Urinary excretions of Purine Derivatives (PD) as a predictor of nutritional status of local Zebu cattle and their crosses

Seresinhe T., Pathirana K.K. and Kumarasiri A.
Faculty of Agriculture, University of Ruhuna, Mapalana, Kamburupitiya, Sri Lanka

Abstract

The objective of the present study was to evaluate the nutritional status of Indian X indigenous Sri Lankan zebu cattle by using the Purine derivative excretion technique (PD). In the preliminary phase of the study, the voluntary intake (VI) was estimated as 3.11 DM/day for local cattle (body weight 100 kg). In the main experiment four male zebu cattle were used to determine the response of PD excretion at four levels of intake (95%, 80%, 60%, 40%). Digestibility of dry matter and organic matter were not affected (P>0.05) but nitrogen retention was increased with increasing levels of feed intake, The endogenous PD excretion rates were 1.91, 1.46, 1.21 and 0.66 mmol/kg $^{0.75}$/day for 95%, 80%, 60% and 40% intake levels respectively. The proportion of allantoin from total PD accounts 82.6% and was comparable to the range previously observed for other breeds of cattle. The excretion of creatinine was 1.05, 1.04, 0.92 and 0.84 mmol/kg $^{0.75}$/day, respectively. Daily output of total PD showed a positive response to the level of feed intake while creatinine(CR) excretion was independent of dietary treatments. The correlation between PD excretion rate and digestible organic matter intake (DOMI) was significant (r^2=0.70). Nevertheless PDC index was affected (P>0.05) by the level of feed intake and the correlation of PDC index and DOMI was significant as well (r^2 =0.63). A preliminary banding system was developed based on the above results. Results suggest that it is possible to use the average PDC index as a tool to estimate the nutritional status of local cattle.

Introduction

Low intake particularly during the dry season when feed availability is low is one of the major factors limiting animal production from native feed resources in many regions of Sri Lanka. Therefore, the strategy for improving production has been to maximize the efficiency of utilization of available feed resources in the rumen by providing optimum conditions for microbial growth.
Ruminants meet 50% to 100% of their total crude protein requirements from ruminal microbial synthesis[1]. The catabolism purine bases usually yields purine derivatives (PD), which are principally allantoin, uric acid, xanthene and hypoxanthene. While allantoin is quantitatively the most abundant, the other three in the urine of ruminants vary from one species to another[2]. It is therefore not surprising that a close relationship exists between urinary excretion of PD and duodenal supply of purines[3-5]. Osuji *et al.*[6] and others[7-8] demonstrated the potential of PD concentration in total urine as a predictor of intake and nutrient status of sheep.
The objective of the present study was to determine the urinary excretions of PD in indigenous Sri Lankan cross - bred animals at 4 levels of feed intake. The diurnal variation in the excretion of PD by means of spot urine samples who also examined.

Materials and Methods

Examine the urinary excretions of PD at four levels of feed intake

Animals and diets
Four mature animals (Indian x indigenous Sri Lankan breed) of average live weight (100 kg) were kept individually in metabolic cages for about one month and being maintained on a diet of natural grasses and concentrates. Diet per animal consisted of (on dry matter basis) 70% grass (mixture of *Bracharia brizantha, B. rusisiensis* and *Panicum maximum*) and 30% of commercial cattle feed (95%DM) to obtained. Diet containing 12.5% CP (on DM basis) was fed *adlibitum* twice daily in equal parts to achieve an intake of at least 3% live weight. *Adlibitum* intake was estimated as 3.105 kg DM /head/day. Fresh water was available freely. After the preliminary period, animals were fed at 4 fixed levels as 95, 80, 60 and 40% of the *adlibitum* intake using a 4 x 4 Latin Square design. Each feeding period lasted 3 weeks.

Sample collection

Samples were collected during last the 10 days of each feeding period. Daily feed intake was monitored with total collection of feces and urine during the first seven days of the experimental period. During the last three-days, spot urine samples were collected between 08 – 12 hrs, 17 – 20 hrs and 21 – 08 hrs the next morning within 24 hrs.

Measurements

Feces and feed were analyzed[9] for dry matter, organic matter and nitrogen (Kjeldhal method) to calculate of dry matter digestibility (DMD) and organic matter digestibility (OMD) and nitrogen intake. The urine was analyzed for total nitrogen (Kjeldhal method); creatinine and purine derivatives (allantoin and uric acid) were determined following the procedures of IAEA TECDOC 945[10].

Statistical analyzes

Statistical analyzes of the experimental were done using the soft ware packages EXCEL 2000 and SAS windows system

Results and Discussion

Feed intake and nutrient digestibility

Feed intake, DMD and OMD and digestible organic matter intake (DOMI) of the diet (70% grass and 30% cattle feed) are given in Table I. DMD and OMD ranged from 69.94 to 65.02 and 71.08 to 68.22 for highest and lowest levels of intakes respectively. The reported DMD and OMD in this study were higher than that of the reported values under Sri Lankan conditions, presumably due to feeding of concentrates in addition to grass[11]. Dry matter intake and DOMI ranged from 1.31 to 2.95 and 0.85 to 2.04 kg/d respectively. Although there were significant differences in the level of feed intake and DOMI, the digestibilities of dry matter (DMD) and organic matter (OMD) were not affected ($P<0.05$) by the level of feed intake. These differences were intentional in an attempt to maximize differences in the production of ruminal microbial nitrogen. Mean body weights were not significantly different with in the experiment (Table II). Therefore, it can be suggested that the PD excretion rate depended mainly on the DOMI rather than DMD or OMD.

Table 1. Feed intake, DM and OM digestibilities and DOMI (kg/day) of the diet fed to local Zebu cattle at different levels of intake

Level of feed intake (%)	95	80	60	40	Sig.
Feed intake(Kg/day)	02.95 ± 0.25	2.42 ± 0.35	01.91 ± 0.24	01.31 ± 0.23	0.001
DMD (%)	69.05 ± 2.97	69.94 ± 2.54	66.06 ± 2.57	65.02 ± 2.25	NS
OMD (%)	71.08 ± 4.44	70.34 ± 4.09	68.68 ± 3.33	68.22 ± 3.00	NS
DOMI (kg/day)	02.04 ± 0.20	01.69 ± 0.29	01.26 ± 0.36	00.85 ± 0.32	0.001

Response of PD excretion to feed intake

Urinary PD excretion in local cattle fed at different levels of intake is shown in Table 2. The excretion of allantoin and total PD ranged from 15.49 to 52.48 and from 21.30 to 60.07 (mmol per day) for the lowest (40%) and highest (95%) levels of intake respectively. As expected the daily outputs of allantoin and uric acid in local cattle showed a positive response to the level of feed intake. However, the excretion of creatinine was independent of the dietary treatments. It was also observed that the profile of PD excretion in the urine of local zebu cattle was similar to the other breeds of cattle [12,13]. Allantoin was found to be the principal PD in the urinary samples and was within the range previously observed in cattle by Verbic *et al* [14].

The PD excretion (Y) of local cattle was correlated to the digestible organic matter intake (DOMI =X) according to the following equation

$$Y = 22.595\ X + 10.794 \quad (R^2 = 0.70,\ n = 16)$$

The mean value for the intercept of above equation indicated an excretion of 22.59 mmol PD /day. Therefore, it can be suggested that the calculated PD excretion per unit digestible organic matter. Intake of local cattle is comparable to the values obtained for Keda-Kelantan[12] and Ongole[13] cattle.
Moreover, the correlation of PDC indexes to digestible organic matter intake with the following equation (Figure 1.).

$$Y = 24.428\ x + 1.9323 \quad (R^2 = 0.6365,\ n = 16)$$

It was also evident that, endogenous PD excretion rate of zebu cattle (Table 3) in this study was comparable but somewhat higher (1.91-0.66 mmol/ kg $^{0.75}$) than that of Bali cattle (0.46 mmol /kg$^{0.75}$) and Ongole cattle (0.54 mmol /kg$^{0.75}$) in Indonesia respectively. These findings provided satisfactory variation upon which to test the predictability of intake using urinary PD.

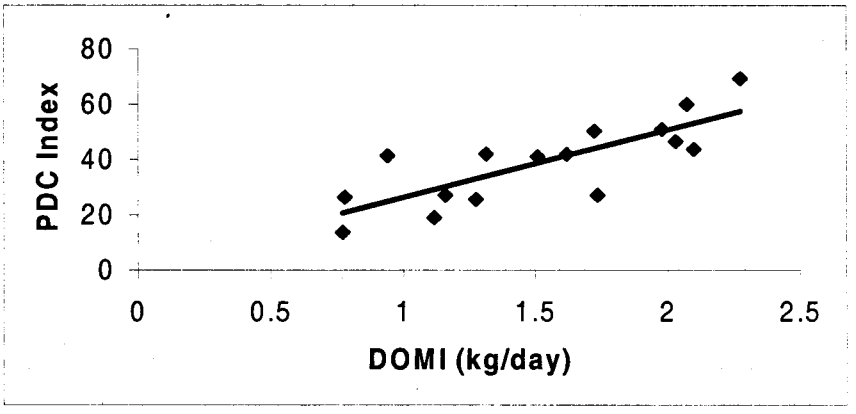

Figure 1. Relationship between PD/C index and digestible organic matter intake (DOMI) in local Zebu cattle.

Table 2. Urinary PD and creatinine excretion in local Zebu cattle

	Level of feed intake (%)				
	95	80	60	40	Sig
Allantoin (mmol/day)	52.48 ± 4.69	39.08 ± 4.84	27.31 ± 4.75	15.49 ± 4.75	0.001
Uric acid (mmol/day)	07.27 ± 0.17	06.55 ± 0.17	06.31 ± 0.18	05.81 ± 0.18	0.001
Creatinine (mmol/day)	32.85 ± 4.65	32.59 ± 4.09	28.15 ± 4.43	25.48 ± 4.06	NS
PDC Index	56.00 ± 4.02	41.77 ± 4.06	35.04 ± 3.99	26.61 ± 3.87	0.001
AL % from TPD	87.40 ± 2.87	85.50 ± 2.49	81.30 ± 2.89	76.10 ± 3.80	NS
Total PD(mmol/day)	60.07 ± 5.68	45.71 ± 5.36	33.59 ± 5.43	21.30 ± 4.82	0.001

Table 3. Urinary PD and creatinine excretion in local Zebu cattle

	Level of feed intake (%)				
	95	80	60	40	Sig.
Body weight (kg)	100	100	100	100	100
Allantoin (mmol/kg $^{0.75}$)	1.68 ± 0.034	1.25 ± 0.037	1.01 ± 0.038	0.51 ± 0.038	0.001
Uric acid (mmol/ kg $^{0.75}$)	0.23 ± 0.005	0.21 ± 0.005	0.20 ± 0.004	0.15 ± 0.005	0.001
Creatinine (mmol /kg $^{0.75}$)	1.05 ± 0.100	1.04 ± 0.090	0.92 ± 0.090	0.84 ± 0.090	NS
Total PD (mmol /kg $^{0.75}$)	1.91 ± 0.170	1.46 ± 0.180	1.21 ± 0.170	0.66 ± 0..170	0.001

Nitrogen balance

Nitrogen balance of local zebu cattle was affected by the level of feed intake is shown in Table 4. The nitrogen balance was 40.30, 29.95, 19.74 and 11.27 g/day for 95%, 80%, 60% and 40% intake levels respectively. All the treatments had positive nitrogen balance, although it was affected by the level of feed intake (Table 4). It was also observed that the nitrogen balance and intake of digestible organic matter were linearly related.

Table 4. The effect of level of feed intake on nitrogen balance on local Zebu cattle

	Level of feed intake (%)				
(g/day)	95	80	60	40	Sig
Nitrogen intake	62.42 ± 0.50	53.22 ± 0.48	40.09 ± 0.34	26.73 ± 0.40	0.001
Faecal nitrogen	10.93 ± 0.82	11.19 ± 0.74	08.59 ± 0.75	06.18 ± 0.77	0.05
Urinary nitrogen	11.18 ± 0.78	12.07 ± 0.74	11.75 ± 0.73	09.29 ± 0.72	0.05
Nitrogen balance	40.30 ± 2.96	29.95 ± 2.09	19.74 ± 1.84	11.27 ± 1.28	0.001

As suggested by Chen *et al* [16] following banding system can be used for Sri Lankan cross bred cattle to predict the nutritional status.

Table 5. The corresponding values for the daily PD excretion and microbial N supply in indigenous Sri Lankan crossbred cattle at five different ranges of PDC index.

Band	PDC Index ($kg^{0.75}$)	PD excretion (mmol/day)	Estimated microbial N (g/day)	Nutritional status
1	< 19	< 11	08 – 15	Under feeding
2	19 - 27	20 – 24	15 – 22	Under feeding
3	27 - 44	29 – 44	22 – 32	Sufficient
4	44 - 59	44 – 58	32 – 43	Good
5	> 69	58 – 78	43 – 57	Very good

PDC index and corresponding microbial N supply are divided into 5 bands. The corresponding microbial N values to PDC index of groups II and I are 8-22 g/day. This is an indication of insufficient feeding and the value matches with 40% feeding level (Table 5). Experimentally estimated DOMI at 40% feeding level was 0. 85 kg/day.

In order to use of PDC index to estimate DOMI (kg/day) of Sri Lankan crossbred cattle, DOMI values obtained from experiment I were plotted against PDC index that were obtained from spot urine analyzes. By using the equation derived in figure I, DOMI (kg/day) were estimated as 1.05±0.3 and1.253±.0.34 for band II and I respectively. Results suggest that the feeding values estimated experimentally and by using the equation was did not differ much. Therefore, it is possible to use the average PDC index as a tool to estimate the nutritional status of local cattle.

Acknowledgements

Authors are grateful to International Atomic Energy Agency for providing financial assistance. Special Thanks are also due to Mr. H. Karunathilaka, Ms S. Narangoda and Ms I. Udulanayani for their assistance in data collection and sample analyzes.

References

1. Johnson, L.M., Harrison, J.H. Riley, R.E. 1996. Estimation of the flow of microbial nitrogen to the duodenum using urinary uric acid and allantoin. *J. Dairy Sci.* 81: 2408-2420

2. Balcells, J., Guada, J.A., Castrillo, C. and Gasa, J. 1991. Urinary excretion of allantoin and allantoin precursors by sheep after different rates of purine infusion into the duodenum, *J. Agric. Sci.* Cambridge 16: 309-317

3. Balcells, J., Fondivila, M., Guada, J.A., Castrillo, C. and Surra, J.C.E. 1993. Urinary excretion of purine derivatives and nitrogen in sheep given straw supplemented with different sources of carbohydrates. *Anim. Prod.* 57: 287-292

4. Fugihara, T., Ørskov, E.R. and Reeds, P.J. 1987. The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intro-gastric nutrition, *J. Agric. Sci.* Cambridge. 109: 7-12.
5. Giesecke, D., Stangassinger, M. and Tiemeyer, W. 1984. Nucleic acid digestion and urinary purine metabolite in sheep nourished by intro-gastric nutrition. *Can. J. Animal Sci.* 64: 144-145
6. Osuji, P.O., Nashlai, I.V. and Khalili, H. 1996. Effect on fasting on the urinary excretion of nitrogen and purine derivatives by zebu (*Bos indicus*) and crossbred (*Bos indicus* x *Bos Taurus*) cattle. *J. Appl. Anim. Res.* 10: 3-47
7. Nsahlai, I.V., Osuji, P.O. and Umunna, N.N. 2000. Effect of form and of quality of feed on the concentrations of purine derivatives in urinary spot samples, daily microbial N supply and predictability pf intake. *Anim. Feed Sci. Techno.* 85: 223-238
8. Chen, X.B. and Gomez, M.J. 1995. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives. An overview of the technical details. *Occasional publication International Feed resources unit,* Rowett Research Institute Bucksburn, Aberdeen, U.K.
9. Association of Official Agricultural Chemists. 1980. Official methods for analysis (13th Ed.) Association for the official analytical chemists, Washington, DC.
10. International Atomic Energy Agency 1997. Estimation of rumen microbial protein production from purine derivatives in urine. IAEA-TECDOC-945. IAEA, Vienna.
11 Chen, X.B., Samaraweera, L., Kyle, D.J. and Ørskov, E.R. 1996. Urinary excretion of purine derivatives and tissue xanthine oxidase (*EC* 1.2.3.4) activity in buffaloes (*Bubalis bubalis*) with special reference to differences between buffaloes and *Bos Taurus* cattle, *Br. J. Nutr.* 75: 397-407
12. Liang, J.B., Pimba, O., Abdullah, N.A., Jelan, Z.A. and NOLAN, J.V. 1999. Estimation of rumen microbial protein production from urinary purine derivatives in Zebu cattle and water buffalo, *Proc. of the second research coordination meeting of Nuclear based technologies for estimating microbial protein supply in ruminant livestock* Vienna, Austria, 24-28 Aug, 35-42 pp.
13. Soejono, M., Yusiati, L.M., Budhi, S.P.S., Widyobroto, B.P. and Bachrudin, Z. 1999. Estimating microbial protein supply for indigenous ruminants using nuclear and purine excretion techniques in Indonesia, *Proc. of the second research coordination meeting of nuclear based technologies for estimating microbial protein supply in ruminant livestock.* Vienna, Austria, 24-28 Aug, 43-58 pp.
14. Verbic, J., Chen, X.B., Macleod, N.A. and Ørskov, E.R. 1990. Excretion of purine derivatives by ruminants. Effect of microbial nucleic acid infusion on purine derivatives excretion by steers. *J. Agric. Sci.* Cambridge 14: 243-248
15. Chen, X.B., Susmel, P., Stefanon, B. and Ørskov, E.R. 1995. On the use of purine derivatives I spot urine plasma and milk samples as indicators of microbial protein supply in sheep and cattle, *Proc of the 7th International symposium qn protein metabolism and nutrition* Portugal 24-27 May, 325-329 pp.
16. Chen, X.B., Mejia, A.T., Kyle, D.J. and Ørskov, E.R. 1995. Evaluation of the use of purine derivative; creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: in sheep. *J. Agric. Sci.* 125: 137-143