Effects of Rumen Acid Load from Feed and Forage Particle Size on Ruminal pH and Dry Matter Intake in the Lactating Dairy Cow

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ABSTRACT

The objective of this study was to evaluate the effects of level of concentrate acidogenic value (AV) and forage particle size on ruminal pH and feed intake in lactating dairy cows. Two isoenergetic (net energy for lactation = 1.5 ± 0.01 Mcal/kg) and isonitrogenous (crude protein = $17.4 \pm 0.1\%$ dry matter) concentrates with either a low AV or high AV were formulated and fed in a total mixed ration with either coarsely or finely chopped corn silage and alfalfa haylage ad libitum. Four rumen-fistulated cows (114 ± 14 d in milk) were randomly assigned to 1 of the 4 treatments in a 4×4 Latin square with a 2×2 factorial treatment arrangement. Each period consisted of 3-wk (14-d treatment adaptation and 7-d data collection). Increasing the concentrate AV decreased the mean pH (from 6.07 to 5.97) and minimum pH (from 5.49 to 5.34). Cows fed high-AV diets spent a longer time below pH 5.6 (135.1 vs. 236.7 min/d; low-AV diet vs. high-AV diet, respectively) and pH 5.8 (290.0 vs. 480.6 min/d; low-AV diet vs. high-AV diet, respectively) than cows fed low-AV diets. Increasing forage particle size had no effect on the mean and minimum ruminal pH. There was an interaction between concentrate AV and forage particle size on maximum ruminal pH. Increasing forage particle size increased the maximum pH for cows fed the high-AV concentrate (6.69 vs. 6.72; low-AV diet vs. high-AV diet, respectively) and had no effect on the maximum pH for cows fed the low-AV concentrate (6.98 vs. 6.76; low-AV diet vs. high-AV diet, respectively). Increasing the concentrate AV did not affect dry matter intake but reduced neutral detergent fiber intake from 9.7 to 8.8 kg/d. Milk fat content was negatively correlated with time and area below pH 5.6 (time below, $r = -0.51$; area below, $r = -0.56$) and pH 5.8 (time below, $r = -0.42$; area below, $r = -0.54$). These results suggest that coarse forage particle size can attenuate drops in ruminal pH. However, the ameliorating effects of forage particle size on drops in ruminal

pH were more apparent for high-AV diets than for low-AV diets. The AV approach combined with physically effective neutral detergent fiber would therefore improve the formulation of diets and help to mitigate subacute ruminal acidosis in dairy cows.

Key words: acidogenic value, dry matter intake, forage particle size, ruminal pH

INTRODUCTION

Rumen acidosis is related to the amount of acid produced from fermented feed and the capacity of the feed to support salivary buffer production. Subacute rumen acidosis (**SARA**) is characterized by repeated bouts of depressed ruminal pH between 5.2 and 5.6, often resulting from a large intake of rapidly fermentable carbohydrates, which leads to an increase of organic acids in the rumen (Owens et al., 1998). The length of time per day when ruminal pH is below 5.6 (Keunen et al., 2002) or below 5.8 (Krause et al., 2002b; Krause and Combs 2003) is a more important determinant of rumen acidosis than the mean daily ruminal pH.

Previously, Rustomo et al. (2006a) evaluated the acidogenic value (**AV**) of feeds using an in vitro laboratory technique. The AV varied with the protein, starch, NFC, and fiber contents of feedstuffs, being highest for NFC-rich feeds, intermediate for forages, and lowest for feeds high in protein. The rate at which rumen fluid pH changed followed a pattern similar to changes in the AV (Rustomo et al., 2006a), and the differences in AV and pH changes likely were associated with the fermentability of the feeds (de Smet et al., 1995). A subsequent in vivo study evaluated the effect of concentrate AV on in vivo ruminal pH (Rustomo et al., 2006b). The ruminal pH of cows fed low-AV (**LA**) diets remained below pH 5.6 for 170 min/d during the day compared with 342 min/d for the high-AV (**HA**) cows, suggesting that the LA diets possessed higher buffering capacity.

Ruminal pH is determined by the equilibrium between acid production and acid disappearance from the rumen (neutralization and absorption). Fiber intake affects acid production and saliva secretion (Allen, 1997). Physically effective NDF (**peNDF**) has been defined as the fraction of feed that stimulates chewing and saliva

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production (Mertens, 1997), and the peNDF value indicates the physical characteristic of the fiber, such as particle size. Although the peNDF concept was developed to reduce the problems associated with an increased rumen acid load from feeding highly fermentable feeds, the peNDF value does not take into account acid production. The effect of forage particle size (**FPS**) on intake and ruminal pH is well documented (Kononoff and Heinrichs 2003a,b; Kononoff et al., 2003a; Beauchemin and Yang, 2005). Although works have been done to evaluate the effects of feed fermentability (Krause et al., 2002b; Krause and Combs, 2003) or AV (Dewhurst et al., 2001; Rustomo et al., 2006b) on intake, to our knowledge, no work has evaluated the relationship between concentrate AV and FPS on ruminal pH and performance in lactating dairy cows. The present study was conducted to test the hypothesis that the effect of increasing the concentrate AV on ruminal pH would depend on the degree of fineness of the FPS. Therefore, the objective of the present study was to determine the effects of the concentrate AV and FPS on the ruminal pH and DMI of dairy cows.

MATERIALS AND METHODS

Animals

Four multiparous Holstein dairy cows (114 ± 14 DIM) were used in this trial. The cows were fitted with a ruminal fistula and averaged 600 ± 66 kg of BW at the beginning of the experiment and 652 ± 92 kg at the end of experiment. The experiment was conducted at the Elora Dairy Research Center (University of Guelph, Guelph, Ontario, Canada) from June 17 to September 8, 2005. Animals were cared for and handled in accordance with the Canadian Council on Animal Care regulations, and the University of Guelph Animal Care Committee approved their use for this experiment.

Cows were fed ad libitum, allowing 10% refusals, and the feed was offered twice daily at 0900 and 1300 h in equal portions. Orts from individual cows were weighed each morning prior to feeding. Representative feed and ort samples were taken 3 times per week and stored at -20°C for later analysis. The samples were then composited over the recording week of each period. Feed intake and milk yield were monitored daily throughout the experiment. The animals had unlimited access to fresh water and were milked in their stalls twice daily at 0500 and 1500 h. Milk samples were collected 3 times per week from morning and afternoon milkings and preserved using 2-bromo-2-nitropropane-1,2-diol. The milk samples were pooled 3 times each week based on milk yield, and the pooled samples were immediately submitted to the Central Milk Testing Laboratory (Lab-

oratory Services Division, University of Guelph) for compositional analysis.

Experimental Design

The experiment was conducted as a 4×4 Latin square with a 2×2 factorial treatment arrangement. Each period was 3 wk (14-d treatment adaptation and 7-d data collection) in duration. Feed ingredient AV was used to formulate 2 isoenergetic ($\text{NE}_L = 1.5 \pm 0.01$ Mcal/kg) and isonitrogenous ($\text{CP} = 17.4 \pm 0.1\%$ DM) concentrate diets (LA vs. HA) fed ad libitum in a TMR alongside a coarse or finely chopped corn silage and alfalfa haylage. The concentrate ingredients were ground in a Schutte pulverizing rotary hammer mill (model 44-24-301B; Schutte Pulverizer Company, Inc., Buffalo, NY) through a 3-mm screen and pelleted (4-mm diameter). First-cut wilted alfalfa haylage and corn silage harvested (John Deere harvester, model 7200; John Deere, Moline, IL) at 1.3 and 1.9 cm theoretical length of cut, respectively, provided the coarse fraction of the diets (**CS**). The finely chopped silage and haylage (**FS**) was obtained by rechopping CS at 1,600 rpm for 36 min daily in a mixer (model 1350; Taylor, Orton, Ontario, Canada). The geometric particle sizes of the mixed forages are given in Table 1. The 2 levels of FPS (CS and FS) were combined with the 2 levels of concentrate AV (HA and LA) to prepare 4 TMR in a data ranger (American Calan, Northwood, NH): **HACS**, HA concentrate + CS; **HAFS**, HA concentrate + FS; **LACS**, LA concentrate + CS; and **LAFS**, LA concentrate + FS. All diets were formulated to meet or exceed the requirements of a 620-kg multiparous cow producing 30 kg/d of milk using the Cornell–Penn–Miner dairy model, version 3.0 (CPM-Dairy). Diets were fed as a TMR with a forage-to-concentrate ratio of 50:50. The DM of the feeds and Orts was determined by oven-drying at 60°C for 48 h using standard AOAC procedures (AOAC, 1990), and the diet ingredients were adjusted weekly to account for changes in the DM content. Diet composition and chemical analyses are shown in Tables 3 and 4.

Particle Size Distribution

The new compact, manually operated Penn State particle size separator (**PSPS**) with 3 sieves and a solid bottom pan (model C24682N, Nasco, Fort Atkinson, WI) was used to assess the particle sizes of both forages and TMR as described by Kononoff et al. (2003b). The material remaining on each sieve and pan was then removed, weighed (scale accurate to 1 g), and oven-dried at 60°C to determine the distribution of feed DM retained on each sieve and in the pan. The percentage of DM retained on each sieve, geometric mean, and

Table 1. Percentage DM, NDF content of forage, NDF content of particle fraction retained on each screen, particle sizes, physical effectiveness factors (pef), and physically effective NDF (peNDF) before and after rechopping¹

Dependent variable	Alfalfa haylage		Corn silage		Mixed forage		SED	P-value
	Mean	SEM	Mean	SEM	Before rechopping	After rechopping		
					Mean	Mean		
DM retained on each screen, %								
>19 mm	20.1	1.0	9.0	1.1	12.1	6.3	0.84	<0.01
19.0 to 8.0 mm	56.3	1.5	67.4	2.2	60.6	59.7	1.27	0.47
8.0 to 1.18 mm	19.9	1.2	22.1	1.3	24.1	28.7	0.79	<0.01
<1.18 mm	5.2	1.4	1.5	0.2	3.2	5.3	0.46	<0.01
X _{gm} , mm	14.5	0.5	13.4	0.2	12.8	10.6	0.31	<0.01
S _{gm} , mm	2.6	0.1	2.2	0.05	2.4	2.5	0.05	0.04
NDF, % DM	55.1	0.6	47.0	1.8	51.1	51.4	1.09	0.79
NDF on each screen, % DM								
>19 mm	50.0	0.7	56.7	1.5	55.5	54.2	0.68	0.07
19.0 to 8.0 mm	52.0	0.8	49.5	2.0	50.8	50.5	0.70	0.69
8.0 to 1.18 mm	49.8	1.2	35.0	1.7	47.7	48.7	1.07	0.37
<1.18 mm	42.7	1.6	38.5	2.5	40.0	44.5	1.25	<0.01
pef _{>8}	0.76	0.01	0.76	0.01	0.72	0.66	0.01	<0.01
pef _{>1.18}	0.96	0.005	0.98	0.002	0.97	0.95	0.004	<0.01
peNDF _{T>8} , % DM	42.1	1.0	35.9	1.3	36.9	34.0	1.23	0.03
peNDF _{F>8} , % DM	38.2	2.0	38.3	3.0	37.5	33.6	0.71	<0.01
peNDF _{T>1.18} , % DM	53.1	0.6	46.3	1.4	49.4	48.7	1.23	0.55
peNDF _{F>1.18} , % DM	47.0	1.6	46.0	2.2	49.0	47.5	0.58	0.02

¹SEM = Standard error of means; SED = standard error of the difference; X_{gm} = geometric mean length as calculated by the ASAE standard (2001); S_{gm} = standard deviation as calculated by the ASAE standard (2001); pef_{>8} = physical effectiveness factor measured as the proportion of DM retained on the top and middle screens; pef_{>1.18} = physical effectiveness factor measured as the proportion of DM retained on the top, middle, and bottom screens; peNDF_{T>8} = physically effective NDF > 8.0 mm = pef_{>8} multiplied by the NDF content of forage; peNDF_{F>8} = physically effective NDF > 8.0 mm, estimated by multiplying the proportion of DM retained by the 19- and 8-mm screens and the NDF content of each fraction of particles retained on the relative screen; peNDF_{T>1.18} = physically effective NDF > 1.18 mm = pef_{>1.18} multiplied by the NDF content of forage; peNDF_{F>1.18} = physically effective NDF > 1.18 mm, estimated by multiplying the proportion of DM retained by the 19-, 8-, and 1.18-mm screens and the NDF content of each fraction of particles retained on the relative screen.

standard deviation were then calculated as described in Standard S424 of the American Society of Agricultural Engineers (ASAE, 2001). The physical effectiveness factor (**pef**) of particles >8.0 mm (pef_{>8}) was determined using the proportion of DM of particles retained on the top and middle screens of the PSPS (Lammers et al., 1996), whereas the pef of particles >1.18 mm (pef_{>1.18}) was determined using the proportion of DM of particles retained on the top, middle, and bottom screens of the PSPS (Mertens, 1997; Heinrichs and Kononoff, 2002; Kononoff and Heinrichs, 2003b). The peNDF_{>8} and peNDF_{>1.18} were estimated using both the total NDF content of the forage or TMR multiplied by the pef_{>8} and pef_{>1.18}, respectively, and using the sum of the percentage of DM retained on the top, middle, and bottom screens multiplied by the NDF content of the forage or TMR retained on each respective screen of the PSPS. Values of both pef and peNDF of particles >8.0 and >1.18 mm were measured, because the original PSPS used 2 screens (>8.0 mm) whereas the new PSPS used 3 screens (>1.18 mm). All feed samples were sieved in triplicate.

Ruminal pH Measurements

Ruminal pH was measured continuously for 4 d in each period using an industrial electrode as described by AlZahal et al. (2005). Briefly, a heavy-duty pH probe designed for industrial applications (PHE-7352-15-PT100; Omega Engineering Inc., Stamford, CT) was placed through the cannula into the anterior region of the ventral sac of the rumen. A stainless-steel weight (approximately 0.5 kg) was attached to the proximal end of the probe via an ultrahigh molecular weight (food-grade plastic) connector to maintain the probe in place and the probe was connected directly to a compact lightweight (120 g) data logger (pH Temp101; Monarch Instrument, Amherst, NH) mounted on the animal's back. Ruminal pH readings were taken every minute and were downloaded onto a computer for subsequent analysis. The position of the pH electrode was examined 3 times each day (before the morning feeding, 3 to 4 h after the morning feeding, and 3 to 4 h after the afternoon feeding) by palpation through the rumen fistula to ensure that the probe remained in place. Electrodes and the pH transmitter were calibrated at least once

during each recording period using standard pH 4 and pH 7 buffer solutions (Fisher Scientific, Fairlawn, NJ). Rumen fluid was also sampled 3 times each day (before the morning feeding, 3 to 4 h after the morning feeding, and 3 to 4 h after the afternoon feeding) for pH measurement using a handheld pH meter (pH 310; Oakton Instruments, Vernon Hills, IL) to verify that the continuous pH recordings were accurate. The daily ruminal pH data were recorded using data-recording software (version 2.0; Monarch Instruments) that automatically calculates the average, minimum, and maximum pH and standard deviation. The continuous ruminal pH data were summarized for each 24-h period by calculating the minimum, maximum, and mean pH; the amount of time below pH 5.0 to 6.8; and the area (time $\times$ pH) below pH 5.0 to 6.8. Using this data set, mean, minimum, and maximum pH; time below pH 5.0 to 6.8; and area below pH 5.0 to 6.8 for each cow were analyzed. The area below pH 5.0 to 6.8 was calculated as the product of time (min) and deviation (0.1 pH unit) from the designated pH value (Keunen et al., 2002).

Chemical Analysis

Dried feed samples were ground to pass through a 1-mm screen (Wiley mill; Arthur H. Thomas, Philadelphia, PA) and chemical composition was determined in duplicate using standard AOAC procedures (AOAC, 1990) at a commercial laboratory (Agri-Food Laboratories, Guelph, Ontario, Canada). The analytical DM contents of feeds were determined by oven-drying at 135°C for 2 h; OM was determined by ashing at 500°C for 16 h; and CP, soluble protein, and nonprotein nitrogen were determined by using the macro-Kjeldahl method. Starch was analyzed using the starch gelatinization and hydrolysis method (Hall, 2000), and Ca and P were analyzed by inductively coupled plasma spectroscopy. The samples were also analyzed for ether extract, ADF, and lignin (AOAC, 1990) and NDF (AOAC, 2002) using α -amylase (A-3306; Sigma-Aldrich, St. Louis, MO). Nonfiber carbohydrates were calculated as $\text{NFC} = 100 - (\% \text{ NDF} + \% \text{ CP} + \% \text{ fat} + \% \text{ ash})$. The AV (mg of Ca/g of DM) of the concentrate and TMR were determined as previously described by Rustomo et al. (2006a). Briefly, 1-g samples (DM basis) were weighed directly from the freeze-drier into 100-mL incubation tubes held at 39°C in a water bath, and the samples were incubated in duplicate with 30 mL of buffered rumen liquor comprising 60% buffer and 40% rumen liquor, as described by Wadhwa et al. (2001). Apparent AV was calculated as the product of Ca concentration from the analysis and fluid volume (30 mL) divided by the sample weight (1 g; Wadhwa et al., 2001). Milk samples were analyzed for CP, fat, and lactose

Table 2. Ingredient composition (% DM) and acidogenic value (AV; mg Ca/g of feed DM) of the concentrate

Ingredient	Treatment ¹			
	HACS	HAFS	LACS	LAFS
Barley	24.2	24.2	—	—
Wheat	24.4	24.4	—	—
Oats	—	—	21.6	21.6
Wheat bran	—	—	46.1	46.1
Roasted soybeans	16.3	16.3	—	—
Canola meal	16.3	16.3	—	—
Corn gluten meal	—	—	9.9	9.9
Fish meal	—	—	2.9	2.9
Soybean meal	14.9	14.9	14.8	14.8
Tallow	—	—	1.58	1.58
Salt	0.99	0.99	0.59	0.59
CaCO ₃ powder	1.29	1.29	2.18	2.18
Dicalcium phosphate	1.29	1.29	—	—
Selenium	0.30	0.30	0.30	0.30
Mineral and vitamin premix	0.10	0.10	0.10	0.10
AV	9.0	9.0	6.5	6.5

¹Treatments: HACS = high-AV concentrate and coarse silage; HAFS = high-AV concentrate and fine silage; LACS = low-AV concentrate and coarse silage; LAFS = low-AV concentrate and fine silage.

using a near-infrared analyzer (Foss System 4000; Foss Electric, Hillerød, Denmark).

Statistical Analysis

Data on the response variables were analyzed using PROC MIXED of SAS (v. 9.1; SAS Institute Inc., 2004) using the model

$$Y_{ijkl} = \mu + C_i + P_j + A_k + F_l + (A \times F)_{kl} + \varepsilon_{ijkl},$$

where Y_{ijkl} is the dependent variable, μ is the overall mean, C_i is the effect of cow ($i = 1, 2, 3, 4$), P_j is the effect of period ($j = 1, 2, 3, 4$), A_k is the effect of concentrate AV ($k = 1, 2$), F_l is the effect of FPS ($l = 1, 2$), $(A \times F)_{kl}$ is the effect of the interaction of A_k and F_l , and ε_{ijkl} is the random residual error. The effects of concentrate AV and FPS were analyzed as fixed effects. For the repeated measurements of continuous ruminal pH data, day of sampling was analyzed as a fixed effect with repeated measurement. The correlations between ruminal pH and either rumen acid load or nutrient intakes were determined using PROC CORR of SAS. Effects were considered significant at $P < 0.05$.

RESULTS AND DISCUSSION

Particle Size Distribution

Analysis of the FPS showed that the alfalfa haylage and corn silage were coarsely chopped (Table 1). Ingredient composition and AV of the concentrates and the dietary composition, chemical analysis, and AV of the TMR are presented in Tables 2 and 3, respectively.

Table 3. Dietary composition, chemical analysis, and acidogenic value (AV) of the TMR

Item	Treatment ¹			
	HACS	HAFS	LACS	LAFS
Ingredient, % (DM basis)				
Concentrate	50.1	50.1	50.1	50.1
Corn silage ²	26.8	26.8	26.8	26.8
Alfalfa silage ³	23.1	23.1	23.1	23.1
DM, %	44.4	44.2	44.7	44.7
OM, % of DM	92.7	91.7	92.8	92.8
NE _L , Mcal/kg	1.5	1.5	1.5	1.5
Composition, % (DM basis)				
CP, % of DM	17.6	17.6	17.1	17.3
Soluble protein, % of DM	5.0	5.0	4.5	4.7
Undegradable protein, % of CP	35.0	35.9	36.9	36.6
NDF, % of DM	41.7	42.6	44.0	44.0
ADF, % of DM	26.6	25.5	26.3	26.1
NFC, % of DM	29.9	28.7	28.6	27.9
Starch, % of DM	15.6	15.4	14.2	13.8
Ca, % of DM	0.7	0.7	0.7	0.7
P, % of DM	0.5	0.5	0.5	0.5
K, % of DM	1.8	1.8	1.8	1.8
Mg, % of DM	0.2	0.2	0.3	0.3
Na, % of DM	0.2	0.2	0.1	0.1
AV, mg of Ca/g of DM	10.0	10.0	8.7	8.7

¹Treatments: HACS = high-AV concentrate and coarse silage; HAFS = high-AV concentrate and fine silage; LACS = low-AV concentrate and coarse silage; LAFS = low-AV concentrate and fine silage.

²DM, 31.0%; CP, 7.1%; ADF, 27.9%; NDF, 47.0%; starch, 24.6%; and NFC, 39.5%.

³DM, 41.3%; CP, 16.3%; ADF, 39.9%; NDF, 55.1%; starch, 0.4% and NFC, 16.2%.

Rechopping the forages reduced the geometric particle length, reduced the percentage of DM retained on the top sieve (>19 mm), and increased the percentage of DM retained on the bottom sieve (1.18 mm) and in the pan (Table 1). The percentage of DM retained on the second sieve was not reduced with rechopping (60.6 vs. 59.7). Rechopping the mixture of alfalfa haylage and corn silage reduced the DM of the TMR retained on the 19- and 8.0-mm screens and increased the DM of the TMR retained on the 1.18-mm screen and in the pan. This reduced the geometric mean particle size and pef of the mixed fine alfalfa haylage and corn silage diets (Table 1) and the pef and geometric particle size of the fine diets (HAFS and LAFS; Table 4). The pef_{>8} of both the coarse and fine diets were lower than the pef_{>1.18} because a high proportion of particles of both diets was retained on the bottom screen (>1.18 mm). Thus, the peNDF_{>8} of both the coarse and fine diets were lower than the peNDF_{>1.18} (Table 4).

The NDF contents of the forages and TMR feed particles retained on each screen are shown in Tables 1 and 4. The NDF content of TMR particles retained on each sieve was highest on the top sieve (>19 mm) and lowest in the pan (<1.18 mm; Table 4). Both the original and new PSPS assume uniformity of the NDF content from

the different fractions of the PSPS (Lammers et al., 1996; Kononoff et al., 2003b). Reducing the FPS reduced the total (T) dietary peNDF_{T>8} of the TMR (25.9 vs. 21.7% DM, CS vs. FS diet, respectively) and reduced the peNDF_{T>1.18} of the TMR (40.4 vs. 38.5% DM; CS vs. FS diet, respectively). By estimating the peNDF using the fractional (F) NDF content, the finer FPS reduced the peNDF_{F>8} of the TMR (26.8 vs. 21.5% DM, CS vs. FS diet, respectively) and reduced the peNDF_{F>1.18} of the TMR (38.2 vs. 34.9% DM, CS vs. FS diet, respectively; Table 4). Assuming uniformity of the NDF content from the different fractions, the PSPS underestimated the peNDF_{>8} of the coarse fraction of the TMR by 3.4% and overestimated the peNDF_{>1.18} of both the coarse and fine fractions of the TMR by 5.6 and 10.5%, respectively. However, both methods gave similar rankings of the diets based on the peNDF contents. The method using the total NDF content would be more applicable on farm because it costs less compared with using the fractional NDF content. Zebeli et al. (2006) suggested that estimating the peNDF_{>1.18} would give a better estimate of the TMR actually consumed by cows and would lower the estimation bias caused by sorting consumption, as compared with the estimate of peNDF_{>8}.

As expected, all diets had similar energy and protein contents. The NDF contents of the HA diets were lower than those for the LA diets (Table 3). However all diets were relatively high in NDF compared with the minimum requirement of 25% (NRC, 2001). The geometric mean particle sizes of 9.8 and 7.5 mm for the CS and FS diets, respectively, were above the threshold of 6.4 mm, which has been reported to decrease rumination and the milk fat content (Woodford et al., 1986). Despite a higher proportion of the CS diets being retained on the top (19 mm) and middle screens (8.0 to 19.0 mm) compared with the FS diets (6.6 vs. 3.7%, and 53.9 vs. 46.4%, CS vs. FS, top and middle screens, respectively), both the CS and FS diets met the standards of 3 to 7% of the TMR particles being retained on the top sieve and 30 to 50% of the TMR being retained on the second sieve, as recommended by Heinrichs and Kononoff (2002). Additionally, the peNDF_{>1.18} for all diets, whether estimated based on the total dietary NDF (peNDF_{T>1.18} of 37.8 to 41.2% DM) or based on the fractional NDF (peNDF_{F>1.18} of 34.2 to 40.6% DM), were greater than the critical value of 22% DM recommended to maintain a ruminal pH of 6.0 and were greater than the critical value of 20% DM needed to maintain milk fat at 3.4% (Mertens, 1997). Thus, all diets were expected to be adequate in effective fiber.

Ruminal pH

Diurnal ruminal pH fluctuations for the 4 treatments are shown in Figure 1. All diets resulted in a similar

Table 4. Effect of reducing forage particle size on TMR particle size, percentage of DM retained on each screen, NDF content, physical effectiveness factor (pef), and physically effective NDF (peNDF)¹

Dependent variable	Treatment ²				SEM	P-value	
	HACS	HAFS	LACS	LAFS		AV	PS
DM retained, %							
>19 mm	6.4	4.1	6.8	3.4	0.58	0.78	<0.01
19.0 to 8.0 mm	54.0	45.9	53.7	46.9	0.42	0.48	<0.01
8.0 to 1.18 mm	34.4	38.6	33.1	38.9	0.76	0.56	<0.01
<1.18 mm	5.2	11.3	6.3	10.8	0.21	0.17	<0.01
X _{gm} , mm	9.9	7.5	9.7	7.6	0.09	0.60	<0.01
S _{gm} , mm	2.6	2.9	2.7	2.8	0.03	0.57	<0.01
NDF, % DM							
>19 mm	53.4	53.9	54.6	53.0	0.46	0.67	0.28
19.0 to 8.0 mm	41.2	40.4	45.2	43.4	0.96	0.01	0.23
8.0 to 1.18 mm	32.7	35.0	38.1	34.4	2.30	0.33	0.77
<1.18 mm	30.7	33.8	30.3	29.2	1.50	0.14	0.53
NDF of TMR, % DM	41.7	42.6	44.0	44.0	0.77	0.02	0.17
pef ₋₈	0.60	0.50	0.61	0.50	0.01	0.82	<0.01
pef _{>1.18}	0.94	0.89	0.94	0.89	0.002	0.17	<0.01
peNDF _{T>8} , % DM	25.2	21.3	26.6	22.1	0.40	0.04	<0.01
peNDF _{F>8} , % DM	25.6	20.8	28.0	22.1	0.70	0.04	<0.01
peNDF _{T>1.18} , % DM	39.5	37.8	41.2	39.2	0.40	0.01	<0.01
peNDF _{F>1.18} , % DM	35.8	34.2	40.6	35.5	1.10	0.03	0.02

¹SEM = Standard error of means; AV = acidogenic value of the diet; PS = particle size of the diet; X_{gm} = geometric mean length as calculated by the ASAE standard (2001); S_{gm} = standard deviation as calculated by the ASAE standard (2001); pef₋₈ = physical effectiveness factor, measured as the proportion of DM retained on the top and middle screens; pef_{>1.18} = physical effectiveness factor, measured as the proportion of DM retained on the top, middle, and bottom screens; peNDF_{T>8} = physically effective NDF >8.0 mm = pef₋₈ multiplied by NDF content of TMR; peNDF_{F>8} = physically effective NDF >8.0 mm, estimated by multiplying the proportion of DM retained by the 19- and 8-mm screens and the NDF content of each fraction of particles retained on the relative screen; peNDF_{T>1.18} = physically effective NDF >1.18 mm = pef_{>1.18} multiplied by the NDF content of the TMR; peNDF_{F>1.18} = physically effective NDF >1.18 mm, estimated by multiplying the proportion of DM retained by the 19-, 8-, and 1.18-mm screens and the NDF content of each fraction of particles retained on the relative screen.

²Treatments: HACS = high acidogenic value concentrate, coarse forage; HAFS = high acidogenic value concentrate, fine forage; LACS = low acidogenic value concentrate, coarse forage; LAFS = low acidogenic value concentrate, fine forage.

biphasic pattern related to feed intake. The diurnal patterns of ruminal pH changes shown by the diets are typical for grain-fed dairy cows (Keunen et al., 2002). Ruminal pH declined shortly after feeding, after which it gradually increased and then returned to normal pH (>6.0) overnight. However, the rate and extent of pH decline after feeding increased with the HA diets. The higher and faster rate of decrease in ruminal pH after feeding exhibited by the HA diets is in agreement with our previous study (Rustomo et al., 2006b). The LAFS and HAFS cows maintained a lower ruminal pH longer than the LACS and HACS cows, suggesting that at the same level of concentrate AV, increasing the FPS mitigated the depression in ruminal pH. However, the effect of increasing the FPS on ameliorating ruminal pH depression was more apparent for cows fed the HA concentrate than those fed the LA concentrate. The lower effect of increased FPS on attenuating ruminal pH drops in the LA diets may have been from the higher intrinsic buffering capacity of the LA concentrate compared with the HA concentrate. The LA concentrate was predominantly composed of 46.1% wheat bran (DM

basis; Table 2), which had a relatively low AV and could maintain a relatively high rumen fluid pH after incubation compared with other energy feeds (Rustomo et al., 2006a).

The effects of dietary treatments on ruminal pH are presented in Table 5. There was an interaction between concentrate AV and FPS on the maximum ruminal pH. For cows fed the HA concentrate, increasing the FPS increased the maximum ruminal pH (6.69 vs. 6.72), whereas for cows fed the LA concentrate, increasing the FPS had no effect on the maximum ruminal pH (6.98 vs. 6.76; Table 5). Increasing the concentrate AV decreased the mean pH (from 6.07 to 5.97) and minimum pH (from 5.49 to 5.34). In our previous study (Rustomo et al., 2006b), the mean and minimum pH were not affected by increasing the concentrate AV, but maximum pH was affected. Increasing the concentrate AV increased the time below pH 5.6 to 6.0 and the area below pH 5.6 to 6.0. The additional time below pH 5.6 and 5.8 attributable to increasing the concentrate AV was approximately 2 h/d (2.2 vs. 3.9 h/d) and 3 h/d (4.8 vs. 8.0 h/d), respectively. This result is in accordance

Figure 1. Mean daily ruminal pH pattern for the dietary treatments [HACS = high acidogenic value concentrate, coarse forage; HAFS = high acidogenic value concentrate, fine forage; LACS = low acidogenic value concentrate, coarse forage; LAFS = low acidogenic value concentrate, fine forage].

Table 5. Effect of the acidogenic value (AV) of the concentrate and the forage particle size (PS) on the mean, minimum, and maximum ruminal pH; time below pH 5.0 to 6.8 (min/d); and area below pH 5.0 to 6.8 (min $\times$ units pH/d)

Dependent variable	Treatment ¹				SEM ²	P-value		
	HACS	HAFS	LACS	LAFS		AV	PS	AV $\times$ PS
Mean ruminal pH,	6.01 ^a	5.94 ^a	6.05 ^{ab}	6.08 ^b	0.03	0.01	0.50	0.13
Minimum ruminal pH	5.38 ^a	5.29 ^a	5.49 ^{ab}	5.48 ^b	0.04	0.004	0.27	0.40
Maximum ruminal pH	6.72 ^a	6.69 ^b	6.76 ^{ac}	6.98 ^c	0.06	0.01	0.13	0.05
Time below pH 5.0	0.06	3.25	0.00	0.00	1.69	0.33	0.35	0.35
Time below pH 5.2	14.38	36.73	2.62	14.00	11.02	0.16	0.17	0.65
Time below pH 5.4	70.66	95.72	40.00	51.69	22.05	0.12	0.43	0.77
Time below pH 5.6	227.02	246.42	129.19	140.94	40.09	0.02	0.70	0.92
Time below pH 5.8	423.55 ^a	537.67 ^a	284.00 ^b	296.00 ^b	54.86	0.003	0.26	0.37
Time below pH 6.0	692.32 ^a	811.66 ^a	560.75 ^{ab}	554.06 ^b	60.96	0.005	0.35	0.30
Time below pH 6.2	982.82 ^a	1,083.44 ^a	956.56 ^{ab}	921.31 ^b	49.59	0.07	0.51	0.18
Time below pH 6.4	1,221.06 ^a	1,262.22 ^b	1,262.38 ^{abc}	1,156.31 ^c	30.59	0.30	0.30	0.03
Time below pH 6.6	1,355.24 ^a	1,371.54 ^b	1,396.13 ^{abc}	1,307.75 ^c	19.24	0.58	0.09	0.02
Time below pH 6.8	1,405.49	1,432.49	1,437.13	1,405.94	12.08	0.84	0.86	0.03
Area below pH 5.0	0.005	0.24	0.00	0.00	0.13	0.33	0.35	0.35
Area below pH 5.2	0.61	3.34	0.06	1.04	1.13	0.25	0.14	0.48
Area below pH 5.4	8.03	16.27	4.57	6.57	4.30	0.17	0.28	0.51
Area below pH 5.6	36.56	47.36	15.08	25.21	9.34	0.04	0.29	0.97
Area below pH 5.8	102.71	113.52	76.86	68.01	18.79	0.07	0.96	0.61
Area below pH 6.0	213.88 ^a	227.17 ^a	179.95 ^{ab}	150.82 ^b	27.36	0.05	0.77	0.44
Area below pH 6.2	351.84	398.04	347.75	298.81	38.33	0.18	0.97	0.22
Area below pH 6.4	557.79	626.83	580.79	508.96	45.97	0.31	0.98	0.14
Area below pH 6.6	800.97	890.73	849.56	755.88	52.52	0.42	0.97	0.10
Area below pH 6.8	1,061.28	1,173.49	1,133.88	1,028.36	59.24	0.54	0.95	0.08

^{a-c}Means within a row with different superscripts differ ($P < 0.05$).

¹Treatments: HACS = high-AV concentrate, coarse forage; HAFS = high-AV concentrate, fine forage; LACS = low-AV concentrate, coarse forage; LAFS = low-AV concentrate, fine forage.

²SEM = Standard error of means.

with our previous study (Rustomo et al., 2006b) in which increasing the concentrate AV increased the time spent below pH 5.6 and 5.8 by approximately 3 h/d (2.8 vs. 5.7 h/d and 6.4 vs. 9.3 h/d), respectively. The higher ruminal pH depression exhibited by cows fed the HA diets likely resulted from the higher fermentability of the feed ingredients used in the HA concentrates, as shown by the higher acid load produced by the HA diets compared with the LA diets (190.7 vs. 211.8 g Ca/d, data not shown). Furthermore, the HA concentrate was composed of 24.4% wheat and 24.2% barley (DM basis; Table 2), which had high AV compared with other energy feeds (Rustomo et al., 2006a) and could therefore cause large drops in rumen fluid pH after incubation (de Smet et al., 1995; Rustomo et al., 2006a).

Krause et al. (2002b) and Krause and Combs (2003) also reported that increasing the dietary fermentable carbohydrates considerably increased the time spent below pH 5.8. Based on the time below pH 5.6 or 5.8 rather than the mean or minimum pH, all cows suffered SARA; however, the SARA in cows on the HA diets was more severe. Subacute ruminal acidosis has been defined as a pH drop below 5.6 (Owens et al., 1998) or below 5.8 (Krause et al., 2002b; Krause and Combs, 2003). However, the severity of SARA is characterized by the duration of ruminal pH below 5.6 or 5.8 (Keunen et al., 2002). Although it is commonly believed that increasing the FPS will stimulate chewing activity and increase salivary buffering, in the current study, ruminal pH was not affected by FPS (Table 5). This is in agreement with several previous studies (Kononoff et al., 2003a; Kononoff and Heinrichs, 2003a,b; Beauchemin and Yang, 2005).

Dietary Intake

The intake of DM, OM, and other nutrients is presented in Table 6. Dry matter intake was not affected by increasing the concentrate AV (21.9 vs. 21.2 kg/d; LA vs. HA concentrate, respectively). This is in agreement with our previous study (Rustomo et al., 2006b). Decreasing the FPS had no effect on DMI (21.4 vs. 21.7 kg/d). Allen (2000) suggested that the effect of physical distention in the reticulorumen could limit feed intake at the low-concentrate inclusion, but the effect would not be expected on the high-concentrate diet. Beauchemin and Yang (2005) found that reducing the $\text{peNDF}_{>8}$ of corn silage-based diets by a high inclusion of concentrate (60% of ration DM) did not affect DMI. The concentrate level of the current study was high (50% in ration DM; Table 3); hence, the effect on DMI of reducing FPS was not observed. There was no interaction between concentrate AV and FPS on DMI (Table 6). This result is in agreement with other studies in

which reducing the particle size of alfalfa silage (Krause et al., 2002a) or corn silage (Kononoff and Heinrichs, 2003a) did not affect DMI. Conversely, positive effects of reducing the corn silage particle size on DMI have been observed (Kononoff et al., 2003a). Although reducing the FPS is commonly believed to reduce chewing activity and salivary buffer secretion and therefore reduce ruminal pH (Mertens, 1997), the effects of FPS on DMI have remained less clear.

We had originally hypothesized that increasing the concentrate AV would increase the time ruminal pH was below a suboptimal pH and that the animals would adjust their feed intake to avoid excessive rumen acid load. Furthermore, the effects of increasing the concentrate AV on ruminal pH depression would depend on the FPS of the diet. Increasing the concentrate AV increased the time spent below pH 5.6 from 2.2 to 3.9 h/d and the time spent below pH 5.8 from 4.8 to 8.0 h/d. Reducing the FPS had no effect on the time below pH 5.6 (3.0 vs. 3.2 h/d) or the time below pH 5.8 (5.9 vs. 6.9 h/d). Dry matter intake was not affected by diet. The significant reduction in DMI with increasing dietary fermentable carbohydrates reported by Krause et al. (2002a) and Krause and Combs (2003) were likely because of the more severe ruminal pH depression demonstrated by the longer time spent below pH 5.8 (7.4 to 10.8 h/d; Krause et al., 2002b) and (7.8 to 12.7 h/d; Krause and Combs, 2003). These results underscore the importance of the relationship between DMI and the extent of ruminal pH depression below pH 5.6 to 5.8. However, the critical period spent per day below a suboptimal pH that would affect DMI remains to be determined.

The intake of OM decreased from 20.5 to 19.5 kg/d and that of NDF decreased from 9.7 to 8.8 kg/d with increasing concentrate AV. Forage particle size had no effect on OM and NDF intakes. The intake of ADF, starch, NFC, and CP was not affected by diet (Table 6). The nonsignificant differences in the intake of starch, NFC, and CP were expected because the diets were formulated to have an isoenergetic and isonitrogenous content. The difference in NDF intake may have contributed to the ruminal pH response, but the effect may be smaller than that of feed acidogenicity. This is because all diets were above the critical NDF value of 22% DM needed to maintain a ruminal pH above 6.0 (Mertens, 1997). Thus, the effects of dietary treatments on ruminal pH depression we observed were likely related to differences in feed fermentability reflected by differences in the estimated total acid load from feed fermentation. Decreasing the FPS decreased the $\text{peNDF}_{T>1.18}$ from 40.4 to 38.5% DM but not the acid load (199.6 vs. 202.8 g Ca/d; CS vs. FS diet, respectively). Increasing the concentrate AV increased the estimated

Table 6. Effect of acidogenic value (AV) of the concentrate and particle size of forage (PS) on the intake of DM, OM, CP, ADF, NDF, starch, and nonfiber carbohydrates (NFC, kg/d) and on acid load (g Ca/d)¹

Dependent variable	Treatment ²				SEM ³	P-value		
	HACS	HAFS	LACS	LAFS		AV	PS	AV × PS
Intake								
DM	21.14	21.21	21.58	22.25	0.60	0.26	0.56	0.63
OM	19.57 ^a	19.51 ^{ab}	20.64 ^b	20.37 ^{ab}	0.29	0.02	0.59	0.73
CP	3.70	3.70	3.81	3.79	0.06	0.19	0.89	0.81
ADF	5.61	5.35	5.83	5.73	0.14	0.07	0.24	0.58
NDF	8.83 ^a	8.82 ^a	9.77 ^b	9.96 ^b	0.26	0.01	0.74	0.72
Starch	3.27	3.03	3.19	3.04	0.15	0.79	0.25	0.77
NFC	6.30	6.09	6.37	6.11	0.23	0.86	0.35	0.93
Acid load	211.40 ^a	212.10 ^a	187.75 ^b	193.54 ^{ab}	5.40	0.01	0.57	0.65

^{a,b}Means within a row with different superscripts differ ($P < 0.05$).

¹Acid load = AV of feed multiplied by the DMI.

²Treatments: HACS = high-AV concentrate, coarse forage; HAFS = high-AV concentrate, fine forage; LACS = low-AV concentrate, coarse forage; LAFS = low-AV concentrate, fine forage.

³SEM = Standard error of means.

acid load from 190.7 to 211.8 g Ca/d. However, the increased acid load could not explain the nonsignificant response in DMI. This is consistent with the report by Rustomo et al. (2006b), in which increasing the acid load from feed fermentation did not affect the DMI. However, Dewhurst et al. (2001) reported a significant effect of concentrate AV on decreasing the DMI in corn silage-based diets as compared with grass silage-based diets (17.4 vs. 22.2 kg DM). These authors suggested that the depression in DMI of the corn silage-based diets was more likely affected by the acidogenicity of the forage than the effect of peNDF of the diets on DMI. Because we used mixed (50:50) alfalfa haylage and corn silage in the TMR, the effect of forage acidogenicity on DMI was expected to be lower than that reported by Dewhurst et al. (2001) when corn silage was fed as the only forage. Feeding methods (TMR or components) could also be a factor influencing DMI; hence, DMI depression in the current study was expected to be lower than that found by Dewhurst et al. (2001).

Milk Yield and Composition

Milk yield and composition are presented in Table 7. Milk yield, milk composition, and 4% FCM were not affected by dietary treatment. Increasing the concentrate AV did not affect the milk fat content or fat yield (Table 7), which is consistent with our previous study (Rustomo et al., 2006b). Milk fat content and fat yield were not affected by decreasing the FPS, which is in agreement with the results of Kononoff and Heinrichs (2003b), Krause et al. (2002a), and Krause and Combs (2003), although their ranges of particle size were wider than those in the present study. All diets were above the critical peNDF value of 20% DM needed to maintain milk fat at 3.4% (Mertens, 1997), and therefore would

be expected to sustain the milk fat content. There was a tendency for the concentrate AV to interact with FPS on the milk fat percentage. Increasing the FPS for cows fed the HA concentrate resulted in a 0.32% increase in the fat content (3.87 vs. 4.19%) compared with cows fed the LA concentrate, in which a relatively constant fat content (4.01 vs. 4.03%) was observed. Dietary treatments had no effect on the milk protein percentage, protein yield, lactose percentage, and lactose yield, in agreement with Krause et al. (2002a). As noted in our previous study (Rustomo et al., 2006b), milk yield and composition may not be explained by acid load from feed fermentation but are more likely because of complex interactions among nutrients in a diet.

Correlations between ruminal pH and acid load; ruminal pH and intake of DM, OM, starch, NFC, ADF, NDF, and CP; ruminal pH and $\text{pef}_{>8.0}$ and $\text{pef}_{>1.18}$; ruminal pH and $\text{peNDF}_{T>8.0}$ and $\text{peNDF}_{T>1.18}$; and ruminal pH and milk fat content are presented in Table 8. The correlation between rumen acid load and ruminal pH was stronger than the correlation between rumen acid load and the intake of starch, NFC, NDF, ADF, or CP. A previous in vitro study showed that ruminal pH changes were more highly correlated with the feed AV ($R^2 = 0.74$) than with the starch content ($R^2 = 0.35$) or the NFC content ($R^2 = 0.56$; Rustomo et al., 2006a). The correlations between dietary particle sizes, $\text{pef}_{>8}$, $\text{pef}_{>1.18}$, $\text{peNDF}_{T>8}$, and $\text{peNDF}_{T>1.18}$ and ruminal pH were not strong (Table 8), suggesting that dietary AV could be used as an additional factor when considering the requirement of peNDF for dairy cows. Despite the nonsignificant effect of diet on the milk fat content, the milk fat content was negatively correlated with the time below pH 5.6 and the area below pH 5.6 (time below, $r = -0.51$; area below, $r = -0.56$) and 5.8 (time below, $r = -0.42$; area below, $r = -0.54$; Table 8), suggesting that

Table 7. Effect of acidogenic value (AV) of concentrate and particle size of forage (PS) on milk yield (kg/d), 4% FCM (kg/d), milk composition (%), and yield of milk component

Dependent variable	Treatment ¹				SEM ²	P-value		
	HACS	HAFS	LACS	LAFS		AV	PS	AV × PS
Milk yield	26.95	27.65	27.85	28.58	2.28	0.84	0.87	0.99
4% FCM	28.01	26.87	27.61	28.98	4.23	0.85	0.98	0.78
Fat	4.19	3.87	4.03	4.01	0.09	0.88	0.16	0.09
Protein	3.48	3.42	3.45	3.46	0.03	0.99	0.51	0.30
Lactose	4.74	4.67	4.68	4.68	0.04	0.51	0.45	0.48
Fat yield	1.15	1.05	1.10	1.17	0.17	0.85	0.95	0.64
Protein yield	0.93	0.94	0.95	0.99	0.15	0.83	0.87	0.92
Lactose yield	1.27	1.30	1.32	1.33	0.21	0.85	0.91	0.96

¹Treatments: HACS = high-AV concentrate, coarse forage; HAFS = high-AV concentrate, fine forage; LACS = low-AV concentrate, coarse forage; LAFS = low-AV concentrate, fine forage.

²SEM = Standard error of means.

ruminal pH depression might have a negative effect on milk fat content.

CONCLUSIONS

This study showed that cows consuming forage of longer particle size were able to improve the ruminal pH, but the effect was more pronounced in the high-AV diets than with the low-AV diets. Ruminal pH was more likely affected by the direct effect of increased acid load from feed fermentation rather than by the indirect effect of rumen buffering from FPS when dietary NDF met requirements. The chop length of the mixed alfalfa haylage–corn silage was less critical than acid load in affecting the ruminal pH, likely because of the high dietary NDF. Hence, the rumen acid load may be useful to estimate ruminal pH changes in dairy cows.

The dietary AV could be used as an additional tool for determining the effective fiber requirement of dairy cows, because peNDF is not dependent on differences in the rumen acid load. The AV approach predicts differences in ruminal pH associated with acid production from feeds and their intrinsic buffering capacity. This approach, combined with peNDF, would improve diet formulations to minimize SARA in dairy cows.

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Table 8. Correlations between ruminal pH and acid load; intake of DM, OM, starch, NFC, ADF, NDF, and CP; physical effectiveness factors (pef); physically effective NDF (peNDF); and milk fat content¹

Item	Mean pH		Time < pH 5.6		Time < pH 5.8		Area < pH 5.6		Area < pH 5.8	
	r	P-value	r	P-value	r	P-value	r	P-value	r	P-value
Acid load, g of Ca /d	-0.57	0.02	0.58	0.02	0.60	0.01	0.29	0.3	0.38	0.1
DM intake, kg/d	-0.44	0.09	0.33	0.2	0.42	0.1	0.11	0.7	0.31	0.2
OM intake, kg/d	-0.35	0.2	0.20	0.5	0.30	0.2	-0.03	0.9	0.19	0.5
Starch intake, kg/d	-0.48	0.06	0.40	0.1	0.49	0.06	0.12	0.6	0.40	0.1
NFC intake, kg/d	-0.20	0.4	-0.04	0.9	0.18	0.5	-0.30	0.3	-0.09	0.7
NDF intake, kg/d	-0.24	0.4	0.20	0.5	0.18	0.5	0.03	0.9	0.23	0.4
ADF intake, kg/d	-0.25	0.3	0.23	0.4	0.19	0.5	0.07	0.8	0.25	0.3
CP intake, kg/d	-0.34	0.2	0.16	0.5	0.30	0.3	-0.06	0.8	0.13	0.6
X _{gm}	0.03	0.9	0.03	0.9	-0.05	0.9	-0.07	0.8	0.06	0.8
pef ₋₈	0.01	0.9	0.005	0.9	-0.05	0.8	-0.10	0.7	0.02	0.9
pef _{>1.18}	0.08	0.8	-0.01	0.9	-0.08	0.8	-0.12	0.7	0.01	0.9
peNDF _{T>8} , % of DM	0.09	0.7	-0.001	0.9	-0.14	0.6	-0.08	0.8	0.04	0.9
peNDF _{T>1.18} , % of DM	0.17	0.5	0.03	0.9	-0.17	0.5	0.04	0.9	0.10	0.7
Milk fat, %	0.45	0.08	-0.51	0.04	-0.42	0.1	-0.56	0.02	-0.54	0.03

¹Acid load = AV multiplied by DMI; X_{gm} = geometric mean length as calculated by the ASAE standard (2001); pef₋₈ = physical effectiveness factor, measured as the proportion of DM retained on the top and middle screens; pef_{>1.18} = physical effectiveness factor, measured as the proportion of DM retained on the top, middle, and bottom screens; peNDF_{T>8} = physically effective NDF >8.0 mm = pef₋₈ multiplied by the NDF content of the TMR; peNDF_{T>1.18} = physically effective NDF >1.18 mm = pef_{>1.18} multiplied by the NDF content of the TMR.

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