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The Effect of Reducing Alfalfa Haylage Particle Size on Cows in Early Lactation

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ABSTRACT

The objective of this experiment was to evaluate effects of reducing forage particle size on cows in early lactation based on measurements of the Penn State Particle Separator (PSPS). Eight cannulated, multiparous cows averaging 19 ± 4 d in milk and 642 ± 45 kg BW were assigned to one of two 4×4 Latin Squares. During each of the 23-d periods, animals were offered one of four diets, which were chemically identical but included alfalfa haylage of different particle size; short (SH), mostly short (MSH), mostly long (MLG), and long (LG). Physically effective neutral detergent fiber (peNDF) was determined by measuring the amount of neutral detergent fiber retained on a 1.18 mm screen and was similar across diets (25.7, 26.2, 26.4, 26.7%) but the amount of particles >19.0 mm significantly decreased with decreasing particle size. Reducing haylage particle size increased dry matter intake linearly (23.3, 22.0, 20.9, 20.8 kg for SH, MSH, MLG, LG, respectively). Milk production and percentage fat did not differ across treatments averaging 35.5 ± 0.68 kg milk and $3.32 \pm 0.67\%$ fat, while a quadratic effect was observed for percent milk protein, with lowest values being observed for LG. A quadratic effect was observed for mean rumen pH (6.04, 6.15, 6.13, 6.09), while A:P ratio decreased linearly (2.75, 2.86, 2.88, 2.92) with decreasing particle size. Total time ruminating increased quadratically (467, 498, 486, 468 min/d), while time eating decreased linearly (262, 253, 298, 287 min/d) with decreasing particle size. Both eating and ruminating per unit of neutral detergent fiber intake decreased with reducing particle size (35.8, 36.7, 44.9, 45.6 min/kg; 19.9, 23.6, 23.5, 23.5 min/kg). Although chewing activity was

closely related to forage particle size, effects on rumen pH were small, indicating factors other than particle size are critical in regulating pH when ration neutral detergent fiber met recommended levels. Feeding alfalfa haylage based rations of reduced particle size resulted in animals consuming more feed but did not affect milk production.

(**Key words:** rumen, effective fiber, pH, and rumination)

Abbreviation key: BWCH = body weight change, DMD = dry matter digestibility, LDR = liquid dilution rate, LG = long treatment containing long harvested haylage, MSH = intermediate short treatment composed of 1/3 parts LG, 2/3 parts SH, MLG = intermediate long composed of 2/3 parts LG, 1/3 parts SH. peNDF = physically effective neutral detergent fiber, PSPS = Penn State Particle Separator, RDOM = rumen degradable organic matter, S_{gm} = geometric standard deviation, SH = short treatment containing rechopped alfalfa haylage, TC = total chewing activity, TLC = theoretical length of cut, X_{gm} = geometric mean length.

INTRODUCTION

Ruminants require forage fiber in coarse physical form (NRC, 2001). Increasing fiber level and forage particle size has been shown to effectively increase chewing activity resulting in increased saliva flow, rumen pH, acetate-to-propionate ratio and milk fat levels (Norgaard 1983; Beauchemin et al., 1997). Although impaired rumen fermentation and function can result when cattle are fed rations lacking in physical structure, excessive amounts of long, coarse fiber may also limit intake and digestibility, ultimately affecting energy balance of the animal (Allen, 2000).

Energy requirements are highest for cows in early lactation (NRC, 2001). During this time attempts are made to elevate energy intake through either feeding a ration of higher energy density or by increasing DMI (Woodford and Murphy, 1988; Kertz et al., 1991). Distension of the reticulo-rumen has been identified as one factor associated with satiety in ruminants with the magnitude of its effect dependent upon the energy re-

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quirement of the animal or the filling effect of the diet (Allen, 2000). Feeds of longer particle size usually result in greater fill because of a slower rate of passage limiting DMI through distension (Gherardi, et al., 1992). It has been suggested that reducing diet particle size during this time could positively affect DMI because the time required for rumination decreases (Allen, 2000). Decreasing forage particle size as a means to increase intake in cows during early lactation has been evaluated (Woodford and Murphy, 1988) using different forms of alfalfa hay, but the effects of haylage chop length is less understood and potential negative effects associated with rumen fermentation are not known.

Current NRC guidelines (NRC, 2001) have proven useful in defining animal requirements and feed composition but do not provide detailed recommendation of ration physical form. The concept of effective fiber was created to amalgamate the chemical characteristics and particle size of the forage, and to quantify its value to rumen function (Mertens, 2000). Although there have been numerous studies designed to examine the effective fiber requirements of dairy cattle, relatively few have been designed using cows in early lactation. In addition, in order to formulate ration particle size recommendations, studies must be designed to determine how reduction in particle size may affect intake and rumen function. Chewing and rumination are known accurate measurements of the roughage characteristics for ruminant diets (Balch 1971; Sudweeks et al., 1981). Physically effective NDF (**peNDF**) is defined as that dietary fiber source which effectively stimulates rumination and salivation (Mertens, 1997). Poppi et al. (1985) determined that particles that were retained on a sieve measuring 1.18 mm pass out of the rumen slower than those, which are not retained. Mertens (1997) suggests that in order for particles >1.18 mm to pass out of the rumen they would have to be reduced through comminution and as a result these particles would stimulate more saliva secretion than those <1.18 mm.

The ability to routinely measure ration particle size has been difficult until introduction of The Penn State Particle Separator (**PSPS**). Based on properties of the ASAE Standard (S424) of forage particle size determination, the PSPS is a quick and cost effective method of particle size analysis. Using the PSPS a particle distribution is determined by separating particles according to size; those >19.0 mm, those between 19.0 and 8.0 mm, and those <8.0 mm (Lammers et al., 1996). With the construction of an additional square meshed screen comprised of nominal size openings of 1.18 mm the PSPS is capable of making the peNDF measurements proposed by Mertens (1997).

The objective of the following experiment is to determine the effect of forage particle size on DMI, chewing

Table 1. Nutrient composition and fermentation measures for alfalfa haylage.

	Mean	SEM
DM	33.5	2.8
CP	21.9	2.1
Soluble Protein	15.1	1.1
Ether Extract	3.9	0.7
NDF	42.9	3.2
NDIN	2.3	3.0
ADF	36.9	3.4
Lignin	7.0	1.2
TNC ¹	1.6	0.4
NFC ²	22.7	1.6
Ca	2.3	0.6
P	0.25	0.01
Mg	0.23	0.007
K	2.9	0.03
pH	4.7	0.3
Ammonia	2.8	0.8
Lactic acid	5.9	2.2
Acetic acid	0.82	0.04
Butyric acid	<0.01	...
Propionic acid	<0.01	...
Isobutyric acid	<0.01	...

¹TNC = Total nonstructural carbohydrates (Smith, 1981).

²NFC = Nonfiber carbohydrate calculated by difference $100 - (\%NDF + \% CP + \% Fat + \% Ash)$.

activities, and rumen fermentation in early lactation cows. It is hypothesized that rations of finest particle size may be consumed at greatest amounts but result in lowest chewing activity and rumen pH.

MATERIALS AND METHODS

Forage, Animals and Experimental Design

Alfalfa haylage was harvested in midbloom stage using a self-propelled forage harvester (John Deere, model 6750) set at 22.3 mm theoretical length of cut (**TLC**). The chopped material was then ensiled in a bunker type system for approximately 100 d and designated "long forage." Every second day during the course of the experiment, haylage was re-chopped twice using a pull type forage harvester (New Holland, model 900) set at 4.8 mm TLC, stored at 4°C and was designated "short forage." Table 1 contains chemical composition and physical characteristics of the alfalfa haylage.

Eight cannulated lactating multiparous Holstein cows averaging 19 ± 4 d DIM and weighing 642 ± 45 kg were randomly assigned to one of two 4×4 Latin Squares. During each of the 23 d periods animals were offered one of four TMR that were chemically identical but differed in forage particle size. Diets were composed of either long (**LG**) or short (**SH**) haylage or as in the case of intermediate treatments, mixtures of these diets. Mostly long (**MLG**) was composed of 2/3 parts LG, 1/3 parts SH TMR (DM basis); while mostly short

(MSH) was composed of 1/3 parts LG, 2/3 parts SH TMR (DM basis). Animals were housed in individual stalls and milked at 0700 and 1900 h. Cows were fed at 0800 h for ad libitum consumption to allow for approximately 5.0% refusal. The experimental cows were cared for and maintained according to the guidelines stipulated by the Pennsylvania State University Animal Care Committee.

Forage and TMR Chemical and Analysis

Samples of feed were collected twice weekly and orts were collected on d 15 and 16 as well as the last 3 d of each period. Collected samples were immediately frozen (-20°C) and stored for further analysis. Samples were then dried at 55°C in a forced air oven and ground (1 mm screen; Wiley mill, Aurthur A. Thomas Co., Philadelphia, PA). All feed and forage samples were analyzed in duplicate for moisture (AOAC 1990), Kjeldahl nitrogen (CP) (AOAC 1990) using a Kjetec 1030 auto analyzer, ether extract (AOAC 1990), calcium and phosphorus (AOAC 1984) and percent organic matter (OM) (AOAC 1984). Neutral detergent fiber (NDF) (Van Soest et al., 1991), acid detergent fiber (ADF) (AOAC 1990), acid detergent lignin (ADL) (AOAC 1990) were analyzed according to the procedure of Van Soest et al. (1991). Neutral detergent insoluble nitrogen (NDIN) and acid detergent insoluble nitrogen (ADIN) were analyzed on NDF and ADF residues (AOAC 1990). Heat stable alpha-amylase (number A3306; Sigma Chemical Co., St. Louis, MO) was included in the NDF procedure (100 μl per 0.50 g of sample). Total nonstructural carbohydrates (TNC) was determined according to the procedure of Smith, (1981) but modified to use ferricyanide as a colorimetric indicator. All ort samples were analyzed in duplicate for ADF, ash, CP, ether extract, moisture, NDF, and TNC.

Chewing Activity

Eating and ruminating activities were measured during d 15 and 16 of each period using Graze Jaw Movement Analysis Software of the IGER Behavior Recorder (Ultra Sound Advice, London) described by Rutter et al. (1997). Data were expressed as daily eating, ruminating, or total chewing activity (TC) by calculating the sum of eating and ruminating time over a 24 h period. Activities per unit of DM and NDF intake were calculated by dividing total minutes or number of bites by the mean of the activity measured. Total number of eating and ruminating bouts were also measured. Ruminating bouts were easily determined as regular activity pattern characterized with a 5- to 10-s period between boli when no jaw movements occurred. An

eating bout was defined as eating activity occurring for at least five minutes. Eating bouts were separated if ten or more minutes of inactivity was observed between adjacent observations.

Flow and Digestibility Markers and Apparent Digestibility

Particulate (forage) and liquid passage rates were determined using $\text{Yb}_3\text{Cl} \cdot 6\text{H}_2\text{O}$ and Co-EDTA as described by Lykos et al. (1996). Prior to the AM feeding on d 17 of each period, 5 g of bound Yb marked forage (average Yb concentration of 22 mg/g of DM) was pulse dosed through the ruminal cannula, mixed with ruminal digesta by hand, and followed by a pulse dose of Co (10 g of Co-EDTA dissolved in 1 L of tap water) for measurements of liquid passage. Solid and liquid passage rates were then calculated according to Grovum and Williams (1973).

Beginning on d 9 of each period, at 0800 and 2000 h, one capsule containing 5 g of Cr_2O_3 was placed in the dorsal area of the rumen via the cannula for measurements of apparent total tract digestibility. Beginning at on d 17, approximately 250 g of fecal material was collected at 0, 6, 12, 18, 24, 25, 32, 38.5, 43, 50, 52, 58, 70, 76, 78, 84, 90, 96, 102, 118 h and stored at -20°C . Samples were then dried at 55°C in a forced air oven, ground (1 mm screen; Wiley mill, Aurthur A. Thomas Co., Philadelphia, PA), and composited by cow and period. All fecal samples were analyzed in duplicate for moisture, ash, CP, NDF, ADF, TNC, EE, and NFC. Whole diet TDN concentration was determined using apparent digestibility of CP, EE, NFC, NDF components of the whole diet (Weiss et al., 1992). Based on these values, production levels of DE, ME and NE_L were calculated as outlined by NRC (2001).

Ruminal and Blood Sampling

Beginning on d 17 of each period ruminal contents were collected from the dorsal, ventral, and caudal area in the rumen at 0, 1.5, 3.5, 5.5, 8.5, 11.5, 14.5, 18, 21.5, and 24.5 h, beginning prior to Co dosing (0 h). Collected digesta was mixed by hand and filtered through four layers of cheesecloth. Ruminal liquid pH determination was immediately determined by using a hand held pH electrode (model M90, Corning Inc., Corning NY). Approximately 15 ml of filtered liquid was then placed into bottles containing 3 ml of 25% metaphosphoric acid and 3 ml of 0.6% 2-ethyl butyric acid (internal standard) and stored at -20°C . Samples were later centrifuged three times at $4,000 \times g$ for 30 min at 4°C to obtain a clear supernatant that was analyzed for ammonia using a phenol-hypochlorite assay (Broderick and Kang,

1980) and VFA concentration using gas chromatography (Yang and Varga, 1989). Additionally, 50 ml of rumen liquid was collected for Co analysis using the procedure described by Hart et al. (1984) (atomic absorption; Instrumentation Laboratories, model 22, aa/ae spectrophotometer, Allied Analytical Systems, Waltham, MA). Ruminal samples for Yb determination were collected from the dorsal, ventral and caudal areas in the rumen at 0, 1.5, 3.5, 5.5, 8.5, 11.5, 14.5, 18, 21.5, 24.5, 28, 31, 37, 40, 46, 52, 60, 72, 78, 87, and 96 h after dosing, mixed by hand and filtered through four layers of cheesecloth. Any ruminal contents left after sample collection was repacked back into the rumen. Approximately 200 to 250 g of solids were placed in plastic bags and stored at -20°C . Samples were then freeze dried, ground in a Wiley Mill through a 1.0 mm screen (A.H. Thomas, Philadelphia, PA) and analyzed for Yb using atomic absorption spectroscopy.

The last day of each period and 4h after feeding, approximately 500 ml ruminal samples from the dorsal, ventral and caudal areas in the rumen and fecal samples were collected and weighed for determination of DM, NDF and particle size. The rumen of each animal was then emptied into large plastic bins and mixed thoroughly. Total digesta amounts were weighed and three 500 mL samples were collected and frozen at -20°C . All remaining digesta was manually repacked into the rumen of each animal. On d 17 at 1000h of each period blood samples were collected from the tail vein of each animal. Blood samples were collected in tubes with sodium heparin for NEFA analysis (Wako NEFA C kit no. 990-75401; Wako Chemicals USA, Inc., Richmond, VA).

Particle Size Analysis

The PSPS was used to measure particle size for both forage and TMR as described by Kononoff et al. (2002). peNDF was estimated by multiplying NDF concentration of the TMR by the proportion of particles > 1.18 -mm (Mertens, 1997). Particle size of feed digesta and fecal samples was determined using wet sieving techniques as described by Beauchemin et al. (1997) using an Analysette 3 PRO Vibratory Sieve Shaker (Fritsch, Oberstein, Germany). Approximately 30 g of wet material was soaked in 1 L of distilled water for 10 minutes and then placed on a series of stacked sieves arranged in descending size and shaken for 10 min. Subsequent sieving material was removed from each sieve and dried in a forced air oven at 55°C to determine the amount of dry matter retained on each sieve. Percent of dry matter retained on each sieve, geometric mean (X_{gm}) and standard deviation (S_{gm}) were calculated as outlined by the ASAE, 2001 (S424).

Table 2. Particle size distribution, geometric mean (X_{gm}) and standard deviation (S_{gm}) for ensiled and rechopped alfalfa haylage as measured by the Penn State Particle Separator.

	Ensiled	Rechopped	SEM
% DM retained			
>19.0 mm	61.5	13.7	5.9
19.0–8.0 mm	26.2	51.7	4.1
8.0–1.18 mm	11.2	32.5	2.0
<1.18 mm	1.1	2.1	0.3
X_{gm} (mm) ¹	18.1	8.9	1.4
S_{gm} (mm) ²	2.1	2.2	0.1

¹ X_{gm} = Geometric mean length as calculated by the ASAE (2001).

² S_{gm} = Standard deviation as calculated by ASAE (2001).

Milk Production

Milk production was measured and recorded daily from d 15 to 23 of each period. On d 15, 16, 19, and 20 milk samples were collected and preserved using 2-bromo-2-nitropropane-1,3 diol. Milk samples were analyzed for milk fat and true protein by the Pennsylvania DHIA milk testing laboratory (AOAC, 1990) using Infrared spectrophotometry (Foss 605B Milk-Scan; Foss Electric, Hillerød, Denmark).

Statistical Analyses

Data were analyzed as a replicated 4×4 Latin Square with model fixed effects for square, cow within square, period, and treatment included in the model. The first order autoregressive covariance structure (AR(1)) and the MIXED procedure of SAS (SAS/STAT, 1999; Version 8.1) were used to analyze all data. Linear, quadratic, and cubic orthogonal contrasts were tested using the CONTRAST statement of SAS. Repeated measurements of rumen ammonia, pH, and VFA concentration were analyzed by including a REPEATED model statement, as well as a term for time and interaction for treatment by time. Square by treatment interaction was tested but was not significant therefore was dropped from the model. Significance was declared at $P \leq 0.05$ and trends are discussed at $P \leq 0.10$. All means presented are least squares means.

RESULTS

TMR Particle Size and Effective Fiber

Chemical and physical description of alfalfa haylage included in the experiment is presented in Tables 1 and 2; total ration chemical composition and physical analysis is presented in Tables 3 and 4. Ration particle size reflected the amount of rechopped forage included in the treatments. Rechopping forage resulted in less material being retained on the 19.0 mm screen but

Table 3. Ingredient and nutrient composition of total mixed rations.

	SHORT	LONG	SEM
Diet Ingredients			
Alfalfa haylage	50.0	50.0	...
Ground Corn	30.1	30.1	...
Soybeans	1.75	1.75	...
Distillers Corn	5.75	5.75	...
Wheat middlings	5.28	5.28	...
Soyhulls	3.43	3.43	...
Gluten Meal	0.88	0.88	...
Feather Meal	0.29	0.29	...
Blood Meal	0.29	0.29	...
Fish Meal	0.29	0.29	...
Tallow	0.73	0.73	...
Salt	0.46	0.46	...
Calcium Phosphate	0.31	0.31	...
Potassium Chloride	0.07	0.07	...
Magnesium Oxide	0.24	0.24	...
Limestone	0.04	0.04	...
Trace Mineral Mix ¹	0.02	0.02	...
Vitamin ADE ²	0.07	0.07	...
Chemical			
Moisture, %	48.7	46.6	0.36
CP, %DM	17.6	17.9	0.20
Soluble CP, % DM	9.3	9.7	0.35
TNC, % DM ³	28.0	26.6	0.59
NDF, % DM	32.4	31.0	0.44
ADF, % DM	26.1	24.2	0.42
Ether extract, % of DM	3.4	3.5	0.08
NFC, % DM ⁴	42.9	42.1	0.97

¹Contained 0.57% calcium, 1362.2 mg/kg cobalt, 40,816.3 mg/kg copper, 2,724.5 mg/kg iodine, 10,204.1 mg/kg iron, 1,222,449.0 mg/kg manganese, 15.8 % sulfur, 122,450.0 mg/kg zinc.

²Contained 28,792.5K IU/kg vitamin A, 7,198.5 KIU/kg vitamin D, 179,959.6 IU/kg vitamin E.

³TNC = Total nonstructural carbohydrates (Smith, 1981).

⁴NFC = Nonfiber carbohydrate calculated by difference $100 - (\%NDF + \%CP + \%Fat + \%Ash)$.

Table 4. Effects of reducing alfalfa haylage particle size on TMR particle size distribution, NDF content and physically effective NDF (peNDF) values.

	Treatment ^{1,2}				P-value	SEM
	SH	MSH	MLG	LG		
% DM retained						
>19.0 mm	3.0 ^d	12.3 ^c	21.9 ^b	31.4 ^a	<0.01	1.66
19.0–8.0 mm	28.3 ^a	24.8 ^b	21.1 ^c	17.5 ^d	<0.01	0.72
8.0–1.18 mm	49.0 ^a	43.7 ^b	38.4 ^c	33.0 ^d	<0.01	0.80
<1.18 mm	19.7	19.2	18.6	18.1	0.44	0.73
X _{gm} (mm) ³	4.1 ^a	4.8 ^b	5.7 ^c	6.8 ^d	<0.01	0.25
S _{gm} (mm) ⁴	2.7 ^a	3.1 ^b	3.4 ^c	3.6 ^d	<0.01	0.02
% NDF (DM)						
>19.0 mm	46.8 ^a	45.6 ^a	43.4 ^{a,b}	41.6 ^b	0.01	1.10
19.0–8.0 mm	39.2 ^a	38.2 ^{a,b}	36.6 ^{b,c}	35.3 ^c	<0.01	0.52
8.0–1.18 mm	30.1 ^a	29.1 ^{a,b}	27.9 ^{b,c}	26.8 ^c	<0.01	0.60
<1.18 mm	24.1	24.5	24.6	24.7	0.73	0.35
peNDF ⁵	25.7	26.2	26.4	26.7	0.52	0.51

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

²Means in the same row with different letters differ ($P \leq 0.05$).

³X_{gm} = Geometric mean length as calculated by the ASAE (2001).

⁴S_{gm} = Standard deviation as calculated by ASAE (2001).

⁵peNDF = Physically effective NDF = Ration NDF multiplied by amount of DM > 1.18 mm.

Table 5. Effects of reducing alfalfa haylage particle size on body weight, body weight change, and intake on cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
BW, kg	667	667	665	661	16.6	0.21	0.65	0.99
BWCH, kg/d ²	0.52	0.40	0.30	0.08	0.19	0.06	0.84	0.87
DMI, kg/d	23.4	21.8	20.7	20.1	0.85	<0.01	<0.01	0.49
DMI, % BW	3.54	3.28	3.11	3.19	0.14	<0.01	0.15	0.63
NDF intake, kg/d	7.33	6.83	6.50	6.59	0.27	<0.01	0.02	0.64
NDF intake, % BW	1.11	1.03	0.978	0.998	0.05	<0.01	<0.01	0.59

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

²BWCH = Body weight change.

increased the amount of particles being retained on both the 8.0 and 1.18 mm screens of the PSPS. Although the proportion of DM retained on the pan increased with decreasing particle size, differences were not significant. Geometric mean decreased linearly as the amount of rechopped forage in the TMR decreased. The peNDF value, as measured by the PSPS decreased numerically with decreasing particle size but these differences were not significant.

Intake and Chewing Activities

Intake data for DM and NDF are presented in Table 5. As particle size decreased there was a significant increasing linear effect on DMI and NDFI when expressed as total amount per day or percentage of BW. The largest difference between diets in DMI occurred between the SH and LG diets (3.37 kg/d; 17%) and between SH and MLG diets for NDFI (0.83 kg/d; 13%). Feed refusal samples were analyzed for NDF to evaluate sorting in the feed bunk and particle size but no effect was observed ($P > 0.05$; data not shown).

The effect of haylage physical form on eating and ruminating activity is presented in Table 6. The total number of eating bouts and ruminating bouts was not significantly affected by forage particle size however total number of ruminating bouts per kilogram of DM and NDFI significantly decreased with decreasing particle size. Daily total time spent eating significantly decreased linearly with decreasing particle size but a significant quadratic effect was observed for total time spent ruminating. Eating, ruminating, and TC activity per kilogram of DM and NDFI decreased linearly with particle size. Total number of eating chews per day and per kilogram of DM and NDF intake was not significantly affected by forage particle length. In contrast, a quadratic trend in the total number of ruminating chews per day was observed.

Daily eating patterns are illustrated in Figure 1. Diurnal eating patterns for SH, MSH, and MLG treat-

ments appeared similar with highest activity at the hour of feeding (0800 h) however animals consuming LG treatments exhibited pronounced meal bouts at 1400, 1500, and 2300 h when compared to the other treatments.

Apparent Digestibilities and Energy Utilization

Apparent digestibility, measured TDN and NE_L is presented in Table 7. Highest dry matter digestibility (DMD) was observed on rations of shortest particle size (66.5%) and decreased linearly with lowest DMD (63.1%) observed on diets of longest particle size. More specifically, apparent digestibility of CP, TNC, ether extract, NFC increased linearly and NDF and OM digestibility tended to increase linearly with decreasing particle size. As a result of differences in digestibility between treatments, dietary content of both TDN and NE_L tended to decrease linearly from the shortest ration (65.8%, 1.57 Mcal/kg) to the longest (62.9%, 1.47 Mcal/kg).

Rumen Fill, Rate of Passage, Particle Size

Rumen fill of total digesta, NDF and total DM and rate of passage is presented in Table 7. No effect was observed in amount of rumen DM or NDF mass across treatments. No effect was observed for rumen liquid dilution rate (LDR) or solid passage rate averaging 16.4 and 4.15 percent per hour.

The effects of rechopping forage on the particle size of TMR, rumen digesta and fecal material is presented in Table 8. Rechopping forage had large effects on the particle size of TMR as X_{gm} and the amount of DM > 1.18 mm decreased with reducing particle size. Effects on rumen digesta particle size were less pronounced however X_{gm} tended to decrease with reduced particle size. In comparison, fecal X_{gm} and percent of material retained on a 1.18-mm sieve was unaffected by ration particle size averaging 1.0 mm and 46.1% respectively.

Table 6. Effects of reducing alfalfa haylage particle size on chewing activities on cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
Bouts/d								
Eating	13.4	13.9	16.4	12.9	1.73	0.88	0.22	0.25
Ruminating	15.3	16.5	16.3	17.2	1.23	0.26	0.88	0.58
Bouts/ kg DMI								
Eating	0.59	0.64	0.81	0.65	0.09	0.35	0.19	0.22
Ruminating	0.67	0.76	0.80	0.85	0.07	0.04	0.54	0.85
Bouts/ kg NDFI								
Eating	1.88	2.05	2.60	2.09	0.31	0.35	0.20	0.24
Ruminating	2.13	2.43	2.57	2.71	0.24	0.04	0.58	0.82
Min/d								
Eating	261.7	254.0	290.8	297.8	20.9	0.02	0.39	0.13
Ruminating	460.2	504.7	477.9	479.1	21.8	0.54	0.02	0.03
TC ²	723.4	758.9	768.3	776.7	31.7	0.08	0.35	0.74
Min/Kg DMI								
Eating	11.2	11.5	14.1	14.3	0.9	<0.01	0.77	0.05
Ruminating	19.9	23.6	23.5	23.5	1.2	<0.01	0.13	0.35
TC ²	31.2	35.2	37.5	37.9	1.6	<0.01	0.04	0.98
Min/Kg NDFI								
Eating	35.8	36.7	44.9	45.6	2.9	<0.01	0.92	0.06
Ruminating	63.4	75.1	74.5	75.7	3.9	<0.01	<0.01	0.09
TC ²	99.7	112.1	119.2	120.8	5.2	<0.01	0.06	0.99
Chews/d								
Eating	6,140	6,172	7,444	6,904	1,090	0.33	0.69	0.38
Ruminating	27,680	31,573	30,122	29,483	1,667	0.53	0.07	0.29
Chews/ Kg DMI								
Eating	264.5	287.0	359.5	332.5	44.4	0.12	0.45	0.35
Ruminating	1,213	1,476	1,470	1,460	77	0.07	0.24	0.51
Chews/Kg NDFI								
Eating	841.6	908.6	1,138	1,066	141	0.11	0.50	0.36
Ruminating	3,883	4,690	4,669	4,666	245	0.03	0.07	0.36

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

²TC = Total chewing activity (minutes eating + minutes ruminating).

Rumen pH, VFA, ammonia and Blood NEFA

Effects of alfalfa haylage particle size on ruminal pH, VFA and ammonia concentrations are presented in Table 9. Total concentration of VFA significantly increased linearly with reducing particle size from 149.3 mM/L on SH to 136.0 mM/L on LG. The same significant effect was observed for concentrations of acetate, propionate, and butyrate while acetate to propionate ratio decreased linearly from 2.92 on LG to 2.75 on SH. There was a significant quadratic effect observed for mean rumen pH with highest values, 6.15 and 6.13 observed on MSH and MLG diets. Diurnal rumen pH appeared to be highest just prior to feeding and reached a minimum approximately two hours after evening milking and return to the barn (Figure 1). Rumen pH measurements for the MSH and MLG rations followed a similar pattern while animals consuming SH diets appeared to be flatter with less fluctuation compared to when consuming LG diets. Ruminal NH₃-N was observed to be unaffected by diet, averaging 11.24 mg/dl. No significant differences were observed in blood NEFA concentration.

Milk Production, Composition and Body Weight Change

Milk production, composition and body weight change (BWCH) are presented in Tables 5 and 10. Milk yield and 3.5% FCM were similar across diets and averaged 35.5 and 35.2 kg, respectively. Forage particle size did not affect either percent fat or yield averaging 3.31% and 1.2 kg. Although no effect of forage particle size was observed on milk true protein yield, a quadratic effect was observed in percent true protein with LG resulting in lowest (2.82%) values. Lastly, feeding rations of reduced forage particle size tended to result in a positive increase in body weight during experimental periods.

DISCUSSION

Based on a survey composed of over 800 samples, Heinrichs et al. (1999) reported that although TMR samples fed on commercial dairy farms typically contain 7% of the particles greater than 19.0 mm variation across herds is large. In the same study, a minimum

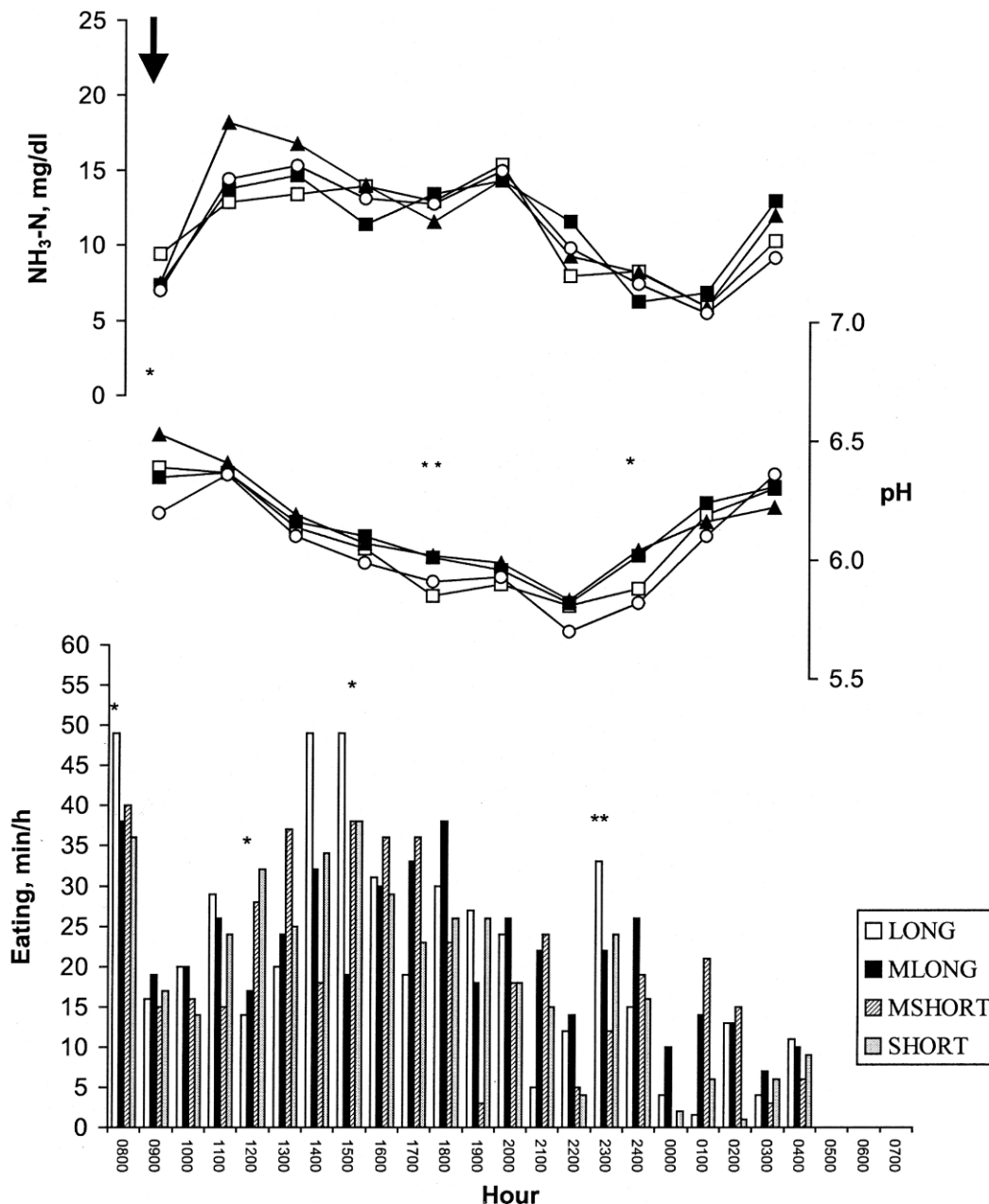


Figure 1. Effects of reducing alfalfa haylage particle size on daily rumen pH and NH₃-N concentration and eating pattern in min/h for a 24-h period for dairy cows LONG (□), MLONG (■), MSHORT (▲) or SHORT (○). Arrow indicates feeding time (**P* ≤ 0.05; ***P* ≤ 0.10).

of 1% and a maximum of 43% of the particles were observed to be greater than 19.0 mm, thus the range of TMR particle size used in the current study is typical of that in use of commercial dairy farms.

Results of this study indicate that intake during early lactation may be increased by reducing forage particle size. These results are similar to some studies in which reducing forage particle size resulted in increased intake (Rodrique and Allen 1960; Jaster and Murphy

1983; Woodford and Murphy 1986; Fisher et al., 1994; Beauchemin et al., 1997), but are in contrast to others which report no observed differences (Grant et al., 1990; Colenbrander et al., 1991). Most studies reporting no effects include cows in midlactation that were likely meeting their energy requirements. In the current experiment cows in early lactation were used and energy status was improved, presumably through increased intake and digestibility, as feeding diets of reduced par-

Table 7. The effect of reducing alfalfa haylage particle size on nutrient digestibility, calculated energy values, rumen fill and solid and liquid passage rate in cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
Digestibility								
DM, %	66.5	63.7	64.4	63.1	1.0	0.03	0.29	0.14
OM, %	68.4	66.0	66.9	65.4	1.0	0.06	0.52	0.15
CP, %	58.6	54.0	53.6	53.2	1.4	0.01	0.09	0.46
NDF, %	48.1	45.6	46.9	44.7	1.1	0.06	0.84	0.11
ADF, %	54.0	50.4	52.8	52.2	1.8	0.70	0.42	0.26
TNC, %	89.8	88.1	87.9	86.9	0.8	0.02	0.55	0.46
EE, %	58.4	54.1	53.5	52.9	1.3	0.01	0.09	0.47
NFC, % ²	88.4	87.5	87.5	86.5	1.5	0.39	0.96	0.78
TDN, % ³	65.8	63.1	64.1	62.9	1.1	0.09	0.38	0.18
NE _{Lp} , Mcal/kg ⁴	1.57	1.48	1.51	1.47	0.03	0.07	0.33	0.16
Rumen Fill ⁵								
Wet weight, kg	73.3	70.08	76.6	71.9	4.5	0.87	0.86	0.23
DM, %	18.9	18.4	19.3	18.5	0.53	0.88	0.79	0.21
Kg	13.5	14.2	13.6	13.6	0.96	0.91	0.77	0.69
NDF, %	60.4	58.2	57.6	58.6	1.1	0.25	0.22	0.96
Kg	8.06	8.23	7.78	7.88	0.47	0.63	0.94	0.58
Liquid passage rate, %/h	16.5	16.2	17.2	15.7	1.0	0.73	0.53	0.35
Forage passage rate, %/h	4.06	4.39	4.0	4.06	0.23	0.54	0.47	0.15

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

²NFC = Nonfiber carbohydrate calculated by difference.

³TDN (%) = tdNFC + tdCP + (tdEE × 2.25) + tdNDF; where td denotes truly digestible fraction.

⁴NE_{Lp} (Mcal/kg) = 0.703 × ME_p (Mcal/kg) (NRC, 2001).

⁵Manual empty.

particle size tended to increase body weight gain and numerically decreased plasma NEFA concentration. Due to the experiment's cross over design it is not possible to completely attribute effects on BWCH to dietary treatments however together these observations suggest increased energy balance with feeding rations of reduced particle size.

In the present study, reducing alfalfa haylage particle size resulted in decreased chewing activities per unit of DM and NDF consumed and is similar to results reported by Beauchemin et al. (1994) and Grant et al. (1990). Chewing activity is the primary mechanism to reduce feed particle size and is central to both the nature of digestion and passage through the gastrointestinal tract. It is well established that increasing forage particle size increases chewing activity but relatively few studies have related these measurements to the PSPS. Our data suggests that increasing the proportion of particles > 19.0 mm may be a primary factor affecting chewing activity in dairy cattle fed diets containing alfalfa haylage as the forage source. In the current study the proportion of feed particles > 19.0 mm increased with increasing particle size while the proportion of particles 8.0–19.0 mm and 8.0–1.18 mm decreased with increasing particle size. The LG ration containing 31% of the particles > 19.0 mm resulted

in the highest eating, ruminating, and total chewing activity per unit of DM and NDF intake as well as pronounced eating bouts at 1400, 1500 and 2300 h. Although it follows that less time would be required for ruminating when a diet of short particle size is consumed, we are unable to explain why greatest time spent ruminating was observed on the MSH ration.

Changes in physical characteristics of the ration also resulted in effects on rumen VFA patterns. In animals consuming SH rations containing 3% of the particles > 19.0 mm, digestibility and VFA concentration were highest but mean rumen pH was lowest indicating that substrate availability to rumen microbes increased with reduction in particle size. In the current study, reduction in particle size increased digestibility of all nutrients except ADF and NFC. Observed effects were likely a result of increased surface area available for microbial attack, ultimately resulting in a more rapid rate of rumen fermentation and increased intake (Chesson et al., 1995). Although depressed fiber digestibility has been observed in some studies when forage particle size is reduced, this is usually observed when a severe elevation in rate of passage is much greater than changes in rate of digestibility (Woodford and Murphy, 1988; Le Liboux and Peyraud, 1998). In the current study, reduction in haylage particle size did not affect

Table 8. The effect of reducing alfalfa haylage particle size on particle size of feed, rumen digesta, and feces in cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
Feed								
Sieve, mm								
13.2	10.8	18.8	27.7	34.9	3.20	<0.01	0.90	0.86
6.7	10.4	9.0	7.6	6.1	0.53	<0.01	0.97	0.96
3.35	9.5	8.1	6.7	5.3	0.45	<0.01	0.99	0.99
1.18	38.0	34.1	29.6	26.2	1.99	<0.01	0.90	0.87
0.6	15.3	14.6	13.8	13.3	0.63	0.02	0.88	0.84
0.15	16.0	15.4	14.6	14.2	0.37	<0.01	0.82	0.76
> 1.18	72.5	71.6	70.0	68.7	0.96	<0.01	0.85	0.80
X _{gm} (mm) ²	2.1	2.4	2.9	3.4	0.25	<0.01	0.78	0.82
S _{gm} (mm) ³	3.3	3.6	3.8	3.9	0.10	<0.01	0.29	0.98
Digesta								
Sieve, mm								
13.2	17.9	8.2	18.6	24.7	3.67	0.06	0.06	0.09
6.7	7.5	9.9	8.0	8.5	1.06	0.78	0.43	0.11
1.18	39.1	50.4	37.1	32.6	3.1	0.02	0.03	0.02
0.6	13.0	10.1	12.2	11.4	1.3	0.61	0.43	0.13
0.15	22.4	21.5	24.2	22.8	0.51	0.09	0.64	0.003
> 1.18	64.6	68.4	63.7	65.8	1.10	0.81	0.47	0.003
X _{gm} (mm) ²	2.1	2.1	2.3	2.5	0.16	0.06	0.36	0.73
S _{gm} (mm) ³	3.9	3.4	4.0	4.3	0.15	0.01	0.05	0.02
Feces								
Sieve, mm								
6.67	2.75	3.27	4.44	2.96	1.1	0.13	0.96	0.45
1.18	48.4	48.5	49.2	36.4	2.6	0.43	0.96	0.89
0.6	9.46	10.8	10.1	13.2	1.3	0.33	0.70	0.18
0.15	39.4	37.4	36.3	47.7	2.1	0.44	0.78	0.31
> 1.18	46.4	46.0	46.3	45.7	2.7	0.89	0.98	0.87
X _{gm} (mm) ²	1.0	1.0	1.1	1.0	0.06	0.92	0.91	0.40
S _{gm} (mm) ³	2.9	2.9	3.0	3.0	0.08	0.24	0.85	0.89

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

²X_{gm} = Geometric mean length as calculated by the ASAE (2001).

³S_{gm} = Standard deviation as calculated by ASAE (2001).

rate of rumen outflow of either liquid or solid particles. These results are consistent with those of Yang et al. (2001) who reported that rechopping forage did not al-

ways affect solid or liquid passage rates. Although it has been suggested feeds of longer particle size may limit intake as a result of a slower rate of passage and

Table 9. Effects of reducing alfalfa haylage particle size on ruminal pH, concentration ammonia, VFA, and blood NEFA level on cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
pH	6.04	6.15	6.13	6.09	0.1	0.28	<0.01	0.46
Total VFA (mM/L)	149.3	141.4	140.2	136.0	2.3	<0.01	0.42	0.37
VFA (mM/L)								
Acetate	92.2	88.3	88.1	85.9	1.5	<0.01	0.55	0.37
Propionate	33.9	31.5	31.0	29.8	0.9	<0.01	0.29	0.32
Isobutyrate	1.43	1.41	1.37	1.30	0.1	<0.01	0.37	0.86
Butyrate	16.6	15.3	14.9	14.5	0.4	<0.01	0.13	0.37
Isovalerate	2.30	2.28	2.19	2.07	0.1	<0.01	0.28	0.83
Valerate	2.94	2.68	2.58	2.49	0.1	<0.01	0.15	0.56
Acetate: Propionate	2.75	2.86	2.88	2.92	0.1	<0.01	0.08	0.24
NH ₃ -N (mg/dl)	10.9	11.8	11.2	11.0	0.6	0.90	0.30	0.47
NEFA (μeq/L)	136.5	145.8	146.8	148.9	19.6	0.64	0.84	0.91

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

Table 10. Effects of reducing alfalfa haylage particle size on milk production and composition on cows in early lactation.

	Treatment ¹				SEM	Contrast		
	SH	MSH	MLG	LG		Linear	Quadratic	Cubic
Milk Yield	36.0	35.3	34.8	36.0	1.6	0.68	0.11	0.39
3.5% FCM	35.6	35.0	34.5	35.5	2.0	0.84	0.26	0.68
Fat %	3.38	3.27	3.31	3.31	0.14	0.67	0.47	0.65
Fat, Kg/d	1.23	1.20	1.17	1.20	0.08	0.58	0.49	0.76
Protein %	2.90	2.91	2.93	2.82	0.04	0.15	0.03	0.32
Protein, Kg/d	1.05	1.07	1.03	1.03	0.05	0.28	0.65	0.28

¹SH = TMR containing short alfalfa haylage, LG = TMR containing long alfalfa haylage, MSH = TMR composed of 1/3 parts LG, 2/3 parts SH TMR, MLG = TMR composed of 2/3 parts LG, 1/3 parts SH TMR.

greater rumen fill, in the current study no effects were observed in either passage rate or rumen DM and NDF pool suggesting that digestibility was the governing factor of intake (Gherardi, et al., 1992).

It is well understood that sufficient amounts of coarse fiber is necessary to maintain proper rumen fermentation and function (Yang et al., 2001). peNDF is believed to be that portion of a diet that stimulates chewing activity and results in the formation of the rumen mat. One animal response used to measure peNDF is TC expressed as minutes per kilogram of NDFI. As particle size of the ration increases the peNDF content is believed to also increase, resulting in elevated TC, salivary buffer secretion and ruminal pH (Mertens, 2000). In the current study, diets of very different particle size were fed and although peNDF content of diets numerically increased with increasing particle size, differences were not significant. In contrast, percent of large particles (> 19.0 mm) significantly increased and resulted in linear increases of TC an effect similar to that reported by others (Krause et al., 2002). Although we originally believed that trends in TC would also result in similar trends in rumen pH, a quadratic effect was observed with diets of intermediate particle size having highest values, indicating that factors other than TC influence rumen pH. Even though shortest particle size resulted in lowest TC, higher acid production, presumably less salivary secretion and lower mean rumen pH, highest chewing activity in animals consuming diets of longest particle size was also paired with low mean pH. Although higher rumen pH is indicative of rations resulting in higher ruminating activity, it is also highly responsive to the introduction of new substrate from the consumption of meals. We suggest that the different meal patterns with animals consuming excessively long rations may have resulted in a marked decrease in rumen pH that characteristically persisted for several hours before recovery to original levels (Le Liboux and Peyraud, 1999). Related to this was the observation that highest NDF digestibility occurred on the shortest diet that also resulted in lowest mean rumen pH. This

was somewhat surprising given that it is commonly believed that decreased pH lowers fiber digestion (Russell and Wilson, 1996). Although we cannot completely explain this effect, we speculate that pH did not reach the point, nor spend enough time at that point, necessary to impede activity of cellulolytic bacteria. Thus, even though it has been suggested that the predominant species of these bacteria do not grow below 6.0, we speculate some fiber digestion persisted, furthermore even though SH rations resulted in a rumen pH below 6.0, it was short in duration.

Practically, it is useful to note that the amount of feed >19.0-mm is a more accurate indicator of chewing activity than peNDF, when estimated by the PSPS. Although the additional sieve measuring 1.18-mm was not useful in predicting chewing activity, its application in measuring particle size of diets is warranted as TMR's typically contain 40 to 60% concentrate, most of which passes through the 8.0-mm screen. Although the PSPS has resulted in a useful analytical method for measurement of particle size, further partitioning of smaller particles will more accurately describe sample fineness (Kononoff et al., 2003). Although demonstrated by this study, reducing haylage particle size decreased time spent chewing, the dataset was small, consisting of only 32 observations. As a result of these observations, further work should be carried out in attempt to further examine the relationship between particle size measurements using the PSPS and chewing activity.

Because saliva contains important buffers for the rumen environment, it is believed that the ability of a diet to stimulate chewing activity is critical in the regulation of ruminal pH levels. Our data suggests that ration particle size may result in large effects on TC; however, only small changes in rumen pH were observed. Recently Yang et al. (2001) noted that contribution of increased total daily saliva output due to increased TC on rumen pH is often overestimated. Although reduced particle size may decrease TC, changes in total saliva production are small (approximately 4%), as resting saliva secretion will increase (Yang et al.,

2001). It therefore seems likely that the physical characteristics of the feed may have a smaller influence on rumen pH than we originally hypothesized. This suggestion is consistent with Allen, (1997) who noted that variation of rumen degradable organic matter (RDOM) may have a greater effect than particle size on the variation of pH. Furthermore, our data suggests that the proportion of NDF ≥ 1.18 mm may not differ in rations containing forage of different cut length and as a result, when used alone, is a poor measurement of effective fiber. More specifically, accounting for larger particles in the ration may result in a more accurate estimate in the ability of the ration to stimulate TC and accounting for RDOM may have greater effects on the variation of rumen pH.

Total milk yield, FCM, fat percentage and yield were not significantly affected by particle size reduction; however, a quadratic decrease was observed for percent milk protein but not yield. Although our original hypothesis speculated that milk fat percentage would increase with increasing particle size as observed by Grant et al. (1990) and Fisher et al. (1994), no significant effect was observed, similar to the results of Colenbrander et al (1991) and Belyea et al. (1989). The use of milk fat as a measure of fiber effectiveness has been questioned, especially for cows in early lactation, which are less responsive to dietary changes (Allen, 1997). The lack of response of milk fat to reduced particle size may also be due to the fact that rations met NRC requirements. It has been suggested that depressions in milk fat are more likely when NDF is below minimum requirements (Beauchemin and Rode, 1997).

Based on herd averages, mean average milk production (35.5 kg) and percent milk protein (2.9%) were below that expected. All diets were evaluated using the NRC (2001) model for nutrient requirements of dairy cattle. Based on simulations, cows were predicted to consume 22 kg of feed containing 1.62 Mcal/kg of NE_L , resulting in an NE_L and MP allowable milk of 38.0 and 32.3 kg respectively. Based on the simulation and recommendations of Schwab et al. (1996), experimental diets were adequate for flow of LYS and MET into the duodenal digesta pool (15 and 5% of the total essential amino acid (EAA) pool) but RDP balance was in slight excess (672 g/d) while RUP was deficient (-182 g/d). Taken together this suggests that intake of NE_L was not limiting and is supported by the observation that plasma NEFA levels were below 200 $\mu\text{eq/L}$ across treatments. It is possible that milk production may have been in part limited due to the cost of metabolizing excess RDP, but more likely due to inadequate RUP in the ration (Schwab, 2002).

Published studies are only beginning to report particle size distributions based on measurements using the

PSPS, thus making recommendations difficult. Current NRC (2001) recommendations state that a minimum mean particle length of 3 mm for alfalfa diets is necessary to maintain rumen pH, chewing activity, and milk fat percentage, however this recommendation is based on measurements using a vertical sieve shaker containing a profile of wire mesh sieves between 0.30 and 9.5 mm. Because differences between methods of measurements have been reported and the method of measurement is more applicable to spherical shaped particles, this recommendation is not applicable to measurements made by the PSPS (Murphy and Zhu, 1997). Results of the current study suggest that alfalfa haylage based rations containing at least 30.0% NDF with 3.0% of the particles > 19.0 mm may be consumed in greater amounts, more rapidly, and fermented more extensively than those of longer particle size, and that passage rates did not reduce either fermentation or digestibility (Van Soest, 1994). Although further work is needed to evaluate possible interactions between particle size and ration NDF or NSC level on chewing activity and rumen pH this study suggests that peNDF is not an accurate indicator when estimated by the PSPS.

CONCLUSIONS

In the present study, reducing forage particle size increased DMI of TMR of dairy cattle in early lactation. Although chewing activity was closely related to forage particle size, effects on rumen pH were small, indicating factors other than particle size are critical in regulation when ration NDF meets recommended levels. Feeding alfalfa based rations with 3% compared to $> 30\%$ of the particles ≥ 19.0 mm increased DMD 3.4%, increased total VFA concentration 13.3 mM/L, and resulted in animals consuming 3.3 kg more and did not affect milk fat.

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