Endogenous Losses of Nitrogen and Protein Requirement for Maintenance of Sheep

by

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SUMMARY

FOUR fistulated and four intact West African dwarf wether sheep, maintained on hay and concentrate supplements were used for a study of metabolic faecal nitrogen (MEN) and endogenous urinary nitrogen (EUN). The composition of the faecal losses was examined. The values obtained enabled calculation of nitrogen requirement of the sheep for maintenance, as well as the intake of the experimental rations in the nutrition of the sheep.

Values of MEN obtained by two methods were 3.31 and 3.10 g/kg dry matter (DM) intake. Endogenous urinary nitrogen value was 0.024 g/day per metabolic weight (Wkg 0.75).

Analysis of the faeces of the sheep showed that 21.7% of faecal nitrogen (N) were present as microbial and endogenous nitrogen (MEN) and 17.3%, was present as water-soluble nitrogen (WSN).

The biological values (BV) of the rations ranged from 85.7% to 100.0% and the digestible crude protein (DCP) requirement for maintenance, were 1.20 and 0.41 g/day/Wkg 0.75 during the feeding trials.

Values obtained for endogenous N losses and N requirement for maintenance were lower compared with reported values and this may indicate adaptation of the dwarf sheep for survival under inadequate dietary protein supply.

INTRODUCTION

There have been abundant information on the nutrient requirement of sheep. The West African dwarf sheep, however, have not been well represented. The results of other breeds of sheep can be applied to the dwarf sheep. However, the reports of Elliott and Topps (1964) have shown that they generally accepted standards for digestible nitrogen for maintenance appeared to be excessive by a factor of 3 when applied to African cattle and sheep given diets adequate in energy. It was suggested that this is an adaptation to low protein supply. The metabolic faecal and endogenous urinary losses are expected to be low if the suggestion is true.

The present reports have shed light on the quantitative importance of endogenous losses, the composition of such losses and the requirement of the dwarf sheep for protein. The efficiency of dietary protein in the nutrition of the sheep was also investigated.

MATERIALS AND METHODS

Animals and their management

Four intact and four rumen-fistulated West African dwarf wethers, 1.0 - 1.5 years old and with live weights ranging from 13.2 to 20.3 kg were used. Each sheep was kept in a metabolism cage. The animals were usually fed once daily at 08:00 hr and were supplied with salt licks and fresh clean water ad lib.

Diets and plan of experiment

The experimental diets consisted of Cynodon nlemfuensis/Centrosema pubescens hay (Dict A) supplemented with five concentrates, (concentrates 1 - 5) varying in crude protein content from 1.6% to 17.5%. Diets, B, C, D, E and F, each consisted of 0.5 kg hay and 0.5 kg of concentrates 1, 2, 3, 4, 5, respectively, while diet A was 1.0 kg of hay. The components of the

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concentrates as well as the proximate analysis are shown in Table 1 and 2.

In the first experiment, the eight animals were divided into two groups of four animals, each group consisting of two intact and two fistulated sheep, and the two groups of animals were used to test diets A and B in a double reversal design. During the second experiment, the animals were divided on a live weight basis into four groups of two animals, each group consisting of one intact and one fistulated animal and each pair was allocated to each of diets C, D, E, and F in a 4 x 4 Latin square design. There were preliminary periods of 14 days and collection period of 6 days.

**Collection of faeces and urine**

Faecal collection was done by means of collection bags fitted to harnesses. The total wet faeces excreted daily were weighed and dried in a forced-draught oven at 80°C for 24 hours. The daily dried faeces for each animal were bulked, milled with Christy and Norris mill and stored in airtight glass bottles until required for analysis.

Urine was collected in small polythene buckets placed underneath the metabolism cages. Each bucket was wetted with 5 ml of 10% mercuric chloride to prevent microbial breakdown of nitrogenous components of urine. About 10% of daily

### TABLE 1

<table>
<thead>
<tr>
<th>COMPOSITIONS</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Groundnut meal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cassava flour</td>
<td>92.5</td>
<td>88.5</td>
<td>82.5</td>
<td>74.5</td>
<td>64.5</td>
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<tr>
<td>Molasses</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
<td>5.0</td>
</tr>
<tr>
<td>Mineral mixture</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
<td>2.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**+1 kg of mineral and vitamin mixture supply the following:**

- Vit. A (M.I.U.) = 0.50
- Vit D = 0.252
- Manganese (g) = 16.00
- Zinc = 12.00
- Iron = 6.00
- Copper = 4.00
- Cobalt = 9.30
- Iodine = 1.20
- Magnesium = 200.00

### TABLE 2

<table>
<thead>
<tr>
<th>CONSTITUENTS</th>
<th>CONCENTRATES</th>
<th>HAY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude protein</td>
<td>1.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Crude fibre</td>
<td>4.8</td>
<td>5.1</td>
</tr>
<tr>
<td>Ether extract</td>
<td>0.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Ash</td>
<td>6.9</td>
<td>6.4</td>
</tr>
<tr>
<td>Nitrogen-free extract</td>
<td>89.5</td>
<td>79.5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>100.0</td>
<td>100.0</td>
</tr>
</tbody>
</table>

**HAY**

- Crude protein = 1.7
- Crude fibre = 4.9
- Ether extract = 0.7
- Ash = 5.8
- Nitrogen-free extract = 71.9
- TOTAL = 100.0
urine collection, was retained, and daily samples were bulked and stored in a deep-freezer at -5°C until required for analysis.

Analytical procedure

The nitrogen content of feeds, faeces, and urine as well as crude fibre, other extracts and ash contents of the feeds were determined according to AOAC (1975) method. Nitrogen of non-dietary origin was determined by the neutral detergent method of Mason (1969).

RESULTS

Two methods were used for determining the values of metabolic faecal nitrogen. These are the regression method and the neutral detergent method Mason, (1969). The regression of faecal N (Y g/kg DM intake) on N intake (X g/day) gave the following equation:

\[ Y = 3.13 + 0.16X, \text{SE}_b = 0.02 \] (1)

Faecal N at zero N intake was given by the equation to be 3.13 g/kg DM intake.

Similarly, when faecal N (Y) expressed in g/kg DM intake was regressed on the percent crude protein in the ration (X), the equation obtained was:

\[ Y = 3.68 + 0.14X, \text{SE}_b = 0.04 \] (2)

which gave the value of MFN to be 3.68 g/kg DM intake.

Two other regression equations were used to estimate the values of MFN. These are the regression of digestible N, per kg DM intake (Y) on N intake per kg DM intake (X), giving the regression equation:

\[ Y = 0.896X - 3.45, \text{SE}_b = 0.025 \] (3)

and the regression of digestible crude protein (Y kg) on the percentage crude protein of the ration (X), giving the equation \[ Y = 0.062X - 1.86, \text{SE}_b = 0.020 \] (4) From equation 3, the value of MFN was 3.45 g/kg DM intake, while the value from equation 4 was 1.86 crude protein per 100g DM intake or 2.98 g MFN/kg DM intake. The values obtained from the four equations were 3.13, 3.68, 3.45 and 2.98 g/kg DM intake, giving a mean value of 3.31 - 0.16 g MFN/kg DM intake.

The values of MFN as obtained by the neutral detergent method of Mason (1969) are given in Table 3, and these ranged from 1.46 to 4.20 g/kg DM intake with a mean value of 3.10 g/kg DM intake. The mean value of MFN obtained by the neutral detergent method is in very good agreement with the value obtained from the regression equations and this indicates that the values of MFN is about 3g/kg DM intake for West African dwarf wether sheep weighing 17 to 27 kg and maintained on forage with or without concentrate supplement.

The value of endogenous urinary N was obtained by regression of urinary N (Y g/day/WKg 0.75) on N intake (X g/day). The relationship is given by the following equation:

\[ Y = 0.0096X - 0.0238, \text{SE}_b = 0.0011 \] (5) From equation 5, urinary N zero N intake calculated to be 0.0238 g/day/WKg 0.75 and this is EUN excretion of the sheep.

The percentage of undigested dietary N (UDN), microbial and endogenous N (MEN), water-soluble N (WSN) and non-dietary faecal N (NDFN or MEN + WSN) in the faeces of sheep are given in Table 3. The values of UDN recovered in the faeces ranged from 17.6% to 24.3% with a mean value of 21.1%. Mean values for other components are: Microbial and endogenous N, 61.6%, water soluble N, 17.3% and non-dietary faecal N, 78.9%. The percentage of non-dietary faecal N contributed by the microbial and endogenous N was 78%.

The apparent and true digestibility, biological values (BV) and net protein utilization (NPU) values of the rations for the sheep are presented in Table 4. The biological value was determined using MFN value of 3.13 g/kg DM intake and EUN value of 0.0238 g/day/WKg 0.75. The equation used was

\[ \text{BV} = \frac{\text{NI} - (\text{FN} - \text{MFN})}{(\text{UN} - \text{EUN})} \] (6)

where NI is the N intake, FN is faecal N, UN is urinary N, EUN is endogenous urinary N, MFN is metabolic faecal N and BV is the biological value of the ration.
TABLE 3

The Percentages of Undigested Dietary N, Microbial N and Water-soluble N in the Faeces of the Sheep (Neutral Detergent Method)

<table>
<thead>
<tr>
<th>Ration</th>
<th>Undigested dietary N (UDN)</th>
<th>Microbial and endogenous N (MEN)</th>
<th>Water-soluble N (WSN)</th>
<th>Non-dietary Faecal N (NDFN)</th>
<th>MEN</th>
<th>Apparent digestibility</th>
<th>True digestibility</th>
<th>Faecal N g/day</th>
<th>Dry matter intake kg/day</th>
<th>Metabolizable N (g/kg)</th>
<th>DM intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>24.3</td>
<td>54.2</td>
<td>21.4</td>
<td>75.8</td>
<td>0.72</td>
<td>55.7</td>
<td>83.3</td>
<td>2.46</td>
<td>0.44</td>
<td>1.87</td>
<td>4.20</td>
</tr>
<tr>
<td>B</td>
<td>22.6</td>
<td>47.9</td>
<td>18.5</td>
<td>76.4</td>
<td>0.76</td>
<td>63.6</td>
<td>91.4</td>
<td>1.59</td>
<td>0.53</td>
<td>1.14</td>
<td>2.15</td>
</tr>
<tr>
<td>C</td>
<td>22.6</td>
<td>61.8</td>
<td>15.6</td>
<td>77.4</td>
<td>0.80</td>
<td>64.1</td>
<td>91.9</td>
<td>2.64</td>
<td>0.61</td>
<td>2.04</td>
<td>3.36</td>
</tr>
<tr>
<td>D</td>
<td>18.9</td>
<td>64.5</td>
<td>16.6</td>
<td>81.1</td>
<td>0.80</td>
<td>70.1</td>
<td>94.3</td>
<td>1.69</td>
<td>0.72</td>
<td>1.37</td>
<td>3.24</td>
</tr>
<tr>
<td>E</td>
<td>19.5</td>
<td>63.9</td>
<td>16.2</td>
<td>80.1</td>
<td>0.79</td>
<td>72.8</td>
<td>94.6</td>
<td>2.56</td>
<td>0.51</td>
<td>2.13</td>
<td>4.20</td>
</tr>
<tr>
<td>F</td>
<td>17.6</td>
<td>67.3</td>
<td>15.3</td>
<td>82.4</td>
<td>0.82</td>
<td>77.0</td>
<td>96.6</td>
<td>1.19</td>
<td>0.66</td>
<td>1.38</td>
<td>2.95</td>
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<tr>
<td>Mean</td>
<td>21.1</td>
<td>61.6</td>
<td>17.3</td>
<td>78.9</td>
<td>0.78</td>
<td>67.2</td>
<td>92.9</td>
<td>2.02</td>
<td>0.52</td>
<td>1.38</td>
<td>3.10</td>
</tr>
<tr>
<td>Standard error</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>0.8</td>
<td>0.03</td>
<td>2.8</td>
<td>0.9</td>
<td>0.20</td>
<td>0.08</td>
<td>0.18</td>
<td>0.29</td>
</tr>
</tbody>
</table>

TABLE 4

Digestibility, Biological Value and Net Protein Utilization Values of Rations Fed to West African Dwarf Sheep

<table>
<thead>
<tr>
<th>Ration</th>
<th>Nitrogen intake (g/day/Wkg@0.75)</th>
<th>Apparent digestibility</th>
<th>True* digestibility</th>
<th>Biological value</th>
<th>Net protein utilization</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.62</td>
<td>55.9</td>
<td>80.9</td>
<td>85.7</td>
<td>69.3</td>
</tr>
<tr>
<td>B</td>
<td>0.59</td>
<td>61.2</td>
<td>80.2</td>
<td>100.0</td>
<td>80.2</td>
</tr>
<tr>
<td>C</td>
<td>0.64</td>
<td>63.3</td>
<td>87.5</td>
<td>96.3</td>
<td>84.3</td>
</tr>
<tr>
<td>D</td>
<td>0.31</td>
<td>69.4</td>
<td>92.9</td>
<td>96.0</td>
<td>86.2</td>
</tr>
<tr>
<td>E</td>
<td>1.28</td>
<td>71.0</td>
<td>88.2</td>
<td>96.8</td>
<td>87.2</td>
</tr>
<tr>
<td>F</td>
<td>1.55</td>
<td>76.3</td>
<td>99.4</td>
<td>92.6</td>
<td>89.7</td>
</tr>
<tr>
<td>Mean</td>
<td>0.95</td>
<td>66.2</td>
<td>86.7</td>
<td>94.2</td>
<td>82.3</td>
</tr>
<tr>
<td>Standard error</td>
<td>0.12</td>
<td>2.8</td>
<td>1.9</td>
<td>1.9</td>
<td>2.8</td>
</tr>
</tbody>
</table>

*True digestibility values obtained from regression equations.

TABLE 5

Digestible Crude Protein Requirement for Maintenance of the West African Dwarf Sheep

<table>
<thead>
<tr>
<th>Trial</th>
<th>Regression Equation</th>
<th>N intake at Zero N balance (g/day/Wkg@0.75)</th>
<th>Crude protein intake at Zero N balance (g/day/Wkg@0.75)</th>
<th>Digestible crude protein Requirement for Maintenance (g/day/kg live weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. (i)</td>
<td>Y=0.91X+0.32</td>
<td>0.35</td>
<td>2.20</td>
<td>1.28</td>
</tr>
<tr>
<td>1. (ii)</td>
<td>Y=0.83X+0.20</td>
<td>0.24</td>
<td>1.51</td>
<td>1.06</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.29</td>
<td>1.75</td>
<td>1.23</td>
</tr>
<tr>
<td>2. (iii)</td>
<td>Y=-0.75X+0.07</td>
<td>0.093</td>
<td>0.48</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.093</td>
<td>0.59</td>
<td>0.41</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>0.095</td>
<td>0.59</td>
<td>0.41</td>
</tr>
</tbody>
</table>
The Biological values obtained were high ranging from 85.7% for hay to 100% for ration B (Hay/cassava flour) which supplied the lowest amount of crude protein.

The net protein utilization (NPU) values were obtained as the product of true digestibility (TD) and biological value (BV). The NPU values were high for the mixed rations, ranging from 89.2 to 96.2 for ration D. A much lower value of 69.3 was obtained for the forage ration. The presence of concentrate appeared to increase the biological value and net protein utilization of basal hay ration.

The digestible crude protein (DCP) required for maintenance of the wethers is the amount of digestible crude protein intake required to keep the wethers at zero N balance or at N equilibrium. Values for N requirement were obtained from regression of N balance on N intake. The results for the trials are summarized in Table 5. The value for trial 1 was 1.28g DCP/day/Wkg 0.75, and the values for the first two periods of trial 2 were 1.06 and 1.23g DCP/day/Wkg 0.75. The mean value was 1.15g DCP/day/Wkg 0.75. There was a sharp decrease in the value of DCP requirement in the third and fourth periods of trial 2; the mean value was 0.41g DCP/day/Wkg 0.75. The sharp decrease in DCP requirement for maintenance during the last two periods indicates that requirement is influenced by the stage of growth of the sheep.

**DISCUSSION**

There was a good agreement between the values of MFN obtained from regression equations and those obtained by the detergent method.

The values obtained in the present report are lower than the value of 50g/kg DM intake often quoted for ruminants (Maynard & Loosli, 1969), but are comparable to those of Deit, El-shazy and Abou Akkada (1968) who reported MFN value of 3.58g/kg DM intake, and Elliott and Topps (1963) who obtained 3.66g/kg DM intake. A wide variation in MFN values have been obtained by some investigators. The estimate of Robinson and Forbes (1970) was 6.5g/kg DM intake while Mason (1969), using the detergent method obtained a range of 4.4 to 7.2 g/kg DM intake. It is evident from the reports of investigators, that a number of factors influence excretion of MFN. The composition of the feed influences MFN excretion. Most of the investigators who obtained low MFN values used highly digestible feeds, while those who obtained high values used highly fibrous rations (Mason, 1959).

Schneider (1935) showed that excretion of MFN was influenced by body size as well as level of feed intake, MFN tending to increase with increasing live weight of the animal. Blaxter and Wood (1951) showed that MFN excretion increased on rations of low digestibility. Walker and Faichney (1964a) obtained MFN value of 2.90g/kg DM intake using protein-free diets. However, Waterlow (1968) showed that protein metabolism of ruminants given nitrogen-free diets are substantially altered and that the results obtained may not apply under normal feeding conditions. The results obtained in the present report showed that MFN values of dwarf sheep is about 3.0g/kg DM intake on forage and mixed rations.

The value of EUN obtained in the present report as 0.20g/kg/day/Wkg 0.75 is lower than values of 0.170g/day/Wkg 0.75 obtained by Walker and Faichney (1964a) of giving protein-free diets, and 0.056g/day/Wkg 0.75 obtained by Black, Pearce and Tribe (1973) using lambs with live weights ranging from 7.8 to 30 kg. It is also less than 0.038g/kg/day/Wkg 0.75 obtained by Singh and Mahadevan (1970) who used adult rams.

The low EUN value obtained for West African dwarf sheep may be an adaptation to subsistence on low quality forage, the condition prevalent in the tropics where natural grass species rapidly decline in nutritional value. Mugerwa and Conrad (1971) have shown that urinary urea excretion is indicated by the levels of blood urea. It may be that in the dwarf sheep, blood urea is preserved by re-cycling into the rumen thus decreasing urinary loss.

The proportion of nitrogenous components of faeces has been shown by Mason (1969) to be influenced by the type of
rations. In the present report, most of the faecal N is present in the microbial component of the faeces. The presence of readily fermentable sources of energy would tend to increase the microbial portion of the faeces. The values obtained in the present report are all comparable to that of Mason (1969) and this shows that for conventional type of rations, the proportions of undigested dietary N, microbial and endogenous N and water-soluble N do not differ much from other reported values.

The Biological values obtained for the rations were very high indicating that dietary protein was well utilized by the sheep. The high biological value of the rations may be due to low excretion of urinary N, and this may in turn be caused by the presence of readily fermentable cassava flour in the ration causing a depression of ruminal ammonia, plasma urea and urinary urea levels. However, the fact that maximum biological value was obtained on a ration supplying the least protein is in line with well-established findings that maximum BV is obtained at minimum protein supply. The BV obtained in the present report is higher than the value of 86.9% obtained by Singh and Mahadevan (1970) and 83.1% obtained by Stobo and Roy (1973) who also fed ground nut meal-based rations to sheep. The findings of Barnes, Bates & Maack (1946) showed that age, class of animal and the production involved would tend to influence the effective apparent BV of a protein in addition to amino acid balance. The fact that ruminal microorganisms are able to synthesize good quality microbial protein from low quality feed makes amino acid balance of diet less importance to ruminants.

The mean value of DCP requirement for maintenance in the Trial 1 and first two periods of Trial 2 was much higher than the value for periods 3 and 4.

The very sharp decline in DCP requirement in periods 3 and 4 shows that protein requirements of growing animals change rapidly with age or live weight (Black et al., 1973). The value of 1.2-0.6kg/day/Wkg 0.75 obtained in the present report is similar to values of 1.25 and 1.16kg/day/Wkg 0.75 obtained respectively by Black et al (1973), and Robinson and Forbes (1966), higher than 0.875 and 0.893kg/day/Wkg 0.75 obtained by Singh and Mahadevan (1970) but lower than 3.6kg/day/Wkg 1.075 recommended by Brody (1945).

Eliott and Topps (1964) showed that the proportion of roughage to concentrate in the ration influenced maintenance requirement of sheep. They showed that the maintenance requirement increased with increasing roughage to concentrate in the ration.

The value of DCP requirement of the dwarf sheep is about 33% of Brody's (1945) recommendation. This agrees with Elliott and Topps's (1964) observation that the generally accepted standards for digestible N for maintenance appear to be excessive by a factor of 3 when applied to African cattle and sheep. The low protein requirement for maintenance of the dwarf sheep may be due to low endogenous N losses as well as to the fact that an appreciable amount of plasma urea are recycled to the rumen, and these are efficiently utilized in the presence of readily fermentable sources of energy. The low protein requirement for maintenance is a distinct advantage in a protein-deficient environment.

ACKNOWLEDGEMENT

The authors wish to thank the technical staff of the Department for their co-operation. We also acknowledge the help rendered by Drs. J. U. Akpokodje and O. Oladosu of the Department of Veterinary Medicine and Surgery who fitted the cannulae on the sheep.

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