Indian Journal of Animal Sciences **83** (2): 143–145, February 2013/Article

Influence of dietary protein levels on urinary purine derivatives excretion in Murrah buffaloes

U R MEHRA[1], A K VERMA[2], K Y DESHPANDE[3] and P SINGH[4]

Indian Veterinary Research Institute, Izatnagar, Uttar Pradesh 243 122 India

Received: 17 April 2012; Accepted: 11 June 2012

ABSTRACT

Growing bulls (30), 1-year-old, weighing 80.3 kg BW were divided into 3 equal groups following completely randomized design and were fed on isocaloric (2.01 ME Mcal/kg diet) diets containing 3 levels of protein i.e. standard protein (SP) ration at 100 (SP), 90 (MP) and 80 (LP)%, respectively, of requirements as per Kearl feeding standards. After 21 days of feeding a metabolism trial was conducted for last 8 days, for which total urine excreted and faeces voided were collected daily. Urine collection for estimation of purine derivatives was made during last 2 days of metabolic trial. The intake (g/kg$^{0.75}$) of CP and OM fulfilled 98, 91 and 83%, respectively, of Kearl's CP requirements. Though other parameters reflecting nutrient utilization were comparable among the various protein levels, DCP intake (g/d) was higher in SP as compared to MP and LP groups. No significant response was noticed in purine derivatives with respect to levels of protein intake. It may be deduced that protein levels (80 to 100% in the ration) did not exhibit any variation in the excretion of urinary purine derivatives in buffaloes.

Key words: Buffalo, Protein levels, Urinary purine derivatives

The small and medium farmers in developing countries like India have limited available resources for feeding their livestock. Thus, any strategy for improvement in livestock production requires efforts to maximize the efficiency of utilization of available feed resources that chiefly depends on the efficiency of microbial fermentation in the rumen. In roughage based diets, rumen microbes constitute main source of digestible protein to the host animal (Soejono *et al*. 1999). Urinary excretion of PD (allantoin and uric acid) was successfully used to estimate the microbial protein synthesized in the rumen vis-à-vis nutritional status of ruminants (Chen *et al.* 1990, Verbic *et al.* 1990, Dipu *et al.* 2006, George *et al.* 2007, George *et al.* 2007, 2011). Since microbial enzymes in rumen rapidly degrade purines of dietary and exogenous origin, any purines present in the digesta at the small intestine are expected to be only of microbial origin and can be considered to be specific markers of or the microbial fraction (Nolan 1999). 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 as well as with feeding regime (IAEA 1999). Many studies assessed the effect of feeding ruminants at different levels of intake (George *et al.* 2007, 2011, Dipu *et al.* 2006) and dietary energy levels (Deshpande *et al.* 2011) on urinary excretion of PD. However, the literature on effect of feeding varying protein levels in diet on PD excretion in Indian buffalo is scarce. Hence, the present study is an effort to study the effect of feeding varying dietary protein levels on rumen microbial protein production using urinary purine derivatives excretion in Murrah buffaloes.

Present address: [1]Formerly Scientist Emeritus, [2,4] Principal Scientist, Division of Animal Nutrition.[3]Subject Matter Specialist (Animal Science) (kuldeepydeshpande@gmail.com), KVK, Tandwal Rajouri, SKUAST-J, Jammu & Kashmir 185 131 India.

MATERIALS AND METHODS

Animals and feeding: Growing male Murrah buffalo calves (30), 1-year-old, weighing 80.3 kg were randomly divided into 3 equal groups following completely randomized design. The animals were housed in well ventilated shed with facilities for individual feeding under hygienic and uniform management conditions. Adequate clean and fresh drinking water was made available daily. During the preliminary feeding period (1 month), all the animals were fed fixed quantity (2 kg/d) of concentrate mixture containing maize 33, wheat bran 32, soybean meal 32, mineral mixture 2 and common salt 1 part, and wheat straw *ad lib*. Animals in group 1, 2 and 3 were fed on isocaloric diets with varying protein levels such as 100% (SP), 90% (MP) and 80% (LP) of protein requirement (Kearl 1982), respectively. After 21 days of feeding, a metabolism trial was conducted for last 8 days,

for which total urine excreted and faeces voided were collected daily. The experimental animals were weighed (before feeding and watering) in the beginning and the 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 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 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 2 plastic vials and stored at –20°C for further analyses.

Analytical techniques used: Representative sub samples of the feeds offered, residue leftover and faeces voided were oven dried (100°C, 24 h), ground to pass through 1 mm screen and then analyzed for the dry matter, organic matter, crude protein and ether extract (AOAC 1995). Urinary purine derivatives (allantoin and uric acid) and creatinine contents were determined by HPLC (Resines *et al.*1992).

Data analysis and interpretation: Statistical analysis of data was done as per Snedecor and Cochran (1994), and suitable superscripts were attributed to mean values for statistical significance. The data were processed as per SPSS software (16.0) to compare the target parameters.

RESULTS AND DISCUSSION

Physical and chemical composition of concentrate mixture and wheat straw are presented in Table 1. The intake and digestibility of nutrients were reported by Verma *et al.* (2009). The DM intake (g/kg $W^{0.75}$ or % of body weight) was significantly (P<0.05) lower in group 3 (Table 2) where animals fed on 80% protein levels. This might be due to significantly (P<0.05) lower DCP intake (Table 2). The intake of CP was fulfilled 98, 91 and 83%, respectively, of Kearl's CP requirements. Significantly (P<0.05) higher DCP intake (g/d) in group 1 may be due to higher CP intake through concentrate mixture. The TDN intake (g/d) was comparable among all groups, however, when TDN intake expressed as g/kg $W^{0.75}$, it was significantly (P<0.05) lower in group 3 as compared to other groups.

Table 1. Physical and chemical composition of concentrate mixtures and wheat straw

Particulars	CM 1 (SP)	CM 2 (MP)	CM 3 (LP)	WS
Physical composition (% as such basis)				
Crushed maize	35	40	45	—
Wheat bran	32	37	42	—
Deoiled soybean meal	30	20	10	—
Mineral mixture	2	2	2	—-
Common salt	1	1	1	—
Chemical composition (% DM basis)				
OM	90.16	90.61	91.42	92.09
CP	22.43	19.31	17.94	3.58
EE	2.79	2.88	2.72	1.23

CM, Concentrate mixture; WS, wheat straw.

Table 2. Body weights and plane of nutrition in various groups

Attributes	Group 1 (SP)	Group 2 (MP)	Group 3 (LP)
Body weight (kg)	83.62±3.91	87.10±1.80	93.85±4.63
Body weight ($kgW^{0.75}$)	27.62±0.96	28.51±0.44	30.12±1.11
DM intake ($g/kg^{0.75}$)*	73.17±2.36^b	76.52±2.59^b	66.34±2.77^a
DM intake (% of Body weight)*	2.42±0.08^b	2.51±0.09^b	2.14±0.10^a
CP intake ($g/kgW^{0.75}$)*	7.31±0.24^c	6.68±0.14^b	5.79±0.18^a
OM intake ($g/kgW^{0.75}$)*	66.90±2.16^b	70.34±2.35^b	60.92±2.55^a
DCP (g/day)*	123.77±3.70^b	107.43±2.05^a	101.98±1.07^a
DCP ($g/kgW^{0.75}$)	4.50±0.18	3.77±0.08	3.41±0.15
TDN (g/day)	1117.27 ±51.06	1205.23 ±22.47	1108.36 ±24.22
TDN ($g/kgW^{0.75}$)	40.54±1.77	42.34±1.05	36.96±1.11

$^{a, b, c}$ Means having different superscripts in a row differ significantly, * (P<0.05).

Response of PD excretion to protein intake: The daily urinary purine derivatives and creatinine at different levels of protein intake are presented in Table 3. A nonsignificant (P>0.05) response in allantoin, creatinine and uric acid excretion in urine was observed with respect to decrease in protein intake. This may be because of less difference in crude protein level in diets, although the digestibility was affected significantly (P<0.05) as reported by Verma *et al.* (2009). The presence of xanthine and hypoxanthine in buffalo urine could not be detected, might be due to very low concentration levels owing to higher activity of xanthine oxidase in the intestine and plasma (Chen *et al.* 1996, Nolan 1999, Pimpa *et al.* 2003). 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). Danils (1993) also found similar value (18.4 mmol PD/Kg DOMI) for European cattle. Our results 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 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). Daily allantoin excretion corroborated well with the values reported by Liang *et al.* (1994) of swamp buffaloes, Chen *et al.* (1996) for Murrah swamp buffaloes and by Pimpa *et al.* (2003) for Malaysian swamp buffaloes. Daily uric acid excretion in the present study (1.96–2.24 mmol) was also within range (1.5

Table 3. Excretion of urinary purine derivatives and creatinine in buffalo calves

Attributes	Group 1 (SP)	Group 2 (MP)	Group 3 (LP)
Urine volume (L)	2.13±0.33	2.17±0.30	1.88±0.27
Allantoin (mg/L)	478.52±46.80	627.89±110.57	614.71±66.67
Allantoin (mg/d)	969.18 ±138.35	1250.67 ±205.80	1026.06 ±143.28
Allantoin (mmol/L)	3.03±0.30	3.97±0.70	3.82±0.41
Allantoin (mmol/d)	6.13±0.87	7.91±1.30	6.88±0.86
Creatinine (mg/L)	289.89±38.31	353.93±33.21	250.94±46.83
Creatinine (mg/d)	564.93±49.14	617.63±91.68	468.34±70.76
Creatinine (mmol/L)	2.56±0.34	2.77±0.46	2.54±0.35
Creatinine (mmol/d)	5.00±0.43	5.46±0.81	4.61±0.59
Uric acid (mg/L)	171.48±29.77	219.23±34.03	161.84±10.54
Uric acid (mg/d)	328.81±37.95	429.88±37.64	391.93±49.94
Uric acid (mmol/L)	1.02±0.18	1.31±0.20	0.97±0.06
Uric acid (mmol/d)	1.96±0.23	2.24±0.27	2.13±0.42
Purine derivatives (mmol/L)	4.05±0.41	5.28±0.85	4.85±0.37
Purine derivatives (mmol/d)	8.09±0.90	10.47±1.38	8.84±1.05

to 4.2 mmol) as reported earlier for buffaloes (Liang *et al.* 1994, Mos Cardini *et al.* 1999).

Urinary excretion of creatinine (mmol/d) did not differ due to various protein levels. Narayanan and Appleton (1980) also reported that the total daily creatinine excretion in urine is breed/ species specific and more closely correlated with muscle mass than body weight.

In this experiment nitrogen (g/d) declined with the decrease in dietary N level as animals tried to adjust their nitrogen output at lower levels of intake to reach equilibrium (Verma *et al.* 2009) and the difference in protein levels did not exert any significant variation in purine derivatives and creatinine in buffalo calves might be either due to the comparatively narrow difference in the protein level or due to the species difference.

It may be deduced that protein levels (80 to 100% in the ration) did not exhibit any variation in the excretion of urinary purine derivatives in buffaloes.

REFERENCES

AOAC. 1995. *Official Methods of Analyses*. 16th edn. Association of Official Analytical Chemists, Washington, D C.

Chen X B, Ørskov E R, and Hovell F D and De B. 1990. Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep. *British Journal of Nutrition* **63**: 121–29.

Chen X B and Ørskov E R. 2003. Research on urinary excretion of purine derivatives in ruminants: past and future. Development, standardization and validation of nuclear based technologies for estimating microbial protein supply in ruminant livestock for improving productivity. (Eds) Makkar H P S. IAEA-2003, Vienna.

Chen X B, Samarweera L, Kyle D J, Ørskov E R and Abeygunawardene H. 1996. Urinary excretion of purine derivatives and tissue xanthine oxidase activity in buffaloes, with special reference to differences between buffaloes and Bos taurus. *British Journal of Nutrition* **75**: 397–407.

Deshpande K Y, Mehra U R, Singh P and Verma AK. 2011. Purine derivatives concentration in body fluids as influenced by different energy levels in dairy cows. *Indian Journal of Animal Sciences* **81 (12)**: 1244–47.

Dipu M T, George S K, Singh P, Verma A K and Mehra U R. 2006. Measurement of microbial protein supply in Murrah buffaloes (*Bubalus bubalis*) using urinary purine derivatives excretion and PDC index. *Asian-Austrailasian Journal of Animal Sciences* **19**: 347–55.

George S K, Dipu M T, Mehra U R, Verma A K and Singh P. 2007. Effect of levels of feed intake on plasma concentration and urinary excretion of purine derivatives in crossbred bulls. *Journal of Animal and Feed Sciences* **16**: 26–36.

George S K, Verma A K, Mehra U R, Dipu M T and Singh P. 2011. Evaluation of purine metabolites - creatinine index to predict the rumen microbial protein synthesis from urinary spot samples in Barbari goats. *Journal of Animal and Feed Sciences* **20**: 509.

IAEA-TECDOC-1093. 1999. *Nuclear based technologies for estimating microbial protein supply in ruminant livestock*. International Atomic Energy Agency. Vienna.

Kearl L C. 1982. *Nutrient requirement of ruminants in developing countries.* Utah Agricultural Experimental Station, Utah State University, International Feed Stuffs Institute, Logan, Utah.

Liang J B, Matsumoto M and Young B A. 1994. Purine derivative excretion and ruminal microbial yield in Malaysian cattle and swamp buffalo. *Animal Feed Science Technology* **47**: 189–99.

Liang J B, Matsumoto M, Ishida M and Young B A. 1993. Urinary allantoin excretion in Malaysian cattle and buffaloes. *Proccedings of 7th World conference on Animal Production.* Pp 58–59. University of Alberta, Alberia, Canada.

Mos Cardini S, Haddi M L, Stefon B and Susmel P. 1999. Measurement of purine derivatives in the urine of some ruminant species. *IAEA-TECDOC*- 1093. Pp.111–18.

Narayanan S and Appleton H D. 1980. Creatinine : A review. *Journal of Clinical Chemistry* **26**:1119–26.

Nolan J V. 1999. Prediction of rumen microbial outflow based on urinary excretion derivatives. *IAEA-TECDOC*- 1093. Pp: 9–19.

Pimpa O, Liang J B, Jelan Z A and Abdullah N. 2003. Urine purine derivative excretion in swamp buffaloes after duodenal purine base infusion. *Animal Feed Science and Technology* **104**: 191.

Snedecor G W and Cochran W G. 1994. *Statistical Methods*. 8th edn. Iowa State University, Iowa (USA).

Soejono M, Yusiati L M, Budhi S P S and Widyobroto B P. 1999. Estimating rumen microbial protein supply for indigenous ruminants using nuclear and purine excretion techniques in Indonesia. *IAEA- TECDOC- 1093.* Pp: 43–58.

Vercoe J E. 1976. Urinary allantoin intake and digestible organic matter intake in cattle and buffaloe. *Journal of Agricultural Sciences (Cambridge)* **86**: 613–15.

Verma A K, Singh P, Deshpande K Y, Verma Vinay and Mehra U R. 2009. Influence of dietary protein levels on nutrient utilization and blood parameters in buffaloes. *Animal Nutrition and Feed Technology* **9**: 21–28.