

Effect of Alfalfa Hay Particle Size and Water Addition to Barely Base Diets on Dairy Cows Performance in Early Lactation

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Abstract: A study was conducted to evaluate the effect of two levels of hay particle size and diet DM on dairy cows performance in barley base diets. Eight cows in early lactation stage were allocated to four dietary treatments in a change over design with factorial arrangement of 2×2 including two levels of alfalfa particle size (5 and 20 mm) and two levels of TMR dry matter (without and with adding water up to 50% DM). Diets were similar in net energy content and chemical composition. Water addition resulted in reduction in percentage of diet particles in the pan and increasing physical effective factor (pef) in both levels of alfalfa particle size ($p < 0.01$). Experimental treatments had no effect on milk production although a trend of milk fat reduction was observed with reduction of the hay particle size ($p = 0.06$). Milk protein, Lactose and SNF content of the produced milk were not influenced by the treatments. Milk production efficiency was showed a trend to change with diet DM changes ($p = 0.06$). Blood pH and bicarbonate content, NH₃-N concentrations and fecal pH were not affected by the treatments but water addition resulted in significant reduction in ruminal pH ($p = 0.02$). TMR particle size had no effect on eating time, but in the wet diets a significant reduction in eating time of peNDF (min kg^{-1}) ($p = 0.02$) and eating time of NDF (min kg^{-1}) ($p = 0.05$) was found. Conversely hay particle size reduction significantly decreased rumination time when expressed as min day^{-1} ($p = 0.02$) and min kg^{-1} of diet NDF ($p < 0.01$). Treatments had no effect on feces particle size, body weight, DMI and NDF intake by the cows.

Key words: Hay particle size, diet dry matter, rumination, physical effective factor (pef)

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/propionate ratio and milk fat levels (Kononoff and Heinrichs, 2003). Although impaired rumen fermentation and function can result when cattle are fed ration lacking in physical structure. Excessive amounts of long coarse fiber may also limit intake and digestibility and ultimately energy balance of the animal (Allen, 2000).

It is well understood that sufficient amounts of coarse fiber is necessary to maintain proper rumen fermentation and function (Yang *et al.*, 2001). Physical effective NDF (peNDF) is believed to be that portion a diet that stimulates chewing activity and results in formation of the rumen mat. The Total Chewing (TC) expressed as minutes per kg of NDF Intake (NDFI) has

been used as a measure for peNDF. As particle size of a ration is increased, its peNDF content also increase and resulting in elevated in TC, salivary buffer secretion and ruminal pH (Mertens, 2000).

Any recommendation of minimum dietary fiber or particle size requirements, such as NRC data (2001) assumed that an animal ingests a diet similar to the diet offered. However, it has been recently shown that lactating dairy cattle ingest a diet that could differ from the offered diet (Leonardi and Armentano, 2003). Selective consumption (sorting) of a diet by cow is the key reason for this difference. There are several techniques to minimize the sorting of diet by dairy cow including frequent diet pushups, water addition or feeding a low dry matter by-product and applying an appropriate forage processing (Stone, 2004).

The objective of this study, thus was to evaluate the relationship between the sorting of diet and performance of dairy cow.

MATERIALS AND METHODS

Animals and diets: This experiment was conducted at the Ferdowsi University of Mashhad Dairy Research Farm. Eight multiparous Holstein cows in early lactation period were allocated to the treatments in a change-over design with periods of 21 days. The average DIM and milk production of the cows were 28±12 (mean±SD) days and 43±3.5 kg d⁻¹, respectively. Cows were housed individually in a tie-stall and fed ad-libitum twice daily and had free access to drinking water. The balanced diets (according to NRC, 2001 recommendation) had the same chemical composition (Table 1). Two particle size of Alfalfa hay (5 and 20 mm theoretical cutting size) and two levels of TMR dry matter (without and with water addition up to 50% of DM) were applied in the treatments. Water was sprinkled to diet during every day diet preparation. The amount of feed offered was adjusted daily to obtain around 10% orts (as fed basis).

Sampling and analyses: Diets were adjusted weekly to account for silage DM fluctuation. Feed, orts and feces samples were collected during the last week of each period. Particle size distribution of forages, TMR and orts was determined by the American Society of Agricultural Engineers (ASAE, 1992) sieves and reported on a 60°C DM basis.

Feed samples were dried at 60°C for 48 h, grounded to pass through a 1 mm screen and analyzed for DM, OM, CP, NDF and ADF (AOAC, 1990). Orts were sampled during the last week of each period and composited by animal in proportion to the wet weight of orts in each period.

Feces samples were wet-sieved to obtain estimates of the particle size distribution. The samples (300 g) were stirred into 5 L of water and then applied to the top sieve to effect a preliminary separation and to prevent mat formation, 50 L of sprinkled water was used in the collection of particles passing through the sieves. The screens used for wet sieving were 6, 4, 2, 1.18, 0.5, 0.25, 0.1 and 0.05 mm apertures (20 mm diameter, 8 mm depth). The amount of dry matter passed through the 0.05 screen was calculated from the difference between the total DM weight of feces and the total DM weight above the 0.05 mm screen. It was assumed that material smaller than 0.5 mm screen was soluble. Particle size distributions were expressed as a percentage of the total DM.

Chewing activities of the cows were monitored visually every 5 min for a 24 h period during the 14-15th days of experimental period. The assumption was made that the particular chewing activity persisted for the entire 5 min period between each visual observation. Chewing activities were expressed as total hours for the 24 h period or on the basis of DMI and NDF intake by dividing minutes of eating or ruminating by intake.

Table 1: Ingredients and chemical composition of the experimental diets

Feedstuffs	%DM
Alfalfa hay	20.0
Corn silage	15.0
Barely	31.0
Cottonseed	9.0
Soybean	12.0
Safflower	6.0
Wheat bam	3.0
Limestone	0.7
Salt	0.3
Vitamin- mineral premix	1.0
Protected fat ¹	2.0
Chemical composition	
DM, %	64.6
OM, % DM	91.3
CP, % DM	17.9
CP-RUP, % DM ²	4.9
CP-RDP, % DM ²	13.0
NDF, % DM	33.0
Forage NDF, % DM	17.2
NFC, % DM	39.2
EE, % DM	5.4
DCAD, meq	136.0
NE _L ² , Mcal/kg	1.62

¹Energizer RP-10. IFFCO. Malaysia SDN. BHD. Company No 485777-W. PLO 406-Jalan Emas, 81700, Pasir Gudang, Johor Malaysia. ²-values were estimated from NRC Tables (2001)

Blood samples were taken 4 h after morning feeding into the sodium-heparin tubes. They were placed in an ice water bath immediately and analyzed for pH and blood gasses exchanges (Radiometer ABL50).

Statistical analysis: Data on all variables were analyzed using the mixed model procedure of SAS (1998). The model used for forage particle size and diet DM was as follows:

$$Y_{ijkl} = \mu + P_i + C_j + A_k + B_l + (AB)_{kl} + e_{ijkl}$$

Where,

- μ = Overall mean.
- P_i = Effect of period (i = 1-4).
- C_j = Effect of cow (j = 1-8).
- A_k = Fixed effect of forage particle size (k = 1-2).
- B_l = Fixed effect of TMR DM (l = 1-2).
- A×B_{kl} = Fixed effect of interaction of A_k and B_l.
- e_{ijkl} = Random residual error, assumed to be normally distributed.

The effect of cow and period considered random. Significance was declared at p = 0.05. A trend was considered to exist if 0.05 < p = 0.1.

RESULTS AND DISCUSSION

Particle size distribution of experimental diets is shown in Table 2. Alfalfa hay particle size reduction

Table 2: TMR particle size distribution (%)

Screen size Mm	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
19	1.13	2.57	0.59	0.44	0.18	<0.01	0.02	<0.01
12.7	3.24	2.71	0.96	1.27	0.27	<0.01	0.20	<0.01
6.3	24.51	21.68	18.96	18.27	0.56	<0.01	0.70	<0.01
3.96	11.37	13.52	10.72	13.65	0.46	0.07	0.03	0.30
1.18	42.36	46.59	46.62	52.62	0.56	<0.01	<0.01	ns
Pan	17.53	13.11	22.32	13.94	0.51	<0.01	<0.01	0.02
GMP ²	2.84	2.87	2.32	2.65	0.05	<0.01	0.02	0.03
Pef ³	82.60	87.08	77.85	86.26	0.51	<0.01	<0.01	0.02
peNDF _{1.18} ⁴	31.39	33.96	30.36	35.29	2.15	0.41	0.48	0.65

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of Particle Size and Dry Mater (PS×DM), ²-GMP: Geometrical Mean Particle size, ³-Physical effectiveness factor determined as the cumulative proportion of particles (DM basis) retained by ASAE sieves above pan, ⁴-Physically effective NDF (peNDF) estimated as the NDF content of TMR multiplied by pef

Table 3: Milk production and composition

	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
Milk yield (kg d ⁻¹)	44.45	44.36	40.93	42.27	3.25	0.58	0.56	0.12
3.5% FCM (kg d ⁻¹)	40.34	41.95	37.45	39.16	2.95	0.09	0.81	0.51
Fat (%)	3.46	3.65	3.51	3.51	0.14	0.61	0.26	0.90
Fat (g d ⁻¹)	1540.8	1614.1	1404.1	1485	113.9	0.06	0.80	0.52
Protein (%)	3.08	3.07	3.07	3.08	0.02	0.52	0.56	0.29
Protein (g d ⁻¹)	1362.7	1367.9	1253	1314	102	0.13	0.66	0.62
Lactose (%)	4.59	4.58	4.61	4.6	0.03	0.87	0.43	0.64
SNF (%)	8.4	8.38	8.4	8.37	0.05	0.52	0.63	0.35
Milk/DMI	1.88	1.86	1.74	1.89	0.07	0.36	0.06	0.20

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of Particle Size and Dry Mater (PS×DM)

resulted in significant changes in TMR particle size distribution. Water addition led to significant changes in the TMR particle size distribution as well (Table 2). This was probably the result of attaching smaller particles to the coarser particles of diets.

Leonardi *et al.* (2005) suggested that water addition to dry diets appears to be a cost-effective management practice that could be implemented on dairy farms to reduce the adverse effect of sorting. They concluded that feeding dry diets caused cows to avoid consuming long particles. The inclusion of water to a dry diet partially reduced sorting however, sorting of longer particles varied across animals even when water was added (Leonardi *et al.*, 2005). In the present study, water addition to the diet reduced consumption from the pan especially in the diets with coarse hay but selective consumption from upper sieves were increased.

Milk production and composition: Milk production was not affected by the treatments (Table 3). However, TMR particle size reduction showed a trend to decrease 3.5% FCM production. Leonardi *et al.* (2005) found no effect of water adding to the diet on milk yield.

Particle size reduction of diet had no effect on milk fat percentage or fat yield although a trend of milk fat reduction was observed (p = 0.06) (Table 3). It was surprising that no reduction in milk fat percentage was observed when forage particle size was reduced

considering the consistent response to reducing forage particle size found in other studies (Woodford *et al.*, 1986; Woodford and Murphy, 1988) and the significant reduction in time spent chewing when forage particle size was reduced (Krause *et al.*, 2002). Milk fat percentage is the animal response that often is associated with effective NDF (eNDF) content of a ration (Mertens, 1997). The NDF content of the diet in the present study was higher (33%) than the minimum (25%) recommendation of NRC (2001) but the proportion of forage NDF in the diets (17.2) was less than the recommended level of 19%. Beauchemin *et al.* (1994) and Mertens (1997) concluded that effects of particle size on milk fat content were likely to be observed when NDF levels below minimum requirements recommendation by NRC (1989). Based on the milk fat percentages in this study and the lack of diet effect on milk fat percentage it can be concluded that all 4 diets provided adequate amount of eNDF to sustain milk fat percentage.

Rumen, blood and fecal measurements: Particle size of diet had no effect on rumen pH but adding water to the TMR resulted in significant reduction in rumen pH (Table 4). Some part of this reduction can be explained by reduction in eating time per kg of NDF and peNDF_{1.18} and consequently decrease of saliva secretion. Because saliva contains important buffers for the rumen environment, it is believed that the ability of a diet to

Table 4: Ruminal, blood and fecal measurements

	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
pH								
Rumen	6.22	5.93	6.18	5.96	0.08	0.61	0.02	0.35
Blood	7.26	7.27	7.26	7.28	0.01	0.93	0.28	0.83
Feces	6.80	6.80	6.78	6.78	0.08	0.83	0.80	0.41
Blood bicarbonate (mmol L ⁻¹)	20.9	21.1	21.1	21.57	0.92	0.32	0.19	0.33
Rumen N-NH ₃ (mg dL ⁻¹)	18.1	18.3	19.1	17.2	1.82	0.88	0.33	0.34

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of particle size and dry mater (PS×DM)

Table 5: Time spent for eating, ruminating and total chewing

	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
Eating time								
min d ⁻¹	350.6	343.8	346.3	321.4	12.36	0.31	0.23	0.37
min kg ⁻¹ DM	14.96	14.52	14.84	14.45	0.82	0.19	0.17	0.55
min kg ⁻¹ total NDF	39.37	37.24	38.04	35.25	2.16	0.06	0.05	0.43
min kg ⁻¹ peNDF _{1.18}	47.57	42.83	48.84	44.07	2.73	0.43	0.02	0.52
Ruminating time								
min d ⁻¹	465.5	463.3	401.3	413.8	22.89	0.02	0.92	0.85
min kg ⁻¹ DM	20.06	19.52	17.24	18.63	0.99	0.06	0.84	0.59
min kg ⁻¹ total NDF	52.77	50.06	44.19	45.44	3.19	<0.01	0.27	0.97
min kg ⁻¹ peNDF _{1.18}	63.76	57.58	56.74	56.82	3.97	0.06	0.11	0.90
Total chewing time								
min d ⁻¹	816.3	806.9	747.5	735.0	50.98	0.03	0.25	0.46
min kg ⁻¹ DM	35.07	34.04	32.07	33.08	1.87	0.03	0.28	0.83
min kg ⁻¹ total NDF	92.14	87.29	82.24	80.69	4.71	<0.01	0.09	0.69
min kg ⁻¹ peNDF _{1.18}	111.3	100.4	105.6	100.9	5.93	0.09	0.03	0.82

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of particle size and dry mater (PS×DM)

stimulate chewing activity is critical for regulation of ruminal pH levels (Kononoff and Heinrichs, 2003). Beauchemin *et al.* (2003) showed that the quality of the bolus in terms of lubrication and ease of swallowing may be more a function of free moisture than total moisture content. Thus a smaller amount of saliva is able to lubricate a coarse forage than a fine forage. Kononoff and Heinrichs (2003) suggested that ration particle size may result in large effects on Total Chewing time (TC) however, only small changes in rumen pH were observed. 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).

The other part of rumen pH reduction can be attributed to attachment of diet fine particles (mainly rumen degradable organic matter) to the coarser parts and their more utilization few hours after feeding by the cows. Allen (1997) noted that variation of Rumen Degradable Organic Matter (RDOM) may have a greater effect than particle size on the variation of pH. Furthermore, Kononoff and Heinrichs (2003) suggested 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.

Blood pH and bicarbonate-as an internal buffer-were not affected by treatments. Also treatments had no effect on fecal pH and rumen ammonia nitrogen production. Yang *et al.* (2001), Beauchemin *et al.* (2003) and Kononoff and Heinrichs (2003) found no effect on rumen ammonia when forage particle size was reduced. As a result it is believed that differences in protein contents between diets, rather than differences in dietary particle size were responsible for differences in rumen ammonia content (Einarson *et al.*, 2004).

Chewing activities: Chewing activities are reported in Table 5. Cows spent 5.4-5.8 h for eating daily, which is within the normal range for cows consuming 4-6 kg of NDF per day (Beauchemin, 1991). Hay particle size or TMR dry matter did not affect the eating time either expressed as min d⁻¹ or min kg⁻¹ DM but when expressed as min kg⁻¹ total NDF and min kg⁻¹ peNDF_{1.18} adding of water to TMR reduced eating time significantly (Table 5). Diet particle size reduction also tended to reduce eating time when expressed as the min kg⁻¹ total NDF (p = 0.6). Water addition to the diet resulted in significant reduction in eating time when expressed as the min kg⁻¹ NDF (p = 0.05) and min kg⁻¹ peNDF_{1.18} (p = 0.02). Krause *et al.* (2002) reported that eating time was higher for Dry Corn (DC) diet than for High Moisture Corn (HMC) diet due to the higher DM content of DC in comparison with HMC.

Table 6: Body weight changes and dry matter intake of the experimental cows

	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
BW(kg)	611.6	614.5	608.6	609.3	41.5	0.40	0.30	0.27
BW changes (kg d ⁻¹)	0.66	0.67	0.72	0.73	0.15	0.60	0.73	0.34
DMI (kg d ⁻¹)	23.64	23.80	23.48	22.34	0.50	0.61	0.53	0.22
DMI (% of BW)	3.87	3.90	3.86	3.68	0.18	0.20	0.40	0.33
NDF intake (kg d ⁻¹)	8.98	9.28	9.16	9.16	0.59	0.58	0.54	0.29
NDF intake (% of BW)	1.47	1.52	1.51	1.51	0.04	0.40	0.15	0.73

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of Particle Size and Dry Mater (PS×DM)

Table 7: Feces particle size distribution (%DM)

Screen size (mm)	Long hay		Short hay		SEM	Effect ¹		
	Dry	Wet	Dry	Wet		PS	DM	PS×DM
6	3.14	4.45	4.39	3.52	0.87	0.95	0.90	0.21
4	7.36	6.95	5.04	4.53	1.51	0.33	0.81	0.71
2	19.26	20.51	26.62	23.55	2.14	0.19	0.69	0.86
1.18	4.17	4.46	5.62	3.93	0.56	0.37	0.27	0.10
0.5	13.27	14.62	14.89	13.35	0.69	0.88	0.81	0.40
0.2	6.70	6.95	7.93	7.19	0.5	0.18	0.59	0.3
0.1	4.56	4.78	4.67	4.41	0.28	0.63	0.91	0.39
0.05	3.86	4.09	3.99	3.89	0.26	0.88	0.81	0.56
Soluble part	37.68	33.18	30.85	35.63	2.75	0.51	0.87	0.07

¹-Probability of the main effect of hay Particle Size (PS), TMR Dry Mater (DM) and the interaction of particle size and dry mater (PS×DM)

Time spent ruminating ranged from 6.6-7.7 h d⁻¹ (Table 5). Time spent ruminating per day increased when forage particle size was increased. When expressed per kg of NDF intake per day, increasing forage particle size increased time spent ruminating but diet DM had no effect on rumination time. Ruminating time for all diets were in normal range. High producing dairy cows consuming large quantities of DM tended to ruminate more than 6 h daily, except when digestive upset occurred (Beauchemin *et al.*, 1994). This is equivalent to a minimum of 16 min kg⁻¹ of DM for 22 kg d⁻¹ of DMI. (Beauchemin *et al.*, 2003).

Reducing alfalfa hay particle size resulted in decreased chewing activity per unit of DM and NDF consumed and it is similar to the results reported by Beauchemin *et al.* (1994) and Grant *et al.* (1998). Chewing activity is the primary responsible mechanism for reducing feed particle size and is central to both the nature of digestion and passage through the gastrointestinal tract (Kononoff and Heinrichs, 2003). Sudweeks *et al.* (1981) reported that the minimum time required for chewing per kg of DMI in order to producing a milk with butter fat content of 3.5 % was 31 min for cows producing 19-20 kg milk d⁻¹. However, the minimum time spent chewing per kg of DMI required for a certain milk fat percentage depends on the total DMI (Sudweeks *et al.*, 1981).

Body weight, DMI and NDF intake: Treatments had no effect on BW and BW changes. Also DMI and NDF intake were not changed by changes in particle size and diet DM (Table 6).

Failure to observe an effect of particle length on intake of DM and NDF is in agreement with some other observations (Soita *et al.*, 2000; Beauchemin *et al.*, 2003; Calberry *et al.*, 2003; Yang and Beauchemin, 2006) but in contrast with other studies in which positive effects on DMI and NDF intake from the reduced particle size had been reported (Kononoff *et al.*, 2003; Einarson *et al.*, 2004). There may be an interaction of diet particle size with forage-to-concentrate ratio on DMI. When high forage diets are fed to lactating dairy cows, a reduction of particle size that results in higher particulate passage rate from the rumen would allow for a greater feed intake (Allen, 2000). But for high concentrate diets (>50%) similar to those used in the present study, a metabolic rather than physical, constraint on feed intake is expected to be rate limiting (Allen, 2000).

Feces particle size distribution: Fecal particle size distribution is shown in Table 7. There was no difference in fecal particle size distribution between the treatments. Other experiments have indicated that after the particles have left the rumen, almost no further reduction in particle size takes place (Poppi *et al.*, 1980). The mean size of the particles leaving the rumen can thus be measured in fecal samples. Kennedy and Poppi (1984) defined the Critical Particle Size (CPS) as the nominal sieve aperture that would retain the top 5% of fecal particulate DM, being 1.18 mm for cattle. Oshita *et al.* (2004) reported that CPS was greater than 1.18 and it was about 2 mm in their experiment. In the present study, the proportions of fecal DM retained on 4-mm screen were near to 5%. These results are in agreement with results of Shaver *et al.* (1988)

and Teller *et al.* (1990) who indicated that dairy cows or steers passed coarser DM from the rumen than the previously been reported for sheep and cattle.

Our results showed that the different particle size of the forage as well as DM content of the diet had no effect on digesta particle size after omasum. Therefore, increasing alfalfa hay particle size led to increasing chewing time hence, the fecal particle size distribution did not differ between the treatments.

CONCLUSION

Decreasing forage particle size in the diet had no effect on ruminal pH and milk fat percentage although rumination time was reduced ($p = 0.02$) and a decreasing trend for milk fat production (kg d^{-1}) observed. Moreover, water addition to the TMR decreased rumen pH, however, pH was in the physiological range for a healthy rumen and normal fermentation.

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