THE INFLUENCE OF THE ABSENCE OF RUMEN PROTOZOA ON RUMINANT PRODUCTION

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SUMMARY

The results of a 6yr research programme designed to investigate the effects of removing protozoa from the rumen of sheep and cattle are discussed in relation to recently published results of similar studies conducted in other laboratories. Evidence is presented which suggests that the removal of protozoa from the rumen is associated with an increase in the availability of protein for digestion in and absorption from the intestines. This response has been demonstrated in animals held under laboratory and grazing conditions.

INTRODUCTION

Although protozoa are normally present in the rumen of sheep and cattle their nutritional value to the host is still the subject of debate. A survey of the results reported in the literature suggested that the maintenance of a protozoal population in the rumen is likely to reduce the amount of protein available for digestion by the host animal particularly since protozoa appear to be preferentially retained in the rumen (see Weller & Pilgrim 1974) and also engulf bacteria (Coleman 1975). Previous experiments reported in the literature compared animals with and without protozoa in the rumen, given high quality forage and concentrate diets. These studies demonstrated no beneficial responses to defaunation. The apparent retention of protozoa in the rumen suggested to us that responses to defaunation were only likely to result where ruminants were given low protein diets and a research programme to study the effects of defaunation was initiated in our laboratories in 1977. The programme has involved; pen feeding trials, investigation of metabolic changes associated with the removal of protozoa from the rumen, a study of the turnover rate and pool size of protozoa in the rumen and more recently comparative grazing trials.

Pen feeding trials

Our early short term studies compared the production rates of sheep and cattle under animal house conditions (faunated vs defaunated) given low protein, high energy diets. Increases in body weight gain of young steers and large increases in wool growth and body weight gain of lambs were associated with the absence of protozoa in the rumen (Bird & Leng 1978, Bird et al.1979). In response to these results a pen feeding trial was initiated to examine the long term effects of defaunation on the productivity of lambs. A brief report on the progress of this experiment was presented at the last school (Bird & Leng 1981), the complete results of the 26 week trial are presented in Table 1. Defaunation was associated with a moderate increase in body weight gain (9%) and a large increase in wool growth (37%). The magnitude of the wool growth response was comparable with the earlier trials using lambs but the body weight gain response was lower. It is suggested that some of the weight gain recorded in the earlier trial was due to an increase in rumen volume and digesta as reported by Burggraaf (1980). Feed intake was only marginally higher in the defaunated groups of lambs and the level of urea in the diet had no apparent beneficial effects. These results dispel any suggestions that the responses associated with

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Table 1. The growth rate (g/d), efficiency of feed conversion (g.D.M.I./g growth and wool growth (g/d) of faunated and de-faunated lambs given a basal diet of oaten & sugar (1:1), 80g fishmeal/kg sugar and either 60 or 100 urea/kg of sugar in the diet (diet A & B respectively).

<table>
<thead>
<tr>
<th>Diet Treatment</th>
<th>Initial live wt.</th>
<th>Protozoal population density (X10^5/ml*)</th>
<th>Feed intake (g/d)</th>
<th>Live wt. gain (g/d)</th>
<th>Feed conversion efficiency (gDMI/g/growth)</th>
<th>Wool growth (g/d) (clean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. faunated</td>
<td>16</td>
<td>12</td>
<td>865</td>
<td>122^a</td>
<td>7.3</td>
<td>8.0^A</td>
</tr>
<tr>
<td>defaunated</td>
<td>16</td>
<td>890</td>
<td>135^b</td>
<td>6.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. faunated</td>
<td>16</td>
<td>13</td>
<td>870</td>
<td>123^b</td>
<td>7.2</td>
<td>7.9^A</td>
</tr>
<tr>
<td>defaunated</td>
<td>16</td>
<td>930</td>
<td>132^b</td>
<td>6.4</td>
<td></td>
<td>11.0^B</td>
</tr>
</tbody>
</table>

Superscripts a & b denote significance of difference P<0.06.
Superscripts A & B denote significance of difference P<0.01.
each value is the mean of 10 animals
* each value is the mean of 40 determinations (10 animals x 4 collection periods.

defaunation are due to the direct action of the chemical defaunating agent on the animal as all animals were initially defaunated and half the animals refaunated at the start of the trial. The results clearly indicate that the amount of protein available for digestion in the intestines of defaunated lambs is increased substantially since wool growth is highly responsive to increased amino acid absorption (Reis & Schinckle 1961; 1963). Recent development of an assay for bypass protein using wool growth as an index suggests that this level of increase of wool growth would require 30-50g/d of extra protein digested in the small intestine (see Leng et al. this symposium).

Comparative grazing trials

Hoggets

At the completion of the pen feeding trial the lambs (now 1 yr. old hoggets) were returned to pasture isolated from other ruminants. As the level of urea in the diet had no effect on productivity, lambs in dietary regimens A and B were combined to give two groups of 20 animals (faunated & defaunated). A paddock (ca 4ha.) was divided into four equal blocks and the predominantly green oat pasture with an understory of rye grass, clover and lucerne was grazed in a 4 x 21d rotation. The blocks were grazed in the order 1,2,3,4 (faunated animals) and 3,4,1,2 (defaunated animals) so there was always one block separating the two groups.

Wool growth and body weight gain were monitored over an 84d period and the results are given in Table 2. Defaunated lambs had a superior rate of body weight gain (23%) and wool growth (1%) with respect to the faunated lambs. At the completion of the grazing trial two wethers from each group were slaughtered. The weight and volume of rumen contents and dressing % were similar for both faunated and defaunated animals.
In the most recent experiment the productivity of pregnant ewes (faunated and defaunated) grazing native pasture was monitored over a 23 week period (the first lambs were born in the 11th week). Eighty joined ewes were drenched with Alkanate 3SL3 to remove protozoa, 16 animals died. All remaining animals were found to be free of protozoa. Thirty two of the remaining ewes were reinoculated with rumen fluid containing protozoa and the faunated and defaunated groups of ewes were returned to pasture. Six paddocks (ca 5ha) were grazed in a 6x2ld rotation. Unfortunately the defaunated group of ewes became contaminated with protozoa or had not been completely defaunated. Small numbers of protozoa were detected in rumen fluid samples collected from 6 of the ewes in the 10th week of the trial, 6 weeks later almost all ewes were contaminated with protozoa. The results of this trial are presented in Table 3. Defaunation was associated with a higher rate of wool production (22%) and a higher birth weight of single lambs (12%) with respect to the faunated group. The early growth rate of lambs with defaunated mothers also tended to be higher but this advantage had disappeared when lambs had reached 12 weeks of age. The average weight of twin lambs and the body weight change of the ewes were similar for both groups.

Metabolism studies

The increased wool growth of sheep in these trials suggested that substantially more protein was available for digestion in defaunated sheep. Therefore it was an obvious extension of the research programme to obtain direct estimates of the outflow of protein-N from the rumen and to quantify the microbial component of that outflow. Mature crossbred wethers fitted with rumen and abomasal cannulae were given a diet containing (g/kg) sugar 455, oat chaff 455, urea 35, fishmeal 35 and minerals and vitamins 20. Animals were fed at 1h intervals. Studies were carried out on the same group of animals in the faunated and defaunated states. Individual animals were offered a constant amount of feed during both experimental periods. The results of this experiment and findings from other similar experiments reported in the literature are shown in Table 4. While not all the reported
Table 3. Effects of defaunation on the productivity of pregnant crossbred ewes grazing native pasture during winter in New England.

<table>
<thead>
<tr>
<th>Ewes</th>
<th>Faunated</th>
<th>Defaunated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body wt. gain (g/d)</td>
<td>-48</td>
<td>-48</td>
</tr>
<tr>
<td>Wool growth (clean) (g/d)</td>
<td>3.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>4.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Lambs - single lambs

| Birth wt. (Kg)              | 4.8<sup>a</sup> | 5.4<sup>b</sup> |
| Body wt gain @ 4 wks (g/d)  | 265        | 293         |
| Body wt gain @ 12 wks (g/d) | 221        | 227         |

- twin lambs

| Birth wt. (Kg)              | 3.6        | 3.8         |
| Body wt gain @ 4 wks (g/d)  | 130        | 149         |
| Body wt gain @ 12 wks (g/d) | 146        | 145         |

Superscripts <sup>a</sup> & <sup>b</sup> denote significance of difference <i>P</i> < 0.05.

differences between the post-ruminal flows of non ammonia-N in faunated and defaunated sheep were significant, defaunation was always associated with a higher flow of NAN. The outflow of microbial-N was also higher in defaunated animals and suggests that removing protozoa from the rumen increases the efficiency of microbial protein synthesis. These findings have been supported by the in vitro work of Demeyer & Van Nevel, (1979) who reported that defaunation increased the net synthesis of microbial cells.

The error associated with the measurement of the microbial-N component in digesta samples collected from the abomasum or duodenum is likely to be large. Most techniques require the separation of a representative microbial fraction from the digesta free of contamination, which is difficult, and variations in estimates have been attributed to the use of different microbial markers (see Siddons et al. 1980). It is therefore suggested that wool growth may be a more sensitive index of an increased flow of protein from the rumen than the more direct but technically difficult marker technique.

Defaunation may be associated with a reduction in the digestibility of diet. Results given in Table 5 all show defaunation to be associated with a lower apparent digestibility of diet in both the forestomach and whole digestive tract, although most of the differences are non significant. It must be remembered however, that the outflow of microbial OM was always higher in defaunated sheep and this component is not accounted for in the apparent digestibility estimate. Therefore, estimates of apparent digestibility of diet will be biased in favour of the faunated sheep.

Protozoal turnover studies

Protozoa are often discussed as though they are a discrete entity rather than a mixed population of organisms which vary considerably in size (10<sup>u</sup>-120<sup>u</sup>), motility and nutritional requirements. Consequently the nature of the diet has a strong influence on the species composition of the protozoal population, while rumen pH (Purser & Moir 1959) and the
Table 4. A comparison of post-ruminal flows of non-ammonia-N (NAN) in faunated and defaunated sheep.

<table>
<thead>
<tr>
<th>Diet#</th>
<th>Nitrogen intake g/d</th>
<th>Number of animals</th>
<th>Site of measurement</th>
<th>N-compound</th>
<th>faunated sheep (g/d)</th>
<th>defaunated sheep (g/d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.a.</td>
<td>25.2</td>
<td>2</td>
<td>abomasum</td>
<td>total NAN</td>
<td>18.3</td>
<td>21.3 ns.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bacterial-N</td>
<td>12.0</td>
<td>14.0 ns.</td>
</tr>
<tr>
<td>b.</td>
<td>33.3/34.4*</td>
<td>2</td>
<td>abomasum</td>
<td>total NAN</td>
<td>29.4</td>
<td>31.7 ns.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Bacterial-N</td>
<td>18.0</td>
<td>19.3 ns.</td>
</tr>
<tr>
<td>2.</td>
<td>25.2</td>
<td>5</td>
<td>abomasum</td>
<td>total NAN</td>
<td>18.0</td>
<td>19.3 ns.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>microbial-N</td>
<td>14.7</td>
<td>16.7 ns.</td>
</tr>
<tr>
<td>3.</td>
<td>20.8/20.4*</td>
<td>4</td>
<td>abomasum</td>
<td>total NAN</td>
<td>17.0</td>
<td>24.8 ns.*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>microbial-N</td>
<td>15.4</td>
<td>19.2 ns.</td>
</tr>
<tr>
<td>4.</td>
<td>25.0</td>
<td>3</td>
<td>duodenum</td>
<td>total-N</td>
<td>19*</td>
<td>22*</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>microbial-N</td>
<td>11.8</td>
<td>15.0</td>
</tr>
<tr>
<td>5.</td>
<td>13.8/12.5*</td>
<td>3</td>
<td>duodenum</td>
<td>total NAN</td>
<td>15.6*</td>
<td>17.4*</td>
</tr>
</tbody>
</table>

* the two values represent av. N-intake (g/d) in faunated and defaunated sheep respectively.
† this experiment was carried out in collaboration with I.C.I. (Melbourne). Values with different superscripts significantly different at P<0.05.

# Diets -
1. Lindsay & Hogan (1972) - a. 1000g. lucerne hay
   b. 1000g. red clover
2. Bird (1982) - 950g. oat chaff/sugar
3. 1980 unpublished I.C.I./U.N.E. - 720g. oat chaff, 100g. casein,
   80g. lucerne, 100g. molasses
4. Rowe et al. (1981) - 500g. hay (medium quality), 225g. oats,
   115g. sugar, 70g. fishmeal,
   40g. (urea, minerals & vitamins
5. Veira & Ivan (1983) - Corn silage 48%, shelled corn 47%, urea 1%,
   mineral mix 4%

turnover rate of digesta (Potter & Dehority 1973) are important factors controlling the establishment and concentration of protozoa in the rumen. To obtain a clearer understanding of the changes associated with defaunation, an in depth knowledge of the contribution of protozoa and possibly protozoa genera to various aspects of rumen function is required. As a part of this objective, studies have been initiated to obtain information on the pool size, the turnover rate of protozoa and the quantity of protozoal protein which leaves the rumen.

A radioactive marker ([Me$^{14}$C]choline) which is exclusively incorporated into protozoal cells was used to measure the pool size and turnover rate of protozoa in the rumen. Protozoal cells were labelled by incubating rumen fluid with [Me$^{14}$C] choline (Coleman et al. 1980). Residual activity was removed by washing with rumen fluid before the labelled cells were returned to the donor animal. The radioactivity in the protozoal pool was monitored by periodic collection of rumen fluid samples and isolation of
Several studies have been conducted with both sheep and cattle and the results are summarised in Table 6. The protozoal population consisted mainly of small Entodinium spp. (≤20 μm) in sheep although in two animals it was possible to obtain information on the turnover of Entodinium spp. and Polyplastron sp. (>120 μm) through differential separation techniques (Leng, 1984). The cattle studies provided additional information on the pool size and turnover rate of the large holotrichs (>100 μm), Epidinium sp. (>20 μm) and Entodinium spp. It is apparent from these studies that the half time of rumen protozoa is considerably longer than that of rumen fluid, indicating a preferential retention of protozoa in the rumen.

If protozoa die in the rumen and are lysed, the label is excreted in methane. Therefore, by estimating the loss of ¹⁴C from labelled protozoa to methane (see Leng 1982) it has been possible to measure the outflow of protozoa from the rumen. Only a small proportion of protozoa apparently moved down the tract (Table 6).

While protozoa make a significant contribution to the total microbial pool in the rumen, the amount of protein they synthesize is small (1.3gN/d in sheep) relative to the total microbial protein synthesized in the rumen (12 - 18gN/d calculated in sheep).

CONCLUSION

The more recent results of defaunation reported in the literature are in accord with findings from our laboratory and strongly support the concept that eliminating protozoa from the rumen increases the availability of protein for digestion by the animal. The nature of this increase appears to be an increased supply of microbial protein leaving the rumen and in some instances additional dietary and/or endogenous protein flowing to the lower digestive tract (Table 4). It is suggested that the retention of
protozoa in the rumen (which will result in a higher maintenance energy requirement of these cells) and the extensive engulfment of bacterial cells by protozoa (Coleman 1975) results in a reduction of the efficiency of microbial protein synthesis in the rumen. Therefore, the end products of fermentative digestion contain a lower protein to energy ratio; the in vitro work of Demeyer and Van Nevel (1979) adds strong support to this concept. It was shown by Veira & Ivan (1983) that defaunation of sheep on high concentrate diets was associated with a lowering of rumen pH and these workers suggested that as a result less dietary protein was digested in the rumen of defaunated sheep.

The effects of defaunation on the availability of energy to the animal are less clear. The results given in Table 5 suggest that the apparent digestibility of diet was lower in defaunated animals, however, the differences in true digestibility may be much smaller when the increased outflow of microbial OM from the rumen of these animals is taken into account. Hungate (1975) suggested that the amount of cellulose digested in the rumen by the protozoa is probably small when compared with the bacteria. In a more recent study however, inoculation of the protozoon

### Table 6. A summary of the dynamics of protozoa in the rumen of cattle and sheep.

<table>
<thead>
<tr>
<th>Animal</th>
<th>Protozoal Species</th>
<th>Rumen fluid t₁/² (min)</th>
<th>Rumen fluid t₂/² (min)</th>
<th>pool size (gN)</th>
<th>apparent production (gN/d)</th>
<th>apparent lysis in the rumen (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep¹</td>
<td>Entodinia</td>
<td>492</td>
<td>846</td>
<td>2.5</td>
<td>2.0</td>
<td>65</td>
</tr>
<tr>
<td>Sheep²(A)</td>
<td>Entodinia</td>
<td>650</td>
<td>797</td>
<td>1.3</td>
<td>1.4</td>
<td>77</td>
</tr>
<tr>
<td>Sheep²(B)</td>
<td></td>
<td>380</td>
<td>667</td>
<td>1.4</td>
<td>2.1</td>
<td>65</td>
</tr>
<tr>
<td>Sheep³</td>
<td>1. Mixed Protozoa</td>
<td>542</td>
<td>997</td>
<td>3.3</td>
<td>3.3</td>
<td>65</td>
</tr>
<tr>
<td></td>
<td>2. Entodinia</td>
<td>542</td>
<td>832</td>
<td>1.8</td>
<td>2.2</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>3. Polyplostron</td>
<td>542 1,362</td>
<td></td>
<td>1.5</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>Cattle⁴</td>
<td>Isotricha + Dasytrich</td>
<td>465 12,240</td>
<td>32.0</td>
<td></td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
<td>Cattle⁵</td>
<td>Entodinia +</td>
<td>619</td>
<td>979</td>
<td>19.0</td>
<td>13.2</td>
<td>84</td>
</tr>
<tr>
<td>Cattle⁶</td>
<td>Epidinium</td>
<td>413</td>
<td>1068</td>
<td>21.8</td>
<td>13.7</td>
<td>64</td>
</tr>
</tbody>
</table>

1. Leng (1982) - diet (g.100g) 720 oaten chaff, 80 lucerne, 100 molasses
2. Leng et al. (1984) - diet (g.100g) 700 oaten chaff, 100 lucerne, 100 molasses
4. Leng et al. (1984) - chopped whole sugar cane
5. Pfoulkes & Leng (1983) - (5a) diet (g/100g) 65 molasses, 35 forage
   - (5b) diet (g/100g) 35 molasses, 65 forage

A. With monensin in the diet (50mg/kg feed)
B. No monensin in the diet
Polyplastron multivesiculatum into defaunated sheep increased cellulose digestion by 3-10% while additions of other protozoal species had no effect (Jouany & Senaud 1979). The defaunation treatment may also reduce the numbers of cellulolytic bacteria in the rumen as was reported by Kurihara et al. (1979).

Results reported in the literature regarding the effects of defaunation on VFA proportions found in the rumen are conflicting. The major fermentation products of rumen protozoa cultured in vitro are acetate, butyrate and lactate, however, defaunation is sometimes associated with a decrease in the proportion of propionate (Luther et al., 1966; Eadie & Gill 1976; Demeyer et al. 1982). In contrast higher plasma glucose levels have been measured in defaunated steers and were attributed to higher propionate levels in the rumen (Whitelaw et al. 1972). More recently Whitelaw et al. (1983) reported that defaunation of steers was associated with an increase in the molar proportion of propionate and a reduced level of methane production. A reduction in the amount of starch digested in the rumen of defaunated sheep has also been observed (of intake defaunated sheep 84 cf. faunated sheep 89) by Veira & Ivan (1983).

The question: does defaunation alter the amount of energy available to the animal?, remains unresolved.

Generally defaunation studies have made no attempt to distinguish between the relative importance of the protozoal species other than their contribution to total biomass. From the studies in these laboratories of the dynamics of protozoa in the rumen, it would appear that while all protozoa had a slower turnover rate than liquid (Table 6) the relative turnover rate of the large holotrichs in the rumen of cattle (Leng et al. 1981) was considerably longer than the rate of turnover of the small Entodinium spp. in the rumen of sheep and cattle (Leng 1982; Ffoulkes & Leng 1984). Also in vitro studies (Leng et al. 1981; Leng 1982) suggested that the large holotrichs were more resistant to lysis than the small Entodinium spp. The detailed work of Coleman and his colleagues established that the rate of engulfment of bacteria by protozoa may not vary in proportion to the size of the protozoal cell. If this is characteristic of protozoa in vivo then the predatory activity of the large ciliates would be small in comparison with the activity of the small entodinias. Small Entodinium spp. generally constitute between 85-100% of the total number of protozoa in the rumen. Therefore, the partial retention and high rate of lysis of the small entodinias coupled with their predatory activity will result in extensive recycling of nitrogen in the rumen. In contrast the physical presence of the large ciliates which may be more easily retained in the rumen, may reduce the biomass of the bacteria and despite their large mass, make only a small contribution to the microbial protein available to the animal.

Defaunation is likely to be associated with a body weight gain response only when the diet does not supply sufficient protein post-ruminally to meet the requirements of the animals. In contrast the grazing trials demonstrated that defaunation stimulated wool growth in animals grazing both high protein and low protein pastures. This finding may have important implications for the wool industry. More research is required in this area but until a safer and more specific defaunating agent is developed progress will be slow. The defaunating agent presently in use cannot be recommended for commercial use because of the risk to animal health. This appears to be a high priority area for research since if the technology has wide application in the sheep industry it could increase wool production to the extent of an extra $10x10^6-100x10^6 worth of product annually.
REFERENCES


