Studies on Milk Allantoin and Uric Acid in Relation to Feeding Regimens and Production Performance in Buffaloes

P. Sikka*, N. K. Saxena, R. Gupta, R. K. Sethi and D. Lall
Central Institute for Research on Buffaloes, Sirsa Road, Hisar 125001, Haryana, India

ABSTRACT : Allantoin and uric acid were estimated in milk to study the association between the levels of these purine derivatives and milk production per day under given feeding regimens. Keeping the stage of lactation, parity and initial milk yield in view thirty lactating buffaloes were randomly selected from early lactating group. All the animals were fed 30 kg green, 2 kg straw and 5 kg concentrate mixture on per animal/day basis at basal level up to 8 l produce. 1 kg concentrate mixture, soaked cotton seed and boiled cotton seed was fed for every 2 l milk, respectively in Group I (control), Group II and Group III animals. Average milk Allantoin and Uric acid levels were 120±11.7 g/ml and 4.03±0.63 g/ml, respectively in milk. Cotton seed feeding enhanced the milk production significantly ($p<0.01$) in comparison to concentrate mixture fed control group animals. A significant difference ($p<0.01$) in milk allantoin levels was found over the different feeding management at higher level of production group animals. Study also revealed a significant negative correlation between the milk allantoin and production per day $r=-0.43$ ($p<0.05$). ***(Asian-Aust. J. Anim. Sci. 2001. Vol 14, No. 11 : 1634-1637)***

Key Words : Allantoin, Uric Acid, Lactation, Buffaloes, Cotton Seed

INTRODUCTION

Dietary crude protein (CP) needs of the ruminants are discussed in terms of rumen degradable proteins (RDP) to meet the N requirement of rumen microbes and undegradable dietary (UDP) protein which is made available to animal whenever microbial protein synthesized in rumen is insufficient to meet the N requirement of the host animal tissue as per the recent concepts (Khandaker and Tareque, 1997). Synthesis of microbial protein in rumen and it's availability to animals remains the central dogma for most of the nutrition studies. It depends upon degraded N (NH_3) incorporation into microbial nitrogen. Biosynthesis and degradation of nucleic acids are the consequent pathways for protein synthesis. Almost 90% of these purines/ pyrimidines derivatives are absorbed and remain in blood plasma (Chen et al., 1990) as an exogenous derivatives. Less than 15% are derived from tissue nucleic acids turnover as an endogenous ones (McAllan, 1982). All the renal (Kanjanapruthipong and Leng, 1998), salivary (Chen et al., 1989) and mammary (Gieseck et al., 1994 and Motojima and Goto, 1990) secretions consist of plasma purine derivatives. Although most of the studies have been conducted in urine. Milk excretions of these purines derivatives have been studied less and are restricted to Sheep and Cattle.

Milk purine derivatives, especially, the Allantoin, Uric acid are known to vary with milk production (Gieseck et al 1994) and any change in exogenous purine supply through rumen microbial protein synthesis and the break down of the nucleic acid material used for the purpose, may be reflected by them (Osuji et al., 1996) in cattle. Present study is an attempt to establish the milk Allantoin and Uric Acid in Buffaloes in relation to performance status under a given dietary regimens.

* Corresponding Author: P. Sikka. Animal Biochemistry, BaB Div. CIRB, Sirsa Road, Hisar 125004, India. Tel: +91-1662-75472, Email: mrs_psikka@yahoo.com
Received May 10, 2001; Accepted July 6, 2001

MATERIALS AND METHODS

Thirty lactating buffaloes of uniform milk yield, parity and lactation stage were randomly selected from early lactation group. The average lactation stage of the animals was 20 weeks. All the animal were fed a farm ration of 30 kg green fodder *(Sorghum vulgare)*, 2 kg wheat straw along with 5 kg concentrate mixture (wheat grains 15 parts; barley grains 15 parts; wheat bran 20 parts; cotton seed cake 30 parts; mustard cake 20 parts) as the basal ration to meet the nutrients requirements of these animals for first 81 milk production as per NRC standards. The metabolisable energy content of the above ration as calculated on the basis of earlier experiments at this Institute was 2-3 M cal/kg DM basis. The total DM intake of these animals ranged from 13.0-13.75 kg/animal/day. The above diet was given as basal level for first eight liters of milk production. Over and above 1 kg concentrate mixture (CM) for every 2 kg of milk produced, thereafter was given in Group I (control) and 1 kg cotton seed, soaked or boiled per 2 kg of milk produced was given to the animals of Groups II (soaked cotton seed fed) and III (boiled cotton seed fed), respectively. Milk samples were collected after 30 days for purine estimations.

Milk purine estimations

Both Allantoin and Uric acid in milk were estimated through HPLC (Waters) as per the method of Tiemeyer and Gieseck (1982). A microparticle reversed phase column (3.8 × 300 mm), μ Bondapak C18 (Waters Associates) was used. The eluant (0.005 molar $NH_4H_2PO_4$, pH 3.5) was filtered through HA 0.45 μm pore size Millipore membrane and used at flow rate of 0.6 ml. Both Allantoin and Uric Acid were detected at 218 nm using 2,500 μ moles/l and 60 μ moles/l as the standard concentration (Sigma chemicals Co., St. Louis, Mo.), respectively, for Allantoin and Uric Acid.

Data recording

Average daily milk production during the experiment period, total lactation milk yield (300 days) and lactation length were recorded for all animals under experiment.

Statistical analysis

Differences in the data on milk allantoin, uric acid and milk yield were analysed by testing the significance of variation amongst the groups. Completely random design was adopted on the three groups of ten animals each, under study. Association of milk purine derivatives and milk production were evaluated by estimating correlation coefficients and their significance was tested (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

All the dietary purines are known to be deaminated by the ruminal microbes (Mcallan and Smith 1973). It has also been revealed from earlier studies that all the exogenous purines i.e. the purines entering the digestive tract, are utilized in intestine and remaining purines are converted to uric acid and allantoin before reaching hepatic tissue (Chen et al., 1990). Subsequently external routes eliminate them. The extent of excretion of purine derivatives as Allantoin and Uric Acid through nonrenal sources has been correlated to the concentration in plasma significantly (r=0.84) in cattle (Giesecke et al., 1994). Keeping above in view the possibility of implicating the variation in milk purine derivatives as markers of rumen microbes flourishment and microbial protein synthesis (Storm et al., 1983) the estimated levels of milk purines have been correlated with lactation performance of the buffaloes in present study.

Milk allantoin

The overall average milk allantoin level in present study was 120±11.7 μg/ml (table 1). The variation in level has been ascribed with change in dietary regimens. The milk allantoin has been a significant ($p<0.01$) variable over the three experimental groups. This variation has been attributed to the difference in energy levels of three diets in study groups. Effect of diet on allantoin levels in milk has been confirmed earlier in terms of protein quality and quantity (Gonda and Lindberg, 1997); OM (Lebzien et al., 1993); energy intake in terms of fatty acids being released during fermentation in rumen and efficiency of microbial N supply (EMNS) (Khandaker and Tareque, 1997).

Milk yield (kg/day) has been found significantly different ($p<0.01$) in three experimental groups (table 1) which obviously is due to the dietary energy changes. Replacement of concentrate mixture with cotton seed, a partially degradable protein improved the milk yield significantly in groups II and III. It is obvious that the ration in control group is not able to meet energy requirement of these animals because of the physiological limits of DMI (Lall and Tripathi, 1995). Adding cotton seed in the ration in place of concentrate mixture partially increases energy content of ration which is known to enhance milk production as shown in groups II and III. Partial degradation of cotton seed is likely to improve the level of nitrogen availability in rumen which facilitates the microflora flourishment and microbial protein synthesis (Tewatia and Paliwal, 1996). Role of partially degradable protein in milk enhancement has been reported in cattle too (Garg, 1998). More availability of microbial protein to the animal at post ruminal stage in turn increases the allantoin excretion in milk (Susmel et al., 1994). These reports might explain the significant differences in milk yield and milk allantoin concentrations revealed in present study. As per the milk allantoin levels the boiled cotton seed fed animals showed improved rumen microbial growth and protein synthesis than in soaked cotton seed fed animals although the milk production is low in this group (table 1). Soaking and boiling cotton seed are known to boost the crude protein digestion significantly without altering the DM intake (Tewatia and Paliwal, 1996). Probably in case of boiled cotton seed fed animals the EMNS is too high and the nutrients available to the microbes are much higher than their utilization limits or the boiled cotton seed regresses the DM digestibility of animals in some way which is consistent with the reports of Coppock et al. (1985).

A significant negative correlation ($p<0.05$) (r=-0.43) between the production per day and milk allantoin levels was revealed. Although the negative relation is inconsistent with the earlier reports (Giesecke et al., 1994) but it explains the origin of milk allantoin from blood plasma which is possibly diluted with increase in milk production as allantoin production is restricted to hepatic tissue due to the limited etiology of the enzyme uricase (Motojima and Goto, 1990) from where the allantoin is excreted to the plasma and thereafter into the milk.

Milk uric acid

The average milk uric acid levels in this study was 4.03±0.63 g/ml. The levels were low in concentrate fed

Table 1. Milk purine derivatives and milk production as affected by feeding of cotton seed (Mean±S.E.)

Groups	n	Allantoin	Uric acid (μg/ml)	Milk (l)	Lactation Yield (1/300 d)	Lactation Length
I (CM)	7	110±16**	1.34±0.15	5.9±0.5**	1416±133	32.0±20
II (Soaked)	10	108±12.3**	3.68±0.2	7.33±0.2**	2408±118	335.0±20
III (Boiled)	7	144±15**	4.45±1.0	6.6±0.73**	2145±96	317.0±22

**=(p<0.01)

Table 2. Analysis of variance of milk purine derivatives and production

Trait	n	MSS	F Values
Milk Production per day (Kgs.)	21	5.5	5.78**
Lactation Milk Yield (300 days)	21	12,045.0	2.445
Allantoin (μg/ml)	19	11,363.0	6.4**
Uric acid (μg/ml)	19	31.0	7.56**

**=(p<0.01)

animals (table 1) than in cotton seed fed animals. The differences in milk uric acid amongst the study groups were significant (p<0.01). However no significant correlation with milk production or allantoin levels was found. It is consistent with earlier findings where any change in exogenous purines supply is expected to be reflected as allantoin and not as uric acid. Possibility of significant variation in milk uric acid levels amongst the study groups is due to the dietary energy variation amongst the groups in present study. Possibly the higher energy boosts the microflora propagation which means more N and amino acids availability to the host mammary tissue for milk protein synthesis and production, hence the more uric acid excretion in milk. Uric acid in milk is said to be originated from mammary purine metabolism within udder probably due to which the total percentage of the nitrogen excretion in the form of purines are covered up in the form of milk uric acid as 28% in comparison to only 4% in urine (Gonda et al., 1996). It supports the role of mammary tissue metabolism in altering the levels of milk uric acid. By enlarge it appears that the soaked cotton seed fed animals performed better than boiled cottonseed fed animals with more metabolic stress on mammary tissue in boiled cotton seed feeding.However, it needs more investigation.

Present study showed a definite relation between the allantoin in milk and the production levels which might prove to be a use full tool in the assessment of production performance, though it needs to be explored over a larger population.

REFERENCES

Chen, X. B., D. J. Kyle, C. C. White, F. D. Deb Hovell and E. R. Orskov. 1989. Uric acid and allantoin in plasma and saliva of sheep. Proc. Nutr. Soc. 48:88A.

Chen, X. B., E. R. Orskov and F. D. Deb Hovell. 1990. Excretion of purine derivatives by ruminant: Endogenous excretion, differences between cattle and sheep. Br. J. Nutr. 63:121.

Coppock, C. E., J. W. West, J. R. Moya, K. G. Thompson, I. D. Jr Rowel, D. H. Nave, J. M. Labore and C. E. Gates. 1985. Effect of amount of whole cotton seed on intake, digestibility and physiological response of dairy cows. J. Dairy Sci. 68:2248.

Garg, M. R. 1998. Role of by pass protein in feeding ruminants on crop residues based diet- Review. Asian-Aus. J. Anim. Sci. 11:107.

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

Gonda, H. L., M. Emanuelson and M. Murphy. 1996. The effect of energy source in nitrogen and purine metabolism in dairy cows. Anim. Feed Sci. Techol. 64:27.

Gonda, H. L. and J. E. Lindberg. 1997. Effect of diet on milk allantoin and it's relationship with urinary allantoin in dairy cows . J Dairy Sci. 80:364.

Kanjanapruthipong, J. and R. A. Leng. 1998. Purine derivatives excreted in urine as an indicator estimating microbial yield from the rumen: - A Review. Asian-Aus. J. Anim. Sci. 11:209.

Khandaker Z. H. and A. M. M. Tareque. 1997. Effect of rumen degradable protein (RDP) in straw based ration on purine derivatives excretion and microbial nitrogen supply in cattle. Asian-Aus. J. Anim. Sci. 10:364.

Lall D. and V. N. Tripathi. 1995. Meeting the requirement of high yielding buffaloes. Indian Dairyman. 47:37.

Lebzien, P., D. Giesecke, S. Wiesmayr and K. Rohr. 1993. Measurement of microbial protein synthesis in rumen of cows by 15 N determination in duodenal contents and excretion of allantoin in milk. J. Anim. Phys. Anim. Nutr. 70:82.

McAllan, A. B. 1982. The fate of nucleic acid in ruminants. Proc. Nutr. Soc. 41:309.

McAllan, A. B. and R. H. Smith. 1973. Degradation of nucleic acids in rumen. Br. J. Nutr. 29:331.

Motojima, K. and S. Goto. 1990. Characterization of liver specific expression of rat uricase using monoclonal antibodies and clonal cDNA. Biochem. Biophys. Acta. 1087:316.

NRC. 1988. Nutrients requirements of dairy cattle. National Research Council, Washington DC.

Osuji, P. O., I. V. Nsahlai and H. Khatili. 1996. Effect of fasting on urinary excretion of nitrogen and purine derivatives by Zebu (*Bos indicus*) and crossbred (*Bos indicus* and *Bos taurus*) cattle. J. Appl. Anim. Res.10:39.

Panse V. G. and P. V. Sukhatme. 1967. Statistical methods for Agricultural Workers. Indian Council of Agri. Res. New Delhi. p. 108.

Storm E, E. R. Orskov and R. Smart. 1983. The nutritive value of rumen microorganisms in ruminants: 3, The digestion of microbial amino and nucleic acids in and losses of endogenous nitrogen from the small intestine of sheep. Br. J. Nutr. 50:479.

Susmel P., M. Spanghro, B. Stefanon, C. R. Mills and E. Plazzolta. 1994. Dgestebility and allantoin excretion in cows fed diets differing in nitrogen content. Livestock Prod. Soc. 39:97.

Tewatia, B. S. and V. K. Paliwal. 1996. Cottonseed for milk animals-A Review. Int. J. Anim. Sci. 11:85.

Tiemeyer, W. and D. L. Giesecke. 1982. Quantitative determination of allantoin in biological fluids by reverse phase HPLC. Analytical Bioch. 123:11.

Verbic, J., X. B. Chen, N. A. Macleod and E. R. Orskov. 1990. Excretion of purine derivatives by recombinants : Effects of microbial nucleic acid infusion on purine derivative excretion by steer. J. Agric. Sci., Camb. 114:243.