Role of rumen protozoa in nitrogen digestion in sheep given two isonitrogenous diets

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1. The effect of protozoa on digestion in the rumen was studied using either defaunated or faunated sheep.

2. Six wethers, each fitted with rumen and simple duodenal cannulas, were given two isonitrogenous diets containing either lucerne (*Medicago sativa*) hay (diet L) or sodium hydroxide-treated wheat straw (diet S). The diets were given in eight equal portions per day at 3-h intervals. The mean intake of dry matter, 53 g/kg body-weight$^{0.75}$ per d, was similar for the two diets and each diet had a similar digestible organic matter content. Diet L promoted a large protozoal population and was rich in nitrogen sources of low rumen-degradability, while diet S supported a smaller protozoal population and was rich in rumen-degradable N.

3. Digesta flow at the duodenum was estimated by means of a dual-marker technique using chromium-mordanted lucerne hay and polyethylene glycol as markers. The microbial flow at the duodenum was estimated using diaminopimelic acid (DAPA), nucleic-acid purine bases (PB) and ^{35}S incorporation simultaneously. The different microbial markers were compared in the defaunated sheep. Protozoal N contribution was estimated in faunated sheep.

4. Defaunated sheep had lower rumen ammonia concentrations and molar proportions of butyric acid than faunated sheep, but they had higher molar proportions of propionic acid.

5. Rumen organic matter digestion was reduced by defaunation, but this decrease was compensated for by increased intestinal digestion.

6. There was a net increase of N flow (approximately 10 g/d) between mouth and duodenum in defaunated sheep. This was explained by increases in both microbial and dietary N flows from the rumen compared with faunated sheep.

7. The influence of protozoa on solid- and liquid-phase retention times in the rumen is discussed, as well as the protozoal contribution to microbial N flow in the duodenum of faunated sheep.

Although some work has demonstrated the proteolytic activity of protozoa (Warner, 1956; Williams *et al.* 1961; Abou Akkada & Howard, 1962), the quantitative contribution of these micro-organisms to the degradation of feed protein in the rumen is still unclear. Moreover, few in vivo experiments have examined the effect of protozoa on rumen bacterial protein synthesis (Lindsay & Hogan, 1972; Ushida *et al.* 1984). Recently, Australian workers (Bird & Leng, 1978; Bird *et al.* 1979) demonstrated the positive effect of defaunation on animal growth when given a high-energy diet with low content of rumen-undegradable protein. These results suggest that defaunation increases protein flow to the small intestine as suggested by Veira *et al.* (1984), and this increase in the protein available to the animal has a marked effect on animal performance under circumstances in which the intestinal protein supply is limiting.

The objective of the present study was, therefore, to investigate quantitatively the contribution of protozoa to nitrogen digestion in sheep, and particularly the effect of defaunation on the passage of dietary and microbial N to the duodenum. The protozoal contribution to duodenal N is also discussed.

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Table 1. *Composition of diets*

Diet...	L	S
Ingredient (g/kg)		
Lucerne (*Medicago sativa*) hay	650	—
Sodium hydroxide-treated wheat straw	—	668
Barley	300	—
Sugar-beet pulp	—	142
Soya-bean cake	—	52
Groundnut cake	—	86
Urea	—	9
Wheat straw	27	—
Vitamin–mineral supplement*	—	19
Chromium-mordanted lucerne hay	23	24
Chemical composition		
Organic matter (g/kg DM)	921	898
Nitrogen (g/kg DM)	20	19
N solubility†	25·0	43·0
Metabolizable energy† (MJ/kg DM)	9·82	6·48

DM, dry matter.

* Contained vitamins and minerals (g/kg DM) as folows: $Ca(H_2PO_4)_2$ 550, NaCl 260, sulphur powder 10, $MgSO_4$ 90, Na_2SO_4 70, trace elements 20 ($ZnSO_4$ 9·5, $MnSO_4$ 4·8, $CuSO_4$ 1·0, $FeSO_4$ 4·7, $CoSO_4$ 20 mg, CaI_2 35 mg, Na_2SeO_3 10 mg); vitamin A 0·3, vitamin D_3 0·1, vitamin E 1·0.

† Calculated with the values from Institut National de la Recherche Agronomique (1978).

MATERIALS AND METHODS

Animals and diets

Six Texel wethers, 1–2 years old and weighing 60 kg body-weight, were used. Each sheep was fitted with a rumen cannula (60 mm internal diameter) and a simple duodenal cannula (20 mm internal diameter). They were defaunated (Jouany & Senaud, 1979*a*) 2 months before the start of the experiment. At the end of the defaunated period (period 1), the sheep were inoculated with rumen contents taken from sheep given various diets (period 2).

Two isonitrogenous diets were given at the rate of 53 g dry matter/kg body-weight$^{0.75}$ per d. One diet (L), which favoured a large protozoal population and which was high in poorly degradable protein, was composed mainly of lucerne (*Medicago sativa*) hay. The other diet (S), which did not promote a large protozoal population and which was rich in highly degradable protein, was composed mainly of sodium hydroxide-treated wheat straw. The details of the diets are given in Table 1. Both diets were pelleted and given in eight equal meals daily at 3-h intervals by means of an automatic feeder.

Experimental procedure

The experiment consisted of two main periods (period 1, sheep defaunated; period 2, sheep refaunated), each divided into two sub-periods. The six sheep were divided into two groups of three (groups 1 and 2). During the first sub-period of periods 1 and 2, group 1 was given diet L and group 2 diet S. The feeding was switched in the second sub-period: group 1 was given diet S and group 2 diet L. Each sub-period lasted for 6 weeks, consisting of a 3-week period of adaptation to the diet and a 3-week period of sampling.

Chromium-mordanted lucerne hay, prepared as described by Uden *et al.* (1980), was incorporated into the diet and given at a rate of 2 g Cr/d. A solution of polyethylene glycol (PEG; molecular weight 4000) was infused via the rumen cannula at a rate of 30 g PEG/d.

These markers were used simultaneously and the introduction began 1 week before the sampling period. $Na_2{}^{35}SO_4$ was included in the solution of PEG 3 d before the collection of duodenal digesta and its infusion continued for 9 d at the rate of 250 μCi/d per animal.

Collection of faeces, duodenal digesta and rumen contents

The 1st week (days 1–7) of the sampling period for each trial was used for faeces collection. Duodenal digesta (70 ml) was taken every 2 h on days 8 and 9. Half of each duodenal sample was filtered through a nylon filter (Blutex XX; Tripette & Renaud, Paris), 250 μm mesh, immediately after being taken. Filtrates and whole digesta were then pooled separately and stored at $-20°$. Rumen contents (400 ml) were taken every 2 h for 10 h on day 10; samples were combined and rumen bacteria obtained by differential centrifugation (Jouany & Thivend, 1972). The isolated bacteria were then freeze-dried immediately. Rumen contents (250 ml) were taken at 1, 2, 4, 8, 12, 24 and 30 h after cessation of marker infusions on day 13 and stored at $-20°$. These samples were used to determine the rate of passage of liquid, solid and microbial phases from the rumen.

During the faunated period, protozoal numbers were determined by the method of Jouany *et al.* (1981) on samples taken 2 h after feeding on at least five successive days.

Chemical analyses

Dry matter (DM) content was determined by drying at 80° for 48 h and organic matter (OM) content was determined by ashing at 600° for 6 h. Total N was determined by a Kjeldahl method and ammonia-N in rumen and duodenal contents as described by Weatherburn (1967). Rumen volatile fatty acid (VFA) analysis was carried out as described by Jouany (1982). PEG was determined turbidimetrically as described by Malawer & Powel (1967) and Cr was determined using a modified method of Mathieson (1970). Nucleic acid content was estimated by measurement of the purine bases (PB) (Ushida *et al.* 1985).

α,ϵ-Diaminopimelic acid (DAPA) was determined using ion-exchange chromatography (Collombier, 1981). Organic ^{35}S was measured as described by Mathers & Miller (1980) using Instagel (Packard Instrument Corp., Grove, USA) as scintillation fluid and a Tricarb 460 CD spectrometer (Packard Instrument Corp.).

Calculations

The amount of each constituent entering the duodenum was calculated using a dual-marker technique (Faichney, 1975), based on 100% recovery of both PEG and Cr. The measured mean recovery rates of PEG in periods 1 and 2 were 0·942 (SE 0·030) and 0·956 (SE 0·026), respectively, while those for Cr were 0·925 (SE 0·027) and 0·957 (SE 0·021). These results indicate that there were no systematic differences in marker recovery between defaunated and faunated sheep.

Estimation of microbial N (MN) flow was made by dividing the amount of microbial marker entering the duodenum by the ratio of the same marker:N content of the bacteria.

Rumen-undegraded feed protein was estimated by subtraction of MN and endogenous N from duodenal non-NH_3-N (NAN). Endogenous N flow was assumed to be 1 g/d (Weston & Hogan, 1967).

Rumen fractional turnover rates were estimated by fitting the determined marker concentrations to the equation $C = C_0 e^{-kt}$ (Faichney, 1975), where C_0 and C are the concentrations of the marker at time 0 and time t respectively, and k is the fractional turnover rate of the selected marker and associated phase. Pool sizes of PEG and Cr were estimated by multiplying the amount of marker introduced into the rumen (g/h) by the retention time of the marker (h). Division of PEG and Cr pool sizes by corresponding C_0 values gave the rumen fluid volume and size of the rumen DM pool respectively.

Table 2. *Volatile fatty acids (VFA) and ammonia concentration in defaunated and faunated sheep*

| | | NH_3-nitrogen (mg/l) | | Total | Molar proportions (mmol/mol) | | | | | |
		Mean	Range	VFA (mmol/l)	Acetate	Propionate	Isobuty-rate	Butyrate	Isovale-rate	Valerate
L	DF	54·1	17·8–93·3	74·5	649·7	222·6	6·2	96·7	9·0	12·3
L	F	139·9	97·8–181·8	82·4	663·7	177·1	7·0	128·3	6·9	13·2
S	DF	98·0	78·8–126·8	57·9	721·2	198·7	5·2	59·6	5·6	8·8
S	F	190·8	125·0–237·0	65·9	707·7	169·9	7·3	95·1	6·7	10·9
SEM		12·9	—	3·8	7·1	6·5	0·3	7·1	0·6	0·5
Statistical significance:										
DF *v.* F		**		NS	NS	**	**	**	NS	*
L *v.* S		**		*	**	NS	NS	**	NS	**

DF, defaunated; F, faunated; NS, not significant.
* $P < 0.05$, ** $P < 0.01$.
† For details, see Table 1.

Table 3. *Organic matter (OM) digestion in defaunated and faunated sheep*

Diet†	Animals	OM intake (g/d)	OM apparently digested (g/d) in the:		Whole-tract digestibility
			Rumen	Intestine	
L	DF	1019	299	302	0·590
L	F	1032	448	186	0·614
S	DF	1059	324	294	0·592
S	F	1064	415	220	0·597
SEM		4	15	13	0·005
Statistical significance:					
DF *v.* F		**	**	**	NS
L *v.* S		**	NS	NS	NS

DF, defaunated; F, faunated; NS, not significant.
** $P < 0.01$.
† For details, see Table 1.

Statistical analyses

The values from one sheep given diet S in the first sub-period in period 1 were eliminated because of feed refusal. The values were analysed, therefore, according to the analysis of variance for unbalanced data (Gill, 1978). Comparison between the MN flow estimation by three marker methods was made using one-way analysis of variance (Snedecor & Cochran, 1971).

RESULTS

There was no protozoal contamination throughout the defaunated period. Sheep fed on diets L and S had 7.6×10^5 protozoa/ml and 3.5×10^5 protozoa/ml respectively during the faunated period. Dietary effects on the numbers of protozoa were highly significant ($P < 0.01$). *Entodinium* sp. accounted for more than 90% of the mixture of protozoa with both diets. *Polyplastron multivesiculatum* accounted for 5–6% of the population, while holotrichs accounted for only 0·5–1%. Diet L favoured the development of *Entodinium* sp. and *P. multivesiculatum* but the holotrichs were not affected by this diet.

Rumen fermentation patterns are shown in Table 2. NH_3 levels in the defaunated animals were lower than those in the faunated animals ($P < 0.01$) and they were lower with diet L than with diet S ($P < 0.01$). Sometimes extremely low rumen NH_3 levels were observed in some defaunated sheep given diet L: for example, 17·8, 28·0, 49·0 mg NH_3-N/l (see Table 2). Rumen total VFA concentration was lower in defaunated sheep than in faunated sheep, but the difference was not statistically significant. Dietary effects on total VFA concentration were also apparent; diet L promoted a significantly higher total VFA concentration than did diet S. In the VFA mixture, defaunation increased the molar proportion of propionic acid (C_3) and reduced the proportions of butyric acid (C_4) and valeric acid (C_5). Diet had a clear effect on the molar proportions of acetic acid (C_2), C_4 and C_5, but it had little effect on C_3 and branched-chain VFA. The C_2 proportion was higher and the proportions of C_4 and C_5 were lower with diet S than with diet L.

The OM digestibilities in the whole digestive tract were similar for the two diets (Table 3). Although defaunation reduced rumen OM digestion, the digestibility in the whole tract was not affected. This suggests that defaunation caused a shift in digestion from the rumen

Table 4. *Nitrogen digestion (g/d) in defaunated and faunated sheep*

Diet†	Animals	N intake	Duodenal N flow		Duodenal MN flow				Rumen-undegraded feed N flow‡				Faecal N output	N digestibility	
			TN	NAN	^{35}S	PB	DAPA	SEM	^{35}S	PB	DAPA	SEM		Whole tract	Intestines§
L	DF	22·1	33·6	32·8	14·7[a]	18·1[a]	17·7[a]	1·1	17·1[a]	13·7[a]	14·1[a]	0·8	8·6	0·611	0·738
L	F	22·8	24·2	23·4	12·6[ab]	15·3[a]	12·1[b]	0·5	9·8[a]	7·1[a]	10·3[a]	0·7	8·0	0·649	0·658
S	DF	21·8	31·1	29·9	18·8[a]	18·5[a]	19·2[a]	1·2	10·1[a]	10·5[a]	9·8[a]	0·9	8·0	0·633	0·732
S	F	23·7	25·1	23·8	16·2[a]	16·1[a]	15·5[a]	0·9	6·7[a]	6·7[a]	7·3[a]	0·7	7·4	0·688	0·689
SEM		0·2	1·1	1·1	0·8	1·0	0·8		1·1	0·9	0·8		0·1	0·007	0·010
Statistical significance:															
DF *v.* F		**	**	**	NS	NS	**		**	**	*		**	**	**
L *v.* S		NS	NS	NS	*	NS	NS		**	NS	**		**	*	NS

[a, b] Values with different superscript letters were statistically significantly different ($P < 0.05$) between microbial markers.

DF, defaunated sheep; F, faunated sheep; NS, not significant; TN, total nitrogen; NAN, non-ammonia-N; MN, microbial nitrogen; PB, nucleic-acid purine bases; DAPA, α, ε-diaminopimelic acid.

* $P < 0.05$, ** $P < 0.01$.

† For details, see Table 1.

‡ Endogenous N estimated as 1 g N /d.

§ NAN digestibility in the intestine.

Table 5. *Efficiency of microbial protein synthesis and feed nitrogen degradability in defaunated and faunated sheep*

Diet†	Animals	Efficiency of microbial protein synthesis (N g/kg OMDR)				Feed N degradability in the rumen (g/kg N intake)			
		^{35}S	PB	DAPA	SEM	^{35}S	PB	DAPA	SEM
L	DF	50·5[a]	63·0[a]	60·6[a]	5·0	225[a]	381[a]	360[a]	38
L	F	28·1[a]	34·2[b]	26·9[a]	1·2	571[a]	690[a]	548[a]	28
S	DF	58·2[a]	57·2[a]	59·2[a]	3·7	538[a]	520[a]	553[a]	41
S	F	38·5[a]	38·7[a]	37·3[a]	1·5	715[a]	716[a]	688[a]	32
SEM		3·2	4·4	3·8		49	41	36	
Statistical significance:									
DF *v.* F		**	**	**		**	**	**	
L *v.* S		NS	NS	NS		**	NS	**	

[a], [b] Values with different superscript letters were statistically significantly different ($P < 0.05$) between microbial markers.

DF, defaunated sheep; F, faunated sheep; NS, not significant; OMDR, organic matter apparently digested in the rumen; PB, nucleic-acid purine bases; DAPA, α, ϵ-diaminopimelic acid.

** $P < 0.01$.

† For details, see Table 1.

to the intestines. N flowing into the duodenum was significantly higher in defaunated sheep than in faunated sheep ($P < 0.01$) (Table 4). A net gain in N between the mouth and duodenum was observed in the defaunated sheep (approximately 10 g N/d). MN flowing into the duodenum was higher in defaunated sheep than that in faunated sheep. However, the differences were not significant, except when MN was estimated using the DAPA method. Therefore, defaunation increases considerably the amount of feed N which escaped rumen degradation. Concerning the effect of diet on MN flow, diet S promoted a slightly larger mean MN flow than did diet L. The amount of rumen-undegraded feed N was also higher with diet L than with diet S. Faecal N output was slightly but significantly higher in defaunated sheep ($P < 0.01$), consequently N digestibility was reduced by defaunation, independent of diet. However, despite this, the amount of NAN digested in the intestines was much greater in defaunated than in faunated sheep ($P < 0.01$).

Efficiency of MN synthesis and feed degradability in the rumen are shown in Table 5. Comparison of the three different microbial markers did not show a significant difference between the methods of estimation, except for the difference between PB and DAPA methods in the faunated sheep given diet L. MN synthesis in defaunated sheep was 1·5 – 2-fold more efficient than those in faunated sheep. Diet S promoted higher efficiency of MN synthesis than did diet L in faunated sheep, while diet had little effect in defaunated sheep. The degradability of feed N in the faunated animals was between 1·3 and 2 times higher than that in the defaunated animals. In accord with the predicted solubility, degradability of N in diet S was about 1·5 times higher than that in diet L (0·43 *v.* 0·25).

The chemical composition of bacteria was affected by defaunation (Table 6). The N, nucleic acid and DAPA contents were higher in bacteria taken from defaunated sheep than in those taken from faunated sheep. Dietary effects on the chemical composition of bacteria were less apparent.

Turnover rates and retention times of the rumen liquid-phase (estimated by PEG dilution

Table 6. *Chemical composition of bacteria (g/kg dry matter)*

Diet†	Animals	OM	Nitrogen	PB‡	DAPA
L	DF	890	84	91·0	3·5
L	F	873	79	72·5	2·8
S	DF	878	87	98·3	3·2
S	F	867	81	85·7	2·5
SEM		4	1	3·6	0·1
Statistical significance:					
DF *v.* F		NS	*	**	**
L *v.* S		NS	NS	*	NS

DF, defaunated sheep; F, faunated sheep; OM, organic matter; PB, nucleic-acid purine bases; DAPA, α, ε-diaminopimelic acid.

$* P < 0.05$, $** P < 0.01$.

† For details, see Table 1.

‡ Purine bases content is expressed as g yeast RNA.

Table 7. *Retention time (h) of liquid-phase, solid-phase and microbial markers in defaunated and faunated sheep*

Diet†	Animals	PEG	Mordanted Cr	Organic ^{35}S	Rumen fluid volume (l)	Rumen DM pool (kg)
L	DF	12·5	15·6	13·9	5·8	0·49
L	F	15·9	19·1	27·6	5·9	0·57
S	DF	10·4	12·7	15·0	5·6	0·42
S	F	11·4	14·5	22·1	6·8	0·51
SEM		0·5	0·8	1·4	0·3	0·03
Statistical significance:						
DF *v.* F		**	**	**	NS	NS
L *v.* S		**	**	NS	NS	NS

DF, defaunated sheep; F, faunated sheep; NS, not significant; PEG, polyethylene glycol; DM, dry matter.

$** P < 0.01$.

† For details, see Table 1.

rate), solid-phase (estimated by Cr-dilution rate), and microbial proteins (estimated by organic ^{35}S-dilution rate) are shown in Table 7 and Fig. 1. All three fractions were retained longer in faunated than in defaunated sheep. A dietary effect was also observed in the liquid- and solid-phase retention times. Organic ^{35}S concentrations decreased at a rate similar to Cr in defaunated sheep, but ^{35}S was retained much longer than Cr in faunated sheep (Fig. 1). There were no significant effects of defaunation or diet on rumen fluid volume and on the size of the rumen DM pool.

DISCUSSION

Diet L, with a higher starch content, promoted a larger protozoal population than diet S. This difference was due mainly to an increased number of *Entodinium* sp.; holotrich numbers were not affected by dietary conditions. *Entodinium* sp. are considered to be the most sensitive to dietary changes (Jouany, 1978; Dennis *et al.* 1983), while holotrich
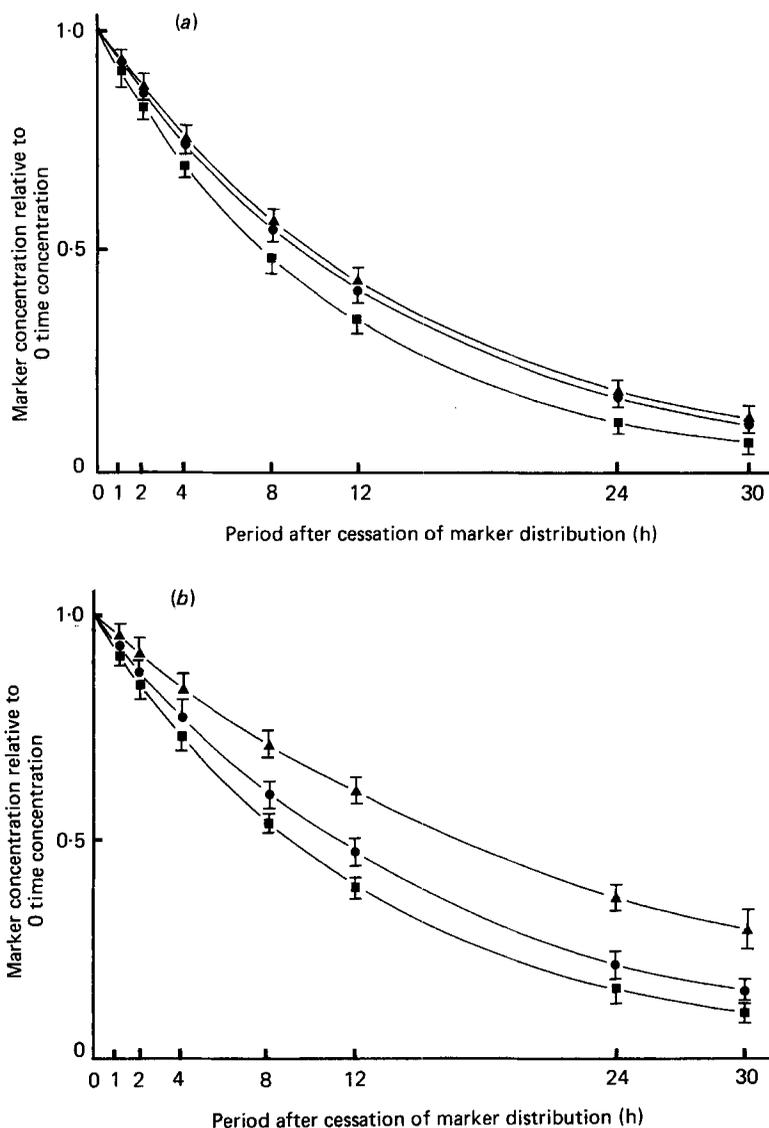
Fig. 1. The mean curves of marker disappearance from the rumen established using the pooled results taken from (*a*) six defaunated sheep given diet L and five defaunated sheep given diet S, and (*b*) six faunated sheep given diet L and six faunated sheep given diet S. Organic [35]S (▲), mordanted chromium (●), polyethylene glycol (■). Points are mean values, with their standard errors represented by vertical bars.

numbers do not normally vary greatly with the diet (Nakamura & Kanegasaki, 1969; Eadie *et al.* 1970; Dehority & Mattos, 1978; Dennis *et al.* 1983). The present results confirm these observations.

Effect of defaunation on end-products of rumen fermentation

The effect of defaunation in decreasing both rumen NH_3 and VFA concentrations agrees with previously published results (Coleman, 1981; Demeyer, 1981; Jouany *et al.* 1981). Protozoal ingestion of bacteria and proteolytic action on dietary protein lead to greater

rumen NH_3 concentrations in faunated animals. Protozoa cannot use ingested protein efficiently; only 50% of ingested bacterial N is retained. The remainder returns to the surrounding environment in the form of amino acids which are catabolized to NH_3 by the bacteria (Coleman, 1975). Diet L promoted lower NH_3 concentrations, especially in defaunated animals, because that diet contained less soluble N and more readily-fermentable carbohydrate than diet S. NH_3 production from dietary N degradation may have been less and the bacterial NH_3 assimilation rate may have been greater with diet L.

The negative effect of defaunation on total VFA concentration in the present study is related to reduced rumen OM digestion (Table 3). The reduction in C_4 concentrations agrees with most observations made in defaunated sheep (Jouany et al. 1981). Since protozoa produce C_2 and C_4 (Hungate, 1966), increased C_4 concentration in faunated sheep is partly due to protozoal C_4 production. The increase observed in molar proportion of C_3 in defaunated animals was probably induced by a change in rumen flora. Bacteriological studies in defaunated calves have shown that C_2-producing species, such as *Ruminococcus*, are not predominant, while succinate producers, such as *Bacteroides*, are the predominant species (Bryant & Small, 1960). Amylolytic species, mainly *B. ruminicola* and *B. amylophilus* which produce succinate, increase in the rumen of defaunated animals (Kurihara et al. 1978). These changes may enhance C_3 production.

Respective roles of bacteria and protozoa in rumen protein degradation

Both MN flow and dietary N which escaped rumen degradation increased in the defaunated sheep. If the endogenous N flow was not greatly affected by defaunation, the extent of the increase in rumen-undegraded dietary N was much greater than that in MN flow. These results show that dietary N degradability is significantly decreased by defaunation, and agree with the observations made using in vitro and nylon-bag techniques (Kayouli et al. 1983; Ushida & Jouany, 1985). Protozoa can ingest directly small particulate matter, such as bacteria, starch granules (Coleman & Hall, 1969) and chloroplasts (Mangan & Pryor, 1969; West & Mangan, 1972), and their proteolytic activity has been widely demonstrated (Abou Akkada & Howard, 1962; Ushida & Jouany, 1985). Therefore higher dietary N degradability in the faunated rumen seems likely, at least in part, to be due to protozoal proteolysis. The change in the composition of the bacterial flora induced by defaunation would also affect proteolytic activity in the rumen. The accessibility of dietary protein to microbial proteases, which can be considered as a main factor in determining the rate of protein degradation (Ganev et al. 1979), was improved by the more intensive digestion of OM or cellulose (Jouany & Senaud, 1979b) in the rumen of faunated sheep. Retention time of feed protein in the rumen is another factor which determines the degradation of rumen-insoluble protein. When the turnover rate of rumen contents is high, low protein degradability is observed (Tamminga et al. 1979; Ørskov et al. 1980). In the present study, the mean retention time of mordanted Cr was longer in the rumen of faunated sheep, which may explain the higher degradation of dietary protein in these animals compared with defaunated sheep.

Measurement of mean retention time of solid particles

The lucerne hay mordanted with Cr was ground through a 4 mm screen before being incorporated in the pelleted diet. After such treatment, lucerne became friable and probably gave very small labelled particles. This assumption is confirmed by the similar retention times of ^{35}S and Cr in defaunated sheep. The mean retention time of the total solid phase might be longer than that measured here.

Influence of protozoa on mean retention times of rumen contents and micro-organisms

After inoculation with protozoa, the mean retention time of PEG and mordanted Cr was increased. These results, obtained with roughage-based diets, suggest that protozoa increase the retention times of liquid and small particles in the rumen. They agree with the results reported by Kayouli *et al.* (1983,1984) obtained with a straw diet, but are contrary to the observations of Faichney & Griffiths (1978) who observed the opposite effect with a concentrate diet. The effect of protozoa on the retention time of either fluid or solid phases of rumen contents is still difficult to explain. Protozoa can be sequestered by their attachment to the rumen wall or to large feed particles (Bauchop & Clarke, 1976; Orpin, 1979 *a, b*; Abe *et al.* 1981). They ingest, and therefore sequester, small feed particles, so this sequestration may lengthen the retention time of small feed particles. Contrary to the present results, Orpin & Letcher (1984) recently reported an increase in retention time of the liquid phase after defaunation. However, their results were obtained from the measurement of marker disappearance rate in the rumen of sheep fed once daily instead of every 3 h, as was the case in the present experiment.

Generally speaking, micro-organisms flow out from the rumen in two ways. They are associated with the liquid phase or attached to the feed particles. Microbial markers would therefore give a value of turnover rate between that of the liquid-phase marker and the solid-phase marker. However, the turnover rate of ^{35}S was close to that of the Cr-mordanted marker in defaunated sheep but slower than that of the Cr-mordanted marker in faunated sheep. The recycling of ^{35}S within the rumen presumably lowered its turnover rate. The magnitude of this recycling is not known but it may be increased in faunated animals because of protozoal predation of bacteria. Although the proportions of bacteria associated with the liquid or solid phases which flow out from the rumen are not known exactly, the results obtained in defaunated sheep may indicate that the larger part of the bacteria which flow out from the rumen consists of small-particle-associated bacteria, because there is little recycling of bacterial matter in the rumen of these animals (Demeyer & Van Nevel, 1979). This could also indicate that the outflow mainly contains liquid-associated bacteria, if it is considered that the mean retention time of large particles is longer than that measured by mordanted Cr. Comparing the retention time of ^{35}S in defaunated and faunated animals, it can be concluded that protozoa increase the retention time of micro-organisms in the rumen, which can be explained by the sequestration of the protozoa themselves (Weller & Pilgrim, 1974; Harrison & McAllan, 1980; Collombier, 1981).

Influence of protozoa on microbial protein synthesis

Bacterial N flow, which was estimated using DAPA, was higher in defaunated sheep than in faunated sheep; the efficiency of bacterial protein synthesis was also higher in the defaunated animals. The efficiency measured in faunated sheep was close to the value adopted in present protein rationing systems (Institut National de la Recherche Agronomique, 1978; Agricultural Research Council, 1980). Similar results were obtained for MN flow and efficiency of MN synthesis when they were estimated by PB and ^{35}S methods. These results indicate that protozoal N supply did not compensate for the decrease in bacterial N flow in faunated sheep. Demeyer & Van Nevel (1979) showed that the efficiency of bacterial protein synthesis was increased by defaunation in an in vitro experiment. They suggested that the lower degradation of bacteria in the defaunated rumen, caused by the absence of predation, would increase the efficiency of microbial synthesis. As discussed earlier, the disappearance of predation and nutritional competition between protozoa and bacteria following defaunation led to a larger bacterial population, which directly explains

the increased duodenal flow of bacterial cells. Moreover, the higher turnover rate of the rumen liquid phase observed in defaunated animals can partly explain the improvement in the efficiency of microbial protein synthesis in the rumen (Harrison *et al.* 1975, 1976; Kennedy & Milligan, 1978).

Comparison of the three methods used to estimate microbial synthesis

The three different markers used in the present study to measure MN showed relatively good agreement, particularly when comparisons were carried out in defaunated animals, where the comparison is strictly valid. No statistical differences were detected, except between PB and DAPA methods in faunated sheep given diet L. However, the variations between animals were sometimes rather high. The reliability of microbial markers has been intensively discussed in the past decade (e.g. Harrison & McAllan, 1980). Since it is impossible to verify the results obtained by any marker method by means of an absolute reference, the reliability of a marker can only be assessed in comparative terms. Many simultaneous comparisons between markers have been conducted (Ling & Buttery, 1978; Smith *et al.* 1978; Siddons *et al.* 1979, 1982; Mercer *et al.* 1980), but it is still difficult to draw a definitive conclusion because of discrepancies between the results. For the nucleic-acid method, the problem of dietary contamination of duodenal nucleic acids has been pointed out, and MN flow estimated by the RNA method usually gives high values. In the present study, the protozoal N contribution to MN in the duodenum was 20% with PB–DAPA comparison in the sheep given diet L, while 4% of MN was attributable to the protozoa in the sheep given diet S. The value observed with diet L is similar to that of previously published results from experiments on sheep with a similar size of rumen protozoal population (Harrison *et al.* 1979; Sutton *et al.* 1983), but further investigation is needed to verify these estimations by use of markers specific for protozoa, such as phosphatidyl choline (John & Ulyatt, 1984).

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