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Effects of feed intake on composition of sheep rumen contents and their microbial population size

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The present study was conducted to determine the effect of feed intake on the composition of the rumen contents of sheep and on their bacterial densities. Whole rumen contents were sampled after a period of continuous inter-rumen infusion of $^{15}NH_3$ from four rumen-cannulated wethers successively fed on a hay–concentrate diet (2:1, w/w on a DM basis) at two rates of feed intake: 40 and 80 g DM/kg body weight$^{0·75}$. Total weight and chemical composition of rumen contents, as well as the distribution by size and chemical composition of particles, were determined. The populations of bacteria associated with the liquid (liquid-associated bacteria, LAB) and solid (solid-associated bacteria, SAB) fractions of rumen digesta and the distribution of SAB according to feed particle size were also examined. The greater feed intake caused an increase in the mass of the rumen contents, while its chemical composition did not change, except for a higher content of organic matter ($P=0·023$). The distribution of feed particles by size was similar at both levels of intake. The concentrations of neutral- and acid-detergent fibre in feed particles decreased and those of total, dietary, and microbial N increased, both with a quadratic response ($P=0·001$), as particle size decreased. The proportion of LAB in the microbial biomass of rumen digesta reached only 8·0 %. This proportion and the density of LAB were unaffected by the level of feed intake, whereas an apparent reduction (10·4 %) occurred with the SAB biomass in whole rumen contents. A systematic, but not significant, reduction (mean value 11·9 %) in the level of microbial colonisation in the different particle fractions with the increase of feed intake was also observed.

Rumen contents: Chemical composition: Feed intake: Microbial population size

Increasing levels of feed intake raises the weight of the rumen contents and/or its rumen outflow rate (Ellis *et al.* 1984). In addition, the level of feed intake affects the rumination and, therefore, the particle distribution of rumen digesta (Bae *et al.* 1979). Rumen outflow rate, composition of rumen contents and particle distribution are factors that may affect the microbial growth and density, and therefore the efficiency of the microbial synthesis and the microbial contribution to the post-rumen nutrient flow. The effect of the intake level on the efficiency of microbial synthesis is considered in the metabolisable protein system (Agricultural and Food Research Council, 1992) through the influence of the plane of feeding. Nevertheless, the effects of feed intake on the microbial density of rumen contents have received little attention, in spite of their possible influence on the microbial growth and on microbial degradative actions on the feeds.

Bacteria in rumen digesta are composed of two different populations: liquid-associated bacteria (LAB) and solid-associated bacteria (SAB), with different chemical compositions (Merry & McAllan, 1983). Some of the differences are related to compounds used as internal (diaminopimelic acid, nucleic acids or purines) or external (^{15}N, ^{35}S) microbial markers. Greater marker:N ratios have been observed systematically for LAB compared with SAB for all these markers (Merry & McAllan, 1983; Martin *et al.* 1994; Benchaar *et al.* 1995; Rodríguez *et al.* 2000). For practical reasons, microbial synthesis in the rumen has usually been calculated using marker:N ratios determined in LAB, even though these bacteria only represent a small fraction of the total bacterial population (Czerkawski & Breckenridge, 1982; Legay-Carmier & Bauchard, 1989). The importance of the resultant under-evaluation of microbial synthesis is logically dependent on the LAB:SAB ratio in

Abbreviations: ADF, acid-detergent fibre; ADL, acid-detergent lignin; LAB, liquid-associated bacteria; NAN, non-ammonia-nitrogen; NDF, neutral-detergent fibre; OM, organic matter; SAB, solid-associated bacteria.

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the rumen outflow. Therefore, this under-evaluation can be derived from the same ratio in rumen digesta and from the ratio between the outflow rates of its respective associated phases. The proportions of LAB and SAB in the rumen show a large variation (Legay-Carmier & Bauchart, 1989; Martin & Michalet-Doreau, 1995), which suggests the need for further research on the proportions of both bacterial populations.

The purpose of the present study was to determine the effect of the feed intake on: (1) the weight, chemical composition and particle distribution by size of the rumen contents; (2) the extent of microbial attachment in relation to particle size; (3) the microbial cell density in the liquid and solid phases of rumen digesta.

Materials and methods

Animals and feeding

Four wethers (average body weight 62·2 kg) equipped with detachable rumen fistulas (inner diameter 80 mm) were fed a diet composed of chopped vetch (*Vicia sativa*)–oat (*Avena sativa*) hay (55:45, w/w) and concentrate (hay–concentrate 2:1, w/w on a DM basis). The concentrate contained (g/kg): maize 607, dehydrated beet pulp 300, soyabean meal 45, fishmeal 20, bentonite 15, minerals and vitamins 13. This diet was offered as six meals per d, at intervals of 4 h. Two successive experimental periods comparing two rates of DM intake were carried out. In the first, all wethers were fed at 40 g DM/kg body weight$^{0·75}$ and in the second, at 80 g DM/kg body weight$^{0·75}$, representing 90 % of the voluntary intake of these animals as measured previously. Both rates of feed intake represent 1·1- and 2·2-times energy maintenance requirements. The chemical compositions of hay and concentrate were described in a previous study (Rodríguez *et al.* 2000).

Experimental procedures

After a 14 d period of adaptation to the diet, the rumen micro-organisms were labelled with a 50 % enriched $(^{15}NH_4)_2SO_4$ solution (ICON Service Inc., Mt. Marion, NY, USA) to determine the microbial colonisation of feeds (Rodríguez *et al.* 1999), the chemical composition of bacteria (Rodríguez *et al.* 2000) and the bacterial population size. This solution (200 mg N/l) was continuously infused into the rumen for at least 30 d at a rate of 400 ml/d. After this period, the total reticulo-rumen content was obtained by manual emptying, just before the first morning meal. To avoid a bias between the liquid and solid phases during sampling, when emptying, the rumen contents were squeezed through a nylon cloth (pore size 200 μm) and the liquid and solid fractions were weighed and recorded. Immediately, both fractions were homogenised and grab samples were taken to reconstitute (by weight) three samples of 200 (sample A), 500 (sample B) and 1200 (sample C) g. Finally, the remaining fractions of solid and liquid were reintroduced into the rumen.

Sample A was freeze-dried and used to determine chemical composition. Analyses of organic matter (OM), N, non-NH_3-N (NAN), neutral-detergent fibre (NDF), acid-detergent fibre (ADF), acid-detergent lignin (ADL) and ^{15}N abundance (^{15}N:N) were performed.

Sample B was squeezed through a double layer of nylon cloth (pore size 46 μm). The solid and liquid phases obtained were weighed and their relative proportions recorded. Next, several subsamples were composed (by weight) in accordance with these proportions, as follows: (1) two samples of 2 g each (with the addition of 0·8 ml 0·4 M-HCl as preservative) for analyses of NH_3-N concentration and its ^{15}N abundance; (2) two samples of 50 g each for the determination of the DM content (oven-dried for 24 h at 40°C and subsequently for 24 h at 80°C); (3) two samples of 150 g each, processed through wet sieving, to determine the particle size distribution. Sieves (200 mm internal diameter) with square meshes of 2·500, 1·250, 0·630, 0·315, 0·160 and 0·080 mm on a side were used in an electromagnetic sieve shaker (Model 200; Filtra SA, Barcelona, Spain) with a flow of 2 litres saline solution (9 g NaCl/l distilled water)/min. The different sieves were removed 15, 20, 24, 27, 29 and 30 min respectively after the beginning of the sieving process. The sieves were each dried at room temperature (20–22°C) for 1 h, transferred to an oven (24 h at 40°C then 24 h at 80°C) and weighed to determine the DM retained. Samples from each pore size were analysed for NDF, ADF, ADL, N and ^{15}N abundance.

Sample C was squeezed as indicated earlier and used for bacterial isolation and quantification. Retained particles (enclosed in the cloth) were subjected to three successive cycles of washing and shaking in a total volume of 700 ml saline solution (9 g NaCl/l distilled water) at 4–5°C in order to remove trapped-fluid-associated micro-organisms. The rumen liquid and saline wash fractions obtained were subjected to double centrifugation (Barr *et al.* 1975) to obtain LAB samples. The total bacterial pellets were lyophilised, weighed and analysed for DM and N. SAB were isolated from the washed solid phase and from the precipitate resulting from the first slow centrifugation during LAB isolation. Prior to isolation, one in eight of these samples were lyophilised, pooled and subjected to determinations of DM, N and ^{15}N abundance. The remaining materials were re-suspended in saline solution (9 g NaCl/l; 2 litres/kg) and homogenised. The homogenate was pummelled for 6 min in a stomacher and squeezed as described earlier for samples B and C. SAB were isolated from this liquid by the double centrifugation method described earlier. The bacterial pellets obtained were lyophilised and subjected to the same determinations. Proportions of N and DM from SAB in the total particulate phase, or in its fractions, were determined as follows:

$$\text{SAB-N (\%)} = (^{15}\text{N excess in PP}/^{15}\text{N excess in SAB}) \times 100,$$

and

$$\text{SAB-DM (\%)} = \text{SAB-N (\%)} \times \frac{(\text{N concentration in PP}/\text{N})}{\text{concentration in SAB}},$$

where PP is the particulate phase.

The ^{15}N excess was calculated assuming a natural abundance of 0·3663 atom%.

Analytical procedures

The analytical procedures used to determine the chemical composition of the rumen contents (DM, OM and N) were those described by the Association of Official Analytical Chemists (1984). Fibre fractions were determined as indicated by Robertson & Van Soest (1981). N isotopic proportions in SAB, LAB and rumen digesta were analysed by isotope–ratio MS (VG Prism II IRMS; VS Isotech, Warrington, Ches., UK) linked in series to a Dumas–style N analyser EA 1108 (Carlo Erba Instruments, Milan, Italy). The NH_3 concentration was measured by distillation with 10 ml sodium tetraborate (25 g/l) in boric acid (10 g/l) and titration with 0·01 M − HCl.

Statistical analysis

Results were subjected to ANOVA using a simple complete block experimental design, except for the results of the chemical composition of the different particle size fractions, for which a split–plot arrangement of treatments was used. In this case, the feed intake (low *v.* high) was considered as the whole–plot treatment and was tested using animal × feed intake interaction as the error term. Pore size and its interaction with the feed intake were the sub–plot treatments. Since animals were adult, feed–restricted and maintained in a controlled environment, the period effect was assumed to be negligible. All statistical analyses were performed using the Statistical Analysis System for Windows software (version 6.12; SAS Institute Inc., Cary, NC, USA).

Results

Weight and chemical composition of the rumen contents

The rumen contents, expressed per kg body weight$^{0·75}$, increased significantly with the rise of feed intake both in terms of fresh matter ($P < 0·016$) or DM ($P < 0·028$) (Table 1). DM concentration and the proportion of the solid phase in rumen digesta were not affected by the increase in feed intake.

Most of the differences in the chemical composition of the rumen digesta (Table 2) were not significant, except for a higher content of OM ($P = 0·023$) and a decrease in

Table 1. Effect of feed intake on DM distribution in total rumen contents*
(Mean values with their standard errors for four sheep)

	Feed intake†			
Item	Low	High	SEM	Statistical significance of effect: *P*
Fresh matter (g/W$^{0·75}$)	215	303	12·4	0·016
DM (g/W$^{0·75}$)	25·0	38·5	2·39	0·028
DM (%)	11·7	12·7	0·33	0·121
Solid phase (%)‡	49·5	53·8	2·20	0·261

W, weight.
* For details of diets and procedures, see pp. 98, 99.
† Low, 40 g DM/kg body weight$^{0·75}$; high, 80 g DM/kg body weight$^{0·75}$.
‡ Squeezed through a nylon cloth (pore size 46 μm).

Table 2. Effect of feed intake on the chemical fractions of rumen contents and their mean rumen retention times*
(Mean values with their standard errors for four sheep)

	Feed intake†			
Item	Low	High	SEM	Statistical significance of effect: *P*
Chemical composition (% DM)				
Organic matter	84·9	88·0	0·51	0·023
Neutral-detergent fibre	51·9	55·9	1·10	0·086
Acid-detergent fibre	29·6	33·7	1·44	0·137
Acid-detergent lignin	7·44	7·05	0·30	0·424
Non-NH_3-N (× 6·25)	22·5	21·2	0·40	0·113
^{15}N/Non-NH_3-N (%)	0·496	0·436	0·006	0·005
Mean retention time (h)				
Organic matter	14·5	11·5	0·78	0·076
Neutral-detergent fibre	20·4	16·9	1·34	0·161
Acid-detergent fibre	19·5	17·2	1·66	0·399
Acid-detergent lignin	36·6	26·5	1·42	0·015
Non-NH_3-N	25·0	17·2	0·92	0·010
^{15}N	22·6	16·7	0·90	0·021

* For details of diets and procedures, see pp. 98, 99.
† Low, 40 g DM/kg body weight$^{0·75}$; high, 80 g DM/kg body weight$^{0·75}$.

the ^{15}N:NON-NH_3-N ratio ($P=0{\cdot}005$) at the higher intake. Nevertheless, the decrease was associated with the methodology employed, as the marker infusion dose was the same for both rates of feed intake. Table 2 also shows the mean retention time (h) in the rumen of the corresponding chemical fractions calculated as the amount present in the reticulo-rumen:daily intake. The higher feed intake produced a generally lower mean retention time with the different chemical variables, this effect being significant only for NAN, ^{15}N abundance and ADL ($P = 0{\cdot}010$, $P=0{\cdot}021$ and $P=0{\cdot}015$ respectively).

Distribution by size, chemical composition and bacterial colonisation of rumen particles

The distribution of particle size in rumen contents (Table 3) was not markedly affected by the higher feed intake, although a trend towards a higher proportion of medium-size particles (retained in sieves between 0·630 and 1·250 mm) was detected. This increase was balanced by a trend towards a decrease ($P=0{\cdot}084$) in the proportion in the effluent fraction (<0·080 mm), consisting of very small particles, free micro-organisms and substances dissolved in the liquid phase.

The level of feed intake did not affect the chemical composition or the SAB content of particles, expressed in terms of DM or N. The feed intake × sieve size interaction was not significant either. These results were thus pooled for both levels of intake. In contrast, the sieve size presented a strong and quadratic effect ($P<0{\cdot}001$) on the bacterial colonisation of particles and on most chemical components, except ADL. The evolution of these variables is presented in a logarithmic scale in Figs 1 and 2. The concentration of NDF and ADF in particles (Fig. 1(a)) showed a progressive reduction with the decrease in particle size. In contrast, the concentration of N (Fig. 1(b)) showed an increase, with the minimum value at the 1·250 mm sieve size. A similar progression was detected for the feed N concentration in particles, but with the minimum value corresponding to the 0·630 mm sieve size.

Even if, as indicated earlier, the bacterial biomass was not significantly affected by the level of feed intake, systematically lower values were observed for the high feed intake (mean value 11·9 %). In contrast, no difference was found in the proportion of total N in particles coming from bacteria (mean values 693 *v.* 698 mg SAB-N/g N for low and high feed intake respectively). Bacterial biomass showed a progressive increase with the reduction of the sieve size. However, when this value was expressed in terms of N, the maximum values were recorded for the 0·630 mm sieve size (Fig. 2).

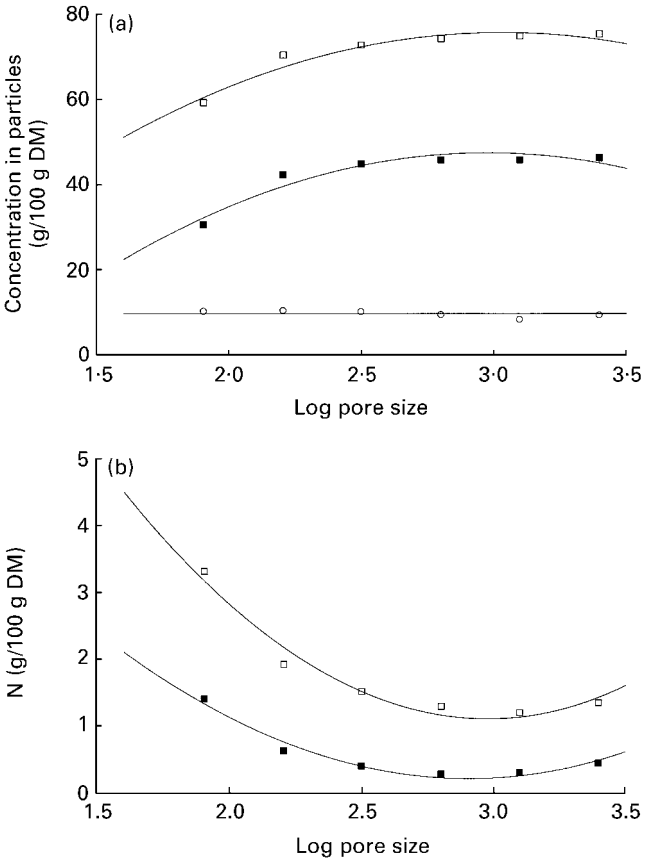

Fig. 1. Relationships between the particle size (logarithm of the pore size (p, μm)) of rumen contents and the concentration in particles (g/100 g DM) of (a) neutral-detergent fibre (NDF, □), acid-detergent fibre (ADF, ■) and acid-detergent lignin (ADL, ○) or (b) total N (□) and feed N (■). For details of diets and procedures, see pp. 98, 99. Regression equations were: NDF $= -36{\cdot}1 + 73{\cdot}2 \times p - 12{\cdot}1 \times p^2$ (RSD 2·95, R^2 0·795, $P<0{\cdot}001$, n 48); ADF $= -72{\cdot}1 + 79{\cdot}5 \times p - 13{\cdot}4 \times p^2$ (RSD 2·66, R^2 0·827, $P<0{\cdot}001$, n 48); total N $= 17 - 10{\cdot}7 \times p + 1{\cdot}79 \times p^2$ (RSD 0·21, R^2 0·927, $P<0{\cdot}001$, n 48); feed N $= 9{\cdot}46 - 6{\cdot}47 \times p + 1{\cdot}12 \times p^2$ (RSD 0·13, R^2 0·909, $P<0{\cdot}001$, n 48).

Table 3. Effect of feed intake on the particle size distribution (% DM) of rumen contents*
(Mean values with their standard errors for four sheep)

	Pore size (mm)						
Feed intake†	<0·080	0·080	0·160	0·315	0·630	1·250	2·500
Low	35·7	7·30	9·00	12·6	11·1	4·41	19·8
High	32·8	6·40	9·15	13·0	13·5	5·77	19·4
SEM	0·81	0·40	0·38	0·31	0·56	0·30	1·14
Statistical significance of effect: *P*	0·084	0·206	0·799	0·393	0·060	0·052	0·838

* For details of diets and procedures, see pp. 98, 99.
† Low, 40 g DM/kg body weight$^{0{\cdot}75}$; high, 80 g DM/kg body weight$^{0{\cdot}75}$.

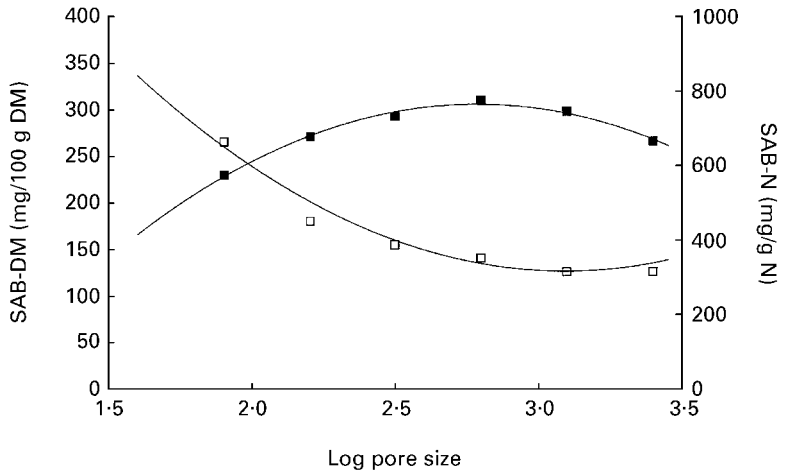

Fig. 2. Relationships between the particle size (logarithm of the pore size (p, μm) of rumen contents and the solid-associated bacteria (SAB) content of particles in terms of DM (mg SAB-DM/ 100 g DM; □) and N (mg SAB-N/g N; ■). For details of diets and procedures, see pp. 98, 99. Regression equations were: $SAB{-}DM = 1040{\cdot}9 - 585{\cdot}9 \times p + 94{\cdot}7 \times p^2$ (RSD 21·8, R^2 0·848, $P<0{\cdot}001$, n 48); $SAB{-}N = -1101{\cdot}3 + 1395{\cdot}4 \times p - 250{\cdot}5 \times p^2$ (RSD 53·0, R^2 0·747, $P<0{\cdot}001$, n 48).

Microbial population distribution and size

Table 4 shows the mean values of the content and density of bacteria in rumen digesta for both rates of feed intake. A significant increase of the absolute SAB fraction, both expressed in terms of DM or N ($P=0{\cdot}015$ and $P=0{\cdot}006$ respectively), was observed with the higher feed intake. The LAB fraction, expressed in the same terms, also gave higher values with the increase in the feed intake, although the differences were not significant in this case. The proportion of the LAB fraction (approximately 8·0 %) and its density in the liquid phase were the same for both feed intakes. In the solid phase, a moderate but non-significant decrease (10·4 %) of the SAB biomass was recorded at the higher feed intake. In contrast, the proportions of N from SAB were similar. The same trend was observed in both variables for the sum of SAB and LAB in whole rumen contents.

Discussion

The higher feed intake led, in the present study, to an increase in the rumen repletion and also of the rumen outflow rate (Rodríguez *et al.* 2000) as a result of the higher rumen pressure and rumination activity. These effects agree with findings by several authors (Minson, 1966; Aitchison *et al.* 1986; Robinson *et al.* 1987).

The only significant effect on the chemical composition was the increase in % OM ($P=0{\cdot}023$), which agrees with results obtained by Robinson *et al.* (1987). This variation can be attributed to the decrease in the ash concentration

Table 4. Effect of feed intake on the bacterial biomass associated with the solid (SAB) and liquid (LAB)-associated bacteria phases of rumen contents*

(Mean values with their standard errors for four sheep)

Feed intake…†	Low	High	SEM	Statistical significance of effect: *P*
Total LAB in rumen contents				
DM (g)	15·4	19·6	2·20	0·268
N (g)	1·06	1·40	0·15	0·614
Total SAB in rumen contents				
DM (g)	174	234	8·39	0·015
N (g)	12·2	17·0	0·48	0·006
LAB proportion (LAB/(LAB + SAB))				
% DM	8·06	7·88	1·19	0·924
% N	7·91	7·68	1·07	0·887
Microbial density				
LAB-DM (g)/kg liquid phase	4·83	4·84	0·71	0·999
SAB-DM (g)/kg solid phase-DM	375	336	16·3	0·188
SAB-N (mg)/g NAN‡	782	773	38·1	0·889
(SAB + LAB)-DM (g)/kg rumen contents DM	336	295	16·0	0·168
(SAB + LAB)-N (mg)/g NAN§	817	813	29·9	0·942

NAN, non-NH_3-N.
* For details of diets and procedures, see pp. 98, 99.
† Low, 40 g DM/kg body weight$^{0·75}$; high, 80 g DM/kg body weight$^{0·75}$.
‡ NAN from the solid phase of rumen contents.
§ NAN from the whole rumen contents.

of bacteria observed in the same experiment (18 mg/g; Rodríguez *et al.* 2000) and to the reduction (Table 4) of the rumen bacterial density. Thus, both factors together explain 92 % of the observed variation in rumen OM.

The lower reductions observed for the rumen mean retention time for ADF (11·8 %) and NDF (17·2 %) than for ADL (27·6 %), which can only disappear from the rumen by transit, showed a reduction in the extent of fibre degradation with the increase in feed intake. This observation was confirmed by a significant depression of the fractional degradation rate of the ADF (39·4 %) and of the NDF (27 %) in the vetch–oat hay in the diet (results not shown). The reduction in mean retention time observed in N, and especially in ^{15}N, is in agreement with a reduction of internal recycling of N in the rumen associated with a lower micro-organism lysis and a higher efficiency of microbial protein synthesis, as observed elsewhere (Hespell & Bryant, 1979; Harrison & McAllan, 1980; Ørskov, 1988) as a consequence of the acceleration of the rumen outflow.

The low variation observed between both rates of feed intake on the distribution of particles by size (Table 3) agrees with the results obtained by Kovács *et al.* (1997). Nevertheless, the trends detected may also indicate that rumination was slightly less efficient at the higher rate, which agrees with results of Bae *et al.* (1979).

The quadratic reductions of the NDF and ADF concentrations with decreasing particle size (Fig. 1(a)) suggests that particles $>0{\cdot}630$ mm come mainly from the hay, while in those of smaller size, the proportion of particles coming from the concentrate increased progressively. This scheme is based on the constancy of the NDF and ADF concentrations in particles $>0{\cdot}630$ mm; these concentrations (ranging from 74·3 to 75·5 % and from 45·8 to 46·3 % for NDF and ADF respectively) were similar to those in the insoluble DM fraction of the original hay (73·9 and 46·2 % for NDF and ADF respectively). The progressive decrease obtained with the reduction of particle size $<0{\cdot}630$ mm is in accordance with the lower concentration in fibre in the raw materials of the concentrate.

The quadratic increase ($P<0{\cdot}001$) in the microbial biomass in particles as their size decreased (Fig. 2) may be attributed to the cumulative microbial colonisation associated with the increase in the rumen residence time derived from the process of particle comminution. On the other hand, this latter process increases the particle surface and the damaged areas on the plant tissues, increasing the possibilities of microbial adhesion and colonisation (Cheng *et al.* 1984; Pond *et al.* 1984). Legay-Carmier & Bauchart (1989) did not detect differences in the microbial content of particles retained by different sieves, but observed a great increase in the bacterial DM content of the effluent not retained by a pore size of 0·100 mm. These results partially agree with our present findings, in which the main part of the detected effect was observed in the smallest particles, which is in turn in agreement with the results obtained *in vitro* by Gerson *et al.* (1988). The main role of comminution on the bacterial content of feed particles was also reinforced by the inverse trend of the bacterial and fibre contents in relation to particle size, in spite of the fact that fibre can be considered as the main substrate for bacterial adherence (Michalet-Doreau & Ould Bah, 1989; Rodríguez *et al.* 1999).

The proportions of free bacteria (mean value 8·0 %) showed that these micro-organisms constituted a small fraction of the rumen biomass, which agrees with the value of 10 % obtained by Czerkawski & Breckenridge (1982). Our present results also support other studies, where SAB were largely predominant in the bacterial biomass (Legay-Carmier & Bauchart, 1989; Martin & Michalet-Doreau, 1995). However, determinations of SAB are always carried out indirectly, due to the impossibility of releasing all adherent bacteria from particles. In this respect, other types of adherent micro-organisms are considered as bacteria in these studies. In addition, in the present experiment, the sample used to calculate the total SAB content included the pellet collected from the first centrifugation of the liquid phase, which contains the non-adherent protozoa. Therefore, in this sample, SAB values tend to be an estimate of the whole microbial biomass of digesta, excluding free bacteria. For the microbial markers most usually employed to measure microbial synthesis (diaminopimelic acid, purines, ^{15}N) the marker:N ratio is higher in LAB than in SAB (Merry & McAllan, 1983; Martin *et al.* 1994; Benchaar *et al.* 1995; Rodríguez *et al.* 2000). Therefore, its use as reference sample of rumen micro-organisms leads to an important underestimate of rumen microbial protein flow and of the efficiency of the microbial synthesis.

The results of the present research also showed that the higher feed intake had no effect on the proportion of free bacteria in the total microbial biomass or on their density in the liquid phase. In contrast, the microbial density of the particulate matter may decrease with the higher feed intake. Thus, the apparent reduction in the microbial density in the total solid phase (10·4 %) was also observed systematically in all the particle size fractions (mean reduction 11·9 %). Nevertheless, the flow of microbial N leaving the rumen increased largely as a consequence of the higher rumen contents (Table 1), but also due to the increase in the liquid and solid outflow rates and in the N content of bacteria determined in the same experiment (Rodríguez *et al.* 2000), which is in agreement with the increase in microbial efficiency usually observed at higher feed intakes.

The microbial N:NAN ratio observed in the whole rumen contents as well as in the total solid phase showed that micro-organisms represented the main part of the nitrogenous compounds of rumen digesta. The value of this ratio was unaffected by the feed intake.

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References

Agricultural and Food Research Council (1992) Technical committee on responses to nutrients. Nutritive requirements of

ruminant animals: protein. Report no. 9. *Nutrition Abstracts and Reviews* **62**, 787–835, (Series B).
Aitchison EM, Gill M, Dhanoa MS & Osbourn DF (1986) The effect of digestibility and forage species on the removal of digesta from the rumen and the voluntary intake of hay by sheep. *British Journal of Nutrition* **56**, 463–476.
Association of Official Analytical Chemists (1984) *Official Methods of Analysis*, 14th ed, Arlington, VA: AOAC.
Bae DH, Welch JG & Smith AM (1979) Forage intake and rumination by sheep. *Journal of Animal Science* **49**, 1292–1299.
Barr GW, Bartley EE & Meyer RM (1975) Feed processing. 8. Estimating microbial protein in rumen fluid with precipitating agents or in incubated mixtures of uncooked grain plus urea or starch with differential centrifugation. *Journal of Dairy Science* **58**, 1308–1319.
Benchaar C, Bayourthe C, Vernay M & Moncoulon R (1995) Composition chimique des bactéries libres ou adhérentes au contenu du rumen et du duodénum chez la vache (Chemical composition of bacteria free or attached to rumen or duodenum content in cows). *Annales de Zootechnie* **44**, Suppl., 139.
Cheng KJ, Stewart CS, Dinsdale D & Costerton JW (1984) Electron microscopy of bacteria involved in the digestion of plant cell walls. *Animal Feed Science and Technology* **10**, 93–120.
Czerkawski JW & Breckenridge G (1982) Distribution and changes in urease (*EC* 3·5.1·5) activity in rumen simulation technique (Rusitec). *British Journal of Nutrition* **47**, 231–348.
Ellis WC, Matis JH, Pond KR, Lascano CE & Telford JP (1984) Dietary influences on flow rate and digestive capacity. In *Herbivore Nutrition in the Subtropics and Tropics*, pp. 269–293 [FMC Gilchrist and RI Machie, editors]. Johannesburg: The Science Press.
Gerson T, King ASD, Kelly KE & Kelly WJ (1988) Influence of particle size and surface area on *in vitro* rates of gas production, lipolysis of triacylglycerol and hydrogenation of linoleic acid by sheep rumen digesta or *Ruminococcus flavefaciens*. *Journal of Agricultural Science, Cambridge* **110**, 31–37.
Harrison DG & McAllan AB (1980) Factors affecting microbial growth yields in the reticulo-rumen. In *Digestive Physiology and Metabolism in Ruminants*, pp. 205–226 [Y Ruckebusch and P Thivend, editors]. Lancaster: MTP Press Limited.
Hespell RB & Bryant MP (1979) Efficiency of rumen microbial growth: influence of some theoretical and experimental factors on Y ATP. *Journal of Animal Science* **49**, 1640–1659.
Kovács PL, Südekum K-H & Stangassinger M (1997) Rumen contents and ruminal and faecal particle size distribution in steers fed a mixed diet at three amounts of intake. *Animal Feed Science and Technology* **64**, 143–154.
Legay-Carmier F & Bauchart D (1989) Distribution of bacteria in the rumen contents of dairy cows given a diet supplemented with soyabean oil. *British Journal of Nutrition* **61**, 725–740.
Martin C & Michalet-Doreau B (1995) Variations in mass and enzyme activity of rumen micro-organisms: Effect of barley and buffer supplements. *Journal of the Science of Food and Agriculture* **67**, 407–413.
Martin C, Williams AG & Michalet-Doreau B (1994) Isolation and characteristics of the protozoal and bacterial fractions from bovine ruminal contents. *Journal of Animal Science* **72**, 2962–2968.
Merry RJ & McAllan AB (1983) A comparison of the chemical composition of mixed bacteria harvested from the liquid and solids fractions of rumen digesta. *British Journal of Nutrition* **50**, 701–709.
Michalet-Doreau B & Ould-Bah MY (1989) Estimation of the extent of bacterial contamination in bag residues and its influence on in sacco measurements of forage nitrogen degradation in the rumen. In *Proceedings of the XVI International Grassland Congress, 4–11 October 1989, Nice, France*, pp. 909–910 [AFPF, editor]. Versailles: Association Française pour la Production Fourragère.
Minson DJ (1966) The apparent retention of food in the reticulorumen at two levels of feeding by means of an hourly feeding technique. *British Journal of Nutrition* **20**, 765–773.
Ørskov ER (1988) *Nutrición proteica de los rumiantes (Protein nutrition in ruminants)*. Zaragoza: Acribia SA.
Pond KR, Ellis WC & Akin DE (1984) Ingestive mastication and fragmentation of forages. *Journal of Animal Science* **58**, 1567–1574.
Robertson JB & Van Soest PJ (1981) The detergent system of analysis and its application to human foods. In *The Analysis of Dietary Fiber in Food*, pp. 123–158 [WPT James and O Theander, editors]. New York: Marcel Dekker Inc.
Robinson PH, Tamminga S & van Vuuren AM (1987) Influence of declining level of feed intake and varying the proportion of starch in the concentrate on rumen ingesta quantity, composition and kinetics of ingesta turnover in dairy cows. *Livestock Production Science* **17**, 37–62.
Rodríguez CA, González J, Alvir MR & Repetto JL (1999) Microbial nitrogen contamination of in sacco ruminal incubated feeds. In *Protein Metabolism and Nutrition. Book of Abstracts of the VIIIth International Symposium on Protein Metabolism and Nutrition*, pp. 68 [GE Lobley, A White and JC MacRae, editors]. Wageningen: Wageningen Pers.
Rodríguez CA, González J, Alvir MR, Repetto JL, Centeno C & Lamrani F (2000) Composition of bacteria harvested from the liquid and solid fractions of the rumen of sheep as influenced by intake level. *British Journal of Nutrition* **84**, 369–376.