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Partition of nitrogenous substances in the urine of sheep on different dietary protein intakes

M Szanyiová *, L Leng, Š Faix

Institute of Animal Physiology, Slovak Academy of Sciences, Palackého, 12, 040 01 Košice, Slovakia

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Summary — The distribution of nitrogenous substances in urine was studied in sheep fed high (28.71 g N daily) and low (9.32 g N daily) protein diets for 6–8 weeks. The concentrations of total nitrogen and urea, allantoin, free amino acids, uric acid, hippuric acid, creatinine, creatine and ammonia were measured in urine. The nitrogen content of these measured substances was calculated. The results showed that the concentrations of total urinary nitrogen and nitrogen of individual nitrogenous substances were lower in sheep on a low protein diet, except for uric acid nitrogen which was unchanged. Urea nitrogen was responsible for up to 74% of the total urinary nitrogen of both groups of animals without any significant difference. The individual nitrogen portions of nitrogenous substances other than urea in total urinary-N did not exceed 7% during the intake of both diets. The intake of a low protein diet resulted in a significant increase in the proportion of nitrogen from uric acid, hippuric acid, creatinine and free amino acids, while the fraction of ammonia nitrogen in the total urinary N was significantly reduced. It is concluded that different protein or nitrogen intake in the diet do not have any significant effect on the urea nitrogen fraction in the total urinary nitrogen of sheep.

sheep / diet / nitrogen / urine

Résumé — Répartition des substances azotées dans l'urine de moutons soumis à différents niveaux d'ingestion de protéines. *La distribution des substances azotées a été étudiée dans l'urine des moutons recevant des régimes riches (28,71 g N/j) ou pauvres (9,32 g N/j) en protéines, pendant 6 a 8 sem. L'azote total, ainsi que l'azote de l'urée, de l'allantoïne, des acides aminés libres, de l'acide urique, de l'acide hippurique, de la créatinine, de la créatine et de l'ammoniac ont été dosés dans l'urine. Les concentrations de l'azote de chaque substance ont été mesurées. Les résultats ont montré que les concentrations de l'azote total et d'azote de chaque substance étaient inférieures chez les moutons ayant reçu un régime pauvre en protéines, excepté celle de l'azote de l'acide urique qui restait inchangée. La proportion d'azote uréique par rapport à l'azote urinaire total s'élevait jusqu'à 74%, sans différence significative entre les 2 groupes d'animaux. La fraction azotée correspondant à chacune des substances autres que l'urée, présente dans l'azote urinaire total, n'a pas dépassé 7% après l'ingestion des 2 régimes. L'ingestion de régimes pauvres en protéines a induit une élévation significative de la proportion de l'azote de l'acide urique, l'acide hippurique, de la créatinine et des acides aminés libres, tandis que la fraction d'azote ammoniacal dans l'azote urinaire total était significativement réduite. En conclusion, les variations dans les quantités de protéines ou d'azote ingérées n'ont aucun effet significatif sur la proportion d'azote uréique dans l'azote urinaire total chez le mouton.*

mouton / régime / azote / urine

* Correspondence and reprints

INTRODUCTION

It is well established that the kidneys of ruminants can change the excretion of urea to a large extent. Urea nitrogen in ruminants under normal nutritional conditions accounts for 60–80% of total urinary nitrogen (Livingstone *et al*, 1962; Topps and Elliot, 1967). It has been reported that the fraction of urea nitrogen in urinary nitrogen drops below 30% during low dietary protein intakes and allantoin excretion increases in such a way that allantoin may even become the main nitrogenous component of urine, in the place of urea (Elliot and Topps, 1963).

Literature opinions on the mechanism(s) by which the kidneys of ruminants spare the urea during low protein intake are varied. Recent results have shown that the substantial decrease in renal urea excretion in sheep on a low protein diet is mainly caused by a reduction in glomerular filtration, and partly by an increase in tubular transport capacity for urea in the tubules and epithelium of renal pelvis (Leng *et al*, 1985; Cirio and Boivin, 1990).

The aim of this experiment was to determine the partition of nitrogenous substances in the urine of sheep and to compare their proportions in the total urinary nitrogen in animals on low and high protein diets.

MATERIALS AND METHODS

Animals and diets

The experiments were carried out on adult female sheep *(Ovis aries)* of the Merino breed with 35–40 kg body weight. The animals were divided into 2 groups. The first group was fed a high protein diet (HP, $n = 12$) and the second a low protein diet (LP, $n = 9$) for 6–8 weeks before collection of urine samples. The animals were given food twice daily. Minerals and water were supplied *ad libitum*. The total daily ration of each sheep on HP diet consisted of 500 g hay, 500 g barley, 250 g wheat bran and 10 g urea. The daily intake of animals on HP diet was 28.71 g nitrogen and 15.2 MJ digestible energy. The animals on LP diet were given daily 1 000 g barley straw, 150 g wheat bran and 300 g maize starch. The daily intake of sheep on LP diet was 9.32 g nitrogen and 14.18 MJ digestible energy.

To avoid the effects of food and water intake on variability in the elimination of nitrogen substances in the urine, the animals were deprived of both food and water the morning before urine sampling and they were housed in metabolic cages. Two samples of urine were collected in 60 min collection periods through a catheter from urinary bladder. Samples of urine from animals with urine flow rate higher than 1 ml/min or lower than 0.3 ml/min were discarded. Urine sampling was made twice for each sheep and there was 1 week left for animals to rest between collections. The measured values from each sheep were averaged.

Analytical procedures

The samples of urine were analyzed for the following: total nitrogen by the Kjeldahl method; urea fluorometrically (Leng *et al*, 1986); allantoin photometrically (Hornawsky and Müller, 1980); hippuric acid fluorometrically (Ellman *et al*, 1961); ammonia photometrically (Cook, 1976); creatinine and creatine photometrically by the Biola test (Lachema Brno); uric acid enzymatically by the Biola test (Lachema Brno); and free amino acids fluorometrically (Roth, 1971). The nitrogen concentrations of the individual substances in urine was calculated on the basis of molecular mass. The difference between the value of total nitrogen and the sum of nitrogen from all determined substances represents unidentified nitrogen. The results were evaluated by an Anova test and are given as the mean ± SEM.

RESULTS

The concentrations of total nitrogen (total-N) and nitrogen of each substance investigated in the urine (urea-N, allantoin-N, hippuric acid-N, creatinine-N, creatine-N, ammonia-N, free amino acid-N) were significantly lower in sheep on the LP diet with the exception of uric acid-N whose concentration did not change (table I).

Table I. Concentrations (g/l) of nitrogen in the urine of sheep on high protein (HP) or low protein (LP) diets.

	Nitrogen concentration in urine (g/l)		*Statistical significance*
	HP diet (n = *12*)	*LP diet* (n = *9*)	
Total	9.42 ± 1.63	1.55 ± 0.14	$P < 0.01$
Urea	7.13 ± 1.30	1.08 ± 0.11	$P < 0.01$
Allantoin	0.41 ± 0.07	0.10 ± 0.02	$P < 0.01$
Uric acid	0.006 ± 0.001	0.005 ± 0.001	NS
Hippuric acid	0.05 ± 0.01	0.03 ± 0.004	$P < 0.05$
Creatinine	0.32 ± 0.05	0.10 ± 0.02	$P < 0.01$
Creatine	0.11 ± 0.03	0.02 ± 0.005	$P < 0.01$
Ammonia	0.40 ± 0.09	0.007 ± 0.001	$P < 0.05$
Free amino acids	0.32 ± 0.05	0.086 ± 0.01	$P < 0.01$

Values are mean ± SEM.

The proportion of urea-N in total urinary nitrogen was the same in sheep on the 2 diets. No changes were observed in the percentage of allantoin-N, creatine-N and unidentified nitrogen (table II).

In sheep fed the LP diet, the percentage of uric acid-N in total urinary nitrogen was significantly larger. The fractions of hippuric acid-N, creatinine-N and free amino acid-N were also larger in animals fed the LP diet. However, the fraction of ammonia-N in total urinary nitrogen was significantly reduced in sheep fed the LP diet (table II).

DISCUSSION

The experiment was designed to study the partition of nitrogenous substances in the urine of sheep fed the LP diet and compare it with sheep fed the HP diet as a control. Urea nitrogen was found in the highest fraction of all the nitrogenous substances in the urine of sheep and there was no difference between the LP and HP diet. This means that urea nitrogen was also the main component of urinary nitrogen during significantly reduced urea excretion due to an LP diet.

Different results were found in similar work with African sheep (Topps and Elliot, 1967) and African cattle (Elliot and Topps, 1963) given a low protein diet. The renal excretion of urea nitrogen was also decreased due to a low protein diet in the experiments of these authors but the excretion of allantoin nitrogen increased and represented an even higher fraction of the total urinary nitrogen than urea nitrogen. Our results showed that the concentration of allantoin nitrogen in the urine of sheep fed LP diet was lower but there was no differences in the fraction of allantoin nitrogen in total urinary nitrogen between animals fed LP or HP diets. Thus the proportion of allantoin nitrogen in total urinary nitrogen was of a relatively low value in sheep on both diets. The explanation for this discrepancy between our results and those of Topps and Elliot (1967) could be more extensive fermentation in forestomachs of African sheep or cows because of a different diet and/or microbial population and, subsequently, a larger production of purine bases. African ruminants are usually better accustomed to intake of grass of low nutritional value and thus the breed differences could also play a role. Similar low values of allantoin excretion like our results in sheep were also

Table II. Proportion of nitrogenous substances (%) in the total urinary nitrogen in sheep fed high protein (HP) or low protein (LP) diets.

	Nitrogen (% of total)		*Statistical significance*
	HP diet (n = *12*)	*LP diet* (n = *9*)	
Urea	74.13 ± 1.56	69.65 ± 3.22	NS
Allantoin	4.57 ± 0.44	6.34 ± 0.86	NS
Uric acid	0.075 ± 0.01	0.35 ± 0.07	$P < 0.01$
Hippuric acid	0.61 ± 0.06	1.98 ± 0.22	$P < 0.01$
Creatinine	3.44 ± 0.27	6.53 ± 1.06	$P < 0.01$
Creatine	1.51 ± 0.30	1.33 ± 0.09	NS
Ammonia	3.75 ± 0.66	0.53 ± 0.16	$P < 0.01$
Free amino acids	3.74 ± 0.43	5.68 ± 0.61	$P < 0.05$
Unidentified nitrogen	8.15 ± 1.65	7.59 ± 1.10	NS

Values are mean ± SEM.

found by Ettala and Kreula (1976) in cows given urea as the only or a partial source of nitrogen. Antoniewicz and Pisulewski (1983) found a decreased allantoin excretion in urine of sheep with a reduction in hay intake. These authors concluded the constancy of allantoin-N yield per unit of energy ingested and stated that the low endogenous allantoin excretion confirms the possibility of using allantoin as an indicator of microbial protein synthesis in the rumen. Urinary excretion of allantoin by ruminants (except for fasting animals) is a reflection of the catabolism of purine bases of the microbial population in the digestive tract (Rys *et al*, 1975).

The considerable reduction in ammonia-N concentration and its fraction in total urinary N of our sheep on the LP diet corresponds with the findings of Elliot and Topps (1963) and Juhasz (1971), who reported that an increase of protein in a diet increased ammonia content in urine. It is obvious that the reduced intake of food protein in sheep on the LP diet resulted in a lower production of ammonia in the rumen and this was consequently reflected in the decreased urinary ammonia excretion.

The concentration and portions of free amino acid nitrogen in urine from our sheep was found to be similar to those of urea-fed dairy cows (Virtanen, 1971). The proportionally higher excretion of free amino acid-N in sheep on low protein diets can be accounted for by an increased protein catabolism (Nolan and Leng, 1970).

Low values were found in excretion of uric and hippuric acid nitrogen. Our findings on uric and hippuric acids correspond with those of Topps and Elliot (1967) in African sheep. The increased proportions of uric and hippuric acid nitrogen in total urinary nitrogen under a protein-reduced diet are due to a concomitant large reduction in total urinary N excretion.

The proportion of creatine-N in total urinary nitrogen was low and did not depend on the dietary nitrogen intake. The concentration of creatinine-N in the urine of sheep on the LP diet significantly decreased but the proportion of creatinine-N in total urinary nitrogen was significantly increased due to reduced total N excretion. There are many different opinions on creatinine excretion. Maloiy *et al* (1970) observed that protein intake in sheep had no effect on creatinine excretion. According to Schiemann *et al* (1965), the addition of protein to the diet decreased the creatinine excretion in sheep.

Van Niekerk *et al* (1963) found that an increase in protein in the diet raised creatinine excretion in sheep. Topps and Elliot (1967) reported that creatinine excretion in African sheep with different protein contents in their diet was directly related to the body weight of the sheep and showed little variation with diet. A constant urinary excretion of creatinine, which is relatively independent on the dietary nitrogen intake in sheep, has also been reported by Antoniewicz and Pisulewski (1983) and Faix *et al* (1988).

Unidentified nitrogenous substances can be considered as some amino acids and peptides that have not been analyzed.

We conclude from our results that urea nitrogen forms the major part of the total nitrogen of the urine of sheep, with the highest percentage up to 74%, independently of protein or nitrogen intake. The other main nitrogenous substances participate only up to 7% of total urinary N. The results of the present study demonstrate that the protein or nitrogen content in a diet did not influence the urea nitrogen portion in the urine of sheep.

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