Methane production and protozoal numbers *in vitro*

Inocula	*Defaunated*	*Isotricha*	*Mixed-faunated*	*Effect of protozoa*
Ciliate number (ml^{-1})	0	5×10^3	1.5×10^5	-
Ciliate volume (ml^{-1})	0	4.8	9.3	-
CH_4 production in 6 hrs (ml):				
$(NH_4)_2SO_4$	205	270	329	S*
Soyabean	273	319	333	S
Fishmeal	228	296	338	S
Peas	260	309	342	S
Effect of N source	S	S	NS*	

*S : significant ($P < 0.05$) ; NS : not significant ($P > 0.05$)

[1]. This work aimed to evaluate the impact of the presence of *Isotricha* in comparison with a mixed B-type fauna or defaunated rumen contents on methane production measured in vitro, in the presence of different nitrogen sources.

The in vitro system [2] received inocula from three defaunated sheep or sheep inoculated with *Isotricha* spp. (three sheep) or a mixed B-type fauna (three sheep). All fermentors were fed with 13g wheat starch + $(NH_4)_2 SO_4$ (187mg N); some of them also received soyabean meal, or fishmeal, or peas supplying the same amount of nitrogen (125mg N). Incubations were repeated six times. Methane was analysed by GLC [3]. Means were compared by analysis of variance. The results are summarized in the table.

Methane production was significantly higher in the presence of *Isotricha* spp. (+20%) or the mixed fauna (+33%) than with the defaunated inocula. Per unit volume, *Isotricha* produced twice as much methane as the mixed B-type fauna. No influence of nitrogen source on methane production was observed with the mixed faunated inoculum. On the contrary, more methane was produced with proteins of medium and high rumen degradability like soyabean meal and peas than with proteins of low rumen degradability like fishmeal with the defaunated and *Isotricha*-inocula. The lowest methane production was observed in fermentors fed with $(NH_4)_2 SO_4$ as sole nitrogen source (Table).

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Effects of defaunation and subsequent refaunation on bacterial numbers in and microbial protein flow from the rumen of sheep. CJ Newbold[1], FM McIntosh[1], XB Chen[1], KM Koenig[2], LM Rode[2], AF Furtado[2] *([1]Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, UK; [2]Research Centre, Agriculture and Agri-Food Canada, Lethbridge, Alberta T1J 4B1, Canada)*

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