RESEARCH ARTICLE

SPECIES DIFFERENCES IN THE CONCENTRATION OF PURINE DERIVATIVES AND CREATININE IN SPOT URINE SAMPLES

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ABSTRACT

A study was conducted to compare the concentration of purine derivatives (PD) and creatinine in spot urine samples of various species of ruminants and rabbit. Urine samples were collected from 80 adult animals of each species (cattle, buffalo, sheep, goat, and rabbit) and were analyzed for PD (allantoin, uric acid, hypoxanthine and xanthine) and creatinine. The concentration of allantoin (mg/L) was highest in the urine of cattle (1487.20) compared to other species. However, the level of uric acid (mg/L) was highest in goat urine (215.80). Xanthine and hypoxanthine were not detected in cattle, buffalo and rabbit urine. Sheep and goat had similar levels of PD in spot urine except for uric acid. The level of creatinine (mg/L) in the urine of rabbit (353.68) was lower compared to ruminants (588.12 to 685.60). The concentrations of allantoin and total PD when expressed as molar proportions of creatinine the values were highest in cattle and goat; and were lowest in buffalo urine. The study revealed that allantoin is the principal PD in spot urine samples of all the species studied while xanthine and hypoxanthine were absent in the urine of cattle, buffalo and rabbit.

INTRODUCTION

Microbial protein production in the rumen is an important function, which provides the necessary good quality protein for the animal. A simple non-invasive technique to estimate the production of microbial protein in the rumen would be of great value. Urinary excretion of purine derivatives (PD) has been successfully used to estimate the microbial protein synthesized in the rumen and subsequently digested in the lower gut of ruminants (Chen *et al*., 1990; Verbic *et al*., 1990). Some studies have also evaluated the use of PD: Creatinine ratio in spot urine samples as an index of microbial protein supply in ruminants (Shingfield and Offer, 1998; Dipu *et al*., 2006). All the four PD (allantoin, uric acid, xanthine and hypoxanthine) are present in the urine of European breeds of sheep (Chen *et al*., 1990) Granadina goats (Belenguer *et al*., 2002), red deer and llama, while the salvageable PD (xanthine and hypoxanthine) are below the detectable levels in the urine of European cattle (Verbic *et al*., 1990), Malaysian buffalo (Liang *et al*., 1994) and yak (Long *et al*., 1999). However, there are reports of the presence of trace amounts of salvageable PD in the urine of swamp buffalo *(Bos bubalus*) and dromedary camel (Guerouali *et al*., 2004). Like ruminants, in single stomached herbivores like rabbits, the urinary excretion of PD may constitute a valuable index to estimate microbial protein intake from caecotrophagy. Since the studies conducted in exotic animals so far, allowed establishing prediction models for estimating microbial nitrogen supply using urinary PD, a preliminary study was conducted to compare the level of PD and creatinine in spot urine samples of various species of indigenous breeds and their cross breds.

MATERIALS AND METHODS

Spot urine samples were collected from 80 unselected adult animals of each species (cattle, buffalo, sheep, goat, and rabbit) from Animal Nutrition Shed/Referral Veterinary Polyclinic, I.V.R.I., Izatnagar. *The urine* samples were transferred into plastic vials containing 10% H_2SO_4 (to maintain the pH below 3) and stored at -20°C till analysis. PD and creatinine in urine

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samples were analyzed by the HPLC method of George *et al*. (2006). The data obtained was subjected to analysis of variance as per Snedecor and Cochran (1994).

RESULTS

The concentration of PD and creatinine in spot urine samples of different species is summarized in Table 1. Allantoin was the major PD in urine for all the species studied. The level of allantoin was highest ($P<0.05$) in the urine of cattle compared to other species, while the concentration of uric acid was highest in goat urine. Sheep and goat had similar levels of PD in spot urine except for uric acid. Buffaloes had much lower level of urinary allantoin than cattle. Similarly, rabbit also had lower level of allantoin in urine. Significant amount of xanthine and hypoxanthine were not detected in cattle, buffalo and rabbit urine. Rabbit had lower urinary creatinine level compared to ruminants. The concentrations of allantoin or total PD when expressed as molar proportions of creatinine i.e. allantoin: creatinine (A/C) and PD: Creatinine (PD/C) the values were highest in cattle and goat; and were lowest in buffaloes.

DISCUSSION

Allantoin was the major PD in urine for all the species studied and thus colloborated well with the findings of previous workers (Verbic *et al*., 1990). Buffaloes had much lower level of urinary allantoin than cattle and this is not related to lower supply of microbial cells from the rumen, nor to absorption of purines from the small intestine, but to differences in tissue metabolism for which mechanisms are not fully understood (Liang *et al*., 1994). Rabbit also had lower urinary allantoin as caecotrophagy contribute only 15 to 38% of total protein intake (Fraga *et al.,* 1991). The absence of salvageable PD (xanthine and hypoxanthine) in cattle, buffalo and rabbit urine might be due to their high ability to oxidise absorbed purine bases to non-re utilizable PD (Chen et al., 1990). This is attributed to higher activity of the enzyme, xanthine oxidase (XO) in the liver and intestinal mucosa of cattle, buffalo (Chen *et al.,* 1996) and rabbits (Balcells et *al*., 1998). High XO activity determined in these species suggests that dietary nucleic acid bases are rapidly degraded to oxidized derivatives across the intestinal mucosa and through hepatic metabolism. *However, i*n sheep and goats reusable-PD (xanthine plus hypoxanthine) accounted for a considerable proportion of total PD as also observed in European counterparts of these species (Chen *et al.,* 1990).

It has been suggested that the excretion rate of creatinine is relatively constant in healthy animals (Chen et al., 1995). The use of creatinine as an internal marker of urinary out put relies on the assumption that the creatinine excretion through urine is affected neither by diet nor by the physiological status of the animal, but is excreted in proportion to body weight. It is conceivable that species differences in musculature and variations in body weight could account for the lower urinary

Table 1. The concentration of PD and creatinine in spot urine samples of different species

Parameters	Cattle	Buffalo	Sheep	Goat	Rabbit
Allantoin (mg/L)	1487.20±176.24^{a}	365.12±42.31^{c}	984.40±78.76^{b}	1139.08±88.38^{b}	382.96±47.45^{c}
Uric acid (mg/L)	143.88±18.75^{b}	55.84±8.60^{cd}	86.36±10.60^{c}	215.80±21.15^{a}	35.32±6.62^{d}
Hypoxanthine (mg/L)	ND	ND	72.40±12.21	92.08±16.00	ND
Xanthine (mg/L)	ND	ND	33.72±7.25	28.00±6.39	ND
Creatinine (mg/L)	668.48±52.79^{a}	588.12±63.97^{a}	685.60±52.90^{a}	659.64±52.77^{a}	353.68±41.89^{b}
A/C (mmol/L: mmol/L)	1.81±0.24^{a}	0.52±0.05^{c}	1.15±0.12^{b}	1.55±0.23^{ab}	1.17±0.26^{b}
PD/C (mmol/L: mmol/L)	1.98±0.24^{ab}	0.61±0.06^{d}	1.40±0.13^{bc}	2.02±0.29^{a}	1.26±0.26^{c}

Values within a row with different superscripts are significantly different, $P<0.05$ ND-Not Detected

creatinine level observed for rabbit in the present study. A number of studies in ruminant species (Daniels *et al.*, 1994; Chen *et al.*, 1995) indicated that purine derivatives: creatinine ratio (PD/C) can predict microbial nitrogen supply with reasonable accuracy as it is little affected by sampling time. Higher A/C and PD/C ratio in cattle and goat obtained in the present study is attributed to higher allantoin/PD level and similar creatinine when compared to other ruminants.

The study revealed that allantoin is the principal PD in spot urine samples of all the species studied followed by uric acid, while xanthine and hypoxanthine were absent in the urine of cattle, buffalo and rabbit.

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