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Short note

Microbial protein production determined by urinary allantoin and renal urea sparing in normal and low protein fed Corriedale sheep

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Abstract – The aim of the present study was to compare the amount of microbial N entering the duodenum and the efficiency of N utilisation for microbial protein synthesis in normal (NP, 17.4 g N/d) and low protein (LP, 7.5 g N/d) fed Corriedale sheep. Renal functional tests for urea handling studies, and determination of urinary allantoin as an indirect method to estimate the microbial protein production were used. Although the N intake was 57% lower in LP sheep, the microbial N production was not very different between both diets (NP: 3.99 ± 0.01 vs. LP: 3.79 ± 0.02 g/d, $P < 0.05$). The efficiency of the microbial protein synthesis in the rumen, expressed as grams of microbial N per kg of digestible organic matter apparently digested in the rumen, was not statistically different for both diets. The urinary elimination of urea was reduced by 84% in LP sheep, essentially due to an important decrease in both renal plasma flow (–63%) and glomerular filtration rate (–71%). These haemodynamic changes would also reduce the filtered load and the urinary elimination of allantoin, thereby leading to an underestimation of the amount of microbial protein entering in the duodenum. Since the renal urea spared by the kidneys remains in the blood, it limits the drop of the available urea for ruminal recycling consecutive to a low nitrogen diet.

microbial protein / allantoin / urea / sheep

Résumé – Production de protéine microbienne mesurée par l'allantoïne urinaire et épargne rénale d'urée chez des moutons Corriedale en sous-alimentation protéique. L'objectif de cette étude a été de comparer la quantité d'azote microbien entrant dans le duodénum et l'efficacité de l'utilisation de l'azote pour la synthèse de protéines microbiennes, chez des moutons Corriedale soumis

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à un régime normoprotéique (NP, 17,4 g N/j) ou hypoprotéique (LP, 7,5 g N/j). Des tests de fonctionnement rénal pour étudier les transferts d'urée, et le dosage de l'allantoïne pour estimer la production de protéine microbienne ont été utilisés. Malgré une ingestion d'azote réduite de 57% chez les moutons LP, la production d'azote microbien n'a pas été très différente entre les deux régimes (NP: 3,99 ± 0,01 vs. LP: 3,79 ± 0,02 g/j, $P < 0,05$). L'efficacité de la synthèse protéique microbienne dans le rumen, exprimée en grammes d'azote microbien par kg de matière organique apparemment digérée dans le rumen, n'a pas été statistiquement différente dans les deux régimes. L'élimination urinaire d'urée a été réduite de 84% chez les moutons LP, grâce essentiellement à une importante diminution du débit plasmatique rénal (–63%) et de la filtration glomérulaire (–71%). Ces modifications hémodynamiques réduisant également la charge filtrée et la quantité d'allantoïne éliminée, peuvent être responsables d'une sous estimation de la quantité de protéine microbienne entrant dans le duodénum. L'urée épargnée au niveau rénal restant dans le sang, limite la baisse de l'urée disponible pour le recyclage dans le rumen, observée lors des régimes pauvres en azote.

protéine microbienne / allantoïne / urée / mouton

1. INTRODUCTION

It is well known that in ruminants, endogenous urea is partly recycled in the forestomach. This process is nutritionally advantageous for ruminants because ruminal bacteria are able to use urea nitrogen to synthesize proteins that will be absorbed in the small intestine. When protein intake is restricted, renal elimination of urea is reduced by (a) a decreased filtered load of urea, consecutive to a reduction in renal plasma flow and glomerular filtration rate, and (b) an enhanced capacity for urea reabsorption from tubules and the renal pelvis [8]. This adaptive process prevents an excessive reduction of the blood urea pool.

A widely used method for the estimation of microbial protein production requires ruminal and duodenal cannulas [16] and microbial and digesta flow markers [11]. However, the microbial protein entering the duodenum can be estimated by quantification of urinary allantoin [21]. The nucleic acids synthesized by rumen microorganisms are enzymatically degraded to purine and pyrimidine bases which are absorbed; their final products are excreted in the urine with allantoin being in the greatest proportion [10]. Several authors have revealed a close relationship between microbial nucleic acids reaching the small intestine and the urinary excretion of purine derivatives, specially allantoin [1, 18].

Corriedale sheep, the main breed of the Uruguayan herd, graze different kinds of pastures with variable levels of nitrogen content throughout the year. To develop feeding strategies for sheep breeding on the basis of local foodstuffs, the objectives of this work were to investigate (a) the microbial protein production in Uruguayan Corriedale sheep under restricted nitrogen intake and (b) the importance of the renal mechanisms to reduce urea losses in this breed.

2. MATERIALS AND METHODS

Six non-pregnant non-lactating ewes weighing 38 ± 2 kg were housed in metabolic cages and successively fed with normal (NP) and low (LP) protein rations (Tab. I), offered in four equal portions at 6 h intervals. Following a 30 days adaptation period to each diet, a retention catheter was placed into the urinary bladder and connected to a peristaltic pump (Masterflex, Cole Parmer Instrument Company, Niles, Illinois, USA) and an experimental period of eight days was started. During the first five days, a 24 h-urine sampling was performed, the urine was preserved (250 mL

Table I. Daily intakes (g/d) on normal (NP) and low protein (LP) diets (DOMI: digestible organic matter intake).

	NP	LP
Diet as-fed	850.0	800.0
Dry matter	754.6	714.6
Organic matter	694.3	643.9
Nitrogen	17.4	7.5
DOMI	454.70 ± 12.23	372.58 ± 25.34

10% sulphuric acid per day) and a sample of 30 mL was frozen until analysed for allantoin.

To study the renal function, four test periods (10 min each, two in the morning and two in the afternoon) were carried out daily during the last three days of the experimental period for each animal and each diet. Both jugular veins were catheterized. One vein was used for perfusion and the other one for blood sampling. In order to measure renal plasma flow and the glomerular filtration rate, a primed constant-infusion (0.5 mL/min) of P-aminohippuric acid (PAH, 85 mg and 8 mg/mL/min) and inulin (1.8 g and 70 mg/mL/min) (Sigma) in 0.9% saline, was made into a jugular vein, starting 20 min before the test periods. Bladder urine was collected for each period and a blood sample (5 mL on heparin iodoacetate) was obtained at the midpoint of each period. Blood samples were centrifuged at 1 350 × *g* for 5 min.

Plasma and urine were analysed for urea (urease kit, Wiener, Austria), inulin and PAH [15]. Renal plasma flow (as PAH clearance), glomerular filtration rate (as inulin clearance) and urea clearance were calculated as the product of urine flow and urinary concentration divided by plasma concentration. Fractional excretion of urea (as urea /inulin clearances) and filtered load of urea (as inulin clearance by plasma urea concentration) were determined. Allantoin was analysed in urine by the method of Young and Conway, modified by Fujihara [12]. Microbial protein synthesis was estimated according to the equation $y = e^{(0.830 + 2.089x)}$ proposed by Puchala and Kulasek [21], where y = microbial N entering the duodenum and x = urinary excretion of allantoin N. The efficiency of the microbial protein synthesis in the rumen was expressed as grams of microbial N per kilogram of digestible organic matter apparently digested in the rumen (DOMR). DOMR was assumed to be equal to 65% of digestible organic matter intake (DOMI) [2]. Statistical significance was evaluated by a Student paired *t* test.

3. RESULTS

After one month on the experimental diets, the kidneys of LP sheep showed an increased ability to spare urea (Tab. II). Plasma urea level, renal plasma flow and glomerular filtration rate were reduced by the LP diet (–45%, –63% and –71% respectively) leading to a reduction in the filtered load of urea (–88%) and in urea elimination (–84%). Changes in fractional excretion of urea were not significant.

The daily urinary allantoin elimination was higher for NP than for the LP diet (747.4 ± 5.4 vs. 676.4 ± 7.6 mg/d respectively, $P < 0.05$). The amount of microbial nitrogen entering the duodenum, estimated from urinary allantoin, was 3.99 ± 0.01 (NP) and 3.79 ± 0.02 (LP) microbial N g/day ($P < 0.05$). DOMR was 0.30 ± 0.02 kg/day for NP and 0.24 ± 0.01 for the LP diet. Although

Table II. Plasma urea level and renal parameters on normal (NP) and low protein (LP) diets.

Parameters	NP diet	LP diet
Plasma urea level (mg/mL)	0.20 ± 0.03	0.09 ± 0.03*
Urine flow (mL/min)	0.83 ± 0.50	0.80 ± 0.30
Glomerular filtration rate (mL/min)	69.5 ± 10.2	20.0 ± 10.5*
Renal plasma flow (mL/min)	803 ± 126	300 ± 70*
Urea clearance (mL/min)	46.9 ± 20.6	17.4 ± 10.2
Filtered load of urea (mg/min)	14.5 ± 3.1	1.7 ± 0.9*
Urea eliminated (mg/min)	9.3 ± 1.9	1.5 ± 0.9*
Fractional excretion of urea	0.61 ± 0.07	0.72 ± 0.18

Values = means ±SD, $n = 6$, * $P < 0.05$.

N intake was 57% lower in LP sheep, their efficiency of microbial protein synthesis was not different from that of NP sheep: 15.64 ± 0.42 (LP diet) vs. 13.52 ± 0.71 (NP diet) g microbial N/kg DOMR (NS).

4. DISCUSSION

As in previous results [8, 22], the kidneys of sheep adapted to restricted protein intake by reducing the amounts of urea eliminated in the urine. This decrease was larger than what we expected from the simple fall of the urea blood produced by the reduction of the N intake. A fall in the filtered load of urea, consecutive to important decreases in renal plasma flow, glomerular filtration rate and plasma urea level, was the main change observed. Changes in the tubular handling of urea (fractional excretion) induced by the LP diet were not found, in coincidence with our previous results concerning the Corriedale breed [23].

Allantoin elimination was higher for the NP diet. This result agrees with that of Chen et al. [7] who found that in sheep receiving constant energy but variable proteins, daily allantoin excretion is positively correlated with the cumulative N-retention, although the magnitude of variation in allantoin excretion is small.

The calculated values for daily microbial N production are lower than those most commonly found in the literature for similar feeding conditions. However, results of the same order as ours and obtained by the allantoin method have been reported: 5.7 g microbial N/day for an intake of 444 g DOMI/day and 45 kg BW [6] and 10.9 g microbial N/kg DOMR/day, corresponding to 4.2 g microbial N/day and an intake of 590 g DOMI/day, in sheep weighing 42 kg [20]. There is no clear explanation for these low values, but factors related to the quality of the ingredients of the diet could be evoked.

Our data on the efficiency of microbial protein synthesis in the rumen indicate that LP sheep were as productive as NP sheep. However, Puchala and Kulasek [21] reported the lowest rates of synthesis of rumen microbial protein in sheep fed low protein diets, independently of energy content.

Obara and Shimbayashi [19] found that the rumen is the main organ of the digestive tract where re-cycled urea is transferred in goats fed low protein diets. Both urea and allantoin are recycled in the rumen via saliva [4, 9] and, at least for urea, through the rumen wall [14], enhancing the availability of N for microbial protein synthesis. Allantoin and other purine derivatives are degraded by rumen bacteria [13, 17] and N could be used for microbial synthesis [3].

Net reabsorption of allantoin from tubular urine occurs in sheep, but this capacity is

limited and appears to be saturated by a tubular load equivalent to endogenous allantoin production [5]. This means that, at a constant glomerular filtration rate, any addition of allantoin to the blood, once filtered at the glomeruli, will be eliminated in the urine. However, sheep fed low protein diets have reduced renal plasma flow and glomerular filtration rates so the filtered load and urine elimination of allantoin should also be reduced. Consequently, the amount of microbial protein entering the duodenum in LP sheep found in the present work may have been underestimated.

In conclusion, a 57% reduction in N intake did not affect the efficiency of microbial protein synthesis in the rumen of Corriedale ewes. Moreover, considering the presumable decrease in urinary allantoin elimination due to the renal heamodynamic response to low protein diets, the calculated values of this efficiency should be higher. Renal adaptation to low protein diets reduced by 84% the urinary losses of urea, thereby contributing to the blood urea pool and increasing its offer for ruminal recycling. The urinary allantoin method has been proved to be a useful tool as an indicator of microbial protein production, but when used in ruminants adapted to a low nitrogen intake, an underestimation of this production may occur.

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