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The role of rumen protozoa in the utilization of paspalum (*Paspalum dilatatum*) hay by cattle

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1. Six Friesian heifers (250 kg live weight) with permanent cannulas in the rumen and abomasum were allocated at random into two groups of three. One group was treated with Teric GN9 (ICI (Aust.) Ltd) to defaunate the animals during the first two of the four periods of the experiment, after which they were refaunated. The second group was treated with Teric at the end of the first two periods. The dietary treatments were: paspalum (*Paspalum dilatatum*) hay (4·1 kg/d) given alone and the hay supplemented with urea (20 g/kg dry matter).

2. Defaunation was not complete but the approximate volume of protozoa in the rumen of treated animals was less than 6% of that in the untreated animals.

3. The amount of organic matter (OM) digested in the stomach was lower ($P < 0·01$) in animals with reduced fauna than in those with normal fauna. There were reductions in both the apparent OM digestibility in the total tract (from 0·56 to 0·52, $P < 0·01$) and the proportion of the digestible OM digested in the rumen (from 0·82 to 0·79, not significant) of animals with reduced fauna. Apparent digestibilities of acid-detergent fibre and neutral-detergent fibre were significantly lower ($P < 0·01$) in animals with reduced fauna.

4. The amount of nitrogen disappearing from the stomach was significantly higher ($P < 0·01$) with the urea supplement; effects due to concentrations of protozoa were not significant. The flow of non-ammonia-N from the abomasum was higher ($P < 0·05$) in animals with reduced fauna than in animals with normal fauna. The flows of bacterial N from the abomasum and the efficiencies of bacterial N synthesis were not significantly affected by the treatments. N retention was higher ($P < 0·01$) in animals receiving the urea supplement but differences due to protozoa were not significant.

5. Protozoal contribution to the microbial N flowing from the rumen of animals with normal fauna was estimated to be 24 and 27% with and without the urea supplement respectively.

6. Concentrations of rumen-fluid ammonia-N were reduced ($P < 0·05$) and those of volatile fatty acids were increased ($P < 0·01$) with reduction in protozoal numbers. Molar proportions of propionic acid increased ($P < 0·05$) and of butyric acid decreased ($P < 0·01$) with reduced rumen fauna.

7. Rumen water volume was lower ($P < 0·05$) and the mean retention time of indigestible acid-detergent lignin tended to be higher in animals with reduced fauna. Rumen dry-matter pool and mean retention time of CrEDTA were not significantly different between treatments.

The microbial population of the rumen may be divided into the microfauna, consisting mainly of ciliated protozoa, and the microflora, which comprises the bacteria. Although the role of protozoa in fermentation and their nutritional significance to the host has been studied for some time (Hungate, 1966), it is still not well understood (Coleman, 1985).

Abou Akkada & El-Shazly (1954) and Christiansen *et al.* (1965) observed better growth in faunated lambs than in protozoa-free lambs. Similar results were obtained in buffalo (*Bubalus bubalis*) calves by Borhami *et al.* (1967). There are many reports in which no effect of defaunation was observed on the growth of lambs (Becker & Everett, 1930; Chalmers *et al.* 1968; Eadie & Gill, 1971) and calves (Pounden & Hibbs, 1950; Hibbs & Conrad, 1958; Eadie, 1962; Williams & Dinusson, 1973). By contrast, higher weight gains in cattle and more wool growth in sheep were reported recently in defaunated animals (Bird & Leng, 1978; Bird *et al.* 1979; Bird & Leng, 1984). These variable responses in performance may

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be due to the nature of the experimental diets as well as the physiological state and age of the animals (Veira *et al.* 1983).

Conrad *et al.* (1950) and Klopfenstein *et al.* (1966) found that the elimination of protozoa from the rumen resulted in reduced dry matter (DM) digestion. Similar results were reported by Jouany *et al.* (1981) and Veira *et al.* (1983). Ushida *et al.* (1984) and Veira *et al.* (1984) also found that the digestion of organic matter (OM) in the rumen was lower in defaunated than faunated sheep.

Lindsay & Hogan (1972) reported higher microbial OM availability as a result of defaunation of sheep on high-protein diets. Jouany & Thivend (1983) showed that the protozoal contribution to duodenal microbial nitrogen was small on a diet rich in highly degradable protein. However, protozoal contribution was significant on a diet high in poorly degradable protein (Ushida *et al.* 1984). Veira *et al.* (1984) reported that the flow of amino acids from the stomach decreased when sheep, fauna-free from birth, were inoculated with ciliate protozoa.

The proportion of protozoa flowing from the rumen to the omasum of cattle is considerable (Punia *et al.* 1984*a*; Punia & Leibholz, 1984). Nutritional significance to the host of microbial protein and OM synthesized in the rumen would also be dependent on the flow to the intestines for absorption.

In the present experiment the influence of protozoa on nutrient utilization and bacterial protein synthesis was studied in cattle fed on a low-quality roughage alone or supplemented with urea.

MATERIALS AND METHODS

Animals, management and defaunation procedure

Six Friesian heifers aged about 2 years (about 250 kg), fitted with cannulas in the rumen (100 mm diameter) and in the abomasum about 50 mm from the pylorus, were housed in ventilated, individual stalls with water available *ad lib.* and in continuous lighting. The six animals were randomly allocated to two identical groups of three heifers for defaunation treatment. At 10 d before each collection period, all animals were fasted for 24 h after which 70–75 g Teric GN9 (ICI (Aust.) Ltd, Sydney) in 800 ml water (Bird & Leng, 1978) were infused into the rumen of the three cattle in one group. The amount of feed given to cattle in the untreated group was similar to that eaten by the treated group.

The three treated animals were refaunated by the transfer of rumen digesta from faunated animals, at the end of the first two of the four periods of the experiment, and the other group of three was then treated with Teric in a similar manner to the first group. Animals treated with Teric were housed in a room separate from untreated animals to avoid cross contamination. Care was taken to change overalls and boots, and hands were washed before entering this room during feeding and sampling.

Dietary treatments and feeding

The basal diet was chopped paspalum (*Paspalum dilatatum*) hay; its composition (g/kg DM) was: OM 937, N 11·1, neutral-detergent fibre (NDF) 734, acid-detergent fibre (ADF) 391, acid-detergent lignin (ADL) 45·7. The hay was sprayed with a solution supplying (g/kg hay): 60 water, 2·0 sodium, 1·2 sulphur, 0·6 chloride, 0·2 iron and (mg/kg hay): 34 zinc, 18 manganese, 10 copper, 0·07 cobalt after which it was sprinkled with dicalcium phosphate supplying (g/kg hay): 2·3 calcium and 1·8 phosphorus. Urea (20 g/kg hay) was also dissolved in the mineral solution when required. Animals were given recommended doses of retinol, cholecalciferol and α-tocopherol intramuscularly, before the experiment and after two collection periods. The treatments were: normal and reduced

fauna with and without urea supplementation of the chopped paspalum hay. The diets were offered at 4·1 kg DM/d in eight equal amounts at 3-h intervals. Animals were allowed 3 weeks to adjust to each dietary treatment.

Markers, feeding and sample collections

CrEDTA and ADL were used to estimate variables relating to digestion. CrEDTA was prepared in solution according to Binnerts *et al.* (1968). The solution was sprayed onto 50-kg batches of feed in a feed mixer to give a chromium concentration of approximately 200 mg/kg feed. Labelled feed was stored in hessian sacks until required.

After the adaptation period on their respective diets, animals were given labelled feed for at least 5 d before digesta, faecal and urine sampling. Rumen and abomasal digesta (90 ml) were collected every 3 h during the day over a 3 d period (nine samples). Some of the rumen digesta was strained through cheesecloth. A portion of the strained liquid was diluted 1:5 (v/v) in formol saline (10 ml formalin plus 90 ml physiological saline (9 g sodium chloride/l)) to fix protozoa for counting. Another portion was acidified to pH < 3 with 10 M-sulphuric acid to stop microbial activity and was used for chemical analysis.

Urine was collected for 4 d during the sampling period through urethral catheters inserted into the bladder. Sufficient hydrochloric acid (5 M) was added to urine containers to maintain pH below 2 so as to avoid N losses. A portion (10 ml/l) of daily urine output was bulked for each animal; faecal samples were taken from the rectum three times daily during the collections and a standard quantity was bulked for each animal. Bulk samples were stored at −10° to await analysis.

After each collection period, labelled feed was withdrawn and animals were given unlabelled feed. Seven rumen digesta samples were collected over the next 31·5 h to determine marker disappearance rates. On the final day of each period, rumen pool size was measured by manual emptying and weighing of digesta. Samples of DM determination were taken after mixing. All the samples were stored at −10°.

Sample preparation and analysis

Protozoa were counted in a standard counting chamber (0·2 mm depth) (Mod-Fuchs, Rosenthal, Weber, England). Pipettes with wide tips were used for protozoal samples and samples were well mixed while handling. Protozoa were differentiated into holotrichs (large) and entodiniomorphs. Entodiniomorph protozoa were grouped as large (> 1000000 μm^3), medium, and small (< 10000 μm^3) on the basis of cell volume. Approximate volume of fauna was calculated assuming the dimensions of 1000000, 100000 and 10000 μm^3 for each of the large, medium and small ciliate cells respectively (Warner, 1962), to compare the biomass of protozoa in normal faunated and Teric-treated animals during the collection periods.

Feed, rumen and abomasal digesta and faeces were dried to constant weight at 100° to determine DM. OM was calculated after igniting the dry samples at 550° for 5 h in a muffle furnace. Subsamples of rumen and abomasal digesta and of faeces were dried at 50° in a forced-draught oven before the analysis of NDF, ADF and ADL (Goering & Van Soest, 1970). The apparent digestibility coefficients of OM, ADF, NDF and N were calculated by the marker (ADL) ratio technique. N was estimated by an automated Kjeldahl technique (Kjel-Foss Automatic 16210; A/S N. Foss Electric, Denmark).

Samples of abomasal digesta were strained through cheesecloth and filtered to obtain liquid fractions for Cr, DM, OM and N analyses. For Cr analyses, samples (500 mg) of feed, rumen and abomasal digesta and faeces were suspended in 4 ml nitric acid overnight; 4 ml nitric–perchloric acid mixture (1:1, v/v) were added next morning and the samples were heated for 3 h or until clear. Digested sample, liquid fractions and urine were filtered

and analysed for Cr by atomic absorption spectrophotometry (Varian Techtron Model AA6DAB; Varian Pty Ltd, Sydney). Flows of digesta were calculated from CrEDTA and ADL concentrations using the double-marker method; all CrEDTA concentrations were corrected for absorption (Faichney, 1975, 1980).

Bacteria were isolated from abomasal samples by differential centrifugation (Mathers & Miller, 1980). Strained fluid was centrifuged at 1750 ***g*** for 10 min to remove protozoa and feed particles. The supernatant fluid was then centrifuged at 27750 ***g*** for 20 min and the pellet thus obtained was washed with Brij saline (2 ml Brij in 4 litres saline) and centrifuged twice to obtain bacterial samples.

Bacterial and digesta samples were freeze-dried and analysed for 2,6-diaminopimelic acid (DAPA) by ion-exchange chromatography using an automatic amino acid analyser (TSM AutoAnalyzer; Technicon Instrument Corporation, Tarry Town, New York) following hydrolysis in 6 M-HCl (110°, 22 h) and oxidation overnight in performic acid (1 ml hydrogen peroxide (300 ml/l) + 9 ml formic acid (880 ml/l)) (Moore, 1963). Norleucine was used as internal standard. The DAPA:N was calculated for each sample of bacteria and digesta. The proportion of bacterial N in the digesta was calculated as the DAPA:N of digesta divided by the DAPA:N of bacteria. The DAPA concentration in bacteria was 30 (SE 0·3) mg/g N. N in the hydrolysates was determined by a micro-Kjeldahl method.

An estimate of the protozoal contribution to the microbial N flow was made using differential (holotrichs, and large, medium and small entodiniomorphs) protozoal counts in the rumen fluid (Table 1) and the relative flow of protozoa to the omasum (0·64 for the holotrichs and 0·57, 0·58 and 0·36 respectively for the large, medium and small entodiniomorphs, taken from Punia *et al.* 1984*a*), assuming that protozoa were suspended in the daily flow of water to the omasum (Table 5, p. 402). The holotrichs and large entodiniomorphs were assumed to have 17·8 g DM/10^8 cells (Leng *et al.* 1981) and the medium and small entodinia 2·0 and 0·2 g DM/10^8 cells respectively based on their size (Broad & Dawson, 1975). N content in this protozoal DM was taken as 35·3 mg/g (Punia & Leibholz, 1984). This is within the range of values given in Hungate (1966) and more recently by Bauchart *et al.* (1986). Approximate DM of protozoa in the rumen fluid was also calculated and compared with the approximate protozoal cell volume (Table 1).

Total volatile fatty acids (VFA) and their molar proportions in rumen fluid were analysed by gas–liquid chromatography using 3-methyl-*n*-valeric acid as an internal standard. Ammonia-N in the rumen and abomasal fluids was determined by the method of Chaney & Marbach (1962).

Mean retention time of CrEDTA in the rumen was calculated as the reciprocal of the rate constant for its disappearance and was corrected for the absorption of Cr from the rumen (Faichney, 1986). Lignin mean retention time was calculated from indigestible ADL content in and flow from the rumen (Faichney, 1980).

Statistical analysis

Experimental values were examined statistically by the analysis of variance for an incomplete Latin-square design. Effects of fauna (F), urea (U) and their interaction (F × U) were tested against the residual mean square (12 df). The least significant difference ($P < 0·05$) was used to compare treatment means (Steel & Torrie, 1980).

RESULTS

Protozoal numbers and the approximate volume and DM of fauna in the rumen fluid are presented in Table 1. Treatment with Teric removed medium and large protozoa but small *Entodinium* spp. reappeared in the rumen of these animals. Thus the volume of protozoa

Table 1. *Protozoal counts ($\times 10^{-4}$/ml) and their approximate volume and dry matter (DM) in the rumen fluid of experimental animals during the collection periods*

(Each value is the mean of six replicates)

Group ...	Normal fauna		Reduced fauna			Statistical significance of		
Urea supplement (g/kg DM) ...	20	0	20	0	SEM (12 df)	Fauna (F)	Urea (U)	F × U
Holotrichs	0·8	1·3	0·2	0·1	0·10	**	NS	*
Entodiniomorphs:								
Large (> 1 000 000 μm^3)	2·7	3·5	0·0	0·0	0·13	**	*	NS
Medium	3·8	4·5	0·7	0·9	0·30	**	NS	NS
Small (< 10 000 μm^3)	9·0	12·4	8·1	16·3	1·19	NS	**	NS
Total ciliates	16·3	21·7	9·0	17·3	1·25	**	**	NS
Approximate volume of total ciliates (μm^3 ($\times 10^8$)/ml)	40·4	53·2	1·5	3·0	1·46	**	**	*
Approximate DM of total ciliates (mg/ml)	7·2	9·7	0·7	0·7	0·28	**	**	*

NS, not significant. * $P < 0.05$, ** $P < 0.01$.

Table 2. *Intake of dry matter (DM) and digestion of organic matter (OM) in heifers with normal and reduced fauna, eating paspalum* (Paspalum dilatatum) *hay with or without 20 g urea/kg DM*

(Each value is the mean of six replicates)

Group ...	Normal fauna		Reduced fauna			Statistical significance of	
Urea supplement (g/kg DM) ...	20	0	20	0	SEM (12 df)	Fauna (F)	Urea (U)
DM intake (g/d)	4185	4132	4115	4057	25·8	NS	NS
OM intake (g/d)	3923	3877	3860	3798	25·0	NS	NS
OM apparently digested in:							
Stomach (g/d)	1847	1734	1591	1581	19·2	**	*
Total tract (g/d)	2249	2115	2026	1979	35·4	**	NS
Apparent digestibility coefficient of OM	0·57	0·55	0·52	0·52	0·009	**	NS
Fraction of digestible OM apparently digested in stomach	0·82	0·82	0·78	0·80	0·011	NS	NS

F × U interactions were not statistically significant.
NS, not significant.
* $P < 0.05$, ** $P < 0.01$.

in the rumen of treated animals was less than 6% (and less than 8% of the DM of fauna) of that present in the rumen of untreated animals.

The DM intake of the heifers was maintained at 4·1 kg/d, which gave OM intakes of 3·80–3·92 kg/d for the four treatments (Table 2). The amount of OM digested in the stomach was lower ($P < 0.01$) in animals with reduced fauna than in those with normal

Table 3. *Digestion of acid-detergent fibre (ADF) and neutral-detergent fibre (NDF) in heifers with normal and reduced fauna, eating paspalum* (Paspalum dilatatum) *hay with or without 20 g urea/kg dry matter (DM)*

(Each value is the mean of six replicates)

Group ...	Normal fauna		Reduced fauna			Statistical significance of		
Urea supplement (g/kg DM) ...	20	0	20	0	SEM (12 df)	Fauna (F)	Urea (U)	F × U
ADF intake (g/d)	1627	1628	1595	1606	9·2	NS	NS	NS
ADF apparently digested in:								
Stomach (g/d)	893	881	787	786	8·3	**	NS	*
Total tract (g/d)	908	871	809	822	15·6	**	NS	NS
Apparent digestibility coefficient of ADF	0·55	0·53	0·50	0·51	0·009	**	NS	NS
NDF intake (g/d)	3063	3058	3008	2977	19·1	NS	NS	NS
NDF apparently digested in:								
Stomach (g/d)	1757	1695	1515	1492	24·0	NS	NS	*
Total tract (g/d)	1865	1786	1686	1643	33·5	**	NS	NS
Apparent digestibility coefficient of NDF	0·61	0·59	0·56	0·56	0·009	**	NS	NS

NS, not significant. * $P < 0·05$, ** $P < 0·01$.

fauna. Apparent digestibility of OM in the total tract was also lower ($P < 0·01$) for the heifers with reduced fauna. There was a tendency for a lower proportion (0·79 *v.* 0·82) of the OM digestion to occur in the stomach of animals with reduced fauna compared with those with normal fauna.

The heifers with reduced fauna digested less ($P < 0·01$) ADF and NDF in the rumen and in the total gastrointestinal tract than did those with normal fauna (Table 3).

Urea supplementation tended to improve the digestibilities of feed components slightly in animals with normal fauna but had no significant effect overall. The supplement of urea increased N intake by 76% (Table 4). The amount of N disappearing from the stomach was higher ($P < 0·01$) with the urea supplement than with paspalum alone. The flow of non-NH_3-N (NAN) from the abomasum was higher ($P < 0·05$) in animals with reduced fauna than in those with normal fauna. The differences in the amounts of NAN digested in the intestines, however, were not significant.

Total bacterial N flows were similar on all the treatments but the bacterial proportion of the NAN flowing from the abomasum was lower ($P < 0·05$) in animals with reduced fauna. Differences due to urea supplementation were not significant. Efficiency of bacterial N synthesis showed no significant difference between treatments.

Protozoal contribution to microbial N flow to the omasum was estimated to be respectively 24 and 27%, in animals with normal fauna with and without urea supplement. Protozoa constituted 2% of the microbial N flow in animals with reduced fauna with and without urea supplement.

Faecal and urinary N excretions were significantly different between treatments (Table 4). N retention was higher ($P < 0·01$) with the urea supplement, but the differences between the groups with normal and reduced fauna were not significant. Concentrations of rumen fluid NH_3-N were lower ($P < 0·05$) in animals with reduced fauna than in those with normal

Table 4. *Intake and digestion of nitrogen, abomasal flows of bacterial and protozoal N and efficiencies of bacterial and total microbial N synthesis in heifers with normal and reduced fauna, eating paspalum* (Paspalum dilatatum) *hay with or without 20 g urea/kg dry matter (DM)*

(Each value is the mean of six replicates)

Group ...	Normal fauna		Reduced fauna			Statistical significance of	
Urea supplement (g/kg DM) ...	20	0	20	0	SEM (12 df)	Fauna (F)	Urea (U)
Total N intake (g/d)	81·5	44·4	79·3	46·2	—	—	—
N intake from urea (g/d)	35·5	0·0	34·0	0·0	—	—	—
Apparent digestibility coefficient of N	0·67	0·40	0·63	0·41	0·007	NS	**
N excretion (g/d):							
Urinary	44·2	15·6	39·4	12·3	0·95	**	**
Faecal	26·8	26·6	29·6	27·3	0·33	**	*
N retention (g/d)	10·5	2·2	10·3	6·6	0·97	NS	**
N apparently digested in stomach (g/d)	26·8	−7·8	18·3	−7·1	1·63	NS	**
Non-ammonia-N (NAN) flow from abomasum (g/d)	50·6	51·0	57·0	52·4	1·36	*	NS
NAN apparently digested in intestines (g/d)	23·8	24·4	27·3	25·0	1·53	NS	NS
Proportion of abomasal NAN flow apparently digested	0·47	0·48	0·47	0·48	0·019	NS	NS
Bacterial N:							
Flow from abomasum (g/d)	32·4	32·0	33·0	28·5	1·26	NS	NS
Proportion in abomasal NAN	0·64	0·63	0·58	0·54	0·019	*	NS
g/kg OM apparently digested in stomach	17·9	18·6	21·2	18·3	0·80	NS	NS
Calculated protozoal N:							
Flow to omasum (g/d)	10·2	11·7	0·7	0·7	0·44	**	NS
Proportion in total microbial N	0·24	0·27	0·02	0·02	0·011	**	NS
Total microbial N:							
Abomasal flow (g/d)	42·9	43·7	33·7	29·3	1·32	**	NS
g/kg OM apparently digested in stomach	22·2	24·3	21·3	18·8	0·78	**	NS

F × U interactions were not statistically significant.
NS, not significant.
* $P < 0.05$, ** $P < 0.01$.

fauna (Table 5). NH_3-N values were significantly higher ($P < 0.01$), both in normal- and reduced-fauna groups, with the urea supplement.

Concentrations of total VFA and the molar proportion of propionate were higher ($P < 0.01$ and $P < 0.05$ respectively) in animals with reduced fauna than in those with normal fauna (Table 5). Animals with reduced fauna exhibited lower ($P < 0.01$) molar proportions of butyrate than those with normal fauna.

Table 5. *Concentrations of ammonia nitrogen and volatile fatty acids (VFA) in rumen fluid, molar proportions of VFA, rumen pool size and mean retention time of markers in the stomach of heifers with normal and reduced fauna, eating paspalum* (Paspalum dilatatum) *hay with or without 20 g urea/kg dry matter (DM)*

(Each value is the mean of six replicates)

Group ...	Normal fauna		Reduced fauna			Statistical significance of	
Urea supplement (g/kg DM) ...	20	0	20	0	SEM (12 df)	Fauna (F)	Urea (U)
Rumen NH_3 (mg/l)	190	23	171	9	5·3	*	**
Total VFA (mmol/l)	61	60	66	75	2·4	**	NS
Acetic (mmol/mol)	756	759	770	775	3·6	*	NS
Propionic (mmol/mol)	146	141	164	152	4·7	*	NS
Butyric† (mmol/mol)	88	91	57	65	2·4	**	NS
Valeric† (mmol/mol)	10	9	9	8	0·4	*	NS
Rumen pool size (kg):							
Water	40·0	39·8	35·6	37·1	1·18	*	NS
DM	5·7	5·6	5·6	5·2	0·17	NS	NS
Mean retention time (h)							
CrEDTA	13·9	16·3	16·4	14·9	0·77	NS	NS
Indigestible acid-detergent lignin	64·5	57·4	72·7	60·2	2·25	NS	**
Water flow from abomasum (l/d)	48·2	48·3	48·6	47·9	1·60	NS	NS
Water flow to omasum (l/d)	69·9	59·9	54·2	61·1	4·36	NS	NS
Apparent water loss in omasum–abomasum (l/d)	21·7	11·6	5·6	13·2	3·18	NS	NS

F × U interactions were not significant.
NS, not significant.
* $P < 0·05$, ** $P < 0·01$.
† Sum of n- and iso-isomers.

The presence of normal fauna was associated with greater rumen water volume ($P < 0·05$) and a tendency towards lower mean retention time of indigestible ADL. The amount of DM present in the rumen, and CrEDTA mean retention time, were not affected by the reduction in protozoal numbers or urea supplementation.

DISCUSSION

Defaunation of the rumen

Defaunation of the rumen appeared to be complete for 2–4 d following dosing of the cattle (with Teric GN9) but low numbers of protozoa, mainly small *Entodinium* spp., appeared over the next 10 d. These protozoa represented a small volume (less than 6%) of fauna compared with that in the untreated group during the collection periods. Similarly, the calculated DM of reduced fauna was also small (about 8% of normal fauna). Animals in the untreated group harboured a normal fauna consisting of the holotrichs and large, medium and small entodiniomorphs.

The dose of Teric was not increased to eliminate protozoa completely because, in

Table 6. *Effects of protozoa on digestion and nitrogen flow to the duodenum*

Main diet	Constituent	Apparent digestibility coefficient of feed constituents		Reference
		Faunated	Defaunated	
Lucerne (*Medicago sativa*) hay	DM	0·66	0·63	Conrad *et al.* (1950)
Pasture grass		0·72	0·72	Conrad *et al.* (1950)
Concentrate		0·78	0·74	Klopfenstein *et al.* (1966)
Concentrate		0·74	0·64	Klopfenstein *et al.* (1966)
Lucerne hay	OM	0·65	0·62	Lindsay & Hogan (1972)
Red clover (*Trifolium pratense*)		0·76	0·75	Lindsay & Hogan (1972)
Meadow hay	ADF	0·71	0·69	Jouany & Senaud (1979)
Meadow hay + barley		0·60	0·56	Jouany & Senaud (1979)
Maize + maize silage	OM	0·53	0·43	Veira *et al.* (1983)*
	ADF	0·42	0·34	Veira *et al.* (1983)*
	Starch	0·89	0·84	Veira *et al.* (1983)*
Grass hay + concentrate	OM	0·54	0·47	Veira *et al.* (1984)*
Lucerne hay + barley	OM	0·61	0·59	Ushida *et al.* (1984)
Concentrate	DM	0·84	0·83	Whitelaw *et al.* (1984)
	N flow to the duodenum (g bacterial N/d) (sheep)			
Lucerne hay		12·0	14·0	Lindsay & Hogan (1972)
Red clover		18·0	19·3	Lindsay & Hogan (1972)
Alkali-treated wheat straw + sugar beet		16·3	16·0	Jouany & Thivend (1983)
Pelleted (lucerne hay + barley)		12·1	17·7	Ushida *et al.* (1984)
	Total amino acids flow (g/kg DM intake)			
Maize + maize silage		101	126	Veira *et al.* (1983)
Grass hay + concentrate		89	124	Veira *et al.* (1984)

DM, dry matter; OM, organic matter; ADF, acid-detergent fibre.
* Digestibility in rumen.

preliminary trials, some animals showed toxic symptoms at higher doses. Burggraaf & Leng (1980) also reported that the dose required for complete defaunation had adverse effects on some animals. Difficulties in defaunation of ruminants have also been reported by Lovelock *et al.* (1982) and Veira *et al.* (1983). In the absence of an ideal defaunating agent, the present experiment was conducted to investigate the effects of substantially reducing the protozoal population.

Feed utilization

The results of the present study showed that the digestibility of OM in the rumen of heifers with reduced fauna was lower than in heifers with a normal fauna. The major cause of this lower digestibility was reduced digestion of fibre in the rumen. Studies with defaunated sheep by Jouany & Senaud (1979) and Veira *et al.* (1983) showed that protozoa aided digestion in the rumen. Veira *et al.* (1983) found that the digestion of OM and starch was significantly improved by the presence of protozoa, while the improvement in the digestion of ADF was not significant. However, the ADF content of their diets was only 74 g/kg compared with 391 g/kg in the present experiment. Jouany & Senaud (1982) showed that the presence of protozoa in the rumen increased the bacterial cellulolytic activity for the diet containing 355 g ADF/kg but not for that containing 204 g ADF/kg.

The effects of protozoa on the digestion of feed constituents in the gastrointestinal tract and N supply to the duodenum as reported in the literature are summarized in Table 6. It is apparent from this table and the present experiment that protozoa improve the digestibility on most diets.

N metabolism and protein synthesis in the rumen

Veira *et al.* (1983, 1984; Table 6) reported higher NAN and amino acid flows from the duodenum in defaunated sheep than in sheep with normal fauna. In the present experiment also, the flow of NAN from the abomasum was higher in animals with reduced fauna, but the bacterial proportion of the NAN was lower than that in animals with normal fauna so that flows of bacterial N from the abomasum in the two groups were similar. However, the flow of microbial N from the abomasum was substantially lower in the animals with reduced fauna so that the higher NAN flows from the abomasum of these animals indicate that the degradation of dietary N in the rumen was reduced. The faecal excretion of N was higher in animals with reduced fauna so that the amount of NAN apparently digested in the intestines was not different between the treatments.

The results suggest that neither the reduction of protozoal population nor the supplementation with urea affected the efficiencies of bacterial protein synthesis. Ushida *et al.* (1984) reported higher efficiencies of bacterial protein synthesis and greater bacterial N flows to the duodenum in defaunated sheep. They concluded that defaunation increased the amount of dietary protein that escaped from the rumen undegraded and allowed the utilization of more recycled N to maintain bacterial protein flow to the duodenum, especially when the diet was rich in proteins of low solubility and supported high numbers of protozoa in the rumen. The lack of an effect of protozoa on bacterial N flows in the present experiment may be due to the difference in the type of diet used since the hay contained only 11·1 gN/kg and protozoal numbers were low compared with those in the experiment of Ushida *et al.* (1984).

Faunation of the rumen has been reported to decrease the bacterial population in the rumen fluid (Eadie & Hobson, 1962; Kurihara *et al.* 1968, 1987; Eadie & Gill, 1971). However, Jouany *et al.* (1981) did not find an increase in total bacterial numbers in defaunated sheep and Jounay & Thivend (1983) reported similar flows of bacterial N to the duodenum in faunated and defaunated animals (see Table 6).

The flow of protozoal N to the omasum was used to calculate the contribution of protozoa in microbial N flow from the abomasum (Table 4); assuming that all the protozoa flowing to the omasum reached the abomasum. There is no direct evidence in the literature that protozoa are destroyed in the omasum (see Smith, 1984).

The calculated protozoal contribution reported in the present paper must be considered as a minimum value because protozoa associated with the solids were not accounted for. This underestimation is partly compensated for by the assumption that the protozoal counts made in the rumen fluid and corrected to apply to rumen fluid outflow (see p. 398) apply to all the water flowing to the omasum. Nevertheless, the estimated protozoal contribution to microbial N flow from the stomach of animals with normal fauna with and without urea in the present experiment was similar to the values reported by Punia & Leibholz (1984) and Punia *et al.* (1984*b*) which were estimated by the ^{35}S and ^{15}N techniques.

Apparent digestibility of N in the total tract was not affected by the presence of protozoa, a finding similar to that of Lindsay & Hogan (1972). Males & Purser (1970) and Takahashi & Kametaka (1976) observed increases in N retention due to defaunation but Whitelaw *et al.* (1984) reported that differences in N retention due to protozoa were not significant. The significant increase in the retention of N with the urea supplement is in agreement with the known effects of urea as a supplement to straws and hays of low N content (e.g. Campling *et al.* 1962; Bird, 1974; Coleman & Barth, 1977).

Effects on rumen fermentation

The observation of lower concentrations of rumen fluid NH_3-N in animals with reduced fauna is in general agreement with the literature (e.g. Abou Akkada & El-Shazly, 1964; Eadie & Gill, 1971; Goetsch *et al.* 1984). Increased rumen NH_3-N in the presence of protozoa results from protozoal action on dietary and bacterial protein (Coleman, 1967) and possibly protozoal protein.

Defaunation of the rumen has been reported to increase the molar proportions of propionate in the rumen fluid and decrease those of butyrate (e.g. Males & Purser, 1970; Whitelaw *et al.* 1984). Similar results were obtained in the present study. However, Bird & Leng (1978) observed lower acetate and higher butyrate proportions in defaunated cattle given molasses and oaten straw which probably reflect the nature of dietary ingredients and the type of microflora sustained on these substrates.

Rumen volume and marker retention time

The lower volume of water in the rumen with reduced fauna in the present study is in agreement with the observations of Kayouli *et al.* (1984). Veira *et al.* (1983), however, did not find changes in rumen water in defaunated sheep and Faichney & Griffiths (1978) and Orpin & Letcher (1984) observed more water in the rumen in the absence of protozoa. Veira *et al.* (1983) and Goetsch *et al.* (1984) did not find changes in the dilution rates of fluid markers due to defaunation and the absence of a significant effect of fauna on the mean retention time of CrEDTA in the present work is in agreement with these observations. By contrast, Faichney & Griffiths (1978) and Faichney (1986) observed increased mean retention times of solute markers and Orpin & Letcher (1984) observed decreases in fluid flow rates in the absence of protozoa.

Conclusion

It may be concluded from the results presented here that the presence of normal fauna in the rumen of cattle fed on low-quality hay enhances the digestion of OM. This may be brought about by increased cellulolytic activity of bacteria in the presence of protozoa (Jouany & Senaud, 1979) as well as by the cellulolytic activity of ciliate protozoa (Jouany & Senaud, 1979, 1982; Orpin, 1984; Coleman, 1985). Further experiments using a variety of diets would help to specify further the role of ciliate protozoa in ruminant nutrition and production.

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