INCORPORATION OF DIETARY NITROGEN INTO MICROBIAL NITROGEN IN THE FORESTOMACH OF THE KANGAROO ISLAND WALLABY PROTEMNODON EUGENII (DESMAREST)

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Abstract—1. Ingested nitrogen is incorporated into microbial nitrogen in the forestomach of the Kangaroo Island Wallaby Protemnodon eugenii (Desmarest).
2. Following feeding, soluble, bacterial and protozoal nitrogen increases in the forestomach digesta as does ammonia nitrogen. Plant nitrogen decreases.
3. Bacterial nitrogen is the major component of the microbial nitrogen fraction.
4. Estimated incorporation of plant nitrogen into microbial nitrogen in the forestomach of the wallaby is 64–85 per cent.

INTRODUCTION

MACROPOD marsupials have an enlarge forestomach which houses a pre-gastric microbiota (Moir et al., 1954; Moir et al., 1956; Brown, 1959). Moir et al. (1956) suggested that macropods, like ruminants (Loosli et al., 1949; Chalupa, 1968), may utilize non-protein nitrogen in the diet as a source of amino acids through initial synthesis of microbial protein. Brown (1969) subsequently demonstrated that urea and casein are utilized equally well as sources of dietary nitrogen by the euro, Macropus robustus (Gould), and this was also demonstrated in the Kangaroo Island Wallaby, Protemnodon eugenii (Desmarest) (Lintern, 1970).

No detailed study has been made of the pre-gastric microorganisms of macropods, although Moir (1965) states that bacteria and protozoa comparable in density (although not diversity) to those found in the rumen of the sheep are also present in the forestomach of the quokka, Setonix brachyurus (Quoy and Gaimard).

The study reported herein investigates the digestion of dietary nitrogen by the pre-gastric microbia of the wallaby. The extent of incorporation of dietary nitrogen into pre-gastric microbial nitrogen was estimated by measuring the changes in the distribution of nitrogen in the forestomach at successive intervals after feeding using the technique of Gray et al. (1958).

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MATERIALS AND METHODS

Animals and diet

Five sexually mature female Kangaroo Island Wallabies obtained from Kangaroo Island, South Australia were kept in individual outdoor pens for 4 weeks and fed a maintenance diet containing 1.4 g N/100 g dry weight (Barker, 1968). During this period they were cage trained. Throughout the experiment the wallabies were given 100 mg of vitamin E each week in the form of 50 mg α-tocopheryl tablets (Ephynal, Roche) as, like the quokka (Kakulas, 1961, 1963), this wallaby also is susceptible to vitamin E deficiency.

Experimental design

Wallabies were held in individual metabolism cages in a temperature controlled animal house (21°C) for 5 weeks and fed a diet containing 0.42 g N/100 g dry weight (Barker, 1968) to which no supplemental nitrogen had been added, thus preventing any loss of undigested dietary nitrogen in the forestomach digesta during subsequent analyses (Pilgrim et al., 1970). The wallabies were trained to consume their daily food ration rapidly by presenting food for 3 hr only each morning. At the end of the 5-week period wallabies were killed at intervals of 3, 5, 8, 12 and 24 hr after commencement of feeding by administering an overdose of Nembutal (Abbott) through the marginal ear vein.

Collections

Immediately after death the enlarged forestomach of the wallaby was ligated at the pyloric and fundic ends and excised. An incision was made along the whole of the lesser curvature of the forestomach and the contents removed and weighed.

Separation of a subsample of forestomach digesta into fluid, bacterial, protozoal, soluble and plant fractions was achieved using the method of Pilgrim et al. (1970) which incorporates sonic stirring to remove bacteria adhering to plant material. Microscopical examination of the products at each step of the fractionation process confirmed that this method was adequate for separation of wallaby digesta. An insignificant fraction of the smaller protozoa and bacteria, however, were present in the bacterial and soluble fractions respectively leading to a small and constant error.

Analyses

Moisture determinations were made gravimetrically after drying at 105°C for 24 hr. Nitrogen was determined by the Conway micro-diffusion technique (Conway, 1962) after acid digestion. Ammonia was determined on fresh forestomach fluid according to Seligson & Seligson (1951).

RESULTS

Dry matter intake, body weight and nitrogen intake are presented in Table 1 for each wallaby on the day changes in the distribution of nitrogen in the forestomach were measured. There were only small differences between these parameters for the five wallabies.

Changes in the distribution of nitrogen throughout the various fractions of forestomach digesta at progressive intervals after feeding are presented in Fig. 1. Although the points have been joined it must be remembered that values for each sampling time have been derived from different animals.

It can be seen that microbial attack on plant nitrogenous compounds was extremely rapid. The forestomach probably contained less than 20 mg of plant nitrogen before feeding and within 3 hr after the ingestion of approximately 190 mg
TABLE 1—Body weight (kg), dry matter intake (g/day) and nitrogen intake (g/day) for each wallaby on the day changes in the distribution of nitrogen in forestomach digesta was measured.

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Time killed after commencement of feeding (hr)</th>
<th>Body weight (kg)</th>
<th>Dry matter intake (g/day)</th>
<th>Nitrogen intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>3</td>
<td>4.71</td>
<td>47</td>
<td>191</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
<td>4.26</td>
<td>50</td>
<td>196</td>
</tr>
<tr>
<td>25</td>
<td>8</td>
<td>4.51</td>
<td>46</td>
<td>193</td>
</tr>
<tr>
<td>32</td>
<td>12</td>
<td>4.49</td>
<td>48</td>
<td>187</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
<td>4.26</td>
<td>50</td>
<td>193</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of nitrogen (g) in the forestomach digesta of five wallabies at progressive intervals after commencement of feeding at time 0. ●, Total nitrogen; ○, bacterial nitrogen; ×, plant nitrogen; △, protozoal nitrogen; ■, soluble nitrogen; □, ammonia nitrogen.

of new plant nitrogen, only 70 mg were left, and at 5 hr only 41 mg. In considering the rate of attack, account must also be taken of the time required to consume the ration. In the present experiment the bulk of the ration was consumed within 90 min after presentation, with a small amount being consumed within the remaining 90 min of the feeding period.

With the loss of plant nitrogen there was a corresponding increase in bacterial, protozoal and soluble nitrogen. Soluble nitrogen reached a maximum within 3 hr after the commencement of feeding, and declined to a minimum after 8 hr and
<table>
<thead>
<tr>
<th>Time killed after commencement of feeding (hr)</th>
<th>Animal No.</th>
<th>Protozoal nitrogen (%)</th>
<th>Bacterial nitrogen (%)</th>
<th>Soluble nitrogen (%)</th>
<th>Ammonia nitrogen (%)</th>
<th>Weight of forestomach digesta (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54</td>
<td>26</td>
<td>63</td>
<td>13</td>
<td>4</td>
<td>272</td>
</tr>
<tr>
<td>5</td>
<td>3</td>
<td>16</td>
<td>82</td>
<td>18</td>
<td>3</td>
<td>252</td>
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<tr>
<td>8</td>
<td>25</td>
<td>14</td>
<td>85</td>
<td>15</td>
<td>3</td>
<td>232</td>
</tr>
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<td>23</td>
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<td>196</td>
</tr>
<tr>
<td>14</td>
<td>23</td>
<td>9</td>
<td>53</td>
<td>11</td>
<td>4</td>
<td>147</td>
</tr>
</tbody>
</table>
increased again during the remaining 16 hr. Ammonia nitrogen followed a similar pattern.

Bacterial nitrogen increased from about 80 mg before feeding to a maximum of 230 mg 5 hr later. Protozoal nitrogen reached a maximum of 35 mg 8 hr after feeding from a pre-feeding level of 10 mg. Therefore, although some of the loss of plant nitrogen may have been due to onward passage of digesta to the smaller part of the stomach and, no doubt, some direct absorption of soluble nitrogenous compounds from the forestomach occurred, the greatest loss was due to incorporation into microbial nitrogen.

Changes in the concentration of nitrogen in the component fractions of forestomach digesta are presented in Table 2 together with the weight of forestomach digesta.

The proportions of bacterial and protozoal nitrogen in the total microbial nitrogen fraction, together with the percentage of microbial nitrogen in the total nitrogen in the forestomach at different times after feeding, are presented in Table 3. It is clear that protozoal nitrogen did not at any time form more than a small part of the microbial nitrogen fraction.

**TABLE 3—CHANGES IN THE PROPORTION OF MICROBIAL NITROGEN IN THE FORESTOMACH DIGESTA OF THE WALLABY AT PROGRESSIVE INTERVALS AFTER FEEDING**

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Time killed after commencement of feeding (hr)</th>
<th>Bacterial nitrogen as percentage of total microbial nitrogen</th>
<th>Protozoal nitrogen as percentage of total microbial nitrogen</th>
<th>Microbial nitrogen as percentage of total digesta nitrogen</th>
</tr>
</thead>
<tbody>
<tr>
<td>54</td>
<td>3</td>
<td>94</td>
<td>6</td>
<td>64</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
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</tr>
<tr>
<td>32</td>
<td>12</td>
<td>86</td>
<td>14</td>
<td>84</td>
</tr>
<tr>
<td>23</td>
<td>24</td>
<td>89</td>
<td>11</td>
<td>74</td>
</tr>
</tbody>
</table>

Weller et al. (1962) point out that, if it can be assumed that all the nitrogen in the forestomach at any particular time is originally of plant origin, then the proportion of microbial nitrogen in the total nitrogen in the forestomach digesta is a measure of the extent of conversion of plant nitrogen to microbial nitrogen. Thus the conversion of ingested plant nitrogen to microbial nitrogen in the forestomach of the Kangaroo Island Wallaby fed this particular type of diet is 64–85 per cent (Table 3).

**DISCUSSION**

The results show that pre-gastric incorporation of dietary nitrogen into microbial nitrogen occurs in the Kangaroo Island Wallaby. This process appears to be extremely rapid and quantitatively similar to the value of 60–82 per cent reported by Weller et al. (1962) for sheep.
The changes in the distribution of nitrogen in the forestomach of the wallaby following feeding paralleled closely those found in sheep (Blackburn & Hobson, 1960, 1961; Weller et al., 1962), but the rate of attack on plant nitrogenous compounds by the forestomach microorganisms was slightly more rapid in the wallaby. In both animals the ingestion of new plant nitrogen is followed by an initial increase in soluble nitrogen which is partly due to an increase in ammonia. Sutherland et al. (1962) and Weller et al. (1962) both noted a secondary rise in ammonia in the rumen between 10 and 12 hr after feeding. This was also found in the wallaby, although the quantities involved were small, probably due to the low nitrogen content or the diet (Gray et al., 1958). The secondary increase in ammonia may be due to the endogenous metabolism of non-growing microbes, the digestion of one microbe by another or the hydrolysis of recycled urea (Lintern, 1970) as has been suggested by Hungate (1966) to explain this phenomenon in sheep.

The initial production of ammonia in the forestomach of the wallaby followed by an increase in bacterial nitrogen suggests that, as in the ruminant (Bryant and Robinson, 1963), ammonia may be intermediate in the assimilation of plant nitrogen by the pre-gastric microorganisms. The increases noted in both bacterial and protozoal nitrogen following feeding were probably due not only to an increase in the nitrogen content of each microorganism but also to an increase in numbers as found in cattle (Bryant & Robinson, 1961) and sheep (Williams et al., 1953).

None of the microorganisms in the forestomach of macropods have been described or studied in detail, although bacteria resembling oscillospiras, ovals, rods and large chain cocci were tentatively identified in both the Kangaroo Island Wallaby (Lintern, 1970) and the red kangaroo, *Megaleia rufa* (Desmarest) (Harrop & Barker, 1972) and the presence of ciliate protozoa in the forestomach of both these species as well as protozoa and bacteria in the quokka has been reported (Moir, 1965; Waring et al., 1966; Lintern, 1970; Harrop & Barker, 1972).

The ability of the Kangaroo Island Wallaby and the euro to utilize urea and casein interchangeably as sources of dietary nitrogen together with the reported pre-gastric incorporation of ingested nitrogen into microbial nitrogen illustrates yet another parallel between macropod and ruminant nitrogen metabolism (Lintern & Barker, 1969; Lintern, 1970). It has been suggested that the nature of the dietary nitrogen ingested is of relatively little importance in ruminant protein nutrition (Johnson et al., 1942, 1944) as most of the protein digested and absorbed is of microbial origin. Although the results indicate that this may also be true of macropod protein nutrition it has yet to be confirmed that macropods are capable of modifying the amino acid composition of dietary nitrogen through microbial protein synthesis in the forestomach.

**SUMMARY**

Ingested nitrogen is incorporated into microbial nitrogen in the forestomach of the Kangaroo Island Wallaby, *P. eugenii* (Desmarest). The extent of incorporation is 64–85 per cent which is similar to the value of 60–82 per cent reported for sheep.
As in sheep, the ingestion of new plant nitrogen is followed by an increase in ammonia, soluble, bacterial and protozoal nitrogen in the forestomach digesta. The rate of attack by the forestomach microorganisms on new plant material is extremely rapid and slightly faster than that reported for sheep. At all times following feeding bacterial nitrogen forms the major portion of the microbial nitrogen fraction.

These results indicate a close parallel between macropods and ruminants in the digestion and utilization of plant nitrogen.

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REFERENCES


*Key Word Index*— *Protemnodon eugenii*; macropod marsupial; forestomach; nitrogen; digestion; microbiota; ammonia; bacteria; protozoa; microbial nitrogen; incorporation.