



Purine derivatives concentration in body fluids as influenced by different energy levels in dairy cows

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ABSTRACT

Purine derivative (PD) concentration in different body fluids (plasma, milk and urine) was studied in 30 dairy cows (*Bos taurus* × *Bos indicus*; 331 to 369 kg mean body weight and 9.8 to 10.1 kg/d average milk yield) fed on the diets having mixed green fodders and concentrate mixtures (CM-1, CM-2, CM-3) containing varying amount of maize and wheat bran in group 1 (control), group 2 and group 3, respectively, following completely randomized design. The concentration of allantoin and total PD (mmol/L) in urine was comparable between groups 1 and 2, but was significantly higher in group 3. Moreover, the concentration of allantoin, uric acid and PD (mmol/l) in milk and plasma was significantly different among 3 groups (1, 2 and 3) indicating that the small variations in TDN and ME intake through diets (1, 2 and 3) could be reflected significantly in allantoin and PD concentration in plasma and consequently in milk but not in urine samples. The correlation coefficient (*r*) between digestible dry matter intake (DDMI, kg/d) and milk allantoin (mmol/L) was 0.67. The correlation of total PD (mmol/l) in milk was better than that of plasma and urine. The diurnal variation in PD secretion in milk (mmol/d) could be explained to a great extent by DDMI (kg/d) ($R^2 = 0.65$ and 0.75 in morning and evening milk respectively). It is concluded that the assay of allantoin and total PD in milk samples can be effectively used to assess the microbial protein supply in dairy cows on account of small variation in energy levels in diets.

Key words: Dairy cows, Milk, Plasma, Purine derivatives, Urine

Purine derivatives (PD) concentration in plasma, their secretion in urine and milk has long been considered as indicator for recovery of microbial protein available at duodenal level; which forms the major portion of metabolizable protein in ruminants (Mota *et al.* 2008). The correlation of various nutrient utilization parameters with urinary PD excretion have been established previously e.g. digestible dry matter (DDM) and organic matter (DOM) in ruminants (Chen *et al.* 1992, Susmel *et al.* 1994); level of feed intake (Singh *et al.* 2007) and levels of CP intake (Ramgaokar *et al.* 2008). So far, influence energy levels on concentration of purine derivatives in different body fluids like milk, urine and plasma have not been studied in Indian dairy cows. Therefore, a study was conducted to understand the purine derivatives concentration in different body fluids in dairy cows fed on diets having various energy levels.

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MATERIALS AND METHODS

Experimental design and diets: Crossbred cows (30) of about similar age and lactation stage (mean body weight range 331 to 369 mg and average milk yield 9.8 to 10.1 kg/d) were selected from the dairy farm of the institute, and randomly distributed into 3 different groups of 10 animals each following completely randomized design. All the experimental animals were housed in well-ventilated sheds under uniform management conditions and fed daily twice on mixed green roughage (maize/cowpea) and concentrate mixtures comprising deoiled soybean meal, deoiled mustard cake, wheat bran, crushed maize, mineral mixture and common salt. The concentrate mixtures were formulated by changing the proportion of crushed maize and wheat bran to get different energy densities but iso-nitrogenous (Table 1). Concentrate mixture was given @1.0 kg/2.5 kg milk produced over and above the maintenance allowance of 1.0 kg to meet out nutrient requirements (Ranjhan 1998). Freshwater was provided *ad lib.* while the rations were given in measured quantity at 06.00 and 17.00 h daily.

Collection and proximate analysis of samples: A 6-day digestion trial was conducted after 21 days of experimental

Table 1. Physical and chemical composition of concentrate mixtures and green fodder used in study

Particulars	Concentrate mixtures			Green fodder
	1	2	3	
Physical composition (%)				
Crushed maize	30	40	50	-
Deoiled soybean meal	20	20	20	-
Deoiled mustard oil cake	10	10	10	-
Wheat bran	37	27	17	-
Mineral mixture	2	2	2	-
Common salt	1	1	1	-
Chemical composition (% DM basis)				
Dry matter	91.67	91.76	92.16	19.67
Organic matter	90.59	90.34	91.34	90.31
Crude protein	21.57	19.94	20.61	11.94

feeding. During the 6 days sampling period, representative samples of feed offered, residues left and faeces voided were collected, aliquoted and brought daily to the laboratory and preserved (Schneider and Flatt 1975) for further analyses. Nitrogen content in feed and faeces was analyzed using the kjeldahl method. Dry matter and organic matter contents were determined (AOAC 1995).

Collection of urine and milk samples: The spot urine samples were collected into clean plastic containers during the early morning hour (6.00 AM) at the time of milking and a fixed sample was strained through glass wool and immediately transferred into plastic vials ensuring final pH below 3.0 by adding few drops of 10% sulphuric acid and stored at -20°C until further analysis. However, representative milk samples were collected in morning (6.00 AM) and evening (4.00 PM) throughout the digestion trial and all the morning and evening collections were pooled separately for each animal and stored at -20°C .

Analysis of purine derivatives: The concentration of allantoin, uric acid and creatinine in urine and plasma samples were estimated using colorimetric methods (IAEA1997-TECDOC-945). Milk was analyzed for allantoin (Chen 1989) and uric acid (IAEA 2002). Clear supernatant solution of 1 ml was used for the assay. Total excretion of allantoin and uric acid was computed as the product of average milk yield (litres) obtained during the trial period (6 days). Estimation of creatinine in urine and plasma was done based on the Jaffe's reaction as per Folin and Wu (Hawk *et al.* 1976).

Statistical analyses: Statistical analyses of data were done as per Snedecor and Cochran (1994). The data obtained were subjected to one-way analysis of variance (ANOVA). The treatment means were compared using the Duncan's multiple range tests to attribute suitable superscripts. The correlation between nutritional constituents and purine derivatives excretion was deduced and estimated by Statistical Analytical System (SPSS version 10.0 for Windows) by processing data for correlation coefficient and linear regression analysis.

RESULTS AND DISCUSSION

Nutrient utilization

The chemical composition of concentrate mixture and green roughage fed to experimental animals is presented in Table 1. All the 3 groups were having similar ($P>0.05$) live weights and metabolic body weights. The digestibility of DM and OM was comparable ($P>0.05$) among 3 groups. In contrary, Fujihara *et al.* (2005) found significant difference ($P<0.05$) in OM digestibility of LE (low energy) and HE (high energy) diets in sheep fed hay and concentrate diets.

PD and creatinine excretion in spot urine samples: In the urine samples of cow the salvageable PD (xanthine and hypoxanthine) could not be detected; hence allantoin and uric acid were accounted as total PD. The urinary allantoin in spot samples (mmol/l) increased linearly ($P<0.01$) in groups 2 and 3. The DOMI and DDMI were significantly correlated ($P<0.05$) with urinary allantoin excretion (mmol/l) with correlation coefficient 'r' 0.63 and 0.66, respectively. Earlier experiments revealed urinary allantoin excretion as a significant predictor of microbial protein synthesis in ruminants (Chen *et al.* 1992, Fujihara *et al.* 2005) wherein, it correlated well to the intake of DDM and/or DOM in ruminants. They attributed this significant correlation between variables to the rationale that number of rumen microbes is generally increased after fermentation of dietary OM in ruminants.

The uric acid and creatinine concentration (mmol/l) in spot samples did not vary significantly ($P>0.05$) with increase

Table 2. Body weights, feed intake and nutrient digestibility in different groups

Particulars	Treatments			SEM
	1	2	3	
<i>Mean body weight during trial period</i>				
(kg)	382.75	362.25	355.25	21.230
(kg W ^{0.75})	86.42	82.89	81.79	3.640
<i>Dry matter intake (kg/d)</i>				
Concentrate	4.73	4.60	4.59	0.170
Green fodder	7.26	7.47	7.26	0.240
Total	11.99	12.07	11.85	0.250
DMI (% B wt)	3.17	3.37	3.37	0.165
DMI (g/kg W ^{0.75})	139.77	146.44	145.77	8.100
<i>Digestibility (%)</i>				
DM	68.20	67.45	69.22	0.990
OM	71.34	70.46	72.35	0.960
<i>TDN and energy intake</i>				
g/kg W ^{0.75}	93.04	95.95	98.83	6.384
DE% of GE**	68.32 ^b	70.45 ^a	71.74 ^a	0.919
ME% of GE**	56.02 ^b	57.77 ^a	58.83 ^a	0.753
ME [†] intake (Mcal/d)**	28.79 ^b	30.93 ^a	31.93 ^a	1.25

The means bearing different superscripts between columns differ significantly* ($P<0.05$); ** ($P<0.01$); [†] ME calculated from DE by using factor '0.82'.

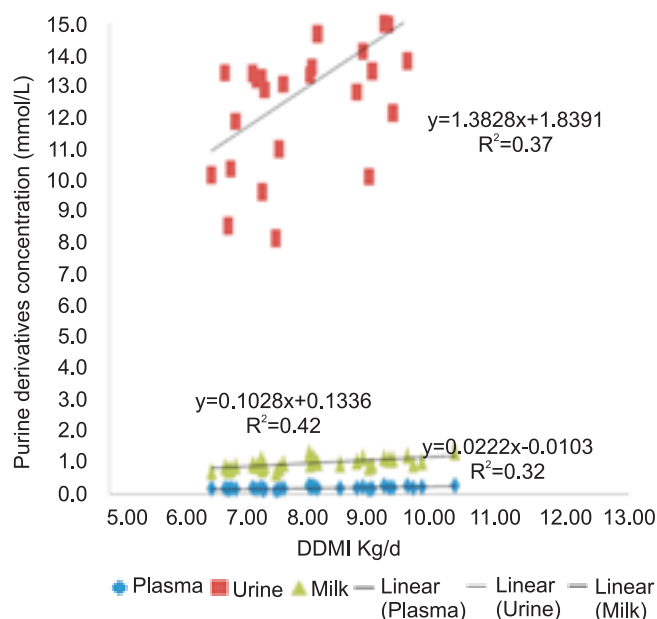


Fig. 1. Total purine derivatives concentration mmol/l in urine, milk and plasma as influenced by digestible DMI kg/d in dairy cows

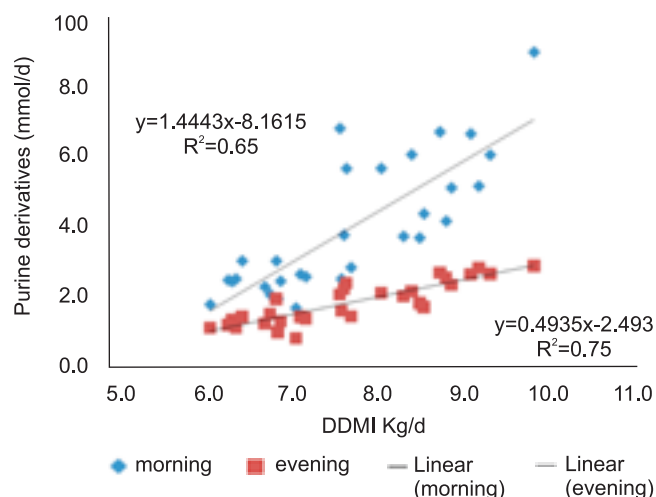


Fig. 2. Purine Derivatives (mmol/d) secretion in morning and evening milk as influenced by digestible DMI (kg/d)

in dietary energy. However, Singh *et al.* (2007) demonstrated a positive correlation between urinary PD excretion and levels of feed intake in crossbred calves. Similar effect of dietary energy levels on the urinary excretion of purine derivatives (PD) was investigated in sheep fed hay and concentrate diets (Fujihara *et al.* 2005).

PD excretion in milk samples

The uric acid and total PD excretion (mmol/d) increased significantly ($P < 0.01$) with increasing energy content of diet (Table 3). The allantoin, uric acid and total PD concentration (mmol/l) showed linear increase with enhancement in dietary

energy levels ($P < 0.01$) among 3 groups. The concentration of allantoin, uric acid and PD in milk sample was significantly ($P < 0.01$) different among 3 groups (1, 2 and 3), which indicates that the small variations in TDN and ME intake through diets (1, 2 and 3) could be reflected significantly ($P < 0.01$) in allantoin and PD concentration in milk but not in plasma and urine samples.

In present study, the diurnal variation observed in total PD secretion in milk (mmol/d) revealed significant correlation with DDMI (kg/d). The R^2 values of 0.65 and 0.75 (Fig. 2) indicated that the diurnal variation in milk PD can be explained very well by nutrient utilization by the dairy animal. Similar findings were reported by Gonza-lez-Ronquillo *et al.* (2003), who evaluated the effect of milk yield and duodenal flow of purine bases (PB) on the PD excretion in milk and concluded that milk PD throughout as reliable as urinary PD excretion. The increase in total PD excretion through milk due to increasing energy level of diet is corroborated with the findings of Gonza-lez-Ronquillo *et al.* (2004) who also suggested that increase in energy or protein content in diet may increase the milk yield and consequently increase PD excretion through milk. They further explained that allantoin is not the only parameter to be used to predict microbial protein synthesis in rumen, but uric acid is also excreted in appreciable amounts in milk and thus it may also be used as predictor of microbial protein status in dairy animals.

PD and creatinine concentration in plasma samples: The concentrations of plasma allantoin and uric acid (Table 3) in present study are comparable to the values reported by Balcells *et al.* (1992) for steers (118.3 and 12.6 $\mu\text{mol/l}$, respectively) and for dairy cows (184.5 and 34 $\mu\text{mol/l}$,

Table 3. Purine derivatives (mmol/l) in urine, milk and plasma and creatinine content in different groups

Particulars	Treatments			SEM
	1	2	3	
<i>Urine</i>				
Allantoin **	10.63 ^b	11.53 ^b	13.03 ^a	0.449
Uric acid	1.39	1.41	1.50	0.380
Creatinine	7.15	6.43	6.01	0.741
Total PD**	12.02 ^b	12.94 ^b	14.53 ^a	0.673
<i>Milk**</i>				
Allantoin	0.57 ^c	0.61 ^b	0.65 ^a	0.007
Uric acid	0.27 ^c	0.39 ^b	0.43 ^a	0.017
Total PD	0.84 ^c	1.00 ^b	1.08 ^a	0.018
<i>Plasma**</i>				
Allantoin	0.11 ^b	0.13 ^a	0.14 ^a	0.007
Uric acid	0.04 ^b	0.05 ^a	0.06 ^a	0.005
Creatinine	0.16 ^a	0.13 ^{ab}	0.11 ^b	0.018
Total PD	0.14 ^c	0.18 ^b	0.20 ^a	0.008

The means bearing different superscripts in a row differ significantly. ** ($P < 0.01$).

respectively) as reported by Giesecke *et al.* (1994). The significantly higher allantoin and uric acid concentrations in groups 2 and 3 are substantiated by the reports of George *et al.* (2007), who observed a significant increase in plasma allantoin and total PD concentration ($\mu\text{mol/l}$) with increase in level of feed intake in crossbred bulls. The creatinine concentration of plasma was significantly higher in group 1 as compared to other groups. This may be attributed to their higher mean body weight as compared to animals in groups 2 and 3. The results are concurrent with Ramgaokar *et al.* (2008), who found that the PD concentration of plasma may prove as index of microbial protein supply in crossbred bulls fed at different levels of CP.

It is concluded that assay of allantoin and PD in milk samples can be used to assess the small variation in energy levels and microbial protein supply of diets in dairy cows.

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