



Urinary excretion of purine derivatives as influenced by GFR and plasma retention of purines in cattle (*Bos indicus* × *Bos taurus*) and buffalo (*Bubalus bubalis*) bulls

K.Y. Deshpande^{1,2,5}, U.R. Mehra¹, P. Singh¹, S.L. Ingale^{1,3}, A.K. Verma¹ and S.K. George^{1,4}

¹ Division of Animal Nutrition, Indian Veterinary Research Institute
Izatnagar, Bareilly – 243122, Uttar Pradesh, India

² Krishi Vigyan Kendra, Sher-e-Kashmir University of Agricultural Science & Technology-Jammu
Tandwal Rajouri, Jammu & Kashmir –185131, India

³ Kangwon National University, Chunchon, 200 701, Republic of Korea

⁴ Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy,
Thrissur, Kerala Veterinary and Animal Sciences University, Kerala, 680 651, India

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⁵ Corresponding author: e-mail:
kuldeepdeshpande@gmail.com

ABSTRACT. A study was undertaken to assess the influence of variation in the glomerular filtration rate (GFR) and plasma retention of purines on excretion of purine derivatives (PD) in crossbred cattle and buffalo bulls. Five male crossbred cattle (*Bos indicus* × *Bos taurus*) and Murrah buffalo bulls (*Bubalus bubalis*) about 2 years and 2 months of age (average body weights: 198.2 and 214.6 kg, respectively) were fed a maintenance ration containing wheat straw and concentrate. Experimental feeding of 40 days was followed by a metabolic trial of six days. The response parameters were compared by the independent 't' test. The plasma allantoin, uric acid, and total PD concentrations were higher ($p < 0.05$) in cattle than in buffalo. Urinary allantoin and total PD excretion were higher in cattle ($p < 0.05$). Creatinine excretion however, was comparable ($p > 0.05$) between the two species. The GFR in both groups remained similar. The tubular loads of allantoin, uric acid, and total PD were numerically higher in cattle, whereas, the uric acid reabsorption percent was higher ($p < 0.05$) in buffaloes. The microbial nitrogen supply was higher ($P < 0.01$) in cattle. The purine nitrogen index remained similar in both species. The present study concludes that the lower urinary PD excretion rate of buffalo was due to their higher reabsorption of PD than cattle; however, the effect of species-specific GFR seems to confound the elucidation of the variation in their individual urinary PD excretion.

Introduction

The urinary purine derivative (PD) excretion rate in ruminants is a function of microbial protein synthesis in the rumen. Microbial protein forms the major portion of protein available at the duodenal level in ruminants that is consequently metabolized and excreted as PD in urine and milk, or retained in

plasma. The plasma PD concentration is monitored by glomerular filtration rate (GFR) (Chen et al., 1991), whereas urinary PD may be of endogenous or exogenous origin (Chen et al., 1990). There is evidence suggesting that the urinary PD excretion rates of small ruminants as well as of zebu cattle and water buffaloes differ from those of European cattle (Thanh and Ørskov, 2006; Mota et al., 2008).

These differences are perceived to be a result of recycling purine bases in small ruminants, which does not take place in their larger counterparts. Mota et al. (2008) deduced that the physiological status of goats had a positive influence on the endogenous contribution to urinary excretion of PD, but could barely affect the urinary recovery of duodenal purine bases. The urinary PD excretion rate is widely used to predict rumen microbial protein production in ruminants. George et al. (2011) concluded that the purine metabolite:creatinine (PMC) index in spot urine samples can serve as a potential tool for the quantification of microbial nitrogen supply in Barbari goats. Moreover, prediction equations have been developed based on European cattle (Chen et al., 1990) and sheep (Verbic et al., 1990). The relationship between the microbial yield of purine from the rumen and urinary excretion of PD may differ, however, among different breeds and species of ruminants (IAEA, 1999). Notwithstanding that detailed accounts of differences in excretion of PD between buffaloes and European cattle have been presented elsewhere, such a comparison between Indian crossbred cattle and buffaloes has hardly been carried out. Thus, the present study is an attempt to investigate whether differences in GFR and the plasma retention of purine derivatives of Indian crossbred cattle and Murrah buffalo bulls are associated with their urinary PD excretion.

Material and methods

The study was conducted at the Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, (India); located at 170 metres MSL (28°22'N and 79°24'E) and with an average annual rainfall of about 900–1200 mm.

Experimental design and diets

Five male crossbred cattle and buffalo bulls about 2 years and 2 months of age (average body weights 198.2 and 214.6 kg, respectively) were fed a maintenance ration containing wheat straw and concentrate. A concentrate mixture was formulated (% DM maize 35, wheat bran 30, deoiled soya bean meal 32, mineral mixture 02, common salt 01) for feeding the animals throughout the trial. The animals were fed a measured quantity of concentrate mixture and wheat straw (40:60) to meet the maintenance requirement for cattle (NRC, 2001) and for buffalo (Kearl, 1989). After 40 days of feeding the experimental diet, the animals were transferred to metabolic cages for an adaptation period of up to four days and a subsequent sampling period of six days.

Collection and analysis of samples

During the 6-day sampling period, representative samples of offered feed, refusals, as well as voided urine and faeces were collected separately, total quantity/volume recorded, aliquoted, and brought daily to the laboratory and preserved (Schneider and Flatt, 1975) for further analyses. Dry matter and organic matter contents of samples were determined (AOAC, 1995) daily and averaged for 6 days. The nitrogen content in feed, faeces, and urine was analyzed following Kjeldahl's method and nitrogen balance was calculated. The spot urine collections were made during the last two days of the trial at 12 h post feeding. For determination of PD, urine samples were filtered and collected into clean plastic containers containing 100 ml of 10% H₂SO₄, so that the final pH remained below 3. All of the urine and plasma samples were preserved at -20°C for subsequent analyses. After thawing, urine samples were diluted with distilled water in a way to maintain the PD concentration in the final sample within the range of standards (5 to 50 mg · l⁻¹), and were further used in the assays for both uric acid and allantoin. Blood samples (10 ml) were collected by jugular venipuncture into centrifuge tubes containing heparin anticoagulant; plasma was separated immediately and preserved. Uric acid, xanthine, hypoxanthine and creatinine were estimated in urine and plasma samples using standard procedures (IAEA, 1997; Joint FAO/IAEA Division, 2003). Allantoin in urine and plasma was determined by colorimetric methods (Young and Conway, 1942). Urine was analyzed directly after filtration (0.22 µm millipore filter) and dilution (1:50). Further, plasma PD, glomerular filtration rate (GFR), tubular load, reabsorption, and purine nitrogen index (PNI) were calculated according to IAEA (1997) with later modifications (Joint FAO/IAEA Division, 2003). For comparison of GFR between the buffalo and cattle, urinary creatinine excretion had to be related to metabolic bodyweight.

Calculations of target parameters

The calculations of different target parameters were performed according to Dipu et al. (2006) as follows:

Glomerular filtration rate (GFR):

$$\text{GFR (l} \cdot \text{d}^{-1}\text{)} = \frac{\text{urinary creatinine excretion (mmol} \cdot \text{d}^{-1}\text{)}}{\text{plasma creatinine concentration (mmol} \cdot \text{l}^{-1}\text{)}}$$

Renal clearance of purine derivatives:

$$\text{tubular load of allantoin (mmol} \cdot \text{d}^{-1}\text{)} = \text{GFR (l} \cdot \text{d}^{-1}\text{)} \times \text{plasma allantoin concentration (mmol} \cdot \text{l}^{-1}\text{)}$$

$$\text{net reabsorption of allantoin (mmol} \cdot \text{d}^{-1}\text{)} = \text{tubular load of allantoin excretion in urine (mmol} \cdot \text{d}^{-1}\text{)}$$

$$\text{tubular load of uric acid (mmol} \cdot \text{d}^{-1}\text{)} = \text{GFR (l} \cdot \text{d}^{-1}\text{)} \times \text{plasma uric acid concentration (mmol} \cdot \text{l}^{-1}\text{)}$$

net reabsorption of uric acid $\text{mmol} \cdot \text{d}^{-1}$) = tubular load of uric acid excretion in urine ($\text{mmol} \cdot \text{d}^{-1}$)

Purine nitrogen index (PNI):

PNI = total urinary PD excretion ($\text{mmol} \cdot \text{d}^{-1}$) * 0.056/nitrogen excreted in urine ($\text{g} \cdot \text{d}^{-1}$) where 0.056 is the conversion factor from PD excretion ($\text{mmol} \cdot \text{d}^{-1}$) to PD nitrogen ($\text{g} \cdot \text{d}^{-1}$).

Calculations and statistical analysis

Calculation of daily purine absorption (X) corresponding to the PD excreted (Y) was done by using the equation of endogenous PD excretion determined by Singh et al. (2007) for crossbred cattle ($Y = 0.83X + 0.296 \text{ BW}^{0.75}$) and by Dipu et al. (2006) for buffalo ($Y = 0.74X + (\text{endogenous PD } \text{BW}^{0.75})$). The slopes of 0.83 and 0.74, respectively, in the equations represent the recovery of absorbed purines as PD in urine (IAEA, 1999) and the value 0.296 in the equation for cattle represents the net endogenous contribution of PD to total excretion.

Microbial nitrogen (MN) supply was determined by multiplying purine absorption (X) by 0.727 as a factor (Chen and Gomes, 1992). $\text{MN} (\text{g} \cdot \text{d}^{-1}) = 70X / (0.116 \times 0.83 \times 1000) = 0.727X$ where it was assumed that:

- The digestibility of microbial purines is 0.83. This is taken as the mean digestibility for microbial nucleic acids from observations reported in the literature.
- The N content of purines is 70 mg N · mmol⁻¹.
- The ratio of purine N:total N in mixed rumen microbes is 11.6:100.

Statistical analyses of the data were done according to Snedecor and Cochran (1994). The means were compared by the independent 't' test. The excretion rates of urinary PD were regressed against their respective digestible organic matter intake (DOMI) using linear regression analysis.

Results and discussion

Intake and digestibility

Body weight (BW), intake, and digestibility of various nutrients and nitrogen balance in experimental animals are presented in Table 1. The average body weight of cattle and buffalo were 198.2 and 214.6 kg, respectively. The intake of DM and OM was higher in cattle than buffalo bulls ($P < 0.05$). Digestibility of a feed is not a fixed trait but is modified by various factors such as the level of feed intake, age, species, and condition of animal production, etc. (Blümmel et al., 2003). Digestibility of DM and OM in the present study was greater

Table 1. The body weights, intake and digestibility of various nutrients, and the nitrogen balance in experimental animals

Parameter	Cattle	Buffalo	P
Body weight			
kg	198.2	214.6	0.207
kg BW ^{0.75}	52.7	56.1	0.203
dry matter			
intake*, $\text{g} \cdot \text{d}^{-1}$	4269.7 ^a	3769.1 ^b	0.046
digestibility, %	56.8	68.6	0.623
Organic matter			
intake*, $\text{g} \cdot \text{d}^{-1}$	3926.0 ^a	3527.6 ^b	0.024
digestibility, %	60.5	70.0	0.017
Nitrogen balance, $\text{g} \cdot \text{d}^{-1}$			
intake	81.4	90.7	0.408
faecal loss	27.9	23.9	0.072
urinary loss	30.9	33.9	0.082
balance	22.6	32.9	0.410

Means bearing different superscripts in a row differ significantly ($P < 0.05$).

in buffalo compared with cattle. This is in agreement with the findings of Kawashima et al. (2006), although the intake was lower ($p < 0.05$) in buffaloes. Studies conducted by various authors have shown that the efficiency of feed utilization and the concentration of volatile fatty acids (VFA) are higher in buffalo than cattle (Chaturvedi et al., 1974); which may be reason for better digestibility in buffaloes.

Nitrogen intake and balance

The N intake and balance ($\text{g} \cdot \text{d}^{-1}$) were comparable ($p > 0.05$) between buffalo and cattle (Table 1) in the present study. These results corroborate well with Kawashima et al. (2006), who conducted a metabolism trial with four each of Brahman cattle and swamp buffaloes fed with ruzi grass hay. They observed similar ($p > 0.05$) energy, nitrogen balance and nutrient digestibility between the two species.

Daily urinary excretion of purine derivatives and creatinine

Daily urinary excretion of PD and creatinine is presented in Table 2. The daily allantoin excretion was higher ($p < 0.05$) in cattle as compared with buffaloes. The uric acid concentration was also higher ($p < 0.05$) in cattle ($3.65 \text{ mmol} \cdot \text{d}^{-1}$) than buffalo ($1.46 \text{ mmol} \cdot \text{d}^{-1}$), but there was no difference ($p > 0.05$) in uric acid excretion when expressed as $\text{mmol} \cdot \text{kg}^{-1} \cdot \text{BW}^{0.75} \cdot \text{d}^{-1}$ between the two species. Also urinary creatinine excretion was comparable between the two species. Total PD excretion also followed a similar trend as urinary allantoin excretion. This may be because allantoin formed the major portion of total PD output in both species and was higher ($P < 0.01$) in cattle.

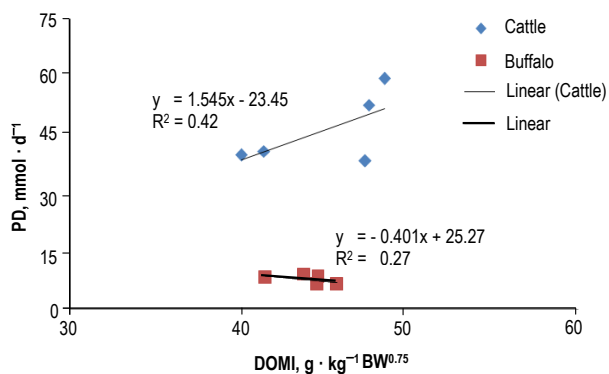
Table 2. Daily purine derivative (PD) and creatinine excretion in urine

Parameter	Cattle	Buffalo	P
Allantoin			
mmol · d ^{-1**}	42.51 ^a	6.13 ^b	0.001
mmol · kg ⁻¹ BW ^{0.75} · d ^{-1*}	0.80 ^a	0.11 ^b	0.012
Uric acid			
mmol · d ^{-1*}	3.65 ^a	1.46 ^b	0.024
mmol · kg ⁻¹ BW ^{0.75} · d ⁻¹	0.07	0.03	0.198
Total PD			
mmol · d ^{-1**}	46.16 ^a	7.59 ^b	0.001
mmol · kg ⁻¹ BW ^{0.75} · d ^{-1*}	0.88 ^a	0.13 ^b	0.015
Creatinine			
mmol · d ⁻¹	28.98	27.79	0.870
mmol · kg ⁻¹ BW ^{0.75} · d ⁻¹	0.55	0.49	0.800

Means bearing different superscripts in a row differ significantly * ($p < 0.05$) and ** ($P < 0.01$).

The relationship between urinary PD excretion rates and DOMI for the two species is shown in the Figure 1. Allantoin and uric acid contributed to total PD, and other compounds such as xanthine and hypoxanthine could not be quantified in either species owing to higher xanthine oxidase activity in their intestines and plasma. These results corroborate with Chen et al. (1996) and Dipu et al. (2006), who reported that the concentrations of hypoxanthine and xanthine were below detectable limits in *Bos taurus* cattle and buffaloes, respectively. Allantoin was found to be the principal PD in the urine samples of both species (Table 2). The results of the present study confirm earlier findings that the urinary PD excretion rate per kg DOMI for cattle is higher than that for buffalo (Vercoe, 1976; Liang et al., 1994, 1998).

The present values were lower, however, than those reported earlier for the same two species by Dipu et al. (2006) and George et al. (2006); this may be due to the lower DOMI and body weights of experimental animals in the present experiment. Hall et al. (2004) deduced that it is also conceptually sound to assume that animal performance will largely depend upon the amount of DOMI, hence the use of PD excretion to predict DOMI would be the most appropriate. The relationship between

**Figure 1.** Urinary purine derivatives (PD) of crossbred cattle and buffalo as affected by *digestible* organic matter intake (DOMI)

DOMI and PD excretion obtained in the present study for both species is given in the Figure 1. The values of the regression coefficient (R^2) in the present study were 0.42 (cattle) and 0.27 (buffalo) in comparison with George et al. (2006) for cattle ($R^2 = 0.76$) and Dipu et al. (2006) for buffalo ($R^2 = 0.77$); the Y and X values were expressed on per kg BW^{0.75}).

Spot urine

The PD and creatinine excretions in spot urine samples are given in Table 3. The allantoin excretion in spot urine was higher ($p < 0.05$) in cattle. This was consequently reflected in the total PD excretion, which followed a similar trend as that of allantoin. Uric acid and creatinine excretion in spot urine was not affected ($p > 0.05$), however, due to species differences.

Variations noted for urinary concentrations of allantoin, uric acid and, consequently, PD and creatinine in spot samples within animals could be due to the influence of urine volumes associated with

Table 3. Purine derivative (PD) and creatinine concentrations in plasma and spot urine, glomerular filtration rate (GFR), tubular load, reabsorption rate, reabsorption percentage, microbial nitrogen supply, and purine nitrogen index (PNI) in experimental animals

Parameter	Cattle	Buffalo	P
Plasma, mmol · l ⁻¹			
allantoin	275.85	113.14	0.064
uric acid	26.49	12.35	0.638
total PD**	302.34 ^a	125.50 ^b	0.005
creatinine	82.09	85.72	0.244
Spot urine, mmol · l ⁻¹			
allantoin**	8.69 ^a	1.06 ^b	0.002
uric acid	0.74	0.25	1.000
total PD**	9.44 ^a	1.31 ^b	0.001
creatinine	5.96	4.79	0.794
GFR			
L/D	352.19	325.01	0.462
l · kg BW ^{0.75} · d ⁻¹	6.67	5.73	0.822
Tubular load, mmol · d ⁻¹			
allantoin	97.14	36.79	0.215
uric acid	9.34	4.01	0.156
total PD	106.48	40.80	0.187
Reabsorption, mmol · d ⁻¹			
allantoin	54.63	30.65	0.928
uric acid	5.69	2.55	0.238
total PD	60.32	33.20	0.946
Reabsorption, %			
allantoin	56.43	83.20	0.224
uric acid*	61.00 ^b	63.29 ^a	0.019
total PD	56.85	81.26	0.243
Microbial N supply			
g · d ^{-1**}	40.17 ^a	7.34 ^b	0.001
g N · kg ⁻¹ DOMI*	16.86 ^a	2.97 ^b	0.012
PNI	0.017	0.002	0.208

Means bearing different superscripts in a row differ significantly * ($p < 0.05$) and ** ($P < 0.01$); DOMI – digestible organic matter intake

drinking water intake (Chen et al., 1992). Dipu et al. (2006) also indicated that better estimates of variability in terms of nutrition were obtained when samples for measurements of PD concentration were taken at 12 h post feeding. Similarly, in the present study, rather than taking mean multiple spot samples, only samples at 12 h post feeding were studied, which might more accurately reflect the standing level of purine excretion for animals on an analogous feeding regime.

Purine derivative and creatinine concentration in plasma

The plasma concentrations of allantoin, uric acid and creatinine estimated for calculation of GFR and renal clearance are shown in Table 3. As in the case of urine, the salvageable PD like xanthine and hypoxanthine could not be detected in the plasma, hence allantoin and uric acid accounted for total PD. The plasma allantoin concentration was higher in cattle (275.85) as compared with buffalo (113.14). The average plasma uric acid concentration was also greater in cattle (26.49) than in buffalo (12.35). The plasma total PD concentration was higher ($p < 0.05$) in cattle (302.34) as compared with buffalo (125.50). The results for plasma creatinine concentration of cattle and buffalo were comparable.

The plasma allantoin concentration in the present study corroborated well with Singh et al. (2007) (259.5; L-95) and George et al. (2007) (290.43; L-100) for cattle and Dipu et al. (2006) (118.81; L-80) for buffalo at the given feeding levels. The uric acid concentration was lower than that reported by Singh et al. (2007) in bulls at all feeding levels; however, it was comparable with the results of George et al. (2007) for cattle and Dipu et al. (2006) for buffaloes, despite different levels of feeding in their studies. Total PD concentrations were also in agreement with L-95 of Singh et al. (2007) and L-100 of George et al. (2007) for cattle and fell between L-60 and L-80 of Dipu et al. (2006) for buffaloes. The creatinine concentrations in the animals in all of the aforementioned studies were higher than in the present study; this might be due to the higher body weight of the experimental animals in them. Brody (1945) found that creatinine excretion is directly proportional to the body weight of animals of different sizes of the same species. It is conceivable that between breeds/species, differences in musculature and variations in body weight could account for these discrepancies in plasma creatinine concentration (George et al., 2007; Deshpande et al., 2011).

Glomerular filtration rate and renal clearance of purine derivative

Measurements of both daily creatinine output in urine and creatinine concentrations in plasma are required to estimate the GFR (Joint FAO/IAEA Division, 2003). Also, as mentioned in that report, inulin can be used to provide an even more accurate measurement of GFR. Nevertheless, creatinine is produced from creatine and creatinine phosphate in muscle, and is excreted in the urine. Creatinine excretion is consequently correlated with muscle mass; creatinine is filtered from the blood by renal glomeruli and is not influenced by either absorption or excretion in the renal tubule. When expressed as mmol per kg BW^{0.75}, daily excretion is relatively constant. Creatinine has been found to be neither reabsorbed from, nor secreted into, the tubule from the primary urine in sheep, cattle, cats and dogs, and may be used as an endogenous internal marker for estimation of GFR in these animals (Thanh et al., 2009).

Filtration through the glomerular capillaries, reabsorption of fluid and solute, and secretion into the lumen of the proximal and distal tubules govern urine formation (White et al., 1968), whereas separation of PD and creatinine from blood is termed renal clearance. The GFR of cattle and buffalo remained comparable ($p > 0.05$). The tubular load of allantoin and uric acid was higher in cattle. The tubular load of total PD consequently followed a similar trend.

The comparatively lower GFR observed in this experiment than in previous reports (Dipu et al., 2006; George et al., 2007) might be due to the lower body weight of experimental animals in the present study. Generally, GFR increased with increased feeding level in both species. Liang et al. (1998) found that the GFR values of swamp buffaloes were generally higher ($p > 0.05$) than KK cattle. They attributed this to the large variation among animals within a species. Marginal differences in GFR, allantoin, uric acid, and total PD recorded between cattle and buffalo in the present experiment corroborate well with the earlier observations of previous workers (Dipu et al., 2006; George et al., 2007). Similarly, a lower GFR in buffaloes than cattle at the same feed intake and body weight was recorded by Norton et al. (1979). Moreover, buffaloes had a mechanism of partitioning plasma PD between renal excretion and non-renal disposal, hence a low GFR was observed (Chen et al., 1996). Thanh and Ørskov (2006) in their detailed study on three each of male Vietnamese cattle and swamp buffalo calves postulated that the difference in PD excretion occurs only after rumen development. They further

suggested that GFR might be lower in buffaloes than cattle, leaving more time for PD to remain in the blood, thus more time for recycling to the rumen for them to be metabolized by bacteria, or that the permeability from the blood to the rumen is greater in buffaloes than cattle.

Reabsorption of purine derivatives and microbial nitrogen supply

Reabsorption of allantoin in $\text{mmol} \cdot \text{d}^{-1}$ was greater in cattle (54.63) when compared with buffalo (30.65), although not significantly so ($p > 0.05$). The case with uric acid was similar (Table 3). Total PD reabsorption was greater in cattle than in buffalo. When reabsorption of PD was expressed as a percentage, uric acid was found to be greater ($p < 0.05$) in buffalo when compared with cattle. Allantoin and total PD reabsorption were also higher in buffalo, confirming the earlier works by Dipu et al. (2006), George et al. (2007) and Singh et al. (2007); moreover, this confirms the hypothesis that the lower urinary PD excretion rate of buffalo was due to the higher recycling of plasma PD as compared with cattle. Measurement of the proportion of plasma PD excreted in urine could provide an explanation for the discrepancy in the urinary PD excretion rates between cattle and buffalo (Liang et al., 1994; Vercoe, 1976). Nevertheless, the higher PD reabsorption in swamp buffaloes provides support for the earlier findings that the lower urinary PD excretion rate of swamp buffalo was due to their higher recycling of plasma PD as compared with KK cattle (Liang et al., 1998).

The microbial nitrogen supply calculated from daily purine absorption values using the equation of Singh et al. (2007) is presented in Table 3. The MN supply ($\text{g} \cdot \text{d}^{-1}$) was higher ($p < 0.05$) in cattle (40.17) as compared with buffalo (7.34). Microbial nitrogen, expressed as $\text{g N} \cdot \text{kg}^{-1}$ DOMI, was also higher ($p < 0.05$) in cattle than buffalo. The PNI was comparable ($p < 0.05$) between the species. In agreement with the results of the present study, Singh et al. (2007) reported that the MN supply, when expressed per kg DOMI, remained similar ($p > 0.05$), irrespective of the intake level in crossbred calves. They compared MN supply, calculated using the established model for European cattle (Verbic et al., 1990), at the intake levels of animals recorded in their experiment and suggested that there is a need to develop a set of equations for different breeds/species of animals, particularly under Indian conditions, where animals are usually kept on a low level of nutrition.

Conclusions

It may be deduced from the present study that urinary purine derivatives (PD) excretion is governed to a great extent by subtle species differences in terms of corresponding glomerular filtration rate and reabsorption rates, and not by the plasma retention of PD in crossbred cattle and Murrah buffalo calves fed identical rations. Nonetheless, a detailed study assessing the microbial protein status of Indian cattle and buffaloes of different age groups, e.g., pre-ruminant to ruminant stage, is necessary.

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