al., 2012). Diets in Exp. 2 resulted in similar concentrations of *trans*-10 C18:1, *trans*-11 C18:1, and *cis*-9, *trans*-11 CLA in milk fat within each phase (Table 6, Figure 6B, Supplemental Figures S3A and S3B; <http://dx.doi.org/10.3168/jds.2014-9249>). In addition, de novo and preformed FA were not different between treatments during any phase and were at expected concentrations indicating no specific limitation in substrate for de novo fatty acid synthesis (Supplemental Figures S4A and S4C; <http://dx.doi.org/10.3168/jds.2014-9249>). However, it is noted that during the challenge, concentrations of 16-carbon FA were greater for SH than LH (27.4 vs. 26.4% of total FA; Supplemental Figure S4B). Low milk fat is also associated with a decline in rumen pH and a shift in the rumen pattern of VFA, resulting in an increase in the production of propionate in the rumen (Bauman et al., 1971). It has been suggested that propionate could contribute to reduced preformed FA incorporation into milk fat, although the mechanism is independent of classic diet-induced milk fat depression (Harvatine et al., 2009). Although the challenge diet successfully reduced ruminal pH in Exp. 2, it did not induce SARA as in Exp. 1. It is possible that the lack of drastic pH response to the challenge in Exp. 2 resulted from the high buffering capacity of the supplemental hay or the low fat content of the diet.

CONCLUSIONS

In 2 experiments, corn silage particle size with or without offering supplemental hay had an effect on rumen fermentation, feed preference, milk yield and milk components, and recovery time from SARA. Increasing corn silage particle size and offering supplemental hay to the long corn silage-based TMR increased A:P, which resulted in the maintenance of a ratio >2 from the start of the rumen challenge through recovery. Offering supplemental hay to cows consuming either long

or short corn silage TMR prevented SARA when cows were challenged with a 50:50 F:C diet. Feeding long corn silage in Exp. 1 decreased milk fat concentration of cows earlier in the challenge phase (third day vs. final day) compared with cows fed short corn silage. Concentrations of *trans*-10 C18:1 increased substantially on d 19 and 23 for both diets compared with baseline d 13, indicating a shift in the BH pathway during the challenge and through recovery. Given long cut corn silage, however, cows fully recovered from SARA within 24 h after the challenge. Cows preferred eating shorter particles despite the rumen challenge in both experiments. Overall, results from these studies indicate that there may be beneficial effects of feeding long particle size corn silage or supplemental hay in corn silage-based diets. Feeding longer corn silage TMR or supplementing the diet with hay when feeding a 50:50 F:C diet can increase rumen pH and A:P in the rumen. In addition, feeding supplemental hay to diets with short cut corn silage can accelerate recovery time from SARA, and when supplemented to both short and long corn silage TMR diets, hay inclusion can blunt the shift of the BH pathway toward *trans*-10 C18:1.

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