CHAPTER 4

THE EFFECT OF REDUCING ALFALFA HAYLAGE PARTICLE SIZE ON COWS IN EARLY LACTATION

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 $\pm$ 4 DIM and 642 $\pm$ 45 kg BW were assigned to one of two 4X4 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 NDF (peNDF) was determined by measuring the amount of NDF retained on a 1.18 mm screen and was similar across diets (27.2, 27.7, 27.9, 28.1%) but the amount of particles >19.0 mm significantly decreased with decreasing particle size. Reducing haylage particle size increased DMI linearly (23.3, 22.0, 20.9, 20.8 kg for SH, MSH, MLG, LG, respectively). Milk production and percent fat did not differ across treatments averaging 35.5 $\pm$ 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 NDFI 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 regulation when ration NDF met recommended levels. Feeding alfalfa haylage based rations resulted in animals consuming more feed, producing milk with 0.08% more milk protein and did not affect percent milk fat. **Key Words:** rumen, effective fiber, pH, and rumination. **Abbreviation key: BWCH** = body weight change, **DMD** = dry matter digestibility, **PSPS** = Penn State Particle Separator, **RDOM** = rumen degradable organic matter, **TLC** = theoretical length of cut, **SH** = short treatment containing rechopped alfalfa haylage, **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, $\mathbf{X_{gm}}$ = geometric mean length, $\mathbf{S_{gm}}$ = geometric standard deviation

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 in cattle 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 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 requirement 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). During this time it has been suggested that reducing diet particle size could positively affect DMI because the density of particles or the time available for rumination increases (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 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 Penn State Particle Separator (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 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 days 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 4-1 contains chemical composition and physical characteristics of the alfalfa haylage.

Eight cannulated lactating multiparous Holstein cows averaging 18 ± 4d DIM and weighing 649 ± 45 kg were randomly assigned to one of two 4X4 Latin Squares. During each of the 23 d periods animals were offered one of four TMR's that were chemically identical but differed in forage particle size. Diets were composed of either long **(LG)** or short **(SH)** forage or as in the case of intermediate treatments, mixtures of these diets. Mostly long **(MLG)** was composed of 1/3 parts LG, 2/3 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.

Experimental Measures and Sample Analysis

Forage and TMR Chemical and Analysis

Samples of feed were collected twice weekly and orts were collected on day 15 and 16 as wells as the last three days 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 fecal and ort samples were analyzed in duplicate for ADF, ash, CP, ether extract, moisture, NDF, and TNC.

Chewing Activity

Eating and ruminating activity was measured during day 15 and 16 of each period using Graze Jaw Movement Analysis Software of the IGER Behavior Recorder® (Ultra Sound Advice, London, UK) described by Rutter et al., (1997). Data were expressed as daily eating, ruminating, or total chewing activity (TCA) by calculating the sum of eating and ruminating time over a 24 h period. Activities per unit of DM and NDF intake was also calculated by dividing total minutes or number of bites by the mean of the parameter measured.

Flow and Digestibility Markers and Energy Determination

Particulate (forage) and liquid passage rates were determined using Yb_3Cl6 H_20 and Co-EDTA as described by Lykos et al., (1996). Prior to the AM feeding on day 17 of each period, 5 g of bound Yb marked forage (averaged 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 1L of tap water) for measurements of liquid passage. Beginning of day 9 of each period, at 0800 and 2000h, one capsule containing 5g of Cr_2O_3 was placed in the dorsal area of the rumen via the cannula for measurements of apparent total tract digestibility. Solid and liquid passage rates were then calculated according to Grovum and Williams (1981).

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 Sampling and Emptying

Beginning on day 17 of each period ruminal contents were collected from the dorsal, ventral, and caudal area in the rumen at 0.0, 1.5, 3.5, 5.5, 8.5, 11.5, 14.5, 18, 21.5, 24.5 h, beginning prior to Co dosing (0h). Collected digesta was mixed by hand and filtered through four layers of cheesecloth. Rumen 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 3ml of 0.6% 2-ethly butyric acid (internal standard) and stored at - 20 °C. Samples were later centrifuged three times at 4,000 X 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 - 250 g of solids are 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 were manually repacked into the rumen of each animal.

Particle Size Analysis

The PSPS was used to measure particle size for both forage and TMR as described by Kononoff et al., (2002). Physically effective NDF (peNDF) was measured by the sum of percent of NDF content being retained on the 1.18, 8.0, and 19.0 mm screens (Mertens, 2000). 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 1L 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).

Statistical Analyses

Data was analyzed as a replicated 4 X 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 (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 4-1 and 4-2; total ration chemical composition and physical analysis is presented in Tables 4-3 and 4-4. 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 increased the amount of particles being retained on both the 8.0 and 1.18 mm screens of the PSPS. Although the amount of particles < 1.18 mm increased with decreasing particle size, differences were not significant ($P > 0.05$). Geometric mean decreased linearly as the amount of rechopped forage in the TMR decreased. The peNDF value, as measured by the proportion of NDF > 1.18 mm, decreased numerically with decreasing particle size but these differences were not significant ($P > 0.05$).

Intake and Chewing Activities

Intakes of DM and NDF intake data are presented in Table 4-5. As particle size decreased there was a significant ($P \leq 0.05$) increasing linear effect on DMI and NDFI as 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 4-6. The total number of eating bouts and ruminating bouts was not significantly affected ($P > 0.05$) by forage particle size however total number of ruminating bouts per kilogram of DM and NDFI significantly ($P \leq 0.05$) decreased with decreasing particle size. Daily total time eating significantly ($P \leq 0.05$) decreased linearly with decreasing particle size but a significant ($P \leq 0.05$) quadratic effect was observed for total time ruminating. Eating, ruminating, and TCA Activity per kilogram of DM and NDFI decreased linearly ($P \leq 0.05$) with particle size. Total number of eating chews per day and per kilogram of DM and NDF intake was not significantly ($P > 0.05$) affected by forage particle length. In contrast, a quadratic trend ($P \leq 0.10$) 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 treatments 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 4-8. Highest 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 ($P \leq 0.05$) increased linearly and NDF and OM digestibility tended ($P \leq 0.10$) 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 ($P \leq 0.10$) 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 4-8. No effect ($P > 0.05$) was observed in total amount of rumen wet weight, DM or NDF mass averaging 73.0, 13.7, and 8.0 kg 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 4-7. 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 ($P \leq 0.10$) to decrease with reduced particle size. In comparison, fecal particle size resulted in no significant effects ($P > 0.05$) on X_{gm} or amount of DM > 1.18 mm averaging 1.0 mm and 46.1% respectively.

Rumen pH, VFA, ammonia and Blood NEFA

Effects of alfalfa haylage particle size on rumen pH, VFA and ammonia concentrations are presented in Table 4-9. Total concentration of VFA significantly ($P < 0.05$) increased linearly with reducing particle size from 149.3 mM/L on SH to 136.0 mM/L on LG. The same significant ($P < 0.05$) 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 ($P < 0.05$) quadratic effect observed for mean rumen pH with highest values, 6.15 and 6.13 observed on MSH and MLG diets. Dirunal 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 short diets appeared to have a more consistent rumen pH compared to those consuming long diets. Ruminal NH_3N was observed to be unaffected ($P > 0.05$) by diet averaging 11.24 mg/dl. No significant ($P > 0.05$) differences were observed in blood NEFA concentration.

Milk Production, Composition and Body Weight Change

Milk production, composition and body weight change (BWCH) is presented in Tables 4-5 and 4-10. 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 ($P > 0.05$) 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 ($P < 0.10$) to result in a linear increase in BWCH 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 of 1% and a maximum of 43% of the particles were observed to be greater that 19.0 mm, thus the range of TMR particle size used in the current study is indicative 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 (Beauchemin et al., 1997; Rodrique and Allen 1960; Jaster and Murphy 1983; Woodford and Murphy 1986; Fisher et al., 1994), but are in contrast to others which report no observed differences (Colenbrander et al., 1991; Grant et al., 1990). 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 as feeding diets of reduced particle size tended (P = 0.06) to increase body weight gain and numerically decrease plasma NEFA concentration. Due to the experiments 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 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. Rations 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 and marked differences in eating patterns.

Changes in physical characteristics of the ration also resulted in effects on rumen VFA patterns. When diets contained 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. Observed effects were likely a result of increased surface area available for microbial attack, ultimately resulting in a more rapidly 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 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 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 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). Physically effective NDF (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 TCA 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 TCA, 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 ($P > 0.05$). In contrast, percent of large particles (> 19.0 mm) significantly ($P \leq 0.05$) increased and resulted in linear increases of TCA. Although we originally believed that trends in TCA 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 TCA influence rumen pH. Even though less chewing activity of shortest rations along with higher acid production resulted in presumably less salivary secretion and lower mean rumen pH, highest chewing activity was 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).

Because saliva contains important buffers for the rumen environment it is believed that a diets ability 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 TCA 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 TCA on rumen pH is often overestimated. Although reduced particle size may decrease TCA, 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. As a consequence, ration NSC level may have a larger effect on pH and that management of ration NSC may be more useful in identifying rations leading to either clinical or sub-clinical acidosis which cause depressions in intake and production. Furthermore, our data suggests that the proportion of NDF $\geq$ 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 TCA 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 it is more likely to see a depression in milk fat 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 μeq/L across treatments. It is possible that milk production may have in part limited due to the cost metabolizing excess RDP, but more likely due to inadequate RUP in the ration (Schwab, 2002). This suggestion is further supported by the observation that diets consumed in least amounts would result in less RUP reaching the small intestine and lower milk protein levels as observed in the experiment.

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 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 this study suggests that although peNDF did not change with forage particle size a level of 27.2 is adequate to maintain rumen health and milk fat percentage for cows in early lactation. These results are consistent with the suggestion of Mertens, (1996) that a peNDF of 22.3 is required to maintain a rumen pH of 6.0.

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 feed producing milk with 0.08% more milk protein and without affecting percent milk fat.

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Table 4-1. 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	.

[1] TNC = Total nonstructural carbohydrates (Smith, 1981)
[2] NFC = nonfiber carbohydrate calculated by difference 100 – (%NDF + % CP + % Fat + % ASH)

Table 4-2. 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)

Table 4-3. 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

[1] Contained 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, 1222, 449.0 mg/lg manganese, 15.8 % sulfur, 12, 2450.0 mg/kg zinc.
[2] Contained 28,792.5K IU/kg vitamin A, 7,198.5 KIU/kg vitamin D, 179,959.6 IU/kg vitamin E.
[3] TNC = Total nonstructural carbohydrates (Smith, 1981)
[4] NFC = nonfiber carbohydrate calculated by difference 100 – (%NDF + % CP + % Fat + % ASH)

Table 4-4. 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]	27.2	27.7	27.9	28.1	0.64	0.53

[1] SH = 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.

[2] means in the same row with different letters differ ($P \leq 0.05$)

[3] X_{gm} = geometric mean length as calculated by the ASAE (2001)

[4] S_{gm} = standard deviation as calculated by ASAE (2001)

[5] peNDF = physically effective NDF

$= (\% DM_{>19.0\ mm} \times \% NDF_{>19.0\ mm}) + (\% DM_{>19.0\ mm} \times \% NDF_{>19.0\ mm}) + (\% DM_{>19.0\ mm} \times \% NDF_{>19.0\ mm})/100$

Table 4-5. 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

[1] SH = 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.

[2] BWCH = body weight change

Table 4-6. 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
TCA[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
TCA[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
TCA[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	1090	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	1213	1476	1470	1460	77	0.07	0.24	0.51
Chews/Kg NDFI								
Eating	841.6	908.6	1138	1066	141	0.11	0.50	0.36
Ruminating	3,883	4,690	4,669	4,666	245	0.03	0.07	0.36

[1] SH = 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.
[2]TCA = Total chewing activity (minutes eating + minutes ruminating)

Table 4-7. 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								
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								
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								
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

[1] SH = 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.
[2] X_{gm} = geometric mean length as calculated by the ASAE (2001)
[3] S_{gm} = standard deviation as calculated by ASAE (2001)

Table 4-8. The effect of reducing alfalfa haylage particle size on nutrient digestibility, calculated energy values, rumen fill and passage rate for four diets containing alfalfa haylage of different particle size on 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^{-1}	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

[1] SH = 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.
[2] NFC = nonfiber carbohydrate calculated by difference
[3] TDN (%) = tdNFC + tdCP + (tdEE X 2.25) + tdNDF; where td denotes truly digestible fraction
[4] NE_{Lp} (Mcal/kg) = 0.703 X ME_p (Mcal/kg)
[5] Manual empty

Table 4-9. 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_3N (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

[1] SH = 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 4-10. 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

[1] SH = 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.

Figure 4-1. Effects of reducing alfalfa haylage particle size on daily rumen pH and NH_3-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 (* $P \leq 0.05$; ** $P \leq 0.10$).