RUMEN PROTOZOA - POTENTIAL FOR MANIPULATION

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Summary

The manipulation of rumen function through the removal of the ciliate protozoa has not resulted in consistent changes in ruminant production. Current research findings are discussed with particular emphasis given to the role of protozoa in fibre digestion in the rumen and the availability of protein for intestinal digestion. Dietary conditions under which positive responses to defaunation can be expected are identified.

I. INTRODUCTION

The majority of the metabolisable nutrients for the ruminant are provided by the end products of fermentation and it is clear that an understanding of rumen function is the key to optimising feed utilisation by ruminants. Manipulation of rumen fermentation provides an important means of increasing the efficiency of feed utilisation and ruminant production. Fermentative digestion and nutrient outflows from the rumen can be adjusted favourably either by protecting dietary components from the microbes or by controlling the balance of microbial species and/or their activities (Chalupa, 1977). The use of chemicals to enhance rumen fermentation has been an integral part of intensive ruminant production for many years. Of course fermentation will only be altered when the chemical agent is present. This necessitates regular dosing and often the dose needs to be accurately controlled. In contrast the removal of protozoa from the rumen (defaunation) can be achieved with a single treatment and further treatment is unnecessary provided defaunated animals are isolated from other ruminants. The cost of controlling protozoa is likely to be low and has an obvious application in the grazing industry,

Although it is well established that protozoa make a significant contribution to fermentation in the rumen (Hungate,1966), it does not necessarily follow that the most efficient combination of microbes will include the ciliates. In support of this statement current research findings will be discussed with particular emphasis given to the role of protozoa in microbial growth and digestion of fibre in the rumen.

II. MICROBIAL PROTEIN SYNTHESIS IN THE RUMEN

The dynamic nature of the rumen population of micro organisms enables the ruminant to successfully adapt to a wide range of diets with the relative proportions of the various microbes changing with the availability'of substrates and changes in the rumen environment (Hungate,1966). It is not surprising therefore to find that when protozoa are removed from the rumen there is an increase in the size of the bacterial population to compensate for this loss (Kurihara et al. 1968). The existence of an inverse relationship between bacteria and protozoa1 numbers is due not only to the direct conpetition for nutrients and space but also to the predatory activity of the protozoa (Colenan, 1975). As with other types of manipulations it is not possible to examine the effect of defaunation in isolation, so that a comparison between faunated and defaunated animals is, in effect, a comparison of the metabolism of the protozoa and the displaced bacteria. It is also apparent that changes in fungal populations associated with defaunation may also be important.

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The rumen ciliates are proteolytic producing ammonia and amino acids as end products (Warner,1956). Their N metabolism is based largely on the digestion of engulfed bacteria (Coleman, 1975) although all rumen ciliates contain enzymes capable of digesting plant proteins (Coleman, 1983). The utilisation of these protein substrates is inefficient as a significant proportion of the total amount engulfed by the protozoa is excreted as amino acids (Coleman, 1975). Degradation of protein or amino acids of microbial origin reduces the net yield of microbial amino acids available for intestinal digestion and hence the net efficiency of microbial growth. Further, a large proportion of the protein incorporated in the biomass of protozoa in the rumen may not be available for digestion in the intestines. Direct enumeration of protozoa in the rumen fluid entering the omasum (Weller & Pilgrim, 1974, Bird et al. 1978), the concentration of a protozoa1 marker (phosphatidyl choline) in abomasal digesta (Neill et al. 1979) and measurement of the turnover rates of the protozoa (Leng et al. 1981, Leng, 1982) all indicate a low outflow rate of ciliates from the rumen in relation to their concentration in the rumen. The studies of Leng et al, (1981) and Leng (1982) show that a large proportion of the ciliates complete their life cycle within the rumen. The apparent turnover of protozoa1 protein was calculated to be 0.67 of the total protein synthesised by protozoa in the rumen of sheep, with only 0.33 of the protein in the protozoa1 biomass leaving the rumen (Leng, 1982). The synthesis of protozoa1 nitrogenous compounds and their breakdown in the rumen therefore contribute considerably to the N recycling within the rumen.

While there is strong evidence indicating protozoa are retained in the rumen, it is obvious that some protozoa move to the lower digestive tract. The proportion of protozoa1 biomass which leaves the rumen appears to vary according to diet and protozoa1 species and in some instances has been reported to make a substantial contribution to the microbial component in duodenal digesta (Smith et al. 1978, Chamberlain & Thomas, 1979, Punia & Leibholz, 1984). However since Lindsay and Hogan (1972) reported that defaunation was associated with an increase in the flow of total non-ammonia N(NAN) and bacterial N at the abomasum of sheep, similar studies have confirmed these findings and the flow of NAN and microbial N from the defaunated rumen was always greater than the flows of protein from the faunated rumen (Table 1). More recently Veira

Table 1. A comparison of flows of microbial N and NAN into the intestines of faunated and defaunated sheep

	N° animals	faunated sheep gN/d	defaunated sheep gN/d	Benefit from defaunation %
Total Non-Ammonia-N* (range)	22	21 (16-29)	26 (17-32)	26
Microbial N* (range)	18	15 (12-15)	17 (15-18)	19

* Mean values obtained from numerous authors

et al. (1984) measured the flow of amino acids to the stomach of defaunated sheep, then following inoculation of these animals with protozoa continued to measure amino acid flows. The flow of amino acids to the stomach was significantly decreased when sheep; defaunated from birth, were inoculated with protozoa (Figure 1). It is apparent from this discussion that more protein should be available from the protozoa-free rumen.

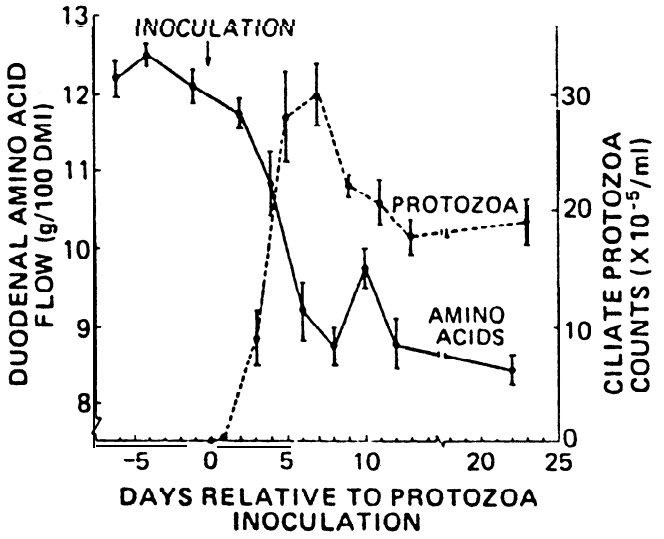

FIGURE 1 Changes in the flow of amino acids at the duodenum associated with the inoculation of protozoa into the rumen of protozoa-free sheep (Veira et al. 1984)

III. FIBRE DIGESTION IN THE RUMEN

Feed intake is often the primary factor limiting 'the productivity of grazing animals and therefore any procedure which stimulates feed intake is likely to be highly beneficial. While apetite control in ruminants is complex (Baile, 1975) bulk-distension of the rumen is considered to have a dominant influence on feed intake of animals consuming relatively indigestible forages. Therefore increasing the rate of fibre digestion should be a prime objective of rumen manipulation.

Although Hungate (1975) suggested that the amount of cellulose digested by the protozoa was likely to be small in comparison with the bacteria, it is apparent that in some situations the ciliates make a significant contribution to fibre digestion in the rumen (Jouany & Senaud, 1979). The effects of defaunation on fibre digestion in the rumen will be influenced by the enzymic activity of the protozoa relative to that of the bacterial and fungal populations that replace the protozoa. Cooperative and competitive interactions between the respective microbial populations and changes in rumen environment associated with defaunation are also discussed.

A number of enzymes that degrade plant cell walls have been isolated from cell-free extracts of rumen protozoa (Orpin,1984). The enzymes, and their activity vary with protozoa1 species (Coleman, 1983) and therefore the polysaccharase activity of the total population of rumen ciliates varies according to the species present. Obviously the protozoa1 can only be important in fibre digestion per se when they make a significant contribution to the microbial biomass in the rumen.

Both cooperative and competitive interactions between microorganisms have been identified in the rumen (Baldwin & Allison, 1983) and therefore it is not sufficient to consider the activity of one group of organisms in isolation. For example it is well known that two or more bacterial species nay function as a consortium with one species growing on the end products of metabolism of another species and these associations appear to be highly beneficial (see Wolin, 1979). While little is known about interactions between bacteria and protozoa, beneficial associations between these organisms cannot be discounted. Yoder et al. (1966) reported a synergistic interaction between bacteria and Epidinium spp. (rumen ciliate) during the digestion of

cellulase in vitro, and the addition of the ciliate Polyplastron multivesiculatum to a defaunated sheep increased the cellulolytic activity of the bacteria (Jouany & Senaud, 1979).

The predator-prey relationship between protozoa and bacteria is an obvious example of a competitive interaction in the rumen. Whether this interaction influences rate of fibre digestion in the rumen will be determined by the relative activities of the protozoa and bacteria. It has been suggested that the predatory activity of the protozoa is unlikely to alter the composition of the bacterial population (Hobson & Wallace, 1982) although in a recent study Orpin and Letcher(1984) reported that the population of bacteria in the fluid phase increased markedly following defaunation while the population of bacteria associated with the fibre fraction of digesta remained unchanged: This suggests that the predatory activity of the protozoa was confined to the fluid phase bacteria.

Recent studies have indicated that the interaction between the ciliates and the phycomycetous fungi may also be important as there appears to be an inverse relationship between their relative population sizes. While the activity of the fungi relative to the protozoa and bacteria is uncertain, it is likely that fungi have a significant role in the digestion of fibre in the rumen. Large populations of fungi rapidly colonise plant material entering the rumen (Bauchop, 1981) and they are capable of digesting a wide range of plant cell wall components (Orpin & Hart, 1980). The invasion of plant material by the fungi results in a decreased tensile strength of fibre (Akin et al. 1983) and an increased fragmentation of plant tissues allowing greater access for other organisms (Bauchop, 1981), and may increase the rate of digestion and removal of the indigestible fibre fraction from the rumen, allowing increased intake.

The life cycle of the anaerobic fungi consists of two distinct stages; a non motile vegetative form and a motile flagellated form (zoospore) which can move freely in rumen liquor. The zoospores are likely to be subjected to predation by the protozoa. While enumeration of a particular stage in the life cycle of the fungi is likely to be a poor index of the extent of the fungal population (Bauchop 1981), currently there are no other methods of determining fungal biomass. The number of fungal zoospores in the rumen appears to be greater on high fibre diets than on high concentrate diets which is the reverse of the cofonisation of the rumen by ciliates (Orpin,1984). On the basis of these findings, Orpin(1984) suggested that protozoa and fungi may be *complementary* in the rumen, but recent defaunation studies carried out in our laboratories indicate a competitive interaction between these organisms, The concentration of viable zoospores in rumen fluid collected from defaunated sheep was two to five fold higher than in rumen fluid collected from faunated sheep given high roughage diets (Soentanto et al. 1984; Romulo et al. 1984) and from weaner lambs grazing pasture (Bird & Leng, 1984) (see Table 2). Viable zoospore counts were determined from in vitro incubation of diluted rumen fluid (Joblin,1981). The results from the roughage-fed animals were also supported by sporangia counts on wheat-stem material held in the rumen of these animals (Soetanto et al. 1984; Romulo et al. 1984).

The apparent increase in size of the fungal population in the defaunated sheep was associated with an increased digestibility of dry matter (determined in sacco) in the rumen (see Table 3). The digestion of cotton wool in nylon

Table 2. Viable zoospore counts determined from in vitro incubation of diluted rumen fluid collected from faunated (+P) and defaunated (-P) sheep

Diet	Viable Zoospores ($X10^{-3}$/ml) faunated sheep (+P)	defaunated sheep (-P)	
Wheat straw	3	17	Soentanto et al. 1984
Ammoniated wheat straw	7	16	Romulo et al. 1984
Wheat straw	4	12	Romulo et al. 1984
Native pasture	7	30	Bird et al. 1984

Table 3. Disappearance of dry matter from nylon bags held in the rumen of faunated (+P) and defaunated (-P) sheep given untreated or ammoniated straw (NH_3-wheat straw) diets supplemented with or without 150 g/d lucerne (Romulo et al. 1984)

Diet	Material in bag	Dry Matter Disappearance % 0-4 hours +P	0-4 hours -P	0-24 hours +P	0-24 hours -P
NH_3 wheat straw	NH_3 wheat straw	15[a]	20[b]	39	43
NH_3 wheat straw+lucerne	NH_3 wheat straw	16	19	44	50
Wheat straw	Wheat straw	5	6	26	29
Wheat straw + lucerne	Wheat straw	6	8	31[a]	34[b]

Values with different superscripts are significantly different at $P<0.05$.

bags was also higher in defaunated sheep which indicates a higher cellulase activity in the rumen of these animals relative to the faunated sheep. It is suggested that the higher digestibility of fibre was due to changes in the activity of the bacteria and/or the fungi.

Our findings appear contrary to earlier work (Christiansen et al. 1965) which indicated that, in the absence of protozoa, the apparent digestibility of organic matter in the rumen was lower. However the early work was carried out with animals sustained on diets containing high amounts of starch-based concentrates whereas the diets used in our work contained little or no soluble carbohydrate, A South African group (Henning et al. 1980) suggested that starch, or sugars derived from starch, may inhibit the activity and/or the synthesis of bacterial cellulases in the rumen. As protozoa rapidly assimilate starch and soluble sugars, their removal from the rumen may result in an increase in the concentration of these carbohydrates and accentuate the inhibition of fibre digestion.

The above discussion highlights the interactive nature of the rumen ecosystem. Irrespective of the rationality of the manipulative procedure, the changes in the rumen and in production are not readily predictable. It is therefore desirable to test the effects of defaunation on productivity of animals in pen feeding and grazing systems,

IV. PRODUCTIVITY OF FAUNATED AND DEFAUNATED SHEEP

From a theoretical viewpoint it appears logical that more protein should be available from the protozoa-free rumen. Our initial work therefore examined the effect of defaunation on the productivity of young ruminants given diets containing suboptimal levels of protein. Defaunation was associated with marked increases of growth rates of cattle (Bird & Leng, 1978) given a diet of straw, molasses and urea, and with higher growth rates of lambs (Bird, Hill & Leng, 1979) given a diet of oaten chaff, sugar and urea. More significantly defaunation consistantly increases wool production (Bird & Leng, 1978) and over a 6 month period ciliate-free sheep grew 37% more wool than their faunated counterparts (Bird & Leng 1984b). This was strong evidence that defaunation increased the amount of protein which is available for intestinal digestion since wool growth is highly sensitive to amino acid absorption (Reis & Schinckel, 1961).

These results were obtained in studies where animals were given specialised diets (ie. rich in soluble CHO to promote large protozoa1 populations but low in protein). More recent studies now suggest that beneficial responses to defaunation may be obtained over a wider range of dietary regimens. Over an 84 d period defaunated hoggets grazing green oats had a faster rate of body weight gain (23%) and higher wool growth rate (19%) with respect to faunated animals (Bird & Leng, 1984a). This work was followed up by a three year study with ewes grazing improved native pasture, six paddocks were grazed in a 6 x 21 d rotation. Pasture growth was very poor in the first year (drought conditions) but was above average and of apparent high quality in the two subsequent seasons. In the first year the ewes were treated for the elimination of protozoa after joining but unfortunately small populations of ciliates were detected in the defaunated group of animals in the 10th week of the experimental period. The same procedure was followed in the second year and the treated ewes remained free of ciliates for the remaining 18 months of the trial. The overall results suggest that defaunation improves the productivity of young animals under grazing conditions (Table 4) which was consistent with the results of the earlier study

Table 4. Wool growth rate and bodyweight gain of faunated (+P) and defaunated (-P) sheep grazing native pasture

	Year	No. of animals	Study period (wks)	Body wt gain (g/d)		Wool growth (g/d)	
				+P	-P	+P	-P
Ewes	1982	32	23	-48	-48	3.6^{A}	4.4^{B}
	1983	39	23	67	73	6.6	7.0
	1984	37	52	8	0	7.5	7.5
Weaner lambs	1983/84	49	16	85^{a}	98^{b}	7.2_{A}	7.6_{B}
	1984/85	62	19	88^{a}	110^{b}	4.9^{A}	5.5^{B}

Values with different superscripts are significantly different at P<0.05.

(Bird & Leng, 1984a). The only beneficial response observed in the defaunated ewes was the higher wool growth recorded in the drought year. It is possible that the additional protein that was available for digestion in the defaunated ewes was used for the maintenance and growth of the foetus rather in for wool growth. The birthweight of the lambs born to defaunated ewes tended to be higher (Table 5). It is important to note that while defaunation was not always associated with a positive response there were no apparent negative effects.

Table 5. Birthweight of lambs born to faunated (+P) and defaunated (-P) ewes

Year		Lamb birthweight (kg)	
		+P	-P
1982	Single	4.8[a]	5.4[b]
	Twin	3.6	3.8
1983	Single	5.5	5.4
	Twin	4.4	4.3
1984	Single	5.2[a]	5.7[b]
	Twin	4.4	4.6

Values with different superscripts are significantly different at P<0.05

As a result of the *in sacco* digestibility studies (see Table 3) it became apparent that increases in the amount of protein available for digestion may not be the only benefit derived from the removal of ciliates from the rumen. Recently a pen feeding study was established to examine the effects of defaunation on the productivity of lambs (9 months of age) given low quality roughage diets. There were two basal rations, wheat straw and ammoniated wheat straw, and each ration was supplemented with 150 g/d lucerne chaff. The untreated wheat straw rations were supplemented with an additional 14 g/d of urea and all rations were supplied with adequate minerals and vitamins. The results are given in Table 6. Intake of the untreated straw by the defaunated lambs was substantially higher, presumably because of the greater digestibility of fibre in the rumens of these animals. In contrast, intake of the treated straw (unsupplemented ration) was lower in the defaunated animals and similar for both groups of animals when lucerne was included in the ration. This result was unexpected as the *in sacco* study had indicated that the digestibility of treated straw was also higher in the defaunated animals. While these results are inconclusive they do suggest that defaunation may be an important means of increasing intake of low quality roughage.

Table 6 Bodyweight change and feed intake of faunated (+P) and defaunated (-P) hoggets given an untreated or an ammoniated wheat straw supplemented with or without 150 g/d lucerne (10 week study)

Diet	Intake of Wheat Straw (g/d)		Bodyweight change (g/d)	
	+P	-P	+P	-P
Wheat straw	410	490	-70	-61
Wheat straw + lucerne	375[a]	560[b]	-20	-2
NH_3 wheat straw	780	695	32	14
NH_3 wheat straw + lucerne	735	735	59	72

Values with different superscripts are significantly different at P<0.05

V. CONCLUSION

Manipulation of rumen fermentation through the removal of the ciliate population appears to have considerable potential for improving ruminant productivity. However while the interaction between bacteria and protozoa apparently reduces the amount of protein available for intestinal ingestion, other changes in the rumen ecosystem, dietary regimens and physiological state of the animal will influence the response to defaunation. For instance the growth rate of lambs consuming a high quality diet rich in concentrates (starch-based) was lowered by defaunation (Christiansen et al. 1965) and it is thought that this may be due to a lower digestion of fibre in the rumen of the defaunated animals. In contrast the digestion of low quality forages was higher in defaunated animals (Soentanto et al. 1984; Romulo et al. 1984) and the intake of wheat straw by unfaunated lambs was considerably higher than their faunated counterparts. A competitive interaction between the fungi and protozoa is indicated in these studies. These findings may have important implications, as the utilisation of crop residues and agro-industrial wastes by ruminants assumes greater significance. Beneficial responses to defaunation have been demonstrated in grazing animals, particularly in young animals. While an increased wool production is perhaps the most important response to defaunation, the faster growth rate of young animals may result in the juvenile females reaching maturity at an earlier age and increase conception rates of maiden ewes which are traditionally low under Australian conditions.

The application of this technology is dependent upon the development of a specific protozoa1 toxin which unfortunately is not imminent.

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