



Estimation of Rumen Microbial Protein Supply Using Urinary Purine Derivatives Excretion in Crossbred Calves Fed at Different Levels of Feed Intake

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ABSTRACT : A study was carried out to study the response of total purine derivatives (PD) excretion in urine to determine microbial N (MN) supply at four fixed levels of feed intake (namely 95, 80, 60 and 40% of voluntary intake). The crossbred (CB) calves were allocated according to a 4x4 Latin Square Design and fed wheat straw and concentrate (1:1). The rate of PD excretion (mmol/d) as a linear function of feed intake was 15.85/kg DMI and 20.12/kg DOMI. Based on the endogenous and PD excretion rates obtained in this study, a relationship between daily urinary PD excretion (Y, mmol) and daily microbial protein supply (X, mmol) was developed for crossbred calves as $Y = 0.83X + 0.296 \text{ kg W}^{0.75}$. The derived microbial N values using this equation differed ($p < 0.001$) among the 4 groups and was the highest in L-95 followed by L-80, L-60 and L-40. The relationship between urinary nitrogen loss (Y, g/d) and DOMI (X, kg/d) was established as: $Y = 6.038X + 21.753$ ($r^2 = 0.663$, $p < 0.01$). When urinary excretion of PD (Y, mmol/d) was plotted against intake of DM and DOM (X, kg/d), the equations obtained were: $Y = 7.1711X + 8.674$ ($r^2 = 0.889$, $p < 0.01$) and $Y = 12.434X + 7.683$ ($r^2 = 0.896$, $p < 0.01$), respectively. The proportional contribution of allantoin and uric acid to total PD remained stable irrespective of level of feed intake. Similarly, urinary excretion of creatinine did not differ ($p > 0.05$) between animals fed at different levels. The MN supply was the highest to animals at intake levels L-95, and decreased linearly with corresponding decrease in feed intake. However, the MN supply when expressed per kg DOMI remained statistically ($p > 0.05$) similar irrespective of level of intake. The results revealed that the excretion of urinary purine derivatives were positively correlated with the level of feed intake as well as rumen microbial supply and thus it could be a good indicator for measuring the microbial protein supply and nutritional status of animals. (**Key Words :** Crossbred Calves, Levels of Feed Intake, Purine Derivatives, Microbial Protein Supply)

INTRODUCTION

The ruminants in developing countries are reared under mixed farming systems especially on a basal diet of cereal straws having poor digestibility, low protein and high fibre content. Hence, any strategy for improvement in livestock production requires efforts to maximize the efficiency of utilization of basal feed resources in the rumen by appropriate supplementation to meet the requirement of rumen microbes. Microbial cells formed as a result of a rumen digestion of fibrous crop residues under anaerobic conditions are a major source of protein for ruminants. They provide the majority of amino acids that the host animal requires for tissue maintenance, growth and production. In roughage fed ruminants, microorganisms are virtually the only source of protein. Therefore, microbial contribution to the nutrition of the host animal is paramount for improving

ruminants' production.

Most of existing methods that are used to measure microbial protein supply are microbial markers ribonucleic acid (RNA) and di-amino-pimilic acid (DAPA) or isotopes (^{35}S , ^{15}N , ^{32}P). These techniques require ruminally and post ruminally fistulated animals. Moreover, the processes of utilizing markers or isotopes are complex, tedious and difficult to practice extensively, especially under field conditions (Broderick and Merchen, 1992). Rumen microorganisms have high concentration of purine containing compounds (RNA and DNA) and the concentration of these compounds in plants and animal cells is negligible. Therefore, any purines present in digesta in the small intestine is expected to be only of microbial origin and can be considered to be specific markers for the microbial fraction. It has been suggested that the estimation of purine derivatives (xanthine, hypoxanthine, allantoin and uric acid) in urine could serve as a simple and non-invasive method of measuring the intestine flow of microbial protein to the animal (Chen et al., 1990; Verbic et al., 1990; Susmel

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et al., 1994). Since only urine is needed, an estimation of microbial protein supply can be incorporated into nitrogen balance and digestibility trials without much additional labour inputs. Appropriate models for the estimation of microbial protein supply by urinary purine derivative (PD) excretion have been established for European breeds of cattle, however, limited available information suggests that the current prediction models may not be wholly suitable for tropical breeds and need to be adjusted to improve the precision of prediction (International Atomic Energy Agency (IAEA), 1997, 2002). The potential of the spot urine sampling technique as an alternative to perform a total urine collection to predict microbial nitrogen supply has been evaluated in crossbred bulls (George et al., 2006). Keeping this background in-view, the present study was undertaken to validate the potential value of urinary PD excretion technique as a tool for estimating microbial protein supply in crossbred calves for its possible adaptation and use in Indian livestock.

MATERIALS AND METHODS

Animal experimentation was carried out at Animal Nutrition Shed of Indian Veterinary Research Institute, Izatnagar in Uttar Pradesh Province of India. It is located at 170 m above sea level (28°22'N and 79°24'E) in the northern upper Gangetic plain, having an annual rainfall of 900-1,200 mm.

Animal, housing and management

Four male crossbred (CB) calves (*Bos indicus* × *Bos taurus*) of about 18 months of age (body weight: 184.5 ± 5.20 kg) were selected for this study. All the experimental animals were housed in a well-ventilated shed under uniform management conditions. All the animals were dewormed for endo and ecto parasites with Fenbendazole (orally) and Butox (Deltamethrin by Hoechst, India) liquid spray, respectively before the start of experimental feeding. Fresh and clean drinking water was made available *ad libitum* twice daily at 10.30 and 14.30 h.

Feeds and feeding

A concentrate mixture was formulated (Maize 33, Wheat bran 32, Soybean meal 32, Mineral mixture 02, Common salt 01 kg/100 kg as fed basis) for feeding of animals throughout the trial. As per the protocol (IAEA, 1997), animals were fed *ad libitum* a mixed diet of wheat straw and concentrate (1:1) individually for a week preliminary period. The 'lowest DM intake' recorded during this period in the animals was considered as 'Voluntary Feed Intake (VFI)'. To enable the animals to consume all the feed offered they were provided 95% DM of the VFI. The observed lowest DM intake was 4.26 ± 0.05 kg/d (VFI).

On the basis of VFI, the quantities offered to animals at different levels were decided as L-95 (95% VFI)-4.04 kg DM, L-80 (80% VFI)-3.40 kg DM, L-60 (60% VFI)-2.55 kg DM and L-40 (40% VFI)-1.70 kg DM.

Experimental design

A 4 × 4 latin square design (LSD) was used for this experiment. The experiment consisted of four 21-day feeding periods and four feeding levels. During last 8 days of each period (test period), total urine and faeces were collected daily. The animals were weighed (before feeding and watering) in the beginning and end of each period to record live weight changes during the study.

Metabolism trial and collection of feed, faeces and urine samples

Two days prior to the collection of samples, animals were placed in individual metabolism cages and the daily out put of urine and faeces was recorded. The animals were offered a mixed diet of roughage and concentrate (1:1) individually in the morning at 10:00 AM. The metabolism cages were having a facility to collect urine under acidic condition by adding 500 ml sulphuric acid (10%) and faecal samples separately. To ensure clean separation of faeces and urine, the screens were cleaned daily and also the angle and position of the collector was suitably adjusted. Moreover, workers were assigned duty round the clock to collect faeces and urine samples as when animals voided them. Representative samples of feed offered were collected daily and dried at 100 ± 2°C in a hot air oven to constant weight to estimate their dry matter content. The samples for each period were pooled and stored in labeled polythene bags at room temperature for further analysis. The acid was added as preservative ensuring that the final pH level remains below 3. At the time of sampling, the pH of the urine was again checked and adjusted accordingly. A representative urine sample was taken as sub-sample and was mixed thoroughly and 20-ml aliquot was taken in two plastic vials for preservation at -20°C until further analysis.

Collection of plasma samples

At least two blood samples (20 ml each) were drawn from jugular vein from each animal during each collection period at 5th and 10th day. The samples were collected in heparinized vacutainers. Tubes were gently inverted a couple of times after sampling. The samples were centrifuged at 1,500 g for 20-30 min for plasma separation. Plasma was transferred into two-labelled storage tubes (5-6 ml in each tube/sample) and stored at -20°C for further analysis.

Feed and faeces analysis : Dry matter in feed and faeces samples was analyzed by drying samples in hot air oven at 100 ± 2°C overnight and organic matter content was

determined by ashing a sample for 4 h in a muffle furnace at 550°C (AOAC, 1995). Accordingly the measurement of digestibility of DM and OM was done.

Chemical analysis of urine and plasma samples : 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 (10-50 mg/L) used in the assays for both uric acid and allantoin. Purine derivatives (allantoin and uric acid) in urine and plasma samples were also estimated using colorimetric methods (IAEA, 1997). Estimation of urine and plasma creatinine was done based on the Jaffe reaction following the method of Folin and Wu as described by Hawk et al. (1976).

Calculations of target parameters

Purine derivatives :

Urine

$$\text{PD (mmol/d)} = \text{Allantoin (mmol/d)} + \text{Uric acid (mmol/d)}$$

Plasma

$$\text{PD (mmol/L)} = \text{Allantoin (mmol/L)} + \text{Uric acid (mmol/L)}$$

PDC index

$$\text{PDC index} = (\text{PD/Creatinine}) \times \text{kg W}^{0.75}$$

Where $\text{kg W}^{0.75}$ represents the metabolic body weight (kg) of the animal. (PD) and (Creatinine) were PD and creatinine concentration in mmol/L in urine.

Glomerular filtration rate :

$$\text{GFR (L/d)} = \frac{\text{creatinine excretion in urine (mmol/d)}}{\text{plasma creatinine concentration (mmol/L)}}$$

Renal clearance of purine derivatives :

Tubular load of allantoin (mmol/d)

$$= \text{GFR (L/d)} \times \text{plasma allantoin concentration (mmol/L)}$$

Net re-absorption of allantoin (mmol/d)

$$= \text{tubular load (mmol/d)} - \text{excretion in urine (mmol/d)}$$

Tubular load of uric acid (mmol/d)

$$= \text{GFR (L/d)} \times \text{plasma uric acid concentration (mmol/L)}$$

Net re-absorption of uric acid (mmol/d)

$$= \text{tubular load of uric acid (mmol/d)} - \text{excretion in urine (mmol/d)}$$

Microbial N supply : Microbial nitrogen supply was calculated using endogenous PD values obtained in phase I and compared with the values derived from the model developed for European Cattle (Verbic et al., 1990), by using equations given below:

$$\text{European cattle } Y = 0.85X + (\text{endogenous PD kg W}^{0.75})$$

Crossbred cattle Y

$$= 0.83X + (\text{endogenous PD kg W}^{0.75})$$

(Ojeda and Parra, 1999)

Where $\text{kg W}^{0.75}$ represents the metabolic body weight (kg) of the animal. The slope of 0.85 and 0.83 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. In European cattle, the endogenous contribution is taken as a constant at 0.385 mmol/kg $\text{W}^{0.75}$ per day (Verbic et al., 1990).

Daily purine absorption and microbial protein supply :

PD absorbed (mmol/d)

$$= (\text{daily urinary PD (mmol/d)} - 0.296 \text{ kg W}^{0.75}) / 0.83 \quad (1)$$

Where, 0.296 (mmol/kg $\text{W}^{0.75}$ per day) was taken as a constant for endogenous contribution as determined by Singh (2004) and 0.83 is the recovery of the absorbed purines as PD in urine (IAEA, 1999).

Then, intestinal flow of microbial nitrogen (N) was calculated using the following equation: (Chen and Gomes, 1992; IAEA, 1997).

Microbial N (g N/d)

$$= 0.727 \times \text{Microbial purine absorbed (mmol/d)} \quad (2)$$

Microbial N (gN/d)

$$= \frac{\text{Microbial purine absorbed (mmol/d)} \times 70}{0.116 \times 0.83 \times 1000}$$

Where,

- i) Digestibility of microbial purines is assumed to be 0.83. This is taken as the mean digestibility value for microbial nucleic acids on observations reported in the literature (IAEA, 1999),
- 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.

The above method for the calculation of microbial N 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

The data obtained from the trials were analysed manually following two-way analysis of variance (ANOVA) ignoring the period effect (Snedecor and Cochran, 1994). The excretion rates of urinary PD and N were regressed against their respective intake of dry matter (DMI) and digestible organic matter (DOMI).

Table 1. Body weight, intake and digestibility of dry matter and organic matter in different groups

Parameter	Level of feed intake				SEM	Significance
	L-95	L-80	L-60	L-40		
Body weight						
kg	192.4	192.2	183.8	176.7	4.75	NS
g/kg W ^{0.75} ¹	51.6	51.6	49.8	48.4	0.97	NS
Intake (kg/d)						
DM	4.0 ^d	3.5 ^c	2.6 ^b	1.7 ^a	0.22	***
DDM	2.5 ^d	2.2 ^c	1.7 ^b	1.1 ^a	0.14	***
OM	3.7 ^d	3.2 ^c	2.4 ^b	1.6 ^a	0.21	***
DOM	2.3 ^d	2.1 ^c	1.6 ^b	1.1 ^a	0.13	***
Intake (g/kg W ^{0.75} /d)						
DM	78 ^d	68 ^c	54 ^b	36 ^a	4.0	***
DDM	48 ^d	43 ^c	34 ^b	23 ^a	3.0	***
OM	71 ^d	62 ^c	49 ^b	33 ^a	4.0	***
DOM	46 ^d	42 ^c	33 ^b	22 ^a	2.0	***
Digestibility (%)						
DM	61.2	63.2	63.5	62.9	0.30	NS
OM	63.7	66.8	67.2	66.8	0.47	NS

¹ Gram per kilogram metabolic body weight.

a, b, c and d Mean values with the different superscripts in the same row are significantly different.

NS: Non significant (p>0.05); *** p<0.001.

Table 2. Nitrogen metabolism (g per day) at different levels of feed intake in various groups

Parameters	Level of feed intake				SEM	Sig.
	L-95	L-80	L-60	L-40		
Intake	77.1 ^d	68.0 ^c	51.9 ^b	33.9 ^a	4.39	***
Faecal loss	22.5 ^d	18.0 ^c	13.4 ^b	9.1 ^a	1.35	***
Urinary loss	35.7 ^b	35.3 ^b	31.0 ^a	28.2 ^a	0.96	**
N retained	18.9 ^c	14.7 ^c	7.5 ^b	-3.5 ^a	2.48	***

a, b, c and d Mean values with the different superscripts in the same row are significantly different.

** p<0.01; *** p<0.001.

RESULTS AND DISCUSSION

Feed intake and digestibility

The average body weight, feed intake of animals and digestibility of dry matter and organic matter are summarized in Table 1. The level of dry matter and organic matter intake was different (p<0.001) among the treatment groups. Similarly, intake of digestible dry matter and organic matter differed (p<0.001) between the groups. However, digestibility of dry matter and organic matter was not influenced by level of feed intake. It has been reported that digestibility of a feed is not a fixed trait but is modified by the level of its intake (Blummel et al., 2003). The digestibility is usually higher under restricted than under *ad libitum* (voluntary) feed intake (Van Soest, 1994). Contrary to this, George et al. (2006) found that the digestibility of DM and OM was significantly (P<0.05) lower in 80% and 60% of voluntary feed intake as compared to 120% and 100% VFI. However, the digestibilities were measured during this trial under restricted intake, and therefore remained independent of level of intake. Similar observations were recorded earlier in Bali and Ongole cattle (Soejono et al., 1999).

Nitrogen balance

The effect of level of feed intake on nitrogen balance of experimental animals is shown in Table 2. Faecal and urinary nitrogen loss (g/d) was affected (p<0.001) by level of feed intake. However, urinary loss of nitrogen was comparable between L95 and L80; L60 and L40, irrespective of level of feed intake. The nitrogen retention (g/d) decreased (p<0.001) when the feed intake was reduced, although no difference (p>0.05) was evident between dietary level L-95 and L-80. Nevertheless, except for the negative N-balance of animals given L-40, the animals remained in positive nitrogen balance. The relationship between urinary nitrogen loss (Y, g/d) and DOMI (X, kg/d) has been established and regressed. The equation obtained was:

$$Y = 6.038X + 21.753 \quad (r^2 = 0.663; p < 0.01) \quad (3)$$

Daily nitrogen intake and its out put in faecal and urine samples declined with level of feed intake. The faecal nitrogen comprises of undigested dietary nitrogen and endogenous nitrogen, and thus the faecal nitrogen losses get altered as per the level of N-intake and microbial nitrogen

Table 3. Excretion of urinary purine derivatives and creatinine at different levels of feed intake

Parameters	Level of feed intake				SEM	Significance
	L-95	L-80	L-60	L-40		
Allantoin						
mmol/d	33.1 ^d	28.5 ^c	23.1 ^b	18.8 ^a	1.50	***
mmol/ kg W ^{0.75} /d	0.63 ^c	0.56 ^{bc}	0.47 ^{ab}	0.39 ^a	0.027	**
Uric acid						
mmol/d	4.7 ^c	4.9 ^c	3.7 ^b	2.9 ^a	0.23	***
mmol/ kg W ^{0.75} /d	0.09 ^c	0.09 ^c	0.07 ^b	0.06 ^a	0.004	**
Total PD						
mmol/d	37.9 ^d	33.4 ^c	26.8 ^b	21.7 ^a	1.70	***
mmol/ kg W ^{0.75} /d	0.72 ^c	0.65 ^{bc}	0.54 ^{ab}	0.45 ^a	0.031	**
Creatinine						
mmol/d	39.3	39.5	35.5	38	0.63	NS
mmol/ kg W ^{0.75} /d	0.77	0.77	0.72	0.79	0.013	NS

^{a, b, c and d} Mean values with the different superscripts in the same row are significantly different.

NS : Non significant (p<0.05); ** p<0.01; *** p<0.001.

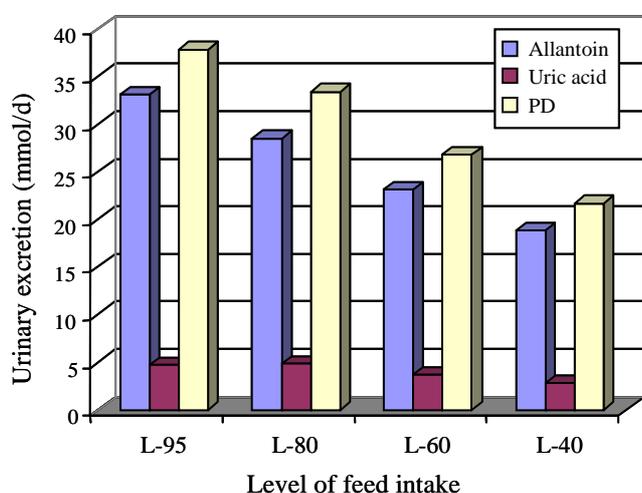


Figure 1. Proportional contribution of allantoin and uric acid to PD.

yield (McDonald et al., 1995). The urinary nitrogen excretion was different (p<0.01) among groups but it was comparable between L-95 and L-80, and L-60 and L-40 that may be due to increased efficiency of nitrogen utilization at corresponding lower level of intake. It is well established that animals try to adjust their nitrogen output at lower levels of intake to reach positive equilibrium (Dutta et al., 1999). However, a positive (p<0.01) correlation ($R^2 = 0.66$) was evident between excretion rates of nitrogen (g/d) and DOMI (kg/d). The extrapolated endogenous nitrogen excretion (g/d) value calculated by the derived regression equation ($Y = 6.038X + 21.753$) was 21.75, which was lower (26.9%) than the nitrogen excretion (29.7) recorded during the fasting period (Singh, 2004). This was probably due to higher nitrogen catabolism during fasting period compared to the level of protein used for production of glucose precursors (Kaneko et al., 1997) and *de novo* synthesis of purines.

Response of PD excretion to feed intake

The daily urinary excretion of PD at different levels of feed intake is shown in Table 3. The PD excretion increased (p<0.001) due to enhanced intake of DMI and DOMI among various groups. Allantoin was the main product of excretion and this was different (p<0.01) among the dietary levels. The urinary excretion of uric acid did not vary between dietary levels L-95 and L-80 but subsequently declined (p<0.001) with feed intake. Total PD excretion (mmol/d) was found to increase linearly with level of intake, however when corrected for metabolic weight basis (mmol/kg W^{0.75}/d), it followed similar trend as observed for allantoin. When urinary excretion of PD (Y) was plotted against DMI (X) and DOMI (Z), the following equations were obtained:

$$Y = 7.1711X_1 + 8.674 \quad (r^2 = 0.89; p < 0.01) \quad (4)$$

$$Y = 12.434Z_1 + 7.683 \quad (r^2 = 0.89; p < 0.01) \quad (5)$$

$$Y = 6.7586X_2 + 0.194 \quad (r^2 = 0.84; p < 0.01) \quad (6)$$

$$Y = 11.877Z_2 + 0.1696 \quad (r^2 = 0.87; p < 0.01) \quad (7)$$

Where Y = PD excretion (mmol/kg W^{0.75}/d), X₁ = DMI (kg/d), X₂ = DMI (kg W^{0.75}/d), Z₁ = DOMI (kg/d) and Z₂ = DOMI (kg W^{0.75}/d).

The proportional contribution of allantoin and uric acid to total PD remained stable irrespective of level of feed intake (Figure 1). This is in agreement with earlier observations recorded in crossbred bulls (George et al., 2006) and Murrah buffaloes (Dipu et al., 2006). Urinary excretion of creatinine did not differ (p>0.05) between animals fed at different levels as also observed by earlier workers (Kanjaputhipong and Leng, 1998, Dipu et al., 2006 and George et al., 2006). A positive relationship between urinary PD excretion rates and feed intake (DM, DOM) for

Table 4. Glomerular filtration rate, creatinine in urine and plasma, tubular load and re-absorption of purine derivatives at different levels of feed intake

Parameters	Level of feed intake				SEM	Significance
	L-95	L-80	L-60	L-40		
Plasma ($\mu\text{mol/L}$)						
Allantoin	259.5	287.5	228.0	193.8	10.88	NS
Uric acid	47.8	51.3	50.8	40.5	2.29	NS
PD	307.3	338.8	278.8	234.3	12.29	NS
Creatinine	96.8	98.8	102.0	99.5	1.42	NS
Urinary creatinine	39.3	39.5	35.5	37.9	10.49	NS
GFR						
(L/d)	406.8	403.5	349.0	381.8	10.49	NS
(L/kg $W^{0.75}$ /d)	7.9	7.8	7.1	7.9	0.19	NS
Tubular load (mmol/d)						
Allantoin	105.2 ^b	115.8 ^b	79.1 ^a	73.8 ^a	5.27	***
Uric acid	19.4	20.5	17.9	15.5	0.92	NS
PD	124.6 ^b	136.3 ^b	97.0 ^a	89.2 ^a	5.93	**
Re-absorption (mmol/d)						
Allantoin	72.0 ^{ab}	87.3 ^b	56.0 ^a	54.9 ^a	4.50	**
Uric acids	14.7	15.6	14.2	12.6	0.80	NS
PD	86.8 ^{ab}	102.8 ^b	70.2 ^a	67.5 ^a	4.97	*
Re-absorption (%)						
Allantoin	63.3	74.7	70.7	73.9	1.15	NS
Uric acid	75.8	75.9	77.3	81.1	1.23	NS
PD	69.5	75.0	72.1	75.2	1.06	NS

^{a, b, c and d} Mean values with the different superscripts in the same row are significantly different.

NS : Non significant ($p>0.05$); * $p<0.05$; ** $p<0.01$.

the CB cattle was obtained in this experiment. Similarly, the daily excretion of allantoin and uric acid (principal constituents of PD) also showed a positive response to the level of intake (Table 3). The response was predictable as nutritional status of animals determines the rate and extent of microbial protein synthesis (McDonald et al., 1995). When the nutritional quality of feed is identical, the efficiency of microbial growth will obviously depend on the level of intake as observed during this trial. The various regression equations developed based on the results of this experiment suggest that urinary PD excretion is closely related with DMI or DOMI ($r^2 = 0.84-0.89$, $p<0.01$) and may be used as an index to predict feed intake in the field. These findings agree with the data reported on the importance of voluntary feed intake (VFI) in forages by Crampton et al. (1960). The extrapolated basal PD excretion (7.68 mmol/d, equation (5)) for CB calves used in this experiment was found to be about 48% lower than the observed value (14.83 mmol/d) obtained from the fasting trial in the same set of animals (Singh, 2004). This may probably be due to higher *de novo* synthesis of purines (*creating purines from amino acids*) during fasting period to replace endogenous purine loss (IAEA, 1997). This is further confirmed by the higher endogenous N loss observed during fasting as compared to corresponding extrapolated values. The relative contribution of allantoin and uric acid remained stable (Figure 1) irrespective of level of feed intake during this trial. As explained earlier,

allantoin is the main component of purine derivatives excreted in urine followed by uric acid within the same cattle and their relative proportion remains constant (Chen and Gomes, 1992).

The average daily urinary creatinine excretion (0.717-0.787 mmol/kg $W^{0.75}$ /d) remained statistically similar irrespective of level of feed offered during this trial. Excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of feed intake (Chen et al., 1992; 1995, Dipu et al., 2006). As described earlier, creatinine excretion is usually independent of the level of intake or duodenal infusion of purine bases (IAEA, 2002). Nevertheless, creatinine excretion (mmol/kg $W^{0.75}$ /d) observed during this trial was close to the values obtained during the fasting trial (Singh, 2004).

Glomerular filtration rate and renal clearance of PD and creatinine

The plasma concentration ($\mu\text{mol/L}$) of allantoin, uric acid and creatinine, estimated for calculation of GFR and renal clearance, are given in Table 4. The formation of urine is the result of filtration through the glomerular capillaries, re-absorption of fluid and solute, and secretions into the lumen of the proximal and distal tubules (White et al., 1968). The renal clearance denotes the removal of substances from the blood i.e. purine derivatives and creatinine. Plasma allantoin, uric acid and creatinine were not affected ($p>0.05$) by level of feed intake. The GFR of

cross-bred cattle remained statistically similar at different levels of intake. This is in agreement with the observations recorded by Cetinkaya et al. (1999) in Yerli-kara cattle. However, tubular load of allantoin (mmol/d) in animals was higher ($p < 0.001$) at intake levels L-95 and L-80 as compared to L-60 and L-40. Nevertheless, tubular load of uric acid remained statistically ($p > 0.05$) similar irrespective of level of feed intake, although tubular load of total PD followed the trend of allantoin. Re-absorption of allantoin (mmol/d) was higher ($p < 0.01$) in animals at intake levels L-95 and L-80 as compared to L-60 or L-40. This is understandable because urinary PD excretion is closely correlated with DMI. The renal excretion of uric acid, a minor component of urinary total PD excretion, however remained similar irrespective of level of feed intake. The uric acid re-absorption did not affect by the level of feed intake. However, total PD re-absorption followed similar trend as observed for allantoin at different levels of feed intake. This corroborated well with the observations recorded earlier in KK cattle (Liang et al., 1999; Soejono et al., 1999).

Microbial nitrogen supply

The estimated microbial nitrogen (MN) supply (g N/d or g N/kg DOMI) to CB calves, calculated from daily purine absorption values derived in present study, at different levels of feed intake is given in Table 5. However, the MN supply when expressed per kg DOMI remained statistically similar irrespective of level of intake. The supply of MN was also calculated using the established model for European cattle (Verbic et al., 1990) using intake levels of animals recorded in this experiment for comparison (Table 5). A significant influence of feed intake level was evident on MN supply (g N/d or g N/kg DOMI) when European model was used. However, the derived MN values of this experiment deviated ($p < 0.01$) and were higher (22.3-63.6%) to the corresponding values obtained with European model. The differences in estimated MN supply became more pronounced at lower level of intake (L-60 and L-40). Available information also indicated substantial differences between species in their responses of PD excretion to purine absorption (IAEA, 1997). It is evident from the results that Indian CB cattle have lower endogenous contribution to urinary PD (0.296 mmol/kg $W^{0.75}/d$) as compared to European cattle (0.385 mmol/kg $W^{0.75}/d$). Similarly, the proportion of total PD in plasma that is excreted in the urine is lower (0.83) for tropical cattle than reported for European breeds (0.85) in the literature (Verbic et al., 1990; IAEA, 1999; Chen and Orskov, 2003). Therefore, it is desirable that research should be continued to develop a set of equations for different breeds/species of animals (IAEA, 2000), particularly under Indian conditions where animals are usually kept on low plane of nutrition.

CONCLUSION

The excretion of urinary purine derivatives found to be positively correlated with the level of feed intake as well as rumen microbial supply and thus, it could be a good indicator for measuring the microbial protein supply and nutritional status of animals.

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