THE ROLE OF RUMEN PROTOZOA IN THE NUTRITION OF RUMINANTS

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SUMMARY

Recent results have tended to support the concept that protozoa are preferentially retained in the rumen. Even though they can constitute a large proportion of the microbial biomass in the rumen they apparently contribute a relatively small proportion of the microbial protein available for digestion in the small intestine 'of ruminants. The large ciliate protozoa may be retained more completely than the small ciliate protozoa. Recent *in* Vitro results suggest that engulfment of bacteria by'protozoa may be the major loss of microbial protein to the animal. Possibly the energy cost of maintenance of protozoa may be the most significant detrimental effect of the presence of large protozoal populations in the rumen.

INTRODUCTION

Bird (1978) in the last issue of these proceedings reviewed the work that had been done on defaunation of ruminants in these laboratories. Bird (1978) reported that the removal of protozoa from the rumen of lambs, mature wethers and cattle on high energy, low protein diets resulted in considerable increases in liveweight gain. Also in one 'study the production of wool was increased considerably in lambs without protozoa in their rumen as compared to similar animals with normal ruminal populations of protozoa. The conclusions from that work were that the presence of large populations of protozoa in the rumen resulted in a decreased availability of both protein and energy from the rumen system and the responses to defaunation were more likely when the diet was suboptimal in bypass protein. In the past studies of the effects of defaunation have not always been carried out in animals with large protozoal biomass. Unfortunately there-has been no attempt to vary the total biomass in relation to the biomass of bacteria, to assess the change in total biomass of microorganisms within the rumen. However, if we assume that the rumen system is limited by nutrient supply then the microbial biomass should be constant. Under these circumstances an increase 'in protozoal numbers must be accompanied by a decrease in the bacterial population within the rumen and vice versa. Using values 'for bacterial and'protzoal size given by Warner (1962), Leng (1976) indicated that the relationships shown in Figure 1 should . apply. Since a small ciliate *(entodinia* spp.) is about 0.1 of the size of the large holotricha protozoa the population of *entodinia* protozoa must be at least 10 times that of the large holotricha before they approximately equal the same biomass within the rumen.

DISTRIBUTION OF PROTOZOA

On dried and preserved forages and grains, (typical diets of animals in research programmes), the small ciliate protozoa (mainly *entodinia* and *epidinium* spp) are predominant in the rumen of sheep and cattle. However, where sugar is a major proportion of the carbohydrates in the diet, large holotrich protozoa tend to become predominant (mainly *isotricha* and *dasytricha* spp.). We basically have two groups of protozoa to consider. On pastures where these contain clovers, the tendency is for the large ciliate protozoa to co-exist. with the smaller

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entodinia (see Clarke 1965). On roughage diets or dry pasture or grasses, *entodinia* protozoa tend to predominate.

EFFECTS OF DIET ON POPULATIONS OF PROTOZOA IN THE RUMEN

In any situation where the pH of the rumen contents falls below about 5.5 at anytime following feeding,protozoa tend to die and if the pH is maintained for long periods of time protozoa1 populations are always small (Purser and Moir 1959). Thus where grain is a high proportion of the diet (i.e. feed-lot situations), protozoa do not usually exist in the rumen. However, where restricted grain/high forage feeding is practised, ruminal protozoa numbers can be extremely high. As a general rule it is suggested that if the pH of the rumen contents is high and there is a readily available carbohydrate source in the diet, protozoa effectively compete with bacteria and represent a large proportion of the microbial biomass in the rumen. Conversely if protozoa are removed by any means, bacterial population will tend to increase. If a chemical is added to the diet which affects bacterial populations, then the protozoa1 numbers may tend to increase, for instance, aureomysin or tylosine increases the number of protozoa but not bacteria in rumen contents (Purser et al. 1964; Klopfenstein et al. 1964).

ASSESSING PROTOZOA NUMBERS

It should be stressed, that protozoa1 numbers as presently assessed by visual counting, are inaccurately determined. 'In order to be sure that a change has occurred with protozoa1 numbers in the rumen a great deal of care must be taken to obtain a representative sample of rumen fluid and in addition to sample the animal over a prolonged period of time. Even under these circumstances it is probably impossible to accurately sample the total protozoa pools because of the changes in protozoa1 numbers that apparently occur over a day. These apparent changes in protozoa1 numbers in the rumen, which fluctuate at times up to a 100 fold within a day, appear to be the result of a sequestering of protozoa on plant materials or settling of the protozoa within the rumen system. Diurnal fluctuations in rumen protozoa numbers are due to changes in distribution of protozoa within the rumen and a lack of ability to sample these protozoa (see Clarke, 1965; Leng and Preston, 1976).

INTRODUCTION OF PROTOZOA INTO THE RUMEN

There appears to be no resistant phase in the life cycle of ciliate protozoa which live in the rumen. In this way animals can only be infected by direct or very close contact with infected animals and therefore the possibility exists for groups of animals to be maintained 'protozoa free.

THE INTERACTION OF RUMEN PROTOZOA AND PRODUCTION OF RUMINANTS

The early studies of Weller and Pilgrim (1974) which showed that protozoa of the *entodinia* spp. were preferentially retained within the rumen have been confirmed by (1) slaughter studies (Minor et al.1978 Bird et al. 1978) (2) studies in which protozoa1 markers have been used (John and Ulyatt 1979 and Harrison et al. 1979). Recent studies from our own laboratory (Leng et al. 1980) have demonstrated, using protozoa labelled with carbon 14, that the turnover of protozoa in the rumen was

FIGURE 1 Calculated protozoal biomass as a percentage of total' biomass for various concentrations of small ciliate protozoa (0) and large ciliate protozoa (0) in rumen fluid

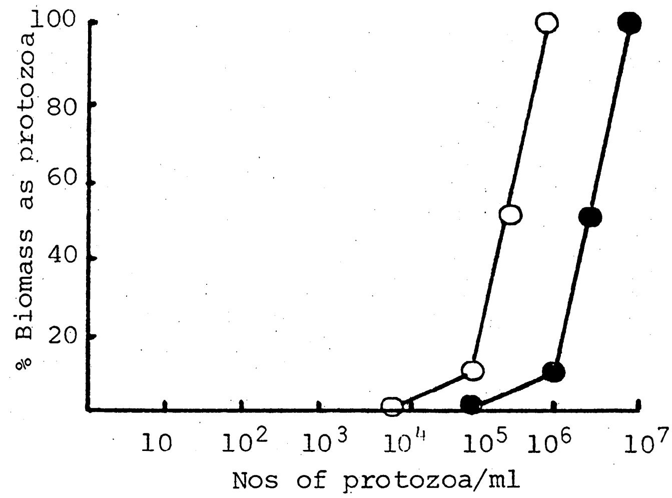
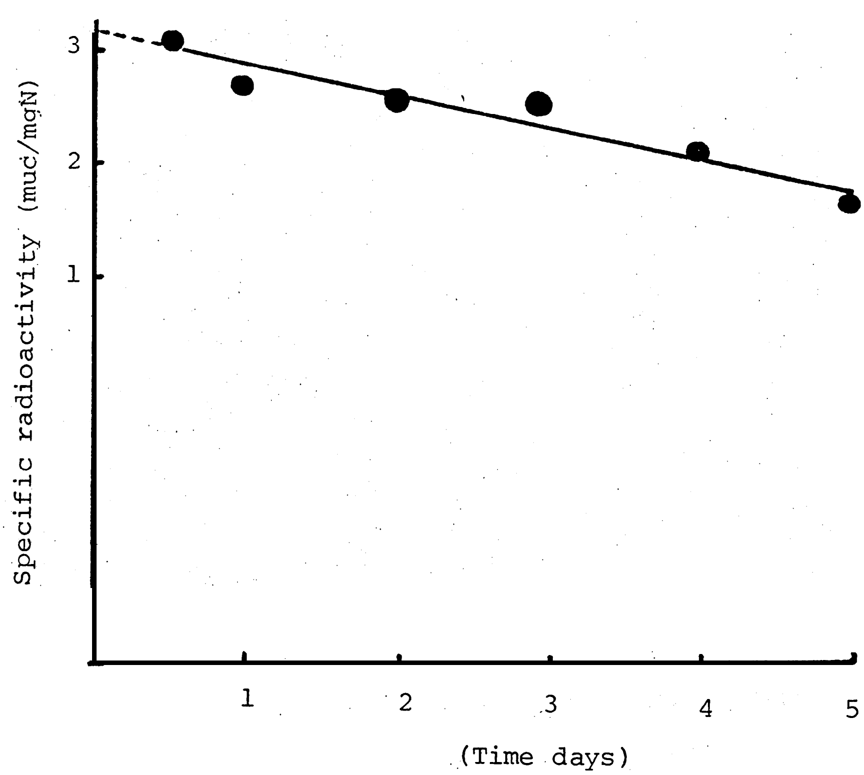


FIGURE 2 The specific radioactivity of protozoa following injection of ^{14}C-labelled protozoa into the rumen of a bull on sugar cane based diets.



very much slower than the turnover of rumen fluid. In these studies protozoa (large ciliates) were isolated from the rumen and incubated with ^{14}C-choline to label their lipids. The labelled protozoa, washed free of bacteria, were reintroduced into the rumen of mature cattle on sugar cane diets, and their specific radioactivity (mμc/mgN) followed for a period of five days.

The results in Figure 2 of the specific radioactivity of protozoa with time indicate the very slow turnover of the large ciliate protozoa in the rumen of cattle on sugar cane based diets. In these animals (700 kg bulls) the protozoa had a five day half life in the rumen indicating that only 2-3 g of protozoal N was irreversibly lost from the protozoal pool. The pool. size of the protozoa was 20-40 g of nitrogen representing about 350 g of dry matter or 2-2½ kg of wet protozoa in the rumen of a bull weighing about 700 kg.

Since these protozoa have a very large surface area and are very active this suggests they could represent some considerable loss of energy to the animal since they appear not to leave the rumen. Over the period of thisexperiment the half time in the rumen of protozoa (5 days) was so much longer than the half time of liquid (12 hours) that there appears to be little doubt that the large protozoa are preferentially retained. Data has already been cited that indicates that the small protozoa are preferentially retained so therefore there now is abundant evidence to indicate the preferential retention of protozoa may have considerable detrimental effects on the efficiency of utilisation of food by ruminants. However, it is stressed that the total biomass of protozoa must be substantial before they can be expected to represent a major constraint.

In our studies the irreversible loss of protozoal nitrogen was small i.e. there was only a small turnover of protozoa either within or through the rumen. Although they are only a small proportion of the microbial protein synthesised in a day they may still markedly reduce the availability of microbial protein to the host animal. Protozoa engulf bacteria in quite large numbers (Coleman 1976) and therefore reduce considerably the availability of bacterial protein output from the rumen. This aspect is under study in these laboratories and it has been confirmed in *in* vitro studies of Demeyer and Van Nevel (1979). The extent of reduction of VFA availability in the presence of protozoa might also be important but its extent is (at present) unknown. The maintenance energy requirements of protozoa in the rumen are met from the breakdown of microbial protein and the energy of carbohydrates.

The effects of the removal of protozoa from the rumen depends 'on a number of factors. Because the rumen is a balanced ecosystem any change brought about by antiprotozoal agents may result in a chain of influences within the rumen, some of which may be detrimental and others which may be advantageous. The ultimate effect of defaunation then depends on the balance of these influences, Protozoa were believed to be beneficial because of their effect on slowing down the rate of fermentation particularly of starches. The entodiniomorphid protozoa ferment starch 'rather slowly, however, they remove the starch from bacterial attack by engulfing the starch grains. This gives the protozoa a competative advantage over bacteria in the rumen ecosystem and allows them to grow. On sugar diets the large protozoa rapidly take up soluble sugars and convert them to starch and in this way they have a similar role to that of the entodiniomorphid protozoa on starch based diets. However, we have been unable to show any metabolic disturbances in the rumen of sheep on sugar based diets in which the protozoa have been removed. It is important to recognise that on starch based diets when protozoa are present there is no accumulation of lactic acid, however, when protozoa are removed, bacteria which produce lactic acid may increase in number in the rumen (Dirksen 1970). Lactic acid is only absorbed slowly through the rumen wall and the result is a drop in rumen pH. A drop in pH allows lactic acid producing bacteria to further. increase in numbers. Fermentation of carbohydrate to lactic acid results in only small availability of ATP for microbial protein synthesis, so a large amount of carbohydrate must be fermented to allow the bacteria to grow. That is the efficiency of microbial protein synthesis is apparently reduced when lactic acid accumulates in rumen fluid. In sheep on sugar diets there is an increase in propionate relative to acetate on defaunation which is beneficial in retaining more substrate energy in the products of fermentation. The above points are described here only to demonstrate that the effects of' defaunation (or any other manipulation of the rumen system) may be complicated and interactive depending on the diet of the animals.

SOME RECENT RESULTS OF THE EFFECTS OF DEFAUNATION OF THE RUMEN

Two areas have been researched quite extensively this year. These include the effects of defaunation on microbial protein synthesis in the rumen. To date no results are available. The other has been to repeat the studies carried out by Bird (1978). The effects of defaunation on weight change in lambs on low protein high energy diets was repeated and similar. results were obtained. In these studies the lambs which were defaunated grew at approximately 25% greater rate than the animals with a normal population of microbes within the rumen (Burggraaf, 1980). These experiments were carried over for 16 weeks and at the end of that time the lambs on high energy/low protein feeds with and without rumen protozoa were slaughtered and comparative body composition data obtained. The lambs which were protozoa free had a considerably larger rumen volume and a smaller killing-out percentage. This removes some of the beneficial effects of defaunation. It appears that the absence of protozoa in some way affects rumen size and turnover and this could be one of the major reasons for the lack of response that has been observed in studies with defaunated lambs at high protein intakes since reduced rumen outflow probably reduces the efficiency of bacterial protein synthesis (see Sutherland 1976). It seems imperative that studies should be undertaken in the future on defaunation where some means of Stimulating rumen throughput is developed.

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