Measurement of Microbial Protein Supply in Murrah Buffaloes *(Bubalus bubalis)* Using Urinary Purine Derivatives Excretion and PDC Index

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ABSTRACT : A study was conducted to predict the rumen microbial protein production based on urinary excretion of purine derivatives in buffaloes fed a diet of wheat straw and concentrate (40:60) at four fixed levels of feed intake. (95, 80, 60 and 40% of preliminary voluntary feed intake) following experimental protocol of IAEA (Phase I). The buffaloes were allocated according to a 4×4 latin square design. The urinary allantoin, uric acid, total PD excretion (mmol/d) in treatments L-95, L-80, L-60 and L-40 was 20.13, 16.00, 12.96 and 9.17; 1.88, 2.12, 2.11 and 2.15; 22.01, 18.12, 15.07 and 11.32, respectively and were significantly ($p<0.05$) different among treatments except for uric acid. The rate of PD excretion (mmol/d) was positively correlated with the digestible organic matter intake. Variations were observed in PD and creatinine concentration in spot samples collected at 6-hour interval. However, daily PD:Creatinine ratio (PDC index) appears to be a reasonably good predictor of microbial-N supply. The contribution of basal purine excretion to total excretion of purine derivatives (PD) was determined in pre-fasting period followed by a fasting period of 6 d (Phase II). Daily PD and creatinine excretion (mmol/kg $W^{0.75}$) during fasting averaged 0.117 and 0.456 respectively for buffaloes. The excretion rates of PD decreased significantly ($p<0.01$) during fasting compare to pre-fasting period, the urinary creatinine excretion remained almost similar. Except for creatinine, plasma concentration of target parameters significantly ($p<0.01$) declined during fasting. Likewise, glomerular filtration rate (GFR) and renal clearance of allantoin and uric acid also decreased. Based on the PD excretion rates during fasting and at different levels of feed intake obtained in this study, a relationship between daily urinary PD excretion (Y-mmol) and microbial purine absorption (X-mmol) was developed for buffaloes as $Y = 0.74X+0.117$ kg $W^{0.75}$. The microbial N supply (g/kg DOMI) remained statistically similar irrespective of dietary treatment. The results showed that excretion of urinary purine derivatives is positively correlated with the levels of feed intake in Murrah buffaloes and thus, estimation of urinary purine derivatives and PDC index could be used to determine microbial nitrogen supply when there is large variation in level of feed intake. ***(Asian-Aust. J. Anim. Sci. 2006. Vol 19, No. 3 : 347-355)***

Key Words : Buffaloes, Levels of Feed Intake, Purine Derivatives, Microbial Protein, PDC Index

INTRODUCTION

Forages are the most important feed resource for ruminants worldwide. The ruminants have to consume the required amount of energy, protein, minerals and vitamins to meet their production potential (Chanjula et al., 2004). However, in tropics most ruminants have been fed on low quality roughages, crop residues and agricultural by-products. There is large variability in the quality of forages so measurement and prediction of feeding value and nutritive value are essential for high level of production (Dynes et al., 2003). Rumen microbial biomass provides the majority of amino acids that are utilized by host ruminants for their tissue maintenance, growth and production (Jayasuriya, 1999). So the knowledge of microbial contribution to nutrition of the host animal is essential to develop feed supplementation strategies for improving buffalo production. The methods generally used for determining microbial protein production depend on the use of natural microbial markers such as RNA (ribonucleic acid) and DAPA (diamino-pimelic acid) or isotopes ^{35}S, ^{15}N or ^{32}P (Martin-orue et al., 2000). These techniques are complex, tedious and difficult to practice extensively under field conditions and require ruminally and post ruminally fistulated animals (Broderick and Merchen, 1992). The method based on measurement of urinary purine derivatives (PD) over comes the problems of earlier methods. It is simple and non-invasive and has the potential to be further simplified for use under farm conditions (Ojeda and Parra, 1999). Since microbial enzymes in rumen rapidly degrade purines of dietary and exogenous materials, so any purines present in the digesta in the small intestine can be expected to be only of microbial origin and can be considered to be specific markers for the microbial fraction (Nolan, 1999). Prediction equations based on urinary purine derivatives (PD) excretion rate as an index to predict rumen microbial protein production have been developed for European cattle (Chen et al., 1990) and sheep (Verbic et al., 1990; Balcells et al., 1991; Fujihara et al., 1991). The important parameters required for this are daily PD excretion through urine, endogenous PD excretion and the proportion of plasma PD excreted in the urine, or the recovery of exogenous PD from the urine. However, the relationships between microbial yield of purines from the rumen and urinary excretion of purine derivatives may differ between different breeds and species of ruminants (IAEA, 1999). The present study was

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conducted to determine rumen microbial protein production at different levels of feed intake using urinary purine derivatives excretion and to explore the possibility of purine derivatives: creatinine (PDC) index in spot urine samples for measuring microbial protein supply in buffaloes.

MATERIALS AND METHODS

The study was conducted in two phases at Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh, India. It is located at 170 metres MSL (28°22'N and 79°24'E) having mean annual rainfall of 900-1,200 mm.

Phase 1: Feeding trial

Animal, housing and management : Four adult male Murrah buffaloes (*Bubalus bubalis*) above two years of age (BW 286.0±10.3 kg) were housed in well-ventilated shed under uniform management conditions. Animals were dewormed for endo and ecto parasites with Panacur (Fenbendazole) and Butox (Deltamethrin), respectively before commencing the experimentation. Fresh and clean drinking water was provided twice daily at 10:30 h and 14:30 h.

Feeds, feeding and experimental design : A concentrate mixture (CM) was formulated for animals feeding (maize 33, wheat bran 32, soybean meal 32, mineral mixture 02, common salt 01%). The CM was having 90.39, 23.75, 2.55, 9.34, 42.47, 12.42 and 9.61% organic matter, crude protein, ether extract, crude fibre, neutral detergent fibre, acid detergent fibre and total ash, respectively, whereas values in corresponding order in wheat straw were 91.72, 2.74, 1.09, 35.48, 86.75, 52.20 and 8.28%, respectively. As per the protocol (IAEA, 1997) animals were fed *ad libitum* a mixed diet of wheat straw and concentrate (40:60) individually for one-week preliminary period. The 'lowest level of intake' recorded during this period among all animals was set as 'Voluntary Intake'. The observed lowest level of feed intake was 4.98±0.15 kg/d (DM basis), which was termed as voluntary feed intake (VFI). Animals were grouped as per 4×4 Latin Square Design (LSD). The animals of different treatments were fed a mixed diet of wheat straw and concentrate mixture (40:60) at four fixed levels viz. 95, 80, 60 and 40% of VFI.

Metabolism trial and collection of feeds, urine and faeces samples : The experiment consisted of 21 days feeding period. Metabolism trial was conducted for last 8 days of each period during which total urine excreted and faeces voided were collected daily. The experimental animals were weighed (before feeding and watering) in the beginning and end of each period to record live weight changes during the study. Representative samples of feed offered were collected daily and stored in labeled polythene bags after drying for further analysis. The daily urine was collected into clean plastic containers containing approximately 500 ml of 10% H_2SO_4 to ensure that the final pH level remains below 3 to avoid precipitation of uric acid. A representative urine sample was taken as sub-sample and was mixed thoroughly and 20 ml aliquot was taken in two plastic vials and stored at -20°C for further analysis.

Spot urine collection : Spot urine collections were made during last 2 d of each feeding trial. Each day (24 h) was divided into four blocks at six hourly intervals, starting immediately before feeding (designated as 0 h). The first urine excreted by animals in each block was collected, measured and a fixed sample (15 ml) immediately transferred into plastic vials (each containing 5 ml of 10% H_2SO_4) and pH was adjusted below 3. Urine samples were stored in plastic vials in a freezer at -20°C for subsequent analysis.

Phase 2: Fasting trial

In phase 2, five adult male Murrah buffaloes (329.9±10.3 kg live weight) were used and fed a mixed diet of wheat straw and concentrate (40:60) at a fixed level of intake (1.70% of body weight) for 2 weeks (adaptation period).

Fasting trial protocol : The 15 days test period consisted of 7 days pre-fasting (1% DMI of the body weight); feed restriction within 2 days (60% and 30% of DMI of test period on 8 and 9 days) and followed by fasting (6 days). Animals were housed in metabolism cages during the test period to facilitate total collection of urine. Towards the end of pre-fasting, 3 blood samples (each about 20 ml) were taken in heparinized vials. Besides, one blood sample was drawn daily from jugular vein at a fixed time (10:00 h) during fasting period.

Chemical analysis

Dry matter, organic matter and ash contents of feeds and faeces were determined as per AOAC (1995). Urine samples were thawed and distilled water was added to dilute urine in such a way that concentration of PD in the final sample would fall within the range of standards (5 to 50 mg/L) used in the assays for both uric acid and allantoin. Purine derivatives and creatinine content in urine samples were analyzed using Schimadzu HPLC system equipped with a UV detector using C_{18} reversed phase column following the method of Resines et al. (1992). Purine derivatives (allantoin and uric acid) in plasma samples were estimated using colorimetric methods as described in IAEA (1997). Estimation of plasma creatinine based on the Jaffe reaction was done following the method of Folin and Wu as described by Hawk et al. (1976).

Measurement of target parameters

Purine derivatives:creatinine (PDC) index :

$$\text{PDC index} = [\text{PD}]/[\text{Creatinine}] \times W^{0.75}$$

Table 1. Mean body weight, DMI, digestible DMI (DDMI), organic matter intake (OMI), digestible OMI (DOMI) and digestibility of DM and OM in different levels of feed intake

Parameters	Treatments				
	L-95	L-80	L-60	L-40	SEM
Body weight					
kg	297.00	293.25	282.63	271.13	15.11
kg $W^{0.75}$	71.50	70.86	68.91	66.78	2.75
Intake (g/d)					
DM**	4747.1[a]	3985.7[b]	3000.6[c]	2015.3[d]	24.9
DDM**	2973.7[a]	2486.6[b]	1859.4[c]	1155.0[d]	70.7
OM**	4317.3[a]	3643.3[b]	2740.8[c]	1829.4[d]	23.1
DOM**	2880.8[a]	2382.1[b]	1769.4[c]	1131.5[d]	61.5
Intake (g/kg $W^{0.75}$/d)					
DM**	66.6[a]	56.3[b]	43.7[c]	30.3[d]	2.1
DDM**	41.8[a]	35.1[b]	27.1[c]	17.4[d]	2.1
OM**	60.6[a]	51.4[b]	39.9[c]	27.5[d]	2.0
DOM**	40.5[a]	33.7[b]	25.8[c]	17.0[d]	1.8
Digestibility (%)					
DM*	62.6[a]	62.4[a]	62.0[ab]	57.3[b]	2.3
OM*	66.7[a]	65.4[ab]	64.5[ab]	61.9[b]	2.1

[a, b, c, d] Values with different superscripts in a row differ significantly: * $p<0.05$; ** $p<0.01$.

Where, $W^{0.75}$ represents the metabolic body weight (kg) of the animal; Purine derivatives and creatinine were concentration (mmol/L) in urine.

Glomerular filtration rate (GFR) :

$$\text{GFR (L/d)} = \frac{\text{Urinary creatinine excretion (mmol/d)}}{\text{Plasma creatinine concentration (mmol/L)}}$$

Renal clearance of purine derivatives :

Tubular load of allantoin (mmol/d)
= GFR (L/d)×plasma allantoin concentration (mmol/L)

Net reabsorption of allantoin (mmol/d)
= Tubular load of allantoin-excretion in urine (mmol/d)

Tubular load of uric acid (mmol/d)
= GFR (L/d)×plasma uric acid concentration (mmol/L)

Net re-absorption of uric acid mmol/d)
= Tubular load of uric acid-excretion in urine (mmol/d)

Microbial N supply : Microbial nitrogen supply was calculated using endogenous PD values obtained in phase II and using that data in equation given below: For Buffaloes, $Y = 0.74X+$(endogenous PD $W^{0.75}$) Where $W^{0.75}$ represents the metabolic body weight (kg) of the animal. The slope of 0.74 in equation represents the recovery of absorbed purines as PD in urine (IAEA, 1999). The component within parenthesis represents the net endogenous contribution of PD to total excretion after correction for the utilization of microbial purine by the animal.

Daily purine absorption : The calculation of daily purine absorption (X, mmol/d) was done by using equation: $X = (Y\text{-Endogenous contribution}\times W^{0.75})/0.74$, Where Y represents daily urinary PD (mmol/d) excretion.

Intestinal flow of microbial N : The following factors were used for the calculation of intestinal flow of microbial N (g N/d) from the microbial purine absorbed (X, mmol/d) as described by Chen and Gomes (1992).

i) Digestibility of microbial purines is assumed to be 0.83.
ii) The N content of purines is 70 mg N/mmol.
iii) The ratio of purine N:total N in mixed rumen microbes is taken as 11.6:100.

$$\text{Microbial N (gN/d)} = 70X/(0.116\times0.83\times1{,}000) = 0.727X$$

The above method for the calculation of protein supply from purine absorption presumes that the purine: protein ratio in mixed rumen microbes remains unchanged by dietary treatments (Chen and Gomes, 1992; IAEA, 1999).

Statistical analysis

Statistical analysis of data was done as per Snedecor and Cochran (1994). The data obtained from trial in Phase I was subjected to analysis of variance (ANOVA) ignoring the period effect. The excretion rates of urinary PD, N and PDC index (*spot urine*) were regressed against their respective digestible organic matter intake (DOMI) using linear regression analysis. Paired samples t-test procedure was used to compare the target parameters during pre-fasting and fasting period.

Table 2. Urinary purine derivatives, creatinine excretion and PDC index at different levels of feed intake

Parameters	Treatments				
	L-95	L-80	L-60	L-40	SEM
Allantoin					
mmol/d**	20.13[a]	16.00[b]	12.96[c]	9.17[d]	0.86
mmol/kg $W^{0.75}$/d*	0.282 [a]	0.226 [b]	0.188[c]	0.138[d]	0.004
Uric acid					
mmol/d	1.88	2.12	2.11	2.15	0.74
mmol/kg $W^{0.75}$/d	0.026	0.030	0.031	0.033	0.011
Total PD					
mmold/d*	22.01[a]	18.12 [b]	15.07 [c]	11.32 [d]	1.40
mmol/kg $W^{0.75}$/d*	0.308[a]	0.256[b]	0.219[b]	0.171[c]	0.002
Creatinine					
mmol/d	52.41	50.88	48.37	43.45	6.56
mmol/kg $W^{0.75}$/d	0.728	0.719	0.703	0.656	0.090
PDC index*	30.47[a]	25.92[b]	21.56[bc]	17.41[c]	2.01

[a, b, c, d] Values with different superscripts in a row differ significantly: * $p<0.05$; ** $p<0.01$.

RESULTS AND DISCUSSION

Intake and digestibility

The total DM and OM intake was significantly ($p<0.01$) different among 4 treatments (Table 1). The digestibility of DM was significantly lower in L-40 compared to L-95 and L-80. Similar observations were recorded earlier in swamp buffaloes (Ngoan et al., 2001). The OM digestibility was significantly ($p<0.05$) higher in L-95 compared to L-40, this might be owing to higher energy consumption in L-95. Similar, observations were also recorded by Sivaiah (1979) in lactating buffaloes fed two levels of energy (100 and 120%) and three levels of protein (80, 100 and 110%) as per NRC standard.

Response of PD excretion to feed intake

The daily urinary PD excretions at different levels of feed intake are presented in Table 2. The PD excretion (mmol/d or mmol/kg $W^{0.75}$/d) responded significantly to intake (kg/d or kg $W^{0.75}$/d) of DM and DOM. A significant ($p<0.01$) increase in allantoin and non-significant response in uric acid excretion in urine was observed with respect to increase in feed intake. This is in agreement with results of earlier workers (Liang et al., 1994; Chen et al., 1996; Nolan, 1999). The presence of xanthine and hypoxanthine in buffalo urine could not be detected and their concentration might have been below the detectable levels owing to higher activity of xanthine oxidase in the intestine and plasma (Chen et al., 1996; Nolan, 1999; Pimpa et al., 2003). Total PD excretion (mmol/d) increased linearly with level of intake (DM and OM) and the following equations were developed: $Y = 3.65X+0.06$ ($R^2 = 0.79$) where, Y = PD excretion (mmol/kg $W^{0.75}$/d), X = DMI (kg $W^{0.75}$/d); $Y = 5.49X+0.08$ ($R^2 = 0.77$) where, Y = PD excretion (mmol/kg $W^{0.75}$/d), X = DOMI (kg $W^{0.75}$/d). The values obtained for buffaloes were lower compared to that of *Bos taurus* cattle (18.5 mmol PD/kg DOMI) as reported by Giesecke et al. (1993). Daniels (1993) also found similar value (18.4 mmol PD/kg DOMI) for European cattle. The results of present study corroborated well with the observations of Vercoe (1976) and Liang et al. (1993) who found lower PD excretion per unit of feed intake in buffaloes in comparison to cattle. This might be due to a higher non-renal route of PD disposal in buffaloes or due to a higher recycling of plasma PD, but the mechanism of buffaloes excreting less PD in comparison to cattle is yet to be understood (Chen and Orskov, 2003). The various regression equations developed based on the results of this experiment suggested that urinary PD excretion is closely related with DMI or DOMI ($R^2 = 0.76$ to 0.82) and may be used as an index to predict feed intake in the field conditions. Daily allantoin excretion corroborated well with the values reported by Liang et al. (1994) for Swamp buffaloes (12.8 mmol) fed 1.5% DM of body weight, by Chen et al. (1996) for Murrah Swamp buffaloes (9.6 to 23.5 mmol) and by Pimpa et al. (2003) for Malaysian Swamp buffaloes (10.9 to 20.6 mmol). The molar proportion of allantoin:uric acid obtained in this study is consistent with values of 0.90:0.10 (Chen et al. 1996) and 0.84:0.16 (Pimpa et al., 2003) previously observed in buffaloes. Daily uric acid excretion in the present study (1.9 to 2.2 mmol) was also within range (1.5 to 4.2 mmol) as reported earlier for buffaloes (Liang et al., 1994; Moscardini et al., 1999).

Urinary excretion of creatinine (mmol/kg $W^{0.75}$/d) did not differ significantly ($p>0.05$) among various treatments. This is in agreement with the findings in Malaysian Kedah Kelantan cattle (Pimpa et al., 2001) and buffaloes (Chen et al., 1996). A similar study suggested that total daily creatinine excretion in urine is breed/species specific and more closely correlated with muscle mass than body weight (Narayanan and Appleton, 1980). The value obtained in the present study occupied an intermediate position (0.66 to 0.73 mmol/kg $W^{0.75}$) compared to the earlier findings of Chen et al. (1996) and Pimpa et al. (2003). The extrapolated

Table 3. Daily glomerular filtration rate (GFR), creatinine excretion in urine and plasma, tubular load and re-absorption of purine derivatives (PD) in different levels of feed intake

Parameters	Treatments				
	L-95	L-80	L-60	L-40	SEM
Plasma (μmol/L)					
Allantoin**	132.72[a]	118.81[b]	102.59[c]	74.13[d]	3.67
Uric acid	11.40	13.38	13.16	11.91	2.10
PD*	144.12[a]	132.19[b]	116.25[c]	86.03[d]	4.03
Creatinine	87.52	92.79	89.76	90.12	6.91
Urine (mmol/d)					
Creatinine	52.41	50.88	48.37	43.45	6.56
GFR					
(L/d)	593.09	546.66	539.72	483.51	50.89
($L/W^{0.75}$/d)	8.28	7.73	7.83	7.28	0.71
Tubular load (mmol/d)					
Allantoin*	78.71[a]	64.51[b]	55.36[b]	35.86[c]	5.24
Uric acid	6.92	7.28	7.30	5.66	1.32
PD*	85.63[a]	71.79[b]	62.66[b]	41.52[c]	5.98
Re-absorption (mmol/d)					
Allantoin*	58.58[a]	48.51[b]	42.21[b]	26.69[c]	4.59
Uric acid	5.04	5.17	5.19	3.51	0.80
PD**	63.62[a]	53.68[ab]	47.60[b]	30.21[c]	5.09
Re-absorption (%)					
Allantoin	74.32	75.02	76.49	74.26	1.42
Uric acid	74.54	70.93	72.83	63.48	7.00
PD	74.22	74.60	75.88	72.68	1.73

[a, b, c, d] Values with different superscripts in a row differ significantly: * $p<0.05$); ** $p<0.01$.

basal PD excretion (4.80 mmol/d) for buffaloes used in this experiment was about 43% lower than the observed value (8.35 mmol/d) obtained from the fasting trial. This may probably due to higher *de novo* synthesis of purines (creating purines from amino acids) during fasting period to replace endogenous purine loss (Chen and Orskov, 2003).

Glomerular filtration rate (GFR) and renal clearance of PD

As in the case of urine, the salvageable purine derivatives like xanthine and hypoxanthine could not be detected in the plasma. The plasma concentration of allantoin, uric acid and creatinine, estimated for calculation of GFR and renal clearance are depicted in Table 3. The concentration of allantoin in plasma at different levels of feed intake was significantly ($p<0.01$) different but uric acid in plasma was similar ($p>0.05$) among the 4 different levels of feed intake. The total PD concentration in plasma was significantly ($p<0.05$) different among 4 treatments. Plasma concentration of creatinine remained non-significant among 4 treatments. Although, the GFR (L/d) of buffaloes remained statistically similar at different levels of feed intake, the values reduced in accordance with reduction in feed intake.

The plasma concentration of allantoin and total PD decreased with the reduction in feed intake. This is corroborated with the findings of Soejono et al. (1999) on Bali and Ongole cattle; Liang et al. (1999) on buffaloes and Singh (2004) on crossbred cattle. However, uric acid concentration in plasma remained similar and was not affected by the levels of feed intake. Similarly, Soejono et al. (1999) also observed in Bali and Ongole cattle and Liang et al. (1999) in Malaysian swamp buffaloes. The GFR was 2 to 2.5 times higher than that recorded in the fasting trial. The value obtained occupied an intermediate position compared to the earlier findings of Liang et al. (1996) (184 to 197 L/d) and Pimpa et al. (2003) (701 to 873 L/d). Norton et al. (1979) observed that buffaloes had a lower GFR than cattle at the same feed intake and body weight. Similarly, Chen et al. (1996) also suggested that buffaloes have a mechanism of partitioning plasma PD between renal excretion and non-renal disposal and hence a low glomerular filtration rate has been observed. However, observations made by Pimpa et al. (2003) contradicted the findings of Norton et al. (1979) and Chen et al. (1996). The tubular load and re-absorption of allantoin and consequently total PD (Table 3) was significantly ($p<0.05$) higher at dietary levels of L-95, L-80 and L-60 compared to L-40. Similar observations were recorded earlier in KK cattle (Liang et al., 1999) and Bali and Ongole cattle (Soejono et al., 1999).

PDC Index in spot urine samples

The relationship of PDC index in urine with DOMI (kg/d) at different levels of feed intake in buffaloes is explained by the linear equation: $Y = 7.32X+8.91$ (R^2 =

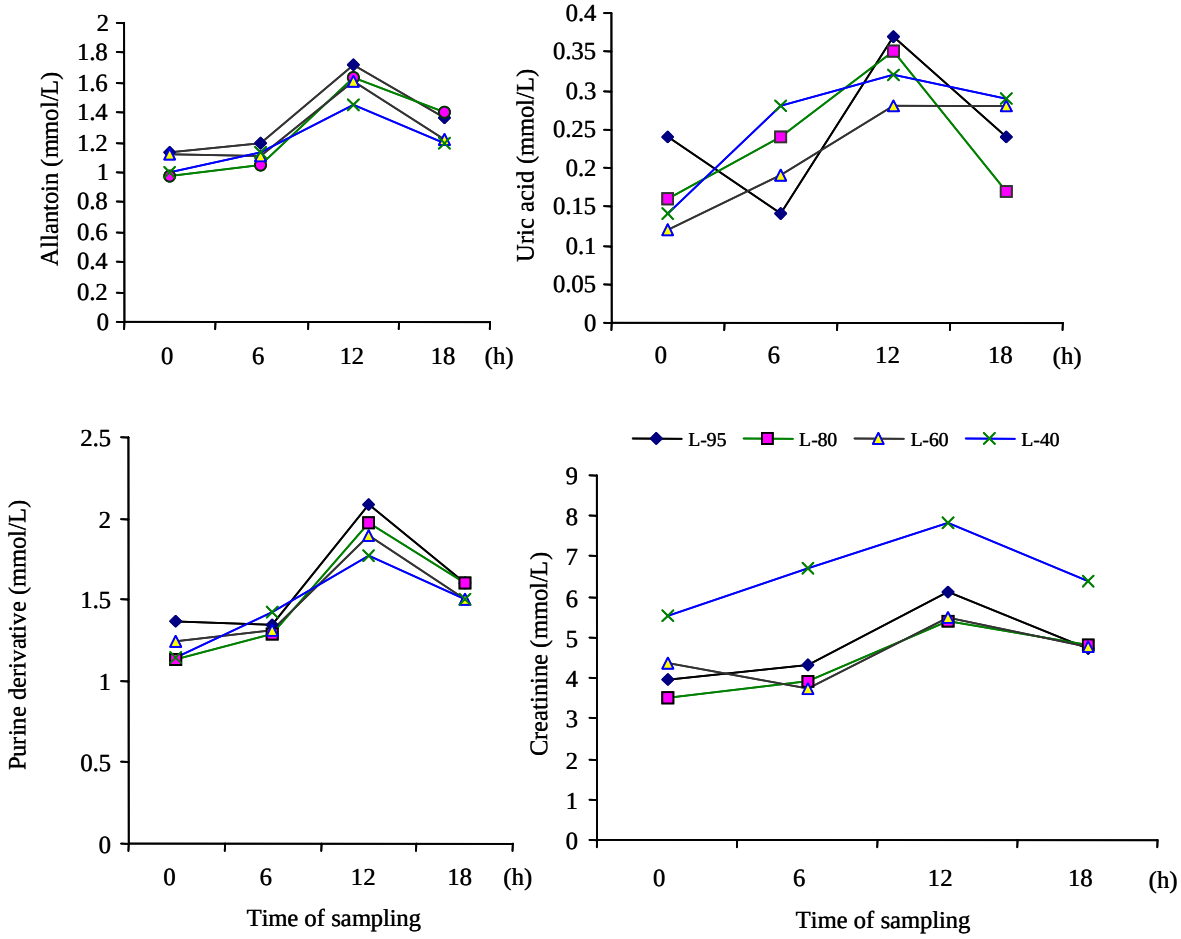

Figure 1. Concentration of urinary allantoin, uric acid, total purine derivative (PD) and creatinine as affected by post feeding at time different levels of feed intake.

0.78). The mean concentration of allantoin, uric acid, total PD and creatinine in spot urine samples of buffaloes at different levels of feed intake is depicted in Figure 1. There was significant interaction of level of feed with period, for values of allantoin ($p<0.01$), uric acid ($p<0.05$), total PD ($p<0.01$) and creatinine ($p<0.01$) during this trial. The concentration of allantoin and total PD was significantly ($p<0.05$) different between L-95 and L-40, however, uric acid concentration remained similar (Figure 1).

The ratio of PD:C (PDC index) was relatively constant. The diurnal fluctuations noted for urinary concentration of allantoin, uric acid and consequently total PD and creatinine in spot samples could have been due to the influence of urine volume associated with that of drinking water intake (Chen et al., 1992). The potential of PDC index in spot urine samples as an index to measure absorption of microbial purines was examined and found that the time of collection of samples did not affect PDC index. Thus, it is a suitable for use as an alternative to measure daily PD excretion and microbial protein supply (Rys et al., 1975; Chen et al., 1991). However, variability attached to these measurements in spot urine determines their sensitivity if they are to be used as an index for comparison between treatments (Shingfield and Offer, 1998). The results obtained indicated that the use of PD in spot samples as an index of microbial protein supply is only suitable for detecting relatively large differences, and more precisely when animals are at low plane of nutrition (Figure 1). The results also indicated that a better estimate of variability in plane of nutrition was reflected when sample for measurement of PD concentration was taken at 12 h post feeding. However, the mean of multiple spot samples may reflect more accurately the standing level of purine excretion for the animal on a particular feeding regime than collecting fewer samples over several days (Shingfield and Offer, 1998). The relationship of PDC index in spot urine with DOMI (kg/d) of experimental animals explained by the linear equation given below: $Y = 4.75X+12.36$ ($R^2 = 0.75$).

Endogenous purine derivatives excretion during pre-fasting and fasting

Endogenous purine derivatives excretion during pre-fasting and fasting has been given in Table 4. The excretion

Table 4. Daily urinary excretion of purine derivatives (PD) and creatinine during pre-fasting and fasting[1]

Parameters	Pre-fasting	Fasting	SEM
Allantoin			
mmol/d**	18.40	7.21	1.90
μmol/ kg $W^{0.75}$/d**	238.40	101.00	26.88
Uric acid			
mmol/d**	2.88	1.13	0.51
μmol/ kg $W^{0.75}$/d*	37.60	16.00	7.90
Total PD			
mmol/d**	21.28	8.35	2.17
μmol/ kg $W^{0.75}$/d**	276.00	117.00	32.30
Creatinine			
mmol/d	38.31	32.65	7.31
μmol/ kg $W^{0.75}$/d	496.4	456.4	106.91

[1] Based on last 5 days of fasting * p<0.05); ** p<0.01.

of PD was higher (p<0.01) during pre-fasting compared to fasting period. This clearly indicated that the excretion of PD was directly proportional to digestible organic matter intake. Chen et al. (1990) hypothesized that the endogenous contribution of PD decreased as the supply of exogenous purines increased, with an associated progressive replacement of *de novo* synthesis from exogenous sources. During fasting period, the excreted allantoin and uric acid has been considered as endogenous in nature, therefore, their measurement is important in modelling of the PD excretion (Walker and Faichney, 1964; McAllan and Smith, 1973). Restriction of feed led to a rapid decrease in urinary excretion of allantoin and total PD, reaching a stable base value after about 3 days. This value (117 μmol/kg $W^{0.75}$/d) was considered as the endogenous contribution to urinary excretion of PD, assuming that duodenal flow of purine bases would represent only a minor fraction (Ojeda and Parra, 1999). The endogenous PD excretion in the animals was slightly lower compared to the reported values (200 μmol/kg $W^{0.75}$/d) obtained for buffaloes (Chen et al., 1996). However, Liang et al. (1999) reported a very high value (370 μmol/kg $W^{0.75}$/d) in Swamp buffaloes in a study conducted in Malaysia. The endogenous PD excretion in buffaloes is very low compared to values previously reported for European cattle (530 μmol/kg $W^{0.75}$/d) (Chen et al., 1990; and 485 μmol/kg $W^{0.75}$/d, Verbic et al., 1990). In Indian crossbred cattle (Singh, 2004), the endogenous PD excretion was lower compared to European cattle (296 μmol/kg $W^{0.75}$/d). The fasting nitrogen excretion measured in the present study was 311 mg/kg $W^{0.75}$/d, however, Chen et al. (1996) reported it as 257 mg/kg $W^{0.75}$/d. These values were lower than endogenous excretion for cattle (350 mg nitrogen/kg $W^{0.75}$/d) (ARC, 1984). This may be due to an inherent mechanism in buffaloes to recycle more nitrogen to rumen via saliva than cattle (Kennedy et al., 1992), thus, PD can also be recycled into rumen in this way and such recycled PD could not be recovered in urine (Chen et al., 1990). Allantoin comprised the major component (80 to 89%) of PD, which is in agreement to the earlier values reported for buffaloes (Chen et al., 1996; Moscardini et al., 1999; Pimpa et al., 2003). The average daily urinary creatinine excretion (0.496 to 0.456 mmol/kg $W^{0.75}$ or 38.31 to 32.65 mmol) remained similar irrespective of level of feed during fasting trial. However, Liang et al. (1999) reported the urinary creatinine value as 27.18 to 21.34 mmol/d in Malaysian swamp buffaloes during pre-fasting and fasting periods. It is presumed that excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of feed intake (Chen et al., 1992; 1995). The creatinine excretion varies widely with the breed or species of animals but usually independent of the level of intake or duodenal infusion of purine bases (IAEA, 2002).

Table 5. Daily glomerular filtration rate (GFR), creatinine excretion in urine and plasma, tubular load and re-absorption of purine derivatives (PD) during pre-fasting and fasting[1]

Parameters	Pre-fasting	Fasting	SEM
Plasma (μmol/L)			
Allantoin**	205.86	133.23	7.06
Uric acid**	27.33	17.43	2.62
PD**	233.19	150.67	9.36
Creatinine**	95.45	138.88	6.40
Urinary creatinine (mmol/d)	38.31	32.65	7.31
GFR			
L/d**	402.42	236.86	62.97
L/kg $W^{0.75}$/d**	5.20	3.31	0.88
Tubular load (mmol/d)			
Allantoin**	82.91	31.21	9.59
Uric acid**	11.03	3.97	1.37
PD**	93.95	35.19	10.82
Re-absorption (mmol/d)			
Allantoin**	64.52	24.00	8.73
Uric acid**	8.15	2.84	1.27
PD**	72.67	26.84	9.72
Re-absorption (%)			
Allantoin	77.76	75.51	4.76
Uric acid	73.53	71.75	7.20
PD	77.26	75.20	4.21

[1] Based on last 5 days of fasting, ** p<0.01.

GFR and renal clearance of PD during pre-fasting and fasting

In the present trial, the plasma concentration of allantoin, uric acid and total PD declined significantly from pre-fasting period to fasting resulting in simultaneous reduction of renal clearance expressed in terms of tubular load and re-absorption of PD (Table 5). Similar findings were reported earlier in studies in Malaysian swamp buffaloes (Liang et al., 1999) and in Indian crossbred cattle (Singh, 2004). The elevated creatinine concentration of plasma during fasting period resulted in variable GFR. As observed, GFR may change with feed intake (Chen et al., 1995; Kagiyama et al., 1996), therefore, plasma PD is not considered an

Table 6. Estimated (developed model) and calculated (spot urine samples) microbial N supply at different levels of feed intake

Parameters	Treatments				
	L-95	L-80	L-60	L-40	SEM
Microbial-N supply (Derived from model developed in the experiment)					
g N/d*	13.41[a]	9.65[b]	6.88[b]	3.44[c]	1.44
g N/kg DOMI	4.67	4.06	3.92	3.01	0.84
Microbial-N supply (Calculated from spot urine sample)					
g N/d*	9.08[a]	9.84[a]	8.03[a]	2.43[b]	2.27

[a, b, c] Values with different superscripts in a row differ significantly: * $p<0.05$.

appropriate index of microbial protein supply unless changes in GFR are also taken into account (Chen and Orskov, 2003). A significant reduction in GFR and PD excretion rates during fasting was also reported for Kedah-Kelantan, Bali, Ongole and crossbred cattle (Liang et al., 1999; Soejono et al., 1999; Singh, 2004) mainly due to increase in creatinine concentration of plasma due to fasting, which is confirmed by present study.

Microbial nitrogen supply

The estimated microbial nitrogen (MN) supply (g N/d or g N/kg DOMI) to buffaloes, at different levels of feed intake is given in Table 6. Based on the PD excretion rates during fasting and at different levels of feed intake, a relationship between daily urinary PD excretion (Y mmol) and daily microbial purine absorption (X mmol) was developed for buffaloes as: $Y = 0.74X+0.117$ kg $W^{0.75}$. The MN supply was the highest in L-95 and decreased significantly ($p<0.05$) with reduction in feed intake. This was further reaffirmed in Indian crossbred cattle (Singh, 2004). However, the MN supply when expressed per kg DOMI remained statistically similar irrespective of level of intake. The microbial nitrogen supply calculated by PD excretion derived from PDC index was compared with MN supply estimated using urinary PD excretion values of total urine. The results indicated that MN supply (g N/d) calculated by either total urine collection or spot urine was almost similar for animals at L-80 (9.65 vs. 9.84) and L-40 (3.44 vs. 2.43). The results showed that urinary PD technique could be used to determine MN supply when there is large variation in feed intake.

CONCLUSION

The results showed that excretion of urinary purine derivatives is positively correlated with the levels of feed intake in Murrah buffaloes and thus, estimation of urinary purine derivatives and PDC index could be used to determine microbial nitrogen supply when there is large variation in level of feed intake.

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