Duodenal flow and digestibility in fauna-free sheep and in sheep monofaunated with *Entodinium caudatum* or *Polyplastron multivesiculatum*

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Three groups of five rumen and duodenum cannulated fauna-free sheep were used in a 28 d experiment. One group remained fauna-free, whereas the second (EN) and third (PP) groups, respectively, were inoculated intraruminally with the protozoan species *Entodinium caudatum* and *Polyplastron multivesiculatum*. Rumen fluid, duodenal digesta and faecal samples were collected during the last 12 d. The flow of digesta to the duodenum was determined using Yb and Co as dual-phase markers. [15]Nitrogen and phosphatidylcholine were used as markers to calculate the duodenal flow of bacterial and protozoal N, respectively. Results showed an increase ($P < 0.1$) in the rumen concentration of NH_3-N and total volatile fatty acids, and a decrease ($P < 0.05$) in the duodenal flow of non-NH_3-N and bacterial N in sheep with EN and PP monofaunas, compared with fauna-free sheep. There were no differences ($P > 0.05$) in these variables between the two monofauna groups. Protozoal N accounted for 8 % of the duodenal non-NH_3-N flow in the EN-monofaunated sheep, whereas no such flow was detected in the PP-monofaunated sheep. Apparent rumen digestibility of organic matter, neutral detergent fibre and acid detergent fibre were similar ($P > 0.05$) in the monofaunated groups of sheep, but rumen acid detergent fibre digestibility was higher ($P < 0.05$) in the monofaunated than in the fauna-free groups. Experimental results suggested that, unlike EN, the PP monofauna might not contribute to the duodenal flow of microbial protein, whereas both monofaunas showed a virtually equal degree of predation on rumen bacteria.

***Entodinium*: *Polyplastron*: Rumen ciliate protozoa: Sheep**

The present work builds on results from our previous experiments in this laboratory on N metabolism by individual or combinations of ciliate protozoal species established in the rumen of fauna-free (FF) sheep (Ivan *et al.* 2000*a,b*). These previous experiments showed that the rumen presence of each protozoal species or their combination decreased the duodenal flow of bacterial N, but to a different degree. In particular, the previous experiments established the magnitude of effects of the individual species of *Isotricha intestinalis, Dasytricha ruminantium* and *Entodinium caudatum* (EN) on the flow of non-NH_3-N (NAN) components from the stomach to the intestinal tract of sheep. The effects on these components of combinations of other species and total fauna in both the type A and type B population (Eadie, 1962) were also established (Ivan *et al.* 2000*a,b*). However, owing to the methodology used in the previous experiments, the effects (including the protozoal flow of N) from the individual cellulolytic species of ciliate protozoa such as *Polyplastron multivesiculatum* (PP) could not be precisely established. It was, however, indicated that the magnitudes of the effects on the duodenal flow of bacterial N from individual cellulolytic protozoa species (PP, *Epidinium ecaudatum* or *Entodinium maggi*) in both types

of population (A or B) were comparable, and such effects appeared to be similar to those of EN when measured in mixed fauna populations (Ivan *et al.* 2000*a*). The objectives of the present experiment were to measure and compare the effects of both PP and EN as a single-species rumen fauna population (mono-fauna) on the duodenal flow of bacterial and protozoal N, and the contribution of these monofaunas (EN and PP) to the rumen fermentation and digestion of feed in sheep.

Materials and methods

All sheep used in the experiment originated from an FF flock (Ivan *et al.* 1986); thus, they were maintained FF from birth. They were cared for according to guidelines of the Canadian Council on Animal Care (1993), and the experimental protocol was approved by the Lethbridge Research Centre Animal Care Committee.

Fifteen 1·5-year-old FF Canadian Arcott sheep (castrated males) were each surgically fitted with rumen (Hecker, 1969) and simple T-type duodenal (Weller *et al.* 1971) cannulas. The duodenal cannula was placed proximal to the common bile and

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pancreatic duct. Approximately 6 weeks were allowed for post-surgical recovery of the sheep before starting the experiment. The sheep were housed in individual pens and were fed the experimental diet *ad libitum*, starting 2 weeks before cannulation.

The experimental diet (DM basis) consisted of (g/kg): barley silage, 602·8; coarsely ground barley grain, 350; soyabean meal, 30; dicalcium phosphate, 4; limestone, 7; trace mineralized salt, 6; vitamin supplement, 0·2. The salt supplied per kilogram dietary DM was: NaCl, 6 g; Zn, 20 mg; Mn, 15 mg; Cu, 2 mg; I, 1·5 mg; Co, 0·6 mg; Se, 0·09 mg. The vitamin supplement contained (per gram vitamin mix): retinol, 3 mg; cholecalciferol, 25 µg; α-tocopherol, 67 µg. The ground barley grain, soyabean meal, minerals and vitamins were mixed and pelleted. Each morning the pelleted concentrate was mixed into the barley silage in a Data Ranger (American Calan, Northwood, NH, USA). The chemical composition of the diet was (g/kg DM): N, 19·8; acid detergent fibre (ADF), 177; neutral detergent fibre (NDF), 324.

Ten days prior to beginning the experiment, each sheep was inoculated intraruminally with 50 ml rumen fluid that had been collected and combined from three rumen-cannulated FF donor sheep containing natural bacterial populations. These bacterial populations were previously established by intraruminal inoculation with rumen contents that had been collected from a cow with a normal microbial population in the rumen, and were frozen to kill the protozoa but maintain the majority of bacteria.

The fifteen cannulated sheep were randomly divided into three groups of five sheep each and used in a 28 d experiment consisting of a microbial adaptation period (16 d) and a sample-collection period (12 d). Each group was housed in an individual pen in a separate room in a aprotozoa barn that was isolated from other barns at the Lethbridge Research Centre. One group of the sheep remained FF, whereas the second group was monofaunated with EN and the third group with PP. The monofaunation was conducted by intraruminal inoculation of each sheep with 50 ml rumen fluid that had been collected and combined from three rumen-cannulated donor sheep, each containing EN or PP as a single-species protozoal population. These populations had previously been established by the anaerobic *in vitro* isolation and cloning of several specific individual protozoa from the rumen contents of cows, and their inoculation into the rumen of FF sheep. The donor sheep received the same type of diet as used during the experiment.

Precautions were taken such that no cross-contamination of protozoal species between the different treatments was observed at any time. The groups of sheep were transferred between individual stalls and metabolism cages, and samples were taken in the order of FF first, PP second and EN (with the largest potential for cross-contamination) last.

During the first 11 d of the microbial adjustment period, the sheep were offered feed twice daily (at 08.30 and 15.30 h) with intake *ad libitum*. Thereafter, the diet was offered at 85 % of the mean daily intake of the sheep with the lowest level of intake. One third of the daily ration was given at 08.30 h and the remainder at 15.30 h. Any unconsumed feed (as occurred in one sheep of the EN group and two sheep of the PP group) was collected and weighed daily. The sheep were weighed at the beginning and the end of the experiment (average body weight for the experiment 67·1 (SD 6·2) kg).

In the middle of the 16 d microbial adaptation period, the sheep were moved from their individual stalls to metabolism cages for 4 days to adjust to the cages. The sheep were then housed in the metabolism cages during the 12 d sample-collection period (days 17–28 of the experiment). For the first 7 d of the collection period, the sheep were fitted with harnesses and faecal collection bags: 2 d for adjustment to the harnesses and 5 d for total collection of faeces. The faecal outputs of each sheep were collected, weighed and mixed; about 10 % of the output was then oven-dried at 55 °C for calculation of DM. Faecal samples were pooled and stored for later analysis of organic matter (OM), NDF and ADF. Thereafter, the harnesses were removed and the sheep were transferred from their metabolism cages to their original individual stalls for a minimum of 4 h (animal care requirement), before being placed back into the metabolism cages to complete the sample-collection period (collection of rumen and duodenal digesta samples).

The flow of digesta to the duodenum was determined using Yb and Co as dual-phase markers. [15]Nitrogen and phosphatidylcholine (PC) were used as markers to calculate the duodenal flow of bacteria and protozoa, respectively. The Yb, Co and ^{15}N markers were incorporated into the concentrate. About 1·3 kg $YbCl_3 \cdot 6H_2O$ (Rhône Poulenc Basic Chemicals Co., Shelton, CT, USA), 2·05 kg CoEDTA (Udén *et al.* 1980) and 300 g ([15]$NH_4)_2SO_4$ (minimum 10 % atom percentage ^{15}N; Isotech Inc., Miamisburg, OH, USA) were dissolved in 10 litres water and sprayed on 350 kg concentrate prior to pelleting. From days 17 to 28 of the experiment, the sheep received the marked concentrate in the diet. Marker consumption approximated 500 mg/d Yb and Co, and 10 mg/d [15]N. On day 17, prior to offering the marked diet, each sheep received a priming dose containing half the amount of markers consumed per day. The priming dose of markers (1·4 g CoEDTA, 0·87 g $YbCl_3 \cdot 6H_2O$, 0·25 g ([15]$NH_4)_2SO_4$) was dissolved in 100 ml distilled water and administered into the rumen. Samples of the marked diet were collected on days 18, 20, 22, 25 and 27 of the experiment, oven-dried at 55 °C to a constant weight for calculation of DM, and stored until analysed for nutrient and marker composition.

Rumen and duodenal digesta samples were collected on days 24–28 of the experiment. Duodenal digesta samples (100 ml) were collected from duodenal cannulas every 6 h, moving ahead 1 or 2 h each day to collect samples at 16 different times over the five collection days. The samples were therefore collected at: 06.00, 12.00, 18.00 and 24.00 h on day 24; 07.00, 13.00 and 19.00 h on day 25; 01.00, 09.00, 15.00 and 21.00 h on day 26; 03.00, 10.00, 16.00 and 22.00 h on day 27; 04.00 h on day 28. The duodenal digesta samples were combined for each sheep as collected and immediately frozen. The pooled duodenal sample was later thawed and centrifuged (500 g and 5 °C for 15 min) to separate it into solid and liquid fractions, which were then freeze-dried and analysed for DM, OM, NDF, ADF, N, ^{15}N and PC. An additional sample of the duodenal contents was centrifuged at 20 000 g and 5 °C for 15 min, and the supernatant was stored frozen until analysed for NH_3-N.

Rumen contents (350 ml) were obtained through the rumen cannulas from different parts of the rumen of each sheep using a 60 ml syringe attached to plastic tube. The samples were taken at 12.00 h on day 24, 07.00 h on day 25, 01.00 and 21.00 h on day 26, and 10.00 and 16.00 h on day 27. The pH of the rumen contents was measured immediately. Part (150 ml) of the rumen sample was then strained through one layer of cheese-cloth. Filtrate (5 ml) was preserved with 5 ml methylgreen-formalin-saline solution (Ogimoto & Imai, 1981) for protozoal counts, and another 10 ml of the filtrate was combined with 2 ml 25 %

(w/v) meta-phosphoric acid and stored frozen until analysis of volatile fatty acids (VFA) and NH_3-N.

The remaining rumen contents (200 ml) were processed to isolate bacteria and protozoa. For bacterial isolation, one half of the rumen contents was blended with an equal volume of saline for 1 min in a blender and then squeezed through four layers of cheesecloth. The filtrate was centrifuged ($800\,g$ and $5\,°C$ for 15 min) to remove feed particles and protozoa, and then the supernatant was centrifuged ($20\,000\,g$ and $5\,°C$ for 40 min) to obtain the bacterial pellet. The bacterial pellet was then freeze-dried and analysed for DM, OM, N and ^{15}N. The other half of the rumen contents was strained through a single layer of cheesecloth, the feed particles were washed with an equal volume of chilled saline and re-strained, and the two filtrates were combined. The combined filtrate was then transferred to a separatory funnel, and the protozoa were allowed to sediment for 1 h. After sedimentation, the protozoal fraction was removed and washed by suspending in 100 ml cold saline and centrifuging at $500\,g$ and $5\,°C$ for 15 min. The protozoal pellet was washed twice, and the washed pellet was freeze-dried and stored ($-20\,°C$) until analysed for DM, OM, N, ^{15}N and PC.

Ciliate protozoa in preserved rumen fluid samples were counted using a Neubauer Improved Bright-Line counting cell ($0·1\,mm$ depth; Hausser Scientific, Horsham, PA, USA). Each sample was counted twice, and if the CV of the two counts was greater than $10\,\%$, the counts were repeated. Rumen fluid samples were analysed for VFA using crotonic acid as an internal standard, and gas chromatography (Model 5890; Hewlett Packard, Little Falls, DE, USA) with a capillary column ($30\,m \times 0·25\,mm$ internal diameter, $1\,\mu m$ phase thickness, bonded polyethylene glycol, Supelco Nukol; Sigma Aldrich Canada, Oakville, ON, Canada) and flame ionization detection. Rumen and duodenal fluid were analysed for NH_3-N concentration by the salicyclate-nitroprusside-hypochlorite method using a flow injection analyser (Sims *et al.* 1995). Ruminal pH, protozoal numbers, and NH_3-N and VFA concentrations were averaged for the six time points for each sheep for statistical analysis.

Feed, duodenal digesta and faecal samples were ground to pass through a $1\,mm$ diameter sieve (Wiley Mill model 4; Thomas Scientific, Swedesboro, NJ, USA) prior to determining analytical DM, OM, NDF, ADF and the digestive flow markers. Feed and duodenal digesta samples were further ground in a ball mill (Mixer Mill MM2000; Retsch, Hann, Germany) to a fine powder prior to determining N and ^{15}N. Analytical DM was determined by drying samples at $135\,°C$ in an oven for 2 h, followed by hot weighing. The OM content was calculated as the difference between 100 and the percentage ash (Association of Official Analytical Chemists, 1995; Method 942.05). The NDF was determined as described by Van Soest *et al.* (1991), and ADF was determined according to the procedure of the Association of Official Analytical Chemists (1995; Method 973.18). N was quantified by flash combustion with gas chromatography and thermal conductivity detection (Carlo Erba Instruments, Milan, Italy), and ^{15}N enrichment was measured by flash combustion with isotope ratio mass spectrometry (VG Isotech, Middlewich, UK). PC was determined according to the method of Neill *et al.* (1992). The concentrations of the digestive flow markers were determined by inductively coupled plasma emission spectrometry (SpectroCiros CCD; Spectro Analytical Instruments, GmbH and Co, Kleve, KG, Germany).

Nutrient intakes were calculated for each sheep based on the DM of the diet offered and refused on days 17–28, and of the chemical composition of the diet and feed refused for the corresponding period. Nutrient flow to the proximal duodenum was determined by mathematical reconstitution of the duodenal digesta based on the Co and Yb marker concentrations and the nutrient content of the solid and liquid fractions (Faichney, 1975). PC was used as a marker to calculate the duodenal flow of protozoal N (Neill *et al.* 1992). The duodenal flow of PC in the FF group of sheep was subtracted from the duodenal flow of PC in the faunated sheep to correct for the flow of feed and endogenous PC. Duodenal N flow and ^{15}N enrichment of the duodenal contents were corrected for protozoal N flow and ^{15}N enrichment in protozoa, respectively. Bacterial N flow to the small intestine was then computed by multiplying the corrected duodenal N flow by the ratio of ^{15}N enrichment (atomic percentage excess) in the duodenal contents and the ^{15}N enrichment in the rumen bacteria. Apparent digestibility of OM, ADF and NDF was calculated as the difference between the nutrient intakes and the amounts passing the duodenum or excreted in faeces.

The data are means for the respective groups of sheep with their standard errors. All data were statistically analysed as a completely randomized design with sheep nested in treatments. To account for the possibility of unequal variances between treatments, the MIXED procedure from SAS (1999*a*) was used to analyse the data with a variance components and a banded covariance structure; the best covariance structure was selected for the final analysis for each dependent variable according to the lowest Akaike's Information Criterion (AIC) value. Least squares means were calculated for each treatment, and Fisher's protected LSD test (Steel & Torrie, 1980) was used to evaluate differences between means for significance effects. The UNIVARIATE procedure from SAS (1999*b*) was used to evaluate the residuals for normality and to check for obvious outliers.

Differences were declared significant at $P<0·05$ and trends were discussed at $P\geq0·1$. One sheep from the FF group was removed from the experiment owing to a significant decrease in feed intake. All experimental results for the FF treatment are based on the four remaining sheep, whereas the results for the other groups (PP and EN) are based on five sheep.

Results

Protozoa and rumen fermentation

There were no protozoa present in any of the sheep of the FF group, whereas the EN in sheep of the EN group averaged over 5 million protozoa per millilitre rumen fluid (Table 1). PP in the sheep of the PP group averaged 46 000 protozoa per millilitre rumen fluid. No ciliates other than the *Entodinium* or *Polyplastron* species were detected in the EN or PP groups of sheep, respectively. The concentration of PC in the *Entodinium* and *Polyplastron* species isolated from the EN and PP groups of sheep (mean 5·31 (SE 0·71) v. 5·99 (SE 1·34) mg/g protozoal DM, respectively) was similar ($P>0·1$). The ratio N:PC was also similar ($P>0·1$) for the *Entodinium* and *Polyplastron* species (10·6 (SE 1·3) v. 11·9 (SE 2·6), respectively).

The mean rumen pH was not affected by the protozoal treatment ($P>0·1$), but the concentration of NH_3-N in the rumen fluid tended to be higher ($P<0·1$) in the EN and PP groups than in the FF group of sheep (Table 1). Total VFA concentration

Table 1. Protozoal number, pH and concentrations of NH_3-N and total volatile fatty acids (VFA) in rumen fluid of fauna-free sheep and of sheep monofaunated with *Entodinium* or *Polyplastron* genera of rumen ciliate protozoa

(Means with their standard errors)

	Fauna-free		*Entodinium*		*Polyplastron*	
Item	Mean	SE	Mean	SE	Mean	SE
Protozoa (cells $\times 10^5$/ml)	0	0	53·18	16·43	0·46	0·18
pH	6·2	0·13	6·2	0·05	6·1	0·07
NH_3-N (mg/100 ml)	5·1[B]	0·66	8·9[A]	1·45	8·4[A]	1·02
Total VFA (mmol/l)	80[B]	5·2	89[AB]	3·7	97[A]	5·4
Acetate:propionate	3·2	0·32	3·4	0·49	3·0	0·42
VFA (mol/100 mol)						
Acetic acid	58·2	2·57	62·5	2·06	62·2	1·88
Propionic acid	18·3	1·05	21·7	3·06	22·9	2·57
Butyric acid	17·2[a]	2·32	11·4[b]	1·14	10·5[b]	0·63
Isobutyric acid	0·9[AB]	0·07	1·0[A]	0·04	0·8[B]	0·05
Valeric acid	2·8[A]	0·74	1·5[B]	0·22	1·4[B]	0·07
Isovaleric acid	1·4	0·38	1·4	0·20	1·6	0·29
Caproic acid	1·02[a]	0·22	0·5[b]	0·11	0·5[b]	0·09
Branched chain and longer acids	4·4	0·58	3·9	0·57	3·3	0·66

[a,b] Mean values in a row with unlike letters were statistically different ($P < 0.05$).
[A,B] Mean values in a row with unlike letters were statistically different ($P < 0.1$).

tended to be higher ($P < 0.1$) in the sheep of the PP group than the FF group, being intermediate for the sheep in the EN group. Acetic acid, propionic acid and acetate:propionate ratio were not affected ($P > 0.1$) by any of the protozoal treatments. In comparison with the FF treatment, the EN and PP treatments decreased ($P < 0.05$) the molar proportions of butyric acid. The responses were variable for the individual branched and longer-chain acids. The molar proportion of isobutyric acid tended to be higher ($P < 0.1$) for the EN group than the PP group, but was intermediate for the FF group of sheep. There was no effect of the protozoal treatments on the molar proportion of iso-valeric acid ($P > 0.1$). Valeric acid tended to be lower ($P < 0.1$) and caproic acid significantly ($P < 0.05$) lower in the EN and PP monofaunated sheep than in the FF sheep. There was, however, no effect ($P > 0.1$) of any protozoal treatment on the total molar proportion of all the branched and longer-chain VFA in rumen fluid.

Duodenal flow

The daily N intake of sheep was almost constant in all three groups (Table 2), but the duodenal flow of NAN was lower ($P < 0.05$) in the EN and PP groups than in the FF group. There was no difference ($P > 0.1$) in the NAN flow between the two monofaunated (EN *v.* PP) groups of sheep. The pattern for the duodenal flow of bacterial N for the protozoal treatments was similar to that for the NAN flow, but the differences were significant ($P < 0.05$) only between the FF and EN means. There was no effect ($P > 0.1$) of the protozoal treatments on the bacterial N flow when it was expressed as a percentage of the NAN flow.

The duodenal flow of PC was highest ($P < 0.1$) in the EN group of sheep (mean 458 (SE 111) mg/d), but the flow did not differ ($P > 0.1$) between the FF and PP groups (259 (SE 57) *v.* 181 (SE 20) mg PC/d, respectively). When the total duodenal flow of PC in the faunated groups of sheep was corrected for the dietary and endogenous flow of PC (determined in FF sheep), the flow of PC was negative for the PP group and assumed to be zero. Thus, the flow of protozoal N in the EN group represented 8·0 % of the NAN flow, whereas no protozoal N flow was detected in the FF and PP groups of sheep. The

total duodenal microbial N flow tended to be lower ($P < 0.1$) in the FF group of sheep (80·1 % of NAN) than in the EN (86·8 % of NAN) or PP (89·3 % of NAN) groups. The flow of non-microbial-NAN (NMNAN) was higher in the FF group than the other groups of sheep (EN and PP) when expressed as g/kg OM intake ($P < 0.05$) and tended to be higher ($P < 0.1$) when expressed as a percentage of the NAN flow. The difference in the duodenal NMNAN flow between the two monofaunated (EN *v.* PP) groups of sheep was not significant ($P > 0.1$).

The duodenal bacterial OM flow (g/d) was not different ($P > 0.1$) between the groups of sheep (Table 3) and ranged between 182 (EN) and 201 (FF). The duodenal protozoal OM

Table 2. Nitrogen intake and duodenal flow in fauna-free sheep and in sheep monofaunated with *Entodinium* or *Polyplastron* genera of rumen ciliate protozoa

(Means with their standard errors)

	Fauna-free		*Entodinium*		*Polyplastron*	
Item	Mean	SE	Mean	SE	Mean	SE
N intake (g/d)	22·9	0·0	22·5	0·39	21·5	0·75
Duodenal flow (g/kg OM intake)						
NAN	28·1[a]	0·57	23·8[b]	0·70	20·4[b]	1·74
Bacterial N	22·5[a]	0·55	18·8[b]	0·60	18·5[ab]	2·34
Bacterial N (% of NAN)	80·1	0·89	79·0	2·46	89·3	4·60
Protozoal N	0	0·0	1·9	1·02	0	0·0
Protozoal N (% of NAN)	0	0·0	8·0	3·99	0	0·0
Microbial N	22·5	0·55	20·7	1·15	18·5	2·34
Microbial N (% of NAN)	80·1[B]	0·89	86·8[A]	3·08	89·3[A]	4·60
NMNAN	5·6[a]	0·26	3·1[b]	0·73	2·1[b]	0·63
NMNAN (% of NAN)	19·9[A]	0·89	13·2[B]	3·08	11·4[B]	4·12

OM, organic matter; NAN, non-NH_3-N; NMNAN, non-microbial non-NH_3-N.
[a,b] Mean values in a row with unlike letters were statistically different ($P < 0.05$).
[A,B] Mean values in a row with unlike letters were statistically different ($P < 0.1$).

flow amounted to 34 g/d in the EN group, but there was no duodenal protozoal OM flow detected in the PP group of sheep.

Digestibility

OM digestion in the stomach (corrected for the flow of bacterial and protozoal OM) was numerically higher in the EN and PP monofaunated sheep compared with the FF sheep (0·71 v. 0·64), but the differences were not significant ($P > 0·1$; Table 3). There were also no differences between the treatments in terms of the apparent intestinal and total tract digestion of OM ($P > 0·1$). The apparent digestibility of ADF in the stomach was lower ($P < 0·05$; Table 3) in the FF group than in other groups of sheep (EN and PP). There was no effect ($P > 0·1$) of the protozoal treatments on intestinal or total tract ADF digestion, although total tract digestion ranged between 0·32 (FF) and 0·43 (PP).

The digestibility of NDF in the stomach and the total digestive tract were both numerically higher in the two monofaunated groups of sheep (EN and PP) than in the FF group, but none of the differences in NDF digestibility was significant statistically ($P > 0·1$; Table 3).

Discussion

The present study was the first known direct comparison of duodenal N flow between EN and PP monofaunas in ruminants. As expected, it showed that the presence of EN or PP monofauna in the rumen of sheep reduced considerably (by 15 % and 27 %, respectively) the duodenal flow of NAN compared with FF sheep. This is similar to the reported effects of the presence in the rumen of total mixed fauna (Veira *et al.* 1983, 1984; Kayouli *et al.* 1986; Ushida *et al.* 1986; Coleman, 1989; Ivan *et al.* 1991). The study also showed an apparent 14 % greater reduction in the flow when the PP monofauna was present in the rumen, compared with the presence of EN monofauna.

Progressive inoculation of FF sheep (Ivan *et al.* 2000*a*) with different species of ciliate protozoa in type A and B populations showed that the cellulolytic PP species in the type A population, and *Epidinium ecaudatum* plus *Entodinium maggi* in the type B population, substantially reduced the duodenal flow of NAN. However, additional significant decreases occurred when EN was added into both populations. It was, therefore, suspected that the effect of EN on the reduction of the NAN flow was larger than that of PP or *Epidinium ecaudatum* plus *Eudoplodinium maggi* (Ivan *et al.* 2000*a*). A follow-up study of direct comparisons of Holotrich (*Isotricha* and/or *Dasytricha*) and EN species (Ivan *et al.* 2000*b*) clearly confirmed previous results. The study also established only a marginal effect of Holotrich protozoa but a substantial effect of EN in reducing the flow of NAN, but the effects of a cellulolytic species of protozoon were not measured. The direct comparison in the present study showed that it was not EN but PP monofauna that were most detrimental to the duodenal flow of NAN. This appears to be contrary to the conclusion derived from previous indirect comparisons (Ivan *et al.* 2000*a*,*b*) and to an earlier indication that EN species might have the largest negative effect on the bacterial synthesis of protein in the rumen (Coleman, 1964). However, in mixed protozoal populations, the number of individual species of protozoa are affected by the presence of other species in the population (Williams & Coleman, 1992). This might affect

Table 3. Intake and apparent digestibility of organic matter, acid detergent fibre and neutral detergent fibre in fauna-free sheep and in sheep monofaunated with *Entodinium* or *Polyplastron* genera of rumen ciliate protozoa

(Means with their standard errors)

Item	Fauna-free		Entodinium		Polyplastron	
	Mean	SE	Mean	SE	Mean	SE
Organic matter						
Intake (g/d)	1086	0·0	1065	17·7	1016	61·7
Duodenal flow (g/d)						
Bacteria	201	2·7	182	9·5	187	23·4
Protozoa	0	0·0	34	16·9	0	0·0
Apparent digestibility (g/g intake)						
Stomach*	0·641	0·024	0·710	0·031	0·712	0·025
Intestine	0·249	0·025	0·223	0·028	0·201	0·035
Intestine (g/g entering intestine)	0·454	0·028	0·447	0·039	0·419	0·061
Total tract	0·705	0·050	0·731	0·010	0·731	0·026
Acid detergent fibre						
Intake (g/d)	205	0·0	201	3·9	204	1·4
Apparent digestibility (g/g intake)						
Stomach	0·201[b]	0·015	0·336[a]	0·057	0·390[a]	0·054
Intestine	0·234	0·096	0·183	0·079	0·220	0·065
Intestine (g/g entering intestine)	0·225	0·097	0·204	0·083	0·278	0·091
Total tract	0·322	0·018	0·381	0·026	0·431	0·079
Neutral detergent fibre						
Intake (g/d)	375	0·0	387	6·9	349	22·9
Apparent digestibility (g/g intake)						
Stomach	0·220	0·056	0·283	0·054	0·267	0·072
Intestine	0·097	0·030	0·090	0·039	0·107	0·055
Intestine (g/g entering intestine)	0·221	0·067	0·198	0·084	0·210	0·104
Total tract	0·402	0·019	0·441	0·027	0·446	0·052

[a,b] Mean values in a row with unlike letters were statistically different ($P < 0·05$).
* Corrected for the flow of bacterial and protozoal organic matter.

the extent of influence of individual protozoal species on the duodenal flow of NAN; such an effect does not exist when only the pure monofauna is present in the rumen, and therefore the former conclusion might be valid.

In addition, the quantitative duodenal flow of bacterial N for the two monofaunas (EN and PP) receiving the same diet in the present experiment was almost identical, indicating an equal predatory activity of the two faunas on rumen bacteria. The diet might, however, affect such activity, and the degree of the dietary effect could be different between monofaunas if the hosts were fed different diets. Thus, compared with FF sheep, EN-monofaunated sheep showed a 25 % reduced duodenal flow of bacterial N when receiving a haycrop-based diet, but the flow was reduced by 46 % when the sheep received a maize silage-based diet (Ivan *et al.* 2000*b*). The reduction was only 16–18 % in the present experiment employing the barley silage-based diet. Although effects of different diets were not measured with the PP-monofaunated sheep, it is reasonable to suggest that such effects are quite substantial, judging from the differences in effect of different diets on the dynamics of establishing PP monofaunas in defaunated sheep (Groliere *et al.* 1980).

John & Ulyat (1984) developed a method for determining PC and concluded that PC was a satisfactory marker for quantifying protozoal flow in naturally faunated sheep. Our modification of the method (Neill *et al.* 1992) improved the PC recovery to 99·4 (SE 0·85) %. The use of PC as a protozoal marker to determine the flow of protozoal N assumes that any PC and protein liberated during the lysis of rumen protozoa is completely degraded by the rumen bacteria. If, however, PC is, during cell lysis, completely degraded in the rumen, but the cellular protein resists complete degradation and a portion passes down the tract, it would be unaccounted for as protozoal N. Such an incomplete degradation of protozoal cells has not, however, been established.

We previously concluded (Neill & Ivan, 1996) that the isolation of mixed-population protozoa from samples of rumen fluid by filtration compared with sedimentation methodology produced a higher recovery of protozoa and a lower bacterial contamination. This was recently confirmed (Sylvester *et al.* 2005). In spite of our earlier conclusion, we selected sedimentation over filtration in the present experiment. This was due to the lack of information on the filtration recovery of the relatively large-size PP as monofauna. Furthermore, because of the similar gravity of cells, separation of the protozoal sediment from monofaunated compared with the mixed fauna population appeared to be more precise, minimizing contamination with bacteria. Although not measured in the present experiment, such a contamination of the monofauna sediment might be lower than that of filtered mixed-species fauna. Also, the filtration technique was known to produce variable results (compare Martin *et al.* 1994 *v.* Volden *et al.* 1999).

There was no duodenal flow of protozoal OM and N detected in PP-monofaunated sheep in the present experiment employing the PC marker discussed earlier. The protozoal N flow in the EN-monofaunated sheep amounted to 8 % of the NAN flow, which is higher than the 5 % obtained with total mixed fauna in sheep (Ivan *et al.* 1992), but lower than the 10–15 % in lactating cows (Robinson *et al.* 1996). The total absence of protozoal N in the duodenal digesta of PP-monofaunated sheep was not expected, but it could be logical. The reason is that PP is a cellulolytic species of protozoon producing enzymes that contribute to the digestion of feed in the rumen (Williams, 1989). Cellulolytic

protozoa attach themselves to plant tissue (Williams & Coleman, 1992). Since such an attachment only lasts 1–2 h (Orpin, 1985) and the generation time appears to be more than 12 h (Dehority, 1998), these protozoa probably move to fresh plant tissues several times during their lifetime before they die and the cells are digested in the rumen. The protozoal protein is then metabolized by rumen bacteria, and the N is recycled within the rumen (Koenig *et al.* 2000). A part of the recycled N might enter the duodenum, but in a form other than that in the protozoal fraction. It has been established that protozoa contribute to the duodenal microbial N flow in a much lower proportion relative to their proportion in the microbial biomass in the rumen (Weller & Pilgrim, 1974). The results of the present study indicated that such a disproportion might be largely due to cellulolytic species of ciliate protozoa. This appears especially so because, as discussed earlier, the proportion of protozoal N flow in EN-monofaunated sheep appeared to be similar to that of a mixed protozoa population (Ivan *et al.* 1992; Robinson *et al.* 1996).

In spite of this evidence, the results of the present experiment indicating the absence of duodenal N flow from PP monofauna should be interpreted with caution. This is because, if it is assumed that PP and EN species flow from the rumen with the fluid phase at the same rate and proportional to the protozoal concentration in rumen fluid, the protozoal flow of PC in the PP-monofaunated sheep in the present experiment would only be about 1·7 mg PC/d (0·02 g protozoal N/d). The variation associated with the measurement of the dietary flow of PC in the FF sheep indicated that PC as a protozoal marker might not be sensitive enough for precise measurements of protozoal N flow in sheep with low rumen protozoal populations, like the PP-monfaunated group of sheep in the present experiment. Additional work is clearly needed to confirm the absence, or to establish the magnitude, of the contribution of PP and other cellulolytic protozoa to the duodenal flow of microbial N.

In spite of a large numerical difference in the proportion of bacterial N in NAN flow, being 15 % higher in the PP- than in the EN-monofaunated sheep, the total microbial N proportion in the duodenal NAN flow was similar in both groups of monofaunated sheep, but 7–9 percentage points higher than in the FF sheep. The similarity was due to the additional N from the protozoal fraction in the EN group, and resulted in similar flows of NMNAN in the sheep with the two rumen monofaunas. However, the results of the present experiment clearly show a higher duodenal flow of both microbial N and NMNAN in the FF group of sheep than in those monofaunated with EN or PP. Compared with the FF sheep, the results also show the increased relative contribution of microbial N by both monofaunas to the NAN flow. This would indicate that both species (EN and PP) of rumen ciliate protozoa metabolize and deaminate both bacterial and feed protein, resulting in higher rumen concentrations of NH_3-N compared with FF sheep. This is in agreement with previous reports describing how an absence of the protozoal predatory activity in FF sheep increases the yield of bacterial protein (Demeyer & Van Nevel, 1979) and eliminates direct protozoal uptake and metabolism of feed protein (Williams & Coleman, 1992). This results in lower rumen protein degradation (Ushida & Jouany, 1985), a lower concentration of NH_3-N and a higher intestinal flow of protein/amino acids (Veira *et al.* 1983, 1984; Kayouli *et al.* 1986; Ivan *et al.* 1991) of both microbial and dietary origin.

Comparing the two monofaunas, VFA concentrations and ADF digestibility in the rumen were numerically higher in the PP- than in

in the EN-monofaunated sheep, which is in agreement with other studies of this kind (Jouany & Senaud, 1982, 1983). As PP is a cellulolytic protozoon whereas EN is not (Williams, 1989), this appears to be logical. However, in comparison with FF sheep, the stomach digestibility of ADF was increased not only by the PP-monofauna, but also by the EN monofauna. Since the EN genus is not cellulolytic (Williams, 1989), such a positive effect of the EN monofauna on stomach ADF digestibility is not direct, and is almost certainly due to the influence of EN inoculation of FF sheep on the rumen bacterial population. It was reported that the number of cellulolytic bacteria in the rumen increased on faunation (Kurihara et al. 1978). The rumen digestibility and apparent total tract digestibility of OM were almost identical for the two monofaunas. However, inoculation of the FF sheep with either of the two monofaunas decreased the molar proportion of butyric, valeric and caproic acids at the expense of acetic acid, without affecting the acetic:propionic acid ratio. The decreased molar proportion of butyric acid owing to the inoculation of the two monofaunas is contrary to the report of Jouany & Senaud (1982).

From the results of the present experiment, it can be concluded that the extents of predation on rumen bacteria by monofaunas of the EN and PP genera of ciliate protozoa are virtually equal, and both monofaunas contribute directly or indirectly to the digestion of ADF in the rumen. The results suggest that PP, and possibly other cellulolytic ciliate protozoal species, might not leave the rumen and therefore might not directly contribute to the post-ruminal intestinal flow of microbial protein.

Acknowledgements

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