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Effect of grinding size and sunflower oil addition on intake, digestibility, rumen function and microbial protein synthesis in sheep fed a dry total mixed ration

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Abstract

An experiment was carried out in which the effect of grinding a dry total mixed ration, based on barley straw, through 6 or 10 mm sieves on intake, digestibility, rumen function and microbial protein synthesis in sheep was assessed. The effect of including 1% sunflower oil as binder was also explored. None of the variables studied was affected by grinding size, hence the 10 mm sieve was recommended due to its lower cost in terms of energy consumed. On the contrary, addition of 1% sunflower oil significantly increased ammonia concentration in the rumen (83 *vs.* 161 mg L^{-1}; $p = 0.002$), potential degradabilities (as proportion) of dry matter (0.65 *vs.* 0.75; $p = 0.0037$), organic matter (0.68 *vs.* 0.75; $p = 0.0005$) and crude protein (0.78 *vs.* 0.90; $p = 0.0004$), and fractional rate of degradation (0.066 *vs.* 0.080 h^{-1}; $p = 0.046$) and effective degradability, as proportion (0.63 *vs.* 0.73; $p = 0.010$) of this latter. Oil addition (1%) is then recommended as binder, although a more economical option than sunflower oil should be explored due to its high cost.

Additional keywords: feeding costs; particle size.

Resumen

Efecto del tamaño de molienda y de la adición de aceite de girasol sobre la ingestión, digestibilidad, fermentación ruminal y síntesis de proteína microbiana en ovejas alimentadas con una ración integral seca

Se llevó a cabo un experimento en el que se estudió el efecto del tamaño de molienda (a través de cribas de 6 ó 10 mm de diámetro de malla) de una ración integral seca, constituida mayoritariamente por paja de cebada, y administrada a ovejas adultas, vacías y secas, sobre la ingestión, digestibilidad, características de la fermentación ruminal y síntesis de proteína microbiana. También se estudió el efecto, sobre las mismas variables, de la inclusión de un 1% de aceite de girasol utilizado como aglomerante. El tamaño de molienda no afectó a ninguno de los resultados, por lo que se recomienda la utilización de la criba de 10 mm, en virtud de su menor coste energético. Por el contrario, la adición de un 1% de aceite provocó un aumento significativo de la concentración de amoniaco en el rumen (83 *vs.* 161 mg L^{-1}; $p = 0{,}002$), de las degradabilidades potenciales de la materia seca (0,65 *vs.* 0,75; $p = 0{,}0037$), materia orgánica (0,68 *vs.* 0,75; $p = 0{,}0005$) y proteína bruta (0,78 *vs.* 0,90; $p = 0{,}0004$), y del ritmo fraccional de degradación (0,066 *vs.* 0,080 h^{-1}; $p = 0{,}046$) y de la degradabilidad efectiva (0,63 *vs.* 0,73; $p = 0{,}010$) de esta última. Por tanto, se recomienda la adición de un 1% de aceite como aglomerante, aunque dado el alto precio del de girasol es necesario buscar opciones más económicas.

Palabras clave adicionales: costes de alimentación; tamaño de partícula.

Introduction

During the last years important changes have occurred in many countries which have led to intensification (milk production) or semiextensification (pasture-based meat production) of sheep production systems. This has led to an improved efficiency of production, although feeding costs (including workforce) have considerably increased

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(Haghdoost *et al*., 2008; Wolfová *et al*., 2009). Total mixed rations (TMR), either wet or dry, have usually been used for small ruminants (Lock *et al*., 2008; Pinos-Rodríguez *et al*., 2008; Gómez-Cortés *et al*., 2009; Tufarelli *et al*., 2009), mainly during lactation, with the aim of reducing feed manipulation, feeding time and hence labour costs. However, one of the main issues when feeding this type of diets is the grinding particle size. An undersize of feed particles (mainly fibre) will dramatically decrease the time devoted to eating and ruminating and the production of saliva, likely to decrease the rumen pH, and increase the occurrence of lessions in the rumen wall (Cassida and Stokes, 1986; Mertens, 2000). On the contrary, an excess of low-quality long fibre may reduce intake and digestibility, compromising the energy balance of the animal (Allen, 1997).

National Research Council (NRC, 2001) guidelines have proven useful in defining dairy cattle requirements and feed composition but do not provide detailed recommendation of ration physical form. Current NRC recommendations only state that a minimum mean particle length of 3 mm for alfalfa diets is necessary to maintain rumen pH, chewing activity, and milk fat percentage. It is also recommended that diet neutral detergent fibre (NDF) be increased if excessively fine forages or high amounts of rapidly fermentable starch are fed. Due to the worldwide importance of the dairy milk industry, a great effort has been dedicated to the aim of solving what is understood as 'excessively fine forages', and how the concept is affected by type of forage, type of animal or forage to concentrate ratio (*e.g.* Lammers *et al*., 1996). However, for beef cattle or other species of ruminants not even an indication about these variables is given, let alone sheep, which economical impact is mainly restricted to marginal areas of the world.

Although it is recognised that there is ample evidence in the scientific literature on effects of dietary particle size on feed intake and rumen fermentation, there is no specific information about the effects of grinding a dry TMR for sheep through sizes commonly used in the Spanish compound feed industry.

On the other hand, the addition of oil to compound feeds is a usual practice which main aims are to contribute energy and to avoid dust production and orts (Wiseman, 1984; Patterson, 1989). For non-dairy sheep, the second objective is the most usual, requiring low levels of oil addition. However, its inclusion may have a series of negative consequences on fibre degradation in ruminants (Busquet *et al*., 2005; Fraser *et al*., 2007).

The present study was planned with the aim of testing the effect of two grinding sizes commonly used in the compound feed industry for sheep in Spain (6 and 10 mm) on intake, digestibility and rumen fermentation variables of a dry TMR based on barley straw. The effect on urinary excretion of allantoin, as an index of the microbial protein synthesis, was also studied. In addition, the influence of adding 1% sunflower oil over the cited variables was also evaluated.

Material and methods

Animals and diets

Four adult non-pregnant, non-lactating Rasa Aragonesa ewes, with an average initial live weight of 48 ± 0.7 kg, fitted with rigid ruminal (5 cm internal diameter) and flexible T-shape duodenal cannulae, were allocated to four treatments following a 4 × 4 latin square design. Treatments consisted on a dry total mixed ration (60% barley straw, 18% barley grain, 10% corn gluten feed, 10% soya-bean meal and 2% premix [370 g kg^{-1} sodium chloride, 1500 mg kg^{-1} zinc oxide, 300 mg kg^{-1} iodine (as calcium iodate anhidrous), 10 mg kg^{-1} sodium selenite, 45 IU kg^{-1} vitamin E and excipients]) ground through sieves of either 6 or 10 mm. Each meal (6 or 10 mm) was given unsupplemented or supplemented with 1% sunflower oil.

Animals were kept in slatted floor individual pens (110 cm × 90 cm) for the whole length of the trial. One month prior to the experiment sheep were treated against internal parasites with Albendazol (10 mL). Water was available at all times throughout the experimental period. Handling was carried out according to criteria from the European Union for care and use of laboratory animals in research, and the experimental protocol was approved by the Ethical Committee for Animal Research of the University of Zaragoza.

Experimental management

The experimental diets were offered twice daily (at 08:30 h and 20:30 h, for 24-h clock) *ad libitum*. Offer

Abbreviations used: ADF (acid detergent fibre); CP (crude protein); DM (dry matter); DOMI (digestible organic matter intake); EE (ether extract); NDF (neutral detergent fibre); OM (organic matter); TMR (total mixed ration); VFA (volatile fatty acids).

and orts were registered daily, and the latter withdrawn before morning meal. Daily samples of the four diets were obtained during the measuring week of each experimental period, pooled by period, ground through a 1 mm sieve and stored in capped plastic containers, at ambient temperature, until analysis of chemical composition. A subsample of the pooled material was for his part pooled for the whole experiment and used for particle size distribution. Mean live weight for each treatment was recorded, for all animals at the same time, at the beginning and the end of each experimental period.

The first period of the latin square started with a 21-day adaptation period to the diets, during which voluntary food intake was set (allowing 15% refusals). The fourth week was for measurements, and along its first day rumen fluid samples were taken just before feeding, and at 2, 4, 6, 8, 12 and 24 h after feeding. Rumen pH was immediately recorded and samples taken to determine ammonia and volatile fatty acids (VFA) concentration.

Digestibility was estimated using hentriacontane (C_{31}) as internal marker. Concentration in feed consumed was estimated from offer and refusals. Spot faecal samples were taken daily, just before morning feeding, for the whole measuring week. The samples from the 7 days were pooled to a single sample per animal and then freeze-dried and ground through a 1 mm sieve for analysis of ashes and n-alkanes.

Rumen degradability of dry matter (DM), organic matter (OM), crude protein (CP) and NDF was studied using the *in situ* method. Polyester bags (0.45 m pore size) were introduced in the rumen after the extraction of the first rumen fluid sample, and incubated for up to 96 hours.

Transit kinetics of solid and liquid phases of the digesta were assessed by introducing in the rumen (via cannula) pulse doses of 15 g of Yb-labelled diets and 50 mL of a Cr-EDTA solution, the first day of the measuring week, just after extraction of the first rumen fluid sample and before introduction of polyester bags. Faecal samples were subsequently taken at 2, 4, 8, 12, 24, 36, 48, 72, 96 and 120 h post dosing. An additional sample was taken before marker dosing for preparation of the analytical calibration curve. All samples were frozen at –20ºC until analysis.

Urinary excretion of allantoin, as an index of the microbial protein synthesis, was estimated from the allantoin/creatinine ratio in spot urine samples collected, at 9:30 h and with the aid of a catheter, on the fourth day of the measuring week, and from individual excretion of creatinine obtained at the end of the experiment. Urine samples were acidified, immediately after extraction, with a few drops of concentrated (95-98 %) sulphuric acid (until the pH was below 3.0), and frozen at –20ºC until analysis.

The last day of the measuring week rumens were emptied, and their contents weighed and sampled for chemical composition (DM, OM, CP, NDF and acid detergent fibre (ADF) and particle size distribution. Samples were frozen at -20ºC until analysis.

Adaptation to the following periods of the latin square was restricted to 10 days, due to scarce differences between diets.

At the end of the experiment animals were placed in metabolism crates (118 cm long, 46 cm wide and 73 cm high) and individual creatinine excretion assessed. Diet consisted in a mixture of 0.25 proportions of the four experimental diets. Three days were allowed for adaptation to the crates and then four for urine daily collection. This was carried out in plastic containers where 100 mL of a 10% sulphuric acid solution were placed to ensure the pH was below 3.0. Daily urine was weighed and its density recorded, and a 10% aliquot was kept at –20ºC until analysis.

Analytical procedures

Dry matter content in feeds, orts, faeces, polyester bags residues and rumen contents was determined by oven drying at 104°C for 24 h, whereas ash content was obtained after incineration at 550ºC for 8 h.

Total nitrogen (N) in feeds, polyester bags residues and rumen contents was determined following the Kjeldahl method using selenium as catalyst and a 2300 Kjeltec Analyzer Unit (Foss Tecator). Neutral detergent fibre in feeds, polyester bags residues and rumen contents, ADF in feeds and rumen contents, and Lignin in feeds were measured with an ANKOM 220 Fiber Analyser (Ankom Technology), as described by Mertens (2002), AOAC (2005; AOAC Official Method 973.18) and Robertson and Van Soest (1981) for NDF, ADF and Lignin, respectively. Either NDF, ADF and Lignin were expressed as ash-free residues. Ether extract (EE) in feeds was analysed according to the procedures described by AOAC (2005; AOAC Official Method 2003.05), using a Soxtec System HT1043 Extraction Unit, and a System 1044 Service Unit heating system.

Particle size distribution in feeds and rumen contents was determined using a wet sieving apparatus as described by Poppi *et al*. (1980), in five replicates per sample.

Rumen fluid VFA were determined by gas chromatography (Jouany, 1982), using an Agilent 6890 gas chromatograph fitted with on-column injector, a 30 m × 0.530 mm HP-FFAP capillary column (1.0 μm thickness), and flame ionization detector. The carrier gas was helium (10 mL min^{-1}) as was the make-up gas to the detector (45 mL min^{-1}). The injector was programmed to track the oven's temperature programme which was as follows: 150°C for 0.2 min, and two ramps of 10°C min^{-1} to 190°C, maintained for 0.2 min, and 25ºC min^{-1} to 240ºC, maintained for a further 25 min. Equilibrium time was set at 5 min. The detector was maintained at 250°C throughout the whole process. Injection volume was 0.2 μL, and peak area data were processed using the HP ChemStation software (version A.08.03). Detector response factors for individual VFA were determined by injecting onto the chromatograph a standard VFA mixture after every eight sample extracts.

Ammonia concentration in rumen fluid samples was analysed by the colorimetric method described by Chaney and Marbach (1962); n-alkanes in samples (0.5 g) of feeds, refusals and faeces were extracted following the technique proposed by Mayes *et al*. (1986), with the modifications described by Keli *et al*. (2008). Alkane analysis was carried out by on-column injection of 0.2 μL of the eluate onto a 30 m×0.530 mm HP-1 capillary column (1.5 μm thickness) in an Agilent 6890 gas chromatograph fitted with an automatic injector and flame ionization detector. The carrier gas was helium (10 mL min^{-1}) as was the make-up gas to the detector (45 mL min^{-1}). The injector was programmed to track the oven's temperature programme which was as follows: 230°C for 0.2 min and a ramp of 6°C min^{-1} to 300°C, maintained for a further 18 min. Equilibrium time was set at 5 min. The detector was maintained at 350°C throughout the whole process. Peak area data were processed using the HP ChemStation software (version A.08.03). Detector response factors for individual n-alkanes were determined by injecting onto the chromatograph a standard n-alkane mixture (C_{21}–C_{36} inclusive) after every eight sample extracts. Alkanes C_{22} and C_{34} were used as internal standards.

Yb-labelled diets were prepared as described by de Vega and Poppi (1997), and Cr-EDTA following the recommendations of Downes and McDonald (1964). Faecal concentration of Yb and Cr was analysed by atomic emission spectrometry-induced coupled plasma (de Vega and Poppi, 1997).

Creatinine and allantoin concentration in urine samples was determined by HPLC following the technique described by Balcells *et al*. (1992).

Mathematical and statistical methods

Hourly individual values of DM, OM, CP and NDF degradability, grouped by ewe and incubation period, were fitted to the equation $y = a + b[1 - exp(-ct)]$ proposed by Ørskov and McDonald (1979), where y represents the actual degradation after time t and a, b and c are estimates of the soluble fraction, the non-soluble degradable fraction and the fractional rate of degradation of fraction b, respectively. Adjustments were carried out using the Marquardt method of the PROC NLIN procedure of the SAS 8.02 programme, with the restriction $a + b \leq 1$. Effective degradability was calculated, for the fractional passage rates (k) of Yb-labelled diets, as $a + b[c/(c + k)]$.

Faecal marker concentrations were fitted to the model developed by Grovum and Williams (1973) $y = A_1 \exp(-k_1*(t\text{-}TT)) - A_2 \exp(-k_2*(t\text{-}TT))$, where y represents the faecal concentration of markers, k_1 and k_2 the slow and fast fractional passage rates, t the sampling time and TT the transit time, calculated as $(\ln A_2 - \ln A_1)/(k_1 - k_2)$. It was considered that only k_1 was of interest in the present experiment.

Mean particle size of feeds and rumen contents were estimated as suggested by Pond *et al*. (1984), where the calculated value indicates the size of the theoretical sieve which would retain 50% of the particles. Only one value was obtained per diet, with the average particle size distribution of the five replicates.

All studied variables (except particle size distribution of the diets) were subjected to analysis of variance following the model:

$$y = \mu + S_i + O_j + SO_{ij} + A_j + P_k + E_{l(ijk)}$$

where S_i represents the effect of the sieve size, O_j the oil-addition effect, SO_{ij} the interaction between sieve size and oil addition, A_j the animal effect, P_k the effect due to each period of the latin square, and $E_{l(ijk)}$ the experimental error. Contrasts between mean values were tested using the Tukey's test, except when there were missing values, where the Scheffe's test was used.

All calculations were made with the PROC GLM procedure of the SAS statistical package (version 8.02).

Results

Chemical composition and particle size distribution of diets

The chemical composition and particle size distribution of the four diets is shown in Table 1. Grinding size had an effect ($p < 0.05$) on NDF and EE contents, whereas oil addition influenced ($p < 0.05$) CP, NDF, Lignin and EE concentrations. The interaction between both variables was significant ($p < 0.05$) for OM and particle size distribution. Diets ground through a 10 mm sieve had a lower NDF and EE content than diets ground through a 6 mm sieve (528 *vs.* 568 g kg DM^{-1} and 20 *vs.* 22 g kg DM^{-1}, respectively). Added-oil diets showed a higher CP (120 *vs.* 95 g kg DM^{-1}) and EE (23 *vs.* 19 g kg DM^{-1}) content, and a lower NDF (533 *vs.* 563 g kg DM^{-1}) and Lignin (37 *vs.* 47 g kg DM^{-1}) content than non-added-oil diets. The OM content was higher for added-oil diets but only when they were ground through a 6 mm sieve.

With respect to particle size distribution, diets ground through a 6 mm sieve had higher proportions of medium size (between 0.15 and 1.2 mm) particles, and a lower proportion of large (>1.2 mm) particles. Added-oil diets had a higher proportion of small (<0.15 mm) and medium size particles, and a lower proportion of large particles ($p < 0.001$).

Intake, digestibility and rumen contents

Table 2 shows the average values of DM and OM intake and digestibility for the four experimental diets. Neither grinding size nor oil addition had an effect ($p > 0.05$) on the studied variables. Also the period ($p > 0.05$; except for DM and OM digestibility: $p < 0.05$) and animal ($p > 0.05$) effects were not significant.

Rumen contents of DM, OM, CP, NDF, ADF, and small (<0.15 mm), medium (>0.15 mm and <1.2 mm) and large (>1.2 mm) particles are given in Table 3. None of the variables was affected by grinding size or oil addition ($p > 0.1$), although animal variability had a highly significant ($p < 0.05$) impact (except for NDF and ADF contents, and mean particle size; $p > 0.05$). The experimental period did not influence the results ($p > 0.1$) in any case.

Rumen fermentation

Rumen contents pH was always close to neutrality (Table 4), and was not affected by grinding size ($p = 0.113$) or oil addition ($p = 0.447$).

Table 1. Chemical composition and particle size distribution of a dry total mixed ration as affected by grinding size (6 or 10 mm sieve) and sunflower oil addition (1%) (n = 4)

Sunflower oil (O)	No		Yes		SEM	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
DM, g kg^{-1}	905	905	898	903	0.3	0.162	0.500	0.452
OM, g kg DM^{-1}	924A	928	933B	930	0.1	0.004	0.596	0.035
CP, g kg DM^{-1}	91	98	120	120	0.4	0.001	0.436	0.417
NDF, g kg DM^{-1}	581	545	555	510	1.2	0.040	0.014	0.720
ADF, g kg DM^{-1}	329	312	305	288	1.0	0.057	0.144	0.970
Lignin, g kg DM^{-1}	49	46	35	38	0.2	0.004	0.919	0.262
EE, g kg DM^{-1}	20	19	25	21	0.1	0.003	0.026	0.190
Particles < 0.15, mm (proportion)	0.24aA	0.26bA	0.31aB	0.28bB	0.005	0.001	0.300	0.001
1.2 > Particles > 0.15, mm (proportion)	0.47aA	0.43bA	0.59aB	0.52bB	0.008	0.001	0.001	0.025
Particles > 1.2, mm (proportion)	0.29A	0.31A	0.10aB	0.20bB	0.007	0.001	0.001	0.001
Mean particle size, mm*	1.05	1.10	0.83	0.94				

SEM: standard error of the mean. P: probability of differences. DM: dry matter, OM: organic matter, CP: crude protein, NDF: neutral detergent fibre, ADF: acid detergent fibre, EE: ether extract. a, b: different lower case letters indicate differences ($p < 0.05$) between grinding sizes within each level of oil addition. A, B: different upper case letters indicate differences ($p < 0.05$) between levels of oil addition within each grinding size. *: ANOVA was not performed as only one value per diet was obtained (see Material and methods).

Table 2. Dry matter intake (DMI), organic matter intake (OMI), digestible organic matter intake (DOMI), dry matter digestibility (DMD) and organic matter digestibility (OMD) of a dry total mixed ration fed to sheep ground through 6 or 10 mm sieves, and with (1%) or without sunflower oil added (n = 4)

Sunflower oil (O)	No		Yes		SEM	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
DMI (g day^{-1})	936	864	791	915	92.0	0.630	0.784	0.329
(g kg (W$^{0.75}$)$^{-1}$)	50.7	46.9	44.9	50.8	5.42	0.874	0.854	0.405
OMI (g day))	864	803	738	853	85.6	0.676	0.760	0.345
(g kg (W$^{0.75}$)$^{-1}$)	46.8	43.5	41.9	47.3	5.04	0.922	0.835	0.423
DOMI (g day^{-1})	419	417	332	416	16.0*	0.213	0.322	0.126
(g kg (W$^{0.75}$)$^{-1}$)	21.8	21.1	20.6	22.6	1.05*	0.243	0.169	0.156
DMD (g kg^{-1})	468	499	462	465	32.8*	0.518	0.669	0.310
OMD (g kg^{-1})	482	518	447	458	34.4*	0.514	0.664	0.306

SEM: standard error of mean. P: probability of differences. W: weigth. *: residual standard deviation (missing data).

Concentration of VFA was affected by grinding size, but only with added-oil diets (116 *vs.* 174 mmol L^{-1} for diets ground through sieves of 6 or 10 mm, respectively; $p = 0.043$), whereas ammonia concentration was affected by oil addition (161 *vs*. 83 mg L^{-1} for diets with or without sunflower oil added, respectively; $p = 0.002$).

Molar proportions of acetic and propionic acids were not affected by either grinding size or oil addition ($p > 0.05$), whereas there were differences due to oil addition in the molar proportions of butyric acid (8.2 *vs.* 9.0 mmol/100 mol VFA; $p = 0.049$).

The experimental period significantly affected pH values ($p = 0.0330$), VFA concentration ($p = 0.0118$) and molar proportions of acetic ($p = 0.0171$) and butyric ($p = 0.0068$) acids, whereas animal variability was only significant for rumen ammonia concentration ($p = 0.0314$), and molar proportions of acetic ($p = 0.0346$) and propionic ($p = 0.0135$) acids.

Transit kinetics, rumen degradability, and urinary excretion of allantoin

Table 5 shows the k_l values of Yb-labelled particles and liquid phase (Cr-EDTA) of the digesta through the gut, as well as urinary excretion of allantoin, as an index of the microbial protein synthesis in the rumen.

None of the variables was affected by either grinding size or oil addition ($p > 0.1$). Experimental period and animal variability had no effect ($p > 0.1$).

Table 3. Rumen contents (g) of dry matter (DM), organic matter (OM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), small (< 0.15 mm) particles, medium (> 0.15 mm and < 1.2 mm) particles and large (> 1.2 mm) particles in sheep fed a dry total mixed ration ground through 6 or 10 mm sieves, and with (1%) or without sunflower oil added

Sunflower oil (O)	No		Yes		SEM	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
DM	606	492	513	554	39.9	0.292	0.154	0.966
OM	503	401	451	481	82.9*	0.564	0.522	0.623
CP	89	79	84	114	14.8*	0.118	0.168	0.265
NDF	333	243	289	276	50.7*	0.351	0.211	0.236
ADF	210	163	193	189	38.7*	0.609	0.534	0.408
Small particles	227	194	205	242	25.3	0.281	0.364	0.823
Medium particles	332	260	270	246	23.6	0.101	0.111	0.483
Large particles	46	39	39	57	6.4	0.334	0.169	0.485
Mean particle size (mm)	0.45	0.41	0.44	0.38	0.039	0.645	0.254	0.828

SEM: standard error of mean; P: probability of differences; *: residual standard deviation (missing data).

Table 4. pH, average concentrations of ammonia (NH_3; mg L^{-1}) and volatile fatty acids (VFA; mmol L^{-1}), and molar proportions of acetic (Ac), propionic (Prop) and butyric (But) acids in the rumen of sheep fed a dry total mixed ration ground through 6 or 10 mm sieves, and with (1%) or without sunflower oil added

Sunflower oil (O)	No		Yes		SEM	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
pH	6.86	6.83	7.02	6.78	0.072	0.447	0.113	0.210
NH_3	84	82	142	179	14.5	0.002	0.275	0.225
VFA	142	139	116a	174b	10.6	0.700	0.043	0.030
Ac	0.73	0.71	0.70	0.71	0.009	0.083	0.465	0.068
Prop	0.17	0.19	0.19	0.17	0.008	0.837	0.742	0.063
But	0.08	0.08	0.09	0.09	0.003	0.049	0.349	0.450

SEM: standard error of mean; P: probability of differences. a, b: different lower case letters indicate differences ($p < 0.05$) between grinding sizes within each level of oil addition.

Degradation parameters of DM, OM, CP and NDF are shown in Table 6. Potential degradability of DM was affected ($p = 0.0037$) by oil addition but not by grinding size ($p = 0.3442$), whereas neither fractional rate of degradation nor effective degradability were affected ($p > 0.1$) by either grinding size or oil addition. Experimental period and animal variability had no effect. Higher OM potential degradabilities were observed with oil-added diets ($p = 0.0005$), together with no effect of grinding size or oil addition on OM fractional rate of degradation or effective degradability ($p > 0.1$).

Oil addition significantly increased CP potential degradability ($p = 0.0004$), fractional degradation rate ($p = 0.0464$) and effective degradability ($p = 0.0102$), whereas no effect of oil addition or grinding size on NDF degradation parameters was observed ($p > 0.1$).

Discussion

Effect of grinding size

Diets ground through a 6 mm sieve had more fibre than diets ground through a 10 mm sieve (Table 1). This is contrary to the findings of Emanuele and Staples (1988), who working with "Tifton 78" bermudagrass and "Florigraze" rhizoma peanut, observed that the smaller the sieve size the higher the CP content and the lower the NDF concentration. However, those authors worked with individualized materials, and in the present experiment TMR were used. It may be speculated that most ingredients (except straw) would produce more dust when passed through the 6 mm than through the 10 mm screen, hence loosing more non-fibrous material. This fact is compatible with the greater amount of small plus medium size particles found in diets ground through the smaller sieve (Table 1). In addition, the differences in physical disruption would have been enough to promote differences in chemical composition, but not enough to modify digestibility (Table 2).

Lack of statistical differences in intake and digestibility between diets ground through 6 mm or 10 mm sieves might reflect the effectiveness of the chewing during eating and ruminating processes in achieving a particle size of the digesta which would not limit the filling capacity of the rumen. This particle size has been established in 1.18 mm in sheep (Poppi *et al*., 1980), and despite the large amounts of particles >1.2 mm found in the diets used in the present work, and the relatively large differences between treatments (Table 1), the amount of large particles in the rumen was considerably reduced (Table 3), and differences between diets considerably diminished (between 7% and 10% of the ruminal dry matter were particles with a size >1.2 mm).

Absence of differences between diets in the rumen contents of NDF and ADF (Table 3) can be seen as another factor enhancing the importante of chewing during eating and ruminating in reducing the particle size of the cell walls (Ulyatt *et al*., 1986). Reduced fibre particles may be evacuated from the rumen and this process may help to explain the non different intakes ($p > 0.05$) found for the four diets used in the present experiment (Table 2). To this respect, it has been well known for a long time that 'fibre' is the fraction of the foods that mainly limit intake (Balch and Campling, 1962; Conrad *et al*., 1964; Aitchison *et al*., 1986).

Values of pH, and concentration of VFA and ammonia found in the present work (Table 4) were com-

Table 5. Slow fractional passage rate of Yb-labelled particles (k_1Yb; h^{-1}) and of Cr-EDTA (k_1Cr; h^{-1}), and urinary excretion of allantoin (ExcrAl: mmol day^{-1}) in sheep fed a dry total mixed ration ground through 6 or 10 mm sieves, and with (1%) or without sunflower oil added

Sunflower oil (O)	No		Yes		RSD	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
k_1Yb	0.041	0.025	0.034	0.045	0.0167	0.362	0.768	0.223
k_1Cr	0.059	0.056	0.051	0.050	0.0224	0.609	0.892	0.943
ExcrAl	10.9	7.8	8.9	8.8	1.04	0.629	0.178	0.203

RSD: residual standard deviation (missing data); P: probability of differences.

parable to those reported by other authors with diets based in high proportions of barley straw and fed to sheep (Fondevila *et al*., 1993; 1994). Unexpected differences were found for VFA concentration, with no differences between the two rations with no oil addition, but significant differences between the two ones with oil addition. No justification can be found for these results. With respect to the proportions of the different VFA produced in the rumen, they were typical of a rumen environment driven by fibre fermentation (Fondevila *et al*., 1994). For its part, ammonia concentration was not affected by grinding size, being always above the minimum level considered to limit microbial synthesis (50 mg L^{-1}; Satter and Slyter, 1974).

In the present experiment there were no statistical differences between diets in transit kinetics. Lack of differences in fractional rate of passage between diets ground at different sizes was already pointed out by Faichney (1983, 1993) and Allen (1996) in animals fed *ad libitum*, and effectiveness of chewing during eating and ruminating in reducing particle size can be, at least in part, responsible for this fact.

Lack of differences between diets in urinary excretion of allantoin (Table 5) were probably a result of the combined effects of a non different digestible organic matter intake (DOMI; Table 2) and a rumen ammonia concentration not limiting microbial synthesis (Table 4). The low values for all four diets seem to agree with the low DOMI recorded in this experiment. It must be taken into account that animals had been fistulated in rumen and duodenum for more than four years, and that although there are evidences that fistulization does not affect intake and performance of the animals (Cruickshank *et al*., 1990), these evidences have been obtained with animals fistulated for not as long as those used in the present work. Also allantoin excretion data

Table 6. Potential (a + b, as proportion) and effective (ED, as proportion) degradability, and fractional rate of degradation (c; h^{-1}) of dry matter (DM), organic matter (OM), crude protein (CP) and neutral detergent fibre (NDF) in sheep fed a dry total mixed ration ground through 6 or 10 mm sieves, and with (1%) or without sunflower oil added

Sunflower oil (O)	No		Yes		SEM	P		
Grinding size (mm; S)	6	10	6	10		O	S	O×S
DM a + b	0.67	0.62	0.75	0.75	0.034	0.004	0.344	0.309
DM c	0.038	0.059	0.046	0.044	0.0063	0.637	0.187	0.121
DM ED	0.43	0.51	0.54	0.50	0.034	0.373	0.706	0.178
OM a + b	0.67	0.69	0.75	0.75	0.015	0.001	0.345	0.613
OM c	0.035	0.041	0.045	0.044	0.0028	0.058	0.434	0.279
OM ED	0.42	0.51	0.53	0.49	0.034	0.407	0.639	0.142
CP a + b	0.76	0.82	0.89	0.90	0.015	0.001	0.159	0.068
CP c	0.071	0.060	0.086	0.074	0.0055	0.046	0.086	0.914
CP ED	0.58A	0.67	0.75B	0.70	0.020	0.016	0.541	0.031
NDF a + b	0.59	0.59	0.70	0.56	0.042	0.354	0.156	0.166
NDF c	0.028	0.059	0.036	0.045	0.0110	0.811	0.119	0.367
NDF ED	0.21	0.33	0.37	0.26	0.034	0.375	0.747	0.080

A, B: different upper case letters indicate differences ($p < 0.05$) between levels of oil addition within each grinding size; SEM: standard error of mean; P: probability of differences.

must be interpreted with caution since it is known that purine bases are not completely degraded in the rumen (Pérez *et al*., 1996; Vicente *et al*., 2004).

Degradation parameters were not affected by grinding size in any case, and this indicates that fermentation, and hence microbial activity, and retention time in the rumen, were not affected by grinding size either. Results shown in Tables 3, 4 and 5 support this statement.

Effect of oil addition

Oil addition elicited, in general, a much more apparent effect than grinding size on most of the variables studied. This is a common practice in compound feeds factories, which main objective is to avoid losses and dust formation. The objective was attained in the present experiment, as demonstrated by the higher proportion of small and medium size (and hence lower proportion of large particles) in added-oil diets (Table 1). The agglomerating effect was then probably responsible for the higher CP and EE, and lower NDF and lignin contents shown by these TMR.

It has been argued (Busquet *et al*., 2005; Fraser *et al*., 2007) that oil addition may have negative consequences on fibre fermentation. However, at the low level used in the present experiment it had no effect on rumen function variables (Table 4), except for ammonia concentration. This latter increased with oil addition, but was probably more related to the indirect effect of the higher CP concentration of the added-oil diets (Table 1), due in turn to the agglomerating effect of oil. In addition, added-oil diets showed a higher CP effective degradability (Table 6). The combined effect of these two factors might explain differences in ammonia concentration between non-added and added-oil diets. The low level of oil addition used in the present experiment (1%) was clearly not sufficient to depress bacterial activity. On the contrary, it increased potential degradability of DM, OM and CP (Table 6), and fractional rate of degradation and effective degradability of the latter. This effect cannot be attributed to an increase in energy availability for the microbes, as oil is not fermented. The higer amount of small and medium size particles, which size would be more rapidly reduced to pass the polyester bags pores, may be the reason. As small particles have more CP (Emanuele and Staples, 1988), the effect of oil addition on protein degradation would be of more importance.

Conclusions

In the present experiment, grinding a dry total mixed ration, based on barley straw, through sieves of 6 mm or 10 mm had no effect on intake, digestibility or rumen function in sheep. On the contrary, addition of 1% sunflower oil significantly increased ammonia concentration in the rumen, potential degradabilities of DM, OM and CP, and fractional rate of degradation and effective degradability of this latter

Due to the high cost of grinding through small sieves, it is recommended that the 10 mm size is adopted for dry total mixed rations based on barley straw and fed to sheep. Oil addition (1%) had a positive effect and its incorporation is recommended. However, and due to the high cost of sunflower oil, a more economical option should be explored.

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