Replacing Chopped Alfalfa Hay with Alfalfa Silage in Barley Grain and Alfalfa-Based Total Mixed Rations for Lactating Dairy Cows

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ABSTRACT

The effects of replacing chopped alfalfa hay with alfalfa silage in a fine barley grain and alfalfa-based total mixed ration (TMR) were evaluated. Diets contained (dry matter basis) 53.0% commercial energy supplement, 10.3% commercial protein supplement, and 9.7% corn silage. Diets varied in inclusion of chopped alfalfa hay and alfalfa silage, and contained either 20.0% chopped alfalfa hay and 7.0% alfalfa silage, 10.0% chopped alfalfa hay and 17.0% alfalfa silage, or 27.0% alfalfa silage. Contents of crude protein, neutral detergent fiber (NDF), acid detergent fiber, and minerals did not differ among diets. Replacing chopped alfalfa hay with alfalfa silage decreased dietary dry matter, and increased dietary soluble protein and physical effective NDF calculated as the proportion of dietary NDF retained by the 8- and 19-mm screens of the Penn State Particle Separator ($\text{peNDF}_{\text{NDF}}$) from 13.3 to 15.6% DM. Replacing chopped alfalfa hay with alfalfa silage did not affect dry matter intake, rumen pH, rumen volatile fatty acids, blood lactate, milk fat, and milk protein percentage, but did decrease blood glucose, tended to increase blood urea, and numerically decreased milk yield and milk protein yield. A wider range in $\text{peNDF}_{\text{NDF}}$ and a higher inclusion of corn silage might have resulted in greater differences in rumen fermentation and milk production among diets. The pH of rumen fluid samples collected 4 h after feeding varied from 5.90 to 5.98, and milk fat percentage varied from 2.50 to 2.60% among diets. These values suggest that mild subacute ruminal acidosis was induced by all diets.

(Key words: physically effective neutral detergent fiber, rumen pH, particle size, dairy cow)

Abbreviation key: AH = alfalfa hay, AHAS = alfalfa hay and alfalfa silage, AS = alfalfa silage, peNDF_{PS} = physically effective NDF measured as proportion of DM

retained by the 8- and 19-mm screens multiplied by the dietary NDF, peNDF_{M} = physically effective NDF measured from tabular values of chewing time (Mertens, 1997), $\text{peNDF}_{\text{NDF}}$ = physically effective NDF measured as proportion of NDF retained by the 8- and 19-mm screens multiplied by the dietary NDF, $\text{peNDF}_{1.18}$ = physically effective NDF measured as the proportion of DFM retained of a 1.18-mm screen multiplied by dietary NDF, **PSPS** = Penn State Particle Separator, **SARA** = subacute ruminal acidosis.

INTRODUCTION

High concentrate diets for dairy cows must contain sufficient physically effective fiber—i.e., fiber that stimulates rumination, saliva production, and rumen buffering—to prevent subacute ruminal acidosis (**SARA**) and the resulting depressions in milk fat, DMI, fiber digestion, and laminitis (Beauchemin and Rode, 1997; Mertens, 1997; Nocek, 1997; Krajcarski-Hunt et al., 2002; Plaizier et al., 2001). Dietary peNDF is affected by NDF content, particle size distribution, and forage-to-concentrate ratio (Mertens, 1997). NRC (2001) recommended a minimum dietary NDF level of 25% DM, of which 75% must be from forage sources, in order to provide sufficient rumen buffering. The NRC (2001) provided no recommendations for dietary inclusion of physically effective fiber, due to the lack of a standard validated technique to quantify the physically effective properties of fiber in a diet. Heinrichs (1996) recommended that between 50 and 60% of particles of silages and between 40 and 60% of TMR particles should be retained by the 8- and 19-mm screens of the Penn State Particle Separator (**PSPS**). Mertens (1997) suggested that physically effective fiber of dairy cow diets should be at least 22% of ration DM in order to maintain an average rumen pH of 6.0 and at least 20% of ration DM to maintain a milk fat percentage of 3.4 in midlactation.

Current recommendations for NDF, physically effective fiber, and particle size distribution have mainly been developed using corn grain-based diets. Barley grain contains more NDF than corn grain (Beauchemin and Rode, 1997), and barley starch is more rapidly fer-

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mentable than cornstarch (McCarthy et al., 1989). Beauchemin (1991), therefore, recommended that diets containing barley grain should contain more NDF in order to maintain a milk fat content of 3.5% than diets containing corn grain.

Different methods for the calculation of physically effective fiber have been used. These calculations are based on dietary NDF and physically effective NDF measured from tabular values of chewing time (**peNDF_M**) (Mertens, 1997), dietary NDF, and the proportion of physically effective NDF measured as a proportion of DM retained by the 8- and 19-mm screens, multiplied by the dietary NDF (**peNDF_{PS}**) (Yang et al., 2001a), dietary NDF and physically effective NDF measured as the proportion of DM retained by a 1.18-mm screen multiplied by dietary NDF (**peNDF_{>1.18}**) (Yang et al., 2001a), and physically effective NDF measured as a proportion of NDF retained by the 8- and 19-mm screens multiplied by the dietary NDF (**peNDF_{NDF}**) (Calberry et al., 2003). These different methods of peNDF calculation result in very different values (Yang et al., 2001a; Beauchemin et al., 2003). Beauchemin et al. (2003) did not find that **peNDF_{PS}**, **peNDF_M**, and **peNDF_{>1.18}** were correlated with average rumen pH, and observed that **peNDF_{PS}** had a higher correlation with time and area below rumen pH 5.8 than the other measures. These authors also found that **peNDF_M** and **peNDF_{>1.18}** were moderately correlated with ruminating or total chewing time, and **peNDF_{PS}** only tended to correlate with ruminating time.

Several studies on the effects of dietary particle size on rumen fermentation, feed intake, milk production, and nutrient digestibility have been conducted in recent years (Kononoff et al., 2000; Yang et al., 2001a, 2001b; Soita et al., 2002; Beauchemin et al., 2003; Calberry et al., 2003; Kononoff et al., 2003; Kononoff and Heinrichs, 2003a, 2003b; Krause et al., 2002a, 2002b; Krause and Combs, 2003; Soita et al., 2003). Results obtained in these studies are inconclusive, as these results are difficult to compare due to differences in measurement and expression of dietary particle size, peNDF, and rumen pH among studies, and interactions between levels of concentrate inclusion, forage source, and concentrate source.

Cereal grain silages and alfalfa silage vary in reticular motility, time spent ruminating, and number of chews per kilogram of DM and kilograms of NDF consumed (Okine et al., 1994) and in intrinsic buffering capacity (McBurney et al., 1983). It can, therefore, be expected that minimum dietary peNDF requirements and the dietary peNDF level below which a reduction in peNDF reduced rumen pH, DMI, and milk fat concentration (Mertens, 1997) depend on the forage source in the diet as well as on the concentrate source.

A recent survey on Manitoba dairy farms showed that alfalfa silages were used on 75% of farms, that alfalfa hay was used on 55% of farms, and that barley grain was the main grain source (Plaizier et al., 2004). Also, in more than 25% of TMR-fed herds included in this survey, the proportion of TMR retained by the 8- and 19-mm screens of the PSPS was less than the minimum recommended level of 40% (Heinrichs, 1996). The impacts of these excessively fine TMR on milk yield and milk composition were not obvious (Plaizier et al., 2004). The effects of deviating from the guidelines of Heinrichs (1996) on milk production and rumen conditions need to be determined in lactating cows fed alfalfa-based TMR in order to develop specific peNDF guidelines for these diets. To achieve this—i.e., the effects on milk production of reducing dietary particle size by replacing alfalfa silage with chopped alfalfa hay, while maintaining a consistent nutrient composition—milk composition, DMI, rumen conditions, and blood plasma levels of glucose, urea, and lactate were determined in lactating dairy cows fed an alfalfa and barley grain based TMR.

MATERIALS AND METHODS

Experimental Procedures

Twelve multiparous lactating Holstein cows, housed in a tie-stall barn at the Glenlea Research Station, University of Manitoba, were used in a 3 × 3 Latin square design with three 3-wk experimental periods. Each experimental period consisted of 14 d of adaptation to the experimental diet and 7 d of data collection. Animals were cared for in accordance with the Canadian Council for Animal Care (CCAC) guidelines. Upon commencement of the experiment, cows averaged 122.8 ± 63.5 DIM (mean ± SD), had an average BCS of 2.84 ± 0.26 on a 5-point scale (Edmonson et al., 1989), and had an average BW of 647.6 ± 86.4 kg. Cows were weighed and evaluated for BCS (Edmonson et al., 1989) at the end of each experimental period.

Cows were assigned one of 3 TMR (alfalfa silage [**AS**], alfalfa hay and alfalfa silage [**AHAS**], and alfalfa hay [**AH**]) during each experimental period (Table 1). Each diet contained (DM basis) 53.0% barley grain-based commercial energy supplement (containing 42% pellets), 10.3% commercial protein supplement (containing 58% pellets), 9.7% corn silage, and 27.0% alfalfa forage. Source of alfalfa forage was 20.0% chopped alfalfa hay and 7.0% alfalfa silage in AH; 10.0% chopped alfalfa hay and 17.0% alfalfa silage in AHAS; and 27.0% alfalfa silage and no chopped alfalfa hay in AS.

Corn and alfalfa were chopped at 10 mm using a New Holland Forage Harvester, model 790 (New Holland Inc., New Holland, PA) and ensiled in upright silos.

Table 1. Ingredients and nutrient composition of experimental diets with equal inclusion rates of corn silage, energy supplement, and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage.¹

Diet ingredients	Diet, % DM basis		
	AH	AHAS	AS
Alfalfa silage	7.0	17.0	27.0
Chopped alfalfa hay	20.0	10.0	0
Corn silage	9.7	9.7	9.7
Energy supplement	53.0	53.0	53.0
Protein supplement	10.3	10.3	10.3
Nutrient composition ²			
DM, %	71.4 (0.09)	63.4 (1.11)	57.3 (0.51)
CP, % of DM	20.5 (0.85)	20.5 (0.70)	20.7 (0.46)
Soluble protein, % of DM	5.5 (0.45)	6.5 (0.15)	7.7 (0.50)
NDF, % of DM	34.6 (2.67)	33.9 (2.18)	34.6 (2.69)
ADF, % of DM	20.2 (1.63)	18.8 (1.23)	19.7 (1.59)
Starch, % of DM	26.1 (2.02)	27.1 (1.89)	25.9 (2.01)
Ether extract, % of DM	5.6 (0.35)	5.7 (0.26)	6.0 (0.47)
Ash, % of DM	10.3 (0.36)	9.8 (0.80)	9.9 (0.37)
Ca, % of DM	1.33 (0.15)	1.34 (0.12)	1.36 (0.07)
P, % of DM	0.54 (0.03)	0.54 (0.08)	0.57 (0.09)
K, % of DM	1.98 (0.11)	1.75 (0.28)	1.75 (0.16)
Mg, % of DM	0.37 (0.01)	0.35 (0.05)	0.39 (0.04)
Na, % of DM	0.41 (0.03)	0.41 (0.06)	0.44 (0.05)
pH	5.67 (0.05)	5.42 (0.12)	5.34 (0.14)

¹Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS) (n = 3 for each treatment).

Alfalfa hay was baled in large round bales with a weight of approximately 700 kg and chopped with a Case/IH model 8610 bale processor (JI Case International, Racine, WI). Second-cut alfalfa was harvested in the bud stage. Corn silage was harvested at second maturity (2/3 milk line). The corn variety was Pioneer 39T68 (Pioneer, Des Moines, IA). The diets were mixed using a Data Ranger mixer (American Calan, Northwood, NH) with a Weigh Tronix weigh head (model 1000, American Calan, Northwood, NH). The TMR were fed once daily for ad libitum consumption, allowing for between 5 and 10%orts. Cows had unlimited access to water. Adequacy of NEL supply was determined using the NRC dairy cattle program (NRC, 2001).

DMI and Feed Analyses

During the collection periods, the amount of TMR offered and refused was recorded daily for each cow. Diet samples were collected daily and pooled for each collection period. Individual cow ort samples were obtained daily during the collection periods and pooled by weight and period. Forages were sampled once per collection period and pooled across collection periods. The DM contents of pooled diets, forages, and ort samples were determined by drying at 60°C for 48 h. Dried feed samples were ground using a Wiley mill through

a 1-mm screen (Thomas-Wiley, Philadelphia, PA) and stored at -20°C until analysis.

All feed samples were analyzed for CP using the CuSO₄/TiO₂ Mixed Catalyst Kjeldahl procedure (AOAC 988.05, 1990), NDF (Van Soest et al., 1991) using α amylase (Sigma no. A3306; Sigma Chemical Co., St. Louis, MO), sodium sulfite and corrected for ash concentration adapted for Ankom200 Fiber Analyzer (Ankom Technology, Fairport, NY), ADF (AOAC 973.18, 1990), ether extract (AOAC 920.39, 1990), and ash (AOAC 942.05, 1990). Soluble protein was determined according to Licitra et al. (1996). Calcium, P, K, Mg, and Na were measured by inductively coupled plasma emission spectroscopy (AOAC 968.08, 1990) using an Atom Scan 25 plasma spectrometer (Thermo Jarrell Ash Corp., Grand Junction, CO) after acid digestion. The pH of the TMR was determined according to Buchanan-Smith and Yao (1981) using an Accumet Basic 15 pH meter and an Accumet gel-filled polymer body combination pH electrode (Fisher Scientific, Fairlawn, NJ), calibrated with pH 4.0 and pH 7.0 buffer solutions (Fisher Scientific).

Particle size distributions were determined for all TMR, pooled refusals, and forage samples using the PSPS (Heinrichs, 1996; Lammers et al., 1996). The PSPS has 2 screens and a bottom pan. The diameters of holes of the screens were 19 and 8 mm for the top and middle screen, respectively. Approximately 150 g of wet sample was placed on the top screen of the PSPS. The PSPS was shaken for a total of 40 times (5 times in each direction, twice) (Heinrichs, 1996). The contents of each fraction were weighed and analyzed for DM and NDF, as described earlier. Physically effective fiber was determined as the proportion of the dietary NDF retained by the PSPS sieves ($\text{peNDF}_{\text{NDF}}$) and the proportion of dietary DM retained by the PSPS sieves multiplied by the dietary NDF content (peNDF_{ps}). The peNDF_{M} was measured using tabular values for the physical effectiveness of feeds, as recommended by Mertens (1997).

Particle size distribution of TMR was measured by dry sieving using a vertical oscillating test sieve shaker (EFL 1 KII, Endecotts Ltd., London, UK) equipped with a stack of 6 brass sieves and a bottom pan with a 200-mm diameter (ASTM E11, Endecotts Ltd., London, UK) arranged in descending mesh size. Sieve mesh sizes were 19, 9.5, 6.3, 4.0, 1.18, and 0.6 mm. Approximately 200 g was placed on the top screen, and the stack of sieves was shaken until the distribution of materials did not change (approximately 10 min). The $\text{peNDF}_{>1.18}$ was determined as the proportion of DM retained on the 1.18-mm screen multiplied by dietary NDF.

Milk Yield and Composition Analysis

Cows were milked twice daily in their stalls, and milk production was determined using Tru Test regulation meters (Westfalia Surge, Mississauga, ON). Milk samples were collected from 4 consecutive milkings in 50 mL vials in each collection period and preserved with 2-bromo-2-nitropropane-1,3 diol. Milk samples were stored at 4°C until analyzed for fat and protein at the laboratory of the Manitoba Milk Producers (Winnipeg, MB) by near infrared analysis using the Milk-O-Scan 303AB (Foss Electric, Hillerød, Denmark).

Rumen pH Measurement and Blood Sample Collection

Rumen fluid and peripheral blood were sampled twice during each collection period (Tuesday and Thursday afternoons) at 4 to 5 h postfeeding. Rumen fluid was aspirated using a Geishausser oral probe (Geishausser, 1993). The first 200 mL of collected rumen fluid were discarded, and the subsequent 50 mL of rumen fluid were kept for subsequent analysis and processing. Rumen fluid pH was measured using an Accumet Basic 15 pH meter and an Accumet gel-filled polymer body combination pH electrode (Fisher Scientific), calibrated with pH 4.0 and pH 7.0 buffer solutions (Fisher Scientific). Rumen fluid samples were centrifuged at 1900 × *g* for 10 min, and the supernatant was stored at -20°C until further analysis. Blood samples were collected by coccygeal venipuncture in heparinized 10 mL Vacutainers, centrifuged at 1900 × *g* for 10 min. Subsequently, the plasma was aspirated and stored at -20°C until further analysis.

VFA, Ammonia, and Blood Plasma Analysis

Frozen rumen fluid samples were thawed at room temperature, and 1 mL of a 25% meta-phosphoric acid solution was added to 5 mL of rumen fluid. The tubes were vortexed and placed in a -20°C freezer for 17 h. Thawed samples were centrifuged for 10 min at 1900 × *g*. Approximately 2 mL of supernatant was decanted into a clean dry vial. The samples were capped and placed into the autosampler device (model 8100, Varian, Walnut Creek, CA) for analysis. Concentrations of VFA were determined by gas chromatography (model 3400 Star, Varian) using a 1.83-m glass column (model 2-1721, Supelco, Oakville, ON) (Erwin et al. 1961). The injector and detector temperatures were set at 170 and 195°C, with initial and final column temperatures set at 120 and 165°C, respectively. The runtime was 4 min, followed by a 2-min thermal stabilization period.

Ammonia nitrogen concentration of rumen fluid samples was determined using the method described by

Table 2. Ingredient composition of the energy and protein supplements (%).

Ingredient	Energy supplement	Protein supplement
Rolled barley	54.0	...
Luprosil salt	0.2	...
Protein pellet ¹	1.9	...
Dairy supplement ²	40.0	...
Tallow (feed grade rendered fat)	4.0	
Dried distillers grain	...	42.0
Fish meal	...	7.0
Canola meal	...	22.8
Soybean meal	...	20.0
Beet molasses	...	3.0
Niacin (Vitamin B3)	...	0.3
Sodium bicarbonate	...	5.0

¹Protein pellets contained 46.1% soybean meal, 2.6% wheat shorts, 40.0% canola meal, 5.0% oat hulls, 0.3% pellet binder, 1.0% cane molasses, and 5.0% corn gluten meal.

²Dairy supplement contained 0.13% vitamin ADE premix, 0.14% trace mineral premix, 2.6% soybean meal, 0.06% selenium, 39.1% wheat shorts, 5.0% distillers grain, 17.5% canola meal, 15.0% ground wheat, 1.7% dicalcium phosphate, 1.6% salt, 2.0% dynamate, 0.3% pellet binder, 1.0% cane molasses, 3.7% calcium carbonate, and 10.0% corn gluten meal.

Novozamsky et al. (1974). Absorbance was read at 630 nm on a Pharmacia Biotech Ultraspec 2000 UV/visible spectrophotometer (Biochrom, Cambridge, UK).

Blood plasma was analyzed for glucose, urea, and lactate using a Nova Stat profile M blood gas and electrolyte analyzer (Nova Biomedical Corporation, Waltham, MA).

Statistical Analysis

Analysis of variance for weekly averages of rumen fluid, blood plasma variables, milk, and intakes was conducted using the SAS MIXED procedure (SAS, 1990). The effect of diet was considered fixed. Cow and period effects were considered random. Statistical significance was set at a *P* value of ≤ 0.05. Differences among treatment means were tested for significance using Tukey's multiple range test (SAS, 1990).

RESULTS AND DISCUSSION

Diets

The nutrient and ingredient composition of the diets, the protein and energy supplements, and the forages are given in Tables 1, 2, and 3. Replacing chopped AH with AS reduced DM, reduced feed pH, and increased soluble protein (Table 1). The CP, NDF, ADF, starch, ether extract, ash, and macro minerals were not affected (Table 1). Chopped alfalfa had less feed particles retained by the 8- and 19-mm PSPS screens than alfalfa silage (Table 4). As a result, replacing chopped AH with

Table 3. Nutrient composition of the forages included in the experimental diets.

Chemical component	Alfalfa silage	Alfalfa hay	Corn silage
DM, %	39.7	86.1	40.5
CP, % DM	20.7	22.1	8.8
SP, % DM	13.5	6.5	3.3
NDF, % DM	39.4	40.0	45.7
ADF, % DM	31.8	26.7	26.1
Ether extract, % DM	2.2	2.5	2.5
Ash, % DM	10.6	10.5	6.13
Ca, % DM	1.65	1.30	0.28
P, % DM	0.22	0.33	0.27
K, % DM	2.58	3.02	1.34
Mg, % DM	0.42	0.34	0.22

AS reduced diet DM passing the 8-mm PSPS screen from 72.7 to 63.3% DM, increased the dietary peNDF_{PS} from 9.2 to 12.5% DM, and increased $\text{peNDF}_{\text{NDF}}$ from 13.3 to 15.6% DM (Table 5). The NDF contents of the 19- and 8-mm screen fractions varied among diets (Table 5). The NDF contents averaged across diets were 51.2, 40.6, and 26.6% DM in the 19-mm screen, 8-mm screen, and bottom pan fraction of the PSPS, respectively. The NDF content differed among PSPS fractions. Results of the dry sieving showed that replacing chopped hay with alfalfa silage reduced the proportion of feed particles passing the 1.18-mm sieve from 25.7 DM to 18.5% DM and increased $\text{peNDF}_{>1.18}$ from 23.4 to 25.5% DM (Table 6).

Heinrichs (1996) recommended that no more than 60% of TMR particles should pass the 8- and 19-mm PSPS screens in order to provide sufficient rumen buffering. All 3 diets in the current experiment had a greater proportion of feed particles passing the PSPS screens than recommended by Heinrichs et al. (1996). Particle size distributions of these diets were similar to the finest diets included in the survey of Plaizier et al. (2004). The peNDF_{M} ranged from 16.0 to 17.6% DM among diets, and were below the minimum of 22% DM to maintain an average rumen pH of 6.0 and the mini-

mum of 20% DM to maintain a milk fat percentage of 3.4 in midlactation recommended by Mertens (1997). Hence, it was expected that the physical effectiveness of diets was insufficient to prevent SARA and milk fat depression.

Dry Matter Intake

Replacing chopped hay with alfalfa silage did not affect DMI. The DMI ranged from 22.0 to 23.1 kg d^{-1} , which represented between 3.4 and 3.6% of BW d^{-1} among diets. In a previous experiment conducted in the same dairy herd in which high concentrate diets were fed without inducing SARA, DMI ranged from 22.0 to 23.9 kg d^{-1} (Calberry et al., 2003). This suggests that the diets used in our experiment did not induce feed intake depression.

If a reduction in dietary particle size induces SARA, then DMI will be reduced (Kleen et al., 2003). Rumen pH below 5.8 is indicative of SARA in dairy cows (Beauchemin et al., 2003). In the studies from Yang et al. (2001a), Soita et al. (2002), Kononoff and Heinrichs (2003a, 2003b), Kononoff et al. (2003), and Soita et al. (2003), rumen pH did not drop below this threshold, and reduction in particle size did not affect DMI. In the studies from Krause et al. (2002a, 2002b), Beauchemin et al. (2003), and Krause and Combs (2003), reduction in forage particle size reduction lowered rumen pH below 5.8, but this was only accompanied by decreased DMI in the study of Krause and Combs (2003). The comparative small particle lengths and high dietary starch contents in the study of Krause and Combs (2003) might explain this difference among studies. In our study, dietary starch was lower, and range in dietary particle size was narrower compared with Krause and Combs (2003) study, which would explain why, in our study, diet did not affect DMI.

Conflicting results on the effects of alfalfa preservation (silage vs. hay) on DMI of dairy cows have been reported. In TMR fed cows, reducing the ratio between

Table 4. Least square means and significance of Penn State particle size analysis of forages included in the experimental diets.¹

	Alfalfa silage	Alfalfa hay	Corn silage
% Retained, as is			
Top screen (>19 mm)	17.2 (2.65)	23.7 (5.42)	4.8 (0.84)
Middle screen (>8 mm)	47.2 (8.27)	22.1 (2.66)	47.3 (6.81)
Bottom pan	35.1 (6.39)	54.1 (6.23)	45.5 (6.42)
% Retained, DM basis			
Top screen(>19 mm)	14.4 (2.78)	21.5 (5.23)	4.3 (0.86)
Middle screen (>8 mm)	46.3 (8.01)	21.0 (2.85)	44.4 (6.46)
Bottom pan	39.4 (6.55)	57.5 (6.32)	51.3 (6.88)

¹Penn State Particle Separator distributions of forages included in experimental diets were calculated for each period (n = 3) for each treatment.

Table 5. Penn State Particle Separator analysis of experimental diets.¹ Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS).

	AH	AHAS	AS
% Retained, as is			
Top screen (>19 mm)	6.2 (1.45)	5.1 (0.88)	2.9 (0.57)
Middle screen (>8 mm)	21.2 (2.11)	28.1 (3.51)	33.2 (4.14)
Bottom pan	72.4 (3.72)	66.6 (4.64)	62.9 (4.38)
% Retained, DM basis			
Top screen (>19 mm)	6.0 (1.21)	5.0 (0.92)	3.7 (0.45)
Middle screen (>8 mm)	21.3 (1.59)	28.2 (2.38)	33.1 (4.07)
Bottom pan	72.7 (2.76)	66.8 (3.33)	63.2 (4.22)
NDF in retained fraction, % of DM			
Top screen (>19 mm)	55.6 (3.19)	50.5 (1.21)	47.4 (1.70)
Middle screen (>8 mm)	40.8 (1.59)	41.7 (1.21)	39.2 (1.22)
Bottom pan	26.2 (2.24)	26.1 (0.36)	27.6 (2.25)
peNDF _{PS} ² , % DM	9.2 (1.89)	11.2 (2.22)	12.5 (2.56)
peNDF _{NDF} ³ , % DM	13.3 (1.22)	15.1 (1.23)	15.6 (2.02)
peNDF _M ⁴ , % DM	16.0 (1.20)	17.1 (1.10)	17.6 (1.36)

¹Penn State Particle Separator distributions of experimental diets were calculated for each period (n = 3) for each treatment and analyzed by analysis of variance.

²peNDF_{PS} = Physically effective NDF measured as proportion of DM retained by the 8- and 19-mm screens multiplied by the dietary NDF.

³peNDF_{NDF} = Physically effective NDF measured as proportion of NDF retained by the 8- and 19-mm screens multiplied by the dietary NDF.

⁴peNDF_M = Physically effective NDF measured from tabular values of chewing time (Mertens, 1997).

alfalfa silage and alfalfa hay from 50:50 to 25:75 increased DMI (Beauchemin et al., 2003). Contrarily, Beauchemin et al. (1997) found that DMI was higher for cows fed AH than for cows fed AS. Broderick (1995) also found that cows fed AH as the sole forage had higher DMI than cows fed AS as the sole forage. Vagnoni and Broderick (1997) found that DMI of cows fed alfalfa hay and high-moisture corn was greater than that of cows fed alfalfa silage and high-moisture corn. Discrepancies among studies might be due to differences in chemical and physical composition between the silages and hays used in these studies. As in previous studies as well as in our study, silages and hays varied in several aspects of chemical and physical composition, including particle size, DM, SP, and pH; it is not possible to isolate the effects of these factors. As DMI did not differ among diets in our study, none of these dietary

Table 6. Particle size distribution of experimental diets as determined by dry sieving and physically effective NDF >1.18 (peNDF_{>1.18}). Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS).

Sieve	AH	AHAS	AS
6 mm, %	3.3 (0.23)	3.3 (0.79)	3.9 (0.59)
4 mm, %	27.8 (3.26)	28.2 (3.24)	30.2 (3.57)
1.18 mm, %	43.2 (1.51)	46.3 (1.59)	47.4 (2.80)
<1.18 mm, %	25.7 (2.15)	22.2 (1.53)	18.5 (0.86)
peNDF _{>1.18} , % DM	23.4 (1.19)	24.0 (1.65)	25.6 (1.95)

Figure 1. Penn State Particle Separator distribution of diets and orts (as fed basis). Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS).

Table 7. Rumen fluid composition for experimental diets with equal inclusion rates of corn silage, energy supplement, and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage.¹

Item ²	Diet			SE	P-value
	AH	AHAS	AS		
pH	5.90	5.99	5.98	0.05	NS
VFA, mM L ⁻¹					
Total	115.0	118.0	122.4	4.57	NS
Acetate	68.2	70.8	74.4	3.42	NS
Propionate	30.4	31.0	30.5	2.16	NS
Butyrate	13.3	14.0	15.2	1.13	NS
Other	3.1 ^a	2.1 ^b	2.3 ^b	0.37	*
Ac/Pr ³	2.29	2.31	2.53	0.15	NS
Ruminal ammonia, mg dL ⁻¹	11.3	12.2	16.0	2.8	†

^{a,b}Means for variables in same row followed by a different superscript letters differ significantly ($P \leq 0.05$).

† $P \leq 0.10$.

* $P \leq 0.05$.

¹Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS).

²Rumen fluid composition variables were averaged for each animal during each period ($n = 12$) for each treatment and analyzed by analysis of variance.

³Acetate:propionate ratio.

factors affected feed intake, or the effects of these factors canceled each other out.

Analysis of particle size distribution of the TMR and diets is shown in Figure 1. The diets did not have a greater proportion of particles that were retained by the 19- and 8-mm PSPS screens compared with the TMR. This contradicts the observations of Calberry et al. (2003) and Leonardi and Armetano (2003) that cows selected against coarse feed particles in favor of fine feed particles. The diets used by Calberry et al. (2003) were coarser than the diets used in our study. Hence, the diet in our study did not contain as many coarse particles to select against compared with the study from Calberry et al. (2003). Leonardi and Armetano (2003) also concluded that diets based on long alfalfa hay are likely to have a larger difference between the TMR offered and the TMR consumed, compared with diets based on chopped alfalfa hay due to the increased presence of long feed particles. This also confirms the observation of Krause and Combs (2003) that sorting by cows decreased when forage was more finely chopped.

Rumen Fermentation

The average rumen pH at 4 h after feeding ranged from 5.90 to 5.99 and did not vary significantly among diets (Table 7). Duration of pH below 5.8, measured by in-dwelling pH probes positioned in the ventral sac, has been used as an indicator of SARA (Beauchemin et al., 2003). In our study, rumen fluid was collected 4 h after feeding using a Geishauser oral probe (Geishauser, 1993), as a low point in rumen pH can be expected at this time postfeeding (Keunen et al., 2002). Duffield et

al. (2004) found in lactating dairy cows that pH of rumen fluid samples obtained with the Geishauser oral probe were on average 0.13 units higher than the pH of rumen fluid samples obtained from the caudal ventral rumen through the rumen cannula. This suggests that the rumen pH of the AH diet was below and the rumen pH of the AHAS and AS diets were just above the threshold for SARA, when corrected for the rumen sampling technique. The average rumen pH did not deviate more than 0.09 units from this threshold for any of the diets. This suggests that mild and not severe SARA was induced. The absence of severe SARA, despite the low peNDF of the diets, could be due to the high intrinsic buffering capacity of alfalfa (McBurney et al., 1983) and the inclusion of 0.52% DM bicarbonate (Tables 1 and 2).

Replacing chopped hay with alfalfa silage increased peNDF_{PS}, peNDF_{NDF}, peNDF_M, and peNDF_{>1.18}, but did not affect rumen pH. Mertens (1997) suggested that reduction in particle size causes a larger reduction in rumen pH in diets with low peNDF compared with diets with high peNDF. As the peNDF of our diets was lower than recommended by Mertens (1997), a negative relationship between peNDF and rumen pH could be expected. Krause et al. (2002b) and Beauchemin et al. (2003) observed that reduction in dietary particle size in fine diets reduced rumen pH, but Krause and Combs (2003) did not. This discrepancy was explained by the relatively narrow range in forage particle length in the Krause et al. (2002b) study. The range in peNDF_{PS}, peNDF_M, and peNDF_{>1.18} was narrower in our study than in the study from Beauchemin et al. (2003). This might explain the absence of an inverse relationship between peNDF and rumen pH in our study. Calberry

Table 8. Concentration of metabolites in blood plasma for experimental diets with equal inclusion rates of corn silage, energy supplement, and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage.¹

Item ²	Diet			SE	P-value
	AH	AHAS	AS		
Glucose (mM L ⁻¹)	4.42 ^a	4.37 ^a	4.17 ^b	0.10	*
Urea (mM L ⁻¹)	6.89	7.29	7.74	0.46	†
Lactate (mM L ⁻¹)	1.14	1.34	1.26	0.09	NS

^{a,b}Means for variables in same row followed by a different superscript letter differs significantly ($P \leq 0.05$).

† $P \leq 0.10$.

* $P < 0.05$.

¹Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay, and 17% DM alfalfa silage (AHAS), or 27%DM alfalfa silage (AS).

²Blood plasma variables were averaged for each animal during each period ($n = 12$) for each treatment and analyzed by analysis of variance.

et al. (2003) used a similar design and rumen-fluid sampling technique and diets with higher peNDF than our study, and observed an inverse relationship between peNDF and rumen pH. Diets in the study from Calberry et al. (2003) contained 30.5% DM corn silage, whereas the diets in our study contained 9.7% DM corn silage. As corn silage has a lower intrinsic buffering capacity than alfalfa (McBurney et al., 1983), it can be expected that rumen pH is more sensitive to changes in dietary particle size in corn silage-based than in alfalfa-based diets, such as those used in our study.

Total rumen VFA, with the exception of other VFA, did not vary among diets (Table 7). Hence, diets did not vary in the production and absorption of VFA, rumen pool size, and rumen turnover, or these effects canceled each other out. Reduction of dietary particle size also did not affect total VFA in the studies from Kononoff and Heinrichs (2003b), Beauchemin et al. (2003), Krause and Combs (2003), and Soita et al. (2003), but increased total VFA in the studies from Krause et al. (2002b), Soita et al. (2002), Calberry et al. (2003), Kononoff and Heinrichs (2003a), and Kononoff et al. (2003), and tended to decrease total VFA in the study from Yang et al. (2001a). Reducing dietary particle size might increase the rate of rumen digestion as the surface area for microbial attachment is increased, but this might not increase the degree of rumen digestion and the supply of VFA, as particulate passage rate can also be increased (Soita et al., 2002, 2003). Reduction in dietary particle size has been shown to decrease liquid passage rate and volume of liquid digesta in the rumen due to reduction in saliva production (Yang et al., 2001a, 2001b; Krause et al., 2002a; Yang et al., 2002). This could increase the concentration of VFA if production and absorption of VFA are not affected. Reduction in dietary particle size might increase DMI (Allen, 2000; Soita et al., 2002; Kononoff and Heinrichs, 2003a; Kono-

noff et al., 2003), which could also increase rumen digestion and the production of VFA. However, this increase in DMI is expected in coarse, high-forage diets, and not in high-concentrate diets, such as those used in our study. If a reduction in dietary particle size lowers rumen pH and induces SARA, then rumen digestion of DM and NDF, and thereby the production of VFA, can be reduced (Plaizier et al., 2001; Krajcarski-Hunt et al., 2002). As dietary particle size can affect rumen VFA in so many different ways, discrepancies on these effects among studies can be expected.

Replacing AH with AS numerically increased the acetate-to-propionate ratio, but this trend was not significant. Increasing forage particle size increased the acetate-to-propionate ratio in the studies of Soita et al. (2002), Krause et al. (2002b), Kononoff and Heinrichs (2003a), Krause and Combs (2003), and Soita et al. (2003), but did not affect this ratio in the studies from Yang et al. (2001b) and Beauchemin et al. (2003). Comparing these studies, it appears that a greater increase of the acetate-to-propionate ratio was observed in studies where increased forage particle size reduced rumen pH, than in studies where rumen pH was not affected. This could be expected, as rumen pH affects species of rumen microbes differently (Van Soest, 1994). In our study, diets did not affect rumen pH (Table 7). This might explain why a significant difference in acetate-to-propionate ratio among diets was also not observed.

Replacing chopped hay with alfalfa silage tended to increase ($P = 0.10$) rumen ammonia (Table 7). Rumen ammonia was increased by an increase in dietary particle size in the studies from Yang et al. (2001b) and Soita et al. (2003), possibly due to increased ruminal digestion of dietary protein. However, in the studies from Beauchemin et al. (2003), Calberry et al. (2003), Kononoff and Heinrichs (2003a, 2003b), and Kononoff et al. (2003), forage particle size did not affect rumen

Table 9. Feed intake and milk production of experimental diets, with equal inclusion rates of corn silage, energy supplement, and protein supplement and varying inclusion rates of chopped alfalfa hay and alfalfa silage.¹

Item ²	Diet			SE	P-value
	AH	AHAS	AS		
DMI, kg d ⁻¹	23.1	22.0	22.2	1.04	NS
Milk yield, kg d ⁻¹	39.0	38.3	37.6	0.45	0.13
Milk components					
Fat, %	2.50	2.63	2.60	0.13	NS
Fat yield, kg d ⁻¹	0.97	1.00	0.98	0.08	NS
Protein, %	3.17	3.16	3.15	0.08	NS
Protein yield, kg d ⁻¹	1.21	1.19	1.16	0.05	0.11

¹Diets contained 20% DM chopped alfalfa hay and 7% DM alfalfa silage (AH), 10% DM chopped alfalfa hay and 17% DM alfalfa silage (AHAS), or 27% DM alfalfa silage (AS).

²Feed intake and milk production variables were averaged for each animal during each period (n = 12) for each treatment and analyzed by analysis of variance.

ammonia. In our study, the soluble protein content of alfalfa silage was higher than that of chopped alfalfa hay. This is thought to be mainly responsible for the differences in rumen ammonia among diets.

Blood Metabolites

Replacing chopped AH with AS decreased ($P \leq 0.05$) blood glucose concentration, tended ($P \leq 0.10$) to increase blood urea concentration, and did not affect blood lactate concentration (Table 8). It is not expected that this decrease in glucose concentration was caused by a reduction in the supply of propionate for gluconeogenesis, as the concentration of propionate in rumen fluid did not differ among diets (Table 7), and an increase in dietary particle size is expected to increase the volume of liquid digesta in the rumen due to increased saliva production (Yang et al., 2001a, 2001b; Krause et al., 2002a; Yang et al., 2002). This decrease in blood glucose concentration could be explained by a shift in starch digestion from the intestine to the rumen, as an increase in dietary particle size has been shown to shift starch digestion from the intestine to the rumen (Yang et al., 2001a; Yang et al., 2002), which would decrease the absorption of glucose in the small intestine. Dietary differences other than particle size could also have contributed to the decrease in blood glucose concentration. The AH had a lower ADF content than AS. This could have resulted in lower digestibility for the silage, compared with the hay and differences in NE_L intake and digestibility among diets (Weiss, 1998).

The numeric increase in blood urea concentration was expected, as the alfalfa silage had a higher soluble protein content (Table 1), and replacing chopped alfalfa hay with alfalfa silage also tended to increase rumen ammonia (Table 7). Hence, the differences in blood urea concentration are attributed to higher rumen degrada-

bility of the CP of the AS, which led to increased ammonia production in the rumen and increased urea production in the liver (Van Soest, 1994), and not due to differences in dietary particle size.

Milk Production, BW, and BCS

Replacing chopped hay with alfalfa silage did not affect milk fat content, milk fat yield, and milk protein content, but numerically decreased milk yield ($P = 0.13$) and protein yield ($P = 0.11$) (Table 9). Body weight and BCS were not affected by diet. Cows did not significantly change in BW and BCS during the experiment.

Percentages of milk fat were low, as none of the diets resulted in milk fat percentages greater than 3%. For all diets, the percentage of milk protein was greater than the milk fat percentage. The milk fat depression was expected, as was the dietary particle size, and the proportion of TMR retained by the 8- and 19-mm PSPS screens and peNDF_M were well below the recommendations of Heinrichs (1996) and Mertens (1997). Mertens (1997) compiled data from 36 citations and observed a curvilinear relationship between peNDF and milk fat percentage, with a larger reduction in milk fat percentage due to a decrease in peNDF below a peNDF level of 20% DM, compared with higher peNDF levels. A reduction in dietary particle size significantly decreased milk fat percentage in the studies from Krause and Combs (2003), and numerically decreased milk fat percentage in the studies from Yang et al. (2001a), Calberry et al. (2003), and Kononoff and Heinrichs (2003b). However, in our study, as well as in the studies of Kononoff et al. (2000), Krause et al. (2002a), Beauchemin et al. (2003), Kononoff and Heinrichs (2003a), and Kononoff et al. (2003), dietary particle size did not affect milk fat percentage. The dietary particle sizes in the study from Krause and Combs (2003) were lower than

the dietary particle sizes of studies in which such an effect was not observed.

On average, the cows in the Krause and Combs (2003) study were 99 DIM. This is low compared with most other studies. Only in the studies from Kononoff and Heinrichs (2003a, 2003b) did cows have, on average, less DIM—i.e., 65 and 63 d, respectively. The effect of increasing the concentrate-to-forage ratio from 50:50 to 75:25 caused a larger reduction in milk fat yield in late lactation cows (average 240 DIM) compared with early-lactation cows (average 100 DIM) (Kennelly et al., 1999; Khorasani and Kennelly, 2001). This suggests that the effect of particle size on milk fat yield could also depend on stage of lactation. This does not, however, explain why dietary particle size affected milk fat in the study from Krause and Combs (2003) and not in studies in which cows had more DIM.

Krause and Combs (2003) also observed that the effect of particle size reduction on milk fat percentage tended to be greater for diets that contained a mix of alfalfa and corn silage, compared with diets that contained only alfalfa silage. This could be explained by the higher starch content and, therefore, the greater need for rumen buffering of corn silage compared with alfalfa silage, and a lower rumen intrinsic buffering capacity of corn silage compared with alfalfa silage (McBurney et al., 1983). The difference in intrinsic buffering capacity between corn silage and alfalfa silage could mean that the level of peNDF below which a reduction in particle size has a large effect on milk fat percentage is lower for alfalfa-based diets than for corn silage-based diets. This might explain why, despite the small dietary particle sizes, in our experiment, in which the forage consisted of 37% DM alfalfa and only 9.7% DM corn silage, no effect of particle size on milk fat percentage was observed.

The numeric decrease in milk protein yield due to replacing chopped alfalfa hay with alfalfa silage might have been due to the higher NPN content of alfalfa silage compared with alfalfa hay. As a result, absorbed protein might be more limiting for AS-based diets than for AH-based diets (Broderick, 1995; Vagnoni and Broderick, 1997). This would explain why increasing dietary content of high moisture corn and fish meal increased milk protein yield more in cows fed AS compared with cows fed AH (Broderick, 1995; Vagnoni and Broderick, 1997). In the latter 2 studies, the effect of alfalfa preservation on milk protein yield was not consistent. The decreases in milk yield and milk protein yield might also be explained by a higher digestibility of the hay compared with the silage, which was also suggested by the differences in blood glucose and ADF among diets.

Evaluation of the diets using the NRC dairy cattle program (NRC, 2001) showed that NEL supply was in

excess of requirements for all diets, ranging from 4.7 to 5.5% for the AS and the AH diet, respectively. Hence, energy supply was not limiting for milk production, but the low milk fat percentages contributed to this excess.

CONCLUSIONS

Replacing 20% DM of chopped alfalfa hay with alfalfa silage in an alfalfa and barley grain-based TMR increased peNDF_{NDF}, calculated as the NDF retained by the 8- and 19-mm screens of the PSPS from 13.3 to 15.6% DM, increased blood glucose, tended to increase rumen ammonia and blood urea, did not affect DMI, rumen pH, rumen VFA, blood lactate, and milk fat, and numerically decreased milk yield and milk protein. The absence of a diet effect on rumen pH and milk fat might be due to the narrow range in peNDF_{NDF} among diets and the high inclusion of alfalfa. The low milk fat content and rumen pH suggested that SARA was induced across all diets, but its severity was not affected by treatment.

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