Digestion and nitrogen metabolism in sheep and red deer given large or small amounts of water and protein

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1. The interaction between nitrogen and water intake was studied in two ewes and two red deer hinds. They were given pelleted diets, at maintenance level, containing equal amounts of roughage but either rich or poor in nitrogen. The deer received 50 % more food than the sheep. Water was given either in large amounts (sheep 5·0 l, deer 7·0 l daily) or in small amounts (sheep 1·1 l, deer 2·4 l daily).

2. Nearly three-quarters of the nitrogen of the high-nitrogen rations but less than half of that of the low-nitrogen rations was excreted in the urine. Restriction of water intake reduced urinary nitrogen excretion by only about 1 g daily, mainly as a result of decreases in the excretion of urea and ammonia, but did not affect the excretion of nitrogen in the faeces.

3. The urinary excretions of creatinine, creatine, hippuric acid, uric acid and allantoin were also examined. The excretion of creatinine was not related to either nitrogen or water intake. The excretion of uric acid and of allantoin was greater in the sheep than in the deer.

4. The concentrations of urea in the plasma and of ammonia in the rumen fluid were measured before and after feeding. The plasma urea value was related to dietary nitrogen intake and was higher on the low- than on the high-water regime. The rumen ammonia value also was related to the nitrogen intake but, while it generally increased after feeding when the high-nitrogen diet was given, it fell almost to zero 2 h after feeding when the low-nitrogen diet was given.

5. The sheep digested dry matter, cellulose and nitrogen a little more fully than the deer. The high-water regime slightly increased the digestibility of dry matter and cellulose but did not affect the digestibility of nitrogen.

Schmidt-Nielsen, Schmidt-Nielsen, Houpt & Jarnum (1957), Schmidt-Nielsen & Osaki (1958) and Elliott & Topps (1963) have shown that if the nitrogen intake of camels, sheep or cattle is severely reduced the concentration of urea in the urine falls to a very low level. It appears that, in camels and sheep at least, the concentration of urea is not affected by urine flow. Any change in urine flow is accompanied, therefore, by a change in the small amount of urea excreted.

Livingston, Payne & Friend (1962) found that the amount of urea excreted in the urine of cattle given *ad lib.* a very low-quality roughage diet diminished when their water intake was curtailed. In a subsequent experiment (Payne, 1964) it was shown that water restriction led to a substantial improvement in nitrogen balance. In such experiments, however, water and nitrogen intake are not the only variables influencing the nitrogen economy. This also depends on the intake of digestible energy which affects the growth of rumen micro-organisms and their capacity to utilize ammonia for protein synthesis, and on the loss of nitrogen in the faeces.

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In an attempt to avoid such obstacles to interpretation, an experiment was designed to study the separate effects of nitrogen intake and water intake on nitrogen metabolism. Two sheep and two red deer were given constant amounts of two rations which differed in nitrogen content but provided nearly the same amounts of digestible energy and roughage. Preliminary accounts of this experiment have been given (Maloiy, Kay, Goodall & Topps, 1968; Topps, Goodall, Kay & Maloiy, 1968).

EXPERIMENTAL

Animals

Two Scottish Blackface ewes, Bunty and Katerine, and two red deer hinds, Adija and Hamisi, were used in the experiment. The ewes were 4 years old and the hinds 3 years old. At the beginning of the experiment the ewes weighed 40 and 45 kg and the hinds 53 and 55 kg respectively. All the animals had permanent rumen cannulas and were kept in metabolism cages similar to those described by Duthie (1959) during the experimental periods. Room temperature was maintained at 18 °.

Table 1. *Constituents of the two diets (g/100 g)*

Constituent	High-nitrogen diet	Low-nitrogen diet
Barley straw	53·3	53·4
Maize starch	10·6	17·7
Groundnut meal	32·0	0·0
Maize meal	0·0	24·7
Molasses	2·0	2·0
Mineral–vitamin supplement	2·1	2·1
Dry matter	88·9	90·1
Ash	18·8	18·9
Nitrogen	2·65	0·84
Cellulose	18·0	18·8
Gross energy (kcal/kg)	4100	3994

Diets

Two pelleted diets differing in nitrogen content were used. These will be called the high-nitrogen (high-N) and low-nitrogen (low-N) diets. They were based on barley straw and meals, and their formulation and chemical analysis are shown in Table 1. The sheep received 800 g and the deer 1200 g of food daily, given as two equal meals at about 09.00 and 20.00 hours. These rations were sufficient to allow the animals to maintain their body-weight over the experimental period. The rations were always fully consumed.

Experimental design

The experiment was divided into two parts with each part being subdivided into three periods as detailed in Table 2. Each part began with an initial period of 10–18 d during which water was given *ad lib.* to each animal. During the second period of the experiment, 13–18 d, the animals were given a high water intake, 5·0 l daily to the sheep and 7·0 l to the deer. This was achieved by pouring half of

Table 2. *Plan of the experiment, showing the sequence of diets and the amounts of water (l/24 h) administered in each period*

		Sheep				Deer			
		Katerine		Bunty		Adija		Hamisi	
Part	Period	Diet	Water	Diet	Water	Diet	Water	Diet	Water
	1	Low-nitrogen	*ad lib.*	High-nitrogen	*ad lib.*	High-nitrogen	*ad. lib.*	Low-nitrogen	*ad lib.*
1	2	—	5·0	—	5·0	—	7·0	—	7·0
	3	—	1·0	—	1·0	—	2·4	—	2·4
	1	High-nitrogen	*ad lib.*	Low-nitrogen	*ad lib.*	Low-nitrogen	*ad lib.*	High-nitrogen	*ad lib.*
2	2	—	5·0	—	5·0	—	7·0	—	7·0
	3	—	1·2	—	1·2	—	2·4	—	2·4

these quantities of warm water into the rumen by way of the rumen cannula at each meal time. This procedure did not appear to affect appetite. For the third period, of 14–15 d, drinking water was provided at meal times but was restricted to 1·0 or 1·2 l daily for the sheep and 2·4 l for the deer. These quantities were near to voluntary water intake and although the animals usually lost some weight their appetite was unimpaired. The sheep on the low-N ration failed to drink all their water and the residue was poured into the rumen at the beginning of the next meal.

In the first part of the experiment one animal of each species was given the high-N diet and the other the low-N diet; in the second part the dietary treatments were reversed.

Faeces and urine were collected for analysis during the last 6 d of the periods of high and low water intake, the urine being combined for 2 d periods. As a preservative, 500 ml of water plus 20 ml of concentrated HCl were placed in the urine containers each day.

During each treatment the concentrations of urea in plasma and of ammonia in strained rumen fluid were measured for each animal over a 12 h period between meals, some 8–10 d after imposing the high or low water intake. The jugular vein was catheterized for blood sampling about 15 min before the morning feed. Samples of blood and rumen fluid were drawn before feeding and at various intervals afterwards.

Analytical methods

The concentrations of ammonia and urea in the plasma, rumen contents and urine were measured by the method of Conway (1957). It was necessary to titrate the urine samples to pH 6 before adding the urease preparation; otherwise, falsely low values were obtained. The total-nitrogen content of samples of urine and fresh faeces was measured by the macro-Kjeldahl method. Dry matter in food and faeces samples was estimated by drying at 105° for 48 h, and cellulose in these dried samples by the method of Crampton & Maynard (1938). Calorific value of the diets was measured in an adiabatic bomb calorimeter (A. Gallenkamp & Co. Ltd, London).

In the neutralized urine samples, creatinine and creatine were measured by the Jaffé reaction (Bonsnes & Taussky, 1945), hippuric acid by the method of Hampton (1948), uric acid by the method of Benedict & Francke (1922), and allantoin by the method of Young & Conway (1942).

RESULTS

Health, appetite and body-weight of the animals

The animals remained in good health and maintained a good appetite throughout the experiment. However, the rate of eating declined during periods of low water intake, Hamisi in particular taking up to 90 min to consume a meal, instead of the usual 10–15 min. During the third period of part two of the experiment, the rumen cannula in Katerine broke and was replaced, but this had no ill effect on the sheep.

The body-weights of the sheep and deer varied with the amount of water they received during the experiment. Compared with their weights when water was freely available, all the animals gained 1–2 kg during the periods they received large amounts

of water. Conversely, when water was restricted all the animals lost weight, Bunty and Adija as much as 4 and 5 kg respectively while the weight of the other two animals fell by 2 kg or less. Nitrogen intake had no appreciable effect on body-weight.

The *ad lib.* water intake of the sheep ranged from 1·0 to 3·0 l daily while that of the deer varied from 1·6 to 4·7 l. These variations in water intake could be attributed to differences between individual animals and to changes in nitrogen intake.

Apparent digestibility of dry matter, cellulose and nitrogen

The apparent digestibility of dry matter was greater in the two sheep than in the two deer and was slightly greater on the high-water regimen than on the low-water regimen. Similar but more marked differences were apparent in cellulose digestibility. Nitrogen intake had no appreciable effect.

Table 3. *Influence of nitrogen and water intake on the apparent digestibility of dry matter, cellulose and nitrogen by sheep and deer*

(Mean values are shown for the two sheep and the two deer)

Animals	Treatment	Water administered (l/24 h)	Apparent digestibility (%)			Faecal water content (%)
			Dry matter	Cellulose	Nitrogen	
Sheep	High-nitrogen,	5·0	61	34	75	58
Deer	high-water	7·0	57	21	73	66
Sheep	High-nitrogen,	1·1	60	26	72	50
Deer	low-water	2·4	55	17	73	59
Sheep	Low-nitrogen,	5·0	62	33	39	61
Deer	high-water	7·0	55	22	27	70
Sheep	Low-nitrogen,	1·1	60	30	34	53
Deer	low-water	2·4	53	18	25	67

On the high-N diet the apparent digestibility of nitrogen was about the same in both pairs of animals but on the low-N diet the apparent digestibility was rather higher in the sheep than in the deer. Water intake did not affect the apparent digestibility of nitrogen.

The faeces of the sheep were drier than those of the deer. As a result of this and the smaller output of faecal dry matter, the sheep excreted daily only about half as much water in their faeces as the deer. The low-water regimen led to the faeces becoming drier.

Excretion of nitrogen

Table 4 shows urine volume and urinary and faecal nitrogen excretion in relation to the intake of water and nitrogen. The excretion of nitrogen in the urine was related to the nitrogen intake so that the animals remained close to zero nitrogen balance on all four treatments. However, about 1 g more nitrogen was excreted in the urine on the high-water regimen than on the low-water regimen. Excretion of nitrogen in the faeces was unaffected by water intake but about 1 g more was excreted on the high-N diet than on the low-N diet. The deer excreted about 65% more nitrogen in their faeces than the sheep.

The urinary excretion of certain nitrogenous compounds is summarized in Table 5. The excretion of ammonia was small for all the treatments and was least on the low-N, low-water treatment.

When the high-N diet was given, the deer excreted about 50% more urea than the sheep, as might be expected from their relative nitrogen intakes; water intake had little or no effect. When the low-N diet was given, little urea was excreted and the deer excreted no more than the sheep. When large volumes of water were administered the concentration of urea in the urine was about half the value for the low-water regimen, but the volume of urine was so much greater that nearly four times more urea was excreted.

Table 4. *Urine volume and nitrogen excretion of sheep and deer given high-N or low-N diets with high or low water intake*

(Mean values are shown for the two sheep and the two deer)

Animals	Diet	Water administered (l/24 h)	Urine volume (l/24 h)	Food N (g/24 h)	Urine N (g/24 h)	Faeces N (g/24 h)
Sheep	High-N	5·0	3·7	21·2	15·4	5·4
		1·1	0·5	21·2	14·4	5·9
	Low-N	5·0	4·1	6·7	3·1	4·1
		1·1	0·3	6·7	2·1	4·5
Deer	High-N	7·0	5·1	31·8	22·9	8·7
		2·4	0·8	31·8	21·5	8·7
	Low-N	7·0	4·6	10·1	3·2	7·4
		2·4	0·8	10·1	1·7	7·6

Neither nitrogen nor water intake had any apparent effect on the amount of creatinine excreted. There was a consistent difference between the sheep and the deer which was probably related to their difference in weight. Excretion of creatine was small, especially on the low-N diet, and it appeared to be reduced when water intake was restricted.

The sheep and deer excreted similar amounts of hippuric acid. Rather more was excreted on the high-N than on the low-N diet but water intake had little effect.

The sheep excreted substantially more of the purine derivatives, uric acid and allantoin, than the deer even though their intake of nitrogen was only two-thirds that of the deer. This species difference was more pronounced on the low-N diet and the difference was relatively greater for uric acid than for allantoin. In general, the loss of both uric acid and allantoin was greatest when the high-N diet was given, but water intake had no apparent effect.

Concentration of plasma urea and ruminal ammonia

Concentrations of urea in the plasma and of ammonia in the rumen fluid of Bunty and Hamisi are shown in Figs. 1 and 2. The results for the other two animals were similar, except that on the low-N diet they had lower concentrations of urea in their plasma when water intake was restricted, and this was accompanied by lower rumen ammonia values. Before feeding, the concentrations of both urea in plasma and

Table 5. *Influence of nitrogen and water intake on urinary N excretion (g/24 h) by sheep and deer*

Animals	Treatment	Total urinary N	Ammonia N	Urea N	Creatinine N	Creatine N	Hippuric acid N	Uric acid N	Allantoin N	N recovered (% of total)
Sheep:										
Bunty	High-nitrogen,	16·30	0·19	13·38	0·40	0·20	0·40	0·10	0·45	93
Katerine	high-water	15·10	0·20	10·45	0·36	0·22	0·41	0·07	0·58	81
Deer:										
Adija		21·72	0·17	16·83	0·66	0·08	0·49	0·02	0·40	86
Hamisi		24·08	0·26	14·68	0·65	0·24	0·73	0·01	0·18	70
Sheep:										
Bunty	High-nitrogen,	13·90	0·08	12·14	0·35	0·12	0·33	0·09	0·51	98
Katerine	low-water	14·87	0·06	10·16	0·36	0·20	0·42	0·05	0·42	78
Deer:										
Adija		22·02	0·13	19·38	0·53	0·06	0·41	0·02	0·31	95
Hamisi		20·94	0·12	16·50	0·60	0·14	0·51	0·02	0·16	86
Sheep:										
Bunty	Low-nitrogen,	2·94	0·28	0·91	0·37	0·13	0·28	0·07	0·42	84
Katerine	high-water	3·33	0·16	1·50	0·39	0·17	0·23	0·07	0·47	90
Deer:										
Adija		2·76	0·14	1·04	0·51	0·06	0·29	0·01	0·15	80
Hamisi		3·55	0·25	1·79	0·62	0·02	0·28	0·01	0·20	89
Sheep:										
Bunty	Low-nitrogen,	2·46	0·05	0·57	0·34	0·06	0·21	0·06	0·40	69
Katerine	low-water	1·77	0·05	0·30	0·37	0·10	0·19	0·06	0·46	86
Deer:										
Adija		1·52	0·04	0·16	0·51	0·02	0·28	0·01	0·15	77
Hamisi		1·88	0·06	0·38	0·61	0·01	0·22	0·01	0·19	79

ammonia in rumen fluid were high on the high-N diet and low on the low-N diet, and values for plasma and rumen fluid were approximately equal when expressed as mg N/100 ml. After a meal of the high-N diet was eaten, rumen ammonia values usually showed a moderate increase but they fell almost to zero about 2 h after the low-N diet was eaten. Plasma urea values showed only minor and irregular fluctuations during the day.

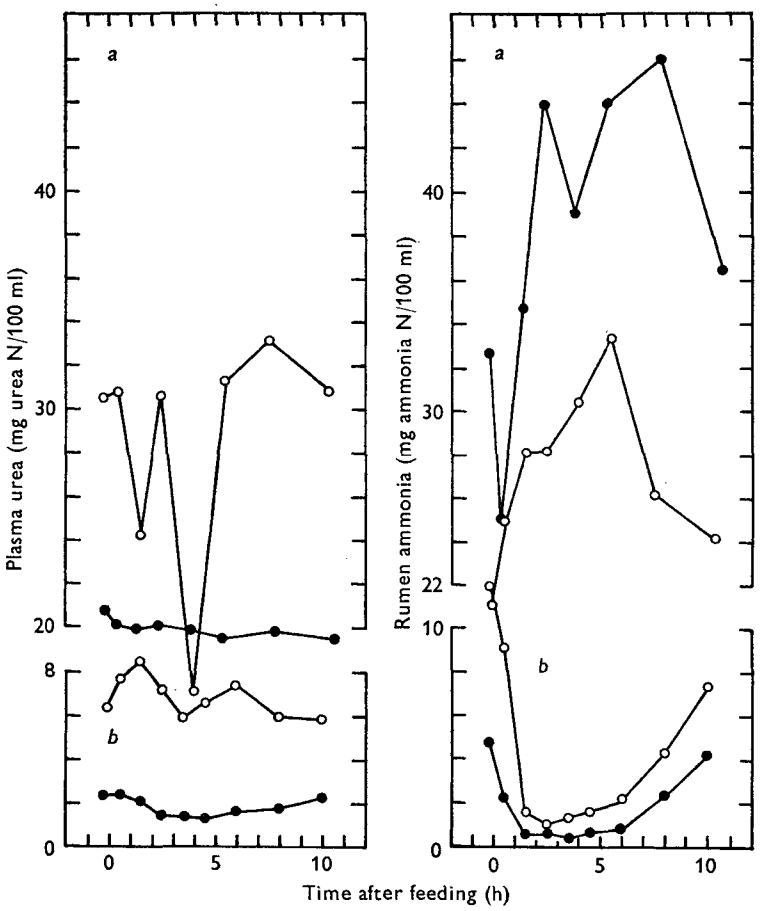

Fig. 1. Sheep Bunty. Effects of nitrogen and water intakes on the concentrations of urea in plasma and of ammonia in rumen fluid. The first samples were taken immediately before feeding. ●, high-water regimen; ○, low-water regimen; *a*, high-N diet; *b*, low-N diet.

DISCUSSION

Criticism of experiment

Any interpretation of experiments involving only two sheep and two deer must be guarded, especially since differences in metabolism between the pairs of animals may have been confounded by differences in body-weight. However clearly the two pairs of animals may have differed in some respects, it is obvious that the work must be

greatly extended before it can be concluded that such differences reflect species rather than individual variation. These limitations will be considered in the discussion that follows.

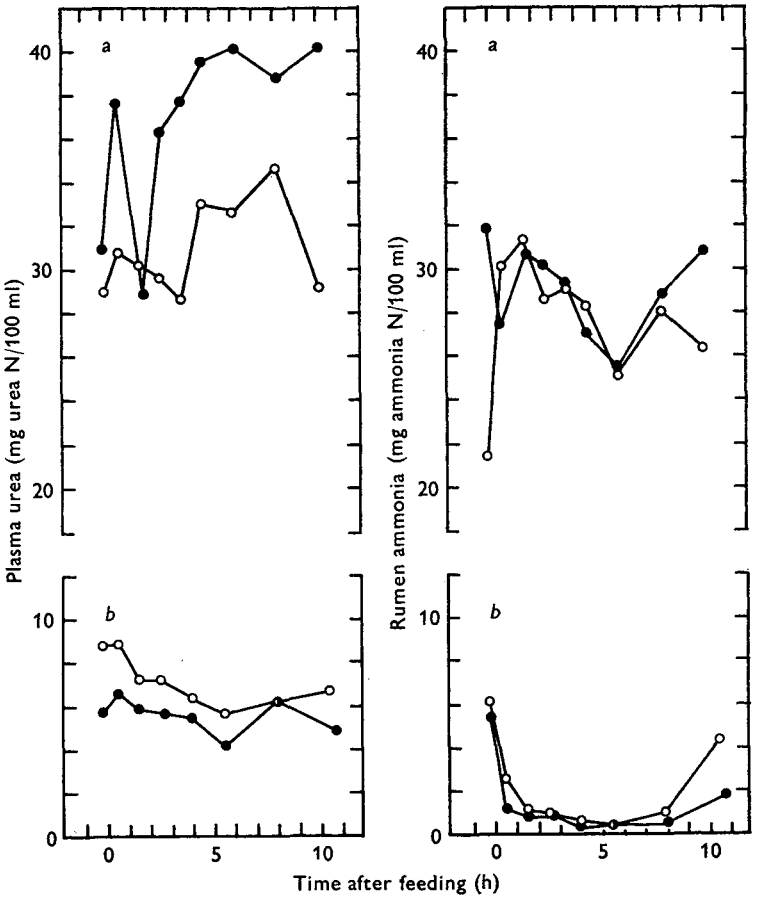

Fig. 2. Deer Hamisi. Effects of nitrogen and water intakes on the concentrations of urea in plasma (*a*) and of ammonia in rumen fluid (*b*). The first samples were taken immediately before feeding. ●, high-water regimen; ○, low-water regimen; *a*, high-N diet; *b*, low-N diet.

Nitrogen metabolism and water intake

The experiment clearly indicates that, when the food intake of the sheep and deer was kept constant in both amount and composition, water intake had only small effects on the excretion of nitrogen in the urine and faeces. On either the high- or low-N diet given with large or small amounts of water, the nitrogen balance of the four animals remained close to zero. If the intake and excretion of nitrogen on the high-N diet is compared with that on the low-N diet it can be seen that about 75 % of the additional nitrogen intake was eliminated as additional urea, 12 % as urinary non-urea nitrogen, and 7 % as faecal nitrogen

The principal effect of water intake appeared to be on the urinary excretion of urea

when the animals received the low-N diet. Water restriction then reduced urea nitrogen excretion from about 1·3 to 0·3 g daily, reducing the contribution of urea to total urinary nitrogen from 42 to 18%, but these amounts were small relative to the total nitrogen excretion. This agrees with results obtained earlier in this laboratory (Goodall & Kay, 1968) showing that water intake has only small effects on the excretion of nitrogen by sheep.

In the last part of the experiments of Livingston *et al.* (1962) and of Payne (1964) in Kenya, cattle given poor-roughage rations and in negative nitrogen balance were allowed water only once every 4 d. The severe reduction in water intake led to a fall in appetite but to an improvement in nitrogen balance. These experiments are not yet fully reported and the relative importance of changes in urinary excretion and of reduced food intake and faecal excretion on the nitrogen economy is not clear. Even so it seems that, despite radical differences in experimental conditions, the changes in urinary urea excretion caused by water restriction in the cattle were similar to those we have found in sheep and deer, when allowance is made for an assumed fivefold to tenfold difference in body-weight.

The design of the experiment was such that the period of low water intake always followed that of high water intake. It is possible that residual effects of hydration, persisting after 8 d of water restriction, may have influenced the results obtained, but the rather mild degrees of hydration and dehydration imposed make this seem unlikely. Although the water intake of the deer was less restricted than that of the sheep, a more detailed study of urea excretion by the same two deer (Maloiy & Scott, 1969) indicated that no marked change in renal function would have occurred had water intake been more severely curtailed.

Excretion of urea accounted for only 16% of the nitrogen of the low-N diet when water intake was high and still less, 4·6%, when water intake was low. Schmidt-Nielsen *et al.* (1957) pointed out that, far from having any ill effect, urea retention by ruminants under adverse dietary conditions may be beneficial since the retained urea can pass into the rumen, by way of the saliva or through the rumen wall, and there encourage microbial activity and protein synthesis. Our reason for measuring the concentrations of urea in the plasma and ammonia in the rumen fluid was to see if our sheep and deer appeared to differ in their ability to cycle urea in this way. In fact, during each of the treatments the values for plasma urea were similar, indicating that the two pairs of animals achieved much the same balance between production, cycling and excretion of urea. Ruminal ammonia values were also alike when the low-N diet was given, suggesting that the amounts of urea cycled to the rumen did not differ greatly. The very low values for ruminal ammonia found shortly after a meal of the low-N diet had been eaten show that the starchy food was allowing almost complete microbial uptake of ammonia. The gradual return to higher ammonia values some 6 h later suggests that by then microbial activity had reached a low ebb although urea was still passing to the rumen from the plasma.

The amounts of hippuric acid and allantoin excreted in the urine were rather greater for the high-N than for the low-N diet. This probably was due to fermentation of the protein-rich diet in the rumen, for Blaxter & Martin (1962) have indicated that

these two metabolites are produced by ruminants partly as a result of bacterial degradation of protein. The method we used to estimate hippuric acid is not specific to this compound and in fact includes other urinary aromatic acids (Martin, 1969), so that our values for hippuric acid must be treated with caution. The constancy of creatinine excretion by each individual animal agrees with the classical work of Brody, Proctor & Ashworth (1934) who found a relationship between creatinine excretion and body-weight. The amount of creatinine excreted by the sheep per kg body-weight was higher than that excreted by African sheep (Topps & Elliott, 1967), while that excreted by the deer was a little higher still. This suggests there may be a difference in body muscle mass between the two types of sheep and between sheep and deer.

The clear difference between our sheep and deer in excretion of uric acid and allantoin was unexpected; it remains to be seen whether this is a true species difference. Ellis & Pfander (1965) have shown that microbial polynucleotide nitrogen represents an appreciable proportion of rumen microbial nitrogen, while Topps & Elliott (1965) found a close relationship between nucleic acid levels in the rumen and purine excretion in sheep. Possibly the amount of microbial polynucleotide synthesized in the rumen was much greater in our sheep than in our deer, though this seems unlikely since the sheep received less food. However, recent work by Smith, McAllan & Hill (1969) has shown that only some of the nitrogen in microbial nucleic acids is excreted in the urine; the remainder is probably found in the faeces. A more credible explanation, therefore, is that there were differences in intestinal digestion and absorption or subsequent metabolism of purine derivatives between the sheep and the deer. The ribonuclease content of the pancreas varies enormously among different species (Barnard, 1969).

Digestion

Our deer appeared to digest dry matter and cellulose less well than our sheep. However, any comparison of the digestion of food by the sheep and deer must take into account their differing food intakes, 800 and 1200 g/d respectively, and body-weights, 43 and 54 kg. The sheep received less food than the deer to allow for their smaller size and low metabolic rate (about 55–60 kcal/kg body-weight$^{0.75}$ daily). Red deer may have a high metabolic rate. Brockway & Maloiy (1968) reported a value of about 90 kcal/kg$^{0.75}$ for two fasted deer and these results have since been extended by single measurements on three other deer, giving a mean value for the five animals of 79 kcal/kg$^{0.75}$ (range 59–93) after 72–96 h fasting (J. M. Brockway, personal communication). On this basis, the rations given to our sheep and deer were calculated to allow maintenance of body-weight, and this was achieved in practice. However, if it is assumed that the sheep and deer had the same metabolic rate then the deer were rationed at 1·3 times maintenance. Blaxter (1962) summarizes experiments in which the decline of digestibility of energy is related to food intake. The decline ranged from 0·98 to 11·07 percentage units for a doubling of food intake relative to maintenance. Since dry matter will be affected similarly, for the highest value (11·07) an increase of 0·3 above maintenance would lead to a fall of about 3·3 units, whereas the difference observed between our sheep and deer was 5·5 units. So even taking two extreme positions one cannot fully account for the difference observed

on the basis of food intake. Still less can one account for the difference (11 units) in cellulose digestibility. The results do not indicate what caused these differences.

One result of the lower digestibility of dry matter by the deer was the excretion of relatively greater amounts of faecal dry matter. The concentration of nitrogen in dry matter was the same in the faeces of all the animals and so the deer lost a greater amount of faecal nitrogen than the sheep, with a consequent lower apparent absorption of nitrogen and a smaller loss of urinary urea. The difference between the sheep and deer in excretion of urea when given the low-N diet may therefore be due to this difference in dry-matter digestibility.

Restriction of water intake depressed the digestibility of dry matter and cellulose by about 2 and 5 units respectively. In contrast, Balch, Balch, Johnson & Turner (1953) found that water restriction slightly increased the digestibility of food by cattle. However, in their experiments long roughages were used and water restriction reduced appetite, whereas we provided a constant ration of pelleted roughage. The difference in the physical form of the diet and in intake may account for the divergence of results. As expected, the water content of the faeces of the sheep and deer diminished on the low-water regime, as it does in cattle. Our sheep lost less water in their faeces than the deer; if this is generally true, it suggests a species difference in adaptation to water restriction similar to that observed between *Bos indicus* and *Bos taurus* (Quarterman, Phillips & Lampkin, 1957; Payne, 1964). A small part of this difference may have been due to the smaller food intake of the sheep, though the results of Blaxter, Graham & Wainman (1956) suggest that the 10% difference in water content we observed would only be found in sheep if there were more than a twofold difference in food intake.

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