

THE ROLE OF PROTOZOA IN THE RUMEN OF SHEEP AND CATTLE  
ON LOW PROTEIN-HIGH ENERGY DIETS

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Summary

Three growth studies were conducted with lambs, mature wethers and young cattle to examine the effects of defaunation on production. The animals were fed a high energy-low protein diet supplemented with several levels of protein. These diets supported relatively high populations of protozoa in the rumen. A single dose of nonyl phenol ethoxylate (trade name Teric GN9) was used to remove protozoa from the rumen.

Defaunation resulted in improved feed conversion ratio and greater growth rates in all animals fed at the low levels of protein supplementation, without an apparent effect on feed intake, and this finding is discussed.

Defaunation also resulted in an increase in wool growth in lambs fed at all levels of protein supplementation, however defaunation of the wethers had no effect on wool growth.

Defaunation of cattle fed a molasses based diet resulted in a shift of the fermentation pattern towards higher butyrate concentration in the rumen. In lambs given a sugar and oaten chaff based diet removal of protozoa resulted in a shift towards higher propionate concentrations in the rumen.

## I. INTRODUCTION

Protozoa are microscopic unicellular animals found (often in large numbers) in the forestomach of ruminants and a large number of other habitats. Many experiments have been carried out to compare faunated and defaunated sheep and cattle to determine the role of protozoa in these animals. The early work of Becker, Shultz & Emmerson (1929) and Becker & Everett (1930) suggested there was no effect of defaunation on the animal. Subsequent work has generally confirmed these early findings and the growth of calves (Pounden & Hibbs 1948, 1950, Conrad & Hibbs 1953, Hardison *et al.* 1957, Bryant & Small 1960, Williams & Dinusson 1973) and of lambs (Eadie 1962, Chalmers *et al.* 1968, Eadie & Gill 1971) was not affected or 'was slightly reduced by defaunation (Abou Akkada & El Shazly 1964, Christiansen, Kawishima & Burroughs 1965).

Interest in the role of protozoa in the rumen in relation to animal production has increased recently with the suggestion that protozoa tend to be retained in the rumen (Weller & Pilgrim 1974, Minor, Macleod, Preston & Leng 1977). It has been known for some time that the concentration of protozoa in the omasum was much less than the concentration of protozoa in the rumen (Pounden & Hibbs 1950, Weller & Gray 1954), and slaughter trials carried out in our own laboratories with both sheep and cattle (Bird, Baigent, Dixon & Leng 1978) agreed with these earlier findings. It was originally thought that the

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protozoa were rapidly broken down in the omasum, however results from several *in vitro* studies carried out in our laboratories, where protozoa were taken from the rumen and incubated in omasal fluid would not support this view. The lack of movement of protozoa out of the rumen has caused Leng (1976) and Bergen & Yokoyama (1977) to suggest that the amount of microbial protein available to the animal may be decreased by the presence of significant populations of these organisms and this may be a limitation to production particularly where protein intake is low and limiting production.

In order to study the effects of defaunation on production, growth trials were conducted using lambs, wethers and young cattle fed on a diet high in energy but containing suboptimal levels of protein. The lambs and adult wethers were fed a basal ration of sugar and roughage and supplemented with several levels of fishmeal. The cattle were established on a molasses based diet which was supplemented with a bypass protein meal as a pellet. Sufficient urea for rumen fermentation (Preston & Willis 1970) was supplied in all diets, and all diets were fed ad libitum.

A number of defaunating agents have been used by previous workers, however, many of these were found to be inadequate in these laboratories. Recently Wright & Curtis (1976) described the effects of the nonyl phenol ethoxylates (trade name Terics ICI (Aust.) Ltd) and teric GN9 was used successfully in these studies to eliminate protozoa from the rumen of sheep and cattle.

## II. EXPERIMENTAL

Defaunation: Animals were defaunated by delivering a dose of teric directly into the rumen. Doses used were: 15 g teric (in 120 ml H<sub>2</sub>O) for lambs, 20 g teric (in 160 ml H<sub>2</sub>O) for wethers and 100 g teric (in 800 ml H<sub>2</sub>O) for cattle.

(i) Experiment 1. Mixed sex Corriedale lambs (6 months, 20 kg) were used. Eight groups of 6 lambs were given diets based on sugar: oaten chaff (50 : 50) supplemented with urea (4%), vitamins and minerals. Two groups were each given this diet supplemented with either 0, 4, 8 or 12 percent fishmeal. Following an equilibration period the lambs were held on these diets for 6 weeks during which feed intake, weight change, wool growth and rumen VFA and protozoa numbers were measured.

(ii) Experiment 2. Cross-bred wethers (4 yrs. 35 kg) were used and were given similar diets to those used in Experiment 1. Two groups (5 animals/group) were given the basal diet supplemented with 0, 6, 12, 18, 24 percent fishmeal. The same measurements were made as in Experiment 1. The experimental period was 8 weeks.

(iii) Experiment 3. Mixed sex Hereford weaners were used and were given either a basal diet of molasses plus 4% urea, minerals and vitamins ad libitum, and 1.5 kg oaten straw/d or the same diet supplemented with 240 g of a pelleted protein meal. One group (9 animals) on each diet was defaunated. The animals without protozoa were isolated from other animals. Feed intake, weight change and rumen VFA's and protozoa numbers were measured over a 7-week period following a 3-week adjustment period.

## III. RESULTS

Presence of protozoa in the rumen: The sugar and oaten chaff based diets supported relatively high populations of protozoa in the rumen of lambs and wethers. The protozoal population in the lambs was of the order of  $5 \times 10^5$ /ml and in the wethers  $8 \times 10^5$ /ml of rumen fluid. The level of protein had no effect on the composition of protozoal species in the rumen. The small Entodinium were the predominant species in both lambs and wethers making up 96% and 93% of the total protozoal population respectively. A notable feature was the absence of Holotricha species in the lambs. The molasses based diet supported a lower population of protozoa in the rumen of cattle, being of the order of  $2 \times 10^5$ /ml of rumen fluid. Holotricha spp. and Epidinium spp. assumed greater importance in the protozoal population accounting for 12% and 11% of the total population respectively. Small Entodinium were still the predominant species making up 76% of the total population.

(a) Food intake and liveweight gain

(i) Lambs. The intake of diet, liveweight gain and food conversion ratio of the lambs on each diet are shown in Fig. 1 (a), (b), (c) respectively in relation to the presence and absence of protozoa. There was a significant difference ( $P < 0.05$ ) in growth rates of the defaunated lambs on the two lowest protein based diets but the difference was not apparent at the higher protein intakes. Feed intake increased as the protein level in the diet increased but there was no significant difference on feed intake between lambs with and without ruminal protozoa. Therefore the feed conversion ratio of lambs on the two low protein diets was improved significantly by removing protozoa from the rumen.

(ii) Wethers. The intake of diet, liveweight gain and food conversion ratio of the wethers on each diet is shown in Fig. 2 (a), (b), (c) respectively in relation to the presence and absence of protozoa. In wethers on the basal diet defaunation had no significant effect on growth rate although there was a trend towards increased growth. However, in wethers given the basal diet supplemented with 4% fishmeal (i.e. ration B) defaunation significantly increased growth rates ( $P < 0.05$ ). The effect disappeared at the higher protein intakes. Feed intake increased as the protein level in the diet increased and was significantly increased by defaunation ( $P < 0.05$ ) on ration B. Defaunation resulted in an improvement in feed conversion ratio at the lower levels of protein intake.

(iii) Cattle. Feed intake, growth rate and feed conversion ratio for each treatment group are given in Table 1. Addition of 240 g/d of a protein meal pellet to the basal diet increased the intake of molasses and the growth rate of cattle. On the basal ration defaunation had no effect on growth rate although there was a trend towards increased growth. However, on the protein supplemented diet defaunation significantly ( $P < 0.05$ ) increased growth rate by 43% without an effect on molasses intake. In both dietary groups feed conversion ratio was improved by defaunation. VFA proportions in rumen fluid - the mean proportions of acetate, propionate and butyrate in rumen fluid samples taken from cattle are shown in Table 1. The level of protein had no effect on the VFA proportions in cattle, however, in the lambs there

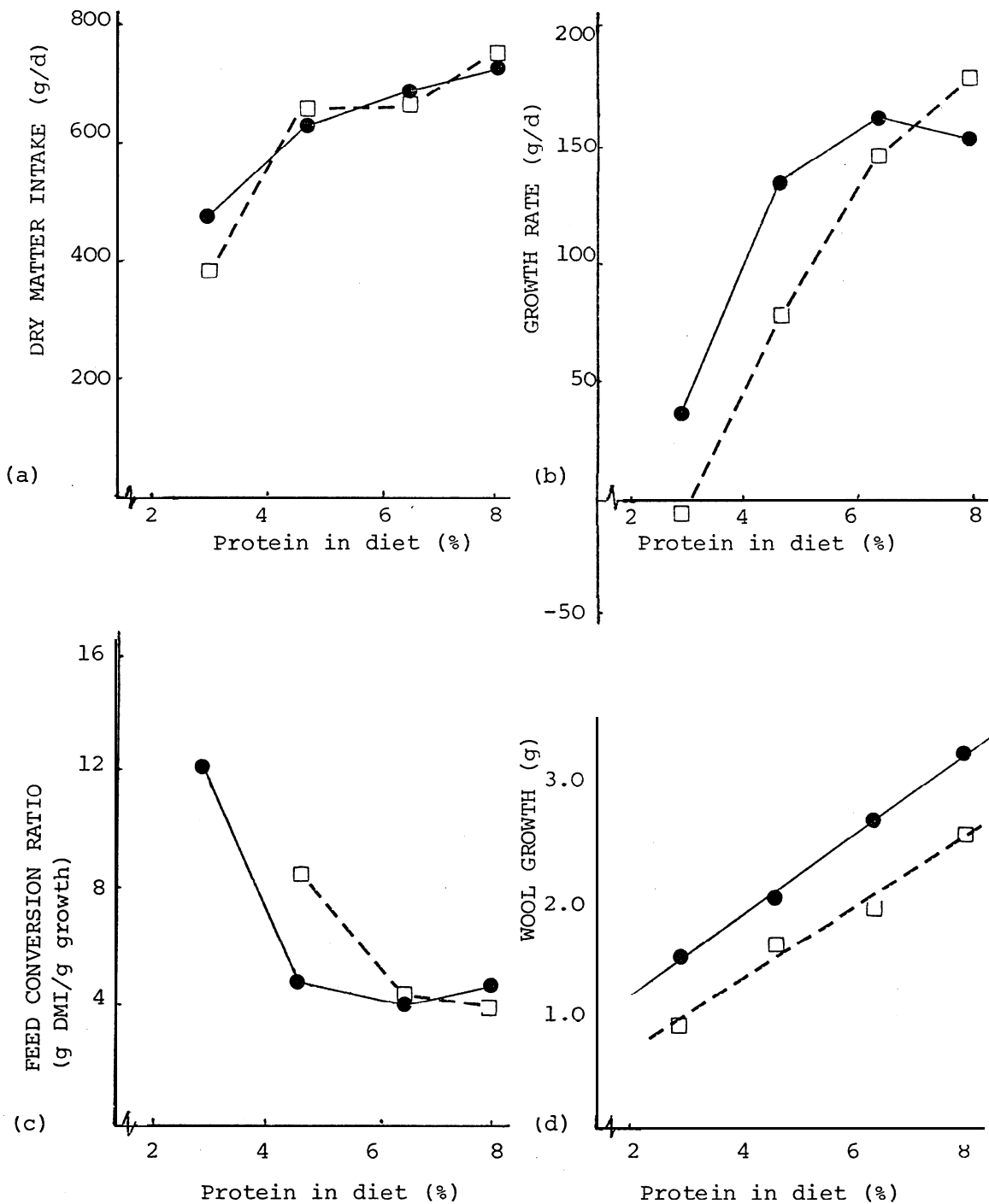


Fig. 1. Relation between the level of protein in the diet and (a) dry matter intake (g/d), (b) growth rate (g/d), (c) feed conversion ratio (g DMI/g growth), and (d) wool growth (dry clean wt. (g) of wool grown on a midside patch) of lambs (● defaunated, □ faunated).

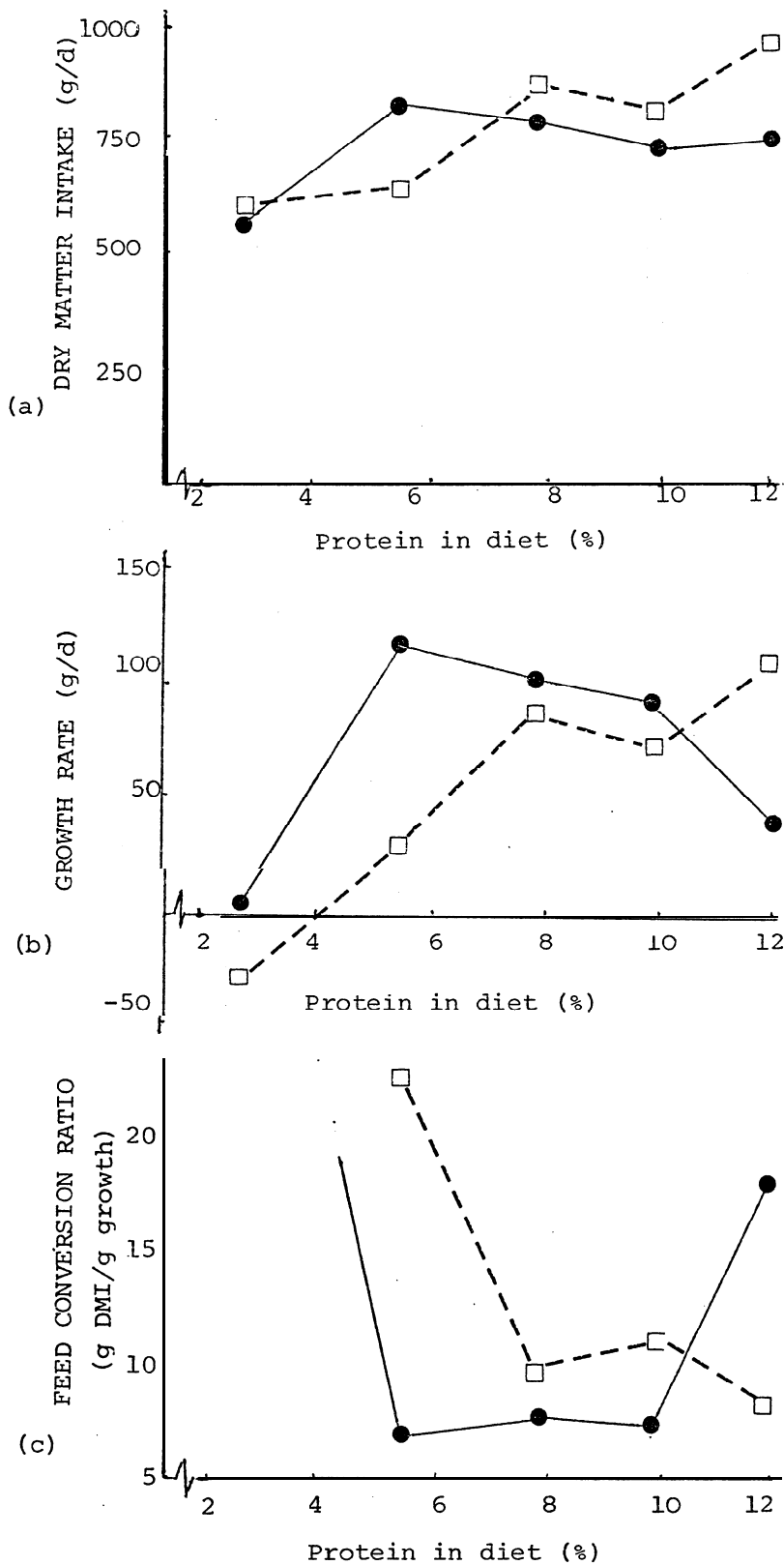


Fig. 2. Relation between the level of protein in the diet and (a) dry matter intake (g/d), (b) growth rate (g/d), and (c) feed conversion ratio (g DMI/g growth) of adult wethers (● defaunated □ faunated)

TABLE 1 The effects of defaunation of cattle on VFA proportions in rumen fluid and on feed intake, growth and feed conversion ratio.  
 (Each value is the mean  $\pm$  SE of nine animals. Animals in Group A were consuming approximately 3.3 kg molasses mixture and 1.5 kg cereal hay. Animals in Group B were each consuming approximately 3.7 kg molasses, 1.5 kg cereal hay and 240 g of protein pellets containing 50% soya-bean meal, 30% cottonseed meal, 10% meat meal and 10% fish meal).

Group	Initial Liveweight (kg)	No. of protozoa in rumen fluid + (10 <sup>-5</sup> /ml)	Percent VFA in rumen fluid as				Mean dry matter Intake DMI (kg/d)	Mean growth rate (g/d)	Feed Conversion ratio (g DMI/g growth)	
			Acetic	Propionic	Butyric	Other				
A faunated	177 $\pm$ 9	2.6	55	17	24	4	3.76	451 $\pm$ 93	]NS	8.3
A defaunated	178 $\pm$ 10	-	50	17	30	3	3.65	490 $\pm$ 59		7.4
B faunated	176 $\pm$ 12	1.7	60	15	21	4	4.15	530 $\pm$ 61	]*	7.8
B defaunated	185 $\pm$ 10	-	49	14	32	5	4.23	757 $\pm$ 61		5.6

\* Significant at P < 0.05

NS, not significant at P < 0.05

<sup>+</sup> The spp. of protozoa present in the rumen were similar for both diets and the composition of the population was as follows: small Entodinium spp. (76%) Holotricha spp. (12%) Epidinium spp. (11%) and other large Entodiniomorphs (1%). Four samples of rumen fluid were taken from all cattle during the experimental period and the results were averaged for each animal and then for each group.

was a tendency towards higher butyrate proportions with higher levels of protein in the diet. Defaunation of the lambs resulted in a decrease in the proportion of butyrate ( $P < 0.05$ ) but in the cattle removal of protozoa resulted in an increase in the proportion of butyrate.

#### (b) Wool growth

(i) Lambs. Wool growth was increased at all protein levels by defaunation and there was a linear relationship between wool growth and protein intake for the animals with or without protozoa, Fig. 1 (d). The slopes of these regressions were not significantly different but the intercepts were ( $P < 0.01$ ).

(ii) Wethers. Defaunation had no effect on wool growth.

#### IV. DISCUSSION

The defaunating agent used in these studies was a nonyl phenol ethoxylate (Terics ICI) and was first shown to have antiprotozoal properties by Wright & Curtis (1974). In the lamb growth studies 15 g of teric given as a single dose removed protozoa from all animals and these remained substantially free for the 6 week experimental period. On retreating the contaminated lambs, protozoa were not observed for the rest of the experiment. Flagellate protozoa were not a problem in any of the ciliate-free animals in these trials, however other workers have reported the development of large populations of these organisms where ciliate protozoa have been excluded from the rumen (Eadie 1962; Luther *et al.* 1966; Eadie & Gill 1971). The treatment of adult sheep and cattle with teric and the maintenance of these animals ciliate-free, was somewhat less successful than the first trial with lambs, with over two-thirds of the animals requiring a second and sometimes a third drench of teric during the experimental period. The lamb growth studies were carried out in winter when the ambient day temperature was usually less than  $10^{\circ}$  whereas the sheep and cattle trials were carried out in the summer when the ambient day temperature was usually in excess of  $25^{\circ}$  and this may have influenced contamination between control and experimental animals which were often only separated by some 6 m. Drenching of animals with teric resulted in a reduction in feed intake for several days and consequently the liveweight change of the defaunated animals can be regarded as minimal values.

Numerous studies have been carried out on the effects of defaunation of the rumen of sheep and cattle or of the effects of maintaining lambs and calves ciliate-free from birth. Interpretation of results has been difficult as workers have used a wide range of diets in their experiments. It has been argued by Church (1974) that in experiments using young ruminants isolated at birth, atypical bacterial population may develop and when these animals are compared with animals which have been inoculated with rumen contents from older animals the differences observed may be a result of the absence of protozoa and a change in the bacterial population. A common feature of all the diets used in previous defaunation studies is that they have tended to be based on concentrates (starch) and have usually had high protein concentrations (in excess of 12-15%). The diets used in these studies were chosen specifically to provide low protein high energy intakes which would support considerable numbers of protozoa in the rumen. To this effect it was consistently observed that the numbers of protozoa in the rumen ranged from 0.5 -

$2.0 \times 10^6$  per millilitre on the sugar based diets and  $1-4 \times 10^5$  per millilitre on the molasses diet which suggests that they were a large proportion of the microbial biomass. The composition of the protozoal population in the rumen was similar on both diets although there were large variations between animals on the same diet. The predominant genus was *Entodinium* constituting 75% of the total population in the molasses-fed animals and > 90% in the sugar-fed animals. The other main genera were *Isotricha*, *Dasytricha* and *Epidinium*.

The results of the lamb defaunation trial demonstrate that in growing lambs on the low levels of protein supplementation of this diet, defaunation increases growth rate but has no effect on intake. At high protein intakes, however, which approach the levels of protein intake that have been used in past studies of the effects of defaunation (Eadie 1962, Abou Akkada & El Shazly 1964, Christiansen, Kawishima & Burroughs 1965 and Eadie & Gill 1971), the effects of the lack of protozoa in the rumen were either not apparent or much reduced. The main effect of defaunation at low protein intakes was to increase the efficiency of utilization of feed. A similar pattern emerged from the wether trial although the differences in growth rates between faunated and defaunated animals did not reach significance at the lowest protein intake level. There appeared to be a feed palatability problem in this trial and nearly all feeding regimes had at least one animal which had a low feed intake. Again the increased growth rates were achieved through an improvement in feed conversion rather than increased feed intake. The cattle also showed a response to defaunation. The ciliate-free animals on the basal diet showed a trend towards increased growth rates although the differences were not significant. When the basal diet was supplemented with 240 g/d of the protein feed pellet defaunation increased growth rates by 43% without an effect on feed intake. The increased feed conversion ratios that were observed in all three experiments and the lack of response of feed intake suggests that concomitant with an increase in protein availability, there was an increase in energy nutrients available to the animal. Thus the protein : energy ratio remained constant but the total amount of protein and energy available increased significantly in defaunated animals on the diets with low protein supplementation. At high protein intakes these differences disappeared suggesting there was some fundamental change in rumen function in animals on low protein intakes.

In the lamb trial the effects of defaunation on wool growth were very clear, with significant differences in the regressions of wool growth against protein intake for the animals with and without protozoa. At the lowest protein intake wool growth was increased by 50% and at the highest protein intake wool growth was increased by approximately 25%, but the quantity of increase was of the same order. Since wool growth is very sensitive to the amount of protein arriving at the duodenum, this is positive evidence of increased protein availability from the rumen in animals where protozoa have been removed. However defaunation of the mature sheep had no effect on wool growth which was an unexpected finding and no explanation can be given at this stage.

The major end products of carbohydrate fermentation by protozoa are acetate, butyrate, carbon dioxide and hydrogen (Hungate 1966). Therefore it would be expected that defaunation would result in an increase in the molar proportion of propionate in the rumen. Higher proportions of propionate in ciliate free animals have been reported



in sheep (Males & Purser 1970; Kurihara *et al.* 1978) and in cattle (Eadie *et al.* 1970; Eadie and Mann 1970; Whitelaw *et al.* 1972). In contrast to these findings lower proportions of propionate have been found in ciliate free lambs (Abou Akkada & El Shazly 1964; Christiansen *et al.* 1965; Luther *et al.* 1966). In the lamb trial, defaunation resulted in a shift in the fermentation pattern towards higher propionate production, however, in the cattle trial the converse occurred (Table 1). From these results and the disparity in the findings of previous workers it is impossible to draw any general conclusions as to the effect of defaunation on the VFA proportions in rumen fluid. It is obvious there is a complex relationship between bacteria and protozoa and the final fermentation pattern will be determined by the species of bacteria present and how the numbers and species composition of the bacterial population change with defaunation.

These studies have demonstrated that on high energy low protein diets, the presence of large populations of protozoa in the rumen are apparently associated with an inefficiency of feed utilization. Although protozoa were eliminated from the rumen, it cannot be discounted here that other changes in the rumen ecosystem brought about by the action of the tetic resulted in some of the increased production. Removal of protozoa obviously results in changes in the rumen ecological system and it has been reported by many workers (Klopfenstein, Purser & Tyznik 1966, Luther, Trenkle & Burroughs 1966 and Eadie & Gill 1970) that when protozoa are removed from the rumen the bacterial population increases. Under these conditions different bacterial species may assume importance in the bacterial population. The overall suggestion is that the presence of a significant population of protozoa in the rumen of sheep and cattle on diets containing suboptimal quantities of protein, reduces the availability of energy nutrient and protein from the rumen. The effect apparently disappears at high protein intakes.

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