Original Research

Plasma Purine Derivatives Concentration in Barbari Goats Fed Diets with Different Oil Seed Cakes

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Abstract

The study evaluated the possibility of measuring the plasma concentration of purine derivatives (PD) as an alternative to urinary PD excretion to predict the microbial protein supply in goats fed different vegetable protein supplements. Four different iso-nitrogenous concentrate mixtures were prepared by using four different oil cakes for the feeding of animals in different groups (groundnut cake/GNC (Group I), mustard cake/MC (Group II), soybean meal/SBM (Group III) and cotton seed cake/CSC (Group IV)]. Eight adult Barbari goats were fed maintenance diets with wheat straw and either one of the concentrate mixture (50:50) in two sets of 4x4 Latin Square Design for a feeding period of 30 days. During the last 8 days of each feeding period animals were placed in metabolism crates for sample collection. Allantoin and total PD excretion (mmol/d) in urine was higher (P<0.05) in groups supplemented with GNC and SBM when compared to groups fed diets containing MC and CSC. The concentration (µmol/L) of allantoin and total PD in plasma was higher (P<0.05) in group III as compared to group II. However, the concentration (µmol/L) of uric acid, salvageable PD (xanthine and hypoxanthine) and creatinine in plasma was similar (P>0.05) among the dietary treatments. The plasma level of PD (Y, µmol/L) was poorly correlated (R^2 = 0.44) with urinary PD (mmol/d) excretion. Therefore, from the present study it can be concluded that the plasma concentration of PD serves as a rough indicator of urinary PD excretion and further investigations are required to use it as a tool for precise calculation of rumen microbial nitrogen synthesis.

Key words: Barbari Goats, Microbial Protein, Plasma, Purine Derivatives

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Introduction

Purine derivatives (PD) in urine (allantoin, uric acid, xanthine and hypoxanthine) have been commonly used as an indicator of the amount of microbial nitrogen arriving at the duodenum (Topps and Elliot, 1965). Allantoin being the final product of purine metabolism in ruminants has the highest proportion in urine because hypoxanthine and xanthine would be converted into uric acid and then to allantoin through enzyme reaction (Hernandez *et al.*, 2014). Application of this method, to quantify rumen microbial protein synthesis however, requires a total collection of urine for a number of days (Thirumalesh and Krishnamoorthy, 2013). If only blood samples are required, the method can be used as a practical indicator of microbial protein supply in ruminants under field conditions. In steers, Chen *et al.* (1992) established that plasma PD level could be valid as a simple index of microbial protein supply, as it was correlated with daily urinary PD excretion. However, in calves, Kagiyama *et al.* (1996) concluded that plasma allantoin concentration is not a proper estimator of intestinal flow of microbial protein. Sheep and goats, when fasted, Fujihara *et al.* (2007) observed positive relation between plasma allantoin level and urinary allantoin excretion. There is a lack of information regarding the relationship between the sources of protein with plasma concentration of PD in goats. Therefore, this study examined the influence of source of dietary protein on concentration of PD in plasma, urinary PD excretion, daily glomerular filtration rate (GFR), tubular load and re-absorption of PD in Barbari goats.

Materials and Methods

Feeds and Feeding

Four different iso-nitrogenous concentrate mixtures (CM) were prepared by using four different oil cakes as protein supplements *viz.* CM-I (Groundnut cake/GNC), CM-II (Mustard cake/MC), CM-III (Soybean meal/SBM) and CM-IV (Cotton seed cake/CSC). Details of composition of concentrate mixtures fed to different groups is given in Table 1.

Table 1: Composition of concentrate mixtures

Particulars	CM I	CM II	CM III	CM IV
Crushed maize	48	48	48	48
Groundnut cake (GNC)	13	-	-	-
Mustard cake (MC)	-	15	-	-
Soybean meal (SBM)	-	-	12	-
Cottonseed cake (CSC)	-	-	-	17
Wheat bran	36	34	37	32
Mineral mixture	2	2	2	2
Salt	1	1	1	1
Vitamin premix (g/100kg)	25	25	25	25

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Eight adult Barbari goat bucks (19.71±0.67 kg mean body wt.) about 2 years of age were used for this study. Two sets of 4x4 Latin square designs (LSD) were used for this experiment. The experiment consisted of four 30-day feeding periods and four feeding groups. During the last 8 days of each feeding period animals were placed in metabolism crates for sample collection. The body weights were recorded at the beginning and end of collection period. Four different iso-nitrogenous concentrate mixtures prepared by using four different oil cakes as protein supplements were offered to different groups (CM I with GNC for Group I, CM II with MC for Group II, CM III with SBM for Group III and CM IV with CSC for Group IV respectively) along with wheat straw (50:50) at maintenance requirement (NRC, 1981).

Sample Collection

During the last 8 days of each feeding period animals were placed in metabolism crates and representative samples of feed offered, residue left (wheat straw) and urine voided were brought daily to the laboratory for further analyses. Jugular blood samples (10 ml) were also obtained on the first and eighth day before the animals received their ration in the morning. Blood was equally aliquoted in 5 ml-heparinized tubes, centrifuged at 4^0C and plasma was separated and stored at -20^0C until analysis.

Measurements and Chemical Analysis

The DM content of feeds was estimated as per AOAC (199). Concentration of allantoin in urine and plasma was determined colorimetrically by the method of Young and Conway (1942). Uric acid level was estimated colorimetrically by phosphotungstic acid method and the level of salvageable PD (hypoxanthine and xanthine) were analyzed by the enzymatic method (Chen and Gomes, 1992). Creatinine in urine and plasma was analyzed colorimetrically by Jaffe alkaline picrate reaction.

Calculation of Target Parameters

Various parameters were calculated as detailed below-

Urinary PD

PD (mmol/d) = Allantoin (mmol/d) + Uric acid (mmol/d) + Xanthine and hypoxanthine (mmol/d)

Plasma PD

PD (µmol/L) = Allantoin (µmol/L) + Uric acid (µmol/L) + Xanthine and hypoxanthine (µmol/L)

GFR

GFR (L/d) = Urinary creatinine (mmol/d) / Plasma creatinine (mmol/L)

Renal Clearance of PD

Tubular load (mmol/d) = GFR (L/d) X Plasma concentration (mmol/L)

Reabsorption (mmol/d) = Tubular load (mmol/d) – Urinary excretion (mmol/d)

Reabsorption (%) = Reabsorption (mmol/d) X 100/Tubular load (mmol/d)

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The same calculation procedures were used in the estimations of renal clearance of allantoin, uric acid, salvageable PD and total PD.

Statistical Analysis

The data obtained in various sets were subjected separately to Analyses of variance (ANOVA) procedure according to a Latin square design using the General Linear Model (GLM) of the SAS system for windows. Treatment means was compared by using Duncan's New Multiple Range Test. The statistical model used is shown below-

$$Y = \mu + \alpha + \beta + \gamma(\alpha) + t + \varepsilon,$$

where μ is the overall mean, α is the random effect of the square, β is the random effect of period, $\gamma(\alpha)$ is the random effect of goat within the square, t is the fixed effect of treatment, and ε is the random error.

Results and Discussion

PD and Creatinine Levels in Urine and Plasma

Purine derivatives and creatinine in urine and plasma, daily glomerular filtration rate, tubular load and re-absorption of PD in different groups is presented in Table 2. The DM intakes were similar ($P<0.01$) among the treatment groups fed with different oil seed cakes. When feed is identical quantitatively, the efficiency of utilization of nitrogen will depend upon the quality of the diet offered. In this experiment, four different vegetable protein sources were used to formulate iso-nitrogenous concentrate mixtures to study the variability in utilization of feed nitrogen under a variety of practical conditions. Dietary treatments influenced ($P<0.05$) the urinary output of PD whereas no difference was observed in the creatinine excretion (Table 2). Allantoin excretion (mmol/d) was significantly ($P<0.05$) higher in groups GNC and SBM when compared to groups MC and CSC. The urinary excretion of uric acid and salvageable PD (mmol/kgW$^{0.75}$/d) was higher ($P<0.05$) in group SBM when compared to group MC. Total PD excretion (mmol/d or mmol/ kgW$^{0.75}$/d) followed similar trend as that of allantoin.

In a similar study (Liu and McMeniman, 2006) in crossbred Merino sheep, fed with oat straw and different legume seeds, it was observed that, with the same intakes of DM and nitrogen there was different ($P<0.05$) excretion of urinary PD. Similarly, Santoso *et al.* (2004) observed that, Cheviot wethers fed on similar diets when supplemented with various feed additives showed variations in the level of urinary PD. These findings indicate that the concentration of urinary PD may be affected not only by the intake of DM and nitrogen but also by factors like the quality of protein as well as synchronization between nitrogen released in the rumen with that of available carbon skeleton from fermented carbohydrates.

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Table 2: Purine derivatives (PD) and creatinine in urine and plasma, daily glomerular filtration rate (GFR), tubular load and re-absorption of PD in different groups.

Parameters	Group I (GNC)	Group II (MC)	Group III (SBM)	Group IV (CSC)	SEM	P Value
Body weight (kg)	19.79	19.58	19.83	19.76	0.998	0.3
DM Intake (g/d)	483.78	479.11	479.86	482.79	17.964	0.21
Urine (mmol/d)						
Allantoin	5.68^{a}	4.77^{b}	5.86^{a}	5.08^{b}	0.253	0.03
Uric acid	0.71^{ab}	0.64^{b}	0.75^{a}	0.70^{ab}	0.043	0.04
Xanthine & hypoxanthine	0.62^{ab}	0.55^{b}	0.66^{a}	0.61^{b}	0.05	0.04
Total PD	7.01^{a}	5.96^{b}	7.27^{a}	6.38^{b}	0.241	0.03
Creatinine	4.09	3.96	3.96	4.16	0.124	0.12
Plasma (µmol/L)						
Allantoin	163.22^{ab}	158.61^{b}	168.38^{a}	162.15^{ab}	4.581	0.03
Uric acid	19.2	18.07	19.68	18.72	1.554	0.21
Xanthine and hypoxanthine	16.94	15.81	17.1	16.62	1.257	0.18
Total PD	199.35^{ab}	192.49^{b}	205.16^{a}	197.47^{ab}	5.643	0.04
Creatinine	75.43	74.74	71.01	76.54	3.513	0.11
GFR						
(L/d)	54.67	53.27	56.04	54.44	2.33	0.15
(L/kgW0.75/d)	5.86	5.75	5.99	5.82	0.301	0.14
Tubular load (mmol/d)						
Allantoin	8.91^{ab}	8.42^{b}	9.42^{a}	8.82^{ab}	0.352	0.03
Uric acid	1.04	0.96	1.09	1.02	0.071	0.19
Xanthine &hypoxanthine	0.92	0.84	0.95	0.91	0.062	0.23
Total PD	10.86^{ab}	10.22^{b}	11.45^{a}	10.75^{ab}	0.342	0.04
Re-absorption (mmol/d)						
Allantoin	3.23	3.65	3.56	3.74	0.402	0.13
Uric acid	0.32	0.32	0.34	0.32	0.034	0.18
Xanthine & hypoxanthine	0.29	0.29	0.29	0.3	0.029	0.25
Total PD	3.85	4.25	4.18	4.36	0.412	0.17
Re-absorption (%)						
Allantoin	36.16	42.84	37.46	42.14	3.49	0.14
Uric acid	31.1	32.68	30.75	31.71	2.103	0.22
Xanthine & hypoxanthine	32.08	34.64	30.85	33.42	2.206	0.31
Total PD	35.37	41.28	36.33	40.39	2.925	0.22

Means with different superscripts in a row differ significantly: (P<0.05)

The level of protein degradation in the rumen depends primarily upon microbial access to the protein, retention time in the rumen, solubility of protein and ruminal pH (Stern *et al.*, 1994). Level of anti-nutritional factors present in some oil cakes may also adversely affect the efficiency of nitrogen utilization.

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Variations in PD excretion were accounted by allantoin, the principal PD, whose relative proportion was 80%. Dapoza *et al.* (1999) reported that allantoin constituted the larger fraction of total PD (>80%) in pregnant and lactating ewes fed diet with varying protein content. In a similar study (Xia *et al.*, 2018) in Mutton sheep, fed with 10 different concentrate mixtures the proportion of allantoin, uric acid, xanthine (including hypoxanthine) in total PD excretion were significantly different ($P<0.01$) among 10 treatments, and the ratios were 85.60-92.47%, 2.54-7.18% and 3.20-7.41%, respectively.

It has been suggested that the excretion rate of creatinine remains independent of feed intake and is relatively constant in healthy animals (Chen *et al.*,1992;1995), as also observed in the present study. Moreover, the use of creatinine as an internal marker of urinary output relies on the assumption that the creatinine excretion through urine is unaffected by diet and is proportional to body weight (Brody, 1945). There was no difference in the body weight of the animals used in this study and hence they had similar excretion of urinary creatinine. Ma *et al.* (2014) studied the effect of dietary concentrate:forage ratios and undegraded dietary protein on urinary creatinine excretion in *Dorper x thin tailed han* crossbred lambs and observed that urinary excretion of creatinine (mmol/kg $BW^{0.75}$) was not affected by dietary treatments. On contrary, there are reports of increased excretion of creatinine when the proportion of concentrate was increased in the diet (Gonda *et al.*, 1996). Similarly, Mukminah *et al.* (2015) observed that age and feeding level affected the body weight gain, feed intake and creatinine excretion in Kacang goats.

The plasma concentration (µmol/L) of allantoin, uric acid, salvageable PD, creatinine, estimated GFR and renal clearance are given in Table 2. The concentration (µmol/L) of allantoin in plasma was higher ($P<0.05$) in group SBM as compared to group MC. However, the concentration (µmol/L) of uric acid and salvageable PD in plasma was similar ($P>0.05$) among the four dietary treatments. The level of total PD (µmol/L) in plasma followed a similar trend as that of allantoin while the plasma concentration of creatinine (µmol/L) was similar ($P>0.05$) in all the groups. The GFR (L/d or L/kg$W^{0.75}$/d) of goats remained similar ($P>0.05$) among various groups. Tubular load of allantoin in animals was higher ($P<0.05$) in group SBM when compared to group MC. Tubular load of uric acid and salvageable PD remained similar ($P>0.05$) among the groups. Tubular load (mmol/d) of total PD followed the same trend as that of allantoin. Re-absorption (mmol/d) of allantoin, uric acid, salvageable PD and total PD remained similar ($P>0.05$) irrespective of dietary treatments.

Measurement of urinary excretion of PD for quantifying rumen microbial protein synthesis, requires a total collection of urine for several days, and would be difficult under farm conditions. If only blood samples are required, the method can be extended to provide a practical index of microbial protein supply in animals under farm conditions (Chen *et al.*, 1992). PD once filtered into the glomerulus, are quantitatively excreted in the urine. There is very little secretion of PD in nephric tubes therefore; the tubular load of PD is dependent on the plasma concentration of PD and glomerular filtration rate. Since allantoin is not reutilized

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for the synthesis of purine bases, the urinary excretion of allantoin will be proportional to its plasma concentration, if the clearance and filtration rate of PD are constant. The molar ratio of plasma PD followed almost similar pattern as that of urine with allantoin accounted nearly 80% of total. However, the plasma level of PD (Y, µmol/L) was poorly correlated with urinary PD (X, mmol/d) and the resultant equation obtained is as follows-

$$Y = 124.16 + 11.18\ X\ (R^2=0.44;\ P= 0.084)$$

Fujihara *et al.* (2007) on the other hand observed positive correlation between urinary PD and plasma PD during fasting and re-feeding in sheep and goats. Nejad *et al.* (2017) found that urinary excretion of allantoin was higher in *Corriedale* ewes having free access to water compared to ewes under water deprivation. The GFR also affected the excretion of PD in urine and it was therefore, estimated based on creatinine clearance. The estimated GFR (53-56 L/d) was similar among the groups. However, the value was lower than the previous reports (65-158 L/d) in sheep (Chen *et al.*, 1995). Difference in DM intake observed in both the experiments may explain this variation. Similar GFR in treatment groups in the present experiment may be responsible for the same trend in the urinary excretion and plasma level of PD as also observed in previous studies (Chen *et al.*, 1995; George *et al.*, 2007). However, if GFR and re-absorption of PD is variable, plasma level of PD will be related with neither the influx into the plasma nor the renal excretion.

Conclusion

The results obtained in the present study indicate that even though the plasma level of allantoin and total PD followed almost a similar trend as that of urinary PD excretion, they were poorly correlated ($R^2=0.44;\ P= 0.084$). Therefore, it is concluded that the plasma concentration of PD serves as a rough indicator of urinary PD excretion, however, to use it as a tool for precise calculation of rumen microbial nitrogen synthesis further investigations are required.

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