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Full Length Research Paper

Effect of corn silage particle size and level of soybean oil on ruminal mat composition, distribution and consistency in Zel sheep

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To determine the effects of two corn silage particle size (coarse particle with geometric mean of 5.83 ± 2.47 mm and fine particle with geometric mean of 4.74 ± 2.74 mm) and two levels of soybean oil (0 and 4% of DM) on ruminal mat composition, distribution and consistency, four two years fistulated ruminant Zel ewes (BW = 34 ± 1.4 kg), fed with 50:50 roughage-concentrate diet, were allocated to a 4 × 4 change over design. The experiment was carried out in 4 periods of 28-days trial (adaptation, 14 days; collection period, 5 days; chewing activity measurement, 1 day; rumen evacuation, 8 days). Experimental diets were: 1) basal diet + coarse corn silage and 0% oil; 2) basal diet + coarse corn silage and 4% oil; 3) basal diet + fine corn silage and 0% oil and; 4) basal diet + fine corn silage and 4% oil. Based on calendar time, rumens were evacuated manually at 3, 7.5 and 12 h post-feeding of each period and total ruminal contents were separated into mat and liquids phase. Dry matter (DM) and organic matter (OM) intake were less in treatment 4 and neutral detergent fiber (NDF) intake in treatment 2 was higher than in the other diets. Physically, effective NDF intakes were higher in treatments 1 and 2 than in treatments 3 and 4, but DM and OM digestibility was higher in treatments 1 than in the other diets. The NDF digestibility was higher in coarse silage diets, but the NFC digestibilities were higher in fine corn silage diets. Rumen mat was greater in coarse silage diets than in fine silage diets (1.83 and 1.32 versus 1.06 and 0.76 kg) at 3 h after feeding. Non-escapable pool sizes in rumen content were higher in diets that contained coarse silage. At all time in post feeding, ruminal pH was less in fine silage particle size with oil supplement. Rumen particulate passage rates were higher in fine corn silage diets. However, passage rates from the lower gastrointestinal tract were similar. In addition, ruminal mean retention times were significantly greater in diets that contained coarse silage. Rumination time was shorter in fine corn silage with oil supplement diet but rumination time per kg of NDF and peNDF was longer in fine corn silage diets. In addition, although reduction of particle size decreased size of rumen mat and non-escapable fraction, oil supplementation reduced ruminal digestion and mean retention of ruminal particulate. However, it seems that oil supplementation can be helpful in fine corn silage diets for maintaining ruminal mat.

Key words: Particle size, soybean oil, ruminal mat, Zel sheep.

INTRODUCTION

Forages as the main source of energy and nutrients in ruminants can maintain optimum rumen function (Clark and Armentano, 2002). Thus, it is necessary to add sufficient and 'long' fibers in their rations to provide energy and to prevent some abnormalities such as acute acidosis, lameness and milk fat depression. The coarse fiber of forages is believed to be effective in stimulating chewing activity, salivary secretion, buffering ruminal acid

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production, and ultimately increasing rumen pH, rumen acetate production and milk fat content. In addition, particle size reduction of dry forage from 28.7 to 9.2 mm in the diet with low concentrate (13 %) increased DM intake (13%), but a ration containing high concentrate (40%) had no effect on feed intake of sheep (Mertens, 1997; Zebeli et al., 2006). Alfalfa macerated with fine corrugated rolls caused consumption and daily gain of sheep was increased from 5 to 25%, respectively (Savoie et al., 2001). However, with decreasing particle size of corn silage, together with reducing rumination and total chewing activity, pH also decreased (Lock and Bauman, 2007). At low pH in the rumen, DM intake, digestibility and microbial products were reduced (Allen, 1997). Therefore, ruminants need dietary forage with an adequate particle size to enhance digesta stratification in the reticulorumen, and consequently chewing activity, ruminal buffering and fiber degradation, as well as to prevent digestive disorders (NRC, 2001). Inadequate fiber levels or particle size negatively affect rumen digestion and may reduce the amount of energy that the ration provides.

Fat supplements reduce ruminal fiber digestion (Pantoja et al., 1994; Lewis et al., 1999) by inhibiting microbial fermentation in the rumen. Ikwuegbu and Sutton (1982) found that infusion of 0, 13, 26 and 40 ml oil per day into the rumen of sheep reduced fiber digestibilities in the rumen by 44, 28, 18 and 14%, respectively. Fiber components covered with fat prevent access to rumen microorganisms (Smith, 2000), decrease microbial attachment to fiber particles (biofilm is not formed), reduce fiber breakdown and fermentation (Jordan et al., 2006), and prevent reduction of particle size and particle passage from the rumen, making feed intake to diminish (Onetti and Grummer, 2004; Jenkins et al., 1998). These fat effects cause functional specific gravity, which is the most important factor affecting removal of particles from the rumen; they also cause less increase (Firkins et al., 1998), which prevents the passage of digested particles from the rumen (Jordan et al., 2006). Therefore, this research was designed to investigate the effects of two corn silage particles size and two levels of soybean oil consumption on intake and DM, OM, NDF, crude protein (CP), non-fibrous carbohydrate (NFC) and EE digestion, ruminal mat composition, distribution and consistency and chewing activity in sheep.

MATERIALS AND METHODS

This experiment was carried out in Ruminant Research Center of Sari Agricultural and Natural Resource University (SANRU), Sari, Iran. Four two years ruminal fistulated Zel ewes (BW = 34.3 ± 1.4 kg), individually housed in metabolism cages, were fed *ad libitum* with four diets containing a ratio (50:50) of forage concentrate (DM basis) with two corn silage particles size (coarse particle with 5.83 ± 2.47 and fine particle with 4.74 ± 2.74 mm geometric mean) and two levels of supplemental oil (0.0 and 4.0% of DM) using a 4 × 4 change over design at four periods of 28-days trial (adaptation, 14 days; collection period, 5 days; chewing activity measurement, 1 day; rumen evacuation, 8 days). Experimental diets were: 1) basal diets + coarse corn silage without oil supplement; 2) basal diets + coarse corn silage and 4% oil supplement; 3) basal diets + fine corn silage without oil supplement and 4) basal diets + fine corn silage and 4% oil supplement. Diets were delivered into two equal meals every 12, 8 and 20 h. Oil soluble vitamins were injected in each experimental period. The sheep had access to fresh water *ad libitum*.

Silage was harvested at 10 mm theoretical cut length. The chopped material was then placed in a concrete bunker, covered with black plastic, ensiled for approximately 40 days, and designated "long" forage. Every day during the experiment, silage was rechopped with designated short forage.

Body weights were taken weekly. Intake of TMR was measured daily for all sheep. Samples of diets were collected each day. During the collection period, total wet feces and feed refusals were weighed daily and 10% were subsampled at 08:00 h (days 14 to 20), dried at 55°C, and ground through a Wiley mill (1-mm screen). Feed, feces and orts were analyzed for DM, OM, Kjeldahl N, ether extract (AOAC, 2002), NDF, ADF (Van Soest et al., 1991) with amylase (Sigma A3306; Sigma–Aldrich, Germany); omitted sodium sulfite was used in the NDF procedure (aNDF), and NDF was not corrected for ash content and ash at 605°C. NFC was calculated using 100 − (%CP + %NDF + %ash + %ether extract) (NRC, 2001). Digestibility was calculated for DM, OM and all nutrients.

Feed particle size was determined by dry sieving. The Penn State Particle Separator (PSPS) sieves were used for measuring particle size distribution. The geometric mean and its standard deviation were calculated according to ASAE S424.1 (2002). The NDF content of all materials retained on PSPS sieves was measured (Van Soest et al., 1991). According to Mertens (1997), the physically effective factor (pef) was determined based on proportion of DM retained on the 1.18-mm sieve ($pef_{>1.18}$). By multiplying NDF content of the TMR and silage by the $pef_{>1.18}$, the $peNDF_{>1.18}$ was calculated.

Based on a timetable, the rumens were evacuated manually at 3, 7.5 and 12 h post-feeding and total ruminal contents were separated into mat and liquids phase (Huhtanen et al., 2007; Robinson et al., 1987); evacuation procedure did not interfere with normal rumen functions (Huhtanen et al., 2007). Particle sizes of feed digesta samples were determined using wet-sieving techniques (ASAE, 2002), with seven sieves, having pore sizes of 6.35, 4.75, 3.35, 1.70, 1.18, 0.8 and 0.5 mm, respectively. Materials retained on each sieve were dried for 72 h at 55°C for DM determination (Robinson et al., 1987). In addition, the particles of rumen content greater than 1.18 mm were considered as non-escapable rumen pool sizes of particulate and the particles of rumen content less than 1.18 mm were considered as escapable rumen pool sizes.

Cr-mordanated NDF of wheat straw was used to estimate the passage rate of solid phase in the rumen and lower parts of the digestive system. A single dose of Cr-mordanated wheat straw samples were fed and rectal fecal grab samples were collected at 0, 18, 24, 30, 36, 42, 48, 60, 72, 90, 120 and 144 h, subsequent to single Cr dose (Uden et al., 1980). Fecal Cr excretion curves were fitted to the double compartment model represented by 2 exponential constants and a time delay (Grovum and Williams, 1973):

$$Y = Ae^{-k_1(t-TT)} + Ae^{-k_2(t-TT)},\ K_1 = K_2 \text{ for } t \geq T,\ Y = 0 \text{ for } t = TT$$

Where, Y = marker concentration (ppm), A = scale parameter, k1 = ruminal rate of passage (%/h), K_2 = lower digestive tract rate of passage (%/h), t = sampling time post dosing (h) and TT = transit time or time delay of marker. The TMRT was calculated as the sum of RMRT ($1/k_1$) and, in the lower digestive tract, mean retention time ($1/k_2$) plus the transit time (TT).

On day 20 of each period, ewes were visually monitored for chewing activity recording. Chewing activity was recorded in 5 min intervals assuming the cow activity persisted throughout that 5 min

Table 1. Ingredient and chemical composition of the four experimental diets differing in particles size of corn silage and levels of soy-bean oil.

Parameter	Forage particle size			
	Coarse		Fine	
	0	4% oil	0	4% oil
Ingredient (%DM)				
Ground barley grain	47.7	42.7	47.7	42.7
Corn silage	50.0	51.0	50.0	51.0
Supplemental soy-bean oil[1]	0.0	4.0	0.0	4.0
Salt	0.3	0.3	0.3	0.3
Mineral premix[2]	0.5	0.5	0.5	0.5
Dicalcium phosphate	0.7	0.7	0.7	0.7
Calcium carbonate	0.8	0.8	0.8	0.8
Chemical composition (%DM) and ME (Mcal/kg)				
Dry matter	56.8	56.6	57.0	56.9
Acid detergent fiber	19.9	19.4	19.9	19.4
Neutral detergent fiber	34.4	35.4	34.4	35.4
Crud protein	11.1	10.5	11.1	10.5
Ether extract	2.65	6.62	2.65	6.62
Non fiber carbohydrate	41.9	40.0	41.9	40.0
Ca	0.6	0.6	0.6	0.6
P	0.4	0.4	0.4	0.4
ME	2.75	2.92	2.75	2.92

[1]Fatty acid compositions of soy-bean oil were 0.07, 10.05, 6.21, 23.23, 52.86 and 6.65 for C14:0, C16:0, C18:0, C18:1, C18:2 and C18:3, respectively. [2]Mineral premix contained Mn 7%, Zn 5%, Fe 4%, Cu 0.3%, Mg 3%, sodium bicarbonate 10%, Co 0.00012%, I 0.0005% and Se 0.0001%.

Table 2. Particle sizes distribution of experimental diets using of the Penn State Particles Separator.

Parameter	Forage particle size				SEM	*P*-value
	Coarse		Fine			
	0	4% oil	0	4% oil		
Particle size distribution (%)						
19-mm	26.16[b]	36.14[a]	16.18[c]	11.59[c]	3.37	0.0049
8-mm	25.30	23.82	23.84	25.88	6.04	0.5702
1.18-mm	16.50[b]	16.53[b]	22.47[ab]	25.24[a]	4.60	0.5627
Pan	31.34[a]	22.99[b]	37.49[a]	39.45[a]	3.78	0.0052
Physical effectiveness factor[1]	68.96[a]	76.49[a]	62.51[b]	60.55[b]	2.78	0.0652
Physically effective NDF[2]	25.69[a]	26.39[a]	23.08[b]	21.03[b]	1.79	0.0023
Geometric mean (mm)	4.15[a]	4.83[a]	2.75[b]	2.51[b]	0.91	0.0027
Standard deviation of geometric mean (mm)	0.52[a]	0.46[a]	0.22[b]	0.12[b]	0.372	0.0275

[a, b, c]Means within a row with different superscripts differ ($P < 0.05$). [1]Calculated as cumulative proportion of particles retained on sieves of the Penn State Particles Separator. [2]Determined by multiplying the proportion of DM retained by the 19, 8 and 8-mm screens and the NDF content of each fraction of particles retained on the relative screen.

as described by Clark and Armentano (2002). Total time spent for chewing was calculated as the total time spent eating and ruminating.

The data of particle size were analyzed as a completely randomized design with model effects of forage and two methods of particle size measurement using the REML variance component and PROC MIXED procedure of SAS® (1998) (Table 1). Mean separation was determined using the PDIFF procedure and significance was declared at $P < 0.05$. Using PROC MIXED procedure of SAS® (1998), data were analyzed using the following statistical model: $Y_{(i)k(l)n} = \mu + T_i + S_j + ewe_{k(j)} + period_{j(l)} + e_{(i)j\,k(l)n}$; where, Y_{ijkl} is the dependent variable, μ is the overall mean, T_i is effect of treatment, S_j is random effect of square, $ewe_{k(j)}$ is random effect of ewe, $period_{j(l)}$ is effect of period and $e_{(i)j\ k(l)n}$ is experimental error. Significance was declared at $p < 0.05$.

RESULTS

Ingredients chemical compositions and particle size distributions of the four total mixed rations are presented in Tables 1 and 2, respectively. Percentage of DM that remained on sieve (19-mm) for coarse corn silage diets was higher than that of fine corn silage diets ($p < 0.005$). The diets were not significantly different in the percentage of DM remaining on the sieve of 8, 1.18-mm and pan. The diets with coarse corn silage had greater pef and peNDF than fine corn silage diets. The geometric means were 5.83 and 4.74 mm for coarse and fine corn silage diets, respectively ($P = 0.0275$). The standard deviations of geometric mean for rations that contained fine corn silage were significantly lower than that of rations that contained coarse corn silage (Table 2). There were significant differences among treatment for intake of DM ($P = 0.0185$), OM ($P = 0.0032$), fat ($P = 0.0029$), NDF ($P = 0.0240$) and peNDF ($P = 0.0473$). In coarse corn silage diets, DM and OM intake was increased by adding soy-bean oil supplement, but in fine corn silage diet, DM and OM intake was decreased by adding soy-bean oil. In fact, in fine corn silage diet with supplemental soy-bean oil, the numerical value of DMI was less than that of other diets. In addition, there were significant differences among treatment for digestibility of DM ($P = 0.0284$), OM ($P = 0.0247$), NDF ($P = 0.0308$) and peNDF ($P = 0.0322$). Reduction of corn silage particle size and soy-bean oil supplementation reduced DM and OM digestibility of the diets. In fine corn silage diets with supplemental soy-bean oil, DM and OM digestibility was lower than that of the other diets because of negative effect of oil supplement and reduction of forage particle size ($p < 0.0684$ and < 0.0747, respectively). The results of this study also show that the construction of 4% of soybean oil supplements, DM and OM digestibility of corn silage in the fine corn silage diet decreased more than coarse corn silage diets. Also, the values of NFC digestibility were higher in diets containing supplemental fat ($p < 0.0322$).

Ruminal contents, particle distribution and pH in ewe are presented in Table 4. The ruminal pH was not different at 3 and 12 h after feed intake, but at 7.5 h after feeding, the values of pH were significantly different ($P = 0.0037$). The pH of rumen content at 7.5 h after feeding was higher in coarse corn silage diets than in the fine corn silage diets (Table 4). In coarse corn silage diets, supplementation of oil reduced rumen pH at 7.5 h after feeding without significant effect on fine corn silage diets.

Crude protein, ash and NDF were similar among treatments at 3, 7.5 and 12 h after feeding. In addition, the EE of rumen contents was higher in diets supplemented with oil at 3 and 12 h after diets consumption; however, was similar among treatments at 7.5 h after diets consumption. The NFC content was higher in the rumen containing coarse corn silage at 3, 7.5 and 12 h after feeding than the fine corn silage diets.

The geometric mean of particle size of rumen content was higher in coarse than the fine corn silage diets at 3 h after consumption ($p < 0.0002$). However, there were no differences between the diets containing fine and coarse corn silage and also between diets containing supplemental fat and without it at 7.5 and 12 h after feeding.

The rumen mat size was decreased by reducing the non-escapable rumen pool sizes, increasing escapable rumen pool sizes in fine corn silage diets 3 h after feed intake ($p < 0.1140$) and increasing the solids passage rate in rumen (the solid rumen passage rate was higher numerically in fine corn silage diets, $p < 0.1033$). Increasing the solid passage rate led to reduction of DM, OM and NDF digestibility in fine corn silage diets.

Non-escapable and escapable rumen pool sizes were significantly different among treatments only at 3 h after consumption. The non-escapable rumen pool sizes were higher in coarse silage diets 3 h after feeding ($P<0.0502$). In contrast, escapable rumen pool sizes were higher in fine corn silage diets ($p < 0.0502$). However, the non-escapable and escapable rumen pools sizes were similar among treatments at 7.5 and 12 h after consumption (Table 4).

Parameters of kinetic for ruminal digestion in ewes are presented in Table 5. Ruminal particulate passage rate was significantly lower in diets with coarse particles compared to diets containing fine corn silage. The lower compartment passage rate was similar among treatments. Ruminal mean retention time was significantly higher in coarse diets than in fine diets; however, there was increase in oil supplemented fine diet compared to unsupplemented fine diets. Oil supplementation increased ruminal mean retention time of fine particulate. Mean retention times of lower compartment of both coarse diets were similar with that of fine oil supplemented diet. In addition, oil supplementation increased mean retention times of lower compartment of fine particulate. Marker lag time was also similar between treatments. Total retention time in both coarse diets was similar to that of fine oil supplemented diet. In addition, oil supplementation increased total mean retention times of fine particulate.

Numeric value of eating time was higher in coarse corn silage diets than in fine corn silage diets ($p<0.1037$). With this trend, the numerical value of eating per kg dry matter intake was higher in coarse corn silage diets ($p<0.1308$). Diets differences were significant in rumination activity ($p<0.0399$) and rumination time was lower in the fine corn silage diet with oil supplement than the other diets because of the lower feed intake (Table 3). Rumination time per kg DM, NDF and peNDF in fine corn silage diets was higher than that of coarse corn silage diets (Table 6, $p<0.0936$, $p<0.0336$, $p<0.0098$, respectively). This is because in fine corn silage diets, there was decreased digestibility due to the reduction of ruminal mat (3 h after

Table 3. Intake and digestibility of nutrients of four experimental diets.

Parameter	Forage particle size				SEM	P-value
	Coarse		Fine			
	0	4% oil	0	4% oil		
Intake (kg/day)						
Dry matter	0.985[ab]	1.091[a]	0.991[ab]	0.660[b]	0.21	0.0185
Organic matter	0.895[ab]	0.983[a]	0.926[ab]	0.608[b]	0.21	0.0032
Crude protein	0.106	0.114	0.095	0.066	0.03	0.1651
Ether extract	0.056[c]	0.133[a]	0.059[c]	0.095[b]	0.018	0.0029
Neutral detergent fiber	0.373[ab]	0.455[a]	0.340[b]	0.276[b]	0.06	0.0240
Physical effective NDF	0.253[a]	0.290 [a]	0.216[b]	0.154 [b]	0.06	0.0473
Non fiber carbohydrate	0.235	0.290	0.216	0.154	0.06	0.0673
Digestibility (%)						
Dry matter	0.783[a]	0.720[ab]	0.695[ab]	0.664[b]	0.05	0.0284
Organic matter	0.791[a]	0.732[ab]	0.719[ab]	0.694[b]	0.04	0.0247
Crud protein	0.686	0.607	0.622	0.678	0.12	0.7368
Ether extract	0.909	0.913	0.840	0.916	0.06	0.3063
Neutral detergent fiber	0.630[a]	0.584[ab]	0.473 [b]	0.491[b]	0.08	0.0308
Non fiber carbohydrate	0.968[ab]	0.984[a]	0.881[b]	0.999[a]	0.05	0.0322

[a, b ,c]Means within a row with different superscripts differ ($p < 0.05$).

feeding) and increased passage rate of the non-escapable rumen pool sizes to escapable rumen pool sizes (Table 4). For passage of particle from rumen, particle size was reduced by microbial digestion, which was compensated by increasing rumination activity.

DISCUSSION

Ingredients, chemical compositions and particle size distribution of the four experimental diets are presented in Tables 1 and 2, respectively. The Pef and peNDF for rations were significantly different among treatments. Diets containing coarse particle had significantly greater pef and ultimately peNDF than diets containing fine particle. Based on our hypothesis in this experiment, the differences between treatments that contained coarse and fine particle were as a result of differences in pef and peNDF. However, the differences between treatments containing 0 and 4% oil were as a result of oil supplementation.

In coarse corn silage diets, DM and OM intake was increased by adding soy-bean oil supplement, but in fine corn silage diet, DM and OM intake was decreased by adding soy-bean oil. There was an interaction between corn silage particles size and supplemental soy-bean oil levels. In fact, in fine corn silage diet with supplemental soy-bean oil, the numerical value of DMI was less than that of other diets. This is in contrast with the result of other researches that reported DMI on fine forage diets with supplemental oil, similar with coarse forage diets (Onetti and Grummer, 2004; Lewis et al., 1999; Jenkins et al., 1998). In the present study, by reducing corn silage particle size and oil supplementation, the numerical value of DM and OM digestibility was less than that of the other diets ($p < 0.0684$ and $p < 0.0747$). The NDF intake was higher in the coarse silage diets ($p < 0.0240$), which was different from the results of other researchers. Teimouri et al. (2004) and Kononoff et al. (2003) found that NDF consumption increased by reducing forage particle size due to increasing DMI, because they observed that DM and NDF intake increased when the geometric mean of diets particles reduced. In addition, the value of peNDF consumption was higher in coarse corn silage diets, which was consistent with the results of Beauchemin and Yang (2005) and Kononoff et al. (2003). Analyzing data from many experiments, Zebeli et al. (2006) found that peNDF can regress by 0.29 variations in DMI. Increasing forage particle length increased intake of peNDF, but decreased DMI. The physical fill could limit feed intake at low concentrate inclusion, but a metabolic rather than a physical constraint can be expected to be rate limiting at high concentrate inclusion. However, Beauchemin and Yang (2005) reported that reducing dietary peNDF>8 from 11.5 to 8.9% ration DM was not a rate-limiting step for particulate passage for rumen physical fill and feed intake in Holstein cows fed corn silage-based TMR with a high concentrate level (~60% of ration DM). Tafaj et al. (2007) reported that reducing dietary hay particle size from 28.7 to 9.2 mm increased DMI by 13% only at a low-concentrate level (13% in DM), but when a high-concentrate diet (~40% in DM) was fed, no differences were observed in sheep. In our study, sheep were fed *ad*

Table 4. Ruminal pH, contents, particle distribution, rumen mat, liquid size, non-escapable and escapable pool size.

Parameter	Forage particle size				SEM	P-value
	Coarse		Fine			
	Oil supplement level (%)					
	0	4	0	4		
3 hours post feeding						
Rumen content pH	5.56	5.87	5.77	5.59	0.18	0.1423
Crud protein (%DM)	12.75	11.12	11.7	12.20	1.48	0.5041
Ether extract (%DM)	4.56[b]	9.22[a]	4.39[b]	8.94[a]	1.76	0.0302
Ash (%DM)	7.80	8.17	7.92	7.52	0.54	0.4632
Neutral detergent fiber (%DM)	66.27	61.54	66.43	67.09	5.16	0.4600
Non-fiber carbohydrates (%DM)	8.22[ab]	9.51[a]	8.10[ab]	4.75[b]	2.70	0.1308
Geometric mean (mm)	4.87[a]	4.09[a]	2.50[b]	2.43[b]	0.60	0.0002
Standard deviation of geometric mean	1.22	1.19	1.11	1.06		
Rumen mat size (kg)	1.83[a]	1.68[ab]	1.06[b]	0.74[b]	0.27	0.0040
Liquid phase size (kg)	1.51	1.36	1.61	1.64	0.54	0.8772
Non escapable rumen pool sizes[4] (%DM)	63[a]	64[a]	50[ab]	47[b]	8.00	0.0502
Escapable rumen pool sizes[5] (%DM)	37[b]	37[b]	50[ab]	53[a]	8.00	0.0502
7.5 h post feeding						
Rumen content pH	6.44[a]	6.14[b]	5.86[c]	5.92[bc]	0.14	0.0037
Crud protein (%DM)	13.99	13.51	13.34	11.07	2.21	0.3291
Ether extract (%DM)	5.01	7.60	6.63	6.09	2.06	0.4210
Ash (%DM)	7.82	8.10	8.01	9.56	2.13	0.6581
Neutral detergent fiber (%DM)	68.41	68.17	69.59	71.76	4.82	0.5511
Non-fiber carbohydrates (%DM)	4.75[a]	2.50[ab]	2.43[ab]	1.52[b]	0.62	0.0765
Geometric mean (mm)	0.54	0.15	0.26	0.31	0.30	0.3786
Standard deviation of geometric mean	1.34	1.09	1.12	1.12		
Rumen mat size (kg)	1.18	1.07	1.18	1.06	0.21	0.0056
Liquid phase size (kg)	1.16	1.29	0.88	1.20	0.30	0.2840
Non escapable rumen pool sizes (%DM)	58	47	54	53	14.00	0.7652
Escapable rumen pool sizes (%DM)	43	53	46	47	14.00	0.7652
12 hours post feeding						
Rumen content pH	6.43	6.16	6.12	6.06	0.33	0.4740
Crud protein (%DM)	13.23	13.84	13.68	14.75	3.59	0.8009
Ether extract (%DM)	4.04[b]	7.76[a]	5.44[b]	9.40[a]	0.94	0.00431
Ash (% DM)	10.18	9.36	9.77	10.30	1.14	0.6656
Neutral detergent fiber (%DM)	69.05	65.01	70.12	66.93	7.79	0.7971
Non-fiber carbohydrates (%DM)	2.10[a]	0.87[b]	0.75[b]	0.43[b]	.0.24	0.0002
Geometric mean (mm)	0.19	0.11	0.19	0.04	0.25	0.7904
Standard deviation of geometric mean	1.19	1.07	1.04	1.14		
Rumen mat size (kg)	1.19[b]	1.29[a]	0.88[c]	1.20[b]	0.05	0.3207
Liquid phase size (kg)	1.23	1.93	1.10	1.47	0.78	0.5272
Non escapable rumen pool sizes (%DM)	58	47	54	53	14.00	0.7652
Escapable rumen pool sizes (%DM)	43	53	46	47	14.00	0.7652

[a, b, c]Means within a row with different superscripts differ ($p < 0.05$).

libitum with four diets containing a ratio (50:50) of forage concentrate with two corn silage particles size. Possibly, lower DM and OM digestibility, toxic effects of supplemented oil on protozoa (Giger-Reverdin et al., 2003) and the rapid degradation of starch cause undesirable conditions in the rumen (Hobson and Stewart, 1997), which re-

Table 5. Kinetic of ruminal digestion in ewe fed two different particle size levels of corn silage and two levels of soy-bean oil diets at voluntary feed intake levels.

Parameter	Forage particle size				SEM	*P*-value
	Coarse		Fine			
	Items Oil supplement level (%)					
	0	4	0	4		
Ruminal particulate passage rate (% of DM/h)	3.42[b]	3.35[b]	4.94[a]	4.35[a]	0.341	0.0054
Lower compartment passage rate (% of DM/h)	3.07	3.19	3.52	3.1	0.942	0.3503
Ruminal mean retention time (h)	29.24[a]	29.85[a]	20.24[c]	22.99[b]	1.082	0.0432
Mean retention time of lower compartment (h)	32.58[a]	31.35[a]	28.41[b]	32.26[a]	1.441	0.0125
Marker lag time (h)	25.41	28.11	26.38	31.67	3.393	0.1912
Total retention time (h)	87.23[a]	89.31[a]	75.03[b]	86.92[a]	3.52	0.0004

[a, b, c]Means within a row with different superscripts differ ($p < 0.05$).

Table 6. Effects of two levels of corn silage particle size and two levels of soy-bean oil on chewing activity of fistulated ewes.

Parameter	Forage particle size				SEM[1]	*P*-value
	Coarse		Fine			
	Oil supplement level (%)					
	0	4	0	4		
Eating (min/day)	262.5[ab]	295.0[a]	232.5[ab]	201.25[b]	44.8	0.1037
Rumination (min/day)	338.8[ab]	313.8[ab]	396.3[a]	250.0	52.8	0.0399
Total chewing activity (min/day)	602.5[a]	598.8[b]	607.5[a]	533.8[ab]	47.3	0.0439
Eating time per different nutrients (min/kg)						
DMI	245.6[ab]	256.1[a]	148.1[b]	200.0[ab]	29.5	0.1308
NDF	641.7	717.4	402.5	563.8	89.2	0.1792
peNDF[2]	966.9	961.9	616.1	857.7	107.7	0.8232
Rumination per different nutrients (min/kg)						
DMI	351.6[ab]	285.6[b]	471.1 [a]	378.5[ab]	29.6	0.0936
NDF	908.8[bc]	720.8[c]	1285.0[a]	1128.5[ab]	145.8	0.0336
peNDF	1352.6[b]	1101.8[b]	1961.3[a]	1800.6[a]	178.6	0.0098
Total chewing activity per different nutrients (min/kg)						
DMI	597.3	544.5	652.8	732.8	91.0	0.5455
NDF	1550.5	1381.5	2027.0	2238.0	289.5	0.2285
peNDF	2326.0[ab]	2065.8[b]	3078.5[ab]	3477.5[a]	525.1	0.1104

[a, b, c]Means within a row with different superscripts differ ($P < 0.05$). 1 SEM = Standard error of means. [2]Physically effective NDF, estimated by multiplying the proportion of DM retained by the 19, 8 and 8-mm screens and the NDF content of each fraction of particles retained on the relative screen.

duces feed consumption.

In fine corn silage with supplemental soy-bean oil, DM, OM and NDF digestibility was lower than that of other diets because of the negative effect of oil supplement and reduction in forage particle size. Kononoff et al. (2003) found that DM digestibility decreased by reducing the particle size of corn silage. The results of Leupp et al. (2006) also showed that OM and DM digestibility was decreased by adding more than 4% fat. The result of this experiment also showed that DM and OM digestibility decreased in fine corn silage diet with soy-bean oil supplement more than coarse corn silage diets with soy-bean oil supplement. In addition, supplementation of 4% oil significantly decreased the DM, OM and NDF digestibility of fine corn silage diet in comparison with coarse corn silage diet supplemented with oil. In addition, oil supplementation reduced NDF digestibility because high doses of dietary fat cause fiber coating and had toxic effect on rumen microorganisms (Jenkins et al., 1998; Lewis et al., 1999; Lock and Bauman, 2007). Zebeli et al. (2006) found that four diets with two levels of fine and coarse corn silage and two levels of low and high concentrate had similar NDF digestibility in whole digestive tract. As digestibility is a function of competition between digestion rate and passage rate (Pasha et al., 1994), reduction of particle size increased ruminal particulate passage rate and significantly decreased ruminal mean retention time; therefore, the NDF digestibility for fine corn silage diets was significantly lower than that of coarse corn silage diets. In contrast, the value of NFC digestibility was higher in diets containing supplemental oil. Poly unsaturated fatty acids in soy-bean oil are toxic for protozoa and Gram positive bacteria in the rumen (Giger-Reverdin et al., 2003). Because protozoa were removed from the rumen, starch was not absorbed with the protozoa cells, thus starch degradation was increased by rumen bacteria (Hobson and Stewart, 1997).

3 h after feed intake, fermentation did not start effectively and 12 h after feeding, little nutrients were left for fermentation in the rumen; so at 3 and 12 h after feeding, rumen pH were similar in coarse and fine corn silage diets. Also, in the present study, the peNDF was high in diets (at least 21 percent in DM) (Table 2); preventing the changes. At this level of peNDF, stimulation of rumination and chewing, and consequently buffering of ruminal acids can be done. However, pH of rumen content at 7.5 h after feeding was higher in coarse corn silage diets than in the fine corn silage diets (Table 4). Long forage particles in the diet promote chewing and salivary secretion, which helps to buffer the acids resulting from feed digestion. Thus, particle length of forages and the amount of fiber in the diet can have a significant impact on ruminal pH through the provision of salivary buffers. Long particle forage causes the sheep to spend more time eating and ruminating, which increases the flow of salivary buffers in the rumen. Rumen pH can be affected by changing the physical form in cattle rations and reducing the corn silage particle size along with decreased rumination and total chewing time; pH also declined (Lock and Bauman, 2007). 3 h after feeding, the ether extract was higher in rumen contents of oil supplemental diets ($p<0.0302$), but at 7.5 and 12 h after diets consumption, due to absorption and passage, the EE value was not significantly different in rumen contents.

The values of percentage of NFC were higher in the rumen containing coarse corn silage at 3 and 7.5h after feeding ($p<0.1308$ and $p<0.0765$, respectively) and at 12 h after feeding ($p<0.0002$) than the fine corn silage diets.

This is because grains were ground in fine corn silage, and starch degradation was increased in the rumen. In Yang and Beauchemin's (2006) experiment on increasing barley silage particle size (medium and long) versus fine particle, starch digestion decreased by 10% in the rumen and increased by 19% in the intestine. 12 h after feeding, the amount of NFC in oil supplemental diet was less (0007 / 0 > P). Poly unsaturated fatty acid in soybean oil is toxic to protozoa and gram positive bacteria (Giger-Reverdin et al., 2003). If protozoa are removed from rumen, starch is not absorbed in the protozoa cells, thus starch degradation increases by rumen bacteria (Hobson and Stewart, 1997).

Particle size of the geometric mean of rumen content was higher at 3 h after feed intake in coarse than in the fine corn silage diets ($p<0.0002$). However, at 7.5 and 12 h after feeding, there were no differences between the diets containing fine and coarse corn silage and diets containing supplemental oil and without it. This is because over time, there was uniformity in the rumen with the feed digestion and particle passage from the rumen. The value of rumen mat was higher in coarse than in the fine corn silage diets at 3 h after feeding. Reduced particles size not only reduces particles that are less than threshold size, but also reduces size of rumen mat. Therefore, changes in rumen mat affect persistence passable materials (Allen and Mertens, 1988). So, non-escapable rumen pool sizes were higher in coarse silage diets at 3 h after feeding ($p<0.0502$). In fact, rumen mat acts as a filter for small particles (Firkins et al., 1998). Increasing the rumen mat at 3 h after feeding, the coarse corn silage diets reduced solids passage in the rumen ($p<0.1033$); in contrast, escapable rumen pool sizes were higher in fine corn silage diets ($p<0.0502$). Therefore, the value of the rumen solids passage rate was higher in fine corn silage diets. But these differences were not observed at 7.5 and 12 h after feed intake.

The value of rumen mat was decreased by reducing the non-escapable rumen pool sizes, increasing escapable rumen pool sizes in fine corn silage diets at 3 h after feed intake and increasing the solids passage rate in rumen (the solid rumen passage rate was higher numerically in fine corn silage diets) (Table 5). Increasing the solid passage rate led to reduction of DM, OM and NDF digestibility in fine corn silage diets. In addition, long forage fiber creates a floating mat in the rumen, which stimulates contractions of the rumen. Without these mixing motions, the rumen can become a stagnant pool, and removal of VFA via absorption and fluid passage from the rumen declines, thereby increasing the risk of acidosis. Fiber is more slowly digested than starch and sugar, so including fiber in the diet slows the rate of feed digestion in the rumen. More VFA is produced right after a meal in the case of grain compared with forage. This explains the large depressions in ruminal pH followed by concentrate meals. So, adding forage to the diet not only increases chewing time and saliva secretion, but it even aids VFA production throughout the entire day. Feeding long particle fiber can also shift the site of starch digestion from the rumen to the intestine, which reduces the potential for ruminal acidosis (Yang and Beauchemin, 2006; Zebeli et al., 2006). In this experiment, although reduction of particle size decreased size of rumen mat and non-escapable fraction, oil supplementation reduced ruminal digestion and mean retention of ruminal particulate. However, it seems that oil supplementation can be helpful in fine corn silage diets for maintaining ruminal mat.

The value of eating time was higher in coarse corn silage diets than in fine corn silage diets. With this trend;the value of eating time per kg DMI was higher in coarse corn silage diets. Yansari et al. (2004) found that reducing alfalfa hay particle size in cow diets of 19 to 10 and 2 mm, the time consumption declined per kg DM and NDF. Soita et al. (2000) reported that comparison between four diets with two levels of particles size and two levels of concentrate particle size had a high significant effect on time consumption and time consumption per kg of forage and NDF intake. In the experiment of Schwab et al. (2002), time consuming (minutes per day) and consumption (min) per kg DM and NDF intake did not decrease significantly in corn silage with size 18 mm vs. 32 mm; but differences were significant between diets containing corn silage with a theoretical cut length of 13 mm vs. 19- mm per kg DM. But in some experiments, changes in forage particles size had no significant effect on diets consuming time. Zebli et al. (2007) observed that the hay particles size had no significant effect on the diets consuming time (minutes per day) and time consumption per kg DM and NDF. But the time consumed per kg DM, numerically, was higher in the forage larger than 1.18 mm. Also, in Kononoff et al. (2003)'s experiment on four diets, different sizes of corn silage had no significant effects on consumption time (minutes per day) in lactating cows.

Diets differences were significant in rumination activity ($p<0.0399$) and rumination time was lower in the fine corn silage diet with oil supplement than the other diets because of the lower feed intake (Table 3). Rumination times per kg DM, NDF and peNDF in fine corn silage diets were higher than coarse corn silage diets (Table 6, $p<0.0936$, $p<0.0336$, $p<0.0098$, respectively), because fine corn silage diets decreased digestibility due to the reduction of rumen mat (at 3 hours after feeding) and increased passage rate of the non-escapable rumen pool sizes to escapable rumen pool sizes (Table 4).

For passage particle from the rumen, reducing particle size by microbial digestion was compensated by increasing rumination activity. In normal condition, 80% of diet particles crushed and broken in the rumen is attributed to chewing and the rest to microbial degradation. If microbial digestion is reduced, it is compensated by more rumination and causes more physical break in rumen contents (Zebeli et al., 2006). In this experiment, adding soy-bean oil to diets of alfalfa meal had a synergism effect on reducing DMI, rumination and total chewing activity.

Differences between experimental diets were significant on chewing activity ($p<0.0439$) which cause more chewing in diets without oil supplementation in comparison to supplemented diet with oil. The value of chewing time per kg of diet peNDF in fine corn silage was longer than coarse corn silage diet ($p<0.1104$). Chewing activity is the sum of eating and rumination activity. Therefore, increased chewing activity in the fine corn silage diets (per kg peNDF) was due to more rumination on fine corn silage diets. But Soita et al. (2000) reported that reducing corn silage particle size of 19 to 13 mm has little effect on chewing activity, but decreasing from 13 to 6 mm caused 30% of the chewing activity to decrease. It could be that part of the differences between hay in stimulating chewing activity was due to the differences in forage particle size, density and the physical interaction with other feeds in the rumen.

Conclusion

The results of this experiment highlight the role of the physical characteristics of diet on rumen conditions. However, we suggest that the reduction of particle size can reduce the content of peNDF in rations and change ruminal environment and composition. In addition, although reduction of particle size decreased size of rumen mat and non-escapable fraction, oil supplementation reduced ruminal digestion and mean retention of ruminal particulate. However, it seems that oil supplementation can be helpful in fine corn silage diets for maintaining ruminal mat.

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