Effect of protozoa on bacterial numbers and microbial protein flow

	Faunated	*Defaunated*	*Refaunated*	*SED*
Ammonia-N (mg l^{-1})	279	153	234	27.5
Total culturable bacteria (x10^8 ml^{-1})	6.4	11.0	5.5	2.74
Cellulolytic bacteria (x10^7 ml^{-1})	2.51	1.98	0.94	0.72
Protozoa (x10^6 ml^{-1})	0.86	-	1.22	0.25
Microbial protein flow from the rumen (gN d^{-1})	11.3	16.9	9.7	1.4

It is well established that bacterial numbers in the rumen increase when ciliate protozoa are removed from the rumen. However, it has also been suggested that bacterial numbers may remain elevated after the subsequent reintroduction of ciliates to the rumen [1,2]. The aim of this study was to investigate the effects of defaunation and subsequent refaunation on bacterial numbers in and microbial protein flow from the rumen.

Four rumen cannulated sheep received a diet of 600g alfalfa haylage and 900g barley concentrate each day. Sheep were defaunated, using a rumen washing procedure, 50 days prior to making measurements in the defaunated period. Sheep were refaunated by transferring rumen fluid from a faunated sheep receiving the same diet and a protozoal population was allowed to develop for 35 days prior to making measurements in the refaunated period.

Total culturable bacterial numbers, but not cellulolytic bacterial numbers, in the rumen increased following defaunation. This was associated with a reduction in rumen ammonia concentration and an increase in the flow of microbial protein from the rumen, as estimated from the excretion of purine derivatives in the urine. Following refaunation, protozoa quickly re-established in the rumen to a density that did not differ from that in the original faunated sheep. Bacterial numbers declined following the reintroduction of protozoa and, as a result, neither rumen ammonia concentration nor the flow of microbial protein from the rumen differed between faunated and refaunated animals. In the current study we could find no evidence of an enhanced number of bacteria in the rumen of refaunated sheep.

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The contribution of individual genera in a mixed protozoal population to the breakdown of bacteria in the rumen. CJ Newbold[1], JP Jouany[2], *([1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [2]INRA, Station de Recherches sur la Nutrition des Herbivores, Centre de Clermont-Theix, 63122 Saint Genès-Champanelle, France)*

Predation and digestion by ciliate protozoa can account for 90% of the bacterial protein turnover in rumen fluid *in vitro*. However there is little information on the role of individual protozoal genera in the breakdown of bacterial protein in the ru

Calculated contribution of *Entodinium, Isotricha* and *Polyplastron* spp. to the breakdown of bacteria in rumen fluid containing a mixed fauna.

Bacteria/ciliate	*Breakdown (%) attributed to specific protozoal genera*		
	Entodinium	*Isotricha*	*Polyplastron*
Selenomonas ruminantium	24.7	6.3	58.7
Megasphaera elsdenii	20.2	5.1	52.7
Bacteroides multiacidus	63.0	2.9	42.1
Lactobacillus casei	14.3	3.3	60.1
Ruminococcus albus	27.0	14.7	58.8
Prevotella ruminicola	33.0	9.9	52.1
Streptococcus bovis	34.5	6.1	52.6

Standard error of the difference (SED) for comparing means between ciliates = 3.96 ($P<0.01$, n=42), SED bacteria = 6.05 ($P>0.05$, n=18), SED Bacteria within ciliate = 10.47 ($P<0.05$, n=6).

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Rumen fluid was withdrawn from ciliate-free sheep or sheep mono-faunated with *Entodinium* spp., *Isotricha* spp. or *Polyplastron* spp., or faunated with a mixed population of all three ciliates. Breakdown of washed pure cultures of bacteria in rumen fluid was determined from the release of acid soluble [^{14}C] from [^{14}C]-leucine labelled bacteria. Release of label in defaunated rumen fluid was used to correct for breakdown not attributable to protozoa. Counts of ciliates were then used to calculate the breakdown of bacteria/protozoa in mono-faunated sheep. The mean protozoal density in sheep with a mixed protozoal population was 6.8×10^4 ml^{-1}, comprising 68% *Entodinia*, 14% *Isotricha* and 18% *Polyplastron*. These values were used to calculate the contribution of the individual genera to breakdown of bacteria in the mixed population (Table 1). On average 92% of the observed breakdown of bacteria by protozoa could be attributed to breakdown by the individual genera. *Isotricha* accounted for less than 7% of the total bacterial breakdown. *Polyplastron* was the most active ciliate with the majority of bacteria tested, only with *B. multiacidus* did *Entodinium* play the dominant role. It appears that the relative contribution of individual protozoal genera to bacterial protein breakdown in the rumen will vary dependent on the bacterial species in question.

Purification and characterisation of a lysozyme-like enzyme from the rumen ciliate *Entodinium caudatum.*

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Ciliate protozoa decrease the flow of bacterial protein from the rumen by engulfing and digesting the indigenous bacteria. Removal of protozoa from the rumen has been shown to increase the flow of bacterial protein to the duodenum by up to 50% [1]. Despite their importance in bacterial protein turnover, little is known about the enzymes responsible for the digestion of bacteria in rumen ciliates. The aim of this study was to isolate and characterise a protozoal enzyme capable of degrading bacterial cell walls.

A potent bacteriolytic activity was iso

lated from the rumen ciliate, *Entodinium caudatum,* maintained *in vivo* in monofaunated sheep. The enzyme was purified using a combination of cation exchange chromatography on carboxymethylcellulose and size exclusion gel filtration on Sephadex G-75. The isolated enzyme resembled lysozyme in that it was a basic protein which rapidly degraded *Micrococcus lysodeikticus* cell walls. The enzyme had an isoelectric point of pH 9. It displayed optimal activity at pH 6.5, an ionic strength of 0.05M and at a temperature of 40°C. It had an apparent affinity constant (K_{app}) of 400mg *M. lysodeikticus* l^{-1}. The protein had a molecular weight of 14 kDa as determined by SDS-PAGE. Based on the N-terminal amino acid sequence the protein showed some similarity to a distinct class of lysozymes found in *Streptomyces* species, the fungi *Chararopsis* and the protozoal parasite *Entamaoeba histolytica* [2].

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Molecular cloning of a chitinase gene from the rumen protozoon *Entodinium caudatum*. K Komatani[1], DP Morgavi[1], R Onodera[1], BA White[2], I Cann[2] KM Karrer[3] *([1]Miyazaki University, Laboratory of Animal Nutrition and Biochem, Miyazaki 889-21, Japan; [2]University of Illinois, Department of Animal Science, 1207 W Gregory Dr, Urbana, IL 61801, USA; [3]Marquette University, Deptartment of Biology, Milwaukee, WI 53201, USA)*

The predatory activity of ruminal protozoa profoundly affects the microbial population in the rumen and consequently influences the degradation of food and availability of nutrients to the host animal. It is thought that chitinases, lysozymes and other hydrolytic enzymes are of fundamental importance in the protozoal digestion of engulfed fungi and bacteria, and this process, if well understood, may lead to the design of strategies to improve ruminant performance. The aim of the present study was to clone a gene from the rumen protozoon *Entodinium caudatum* encoding a chitinase in order to better understand the role that this hydrolytic enzyme plays in the interrelationship between protozoa and fungi in the rumen.

E. caudatum, harvested from a monofaunated goat fed a concentrate and hay cube ration, were washed by centrifugation and total RNA was isolated using a single-step guanidinium method. Poly(A)$^+$ RNA was purified on oligo(dT)-cellulose resin and used as template for the construction of a cDNA library in λphage by using a commercial kit (ZAP-cDNA® synthesis kit, Stratagene). The pBluescript phagemids containing the cloned inserts from the entire library were in vivo excised from the λphage vector (Uni-ZAP XR) following the manufacturer's instructions; these phagemids were used to transform *E. coli* strain SORL. Transformants grown on LB ampicillin agar plates in the presence of IPTG were covered with 0.7% agar containing 0.02% glycol chitin and incubated at 37°C for 6 to 8h. After the incubation period, plates were stained with a 1% solution of Congo Red.

Several colonies showing a clear halo, indicating chitin degradation, were isolated and re-screened. After a third screening, the clone producing the most perceptible clearing of chitin was chosen.