

EFFECT OF LEVEL OF FEED INTAKE ON THE EXCRETION OF PURINE DERIVATIVES AND PURINE DERIVATIVES TO CREATININE RATIO IN THE URINE OF SHEEP

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Summary

Urinary purine derivatives and creatinine excretion was measured in a total of 4 white Alpine sheep. They were given diets 718 to 1060 g/kg dry matter (DM) of roughage. The crude protein content of this diets was on average 93.87 ± 5.57 g in kg DM. Purine derivatives-N excretion increased linearly with incremental DM intake and was significantly correlated ($n=16$) with amounts of digestible organic matter (DOM) intake: allantoin-N (mg) = $1.205(\pm 0.070) \times \text{DOM (g)} - 136.709(\pm 37.399)$, $r=0.9770$, RSD = 22.97; uric acid-N (mg) = $0.131(\pm 0.041) \times \text{DOM (g)} + 11.380(\pm 21.881)$, $r = 0.6306$, RSD = 13.44; Hypoxanthine-N (mg) = $0.049(\pm 0.014) \times \text{DOM (g)} - 28.640(\pm 7.708)$, $r = 0.6544$, RSD = 4.73; total purine derivatives-N (mg) = $1.385(\pm 0.083) \times \text{DOM (g)} - 90.261(\pm 44.552)$, $r = 0.9706$, RSD = 27.47. Microbial protein synthesis per kg DOM was estimated of 113 g. The urinary creatinine-N excretion was on average 9.10 mg/kg live weight (LW) with a standard error of 0.12 mg creatinine-N per kg LW. The excretion of creatinine excretion was not related to feed intake. Daily creatinine excretion (mg/d) was calculated from individual LW measurements and the average creatinine excretion (mg/kg LW). It was possible to predict the daily urinary purine derivatives excretion ($r = 0.9720$ for allantoin, $r = 0.9886$ for total purine derivatives) from the ratio of purine derivatives (mg/100 ml) and creatinine (mg/100 ml) in the urine and the daily creatinine excretion.

(Key Words: Purine Derivatives, Creatinine, Microbial Protein, Digestible Organic Matter)

Introduction

Allantoin, the end product of nucleic acid purine bases metabolism, and other purine derivatives in urine appears in sheep to derived mainly from nucleic acids of rumen micro-organisms (Topps and Elliot, 1965; Tiemeyer et al., 1983). This implies that the excretion of purine derivatives in urine could be a possible indicator of microbial protein production in ruminant animals. Exogenous purine components absorbed from the intestine are metabolized within the body and excreted in the mainly in the form of allantoin and other purine derivatives (Condon and Hatfield, 1970; Tiemeyer et al., 1983).

Energy is stored in the muscles in the form

of phosphocreatinine. When there is a need for energy in the body phosphocreatine is converted to creatine. Creatine is converted to creatinine at a fairly constant rate and distributed throughout body water (Finco, 1980). According to Brody (1945) creatinine is excreted in proportion to live weight within a wide range of body weights. Provided that the individual variations are sufficiently small, creatinine could be useful as an internal marker to make quantitative predictions of metabolic processes possible in intact animals.

The aim of this study was to examine the relationship between urinary excretion of allantoin and other purine derivatives and creatinine, and the amount of digestible organic matter (DOM) consumed by sheep. It was also interested to test the possibility of creatinine as an internal indicator to predict urinary metabolic derivatives.

Materials and Methods

Treatment of sheep

A total of four white alpine sheep were used.

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They were 3 to 6 years old and weighed on average 84.65 ± 3.58 kg initial live weight (LW), kept in individual stalls and metabolism cages throughout the experiment. The hay ration (g/kg dry matter, DM: crude protein 93.87 ± 5.57 , crude fibre 293.15 ± 3.860), trace mineral and vitamin mixture were fed in equal portions at 8 h intervals (08:00, 16:00 and 24:00 hours), water was freely available throughout the experiment. Design of the experiment was randomized, in which the animals were fed ranged from 718 g to 1060 g DM/d. Each level of DM was fed during 11 day preliminary and 6 day period of daily faeces and urine collection. The urine was collected into a vinyl bag submerged in ice bag. The animals were weighed at the begin and end of each collection period.

Analytical methods

Urine was analyzed daily. Allantoin (All), uric acid (Urd), hypoxanthine (Hxn) were determined with Reversed-phase HPLC by the methods given previously (Han and Landis, 1991b). Creatinine was determined by enzymatic method (Boeringer Mannheim, No. 883263, FRD) with a automatic spectrophotometer (COBAS MIRA, Roche, Switzerland). Faeces samples were taken daily all of the excreted amount and analyzed. DM and organic matter (OM) content of feeds and faeces samples were determined according to the AOAC methods.

Calculation of predicted purine derivatives excretion

The concentration of creatinine in urine depends on urine volume just as purine derivatives concentration does. With daily creatinine excretion being constant, purine derivatives can be related to creatinine concentration, and urine volume factor cancels. Predicted purine derivatives excretion (mg/d) was calculated for each animal according to the relationship:

$$\text{Purine derivatives} = \text{LW} \times \text{CLW} \times \text{PD/C}$$

where

LW = live weight in kg, CLW = creatinine excretion in mg/kg LW (average value for all animals), PD/C = the ratio of purine derivatives (mg/100 ml) and creatinine (mg/100 ml) in the urine.

Statistical analysis

Least-square means (LS means) were calculated including year, animal and DOM (digestible organic matter) intake in the model. Linear regressions were made conventionally. All statistical calculations were made with BMDP (Dixon, 1985).

Results

The relationship between urinary allantoin (All), uric acid (Urd), hypoxanthine (Hxn), total purine derivatives (Tpd) excretion (mg/d) and the intake (g/day) of DOM were given below:

$$\text{All-N} = 1.205 (\pm 0.070) \times \text{DOM} - 136.709$$

(± 37.399), $n = 16$, $r = 0.9770$, $\text{RSD} = 22.97$, $p < 0.0001$

$$\text{Urd-N} = 0.131 (\pm 0.041) \times \text{DOM} + 11.380$$

(± 21.881), $n = 16$, $r = 0.6306$, $\text{RSD} = 13.44$, $p < 0.005$

$$\text{Hxn-N} = 0.049 (\pm 0.014) \times \text{DOM} + 28.640$$

(± 7.708), $n = 16$, $r = 0.6544$, $\text{RSD} = 4.734$, $p < 0.025$

$$\text{Tpd-N} = 1.385 (\pm 0.083) \times \text{DOM} - 90.261$$

(± 44.552), $n = 16$, $r = 0.9760$, $\text{RSD} = 27.47$, $p < 0.0001$

Recalculation the data on the basis of metabolic body weight ($W^{0.75}$) did not improve the relationships. The relationship between urinary All-N, Urd-N, Hxn-N, Tpd-N and digestible organic matter intake is shown in figure 1.

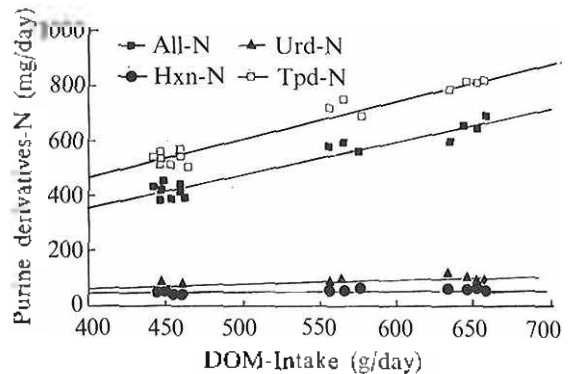


Figure 1. The relationship between urinary purine derivatives-N excretion (mg/d) and digestible organic matter (DOM) intake.

The urinary creatinine-N excretion was on average 9.10 mg/kg LW (SE 0.12 mg creatinine-N). The excretion of creatinine was not related to feed intake. The predicted allantoin-N and

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total purine derivatives-N was positively correlated ($r = 0.9720$ for allantoin-N, $r = 0.9880$ for total purine derivatives-N) to the measured values (figure 2).

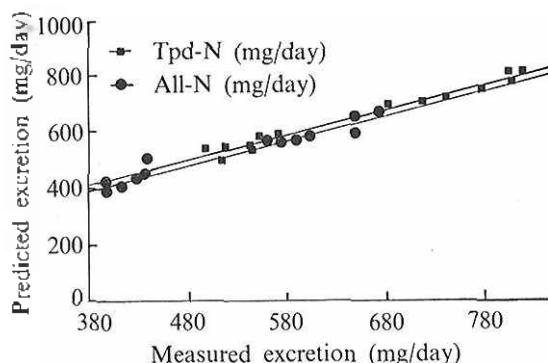


Figure 2. The relationship between measured and predicted urinary allantoin-N (All-N) and total purine derivatives-N (Tpd-N) excretion (mg/d) in sheep.

Discussion

In this experiment the excretion of purine derivatives was linearly related to the intake of DOM within a range of 718 to 1060 g DOM per day. The variation in All-N and Tpd-N excretion (coefficient of variation 5.81% and 5.99%) was of better magnitude than the variation in microbial protein production normally found in studies on caulated animals (Miller, 1982) and the variation in studies on digesta retention time in ruminants (Warner, 1981). The rumen retention time of water is one factor shown to influence the efficiency of microbial protein synthesis (Lindberg, 1984). But the variation in Urd-N and Hxn-N was not so same magnitude as the variation in All-N and Tpd-N (C.V., 30.53% and 28.53%). Assuming that a constant proportion of the DOM is digested in the reticulo-rumen irrespective of the level of intake (Hagemeister et al., 1981), this also indicates a constant microbial protein production per unit of organic matter digested in the rumen. In order to get a quantitative estimate of the amount of microbial nitrogen flowing to the duodenum in this experiment the conversion rate of nucleic acid to urinary allantoin has to be known as well as the content of nucleic acids in micro-organisms

and its digestibility. Very widely differing conversion rates can be found in the literature. Condon and Hatfield (1970) recovered 29.5 to 34.1% of abomasally infused ribonucleic-acid (RNA) as urinary allantoin-N. Antoniewicz et al. (1980) infused yeast RNA into the duodenum of sheep and recovered 11.9% as urinary allantoin-N. Sibanda (1981) found on average 14.1% allantoin-N per unit of microbial NA infused into the abomasum of sheep. This would give an efficiency of microbial protein ($N \times 6.25$) production of 244 g, 407 g, 644 g protein per kg DOM based on the conversion rates given by Condon and Hatfield (1970), Sibanda (1981) and Antoniewicz et al. (1980) respectively. All estimate seem not reasonable in comparison with other data (INRA, 1978; ARC, 1980). New estimation for conversion rate of total purine-N in micro-organisms to urinary purine derivatives-N found on average 67.2 (± 1.0)% allantoin-N and 77.4 (± 0.7)% total purine derivatives-N per unit of microbial purine-N infused into the abomasum of sheep (Han, 1991; Han and Landis, 1991a). This would give an efficiency of microbial protein production of 113 g and 112 g protein per kg DOM based on the conversion rates (10.77 mg allantoin-N and 12.33 mg/g microbial protein) given by Han (1991) and seems most reasonable in comparison with other data (ARC, 1980; Beever et al., 1981; Hume and Purser, 1974; Walker et al., 1975).

The excretion of creatinine-N was on average 9.10 mg per kg LW with a coefficient of variation of 5.27%. The average figure for creatinine excretion agrees well with other data on sheep (Antoniewicz et al., 1981; Walker and Faichney, 1964). An attempt was made to predict the allantoin N and total purine derivatives-N excretion from the ratio of All-N, Tpd-N/creatinine-N in the urine, the average creatinine excretion and the live weight of the animal (figure 2). The predictions scatter closely around the line of unity indicating that it should be possible, at least on a practical basis, to predict urinary purine derivatives excretion and, indirectly rumen microbial protein production from the PD/C ratio in spot samples of urine.

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