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Relationship of severity of subacute ruminal acidosis to rumen fermentation, chewing activities, sorting behavior, and milk production in lactating dairy cows fed a high-grain diet

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ABSTRACT

The objectives of the current study were to evaluate the variation in severity of subacute ruminal acidosis (SARA) among lactating dairy cows fed a high-grain diet and to determine factors characterizing animals that are tolerant to high-grain diets. Sixteen ruminally cannulated late-lactating dairy cows (days in milk = 282 ± 33.8; body weight = 601 ± 75.9 kg) were fed a high-grain diet consisting of 35% forage and 65% concentrate mix. After 17 d of diet adaptation, chewing activities were monitored for a 24-h period and ruminal pH was measured every 30 s for 72 h. Acidosis index, defined as the severity of SARA (area of pH <5.8) divided by dry matter intake (DMI), was determined for individual animals to assess the severity of SARA normalized for a feed intake level. Although all cows were fed the same diet, minimum pH values ranged from 5.16 to 6.04, and the acidosis index ranged from 0.0 to 10.9 pH·min/kg of DMI. Six cows with the lowest acidosis index (0.04 ± 0.61 pH·min/kg) and 4 with the highest acidosis index (7.67 ± 0.75 pH·min/kg) were classified as animals that were tolerant and susceptible to the high-grain diet, respectively. Total volatile fatty acid concentration and volatile fatty acid profile were not different between the groups. Susceptible animals sorted against long particles, whereas tolerant animals did not (sorting index = 87.6 vs. 97.9, respectively). However, the tolerant cows had shorter total chewing time (35.8 vs. 45.1 min/kg of DMI). In addition, although DMI, milk yield, and milk component yields did not differ between the groups, milk urea nitrogen concentration was higher for tolerant cows compared with susceptible cows (12.8 vs. 8.6 mg/dL), which is possibly attributed to less organic matter fermentation in the rumen of tolerant cows. These results suggest that a substantial variation exists in the severity of SARA among lactating dairy cows fed the same high-grain diet, and that cows tolerant to the high-grain diet might be characterized by less sorting behavior but less chewing time, and higher milk urea nitrogen concentration.

Key words: subacute ruminal acidosis, chewing activity, sorting behavior, milk urea nitrogen

INTRODUCTION

Subacute ruminal acidosis (SARA) is a prevalent metabolic disorder found in high-producing dairy herds, mainly caused by feeding excessively fermentable diets. One field survey in the United States indicated that incidences of SARA were 19% in early-lactation dairy cows and 26% in mid-lactation cows (Garret et al., 1997). Subacute ruminal acidosis accounts for substantial economic losses in the dairy industry due to its association with decreased feed intake, liver abscesses (Nagaraja and Lechtenberg, 2007), milk fat depression (Kleen et al., 2003), diarrhea, laminitis (Nocek, 1997), and increased bacterial endotoxins and inflammation (Khafipour et al., 2009). Diet formulation strategies to reduce the incidence of SARA have been extensively studied; however, some cows in a herd still experience SARA even if suggested strategies are implemented. Previous studies indicated that huge variations exist in the extent of severity of rumen acidosis among beef steers (Brown et al., 2000; Schlau et al., 2012), primiparous dry cows (Penner et al., 2007), and sheep (Penner et al., 2009) fed identical diets. But, to our knowledge, similar data have not been demonstrated for lactating dairy cows.

Ruminal pH is determined by the balance between acid production in the rumen and acid removal from the rumen by absorption through rumen epithelial cells, neutralization with buffers, and passage to lower digestive tracts (Allen, 1997). Therefore, the variation in the tolerance to the high-grain diet could be due to variations in any single or any combination of these 4 factors (Penner et al., 2009). Penner et al. (2009) demonstrated that ruminal epithelial cells from acidosis-resistant sheep had a greater capability to absorb VFA in vitro, which suggested that rate of VFA absorption might affect the extent of tolerance to the high-grain

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diet in vivo. Schlau et al. (2012) found that acidosis-resistant steers had lower total VFA concentrations in the ruminal fluid compared with acidosis-susceptible steers, which indicated that higher ruminal pH in tolerant animals might be due to faster VFA absorption, lower VFA production, or both. Besides VFA production and absorption, neutralization is another main factor contributing to regulation of rumen pH. Chewing activities are expected to stimulate salivary secretion (Church, 1988), and Allen (1997) estimated that approximately 37% of protons are removed from the rumen via neutralization by salivary buffers. Therefore, we hypothesized that variation in chewing activities is related to tolerance or susceptibility to high-grain diets.

It has been suggested that milk fat depression is commonly associated with SARA (Kleen et al., 2003; Oetzel, 2003; Stone, 2004). A field study on a large dairy farm found that SARA reduced milk fat production by 0.3% (Stone, 1999). In addition, experimentally induced SARA, either by adding grains to the diet or by replacing alfalfa hay with alfalfa pellets, reduced milk fat concentration (Fairfield et al., 2007; Khafipour et al., 2007). Moreover, Allen (1997) and Enemark et al. (2004) reported a positive relationship between milk fat concentration and ruminal pH ($R^2 = 0.39$ and 0.31 for each study, respectively). Therefore, we hypothesized that cows that are tolerant to highly fermentable diets have higher milk fat content compared with cows that are susceptible to high-grain diets, and expected that milk fat content might be a noninvasive indicator to identify the tolerant and susceptible cows on farm. The objectives of the current study were to evaluate the variation in severity of SARA among lactating dairy cows fed a high-grain diet and to determine factors characterizing cows that are tolerant and susceptible to high-grain diets.

MATERIALS AND METHODS

All experimental procedures used in this study were approved by the University of Alberta Research Centre Animal Care Committee and conducted according to the guidelines of the Canadian Council of Animal Care (Ottawa, Ontario, Canada).

Animals, Diets, and Experimental Design

Sixteen (8 primiparous and 8 multiparous) ruminally cannulated lactating Holstein cows (DIM = 282 ± 33.8; BW = 601 ± 75.9 kg; mean ± SD) were used in this study. Cows were fed a diet containing 35% forage and 65% concentrate mix (Table 1) ad libitum for 21 d, consisting of a 17-d diet adaptation period and a 4-d data and sample collection period.

Table 1. Ingredient, chemical composition, and particle size distribution of the diet

Item	Measurement
Ingredient, % DM	
Barley silage	30.0
Barley grain, dry rolled	25.0
Corn grain, ground	20.0
Canola meal	7.35
Corn gluten meal	5.26
Alfalfa hay	5.0
Beet pulp	3.96
Vegetable oil	1.0
Mineral and vitamin mix[1]	2.43
Nutrient composition, % DM	
DM	60.8
Ash	7.95
CP	15.9
NDF	25.6
Starch	31.1
Ether extract	4.0
NFC	49.8
Forage NDF	14.3
Particle size distribution, % as fed	
>19 mm	20.2
19–8 mm	24.3
1.18–8 mm	39.4
<1.18 mm	16.1
Physical effectiveness factor[2]	44.5

[1]Contained 15.7% Ca, 3.32% P, 14.1% Na, 21.8% Cl, 5.70% Mg, 0.23% S, 0.06% K, 2,867.4 mg/kg of Fe, 468.7 mg/kg of Cu, 902.8 mg/kg of Mn, 11.2 mg/kg of Co, 718.0 mg/kg of Zn, 7.08 mg/kg of Se, 21.0 mg/kg of I, 442.8 kIU/kg of vitamin A, 45.0 kIU/kg of vitamin D, and 1,449.9 kIU/kg of vitamin E.

[2]Determined as the proportion of particles retained on 19- and 8-mm sieves on an as-fed basis (Lammers et al., 1996).

Cows were housed individually in tiestalls bedded with wood shavings, fed the experimental diet as a TMR once daily at 0900 h, and had free access to water. Feed was offered at 105 to 110% of actual feed intake of the previous day. Samples of TMR and feed ingredients were collected daily during sample collection period. The weight of feed offered and refused was recorded daily on d 19, 20, and 21 of the study, and 12.5% of the total daily refusal from each cow was composited to yield one sample per cow per period. The DM concentration of barley silage and alfalfa hay was determined twice weekly and diet formulation was adjusted if necessary. Cows were weighed after the morning milking on 2 consecutive days immediately before the start of experiment. Cows were milked twice daily at 0400 and 1500 h. Milk was sampled from both a.m. and p.m. milkings on d 19, 20, and 21 of the study.

Rumen pH and Rumen Fermentation

Ruminal pH was measured in the ventral sac every 30 s continuously for 72 h (d 19–21) using the pH measurement system evaluated by Penner et al. (2006). Minimum, mean, and maximum pH, and duration and

area below pH 5.8 were determined for each cow daily and averaged over 3-d periods. These data were used to determine acidosis index (area under pH 5.8 divided by DMI; Penner et al., 2009) to assess the severity of SARA normalized for a feed consumption level.

Rumen fluid was collected from cranial, ventral, and caudal sacs, then combined and strained through a perforated screen (Peetex, Sefar Canada Inc., Scarborough, ON, Canada; pore size = 355 μm) every 9 h over a 72-h period. The samples were centrifuged at 3,000 × *g* at 4°C for 20 min immediately after collection, and the supernatants were stored at −20°C until analysis. Rumen fluid samples were composited to yield one sample per cow for further analysis. Ruminal fluid samples were analyzed for VFA profile by gas chromatography according to the method described by Khorasani et al. (1996). Rumen ammonia-N concentration was determined as described by Fawcett and Scott (1960) using a plate reader (SpectraMax 190, Molecular Devices Corp., Sunnyvale, CA).

Chewing Activity and Sorting Behavior

Chewing activities were directly monitored for 24 h on d 18. Eating and ruminating activities were recorded every 5 min and each activity was assumed to last for the entire 5-min interval between observations, as described previously (Beauchemin et al., 2003; Krause et al., 2003; Zhang et al., 2010). Total chewing time was calculated as the sum of eating time and ruminating time.

Particle size distribution of the TMR and orts were determined using a Penn State Particle Separator with 3 sieves (aperture size of 19, 8, and 1.18 mm). Sorting 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). A sorting index of 100, greater than 100, and less than 100 indicate no sorting, selective consumption, and selective refusals, respectively. Physically effective factor was determined as the proportion of particles retained on 19- and 8-mm sieves (Lammers et al., 1996).

Blood Collection

Blood samples were collected every 18 h over a 72-h (d 19–21) period from the coccygeal vessels into tubes containing sodium heparin (Fisher Scientific Company; Nepean, ON, Canada). Samples were centrifuged at 3,000 × *g* at 4°C for 20 min immediately after collection, and plasma was harvested and stored at −20°C until analysis. Plasma samples were composited to yield one sample per cow for further analysis.

Plasma samples were analyzed for glucose concentration using a glucose oxidase and peroxidase enzyme (Sigma, St. Louis, MO) and dianisidine dihydrochloride (Sigma) procedure. Absorbance was determined by a plate reader (SpectraMax 190) at a wavelength of 450 nm. Plasma BHBA concentration was measured by the enzymatic oxidation of BHBA to acetoacetate using 3-hydroxybutyrate dehydrogenase (Roche, Mississauga, ON, Canada) followed by determination of reduction of NAD^+ to NADH at a wavelength of 340 nm. Commercial kits were used to determine concentrations of plasma NEFA (Wako Chemicals USA Inc., Richmond, VA) and insulin (Coat-a-Count kit, Diagnostic Products Corp., Los Angeles, CA).

Milk Composition

Milk samples were analyzed for milk fat, CP, lactose, and MUN by infrared spectroscopy (AOAC International, 2002; method 972.16; MilkoScan 605, Foss North America, Brampton, ON, Canada) at the Alberta Central Milk Testing Laboratory (Edmonton, AB, Canada).

Statistical Analysis

Effect of parity was originally tested using the PROC TTEST procedure of SAS (version 9.2, SAS Institute Inc., Cary, NC), but it was not included in the final model as significant parity effects were not observed for ruminal pH variables (Table 2, $P > 0.05$). Values of mean ± 0.5 × SD of acidosis index were used as criteria to identify groups of extreme animals (i.e., tolerant and susceptible animals), and all response variables were evaluated for the group effect using PROC TTEST. In addition, sorting index data were tested to determine if they are different from 100 by using PROC TTEST. The PROC REG procedure was used to determine the relationships between sorting index versus minimum ruminal pH, sorting index versus acidosis index, and MUN versus acidosis index. Significance was declared at $P < 0.05$ and tendency was declared at $0.05 < P < 0.10$.

RESULTS

No differences were observed in minimum, mean, and maximum ruminal pH between primiparous cows and multiparous cows, as well as duration of pH below 5.8 and area of pH below 5.8 ($P > 0.10$; Table 2). Although a tendency for higher DMI for multiparous cows was noted, acidosis index was not different between primiparous and multiparous cows.

Table 2. Comparison of BW, DMI, and pH measurements between primiparous and multiparous cows

Variable	Primiparous	Multiparous	SE	*P*-value
BW, kg	564	639	23.9	0.04
DMI, kg/d	19.4	22.1	1.02	0.08
Ruminal pH				
Nadir	5.48	5.61	0.10	0.38
Mean	6.20	6.34	0.07	0.19
Maximum	6.85	6.97	0.05	0.15
Duration pH <5.8, min/d	298	154	78.3	0.21
Area pH <5.8, pH × min/d	68.1	27.7	20.4	0.18
Acidosis index, pH × min/kg	3.84	1.21	1.12	0.12

Among all cows, minimum ruminal pH, mean pH, and duration and area of pH below 5.8 ranged from 5.16 to 6.04, 5.94 to 6.57, and 0 to 606 min/d and 0 to 193 pH × min/d, respectively. The acidosis index ranged from 0.0 to 10.9 pH × min/kg of DMI. Acidosis index of 6 cows was lower than the value of mean − 0.5 × SD, and that of 4 cows was higher than the value of mean + 0.5 × SD, and they were classified as animals tolerant and susceptible to the high-grain diet, respectively.

Ruminal pH and VFA Profile

No differences were observed in BW and DMI between tolerant and susceptible animals ($P > 0.10$; Table 3). However, the minimum (5.83 vs. 5.22; $P < 0.01$) and mean ruminal pH (6.47 vs. 6.02; $P < 0.01$) were higher for tolerant animals compared with susceptible animals, whereas maximum pH values were not different between the groups. Duration (10.1 vs. 556 min/d; $P < 0.01$) and area of pH below 5.8 (0.86 vs. 140 pH × min/d; $P < 0.01$) were lower in tolerant animals. Acidosis index was lower in tolerant animals (0.04 vs.7.67 pH × min/kg; $P < 0.01$). Total VFA concentration and VFA profile were not different between the groups ($P > 0.10$; Table 4), whereas the concentration of rumen NH_3-N tended to be higher for tolerant cows ($P = 0.06$).

Sorting Behavior and Chewing Activity

Both groups sorted for short particles, but susceptible animals sorted to a greater extent (sorting index = 105 vs. 102; $P = 0.05$; Table 5). Moreover, susceptible animals sorted against long particles, whereas tolerant animals did not (sorting index = 87.6 vs. 97.9; $P = 0.05$). Eating, ruminating and total chewing time (minutes per day) were not different between tolerant and susceptible animals ($P > 0.10$; Table 6). However, the tolerant cows had shorter ruminating time per unit of DMI (25.4 vs. 33.2 min/kg of DMI; $P = 0.05$) and total chewing time per unit of DMI (35.8 vs. 45.1 min/kg of DMI; $P < 0.05$).

Milk Production

No differences were observed in milk yield and milk component yields between tolerant and susceptible cows ($P > 0.10$; Table 7). In addition, concentrations of milk fat, protein, and lactose did not differ between the groups. However, concentration of MUN was higher for tolerant animals compared with susceptible animals (12.8 vs. 8.6 mg/dL; $P < 0.05$).

Plasma Metabolites and Hormones

Plasma glucose, insulin, BHBA, and NEFA concentrations were not different between tolerant and susceptible cows ($P > 0.10$; Table 8).

DISCUSSION

Subacute ruminal acidosis is a metabolic disorder particularly prevalent in high-producing dairy herds.

Table 3. Comparison of BW, DMI, and pH measurements between tolerant and susceptible cows

Variable	Tolerant	Susceptible	SE	*P*-value
BW, kg	622	566	36.2	0.31
DMI, kg/d	21.6	18.8	1.31	0.17
Ruminal pH				
Nadir	5.83	5.22	0.06	<0.01
Mean	6.47	6.02	0.04	<0.01
Maximum	6.98	6.88	0.08	0.38
Duration pH <5.8, min/d	10.1	556	23.3	<0.01
Area pH <5.8, pH × min/d	0.855	140	9.92	<0.01
Acidosis index, pH × min/kg	0.037	7.67	0.67	<0.01

Table 4. Comparison of ruminal VFA profile and rumen NH_3-N between tolerant and susceptible cows

Variable	Tolerant	Susceptible	SE	*P*-value
Total VFA, m*M*	126	131	7.82	0.66
Acetate, mol/100 mol	54.2	53.5	1.91	0.81
Propionate, mol/100 mol	26.4	29.3	2.08	0.36
Isobutyrate, mol/100 mol	1.07	0.65	0.04	<0.01
Butyrate, mol/100 mol	13.8	11.5	1.25	0.24
Isovalerate, mol/100 mol	1.97	1.26	0.18	0.03
Valerate, mol/100 mol	2.08	2.36	0.19	0.33
Caproate, mol/100 mol	0.56	1.48	0.46	0.20
Acetate:propionate	2.13	1.89	0.23	0.48
Rumen NH_3-N, mg/dL	9.38	4.66	1.51	0.06

The risk of SARA is greater for early- and mid-lactation cows compared with late-lactation cows due to feeding highly fermentable diets and greater feed intake. Garret et al. (1997) indicated that incidences of SARA were 19% for early-lactation dairy cows and 26% for mid-lactation cows. However, late-lactation cows were used in the current study due to the animal availability. Although early- or mid-lactation cows would be a better model for the current study, a substantial variation in the severity of SARA was detected among late lactating cows fed the same high-grain diet, which is consistent with previous studies using different type of animals. Brown et al. (2000) found that, when 5 steers were intraruminally dosed with steam-flaked corn, average ruminal pH ranged from 4.26 to 5.63. In another steer study by Schlau et al. (2012), 17 beef steers were force-fed the same diet consisting of 85% grain through rumen cannulas and the acidosis index ranged from 4.0 to 96.5 pH × min/kg among the animals. In addition, when Penner et al. (2007) provided additional concentrate to primiparous cows during the periparturient period, they found high SEM for ruminal pH variables within the treatment; for example, SEM was 30.7% of the mean for the area of pH <5.8 (mean ± SEM; 766 ± 235 pH × min). The high SEM values indicated that some cows within a treatment were able to cope with diet challenge better than others. Another experiment was conducted to induce SARA in sheep through oral glucose drench (Penner et al., 2009). Although the dose of glucose was same for all sheep, mean rumen pH was higher for resistant animals compared with susceptible ones (5.97 vs. 5.57). These individual variations among animals within the treatment clearly demonstrate that ruminants markedly vary in the extent of tolerance to dietary factors that predispose them to acidosis. However, as the type and intensity of acidosis challenge was not same for the studies mentioned previously, it is not possible to compare variations in rumen pH response and severity of SARA among different types of animals.

The second objective of the present study was to identify factors that are related to cows tolerant and susceptible to high-grain diets. We found that the tolerant cows sorted feed to a less extent than the susceptible ones. Cows have been shown to selectively consume rations even when fed a TMR; they generally sort against long particles and for fine particles (Kononoff et al., 2003; Leonardi and Armentano, 2003; DeVries et al., 2007). The majority of previous studies evaluating sorting behavior of dairy cows focused on management factors, such as effects of feeding frequency and

Table 5. Feed refusal and sorting index between tolerant and susceptible cows

Item	Tolerant	Susceptible	SE	*P*-value
Feed refusal, kg/d	3.7	4.1	0.49	0.61
Sorting index[1]				
>19.0 mm	97.9	87.6*	3.19	0.05
19.0 to 8.0 mm	97.3*	98.8	1.17	0.39
8.0 to 1.18 mm	102*	105*	0.91	0.05
<1.18 mm	101	104*	1.02	0.06
DMI, kg/d				
>19.0 mm	4.29	3.33	0.32	0.07
19.0–8.0 mm	5.12	4.51	0.32	0.23
8.0–1.18 mm	8.73	7.79	0.48	0.22
<1.18 mm	3.51	3.14	0.21	0.26

[1]Sorting index was calculated as the ratio of actual intake to predicted intake for particles retained on each sieve of the separator. A sorting index above 100 indicates sorting for particles, and a sorting index below 100 indicates sorting against particles (Leonardi and Armentano, 2003).

*Different from 100 ($P < 0.05$).

Table 6. Comparison of chewing activity between tolerant and susceptible cows

Variable	Tolerant	Susceptible	SE	*P*-value
Time, min/d				
Eating	223	220	13.7	0.87
Ruminating	544	610	33.7	0.21
Total chewing[1]	768	830	32.8	0.22
Time, min/kg of DMI				
Eating	10.3	12.0	0.70	0.14
Ruminating	25.4	33.2	2.35	0.05
Total chewing	35.8	45.1	2.65	0.04
Time, min/kg of NDF				
Eating	40.3	46.7	2.72	0.14
Ruminating	99.4	130	9.05	0.05
Total chewing	140	176	10.1	0.04

[1]The sum of eating time and ruminating time.

stocking density, and dietary factors, such as effects of DM content, forage content, and particle size of TMR. However, substantial individual variation in sorting behavior exists among animals, even those fed the same diet. Leonardi and Armentano (2003) indicated that although all cows generally sorted against long particles (retained on a sieve of 26.9-mm apertures), intake of long particles as a percentage of predicted intake was <70% for 4 cows, between 71 and 80% for 11 cows, between 81 and 90% for 5 cows, and between 91 and 100% for 2 cows. One extreme cow even failed to consume any of the long particles of TMR. Leonardi et al., (2005) found similar animal variation in sorting; sorting index of long particles (retained on a sieve of 26.9-mm apertures) ranged from approximately 10 to 100 when a dry TMR (89.9% DM) was offered. In another study, Leonardi and Armentano (2007) detected that the sorting index of the long particles were from 40 to 100 among 29 cows fed a diet containing 68% DM. In the current study, we also found that sorting index of long particles (retained on a sieve of 19-mm apertures) ranged from 76.1 to 103.6, even though all cows were fed the same diet, and acidosis-susceptible cows sorted against long particles whereas the tolerant cows did not. The DM content of the experimental diet was 60.8% due to the high-concentrate content, whereas the typical TMR given to high-producing dairy cows ranges from 40 to 60% DM (Eastridge, 2006). The dry TMR used in the current study may have increased sorting behavior of animals, but the effect of DM content on sorting is not conclusive. It is commonly believed that addition of water to a dry TMR would bind particles together and make it harder for cows to sort (Miller-Cushon and DeVries, 2009). Leonardi et al. (2005) indicated a reduction in the extent of sorting against long particles when water was added to a dry TMR (DM reduced from 81 to 64%). However, Miller-Cushon and DeVries (2009) found that sorting was increased when adding water to a TMR (reducing DM content from 58 to 48%). The difference between these 2 studies may be explained by the difference in diet composition and DM content of the diets used. Felton and DeVries (2010) also found that greater amounts of water added to the TMR (DM of diets were 56.3, 50.8, and 44.1% DM) resulted in greater sorting against long-particle diets. Therefore, it is not clear whether the sorting behavior of animals fed a relatively dry TMR in the current study would be different from those fed a TMR with less DM content.

Cows sorting TMR may create problems because sorting not only reduces the particle size of the diet consumed, but also reduces NDF intake, as the longer

Table 7. Comparisons of milk yield and milk composition between tolerant and susceptible cows

Variable	Tolerant	Susceptible	SE	*P*-value
Yield, kg/d				
Milk	28.6	24.2	3.58	0.41
Fat	0.93	0.67	0.16	0.30
CP	1.03	0.87	0.11	0.32
Lactose	1.26	1.11	0.16	0.54
Milk composition				
Fat, %	3.22	2.73	0.33	0.33
CP, %	3.64	3.60	0.14	0.84
Lactose, %	4.42	4.59	0.13	0.40
MUN, mg/dL	12.8	8.60	0.97	0.02

Table 8. Comparison of plasma blood metabolite and hormone concentration between tolerant and susceptible cows

Variable	Tolerant	Susceptible	SE	*P*-value
Glucose, mg/dL	70.2	67.0	3.86	0.57
BHBA, mg/dL	7.73	9.37	1.73	0.52
NEFA, mEq/L	64.9	74.9	4.01	0.12
Insulin, μIU/dL	21.2	19.8	5.04	0.85

particles of TMR contain more NDF than the rest of the ration (Leonardi and Armentano, 2003). Excessive sorting of a TMR can result in overconsumption of rapidly fermentable carbohydrates (relative to the anticipated intake) and refusal of physically effective fiber, which is expected to increase VFA production and decrease acid neutralization by reduced chewing (Cook et al., 2004; DeVries et al., 2008). Therefore, sorting behavior may be one of the factors that increase the risk of SARA. DeVries et al. (2008) found that when early-lactation cows were fed a low-forage diet (45% forage), their sorting activity was related to ruminal pH: the more cows sorted for medium and short particles, the lower their nadir, mean, and maximum ruminal pH were. In the current study, the tolerant cows tended to consume more long particles (>19.0 mm) than the susceptible cows (Table 5). In addition, sorting index of long particles was positively correlated with minimum ruminal pH ($r = 0.60$, $P = 0.01$; Figure 1) and negatively correlated with acidosis index ($r = -0.64$, $P < 0.01$). Therefore, the variation in susceptibility to high-grain diets among animals might be related to the variation in sorting against long particles, and more work needs to be done to confirm these findings.

The current study showed the relationship between sorting behavior and rumen pH, but no differences were observed in ruminal pH in some of the previous studies where significant ration sorting was identified (Kononoff and Heinrichs, 2003; Kononoff et al., 2003; Leonardi et al., 2005; Bhandari et al., 2008; Maulfair et al., 2010). In addition, Maulfair et al. (2010) detected that rumen pH tended to increase quadratically ($P = 0.07$) with increased sorting against long particles. Moreover, a few studies indicated that cows sorted for long feed particles as an attempt to meet physically effective fiber requirements when cows experience low rumen pH (Keunen et al., 2002; Beauchemin and Yang, 2005; DeVries et al., 2008), which means that cows may change their sorting behavior to attenuate the effects of acidosis. Therefore, the speculation that ration sorting decreases ruminal pH is not conclusively supported, and the reasons are not clear. However, effects of sorting on rumen pH might have been confounded by dietary (treatment) effects, and the extent of sorting in these studies might not be severe enough to pose the potential effect on rumen pH. DeVries et al. (2011) speculated that the risk of SARA would be much greater when sorting against long particles is more substantial (i.e., 20–30% refusal of long particles).

We found that cows that tolerant to a high-grain diet sorted feed to a lesser extent than the susceptible cows, and, as such, we expected that the tolerant cows would chew more. It has been suggested that chewing time is a good indicator of rumen health because chewing stimulates salivary buffer secretion (Allen, 1997), which helps neutralization of acids produced from fermentation. Chewing activity is highly influenced by particle length and chemical NDF concentration of the diets (Mertens, 1997; Zebeli et al., 2008). Balch (1971)

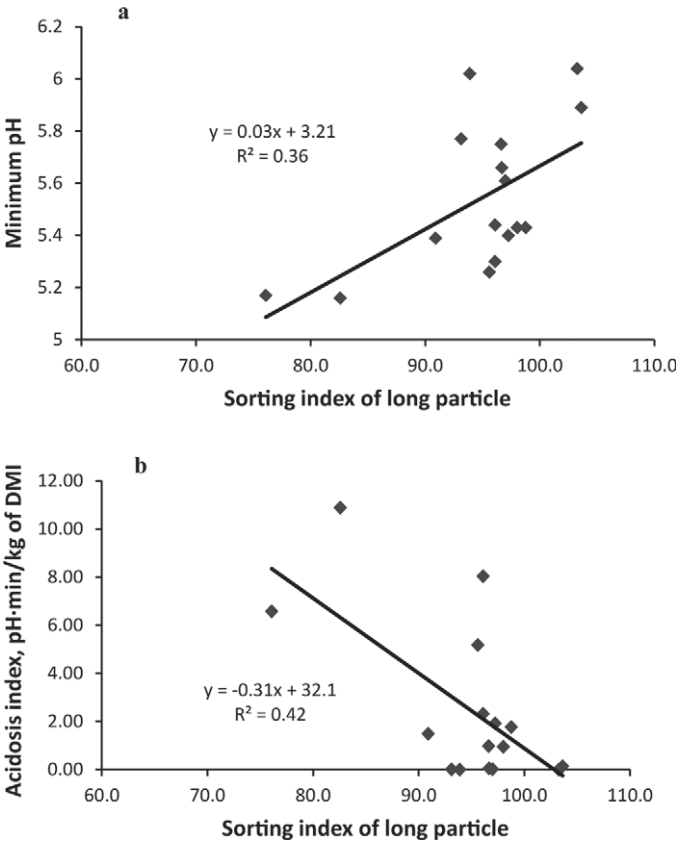

Figure 1. Relationship between sorting index of long particles (retained on a sieve of 19-mm apertures) with (a) minimum ruminal pH ($P = 0.01$), and (b) acidosis index ($P < 0.01$).

proposed using total time spent chewing per kilogram of DMI as an indicator of the physical property of the diet to minimize the confounding effects of different feed intakes. In the current study, we expected that the tolerant cows would have longer chewing time per unit of DMI than the susceptible cows; however, we found the opposite results. Total chewing time was 768 min/d and total chewing time per NDF intake was 140 min/kg for tolerant cows, whereas total chewing time was 830 min/d and total chewing time per NDF intake was 176 min/kg for susceptible cows. Chewing time measured in the current study was longer, regardless the group, than that reported in previous studies; Yang and Beauchemin (2007) reported total chewing was 655 min/d and 101.3 min/kg of NDF intake when diet forage-to-concentrate ratio was 35 to 65. In another study conducted later (Yang and Beauchemin, 2009), similar total chewing time was found (657 min/d and 102 min/kg of NDF intake) for cows fed the diet with a similar forage-to-concentrate ratio. Longer chewing time for the current study might be partly due to different methods of chewing activity monitoring (visual observation vs. automated data collection). In addition, in the present study, the data collection period for chewing behavior was limited to a relatively short period (1 d). Dado and Allen (1994) indicated that considerable day-to-day variation exists in feeding behavior data within cows. As such, the differences observed in chewing activities between 2 groups need to be interpreted with caution. Nonetheless, our findings provided no evidence to attribute higher rumen pH of the tolerant cows to the difference in chewing activity.

A couple of possible explanations exist for our observation that tolerant cows chewed less than susceptible cows. First, it may be possible that cows possess an adaptive response to the reduction in rumen pH. Previous studies have shown that cows would increase the amount of rumination needed per unit of NDF when rumen pH is low (Beauchemin, 1991; Beauchemin et al., 1994); likewise, chewing time per unit of NDF intake was less for high- than for low-NDF diets (Oba and Allen, 2000; Yang et al., 2001; Maulfair and Heinrichs, 2013), which suggests that effectiveness of forage in promoting chewing increases when rumen pH becomes lower. In the current study, susceptible cows had a lower ruminal pH. Therefore, as an adaptive response, chewing time per unit of DMI might have increased to attenuate the reduction in ruminal pH by increased saliva secretion or enhanced particulate and fluid movement from the rumen (Krause et al., 2002). DeVries et al. (2009) dosed 4 kg of ground barley and wheat into the rumen to induce ruminal acidosis before feeding TMR (45:55 of forage to concentrate ratio). Those authors found that rumination time was longer for animals experiencing more severe SARA as a result of the grain dosage. Therefore, although the particle length and chemical NDF concentration of the diets influence chewing activity, additional metabolic mechanisms regulating chewing activity need to be identified (Oba and Allen, 2000).

The second possibility for greater chewing time per unit of DMI for the susceptible cows is that lower rumen pH decreased fiber digestibility in the rumen (Russell and Wilson, 1996; Beauchemin, 2000) and increased the retention time of ruminal digesta for susceptible cows. Expected greater digesta mass in the rumen of susceptible cows may have stimulated chewing activity. Grant et al. (1995) found that total chewing time per kilogram of NDF intake was lower for cows fed brown midrib sorghum silage compared with those fed normal sorghum silage. Brown midrib sorghum silage was greater in NDF degradability than in normal sorghum silage. Therefore, enhanced NDF degradability of forage might have decreased its physical effectiveness at stimulating chewing due to a faster disappearance rate of digesta in the rumen. Oba and Allen (2000) also suggested that forage NDF degradability might affect chewing activities unless a critical amount digesta in the rumen is maintained. In the current study, rate of fiber digestion and digesta mass in the rumen were not determined, but the possibility that low rumen pH increased chewing activities via a greater rumen fill cannot be excluded as a reason for greater chewing activities for the susceptible cows.

The variation in the susceptibility to high-grain diets among animals is a concern because dairy diets are often formulated for the average animal on farms, and the susceptible cows may experience SARA whereas the average animal does not. Therefore, identifying tolerant and susceptible cows and adjusting nutritional management accordingly may reduce this nutritional disorder. In the current study, ruminally canulated cows were used, and the tolerant and susceptible cows were identified by measuring rumen pH. However, it is not practical to measure rumen pH for numbers of cows on farms. Therefore, it is necessary to evaluate an easy indicator of rumen pH to identify the tolerant and susceptible cows. We expected milk fat content might be the noninvasive indicator, but milk fat content did not differ between the 2 groups in the current study. Some previous studies also reported no effect of ruminal pH on milk fat concentration and indicated that milk fat depression does not always accompany SARA (Keunen et al., 2002; Cottee et al., 2004; Gozho et al., 2007). Those authors suggested that the inconsistent response in milk fat in experimentally induced SARA may be related to the duration of SARA; short durations of SARA may not affect milk fat content (Krause and

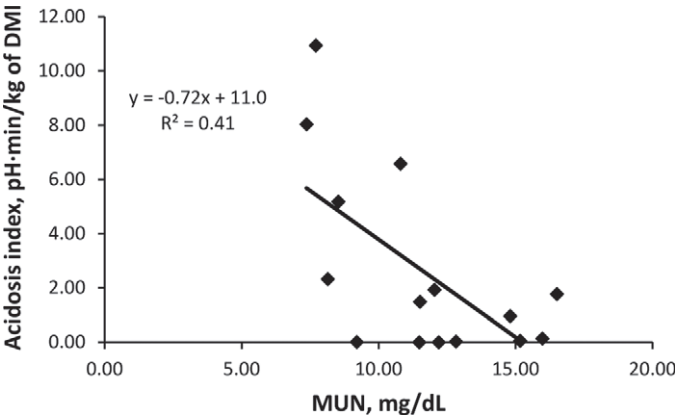

Figure 2. Relationship between acidosis index with MUN concentration ($P = 0.01$).

Oetzel, 2005). Because microbial responses to ruminal acidosis may be slow, multiple acidotic insults are necessary before ruminal biohydrogenation is inhibited to cause milk fat depression (Oetzel, 2007). However, in the current study, cows were fed the high-grain diet for 21 d. As such, the duration of the SARA was expected to be long enough and may not be a possible explanation for this case. However, Oetzel (2007) indicated that the relationship between SARA and milk fat depression is inconsistent and influenced more by other factors, and suggested that many cows and herds with substantially depressed ruminal pH could have no milk fat depression at all. In the current study, we observed a large numerical difference in milk fat concentration between the 2 groups (3.22 vs. 2.73), but we could not detect this as a significant difference due to a substantial variation within groups (SE = 0.33). This indicates that other unidentified factors, besides rumen pH, affected milk fat content, and that milk fat content may not be a sensitive indicator to identify cows that are tolerant or susceptible to high-grain diets. In addition, fat content of tolerant cows was 3.59% immediately before the current study when cows were fed a high-forage diet (60:40 forage-to-concentrate ratio), which indicated that the high-grain diet fed during the current study decreased milk fat content to some extent even for the tolerant cows.

However, we found that the tolerant cows had higher MUN concentration than the susceptible ones. In addition, a negative correlation was observed between MUN and acidosis index ($r = -0.64$, $P = 0.01$; Figure 2). Milk urea nitrogen concentration did not differ between the 2 groups before the start of experiment when all cows were fed a diet containing 60% forage on a DM basis. Therefore, MUN might be potentially used as an indicator to identify tolerant and susceptible cows fed high-grain diets. Concentration of MUN is a good predictor of urinary N excretion and the efficiency of protein utilization in dairy cows (Gustafsson and Palmquist, 1993; Kohn et al., 2002). It has been indicated that MUN can be affected by nutritional factors, such as dietary CP content, ruminally fermentable OM, the ratio of dietary CP to energy, and the extent of CP degradation in the rumen (Carlsson et al., 1995; Hof et al., 1997; NRC, 2001). In addition, it is affected by nonnutritional factors, such as DIM, parity, season, and milking frequency (Carlsson et al., 1995; Hof et al., 1997). Also, MUN concentration is affected by unidentified animal factors (Wattiaux et al., 2005; Cyriac et al., 2008; Rius et al., 2010). In the current study, all cows were fed the same diet and, most likely, were in positive balance for all nutrients, including energy and protein. Due to the numerical difference in DMI, the tolerant cows had 445 g more CP intake; higher MUN and rumen NH_3 appear to be attributable to the difference in CP intake. However, the difference in CP intake between the groups was not significant. In addition, before the start of experiment, when cows were fed a 60% forage diet, the tolerant cows and susceptible cows had similar ruminal NH_3 (9.37 vs. 10.9 mg/dL, respectively; $P = 0.56$) and MUN concentrations (12.5 vs. 11.6 mg/dL, respectively; $P = 0.54$), although DMI was numerically greater for the tolerant cows (23.9 vs. 19.6 kg/d; $P = 0.14$). Therefore, the numerical difference in CP intake may not be the exclusive reason that tolerant cows had higher MUN and rumen NH_3 when cows were fed the high-grain diet. Schlau et al. (2012) suggested that higher ruminal pH for acidosis-resistant steers is partly due to lower VFA production. Greater MUN and ruminal NH_3 concentrations for the tolerant cows in the current study may indicate that OM fermentation is lower for them even if the same diet was fed. However, we did not measure the rate of VFA production or OM fermented in the rumen in the current study; as such, further research is warranted to confirm this preliminary finding and identify if MUN could be used as a noninvasive indicator to identify tolerant and susceptible cows on farms.

CONCLUSIONS

A substantial variation exists in the severity of SARA among lactating dairy cows when fed the same high-grain diet. Cows that are tolerant to high-grain diets sorted to a lesser extent compared with susceptible cows. However, tolerant cows may not necessarily have longer chewing time than susceptible cows. In addition, MUN concentration, rather than milk fat content, might be potentially used as a noninvasive indicator to identify cows that are tolerant to high-grain diets on farm.

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