URINARY NITROGEN EXCRETION OF SHEEP FED NITROGEN-FREE DIETS

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Summary

The urinary nitrogen excretion of sheep offered a low nitrogen diet was lower than when the sheep were fed a nitrogen-free mixture post-ruminally. This result is discussed in terms of Folin's (1905) definition of endogenous nitrogen, and the nitrogen recycling and utilization which can occur in the rumen/reticulum of sheep. Reasons are given why post-ruminal feeding should give an endogenous nitrogen value near to the generally accepted inter-species mean.

I. INTRODUCTION

The classical concepts of endogenous nitrogen excretion in mammals proposed by Folin (1905) have long been the base for determining nitrogen requirements. Empirically determined, endogenous nitrogen excretion is that level of urinary nitrogen attained on a low nitrogen or nitrogen-free diet adequate in all other requirements. In most animals, the concept and experimental definition are compatible, and well conducted experiments give values within a narrow range in relation to metabolic body size (Brody, Procter and Ashworth 1934) or basal energy (Smuts 1935). Within the herbivores, however, experimental values frequently differ appreciably from the normal (Stielau, Owen and Van Ryssen 1967). Arguments may be advanced that the experimental values of ruminants should differ significantly from those for "conventional" animals.

It would be expected that the relative contribution of nitrogenous compounds to the endogenous urinary nitrogen would differ between ruminants and other herbivores. The low urinary urea excretion of ruminants and pseudo-ruminants offered low nitrogen diets (Schmidt-Nielsen et *al.* 1957; Schmidt-Nielsen and Osaki 1958) can be partly attributed to recycling of urea to the rumen/reticulum (for review, Le Bars 1967) ; up to 92 per cent of the urea entering the body pool could be degraded in the alimentary tract of sheep offered low nitrogen diets (Cocimano and Leng 1967). On the other hand, the considerable amounts of nucleic acid formed by microorganisms in the rumen/reticulum (Ellis and Pfander 1965) and the high fibre content of ruminants diets would tend to elevate the purine-nitrogen and hippuric acid-nitrogen excretions.

The following experiments were designed to measure urinarv nitrogen excretion of sheep both when recycling of nitrogen was maintained by offering a low

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nitrogen diet *per* os and, when the utilization of recycled nitrogen was restricted, by infusing a nitrogen-free mixture *per duodenum* or *per abomasum.*

II. MATERIALS AND METHODS

(a) Sheep

Mature Merino wethers were used. They were treated a few days before an experiment with an anthelmintic and vitamins A, D and E. The sheep used were:— experiment 1, one sheep (A) with a duodenal fistula (Phillipson 1952) and one (B) with an abomasal fistula 15 to 20 cm from the pylorus; experiment 2, four sheep (C, D, E and F) each with an abomasal fistula about 5 cm from the pylorus. Sheep were confined in metabolism crates to allow daily collections of urine and faeces separately. Faeces were collected in canvas bags and urine in plastic bottles chilled to about 3°C and containing hydrochloric acid to minimize decomposition.

(b) Diets

Before an experiment, sheep were fed for some months a ration consisting of chopped oaten hay 450 g, chopped lucerne hay 100 g, commercial cubes (crude protein 20 per cent) 225 g and a mineral mixture (modified, Moir and Harris 1962) 8 g.

During experiment 1, sheen were offered *ad libitum* a mixture containing oat hulls 78 per cent, wheat starch 15 per cent, sucrose 4.5 per cent and a mineral mixture (as above) 2.5 per cent (period 1). The nitrogen concentration was 0.23 per cent (dry matter basis). After a number of days of oral feeding, infusion *via abomasum* or *via duodenum* commenced, one fifth of the final quantity being infused while the diet was still available orally. The quantity was increased in steps of one fifth until the whole ration was being infused and then, after some days, oral feeding was discontinued (period 2). The full administered ration, which was prepared daily, consisted of glucose 50 g, sodium propionate 50 g, starch 50 g, olive oil 50 g, a mineral/vitamin mixture containing sodium chloride 12 g, calcium tetrahydrogen diphosphate 16 g, magnesium chloride 4 g, potassium chloride 3 g, zinc sulphate to-provide 20 mg of zinc, two commercial- vitamin/ mineral capsules ("Myadec"; Parke-Davis), calcium pantothenate 20 mg, pyridoxine 2 mg and choline chloride 1 g. The oil was emulsified in warm water with 1 ml of "Tween 80" and added to the previously-cooked starch solution in which was dissolved the glucose, propionate and minerals. The final volume of 3 1. was infused by gravity drip over 24 h. During the latter part of period 2, the amount of glucose was progressively reduced (see Table 2).

During experiment 2, the mixture was infused through a peristaltic pump, and the ingredients were continuously stirred by a magnetic bar. The basic ingredients of the ration were sodium propionate 80 g, wheaten starch 50 g, olive oil 120 g and "Tween 80" 2 ml. Sheep C, D, E and F received respectively 0, 30, 60 and 90 g of glucose in the ration. The mineral and vitamin supplements were as given in experiment 1.

(c) Chemical analysis

Nitrogen concentration was determined by the Kjeldahl method, urinary ammonia and urea by the micro-diffusion method of Conway (1950), and calorific values by combustion in a Baird and Tatlock bomb calorimeter.

TABLE 1

Dry Matter Intake** (DMI), Metabolizable **Energy Intake** (MEI), **Body Weight, Urinary Nitrogen and Urinary Urea Nitrogen plus Ammonia Nitrogen** of **Sheep offered a low nitrogen diet per os or infused with a Nitrogen-free diet per abomasum or per duodenum

Feeding Technique		*Per os*						*Per abomasum or duodenum*				
	Sheep	Period of measurement (days)	DMI (g/day)	MEI (kcal/day)	Body weight (kg)	Urinary nitrogen (g/day)	Urinary urea plus ammonia (g N/day)	Period of measurement (days)	MEI (kcal/day)	Body weight (kg)	Urinary nitrogen (g/day)	Urinary urea plus ammonia (g N/day)
Experiment 1	A	7	895	1225	38.0	1.01	0.35	14	880	28.0	1.60	0.92
	B	5	920	1210	35.0	0.90	0.30	18	860	28.5	1.79	0.86
Experiment 2	C							8	1210	43.5	2.51	1.53
	D							8	1400	42.0	2.68	1.72
	E							8	1265	37.5	2.50	1.81
	F							8	1860	39.5	2.60	1.62

(d) Calculation of *energy intake*

Metabolizable energy intakes during period 1 of experiment 1 were estimated by using the equation of Moir (1961) to calculate digestible energy (kcal/g) from dry matter digestibility per cent, and the conversion of Blaxter (1964) to calculate metabolizable from digestible energy. Metabolizable energy infused during experiment 2 was estimated from measured calorific values and assuming a value of less than 5 kcal/g urinary nitrogen excreted (Martin and Blaxter 1965).

III. RESULTS

The urinary nitrogen excretions, urinary urea nitrogen plus ammonia nitrogen excretions, dry matter intakes, metabolizable energy intakes and bodyweights of the sheep are given in Table 1. For both experiments, bodyweight loss during the periods used to calculate nitrogen excretions was not greater than 1 kg.

For period 1 of experiment 1, urinary collections during the initial 5 to 8 days were not included in the calculations. For period 2 of experiment 1 and for experiment 2, urinary nitrogens have been calculated from collections taken after infusing post-ruminally for at least 40 and 20 days respectively.

The urinary nitrogen excretions during period 2 of experiment 1, when the quantity of glucose was reduced, are given in Table 2. The effect of different amounts of glucose on urinary nitrogen in exoeriment 2 is included in Table 1.

IV. DISCUSSION

The results demonstrate the feasibility of studying some aspects of nitrogen metabolism in sheep by infusing post-ruminally a nitrogen-free diet. In this way, some aspects of ruminant metabolism can be studied, uncomplicated by physiological mechanisms specific to the forestomachs. In this study, the technique was used to estimate endogenous urinary nitrogen excretion of sheep, uncomplicated by nitrogen recycling and utilization in the rumen/reticulum.

Urinary nitrogen excretion of sheep infused with a nitrogen-free diet post-ruminally was higher than when sheep were fed orally, the difference being accounted for mainly in the greater excretion of urea nitrogen plus ammonia nitrogen. The magnitude of the difference will depend on the amount of metabolizable energy received, for two reasons.

TABLE 2

Urinary Nitrogen *(g/day)* of ***sheep infused post-ruminally with a Basal Mixture† and decreasing amounts of Glucose***

Glucose (g/day)	40	35	30	25
Days	4	4	6	4
Sheep				
A	1.48	1.52	1.75	
B	1.84	1.64	1.86	1.60

* Urine collected on the first day of these periods has not been used in these calculations.

† Ration of starch 50 g, sodium propionate 50 g, olive oil 50 g plus minerals and vitamins as given in text.

Firstly, the quantity of recycling urea which can be used for bacterial protein synthesis, and thus the amount of residual urea plus ammonia which remains to be excreted, will depend largely on the ratio of protein to metabolizable energy in the diet. In the present study, the ratio, for sheep fed orally, of about 4 g of protein/100 g of digestible organic matter was conducive to a high utilization of recycling urea, and thus to a net gain of protein entering the abomasum (Hogan and Weston 1967). In sheep fed post-ruminally, the utilization of recycled urea would have been minimal.

Secondly, the amount of urea synthesised in sheep receiving low-nitrogen diets will depend on the amount of tissue protein catabolised to meet energy requirements. In period 1 of experiment 1, the metabolizable energy consumed (82 kcal/$Wt_{kg}^{\frac{3}{4}}$) was close to accepted energy requirements for maintenance of bodyweight of penned sheep (Phillipson 1958) , and higher than that received in period 2 (71kcal/$Wt_{kg}^{\frac{3}{4}}$). In experiment 2, some sheep received more than the maintenance requirement for metabolizable energy (intake ranged from 71 to 118 kcal/$Wt_{kg}^{\frac{3}{4}}$) because of difference in glucose infusion (0 to 90 g/day), yet there were no appreciable or consistent changes in urinary nitrogen excretion. It seems that all these sheep, fed post-ruminally, provided satisfactory conditions for the estimation of endogenous urinary nitrogen excretion.

The mean urinary nitrogen excretion of the six sheep fed post-ruminally was 62 mg nitrogen/Wt_{kg}. This value, determined by adapting the concepts and methods of measuring endogenous nitrogen excretion in non-ruminant animals, is equivalent to 154 mg $Wt_{kg}^{\frac{3}{4}}$ (170 mg $Wt_{kg}^{0.72}$), and conforms closely to the interspecies mean of 146 mg $Wt_{kg}^{0.72}$ (Brody, Procter and Ashworth 1934).

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