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Full Length Research Paper

The effects of alfalfa particle size and acid treated protein on ruminal chemical composition, liquid, particulate, escapable and non escapable phases in Zel sheep

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This study was conducted to investigate the effects of alfalfa particle size (long vs. fine) and canola meal treated with hydrochloric acid solution (untreated vs treated) on ruminal chemical composition, liquid, particulate, escapable and non escapable phases in Zel sheep. Four ruminally cannulated sheep received a mixed diet (% of dry matter) consisting of 23.73 alfalfa, 8.70 canola meal, 39.56 wheat straw, 13.45 beet pulp and 13.45 barley grain and 1 mineral-vitamin mixture. The experimental design was a 4 × 4 Latin square with 22-days periods. The diet was offered twice daily (09:00 and 21:00 h). The rumens were evacuated manually at 3, 7.5 and 12 h post-feeding and total ruminal contents were separated into mat and liquids. Dry matter weight distribution of total recovered particles was determined by a wet-sieving procedure and used to partition ruminal mat and liquids among percentage of large (≥ 6.35 mm), medium (< 6.35 and ≥ 1.18 mm), and small (< 1.18 and ≥ 0.5 mm) particles. Lyophilized ruminal digesta were analyzed for chemical composition especially for CP, NDF and EE. No interactions (*P* > 0.05) between dietary particle size and acid level were observed for ruminal chemical composition, liquid, particulate, escapable and non escapable phase. Treatment of canola meal and increase of particle size reduced the values of CP. Generally, with increase in time after feeding, the values of each nutrient decreased. Particle size and time post-feeding had a pronounced effect on the distribution of different particle fractions, whereas acid level did not influence it. With increase in time after feeding, percentage of particles ≥ 6.35 mm decreased, whereas the percentage of particles < 6.35 mm increased, illustrating intensive particle breakdown in the reticulo-rumen. Different particle size and time post-feeding had pronounced effect on total mass of ruminal digesta, ruminal mat and liquid part, in which fine particles and 12 h post feeding caused the lowest rumen mat. Time post feeding and acid level did not influence the values of pH significantly, whereas with increase in particle size, the values of pH increased.

Key words: Canola meal, particle size, rumen mat, escapable, non escapable phase.

INTRODUCTION

Optimal utilization of diets by ruminant animals is influenced by the chemical composition and physical characteristics of the ration. NDF measures some chemical characteristics, but not physical characteristics of fiber such as particle size. This physical characteristic can influence nutrient utilization, ruminal fermentation, and animal production independently of the amount or composition of NDF (Mertens, 1997).

Particle size accounted for 59% of the variation in

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ruminal mean retention time of plastic particles in sheep (Kaske and Engelhardt, 1990). According to critical size theory, particles longer than 1.18 mm have the greatest resistance to passage and are largely responsible for stimulating chewing and rumination (Poppi et al., 1980). A sufficient supply of long particles or NDF must be in the ration to increase total chewing activity, maintain rumen pH, optimize rumen environment for digestion, increase acetate: propionate ratio, increase milk fat concentration, and avoid metabolic disorders (Mertens, 1997, 2000). Ruminal digestibility of a dietary component is a function of the rate at which it is degraded in the rumen (chemically and physically) and the rate at which it is physically removed from the rumen. Both processes (degradation and physical removal) determine the release of nutrients to the ruminal microbes and the host animal, and the amount of forage that can be eaten by the animal (Faicheny, 1986). Overall rate of physical removal of digesta can be separated into two distinct rates: rate of escape from the reticulo-rimen and rate of large particle breakdown (Woodford and Murphy, 1988). In ruminants consuming all forage diets or diets high in forage of low quality, large particle breakdown was assumed to be an important determinant of passage and feed intake (Balch and Campling, 1962; Campling, 1970; Poppi et al., 1981; Welch, 1982; Okine and Mathison, 1991). On the other hand, canola meal is a readily available supplemental protein that is used extensively in ruminant rations (Aherne et al., 1986; Christensen and McKinnon, 1989). Because glucosinolate and erucic acid contents in rapeseed have been reduced to form canola meal and do not limit ruminant performance, interest in canola meal is increasing (Kozlowska, 1986). The value and optimal proportion of high quality ruminal undegraded proteins for growing ruminants has not been defined conclusively (Kirkpatrick and Kennelly, 1987). Microbial protein production is not markedly affected by diet and has a relatively high quality (Richardson and Hatfield, 1978; Owens and Bergen, 1983). But microbial protein alone may not meet the demand of high-producing ruminants (0rskov, 1982; Owens and Bergen, 1983). A supplemental supply (quantity) of amino acids from sources that escape luminal degradation and that complement the amino acid profile of microbial protein should increase performance or decrease the amount of protein required (Owens and Bergen, 1983). In a comparison of protein evaluation systems, the NRC (1985) found that the undegraded protein proportion recommended for dairy cows at a minimum protein intake ranged from 20 to 55%. In contrast, receiving feedlot cattle requires at least 60% ruminal escaped protein for maximal performance (Eck et al., 1988). Despite an excellent AA profile, canola meal is a poor source of metabolizable AA because it is extensively degraded in the rumen (Kendall et al., 1991; McAllister et al., 1993). Acid decreases solubility of proteins by creating structural changes in canola meal protein (khorasani et al., 1993).

Thus, acid exposure can increase the ruminally undegraded protein (RUP) value of the meal (Mc Kinnon et al., 1991) and potentially increase the contribution of such protein supplements to MP. Unfortunately, few studies have been done in relation to the effects of different particle size and acid treated proteins on ruminal chemical composition, liquid, particulate, escapable and non escapable phases. Complexity existence between feed intake, concentrate nature and act of degradation in the rumen often causes problems to discriminate the quantitative characteristics of forages particle size effects especially in the presence of different sources of protein and fat. It seems that most of these results are due to effects of these treatments on ruminal fermentation, stability of ruminal mat and eventually physically effectiveness of fiber in the ration.

The objectives of this study were to determine the effects of alfalfa particle size (long vs fine) and canola meal treated with hydrochloric acid solution (untreated vs treated) on ruminal chemical composition, liquid, particulate, escapable and non escapable phases in Zel sheep.

MATERIALS AND METHODS

Animals and diets

This experiment was carried out at the farm of Sari Agricultural Science and Natural Resources University, Sari, Iran. 42 year old Zel sheep, weighing 30±2 kg was used in a Latin square design. Treatments included: (1) treated canola meal and long alfalfa hay; (2) treated canola meal and alfalfa meal; (3) not treated canola meal and long alfalfa hay, and (4) not treated canola meal and alfalfa meal. The experiment was done in 22-day periods (adaptation, 14 days; taking out of rumen contents, 7 days; initial chewing activity measurement, 1 day). Each of the four sheep was fitted with a ruminal cannula and housed indoors in individual tie stalls. A mixed diet was formulated based on 23.73, 8.70, 39.56, 13.45 and 13.45% of alfalfa forage, canola meal, wheat straw, beet pulp and barley grain, respectively. Water and mineralized salt stone was available for sheep throughout the duration of the experiment. Sheep were fed at maintenance level during this experiment. The diets had similar chemical composition (Table 1). The daily allotment of feed was offered in two equal meals at 09:00 and 21:00 h. Prior to feeding, all dietary ingredients were completely mixed by hand. Diets were formulated using the sheep CNCPS system (2007). Canola meal was treated with hydrochloric acid solution at 5% level using spraying method. After it was air dried, treated canola meal was dried in an under vacuum oven at 55°C for 24 h. Conventional canola samples were treated with distilled water and then dried at 55°C for 24 h in the oven. All of the samples were sieved via wire screen with 2 mm pore diameter.

Feed intake and chemical composition of rumen contents

Dry matter intake (DMI) was measured daily for all sheep (Table 3). Lyophilized ruminal digesta were analyzed for chemical composition especially for CP, NDF and EE. CP concentration in ruminal digesta was determined by the Kjeldahl procedure (Kjeltec Analyzer Unit, 2300; AOAC, 2002). NDF and EE concentrations

Table 1. Chemical composition (% of DM) of ingredients and TMR containing treated and not treated canola and two different particle size of alfalfa.

Ingredient	DM	Fat	CP	NDF	NFC
Treated canola	91.40	3.43	35.82	25.64	27.61
Not treated canola	91.40	3.50	35.17	25.24	28.63
Long alfalfa	87.30	3.00	23.61	48.52	11.91
Alfalfa meal	87.35	3.12	23.88	48.32	12.59
Barley grain	89.40	2.1	12.4	18.5	64.52
Beet pulp	85.80	1.5	11	39.8	43.4
Wheat straw	85.80	2.1	3.6	78.78	6.73
TMR containing treated and not treated canola and two different particle size of alfalfa					
Long and treated	87.94	2.42	17.28	42.2	29.8
Fine and treated	88.48	2.65	16.94	42.60	31.41
Long and not treated	87.94	2.24	17.95	42.5	30.61
Fine and not treated	88.48	2.46	17.61	42.12	30.91

were determined by Van Soest (1994) and AOAC (2002) procedures.

Sample collection

Representative samples were obtained from each dietary ingredient and stored for subsequent analysis. Total reticulo-ruminal contents were removed manually at 3, 7.5 and 12 h after feed consumption. Because of the time required to empty rumen, rumen emptying was started 15 min before these times and finished 15 min after. The order of animals was changed at each emptying using a blocking system. All ruminal contents that could be removed by hand were emptied into a rectangular insulated tub. This material was referred to as the mat. Material not removable by hand was bailed into an insulated plastic barrel. This material was referred to as the liquid phase (Robinson et al., 1987). Both fractions were weighed, mixed thoroughly, sub sampled [mat (2 to 3 kg) and liquid phase (1 kg] and then liquids were returned to the rumen followed by the mat.

Particle size distribution of ruminal contents and feces

Particle size distribution of reticulo-ruminal contents and feces were determined by a wet sieving technique, using sieves with square apertures of 6.35, 4.75, 3.35, 1.68, 1.18, 0.8 and 0.5 mm on a side. The samples were soaked in cold tap water for 30 min prior to sieving (Luginbuhl et al., 1990), and then sieved with an electromagnetic sieve shaker. Triplicate samples of ruminal mat (30 g) and feces (40 g) were sieved. Material was retained on the sieves, dried at 70°C for 24 h and weighed. Particles were grouped by weight as percentages of large (retained on sieve 6.35), medium (retained on sieves < 6.35 mm and > 1.68), and small (retained on sieves < 1.18 mm and < 0.5). The geometric mean (GM) and the standard deviation of GM were calculated according to ASAE S424.1 (2002; Table 5). Dry residues remaining on the final screens were calculated from the accumulative DM weight of particles remaining on each sieve. Total material remaining on the sieve ≥ 0.5 mm was hereafter referred to as the total particle DM, materials that remained on sieves 0.5, 0.8 and 1.18 mm were considered as escapable phase and materials remaining on sieves 1.68, 3.35, 4.75 and 6.35 mm, as non escapable phase.

Initial chewing effects on particle size distribution

By measurement of the particle size distribution of ruminal contents taken out during the time of measurement of the saliva secretion rate, the effect of initial chewing was determined on the values of particle size reduction.

Statistical analyses

Data were analyzed as a Latin square design according to the GLM procedures of SAS (2001). Model sums of squares were separated into sheep, period, and treatment effects. Because treatments were arranged as a 2 × 2 factorial; the sums of squares for the treatments in the GLM model were further separated into dietary particle size level, acid level and the particle size level × acid level interaction.

$$Y_{ijk(i)lmn} = \mu + S_i + P_j + VS_{k(i)} + PS_m + AL_n + (PSAL)_{mn} + E_{ijk(l)mn}$$

Where, $Y_{ijk(i)lmn}$ = dependent variable; μ = overall mean; S_i = effect of square i (i = 1, 2,3,4); P_j = effect of period j (j = 1,2,…4); $VS_{k(i)}$ = effect of sheep k (within square i) (k(i) = 1,2,3,4); PS_m = effect of particle size; A_n = effect of acid level; $PSAL_{mn}$ = interaction between particle size m and acid level n; $E_{(i)jk(l)n}$ = residual error.

RESULTS

Feed intake and chemical composition of rumen contents

Chemical composition of the two sizes of alfalfa, acid treated canola meal, and consequently TMR was not affected by the reduction of the particle size and increase of acid level (Table 1).

Table 2. Amount of nutrient consumption in sheep fed TMR containing treated and not treated canola meal and two different particle size of alfalfa forage.

Intake (Kg/d)	Treatment					Effect (*P*) [a]		
	Long and treated	Fine and treated	Long and not treated	Fine and not treated	SEM	PS[1]	AL[2]	PS × AL
DM	1.25b	1.33[a]	1.26[b]	1.37[a]	0.37	0.003	0.0002	0.40
OM	1.003 [b]	1.09[a]	1.02[b]	1.11[a]	0.34	0.04	0.0001	0.79
NDF	0.54	0.56	0.56	0.58	0.12	0.02	0.002	0.63
CP	0.21[b]	0.22[ab]	0.22[ab]	0.24[a]	0.07	0.007	0.001	0.16
NFC	0.36[b]	0.41[a]	0.31[b]	0.42 [a]	0.08	0.03	0.0001	0.44
Fat	0.03[c]	0.035[a]	0.028[d]	0.033[b]	0.03	0.0001	0.0001	0.37
Ash	0.096[a]	0.085[b]	0.084[b]	0.094[a]	0.02	0.045	0.42	0.0001

[a,b,c] Means within a row with different superscripts differ at $P < 0.05$. [1]Particle size; [2]acid level.

Table 3. Chemical composition of rumen content of sheep fed with TMR containing treated and not treated canola meal and two different particle size of alfalfa at 3, 7.5 and 12 h post-feeding.

Chemical composition	Long and treated	Fine and treated	Long and not treated	Fine and not treated	SEM	Effect (P)[a]		
						PS[1]	AL[2]	PS × AL
3 h post-feeding								
DM content (%)	19.55[a]	15.25[d]	17.35[b]	15.85 [c]	0.0014	0.0001	0.48	0.15
Rumen contents								
Weight of solid phase(kg)	5.81[a]	4.18[b]	5.08[ab]	4.54[b]	0.1589	0.0001	0.0001	0.77
Weight of liquid phase(kg)	0.5[a]	0.55[a]	0.37 [b]	0.57 [a]	0.0023	0.01	0.0001	0.003
Volume of solid phase(L)	6.85[a]	5[c]	6b	5.4 [c]	0.0408	0.0001	0.98	0.73
Volume of liquid phase(L)	0.54[a]	0.55[a]	0.42 [b]	0.57 [a]	0.0007	0.06	0.0001	0.006
pH	6.80[a]	6.70[b]	6.80a	6.68 [b]	0.0009	0.0001	0.0001	0.77
7.5 h post-feeding								
DM content (%)	16.15[a]	14.9[d]	15.85 [b]	15[c]	0.031	0.0001	0.13	0.49
Rumen contents								
Weight of solid phase(kg)	5.01[a]	3.32[d]	4.66 [b]	3.86 [c]	0.0177	0.001	0.25	0.17
Weight of liquid phase(kg)	0.55[b]	0.79 [a]	0.55 [b]	0.64[ab]	0.0068	0.98	0.0001	0.73
Volume of solid phase(L)	5.95[a]	3.87[d]	5.37 [b]	4.65 [c]	0.0598	0.039	0.001	0.4
Volume of liquid phase(L)	0.52[b]	0.60[b]	0.62[ab]	0.70[a]	0.0024	0.35	0.006	0.31
pH	6.83[a]	6.66[b]	6.70 [b]	6.27 [c]	0.0018	0.0001	0.0001	0.51
12 h post-feeding								
DM content (%)	16[b]	14[c]	16.15 [a]	12.05 [d]	0.002	0.0001	0.2	0.41
Rumen contents								
Weight of solid phase(kg)	3.93[a]	2.34[d]	3.56 [b]	2.99 [c]	0.0215	0.54	0.0001	0.61
Weight of liquid phase(kg)	0.23[b]	0.61 [a]	0.59 [a]	0.61 [a]	0.0027	0.08	0.0001	0.44
Volume of solid phase(L)	4.2[a]	2.75 [c]	3.95 [a]	3.05 [b]	0.0204	0.0001	0.0001	0.37
Volume of liquid phase(L)	0.33[c]	1.17 [a]	0.56 [b]	0.7 [b]	0.0067	0.04	0.0001	0.79
pH	6.88[a]	6.72[c]	6.87 [b]	6.7 [d]	0.002	0.16	0.001	0.77

[a,b,c] Means within a row with different superscripts differ (P <0.05). [a] Main and interaction effects of PS and AL; [1]particle size; [2]acid level.

In treatments containing not treated canola meal, as particle size decreased, there was an increase in DMI and consequently in the daily intake of OM, NDF, ADF, CP, NFC, ether extract, and forage in the fine alfalfa and not treated canola treatments compared with the long alfalfa and treated canola treatments (Table 2). No interaction effects were observed (P > 0.05) between particle size and acid level on intake except for Ash *(P =*

Table 4. Dry matter (DM) content, weight and volume of solid and liquid phases and rumen pH of sheep fed with TMR containing treated and not treated canola meal and two different particle size of alfalfa at 3, 7.5 and 12 h post-feeding.

Parameter	Long and treated	Fine and treated	Long and not treated	Fine and not treated	SEM	Effect (P)[a]		
						PS[1]	AL[2]	PS × AL
3 h post-feeding								
DM content (%)	19.55a	15.25d	17.35b	15.85c	0.0014	0.0001	0.48	0.15
Rumen contents								
Weight of solid phase(kg)	5.81^{a}	4.18^{b}	5.08^{ab}	4.54^{b}	0.1589	0.0001	0.0001	0.77
Weight of liquid phase(kg)	0.5^{a}	0.55^{a}	0.37^{b}	0.57^{a}	0.0023	0.01	0.0001	0.003
Volume of solid phase(L)	6.85^{a}	5^{c}	6^{b}	5.4^{c}	0.0408	0.0001	0.98	0.73
Volume of liquid phase(L)	0.54^{a}	0.55^{a}	0.42^{b}	0.57^{a}	0.0007	0.06	0.0001	0.006
pH	6.80^{a}	6.70^{b}	6.80^{a}	6.68^{b}	0.0009	0.0001	0.0001	0.77
7.5 h post-feeding								
DM content (%)	16.15^{a}	14.9^{d}	15.85^{b}	15^{c}	0.031	0.0001	0.13	0.49
Rumen contents								
Weight of solid phase(kg)	5.01^{a}	3.32^{d}	4.66^{b}	3.86^{c}	0.0177	0.001	0.25	0.17
Weight of liquid phase(kg)	0.55^{b}	0.79^{a}	0.55^{b}	0.64^{ab}	0.0068	0.98	0.0001	0.73
Volume of solid phase(L)	5.95^{a}	3.87^{d}	5.37^{b}	4.65^{c}	0.0598	0.039	0.001	0.4
Volume of liquid phase(L)	0.52^{b}	0.60^{b}	0.62^{ab}	0.70^{a}	0.0024	0.35	0.006	0.31
pH	6.83^{a}	6.66^{b}	6.70^{b}	6.27^{c}	0.0018	0.0001	0.0001	0.51
12 h post-feeding								
DM content (%)	16^{b}	14^{c}	16.15^{a}	12.05^{d}	0.002	0.0001	0.2	0.41
Rumen contents								
Weight of solid phase(kg)	3.93^{a}	2.34^{d}	3.56^{b}	2.99^{c}	0.0215	0.54	0.0001	0.61
Weight of liquid phase(kg)	0.23^{b}	0.61^{a}	0.59^{a}	0.61^{a}	0.0027	0.08	0.0001	0.44
Volume of solid phase(L)	4.2^{a}	2.75^{c}	3.95^{a}	3.05^{b}	0.0204	0.0001	0.0001	0.37
Volume of liquid phase(L)	0.33^{c}	1.17^{a}	0.56^{b}	0.7^{b}	0.0067	0.04	0.0001	0.79
pH	6.88^{a}	6.72^{c}	6.87^{b}	6.7^{d}	0.002	0.16	0.001	0.77

^{a,b,c} Means within a row with different superscripts differ ($P < 0.05$). ^{a} Main and interaction effects of PS and AL; ^{1}particle size; ^{2}acid level.

0.0001). Regardless of the effective system, as particle size decreased, daily intake of NDF increased, (Table 2); this could be as a result of the increased DMI.

Chemical compositions of the rumen contents especially crude protein, ether extract and neutral detergent fiber are shown in Table 3. Treatment of canola meal and decrease of particle size decreased the values of CP. The highest and the lowest value of CP at different hours post-feeding was related to treatments containing alfalfa with long particle size and not treated canola meal and treatments including fine alfalfa and treated canola meal, respectively. There were no effects (P > 0.05) of particle size and acid level on ether extract of the rumen components. Treatments including fine particle size of alfalfa had the highest NDF. General trend of changes showed that after increase of time post-feeding, the value of each nutrient decreased (Table 3).

Ruminal contents characteristics

As particle size increased, total ruminal digesta and solid phase increased, whereas liquid phase decreased (Table 4). Consequently, percentage of mat in total ruminal digesta increased. Time post-feeding had pronounced effect on total mass of ruminal digesta and ruminal mat, all of which were lower at 12 h post-feeding. Thus, percentage of mat was lower at 12 h post-feeding than 7.5 and 3 h post-feeding. Contents of ruminal liquid phase tended to be greater at 12 h post-feeding than at 3 h post-feeding. The results of pH data at different hours of post-feeding are shown in Table 4. Time post feeding did not influence the values of pH significantly, whereas with increase in particle size and acid level, the values of pH increased. The highest and the lowest values for pH were related to treatments including long particles and fine

Table 5. Particle size distribution (% of DM) of rumen content in sheep fed with TMR containing treated and not treated canola meal and two different particle size of alfalfa at 3, 7.5 and 12 h post-feeding.

Parameter	Long and treated	Fine and treated	Long and not treated	Fine and not treated	SEM	Effect (P) [a]		
						PS[1]	AL[2]	PS × AL
3 h post-feeding								
Separator sieves(mm)								
6.35	16.24[a]	12.37[ab]	18.93[b]	10.81[b]	3.43	0.002	0.26	0.16
4.75	8.69 [b]	15.07[a]	5.69b	6.55[b]	8.16	0.03	0.13	0.14
3.35	11.25[b]	18.55[a]	11.72[ab]	9.75[b]	9.67	0.0003	0.16	0.73
1.68	24.95	25.99	25.68	27.41	4.00	0.8687	0.98	0.51
1.18	18.30	20.56	9.32	20.65	0.26	0.710	0.65	0.49
0.8	9.38[b]	9.87[ab]	13.65[a]	11.17[ab]	2.68	0.001	0.39	0.15
0.5	6.41[c]	7.78[c]	14[b]	18.55[a]	3.51	0.032	0.35	0.79
GM	5.95	5.70	5.90	4.58				
SD of GM	3.14	2.99	3.31	3.05				
Escapable material from the rumen (%)	38.87	28.02	37.98	45.48				
Non escapable material from the rumen (%)	61.13	71.98	62.02	54.52				
7.5 h post-feeding								
Separator sieves (mm)								
6.35	13.18[a]	5.93[b]	7.45[b]	4.38[b]	5.54	0.001	0.82	0.22
4.75	5.11	9.35	5.70	7.55	4.77	0.6575	0.17	0.49
3.35	8.09	8.95	8.63	8.07	1.33	0.7718	0.48	0.1
1.68	24.96[b]	33.17[a]	32.08[a]	26.71[ab]	9.76	0.01	0.2	0.93
1.18	17.73	21.73	21.35	22.40	2.69	0.9141	0.34	0.19
0.8	17.55	11.65	12.58	13.62	8.39	0.6147	0.75	0.66
0.5	13.69[ab]	10.68[b]	12.14[ab]	17.22[a]	4.35	0.0001	0.56	0.27
Geometric mean (GM)	4.29	4.21	4.09	3.72				
SD of GM	3.38	2.87	2.94	3.03				
Escapable material from the rumen (%)	48.66	42.6	46.14	53.29				
Non escapable material from the rumen (%)	51.34	57.4	53.86	46.71				
12 h post-feeding								
Separator sieves(mm)								
6.35	6.03	2.60	3.87	3.57	1.00	0.8503	0.26	0.25

Table 5. Continued

4.75	4.65	2.64	2.63	1.92	0.8623	0.9603	0.64	0.12
3.35	7.25	4.21	5.52	3.74	0.85	0.3602	0.52	0.92
1.68	31.27^{ab}	38.15^{a}	23.84^{b}	25.48^{b}	2.95	0.002	0.15	0.65
1.18	17.94^{b}	21.57^{b}	23.15^{b}	34.28^{a}	1.70	0.0001	0.28	0.77
0.8	15.26	14.73	12.67	16.74	6.42	0.3891	0.74	0.86
0.5	12.55^{bc}	16.04^{a}	11.86^{c}	14.22^{ab}	1.19	0.03	0.19	0.71
GM	3.77	3.27	3.17	2.65	1.00			
SD of GM	3.02	2.83	3.06	3.19	0.8623			
Escapable material from the rumen (%)	50.8	52.4	64.14	65.29	0.85			
Non escapable material from the rumen (%)	49.2	47.6	35.86	34.71	2.95			

^{a,b,c} Means within a row with different superscripts differ ($P < 0.05$). ^{a} Main and interaction effects of PS and AL. ^{1}Particle size; ^{2}acid level.

particles. No interactions (P > 0.05) between dietary particle size and acid level were observed for ruminal contents characteristics.

Particle size distribution of rumen contents and feces

Trend of particle size distribution for total hours showed that increase in time post-feeding percentage of long particles was reduced and accordingly, percentage of the remaining particles on down sieves increased (Table 5). Comparison between percentage of the remaining material on different sieves at the same hours for different treatments showed that during the first hours of post-feeding, the most difference was related to top sieves (6.35,4.75 and 3.35 mm) and with an increase in time post-feeding, this difference was more on down sieves (1.68, 1.18, 0.8 and 0.5 mm). In a general trend, with increase in time post-feeding, percentage of escapable phase from the rumen increased. Comparison between percentages of non escapable phase from the rumen during time post-feeding showed that the highest decrease in this part was related to treatments including long particle size and not treated (Table 5). Acid level did not influence particle size distribution of rumen contents at different particle size and time post-feeding. Feces contained no large particles (Table 6). Percentages of medium and small particles were similar between particle sizes and acid levels. Values of GM decreased in the fine alfalfa treatments. As the GM of TMR decreased, the size of the standard deviation of GM of particle size decreased.

The effects of initial chewing on particle size distribution

Particle size distribution of masticated feeds is the indicator of particle size reduction during initial chewing (Table 7). Comparison of particle size distribution of masticated feeds showed that initial chewing reduced long particle size and increased the ratio of fine particles. In the four experimental treatments, the most and the least affluence of masticated feed particle size in treatments including alfalfa with long particle size was related to sieves 6.35 and 0.8 mm and in the treatments including alfalfa meal was related to sieves 1.68 and 6.35 mm. The dry matter weight distribution of feed particle size was determined by dry- sieving procedure, the major part of particles were longer than 6.35 mm (table 7). The original (Lammers et al, 1996), and new (Kononoff, 2002) PSPS sieves were used for measuring particle size distribution. Comparison of these results is the indicator of the reduction in particle size during initial chewing. The most affluence of particle size of sieve 6.35 mm was related to treatment including long alfalfa particle size.

DISCUSSION

Feeds of longer particle size usually result in greater fill because of a slower rate of passage, limiting DMI through distension. During this time, it has been suggested that reducing diet particle size could positively affect DMI because the density of particles increases (Allen, 2000). Shaver et al. (1988) and Beauchemin et al. (1997)

Table 6. Particle size distribution (% of DM) related to faeces collected during measuring of digestibility in sheep fed with TMR containing treated and not treated canola meal and two different particle size of alfalfa.

Separator sieve (mm)	Long and treated	Fine and treated	Long and not treated	Fine and not treated	SEM	Effect (P)[a]		
						PS1	AL2	PS × AL
6.35	0.13[a]	0.00[b]	0.07[ab]	0.02[ab]	0.0026	0.003	0.18	0.63
4.75	0.74[a]	0.10[b]	0.05[b]	0.02[b]	0.0147	0.0005	0.13	0.74
3.35	3.17[a]	0.18[b]	0.15[b]	0.12[b]	1.11	0.001	0.87	0.18
1.68	77.28[a]	63.99[b]	73.28[ab]	51.41[c]	2.79	0.008	0.68	0.13
1.18	10.87	14.68	14.85	26.36	1.93	0.6606	0.24	0.49
0.8	4.26[c]	13.72[a]	7.2[b]	8.78[b]	1.10	0.0165	0.37	0.61
0.5	3.51[b]	7.3ab	4.35[b]	17.24[a]	2.44	0.0002	0.49	0.77
GM	4.61	3.49	4.03	3.20				
SD of GM	1.77	2.33	1.98	2.37				

[a] Main and interaction effects of PS and AL; [a,b,c] means within a row with different superscripts differ ($P < 0.05$); [1]particle size; [2]acid level.

Table 7a. Particle size distribution (% DM), geometric mean, pef and peNDF of feed ingredients and TMR containing treated and not treated canola meal and two different particle size of alfalfa forage (a). original Penn state particle separator, PSPSoriginal (b) new Penn state particle separator, PSPSnew.

Ingredients and TMR	19mm	8mm	pan	GM[1]	SD[2] of GM	$Pef_{PSPSoriginal}$[3]	$peNDF_{PSPSoriginal}$[4]
long alfalfa	31.14[a]	10.78[b]	58.08[c]	12.62	2.96	41.92[a]	24.53[a]
alfalfa meal	0[b]	0[c]	100[a]	8.98	1.72	0[c]	0[c]
wheat straw	0[b]	18.8[a]	81.2[b]	10.22	2.07	18.8[b]	14.80[b]
SEM	2.48	2.46	1.32			1.32	0.72
P-value	0.0001	0.0001	0.0001			0.0001	0.0001
Long and treated	4.88[a]	16.47[a]	78.6[b]	10.66	2.21	21.35	9.22[a]
Fine and treated	0.48[b]	8.3[b]	91.22[a]	9.56	1.90	8.78	3.74[b]
Long and not treated	4.88[a]	16.47[a]	78.6[b]	10.66	2.21	21.35	9.22[a]
Fine and not treated	0.48[b]	8.3[b]	91.22[a]	9.56	1.90	8.78	3.74[b]
SEM	0.24	1.12	1.94			1.94	0.35
P-value	0.0001	0.0001	0.0001			0.0001	0.0001

[a,b,c] means within a raw with different superscripts differ (P < 0.05).
[1]GM, geometric mean of particle size (mm); 2standard deviation; 3 physically effective factor based on DM retained on 19 and 8-mm sieves; 4obtained from multiplying the amount of pefPSPSoriginal to NDF of feeds remained on 19 and 8-mm sieves (lammers et al, 1996).

Table 7b.

Ingredients and TMR	19mm	8mm	1.18mm	pan	GM[1]	SD[2] of GM	$Pef_{PSPSnew}$[3]	$peNDF_{PSPSnew}$[4]
long alfalfa	31.14[a]	10.78[b]	47.06[b]	11.02[c]	12.96	2.93	88.98[a]	52.07[b]
alfalfa meal	0[b]	0[c]	13.18[c]	86.2[a]	5.97	1.24	13.18[c]	8.34[c]
wheat straw	0[b]	18.8[a]	63.32[a]	17.88[b]	9.41	2.00	82.12[b]	64.87[a]
SEM	2.48	2.46	3.81	2.20			2.20	0.58
P-value	0.0001	0.0001	0.0001	0.0001			0.0001	0.0001
Long and treated	4.88[a]	16.47[a]	60.55[a]	18.1[b]	13.30	3.02	81.9[a]	35.38[a]
Fine and treated	0.48[b]	8.3[b]	55[b]	36.22[a]	8.07	1.74	63.78[b]	27.23[b]
Long and not treated	4.88[a]	16.47[a]	60.55[b]	18.1[b]	13.30	3.02	81.9[a]	35.38[a]
Fine and not treated	0.48[b]	8.3[b]	55[b]	36.22[a]	8.07	1.74	63.78[b]	27.23[b]
SEM	0.24	1.12	3.80	3.30			3.30	0.61
P-value	0.0001	0.0001	0/0083	0.0001			0.0001	0.0001

found that when poor quality high fiber diets were fed, reducing the forage particle size significantly increased DMI. Forage particle size has less impact on intake when well-balanced rations are fed to lactating cows (Beauchemin et al., 1997). Voluntary DMI and nutrient supply can be constrained by rumen fill and clearance of digesta from the rumen. Reducing particle size decreases the filling effects of forage and increases ruminal passage rate (Allen, 2000). Hence, forages that occupy larger volumes per unit of DM weight (have lower bulk density) should have a greater ruminal filling effect than more dense forages (Wattiaux, 1990). The reduced protein intake accompanying the decrease in DM intake may indicate that dietary protein concentrations should be increased when sheep are fed acid treated canola meal-supplemented diets. Differences among protein sources in DMI primarily were due to differences in sheep weights. Due to the fact that treatment of canola meal with hydrochloric acid increases bypass protein from the rumen to the intestine, this in turn decreases available protein for ruminal microorganisms. Therefore, in giving attention to bacteria, growth in the rumen will not be motivated, degradation of feed in the rumen will be reduced, resting metabolic rate at thermoneutrality (RMRT) of feed increases and finally, dry matter intake will be reduced.

The protein that reaches the lower digestive tract of the ruminant is the sum of the protein that escaped the rumen fermentation plus the microbial yield. Factors affecting microbial yield involve microbial maintenance, dilution, and the Michaelis-Menten Kinetics of growth. Escape often involves indiscriminate loss from the rumen, the consequence of which is variable, depending on the components and the circumstances. Escaped protein may be beneficial, since it will be utilized efficiently in the postruminal digestion as long as it contains essential amino acids. Dietary nitrogen can escape rumen fermentation and pass to the lower tract in quantities sufficient to significantly modify the ruminant's efficiency. Escape can be altered by manipulating digestion or passage rates. It is likely that rumen escape is variable and depends on the type of protein and its rate of digestion, level of intake, rate of passage, and other factors, although there has been a regrettable tendency to regard rumen escape as a constant for a given diet (Chalupa et al., 1991). The National Research Council (NRC) treats escape as a constant, which is not realistic considering the increase in rumen passage with increase in feed intake. The physical nature of dietary protein- that is, whether it is soluble and moves with liquid or is insoluble and moves with particulate matter- is critical. Particle size is also important. Also, increased feed consumption means faster passage, and therefore greater escape. Treating feeds with chemical treatments may reduce protein solubility and increase the quantity of amino acid nitrogen digested in lower tract (Barry et al., 1973; Nolan, 1975). In those cases, it is difficult to say whether response or lack of response is due to escape, as microbial efficiency and synthesis may be affected by the reduced protein solubility. A moderate reduction in degradation rate should be beneficial, however, because this would provide a steadier supply of ammonia for the fermentation of the slower digesting carbohydrates. On the other hand, if over protection forces rumen organisms to become dependent on recycled urea, which is inadequate as a sole support of fermentation, their growth is generally slowed and intake or digestibility will suffer. Complete protection of dietary protein should lead to rumen nitrogen deficiency and responsiveness to NPN at high protein intakes. Acid decreases solubility of proteins by creating structural changes in canola meal protein (Khorasani et al., 1993). Thus, acid exposure can increase the ruminally undegraded protein (RUP) value of the meal (Mc Kinnon et al., 1991) and potentially increase the contribution of such protein supplements to MP.

As in the results, treatments including fine particle size of alfalfa hay had the highest NDF. This result is due to the fact that NDF has converse relationship with bulk density and has direct relationship with cell components; therefore with increasing particle size and consequently increasing bulk density, the value of NDF reduced. In this study, the decrease of CP degradability during treatment with hydrochloric acid was due to the structural changes in canola meal protein that caused protein to become unavailable to microbial enzymes produced in the rumen and thus digested with enzymes secreted by animal.

In the classic view of the rumen as a three-phase compartment, gas is capped above a floating raft on a liquid pool of particles that are not dense enough to sediment into the ventral sac of the rumen and of particles entrapped by the filter bed. As fermentation proceeds, rumination and digestion reduce particle sizes. These processes result in particles that become denser and tend to sink. Particles that settle to the floor of the rumen and having an optimum density are most likely to be selectively transported through the reticulo-omasal orifice to the omasum (Van Soest, 1994). We supposed that ruminal mat (solid phase); material that could be removed from the rumen by hand was composed mainly of material from the floating ruminal raft. Opposed to that liquid phase principally contains material from the cranial sac of the rumen and from the reticulum, containing also the material from the "zone of potential escape" (Allen and Mertens, 1988). When particles entering this zone have the greatest probability to leave the rumen to the omasum, liquid phase would contain most of the particles available for passage from the rumen. Accepting that liquid phase is a fluid suspension, which allows mixing between the contents of the cranial sac of the rumen and the reticulum, the examination of the liquid phase would be of key importance in the study of particle passage from the rumen. With increase in the particle size, value of liquid phase and proportion of liquid phase of total ruminal digesta decreased. An increasing part of total

ruminal digesta from the ventral region of the rumen that became available for manual emptying, was recovered as mat. It should be considered, however, that mat and liquid phase are defined by the procedure of emptying the rumens. Thus, they are similar but not equivalent to the classical solid and liquid phases in the rumen. The fact that total rumen contents were available for manual emptying indicated that the solid and liquid phases were less separated at the long particle size than at the fine particle size. From the aforementioned, it appeared that following increase in particle size, the mixing or starting pool for particles that are presented to the reticulo-omasal orifice may change. Physical characteristics of mat particles became more important also in the ventral region of the rumen. The increasing DM of rumen contents with increase of particle size would support this process. There is evidence that the consistency of reticular digesta influences outflow and particle separation within the reticulo-rumen (Baumont and Deswysen, 1991).The greater mat proportion could have assisted in entrapping more small particles in the mat (Robinson et al., 1987). Although percentage of small particles in mat DM remained fairly constant with longer particles, which indicates a well balanced process of particle break down (PPB), the increasing proportion of particulate content in total DM and the increasing DM content of mat indicates an increasing value of small particles in the mat. Because the contents of the reticulum and the rumen appeared to be more intimately associated at the higher intake due to decreasing particle size than at lower intake due to longer particle size, a less effective selection of well digested particles at the higher intake could have occurred.

When PPB in the rumen is studied, care must be taken to exclude solubilized DM and microbial from calculations related to ruminal particle size distribution. These fractions do not undergo PPB and do not arise from PPB, respectively (Mertens, 1993). In this study, particles retained on sieves 0.5 and 0.8 mm were defined as ruminal particulate DM according to Mertens (1993), who reported that DM passing through a 0.063 mm-screen can be assumed to be mostly soluble DM and microbial debris. Time post-feeding exerted an effect on particle size distribution of mat and liquid phase materials.

Percentage of particles remaining on top sieves was lower and on down sieves was greater at 12 h than 3 and 7.5 h post-feeding. Only the medium particle fraction in ruminal mat was not affected by time post-feeding. This introduced the medium particle fraction as a turning-point in particle size distribution. The medium particle fraction (< 4.75 and ≥ 1.18 mm) includes particles reported to possess a threshold size for passage out of the rumen: 1.18 mm (Poppi et al., 1980); 1.0 to 2.5 mm (Grent, 1984); 3.6 mm (Cardoza, 1985; Shaver et al., 1988); and 2 to 4 mm (Ulyatt et al., 1986). Value of particles greater than this size decreased, whereas value of particles smaller than this size increased with advancing time post-feeding of sheep fed a 100% forage diet (Luginbuhl et al., 1990) and a mixed diet consisting of 68% forage and 32% concentrate (Kovacs et al., 1997) and a mixed diet consisting of 64% forage and 36% concentrate (this study). This conformity of post-prandial changes of particle size distribution indicates that PPB in sheep on a mixed diet containing moderate proportions of concentrate is similar to that on an all forage diet. Reduction of particle size under a critical size is necessary to increase the probability for escape of particles from the rumen. The relatively small differences observed for particle size distribution of ruminal digesta components between the different intakes of a mixed diet fed to sheep indicates that PPB was constant. Particulate matter, however, influences the physical consistency of the digesta (Martz and Belyea, 1986) and consequently plays an important role in forming the ruminal floating raft. The greater value of ruminal raft with higher intakes could then assist in entrapping more small particles in the raft.

The first stage that affects particle size dynamic is feed chewing and mixing with saliva in the mouth in order to form bolus which can be swallowed easily. Ulyatt et al. (1983) suggested that feeds are chewed until they can be swallowed easily. So it seems that reduction of forages particle size during initial chewing not only depends on the kind of forage, but also on the initial particle size, dry matter contents, NDF and three-dimensional structure of plant tissue (Bailey et al., 1989). The treatments investigated in this study were same from the point of view of DM content and kind of forage; therefore, the only effectiveness factor in the value of particle size reduction during initial chewing was related to initial size of treatments. Longer time spent for feeding in the treatment containing long particle size of alfalfa (another study we have done; data not shown) showed that more time was spent for a bolus to become ready for swallowing. Since other treatments were shorter from the viewpoint of particle size, it seems that initial chewing would have the most effect on this treatment. Bailey et al. (1989) obtained the same results.

Conclusion

The results of this experiment emphasize that particle size of forage or TMR can influence liquid, particulate, escapable and non escapable phases. The acid treatment of canola meal influenced the chemical composition especially CP because it induced changes in rumen and intestine metabolism due to low degradation of its protein in the rumen and its availability in the intestine. Based on his proper physical characteristics, alfalfa can be used as a favorite source in the production of a healthy fermental system in ruminant's rumen. Canola meal did not have any significant effect on physical specifications of the ration. This is due to the fact that concentrates lack particle size which is the most important characteristic affecting physical characteristic. No interactions between dietary particle size and acid level were observed for

ruminal chemical composition and contents characteristics. However, more research is needed to determine how changes in particle size of forage and TMR and acid treated protein affect rumen conditions.

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Abbreviation

CP, Crude protein; **EE,** ether extract; **NDF,** neutral detergent fiber; **DM,** dry matter.

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