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Renal and Salivary Excretions of Plasma Purine Derivatives in Swamp Buffaloes and Zebu Cattle

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ABSTRACT : This study compared the recovery rate of intrajugular-administered allantoin in the urine and saliva between swamp buffaloes and zebu cattle to examine whether it could explain the lower excretion rate of urinary purine derivatives (PD) in the buffaloes. Three male swamp buffalo yearlings, with an average body weight of 349±40.35 kg, and three Thai native cattle (154±3.26 kg) of similar age and sex were used in the study. Animals were kept in individual pens and fed at a maintenance energy level with a diet containing 65% monk bean husk (*Vigna radiata*) as roughage and 35% concentrates. Allantoin solution was infused into the jugular vein in four incremental rates equivalent to 0, 5, 10 and 15 mmol/d and urine was collected daily in acidified form. Daily PD excretion was linearly correlated with intrajugular allantoin infusion in both species. The relationship between daily urinary PD excretion (Y, mmol/d) and intrajugular allantoin infused (X, mmol/d) was Y = 0.75±0.318X+22.45±2.98 (r^2 = 0.36, n = 12, MSE = 38.02, CV = 21.9, $p<0.01$) for swamp buffaloes and Y = 0.96±0.10X+15.93±0.92 (r^2 = 0.91, n = 12, MSE = 3.60, CV = 8.27, $p<0.01$) for zebu cattle. The salivary PD concentration was not correlated with intrajugular allantoin infusion in both species, with values for buffaloes numerically lower than those for cattle. The present study reconfirmed previous studies that buffaloes have a lower plasma PD excretion rate via the renal route and a significant proportion (22%) of the plasma PD loss is via the saliva. However, results of our present and previous studies suggest that differences in purine base (PB) metabolism between buffaloes and zebu cattle occur before the purine compounds reach the plasma pool. (**Key Words :** Swamp Buffaloes, Zebu Cattle, Purine Derivatives, Allantoin)

INTRODUCTION

Excretion rates of purine derivatives (PD) in the urine reflect and, therefore, can be used to predict the duodenal absorption of purine bases (PB) and thus the microbial N yield from the rumen (Topps and Elliott, 1965). The rate of allantoin and total PD excretion were positively correlated with digestible organic matter intake in buffaloes (Pimpa, 2002; Dipu et al., 2006) and crossbred bulls (George et al., 2006). Prediction equations have been developed for European (*Bos taurus*; Verbic et al., 1990) or zebu cattle (*Bos indicus*; Liang et al., 1994; Pimpa et al., 2001) and sheep (Perez et al., 1996). Based on the response of urinary PD to post-ruminal infusion of nucleic acid, published models showed a high and consistant recovery of absorbed purines (0.75-0.84) in cattle, however when similar response model was studied in buffaloes (*Bubalus bubalis*), only 0.12 of the duodenally infused PB were recovered as urinary PD (Pimpa et al., 2003).

Differences in urinary PD excretion between buffalo and zebu cattle only occur after rumen development (Thanh and Orskov, 2005) and differences in rate of apparent PB absorption cannot be demonstrated *in vivo* (Pimpa, 2002) between these two species. It is therefore, suggesting that the lower recovery of duodenal infused purines in buffaloes must be explained by their enhanced ability to recycle more plasma PD, presumably via saliva (Liang et al., 1994; Chen et al., 1996). The objective of the present study was to investigate the partitioning of renal and salivary glands excretions in buffaloes and zebu cattle to examine whether it could help to explain for the lower excretion rate of urinary PD in buffaloes.

MATERIALS AND METHODS

Animals and feeding

Three male swamp buffalo yearlings (349±40.35 kg

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Table 1. Dry matter (DM) and major chemical components[a] of dietary ingredients and feed intake

Dietary ingredients	DM (%)	Crude protein	NDF	ADF	ADL	Ash
		% DM basis				
Monk bean husk	91.6	7.8	52.2	42.2	8.9	7.8
Concentrate[b]	92.8	13.5	14.3	7.8	1.5	6.8
Diet[c]	93.5	9.8	38.9	30.2	6.3	7.5
Daily feed intake (kg/hd/day)						
Buffaloes	6.0	0.58	2.33	1.80	0.37	0.45
Cattle	4.0	0.39	1.55	1.20	0.25	0.29

[a] Values are means of 3 subsamples of each material were assayed.
[b] Consisted of maize grain 23%, soy bean meal 29%, cassava chip 46% and mineral premix 2% on DM basis.
[c] Consisted of 65% Monk bean husk and 35% concentrate.

body weight) and three Thai native cattle (154±3.26 kg body weight) of similar age and sex were used in the experiment. Each animal was fitted with an indwelling vinyl catheter in the jugular vein. The animals were kept in individual pens and fed a near maintenance diet (6 and 4 kg dry matter (DM) per day for buffaloes and cattle, respectively), containing 65% roughage (monk bean husk; *Vigna radiata*) and 35% concentrates (Table 1). The daily feed was offered in two equal portions, the first at 07:00 h and the remainder at 16:00 h for the 30 days study. Clean drinking water was freely available at all times and the body weights of the animals were recorded during the last two days of each period.

Experimental design and allantoin infusion

The experiment was conducted using a double 3×4 Youden Square design (one each for buffalo and cattle) over a period of 30 days (10 days of adaptation and 20 days of test). During the 20 days test period, 4 levels of allantoin solution containing the equivalent of 0 (L0), 5 (L5), 10 (L10) and 15 (L15) mmol allantoin, respectively, were prepared the morning prior to infusion by dissolving the required quantities of allantoin (Sigma® FW 158.1) in 600 ml of commercial medical grade saline solution (0.9% NaCl solution). Allantoin solution (in the saline solution bag) was infused via jugular vein by gravity dripping twice (300 ml/infusion) at a flow rate of 10 ml/min at 13:00 and 20:00 h, respectively. The three animals within each species was randomly allocated to one of the four infusion rates in each period (5 days per period) in a manner that each animals completed the four treatments by the end of the fourth period.

Urine, blood and saliva sampling

Urine, blood and saliva samples were collected daily during the test period. Urine of individual animal was collected in 100 ml of 20% H_2SO_4 to keep the final pH of the urine below 3. After recording the weight, 30 ml of the urine was sampled and diluted with 30 ml distilled water and mixed thoroughly. The diluted urine sample was divided equally into 2 sub-samples and stored at -20°C for later analysis for PD.

About 20 ml of blood was sampled from jugular vein at about 12:00 h in heparinized tubes, which were later centrifuged at 2,200×g for 20 min. The plasma obtained was stored at -20°C for later analysis.

Saliva was collected by mean of self constructed saliva collection tube. Briefly the collection tube was made of a 20 cm household rubber water-hose with small holes along the wall of the tube. The tube was inserted into the mouth of the animals and the saliva produced when the animals chewed the water-hose was collected. Samplings of saliva were done at about 12:00 h. The saliva was later centrifuged at 2,200×g for 10 min and stored at -20°C for later analysis.

Analytical methods

Purine derivatives and creatinine of urine, plasma and saliva samples were determined using reversed phase HPLC, according to the technique of Balcells et al. (1992a).

Urine sample was centrifuged and diluted from 0.5 ml to 5 ml with 4.25 ml of 10 mM $NH_4PO_4H_2$ and 0.25 ml of 1 mM oxypurinol before filtered and analysis. Plasma and saliva (1 ml) were analyzed with procedure of Martin Orue et al. (1995) after acid hydrolysis.

Data analyses

Individual data of glomerular filtration rate (GFR, L/d) was calculated from the relationship between urinary creatinine excretion rate (A, mmol/d) and the plasma creatinine concentration (B, mmol/L) as GFR = A/B. Tubular load (TL) (mmol/d) was estimated as GFR (L/d)× plasma concentration (mmol/L) and reabsorption (RB, mmol/d) TL- urinary excretion (mmol/d) following the procedure of IAEA-TECDOC-945 (1997). Daily urinary PD excretion, concentration of PD in plasma and saliva, GFR, TL and RB of PD were compared among allantoin infusion levels using the mixed procedures of Statistical Analysis Systems Institute Inc. (SAS, 1996). Relationship between the excretion of urinary allantoin, PD (mmol/d) and allantoin infusion levels (mmol/d) was established using the linear regression of SAS (1996). The individual slopes of the linear equations of urinary PD excretion rate

Table 2. Dairy excretion of urinary PD, creatinine and recovery rates of allantoin as derivatives in urine of swamp buffalo and zebu cattle

	Level of plasma allantoin infused (mmol/day)				SEM	Significance of treatment effects
	0	5	10	15		L
Swamp buffalo						
Allantoin (μmol/kg $W^{0.75}$)	215.9	233.0	254.9	299.7	4.12	***
Uric acid (μmol/kg $W^{0.75}$)	63.4	92.7	96.6	130.6	2.71	***
Total PD (μmol/kg $W^{0.75}$)	279.4	325.7	351.5	430.3	5.87	***
Recovery of allantoin[b] (%)	-	28.0	31.7	45.2	28.64	NS[a]
Creatinine (μmol/kg $W^{0.75}$)	594.8	587.2	559.8	633.2	10.55	NS
Zebu cattle						
Allantoin (μmol/kg $W^{0.75}$)	267.7	342.3	474.9	578.4	10.86	***
Uric acid (μmol/kg $W^{0.75}$)	90.9	100.7	106.7	111.7	2.95	*
Total PD (μmol/kg $W^{0.75}$)	358.6	443.0	581.6	690.1	11.51	***
Recovery of allantoin[b] (%)	-	65.0	90.5	90.5	11.20	NS
Creatinine (μmol/kg $W^{0.75}$)	520.4	522.3	500.1	504.1	8.76	NS

[a] NS: not significant, p>0.05.

[b] Calculated as: (Urinary allantoin excreted-urinary allantoin at L0 infused)×100/allantoin infused (mmol/d).

* p<0.05. *** p<0.001.

Table 3. Relationship between plasma allantoin infusion (mmol/day) and urinary allantoin excretion and PD excretion (mmol/day) in cattle and buffaloes

Dependent variable	Model r^2	Intercept			Slope		
		Estimate	SE	p>t^2	Estimate	SE	p>t^2
Urinary allantoin in cattle	0.91	11.35	0.90	0.001	0.82	0.09	0.001
Urinary PD exctretion in cattle	0.91	15.93	0.92	0.001	0.96	0.10	0.001
Urinary allantoin in buffalo	0.25	17.14	2.18	0.001	0.35	0.23	0.09
Urinary PD exctretion in buffalo	0.35	22.45	2.98	0.001	0.75	0.32	0.04

from the two species were analyzed by the analysis of variance (ANOVA) procedures of the SAS (1996) and were subjected to group T-test procedure of SAS (1996).

RESULTS

Condition of animals

The buffaloes and cattle remained in good health throughout the experiment and no substantive changes in their body weights were recorded.

Urinary purine derivatives excretion in relation to allantoin infusion

Allantoin constituted the principal PD in the urine, followed by uric acid while no hypoxanthine or xanthine was detected. Urinary allantoin, uric acid and total PD (allantoin plus uric acid) responded linearly to increasing levels of allantoin infusion. In cattle, the increase was from 267.7 μmol/kg $W^{0.75}$ (control) to 578.4 μmol/kg $W^{0.75}$ (L5) (p<0.01) whereas in buffaloes changes were less pronounced, from 215.9 μmol/kg $W^{0.75}$ (control) to 299.7 μmol/kg $W^{0.75}$ (L5) (p<0.01). The opposite was observed for uric acid which increased from 63.4 to 130.6 μmol/kg $W^{0.75}$ in buffalo (p<0.01) whereas in cattle it remained near constant from 90.0 to 111.7 μmol/kg $W^{0.75}$ (p<0.05). Creatinine excretion was not affected by treatment with an average value of 593.7 and 511.7 μmol/kg $W^{0.75}$, respectively, for buffalo and cattle.

Urinary allantoin and PD excretion (Y, mmol/d) correlated linearly with allantoin infusion (X, mmol/d) as described by the equations presented in Table 2. The slope of the urinary recovery rate of infused allantoin in buffaloes was significantly lower than in cattle, being 0.35 and 0.82 vs. 0.75 and 0.96 (p<0.01), for allantoin and total PD, respectively (Table 3). The relationship between daily urinary PD excretion (Y, mmol/d) and intrajugular allantoin infused (X, mmol/d) was Y = 0.75±0.318X+22.45±2.98 (r^2 = 0.36, n = 12, MSE = 38.02, CV = 21.9, p<0.01) for swamp buffaloes and Y = 0.96±0.10X+15.93±0.92 (r^2 = 0.91, n = 12, MSE = 3.60, CV = 8.27, p<0.01) for zebu cattle.

Concentration of PD and creatinine in plasma and saliva

As in the urine samples, hypoxanthine and xanthine were undetectable in the plasma samples. Allantoin concentration in the plasma samples increased linearly with increased levels of allantoin infusion in both species (p<0.01), but uric acid and creatinine were independent of the experimental treatment (p>0.05) (Table 4). However, allantoin concentration in the plasma samples for buffalo

Table 4. Purine derivatives (PD) and creatinine concentration in plasma, glomerular filtration rate (GFR), tubular load (TL) and net reabsorption (RB) of purine derivatives in swamp buffalo and zebu cattle in different treatments (data were collected every day during 5 days of each test period)

	Level of plasma allantoin infused (mmol/day)				SEM	Significance of treatment effects
	0	5	10	15		L
Swamp buffalo						
Allantoin (μmol/L)	45.7	53.1	58.2	68.4	1.51	***
Uric acid (μmol/L)	79.7	83.1	80.9	80.8	1.93	NS [a]
Total PD (μmol/L)	125.4	136.2	139.1	149.3	3.02	*
Creatinine (μmol/L)	107.4	108.0	112.5	112.5	2.08	NS
GFR (L/d)	444.9	434.9	399.6	453.1	13.34	NS
TL (mmol/d)						
Allantoin	20.6	23.1	23.3	30.8	1.45	*
Uric acid	35.7	36.2	32.3	36.4	1.41	NS
RB (mmol/d)						
Total PD	33.6	32.6	27.4	32.6	3.19	NS
Zebu cattle						
Allantoin (μmol/L)	73.5	79.4	93.3	113.1	1.69	***
Uric acid (μmol/L)	55.9	68.2	60.9	64.9	3.62	NS
Total PD (μmol/L)	129.5	147.7	154.3	178.0	4.48	**
Creatinine (μmol/L)	62.8	69.7	71.7	62.4	2.32	NS
GFR (L/d)	367.5	347.2	329.4	359.1	25.60	NS
TL (mmol/d)						
Allantoin	27.0	27.3	30.9	40.9	2.77	*
Uric acid	20.6	23.2	19.0	23.3	2.36	NS
RB (mmol /d)						
Total PD	31.7	30.9	24.1	33.7	4.31	NS

[a] NS: not significant, $p>0.05$.
* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

(45.7 to 68.4 μmol/L) was at all times lower than the corresponding values for cattle (73.5 to 113.1 μmol/L) while that for uric acid was the opposite (79.7 to 83.1 μmol/L for buffaloes vs. 55.9 to 68.2 μmol/L for cattle).

Concentration of salivary allantoin is less consistent among the treatment groups, while concentration of salivary uric acid of buffalo was less than 10% of that of cattle. However, the differences were not significant for the two parameters (Table 5). Total PD and creatinine were independent of the experimental treatments ($p>0.05$).

Glomerular filtration rate, Tubular load and Re-absorption

Glomerular filtration rate (GFR) did not differ among treatments in both species (Table 4). Similarly, Tubular load (TL) of uric acid and re-absorption (RB) of total PD also did not differ among treatments. However, TL of allantoin increased linearly as the supply of plasma allantoin increased ($p<0.05$).

DISCUSSION

Urinary purine derivatives excretion in relation to allantoin infusion

Allantoin being the main PD in urine followed by uric acid in both, swamp buffaloes and zebu cattle is in agreement with our previous data (Liang et al., 1994; Pimpa et al., 2001; Pimpa et al., 2003). Undetectable level of hypoxanthine and xanthine concentrations in urine samples reconfirming the high activity of xanthine oxidase reported for both species (Al-Khalidi and Chaglassian, 1965; Chen et al., 1996). However, buffaloes showed a higher uric acid concentration in urine (25-40%) than cattle (14-26%).

A linear relationship between urinary excretion of allantoin and plasma administration has also been previously described for cattle (Chen et al., 1990; Verbic et al., 1990) where the slope (averaged 0.85) representing the recovery of incremental intra-jugular infused allantoin. The corresponding value for zebu cattle (0.82±0.10) in the present study was near to the published data, however, in the case of buffaloes the value was only 0.34±0.23. Uric acid excretion also responded linearly to the experimental treatments in both species, but being more pronounced in the buffaloes (63.4 to 130.6 mmol/d; $p<0.001$) than in cattle (90.9 to 111.1 mmol/d; $p>0.05$). The administration of allantoin (being an end product of PB metabolism), could have exerted some kind of feed back mechanism over uricase (EC 1.7.3.3), and thus reduced uric acid oxidation resulted in its higher excretion in buffaloes. It is suggested that total PD instead of allantoin excretion should be used

Table 5. Purine derivatives and creatinine concentration in saliva of swamp buffalo and zebu cattle in different treatments (data were collected every day during 5 days of each test period)

	Level of plasma allantoin infused (mmol/day)				SEM	Significance of treatment effects
	0	5	10	15		L
Swamp buffalo						
Allantoin (μmol/L)	54.7	151.7	140.7	98.9	28.00	NS[a]
Uric acid (μmol/L)	3.5	2.2	3.1	3.2	0.70	NS
Total PD (μmol/L)	58.2	153.9	143.8	102.1	6.72	NS
Creatinine (μmol/L)	35.8	57.7	49.8	44.3	2.08	NS
Zebu cattle						
Allantoin (μmol/L)	95.0	80.8	50.7	37.6	10.95	NS
Uric acid (μmol/L)	43.5	43.0	39.8	47.5	10.58	NS
Total PD (μmol/L)	138.5	123.8	90.5	85.1	16.50	NS
Creatinine (μmol/L)	58.1	55.0	40.6	37.2	8.41	NS

[a] NS: not significant, p>0.05.

as a measurement to test the urinary recovery of the infused allantoin.

Based on total urinary PD, nearly the entire amount of purine (allantoin) administered via the jugular vein was recovered (0.96), which is slightly higher than previous values obtained after a single dose of intrajugular infusion of [8-^{14}C]-uric acid (78%-81%, Sojoeno et al., 1999; Cetinkaya et al., 1999) or the recovery reported using duodenal infused PB (0.84 to 0.93; Vagnoni et al., 1997; Liang et al., 1999; Orellana-Boero et al., 2001). Assuming that the marginal variations among the different studies were due to differences in experimental procedures, diets and animals, our findings reconfirmed that cattle has limited ability to excrete PD though other routes besides the kidneys. On the other hand, only 75% of administered allantoin are recovered as urinary PD in buffaloes. This value is close to the value we previously reported for buffaloes (0.80; Pimpa, 2002). The recovery was, however, significantly higher than the values we reported after administration of purine compounds through the duodenum in buffaloes (0.12; Pimpa et al., 2003). Our findings thus suggest that differences in PB metabolism between buffaloes and cattle occurred before PB compound reach the plasma pool.

Concentration of PD and creatinine in plasma and saliva

Although creatinine concentration was detected in plasma and saliva in both species, it was not affected by experimental treatments. Since no changes were detected in animal's body weight during the study, creatinine was suitable for measurement of the GFR as reported for other ruminant species such as in goats (Valtonen et al., 1982), sheep (Chen et al., 1995; Surra et al., 1997), cattle (Vagnoni et al., 1997; Pimpa et al., 2001) and buffaloes (Chen et al., 1996; Dipu et al., 2006).

As in urine samples, hypoxanthine and xanthine were not detected in plasma samples. Plasma allantoin was linearly correlated with levels of allantoin infusion whereas uric acid was not. Allantoin concentration in plasma was lower than the 118.3 μmol/L reported by Balcells et al. (1992b) for steers and 184.5 μmol/L for dairy cows (Giesecke et al., 1994) but was similar to those reported previously for zebu cattle and buffaloes by us (Pimpa et al., 2001; 2003). The low concentration of allantoin in plasma samples together with the difficulties to obtain a good resolution of the elution peaks in the HPLC analytical process (Balcells et al., 1992a) may explain for the variations in the values reported by the different groups.

Mean salivary concentration of allantoin and total PD were in the range described by Chen et al. (1990) using oral sponges (16 and 120 μmol/L) but much lower than the values reported in sheep using total saliva collection (Surra et al., 1997).

Assuming that saliva production in buffaloes was between 50 to 56 L/day (McDougall, 1948) and did not change with the experimental treatments (Table 5), the proportion of PD lost irreversibly through saliva reach a mean value of 22%. The above estimate suggests that salivary PD forms a significant portion of non-renal excretion of plasma PD and complement well with the 75% loss via urine recorded in this study.

Glomerular filtration rate (GFR), Tubular load (TL) and Re-absorption (RB)

Glomerular filtration rate (GFR) did not differ among treatments in both species. The result is in agreement with our previous study using duodenal infusion of different levels of PB for Kedah-Kelantan cattle and swamp buffaloes (Pimpa et al., 2001; 2003). Similarly, RB of total PD also did not differ among treatments, which is in agreement with the result reported for Indonesian cattle (Soejono et al., 1999). Although Chen et al. (1996) reported that GFR for buffaloes was lower than that for cattle, they did not substantiate their finding with any data. The GFR

values for buffaloes in this study were higher than for cattle (Table 4).

CONCLUSION

Nearly the entire amount of purine administered intrajugularly in zebu cattle was recovered in the urine (96%) suggesting that cattle has limited ability to excrete plasma PD through other routes besides the kidneys. On the other hand, buffaloes excreted only 75% of the infused purine via renal route and a significant proportion (22%) of the remaining via saliva. However, available data seems to suggest that differences in PD metabolism between cattle and buffaloes also occurred before the PB compounds reach the plasma pool.

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