Effects of lucerne particle size and source of dietary carbohydrates on *in situ* degradation and ruminal variables in sheep

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ABSTRACT: The effects of altering forage particle size and source of rapidly degradable carbohydrates on *in situ* degradation and ruminal variables were studied in four Iranian male sheep. The study was designed as a Latin square with a 2 × 2 factorial arrangement of treatments including two carbohydrate sources (pelleted beet pulp vs. maize- and barley-based concentrate) and two lucerne particle sizes (2.38 vs. 0.94 mm). Kinetics of disappearance of lucerne, concentrates and mixed samples was studied *in situ*. Among feed samples, the degradation rate constant of lucerne dry matter was higher ($P < 0.02$) and disappearance of lucerne neutral detergent fibre (NDF) in 4 h of incubation was lower ($P = 0.06$) in diets with reduced particle size. The rapidly degradable fraction of lucerne samples was also affected by treatments. Other degradability components were not affected. The mean ruminal pH was lower in diets containing short hay than in those containing long hay (5.76 vs. 5.86, $P < 0.006$) and pH values were consistently lower immediately after feeding diets with short lucerne hay. The form of carbohydrates did not affect ruminal pH, however, altering the source of carbohydrates changed the pattern of pH over time. Total volatile fatty acid (VFA) concentration and proportions of individual VFA were similar but numerical differences indicated a lower acetate to propionate ratio in diets with short hay. Most of the affected variables were influenced by the particle size of forage to a larger extent than by the source of rapidly degradable carbohydrates or the interaction between them. So, when sheep diets contain no more than 250 g/kg starch, the source of dietary carbohydrates may not interact with forage particle size to affect DM degradability and ruminal fermentation.

Keywords: forage particle size; carbohydrates; rumen; degradation; sheep

Neutral detergent soluble carbohydrates vary in ruminal fermentation pattern, end products and potential to yield microbial cells. Soluble fibre tends to produce more acetate, while the fermentation of sugars and starch tend to produce more propionate (Leiva et al., 2000). Hristov and Ropp (2003) found no differences in fermentation acids and microbial protein synthesis in dairy cows, when diets were compared based on either ruminally fermentable non-structural carbohydrates (barley and molasses) or ruminally fermentable fibre (maize brewers' grain and beet pulp). When sheep were fed high-concentrate diets, i.e. up to 750 to 1 000 g/kg of diet dry matter (DM), rumen pH on a barley-based diet decreased to a larger extent (down to 5.18) than on a sugar beet pulp-based diet (down to 5.57) (Flachowsky et al., 1993).

To maximize an economic forage intake level, indigestible residues must be cleared from the rumen. A reduction of forage particle size has

been shown to increase intake and alter ruminal metabolism by altering volatile fatty acid (VFA) production (Thomson, 1972; Krause and Combs, 2003), organic matter digestibility (Thomson, 1972; Faichney et al., 2004), ruminal pH (Thomson, 1972) and passage rate (Faichney et al., 2004; Faichney and Brown, 2004) in sheep and cattle. On the other hand, feeding forage of proper length is necessary to maintain rumen functionality, balanced metabolism and animal health. This situation is a challenge because in mixed diets, where forage is accompanied by appreciable amounts of rapidly degradable carbohydrates, ruminal pH may drop so quickly that any advantage of reducing the forage particle size in promoting higher intake and productivity is unfavourably negated. For this reason, an alternative to consider is the supplementation of forages with soluble fibre-rich diets or feeds high in digestible neutral detergent fibre (NDF) that usually have a less negative effect on the rumen environment and thus on cellulolysis than the supplementation with starch- or sugar-rich feeds (Bampidis and Robinson, 2006). Studies have been conducted on effects of different non-fibrous carbohydrate sources on performance and ruminal metabolism in sheep (Bhattacharya and Sleiman, 1971; Ben-Ghedalia et al., 1989; Carey et al., 1993, Flachowsky et al., 1993; Cooper et al., 1996). To our knowledge, however, those studies are missing that compare different sources of neutral detergent soluble carbohydrate in mixed diets at different forage particle size. The hypothesis was that ruminally "safer" concentrate feeds such as beet pulp would create an opportunity for further reducing the particle size of the forage portion of the diet without compromising the rumen health and thus allowing for better DM and fibre digestion. Therefore, treatments were arranged in a factorial design to investigate the possible interactions between types of rapidly degradable carbohydrates and forage particle size.

MATERIAL AND METHODS

Animals, diets and treatments

Four Iranian 1-year-old crossbred male sheep weighing 36.1 ± 3.55 kg were used. Sheep were fitted with ruminal cannulae (internal diameter, 58 mm; length, 120 mm) in the dorsal rumen. They were housed in individual 1.5 × 1.2 m cages and offered lucerne hay for *ad libitum* intake and a mineral supplement during the recovery time after cannulation and adaptation to cages.

Full bloom lucerne hay (945 g/kg DM) from a single load was used as a forage source. The chemical composition of lucerne hay DM was as follows: 462 g/kg NDF, 369 g/kg acid detergent fibre (ADF), 82 g/kg acid detergent lignin, 142 g/kg crude protein, and 92 g/kg ash. A half of the load was chopped by a chopper equipped with a 30 × 35 mm screen size. This was considered as long particles (LN). The other half was chopped while the screen size was reduced to 20 mm and considered as short particles (SH). Particle size distribution of chopped lucerne hay was determined by dry sieving using standard sieves as recommended by the American Society of Agricultural Engineers (ASAE, 1996, method S424.1). Diets were formulated with CNCPS version 1.0.20 for sheep (Cannas et al., 2004) to contain 400 g/kg lucerne and 600 g/kg concentrate on DM basis for a 37 kg sheep. In the concentrate, pelleted beet pulp ground to pass a 2 mm screen was replaced for barley and wheat bran to alter the form of neutral detergent soluble carbohydrate. The concentrate containing beet pulp is noted hereafter as high soluble fibre (HiSF) indicating the greater amount of soluble fibre arising from beet pulp, and the concentrate without beet pulp is noted as high starch (HiSt). Four treatments were constructed by combining each size of lucerne hay with each type of concentrate, i.e. LN with HiSt (LNHiSt), LN with HiSF (LNHiSF), SH with HiSt (SHHiSt), and SH with HiSF (SHHiSF).

The experimental design was a 4 × 4 Latin square with a 2 × 2 factorial arrangement of treatments. Periods were 15 days of length with 9 days for adaptation and 6 days for sampling and incubations. Diets were offered for *ad libitum* intake once daily at 08:30 from day 1 to 5 and then restricted to 0.9 of the intake of day 1 to 5 to prevent confounding effects of intake on the rate of passage and estimates of degradability. Average intake in sheep during restricted feeding was 3.41 g/100 g of BW (1.23 kg vs. 0.96 kg predicted DMI based on CNCPS version 1.0.20 for sheep, Cannas et al., 2004) showing that the intake was still representative of intensively fed sheep.

In situ incubation, sampling and analysis

For *in situ* incubations, nylon bags of 10 cm length × 6 cm width having a 45 μm pore size were

used. Bags contained 2 g of air-dried samples, presoaked in 39°C water for 15 min and then inserted in the rumen for 2, 4, 6, 8, 16, 24, and 48 h. For each incubation time, duplicate bags were used for each feed sample. Samples included lucerne hay, concentrate, and a 40:60 mixture (DM basis) of hay and concentrate. For each sheep, the concentrate and mixtures were the same as in the diet being fed in that period. Upon removal, bags were rinsed with a gentle current of tap water until a clear filtrate was apparent. Bags were then oven-dried at 65°C for 48 h. Washing loss was determined by suspending duplicate samples in 39°C water for 15 min and disappearance was calculated. Sample residues of lucerne hay were analyzed for NDF disappearance in 4 and 24 h. The 72-h incubation was not performed but 72-h disappearance was extrapolated for lucerne hay by plotting 1/residues remained against 1/time as described by Udén and Van Soest (1984).

On day 15 of each period, samples of rumen fluid were taken –0.5, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 6, 8, and 19 h relative to the time of feeding, squeezed through 2 layers of cheesecloth and pH was immediately determined with a portable digital pH meter (HI8314, Hanna Instruments, Cluj-Napoca, Romania). Samples collected in 2.5 h post-feeding were acidified with sulphuric acid 1% (5:1 v/v), and stored at –13°C for later analysis. After thawing at room temperature, samples were centrifuged at 3 400 × g for 10 min; the supernatant was recentrifuged at 9 000 × g for 10 min and subsampled. Ammonia nitrogen was determined by the colorimetric phenol-hypochlorite method of Broderick and Kang (1980). The VFA were determined by gas chromatography (Chrompack, Model CP-9002, Chrompack, EA Middelburg, The Netherlands) with a 50 m (0.32 mm ID) silica-fused column (CP-Wax Chrompack Capillary Column, Varian, Palo Alto, CA). Helium was used as a carrier gas and the oven initial and final temperature was 55 and 195°C, respectively, and the detector and injector temperature was 250°C. Crotonic acid (1:7 v/v) was used as an internal standard. The values of NDF and ADF (without correction for ash content; Van Soest et al., 1991), crude protein (955.04), ash (942.05) and acid detergent lignin (973.18) (AOAC, 2002) were determined in lucerne hay and entire diets using dried samples ground through a 1 m screen. Heat-stable α-amylase (Sigma A3306; Sigma-Aldrich, Steinheim, Germany) was used for NDF analysis while sodium sulphite was omitted.

Statistical analysis

Data on DM degradability of feed samples were analysed by non-linear regression using the following model:

$$P = a + b \times (1 - e^{-c(t - L)})$$

where:
P = potential total degradability
a = rapidly degradable fraction
b = slowly but potentially degradable fraction
c = rate constant of fraction b
t = time
L = lag time

Lag time was determined only for lucerne. The parameter estimates from the natural log-linear procedure were used as starting parameter values for iterations in the NLIN procedure of SAS (SAS, 1999). Effective degradability of DM was calculated as:

$$ED = a + bc/(c + k)$$

where:
ED = effective degradability
k = fractional outflow rate
a, b, c = as described above

The ED was reported assuming k of 0.05 and 0.08/h. Data on ruminal parameters and *in situ* degradability components including a, b, c, ED and P were subjected to analysis of variance using PROC MIXED of SAS (1999) and the effect of sheep was considered as random. To estimate the mean rumen pH, time of sampling was included in the model as a repeated measure and the mean squares of two- and three-way interactions of time by particle size, type of concentrate, and period were excluded from the error mean square. Differences were declared significant at $P < 0.05$ and trends were noted at $P < 0.1$.

RESULTS AND DISCUSSION

Diet composition and particle size

Chemical composition of the diets and particle size distribution of lucerne hay are shown in Table 1. The major difference from other trials (Bhattacharya and Sleiman, 1971; Ben-Ghedalia et al., 1989) regarding the diet formulation in which sources of soluble fibre were substituted for grain was that we kept the NDF content of diets constant

Table 1. Ingredients and chemical composition of experimental diets

Ingredients (g/kg of DM)	Treatments[1]			
	LNHiSt	LNHiSF	SHHiSt	SHHiSF
Lucerne hay	405.4	400.2	405.4	400.2
Barley grain, ground	232.0	164.5	232.0	164.5
Maize grain, ground	88.0	94.6	88.0	94.6
Wheat bran	93.8	0	93.8	0
Pelleted beet pulp, ground	0	148.5	0	148.5
Protein-mineral supplement[2]	180.8	192.2	180.8	192.2
Nutrient contents (g/kg of DM)				
Crude protein	175.2	169.4	173.5	168.1
Neutral detergent fibre	346.1	336.4	338.0	342.7
Acid detergent fibre	219.4	207.1	211.2	222.6
Ash	81.2	85.6	82.1	84.7
Starch[3]	231.8	185.2	231.8	185.2
Soluble fibre[3]	117.3	157.1	117.3	157.1
Sugars[3]	65.5	74.3	65.5	74.3

[1]LN = long hay; SH = short hay; HiSF = high soluble fibre; HiSt = high starch;
[2]contained (g/kg of DM): soybean meal = 576.6; canola meal = 294.7; maize germs = 44.4; limestone = 35.6; mineral-vitamin premix = 21.7; salt = 16.4, dicalcium phosphate = 10.6
[3]estimated from Cornell-Penn-Miner (CPM) ver. 3.0.8 tabular values

by including wheat bran in the HiSt diet. Similar NDF concentrations ensured that any changes in measured variables were mostly the result of an altered form of carbohydrates rather than simply a substitution effect. Estimations by Cornell-Penn State-Miner (CPM) version 3.0.8 tabular values (Table 1) showed that the profile of readily available carbohydrates of HiSF and HiSt diets e.g. sugars, soluble fibre, and starch was changed by around 30%. The geometric mean particle size of LN hay

Table 2. Particle size distribution of long and short lucerne hay used in the experiment

Item (g/kg of DM retained on sieve)	Lucerne hay		$P > t$
	long	short	
19 mm	76.8	0	< 0.01
16 mm	13.6	0.3	< 0.01
8 mm	172.0	29.5	< 0.01
4 mm	337.1	304.9	0.65
1.41 mm	210.6	278,0	0.14
0.5 mm	110.1	206.7	< 0.01
Pan	78.7	180.5	< 0.01
Geometric mean (mm)	2.38	0.94	0.03
Geometric standard deviation (mm)	1.45	0.41	

was 2.38 ± 1.45 mm and that of SH hay was 0.94 ± 0.4 mm ($P < 0.03$, Table 2). 260 g/kg of particles were retained on the 8-mm sieve in LN and only 30 g/kg in SH lucerne hay ($P = 0.006$). On the other hand, only 190 g/kg of particles of LN lucerne passed through the 1.41 mm sieve, while 390 g/kg of particles of SH lucerne passed through this sieve size ($P = 0.003$). According to Faichney and Brown (2004), approximately 180 g/kg of lucerne in SH diets and 80 g/kg in LN diets could be categorized as medium particles, the remainder were large particles.

In situ parameters

Among feed samples, only degradation rate and rapidly degradable fraction of lucerne were significantly affected by treatments (Table 3). Fraction *a* of lucerne was affected by both the type of concentrate (CONC) and the particle size (PS), while the rate of degradation was affected by PS only. Grant and Mertens (1992) obtained a similar rate constant for lucerne NDF *in vitro* which ranged from 0.079 to 0.094/h with or without maize starch addi-

Table 3. Effects of lucerne particle size and source of dietary carbohydrates on *in situ* degradation kinetics of lucerne, concentrate and the mixture of lucerne and concentrate in sheep[1]

Parameter[2] / Sample	Treatments				SE	*P* <		
	LNHiSF	SHHiSF	LNHiSt	SHHiSt		PS	CONC	PS × CONC
Lucerne								
$EF_{0.05}$ (g/kg)	560.2	559.3	565.7	562.7	9.7	0.79	0.62	0.85
$EF_{0.08}$ (g/kg)	517.2	519.0	525.0	522.0	8.9	0.95	0.58	0.80
a (g/kg)	334.5	305.8	347.7	327.7	12.6	0.02	0.07	0.59
P (g/kg)	706.0.	675.6	724.0	691.2	18.6	0.17	0.43	0.95
Rate (/h)	0.0782	0.1072	0.0782	0.0955	0.009	0.02	0.41	0.41
Lag (h)	0.30	0.24	0.08	0.00	0.13	0.62	0.15	0.91
NDF disappearance (g/kg), 4 h	163.8	123.0	177.3	121.8	18.9	0.06	0.76	0.71
NDF disappearance (g/kg), 24 h	430.4	419.1	456.0	387.2	21.4	0.14	0.90	0.25
Concentrate								
$EF_{0.05}$ (g/kg)	715.5	727.7	714.5	711.0	16.1	0.68	0.42	0.47
$EF_{0.08}$ (g/kg)	651.5	671.6	648.7	647.5	11.7	0.47	0.32	0.42
a (g/kg)	303.0	294.7	291.1	300.5	8.2	0.90	0.68	0.27
P (g/kg)	901.2	873.4	903.1	894.5	21.4	0.45	0.63	0.68
Rate (/h)	0.118	0.157	0.116	0.114	0.016	0.34	0.24	0.29
Mixture								
$EF_{0.05}$ (g/kg)	640.0	652.0.	651.0	631.0	11.7	0.75	0.70	0.25
$EF_{0.08}$ (g/kg)	584.5	592.3	596.2	576.7	11.7	0.65	0.88	0.31
a (g/kg)	317.5	299.7	296.5	287.2	8.7	0.20	0.13	0.66
P (g/kg)	811.5	834.3	809.0	790.2	19.3	0.87	0.13	0.16
Rate (/h)	0.094	0.099	0.114	0.112	0.011	0.90	0.19	0.78

[1]LN = long hay; SH = short hay; HiSF = high soluble fibre; HiSt = high starch; PS = particle size; CONC = concentrate; PS × CONC = interaction of PS and CONC

[2]$EF_{0.05}$, $EF_{0.08}$ = effective degradability at an outflow rate of 0.05 and 0.08/h, respectively; *a* = rapidly degradable fraction; *P* = potential total degradability; rate = rate constant of degradability of slowly degradable fraction

tion. Udén and Van Soest (1984) found the highest *in situ* degradation rate of hay fibre for sheep compared to small and large heifers, ponies, and rabbits. The reduction of particle size, either through rechopping or grinding and pelleting, can generally increase the rate of digestion or complete the fermentation of soluble fractions of forage in the rumen (Thomson, 1972). A faster rate of degradation in the bags, which should reasonably reflect the degradation rate of lucerne in the rumen, might also imply that the particle size reduction increased bacterial attachment to forage particles by offering a greater surface area per unit weight. Allen and Mertens (1988) suggested that the rate of digestion is related to the fibre matrix and the surface area that is accessible to enzymes is positively related to the rate of digestion. The observation that the potential extent of digestion and effective degradability of lucerne were not affected by treatments ($P > 0.05$, Table 2) could indirectly indicate that positive effects of smaller particle size on the degradation rate of lucerne may be temporary and be negatively offset by a faster reduction in ruminal pH (Thomson, 1972). The lag time of degradation was unaffected by PS or CONC but was slightly longer with HiSF diets. This was in agreement with the result of Carey et al. (1993), who found that the lag times of brome hay DM degradation in the rumen of steers were not affected by supplementation of barley, maize or beet pulp when they were supplemented at around 200 g/kg of dietary DM.

The disappearance of lucerne cell wall in 4 h (a measure of the cell wall early fermentation rate) tended to decrease with reduced particle size (122 vs. 171 g/kg for SH vs. LN hay respectively, $P = 0.06$), but in 24 h (a measure of the extent of disappearance during a reasonable retention time) it was unaffected (403 vs. 443 g/kg). *In situ* 24-h lucerne hay NDF digestibility decreased from 350 to 260 g/kg in cattle induced with subacute ruminal acidosis (SARA; Krajcarsky-Hunt et al., 2002). In that study, the duration of pH below 5.6 was 10 h/day in SARA-induced cows versus 0.5 h/day in control cows. In our trial, SH diets had lower pH than LN diets from 1 h to 19 h post feeding, and differences were significant from 1.5 to 3.5 h and tended to be significant in 4 h (Table 5). Assuming a curvilinear pattern over time in ruminal pH with regard to once daily feeding and based on our pH reading intervals, the time that pH was below 5.6 was 4.5 h in SH and 0 h in LN diets. As depressed NDF digestion was relatively higher than that of Krajcarsky-Hunt et al. (2002), acidosis might have occurred in SH diets. However, the results of 24-h NDF disappearance suggest that as animals recover from short periods of acidosis, depression in fibre

Table 4. Effects of lucerne particle size and source of dietary carbohydrates on ruminal fermentation parameters in sheep

Item	Treatments[1]				SE	*P* <		
	LNHiSF	SHHiSF	LNHiSt	SHHiSt		PS	CONC	PS × CONC
Ammonia (mg/l)	133.70	93.90	73.10	101.30	22.40	0.81	0.25	0.15
Total VFA (mM)	90.74	89.12	86.63	84.07	17.61	0.77	0.94	0.57
Individual VFA (mol/100 mol)								
Acetate	74.44	67.57	72.84	69.61	3.21	0.17	0.94	0.57
Propionate	16.44	19.17	15.61	18.67	2.27	0.25	0.75	0.93
Butyrate	10.76	10.42	10.15	10.65	1.97	0.90	0.91	0.84
Isobutyrate	< 0.20	1.51	< 0.10	0.20	0.72	0.11	0.08	0.07
Valerate	0.70	1.21	0.78	1.11	0.32	0.98	0.80	0.80
Isovalerate	< 0.10	0.85	0.4	0.76	0.41	0.40	0.74	0.61
Acetate: propionate	4.74	3.81	4.59	3.77	0.63	0.19	0.86	0.92

[1]LN = long hay; SH = short hay; HiSF = high soluble fibre; HiSt = high starch; PS = particle size; CONC = concentrate; PS × CONC = interaction of PS and CONC

digestibility may be compensated for; hence the determination of cell wall disappearance in early fermentation hours may better represent treatment differences than the determination of the extent of disappearance.

None of the degradability parameters of concentrate and mixed samples was affected by treatments ($P > 0.05$, Table 3). The rate constant of fraction *b* of concentrate samples in diet SHHiSF was exceptionally high (0.157/h); otherwise the rate constant values of treatments were very similar. In mixed samples, however, HiSF diets had numerically lower rates of degradation compared to HiSt diets (0.097/h vs.0.113/h) and values for the rate constant of diets of different particle size were very similar (0.104/h for LN diets vs. 0.106/h for SH diets). The form of carbohydrate did not interact with PS and did not alter DM or NDF degradation. This was not consistent with the hypothesis of the work and contrasted with the results of other researchers who reported a positive effect of beet pulp, compared to grain, on total tract DM (Carey et al., 1993) or NDF (Carey et al., 1993; Cooper et al., 1996) digestibility of forage. The level of inclusion of beet pulp in lieu of grain in this study was lower than that of Carey et al. (1993), and Cooper et al. (1996). On the other hand, beet pulp used in this study was pelleted and then ground which, due to structural changes associated with pelleting and grinding, might result in a similar impact on DM and NDF degradation when compared to barley grain.

Ruminal fermentation

Except for the molar percentage of isobutyrate which tended to be higher ($P < 0.1$) in HiSF than in HiSt diets and showed a PS × CONC interaction ($P < 0.07$), other fermentation products were similar among treatments, although a numerical difference was observed in the acetate to propionate ratio between SH and LN diets. Bampidis and Robinson (2006) suggested that pectic by-products generally increase acetate and decrease propionate

Table 5. Effects of lucerne particle size and source of dietary carbohydrates on mean pH and changes in ruminal pH in sheep

Time relative to feeding (h)	Treatments[1]				SE	*P* <		
	LNHiSF	SHHiSF	LNHiSt	SHHiSt		PS	CONC	PS × CONC
Mean[2]	5.83	5.78	5.89	5.74	0.08	0.006	0.86	0.18
–0.5	6.84	7.00	6.78	6.79	0.11	0.47	0.23	0.49
0.5	6.11	6.18	6.13	6.13	0.09	0.71	0.88	0.69
1.0	5.91	5.95	6.05	5.89	0.06	0.31	0.51	0.12
1.5	5.79	5.74	5.88	5.75	0.04	0.002	0.03	0.04
2.0	5.68	5.63	5.83	5.66	0.05	0.02	0.06	0.16
2.5	5.65	5.61	5.74	5.59	0.05	0.02	0.28	0.12
3.0	5.66	5.59	5.71	5.55	0.05	0.03	0.83	0.27
3.5	5.68	5.56	5.69	5.56	0.06	0.02	0.91	0.83
4.0	5.70	5.57	5.63	5.57	0.06	0.09	0.46	0.52
6.0	5.66	5.45	5.60	5.47	0.11	0.18	0.81	0.74
8.0	5.96	5.71	5.75	5.67	0.13	0.17	0.29	0.46
19.0	6.20	6.45	6.48	6.17	0.14	0.81	0.91	0.09

[1]LN = long hay; SH = short hay; HiSF = high soluble fibre; HiSt = high starch; PS = particle size; CONC = concentrate; PS × CONC = interaction of PS and CONC

[2]time –0.5 h was not included

production resulting in higher acetate to propionate ratios. However, Leiva et al. (2000) found no difference in total VFA and acetate to propionate ratio between citrus pulp and maize hominy at about 250 g/kg of dietary DM. In support of the results of Leiva et al. (2000), our results showed that like in traditional mixed diets, where pectic- or soluble fibre enriched feeds generally account for less than 250 g/kg of dietary DM, rumen VFA concentrations are affected by the variety of dietary ingredients and results maybe different from those that are expected after fermentation of pectins and soluble fibre.

Ammonia nitrogen was unaffected by treatments (Table 4, $P > 0.05$), however, there was a relatively wide range of ammonia concentrations across treatments without an obvious explanation. Others have observed positive effects of pectin and soluble fibre in capturing ammonia and converting it into microbial protein (Remesy and Demigne, 1989; Hristov and Ropp, 2003).

The mean ruminal pH was significantly lower in SH diets but the concentrate type had no effect (Table 5). The PS × CONC interaction was significant only in 1.5 h post feeding when pH of LNHiSt diet was 0.13 units higher than SHHiSt, whereas the difference was only 0.06 units when LNHiSF diet was compared to SHHiSF (Table 5). As mentioned above, the SH diets had lower pH than LN diets from 1 h to 19 h post feeding and differences were significant from 1.5 h to 3.5 h post feeding. Hay of reduced particle size may promote a rapid ingestion rate which may lessen the contribution of salivation during eating and ruminating to buffer the considerable amount of acids during the period immediately after feeding, thus resulting in a marked reduction in ruminal pH (Thomson, 1972). Altering the source of carbohydrates decreased pH in a manner that changed with time. Feeding HiSF diets significantly decreased pH in 1.5 h and 2 h post feeding, while after 2.5 h there was no difference between CONC. These observations support findings of Leiva et al. (2000), who observed a consistently lower ruminal pH for a pectic diet until 8 h post feeding and after that the starchy diet had lower pH. They attributed those results to the faster degradation of sugars and soluble fibre in early fermentation hours compared to starch. However, as pH decreases, fermentation of soluble fibre is reduced, while starch degradation is continued. The lower pH in HiSF diets was observed for shorter durations and with a slighter degree when compared to Leiva et al. (2000), which may be due to the lower substitution rate of soluble fibre. The observation of significantly higher pH values in LN versus SH diets ($P = 0.01$) and lack of difference in mean pH between HiSF and HiSt diets ($P = 0.8$) suggest that longer particle size may have a stronger effect on maintaining an optimum rumen pH than altering the source of rapidly degraded carbohydrates.

CONCLUSIONS

The reduction of particle size in the diet of sheep significantly affected degradation kinetics of forage but not of concentrate and mixed diets. The mean ruminal pH was significantly affected by particle size while the type of concentrate affected ruminal pH only at some time points after feeding. Affected variables were mainly influenced by particle size and the hypothesis of study that aimed at establishing an interaction between forage particle size and concentrate type was not confirmed. Further research may clarify whether higher substitution rates of soluble fibre for starchy concentrate will modify rumen responses.

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REFERENCES

Allen M.S., Mertens D.R. (1988): Evaluating constraints on fiber digestion by rumen microbes. Journal of Nutrition, 118, 261–270.

AOAC (2002): Official Methods of Analysis. 17th ed. Association of Official Analytical Chemists. Arlington, USA.

ASAE (1996): S424.1. Method of determining and expressing particle size of chopped forage materials by sieving. In: Standards American Soc. Agric. Eng., St. Joseph, USA.

Bampids V.A., Robinson P.H. (2006): Citrus by-products as ruminant feeds: A review. Animal Feed Science of Technology, 128, 175–217.

Ben-Ghedalia D., Yosef E., Miron J., Est Y. (1989): The effect of starch- and pectin-rich diets on quantitative

aspects of digestion in sheep. Animal Feed Science of Technology, 24, 289–298.

Bhattacharya A.N., Sleiman F.T. (1971): Beet pulp as a grain replacement for cattle and sheep. Journal of Dairy Science, 54, 89–94.

Broderick G.A., Kang J.H. (1980): Automated simultaneous determination of ammonia and total amino acids in rumen fluid and *in vitro* media. Journal of Dairy Science, 63, 64–75.

Cannas A., Tedeschi L.O., Fox D.G., Pell A.N., Van Soest P.J. (2004): A mechanistic model for predicting the nutrient requirements and feed biological values for sheep. Journal of Animal Science, 82, 149–169.

Carey D.A., Caton J.S., Biondini M. (1993): Influence of energy source on forage intake, digestibility, *in situ* forage degradation, and ruminal fermentation in beef steers fed medium-quality brome hay. Journal of Animal Science, 71, 2260–2269.

Cooper S.D., Kyriazakis I., Oldham J.D. (1996): The effects of physical form of feed, carbohydrate source, and inclusion of sodium bicarbonate on the diet selections of sheep. Journal of Animal Science, 74, 1240–1251.

Faichney G.J., Brown G.H. (2004): Effect of physical form of lucerne hay on rumination and the passage of particles from the rumen of sheep. Australian Journal of Agricultural Research, 55, 1263–1270.

Faichney G.J., Teleki E., Brown G.H. (2004): Effect of form of lucerne hay on digestion and rate of passage in sheep. Australian Journal of Agricultural Research, 55, 1253–1262.

Flachowsky G., Koch H., Tiroke K., Matthey M. (1993): Influence of the ratio between wheat straw and ground barley, ground corn or dried sugar beet pulp on *in sacco* dry matter degradation of ryegrass and wheat straw, rumen fermentation and apparent digestibility in sheep. Archives of Animal Nutrition, 43, 157–167.

Grant R.J., Mertens D.R. (1992): Influence of buffer pH and raw corn starch addition on *in vitro* fiber digestion kinetics. Journal of Dairy Science, 75, 2762–2768.

Hristov A.N., Ropp J.K. (2003): Effect of dietrary carbohydrate composition and availability on utilization of ruminal ammonia nitrogen for milk protein synthesis in dairy cows. Journal of Dairy Science, 86, 2416–2427.

Krajcarsky-Hunt H., Plaizier J.C., Walton J.P., Spratt R., McBride B.W. (2002): Effect of subacute ruminal acidosis on *in situ* fiber digestion in lactating dairy cows. Journal of Dairy Science, 85, 570–573.

Krause K.M., Combs D.K. (2003): Effects of forage particle size, forage source, and grain fermentability on performance and ruminal pH in midlactation cows. Journal of Dairy Science, 86, 1382–1397.

Leiva E., Hall M.B., Van Horn H.H. (2000): Performance of dairy cattle fed citrus pulp or corn products as sources of neutral detergent-soluble carbohydrates. Journal of Dairy Science, 83, 2866–2875.

Remesy C., Demigne C. (1989): Specific effects of fermentable carbohydrates on blood urea flux and ammonia absorption in the rat cecum. Journal of Nutrition, 119, 560–565.

SAS (1999): User's Guide: Statistics, version 8, SAS Institute Inc., Cary, USA.

Thomson D.J. (1972): Physical form of the diet in relation to rumen fermentation. The Proceedings of the Nutrition Society, 31, 127–134.

Thomson D.J., Beever D.E., Coelho da Silva J.F., Armstrong D.G. (1972): The effect in sheep of physical form on the site of digestion of a dried lucerne diet. The British Journal of Nutrition, 28, 31–41.

Udén P., Van Soest P.J. (1984): Investigation of the *in situ* bag technique and a comparison of the fermentation in heifers, sheep, ponies, and rabbits. Journal of Animal Science, 58, 213–221.

Van Soest P.J., Robertson J., Lewis B. (1991): Methods for dietary fiber, neutral detergent fiber, and total nonstarch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583–3597.

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