# EFFECT OF LEVELS OF FEED INTAKE ON PLASMA CONCENTRATION OF PURINE DERIVATIVES IN BARBARI GOATS

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**ABSTRACT**

The study evaluated the potential of the plasma concentration of purine derivatives (PD) as an alternative to total urine collection method to predict the microbial protein supply in goats. In two sets of 4 x 4 Latin square design, 8 barbari goat bucks were assigned four levels (95, 80, 60 and 40%) of voluntary dry matter intake (VDMI) on a diet of wheat straw and concentrate mixture (50:50) for thirty days. During last eight days of each feeding period, a metabolism trial was conducted. The daily urinary excretion of allantoin and PD (mmol/d) decreased ($P<0.01$) with the reduction in feed intake. The concentration (µmol/L) of allantoin in plasma was higher ($P<0.05$) in goats fed with 95% of VDMI as compared to those fed with 60 and 40% of VDMI. The glomerular filtration rate (L/d) decreased ($P<0.05$) with the reduction of feed intake and the values were higher ($P<0.05$) at 95% of VDMI when compared to goats fed with 60% of VDMI. The plasma level of PD (Y, µmol/L) was poorly correlated ($R^2 = 0.42$) with urinary PD (X, mmol/d). Therefore, if the plasma concentration is to be used to indicate the flow of PD into the blood or their excretion into the urine, the variation in glomerular filtration rate (GFR) needs to be taken into account.

**Keywords**: Barbari goats, purine derivatives, plasma, microbial protein

**INTRODUCTION**

The use of urinary purine derivatives (PD) as a specific marker for rumen microbial protein synthesis was suggested by Topps and Elliot (1965). Urinary excretion of PD (allantoin, uric acid, xanthine and hypoxanthine) has been successfully used as an alternative to existing methods for measuring microbial protein production, which require cannulation of the gut. Application of this technique, however, requires a total collection of urine for several days. If only blood samples are required, the method can be extended to provide a practical indicator of microbial protein supply in animals under field conditions. In steers, Chen *et al.* (1992) demonstrated that plasma PD level could be applicable as a simple index of microbial protein supply, as it was correlated with daily urinary excretion of PD. However, Kagiyama *et al.* (1996) concluded that plasma allantoin

concentration would not be a proper estimator of intestinal flow of microbial protein in calves. In sheep and goats, Fujihara *et al.* (2007) observed positive correlation between plasma allantoin level and urinary allantoin excretion during fasting. There is a lack of information regarding the relationship of levels of feed intake with plasma concentration of PD in goats. Consequently, this study examined the influence of levels of feed intake 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

### Animals, experimental design, diets and feeding

Eight adult Barbari goat bucks about two year's age (20.24±0.74 kg mean body wt) were used for this study. The animals were fed *ad libitum* with a mixed (50:50) diet of wheat straw and concentrate (Table 1) individually for one week during the preliminary feeding period. The lowest level of intake recorded among all animals during this period (484.47±0.05 g/d on DM basis) was set as voluntary feed intake (VFI) to ensure that animals were able to consume all the feed offered to them during the experimental feeding.

Two sets of 4 x 4 Latin square design were used for this experiment. The experiment consisted of four 30-day feeding periods and four feeding levels. The highest level of intake was 95% of VFI (Group I). The other 3 levels were 80% (Group II), 60% (Group III) and 40% (Group IV) of the voluntary intake. Water was made available thrice daily (10.30 h, 14.30 h and 20.30 h) *ad lib*.

### Sample collection

During last eight days of each feeding period, a metabolism trial was conducted. Representative samples of feed offered and residue left (wheat straw) were brought daily to the laboratory for further chemical analysis. Jugular blood samples (10 ml) were also obtained at the start and finish of the metabolism trial before the animals received their ration in the morning. Blood was equally aliquoted in 5 ml-heparinized tubes, centrifuged at $4^0$C, and plasma was separated and stored at –20□C until analysis.

**Table 1.** Ingredients and chemical composition of the roughage and concentrate supplied to experimental goats

| | Concentrate | Roughage |
|---|---|---|
| **Ingredients (%)** | | |
| Wheat straw | | 100 |
| Maize | 38 | |
| Wheat bran | 38 | |
| Soyabean meal | 22 | |
| Vitamin/mineral suppement | 02 | |
| **Chemical composition (%, DM basis)** | | |
| Organic matter | 90.88 | 90.31 |
| Crude protein | 18.12 | 03.07 |
| Ether extract | 02.31 | 01.09 |
| Crude fibre | 08.34 | 37.48 |
| Neutral-detergent fibre | 42.70 | 81.03 |
| Acid-detergent fibre | 11.10 | 51.24 |
| Total ash | 09.12 | 09.69 |

DM = dry matter

† Declared compositon: Ca: 160, Mg: 95, P: 95, S: 27, Mn: 3, Fe: 2.8 (g/Kg DM); I: 77, Co: 15, Se: 5(µg/kg DM); vitamin A: 40 00000, $D_3$: 80 000 (IU/kg DM)

### Measurements and chemical analysis

The DM content of feeds were estimated as per AOAC (1995). Urinary and plasma

allantoin was determined colorimetrically by the method of Young and Conway (1942). Uric acid was determined colorimetrically by phosphotungstic acid method using a commercial kit (Span Diagnostics®, India) and the salvageable PD (hypoxanthine and xanthine) were analyzed by the enzymatic method, according to the procedure of Chen and Gomes (1992). Creatinine in urine and plasma was analyzed based on the Jaffe alkaline picrate reaction using a commercial kit (Qualigens®, India).

**Calculations**

The GFR (L/d) was calculated from the urinary creatinine excretion rate and the plasma creatinine concentration [Urinary creatinine excretion (mmol/d) / Plasma creatinine concentration (mmol/L)]. Tubular load of allantoin (mmol/d) was estimated as the product of GFR and plasma allantoin concentration [GFR (L/d) x Plasma allantoin concentration (mmol/L)]. The same calculation procedures were used in the estimations of tubular load of uric acid, salvageable PD and total PD.

**Statistical Analysis**

The various data sets obtained 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 $\mu$ is the overall mean, $\alpha$ is the random effect of the square, $\beta$ 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 $\varepsilon$ is the random error.

**RESULTS AND DISCUSSION**

**Feed intake**

Table 2 gives the values for mean body weight and the feed intake (DM). The DM intakes were different ($P<0.01$) among the treatment groups as envisaged by the experimental design.

**PD and creatinine levels in urine and plasma**

The variations in daily urinary excretions of PD and creatinine at different levels of intake are presented in Table 2. The urinary PD excretion lowered with feed restriction and differed significantly ($P<0.05$) between the groups. This variation was mainly explained by the significant response in allantoin excretion with regard to feed intake. The urinary excretion of uric acid and salvageable PD (mmol/d) was higher ($P<0.05$) in group I when compared to groups III and IV. However, urinary excretion of creatinine did not differ ($P>0.05$) among groups.

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 and total PD in plasma was significantly ($P<0.05$) higher in group I as compared to groups III and IV. However, the concentration (µmol/L) of uric acid and salvageable PD in plasma were similar ($P>0.05$) among the four different levels of feed intake. The level of total PD in plasma followed a similar trend as that of allantoin

**Table 2.** Mean body weight, DM Intake, purine derivatives (PD) and creatinine in urine and plasma, daily glomerular filtration rate (GFR), tubular load and re-absorption of PD at different levels of feed intake

| Parameters | Group I (L-95) | Group II (L-80) | Group III (L-60) | Group IV (L-40) | SEM |
|---|---|---|---|---|---|
| Body weight (kg) | 21.09 | 20.63 | 19.86 | 19.16 | 1.00 |
| DM Intake (g/d)** | $459.50^{a}$ | $385.98^{b}$ | $294.08^{c}$ | $192.99^{d}$ | 0.90 |
| *Urea (mmol/d)* | | | | | |
| Allantoin** | $5.36^{a}$ | $4.31^{b}$ | $3.10^{c}$ | $2.07^{d}$ | 0.34 |
| Uric acid* | $0.64^{a}$ | $0.60^{ab}$ | $0.56^{b}$ | $0.55^{b}$ | 0.03 |
| Xanthine & hypoxanthine* | $0.53^{a}$ | $0.49^{ab}$ | $0.45^{b}$ | $0.44^{b}$ | 0.03 |
| Total PD** | $6.52^{a}$ | $5.41^{b}$ | $4.11^{c}$ | $3.05^{d}$ | 0.35 |
| Creatinine | 4.02 | 4.05 | 4.10 | 4.20 | 0.14 |
| *Plasma (µmol/L)* | | | | | |
| Allantoin* | $163.45^{a}$ | $150.01^{ab}$ | $133.17^{bc}$ | $129.78^{c}$ | 8.56 |
| Uric acid | 18.07 | 17.91 | 17.26 | 17.75 | 1.67 |
| Xanthine & hypoxanthine | 15.53 | 15.64 | 15.13 | 15.46 | 1.66 |
| Total PD** | $197.04^{a}$ | $183.55^{ab}$ | $165.56^{b}$ | $162.98^{b}$ | 11.28 |
| Creatinine** | $77.37^{b}$ | $79.58^{b}$ | $84.82^{ab}$ | $93.39^{a}$ | 4.34 |
| GFR | | | | | |
| (L/d)* | $52.91^{a}$ | $51.68^{ab}$ | $49.02^{ab}$ | $45.19^{b}$ | 3.69 |
| ($L/kgW^{0.75}/d$) | 5.40 | 5.37 | 5.23 | 4.97 | 0.42 |
| *Tubular load (mmol/d)* | | | | | |
| Allantoin* | $8.49^{a}$ | $7.66^{b}$ | $6.46^{c}$ | $5.84^{c}$ | 0.35 |
| Uric acid* | $0.93^{a}$ | $0.90^{ab}$ | $0.83^{bc}$ | $0.79^{c}$ | 0.05 |
| Xanthine & hypoanthine* | $0.80^{a}$ | $0.79^{ab}$ | $0.73^{ab}$ | $0.69^{b}$ | 0.05 |
| Total PD* | $10.22^{a}$ | $9.36^{b}$ | $8.02^{c}$ | $7.32^{c}$ | 0.38 |
| *Re-absorption (mmol/d)* | | | | | |
| Allantoin | 3.13 | 3.35 | 3.36 | 3.77 | 0.35 |
| Uric acid* | $0.29^{a}$ | $0.30^{a}$ | $0.27^{ab}$ | $0.24^{b}$ | 0.02 |
| Xanthine & hypoxanthine | 0.27 | 0.29 | 0.28 | 0.25 | 0.03 |
| Total PD | 3.69 | 3.95 | 3.91 | 4.27 | 0.35 |
| *Re-absorption (mmol/d)* | | | | | |
| Allantoin* | $37.05^{c}$ | $43.37^{c}$ | $52.15^{b}$ | $64.70^{a}$ | 4.13 |
| Uric acid | 31.20 | 33.07 | 32.47 | 31.06 | 1.16 |
| Xanthine & hypoxanthine | 33.22 | 37.02 | 37.94 | 36.53 | 2.26 |
| Total PD* | $36.21^{c}$ | $41.94^{c}$ | $48.83^{b}$ | $58.36^{a}$ | 2.26 |

Means with different superscripts in a row differ significantly: *($P<0.05$); **($P<0.01$)

The relationship between plasma PD level and urinary excretion of PD has been investigated by a number of workers (Chen *et al*., 1992; Giesecke *et al*., 1994; Fujihara *et al,* 2007; George *et al*., 2007). However, the results on whether plasma PD concentration was correlated with daily urinary PD output were inconsistent. In the present study, the plasma allantoin concentration was higher at increased levels of feed intake (Table 2). The mean plasma level (mmol/L) of allantoin recorded in the present experiment (130-164mmol/L) was higher than the value (69-142mmol/L), reported for crossbred sheep (Chen *et al*., 1991). The higher plasma allantoin concentration in goats may be due to a higher absorption rate of microbial purines from the small intestine into the circulatory system or a lower recycling into the rumen. The molar ratio of plasma PD followed almost similar pattern as that of urine with allantoin accounted nearly 80 per cent of total. However, the plasma level of PD (Y, mmol/L) was poorly correlated with urinary PD (X, mmol/d).

$$Y = 123.8 + 11.20X \qquad (R^2 = 0.42)$$

Plasma concentration (µmol/L) of creatinine was higher (P<0.01) in group IV as compared to groups I and II. The GFR (L/d) decreased (P<0.05) with the reduction of feed intake and the values were higher (P<0.05) in group I when compared to group IV. Although, the GFR ($L/kgW^{0.75}$/d) of goats remained similar (P>0.05) at different levels of feed intake, the values reduced in accordance with reduction in feed intake. Tubular load of allantoin (mmol/d) in animals was higher (P<0.05) at higher levels of feed intake, except for group III and IV, which had similar (P>0.05) values. Tubular load of uric acid and salvageable PD followed almost a similar trend with higher (P<0.05) values at increased plane of nutrition.

Re-absorption of allantoin (mmol/d) was similar (P>0.05) among the groups. However, the re-absorption (mmol/d) of uric acid was lower (P<0.05) in group IV when compared to groups I and II. Nevertheless, re-absorption (mmol/d) of salvageable PD and total PD remained similar (P>0.05) irrespective of level of feed intake. The re-absorption percentage of allantoin and total PD increased significantly (P<0.05) with reduction in feed intake, except for groups I and II which had similar (P>0.05) values. However, the re-absorption (%) of uric acid and salvageable PD remained similar (P>0.05) irrespective of feed intake.

The GFR was calculated based on creatinine clearance as the ratio between daily urinary creatinine excretion and plasma creatinine concentration (IAEA-TECDOC-945, 1997). The estimated GFR (4.97-5.4 $L/kgW^{0.75}$/d) was comparable to the previous reports (4.30-6.96 $L/kgW^{0.75}$/d) in Suffolk sheep (Prasitkusol *et al*., 1999). The studies conducted in European cattle (Kagiyama *et al*., 1996) and sheep (Chen *et al*., 1995) revealed that the GFR in same animal may change with feed intake. The change in GFR with levels of feed intake might be responsible for the poor correlation between the plasma and urinary levels of PD in the present study. The fact that GFR is related to feed intake, it can be inferred that GFR can also vary within the same animal if feed intake changes, as also observed in the present study. Therefore, 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. The implication is that if the plasma concentration is to be used to indicate the flow of PD into the blood or their excretion into the urine, the variation in GFR needs to be taken into account.

## SUMMARY

The results obtained in the present study indicate that if the plasma concentration is to be used to predict the microbial nitrogen supply, the variation in GFR needs to be taken into account. Therefore, the authors consider PD measurement in total urine to be a more precise tool than plasma PD as indicator of microbial nitrogen supply in barbari goats.

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## REFERENCES

AOAC. 1995. Association of Official Analytical Chemists, *Official Methods of Analyses*. 16th ed. Washington, DC.

Chen, X.B. and Gomes, M.J., 1992. Estimation of microbial protein supply to sheep and cattle based on urinary excretion of purine derivatives: An overview of the technical details. The Rowett Research Institute, University of Aberdeen, U. K. 21p.

Chen, X.B., Grubic, G., Orskov, E.R. and Osuji, P. 1992. Effect of feeding frequency on diurnal variation in plasma and urinary purine derivatives in steers. *Anim. Prod*. **55**: 185-191.

Chen, X.B., Mejia, A.T., Kyle, D.J. and Orskov E.R. 1995. Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples as an index of microbial protein supply in ruminants: studies in sheep. *J. Agric. Sci., Camb*. **125**: 137-143.

Chen, X.B., Kyle, D.J., Orskov, E.R. and Hovell, F.D. DeB. 1991. Renal clearance of plasma allantoin in sheep. *Exp. Physiol.* **76**: 59-65.

Fujihara, T., Shem, M.N. and Matsui, T. 2007. Urinary excretion of purine derivatives and plasma allantoin level in sheep and goats during fasting. *Anim. Sci. J.* **78** (2): 129-134.

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. *J. Anim. Feed Sci.* **16**: 26-36.

Giesecke, D., Ehrentreich, L. and Stangassinger, M. 1994. Mammary and renal excretion of purine metabolites in relation to energy intake and milk yeild in dairy cows. *J. Dairy. Sci*. **77**: 2376-2381.

IAEA-TECDOC-94, 1997. Estimation of Rumen Microbial Protein Production from Purine Derivatives in Urine. International Atomic Energy Agency, Vienna.

Kagiyama, R., Funaba, M., Iriki, T. and Abe, M. 1996. Plasma allantoin concentration in response to changes in nutritional status of calves. *Asian Aust. J. Anim. Sci*. **9**: 165-170.

Prasitkusol, P., Chen, X. B., Kyle, D. J. and Orskov, E. R. 1999. Measurement of the proportion of plasma purine derivatives excreted in the urine of sheep. IAEA-TECDOC-**1093**: 81-86.

Topps, J.H. and Elliot, R.C. 1965. Relationships between concentrations of ruminal nucleic acid and excretion of purine derivatives by sheep. *Nature,* **205**: 498-499.

Young, E.G. and Conway C.F. 1942. On the estimation of allantoin by Rimini-Schryver reaction. *J. Biol. Chem*. **142**: 839-852.