Indian Journal of Animal Sciences **80** (7): 674–678, July 2010

Effect of variable rumen degradable nitrogen levels on microbial protein synthesis estimated with purine derivatives in sheep urine and PDC index

M CHANDRASEKHARAIAH [1], A THULASI[2] and K T SAMPAT[3]

National Institute of Animal Nutrition and Physiology, Bengaluru, Karnataka 560 030 India

Received: 11 November 2009; Accepted: 16 January 2010

ABSTRACT

An experiment was conducted to study the effect of varying rumen degradable nitrogen (RDN) levels on microbial protein synthesis in sheep by measuring excretion of purine derivatives in spot urine samples and purine derivatives: creatinine (PDC) index. Sheep (30) were divided into 5 groups of 6 each and the animals in all groups were fed finger-millet straw (FMS) as a basal diet and soybean extraction as a nitrogen source. The animals in group 1 were fed with *ad lib*. FMS. Animals in group 2, 3, 4 and 5 were offered soybean extraction@ 16.16, 20.65, 24.75 and 26.64 g RDN/kg DOM along with FMS. The experimental feeding was continued for 2 months followed by metabolism trial. The purine derivatives (mmol/litre) excretion and PDC index were 18.74 and 34.37; 17.88 and 40.68; 20.82 and 42.62; 15.92 and 49.36 and 16.92 and 45.62; 15.92 and 49.36 and 16.92 and 45.44, in group 1 through 5, respectively. The microbial purine absorption, the microbial nitrogen (MN) supply (g/d) were comparable among the RDN supplemented groups. Purine nitrogen index (PNI) and nitrogen conversion efficiency (NCE) values were 0.10 and 1.17; 0.10 and 0.78; 0.07 and 0.65 and 0.06 and 0.62, respectively, in G 2 through 5. The result indicated that 16.16 g RDN from soybean extraction may be adequate for optimum microbial protein synthesis in sheep fed on FMS based diet.

Key words: PDC index, Microbial protein synthesis, NCE, PNI, Sheep

Indian livestock production system is based mainly on crop residues/agricultural by-products. These low quality feeds are primarily deficient in nitrogen leading to poor performance of animals. So, supplementation of these crop residues with deficient nutrients such as rumen degradable nitrogen (RDN) is necessary to meet protein requirement of rumen microbes and for optimum rumen fermentation thereby maximum microbial protein synthesis on straw/crop residue based diets. Hence, there is a need to assess or predict how much of microbial protein is synthesized in the rumen to rectify the problem and make the best use of expensive protein supplements in ruminant rations.

Purine derivatives (PD) excreted in the urine of the ruminants, originate primarily from the microbial biomass synthesized from ruminal fermentation of ingested feed. The daily excretion has been used as a parameter to estimate supply of microbial protein to the animal. This method provides a simple and non-invasive tool to study factors affecting microbial protein production. Due to the need for complete urine collection, the potential application of the method under farm condition is restricted. Chen *et al*. (1995a) reported that PD levels in spot samples of urine can be used to estimate microbial protein supply and thus provide an alternative method, which has practical application in the field. Hence an attempt was made to study the effect of different RDN levels from soybean extraction on microbial protein synthesis in sheep fed on fingermillet straw based diets by the use of purine derivatives excretion in spot urine samples.

Present address: [1,2]Senior Scientist (e-mail: chandrasekharaiah_m@yahoo.com), [3]Director.

MATERIALS AND METHODS

Bandur sheep (30) were divided into 5 groups of 6 animal each based on body weight (Table 1). The animals in all the groups were fed fingermillet straw (FMS) as a basal roughage source. Soybean extraction is used as a rumen degradable nitrogen (RDN) source. The RDN content of soybean extraction was calculated by estimating dry matter (DM) and crude protein (CP) disappearing from samples in nylon bags (pore size 40 mm) of size 100 mm×170 mm incubated in the rumen of crossbred fistulated steers for 3, 6, 9, 12 and 24 h (Mehrez and Orskov 1977). The residual CP values were transformed to natural logarithms and then subjected to linear regression (Snedecor and Cochran 1989). The degradation constant was calculated at 3 to 12 h period. The effective degradability was calculated for an assumed outflow rate of 5%/h (Miller 1980). The RDN provided by straw was not taken into account for calculating the total RDN of the diet.

Table 1. Effect of different rumen degradable nitrogen levels on intake and digestibility of finger millet straw based diet in sheep

	G1	2	3	4	5	SEM
Body weight						
kg	19.5	18.83	19.18	18.43	18.75	0.66
DM intake (g/day)						
FMS	515.58	434.94	407.31	397.61	387.25	17.58
Soybean extraction	0.00	113.40^{a}	154.72^{b}	196.42^{c}	228.68^{d}	14.75
Total	515.58	548.34	562.03	594.02	615.93	16.66
OM intake (g/day)						
FMS	464.84	392.14	367.23	358.48	349.14	15.85
Soybean Extraction	-	100.86^{a}	137.62^{b}	174.71^{c}	203.41^{d}	13.12
Total	464.84	493.01	504.85	533.19	552.53	14.95
Total intake (g/day W $^{0.75}$)						
DM	56.19^{a}	60.74^{ab}	61.86^{ab}	67.19^{b}	68.15^{b}	1.30
OM	50.66^{a}	54.60^{ab}	55.56^{ab}	60.30^{b}	61.13^{b}	1.16
Digestibility (%)						
DM	53.58^{a}	63.63^{b}	64.65^{b}	63.61^{b}	68.7^{b}	1.41
OM	56.63^{a}	66.16^{b}	67.08^{b}	66.42^{b}	70.78^{b}	1.33

Values with different superscripts within a row differ significantly (P<0.01).

The animals in group 1 (control) were fed with *ad lib.* FMS. Animals in group 2, 3, 4 and 5 were offered soybean extraction to provide 15, 22, 30 and 37 g RDN/kg digestible organic matter (DOM), respectively along with FMS. Fresh clean water was made available at all times. All the experimental animals were dewormed and kept in individual pens with feeding and watering arrangements.

The feeding period was continued for 2 months followed by a 7-day metabolism trial. Representative samples of feeds offered, leftover and faeces were collected for 7 days and preserved for further analysis.

A total of 4 spot urine samples per animal per day were collected at 6 h intervals, starting prior to feeding (0 h). The urine excreted by animals in each block was mixed, collected, measured and a fixed sample (10–15 ml based on volume collected during the period), was immediately transferred into plastic vials containing sufficient quantity of 10% H_2SO_4 to maintain pH below 3. The urine samples were diluted with distilled water in such a way that the concentration in the final samples would fall within the range of standards (5–50 mg/ litre) used in the assays for estimation of purine derivatives. These diluted samples were centrifuged and filtered through surgical gauze and stored at –20°C.

The proximate analysis of the feeds and faeces were carried out as per AOAC (1995). Further the feed samples were analyzed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) (Goering and Van Soest 1970).

Purine derivatives and creatinine content in urine samples were analyzed as per Resines *et al*. (1992) using HPLC system (waters) with minor modification. The thawed urine samples were, sonicated, centrifuged and filtered through a millex- HV 0.45 µm pore size filter and a 5 µl of the filtrate was injected into HPLC column. The PDC index, PD excretion thereby the microbial protein synthesis, was estimated as per the equations of Chen *et al*. (1992a, 1995b). The amount of microbial purines absorbed (X mmol/day) corresponding to the PD excreted (Y mmol/day) was calculated based on relationship derived by Chen *et al*. (1990). The calculation of intestinal flow of microbial N (g N/d) from the microbial purines absorbed (X mmol/d) was based on Chen and Gomes (1992). The efficiency of microbial N supply (EMNS) was expressed as grams of microbial N per kg of digestible OM apparently digested in the rumen (DOMR), which is assumed to be 65% of digestible OM intake (DOMI) (ARC 1984). The purine nitrogen index (PNI) and nitrogen conversion efficiency (NCE) were calculated in RDN supplemented groups (Chen *et al*. 1999).

The statistical analysis of data was carried out (Snedecor and Cochran 1989). The data were subjected to one-way analysis of variance; pair-wise significance was done by Duncan multiple range test using statistical software SPSS.

RESULTS AND DISCUSSION

The FMS contained 90.2, 4.2, 0.8, 75.1 and 44.8% organic matter, crude protein, ether extract, NDF and ADF, respectively, whereas the values in corresponding order for soybean extraction were 88.9, 51.3, 0.9, 21.4 and 12.5 respectively. The RDN content in soybean extraction was 4.4%, whereas RDN content of FMS was not taken into consideration for calculation. Body weight of Bandur sheep used in the study was 18.8 to 19.5 kg.

Though the diets were formulated to supply 15, 22, 30 and 37 g RDN/kg DOM of straw in animals of G 2, 3, 4 and 5; the corresponding actual intakes were 19.58, 27.85, 37.43 and 42.82 g RDN/kg DOM of straw alone and 16.16, 20.65, 24.75, 26.64 g RDN/kg DOM of total ration, respectively.

As the supplementation level increased from G 1 to G 5,

Table 2. Urinary purine derivatives (PD), creatinine excretion and PDC index as affected by different RDN levels in sheep fed finger millet straw based diets

	G1	2	3	4	5	SEM
Allantoin (mmol/lit)	14.665	15.186	18.620	13.609	15.807	0.83
Uricacid (mmol/lit) **	2.746^{c}	2.058b^{c}	1.684^{ab}	1.961^{bc}	0.974^{a}	0.17
Hypoxanthine+xanthine **	1.061^{c}	0.641^{bc}	0.518^{ab}	0.352^{ab}	0.136^{a}	0.09
Total PD (mmol/lit)	18.472	17.884	20.822	15.922	16.917	0.90
Creatinine (mmol/lit)	5.01	3.984	4.576	3.007	3.349	0.26
Creatinine (mmol/kg $W^{0.75}$)	0.12	0.15	0.14	0.13	0.15	0.01
PDC index *	34.374^{a}	40.684^{ab}	42.616^{ab}	49.36^{b}	45.436^{b}	1.63

Values with different superscripts within a row differ significantly * (P<0.05), ** (P<0.01).

the total DMI and OMI increased, hence experimental animals in supplemented groups ate slightly higher DM and OM when compared to control group of animals. However, DMI was similar among the supplemented groups indicating that supplementing soybean extraction levels did not affect the straw intake at different RDN levels. DMI of straw did not increase significantly due to supplementation, as the animals in these groups have already met the DM requirements more than the recommended levels (ICAR 1985).

Dry matter intake and organic matter intake/kg $W^{0.75}$ was significantly higher in G 4 and 5 when compared to G 1, however, it was on par among the supplemented groups. The sheep had mean values of DMI ranging from 56.19 ± 2.28 to 68.15 ± 2.01/kg $BW^{0.75}$ which were comparatively higher than the value of 50 g/kg $BW^{0.75}$ suggested by NRC (1985) for non productive sheep.

The DMD and OMD% were comparable among the supplemented groups but significantly (P< 0.01) higher than the control group indicating that supplementation increased the digestibility. The higher DMD and OMD% observed in supplemented groups when compared to unsupplemented groups (Table 1) might be owing to higher RDN consumption there by availability of required rumen degradable nitrogen for microbial growth in supplemented groups. Increased *in vitro* NDF digestibility was observed when groundnut cake and other locally available protein sources was supplemented @15 g RDN/kg DOM on finger millet straw based diets by Chandrasekharaiah *et al.* (2002, 2003).

Purine derivatives, creatinine, PDC index, microbial nitrogen supply: The mean concentration of allantoin, uric acid, hypoxanthine plus xanthine, total PD and creatinine (mmol/litre) is presented in Table 2.

The allantoin concentration was not significantly different among the experimental groups. The mean uric acid concentration was different (P<0.01) among different groups. G 1 recorded higher uric acid concentration compared to G 5; however the uric acid concentration is comparable among the supplemented groups.

Hypoxanthine plus xanthine concentrations were different (P< 0.01) among different groups (Table 2). Higher values were observed in G 1 and lower values were observed in G 5. As the supplementation level increased, the hypoxanthine and xanthine levels decreased in the experimental animals. The mean PD concentrations were not different among treatments.

The concentration of creatinine was not different among the experimental groups. The observed measurements of creatinine/kg BW $^{0.75}$ of 0.12 ± 0.0 to 0.15 ± 0.02 mmol/kg BW $^{0.75}$ for sheep was lower than the range of values 0.40 ± 0.06 to 0.502 ± 0.10/kg BW $^{0.75}$ observed in European sheep (Chen *et al*. 1991, Lindberg and Jacobsson 1990 and Chen *et al*. 1995a). The lower creatinine concentration observed in the present study when compared to the reported values may be due to lower body weight of the animal (Banur sheep), as the creatinine excretion is a function of protein mass of the animal or metabolic body weight of the animal. It is presumed that excretion rate of creatinine is relatively constant in healthy animals and remains independent of level of feed intake (Chen *et al.* 1992b, 1995b). The creatinine excretion varies widely with the breed or species of animals but usually independent of the level of intake or duodenal infusion of purine bases (IAEA 2002).

The proportions of the individual purine derivatives in total PD excretion were not constant. This observation agrees with the results of the previous studies by Chen *et al*. (1990, 1992b). The fluctuations noted for urinary concentrations of allantoin, uric acid and xanthine plus hypoxanthine and consequently total PD and creatinine in spot urine samples could have been due to the influence of urine volume associated with that of drinking water intake Chen *et al*. (1992a).

The mean PDC index values were comparable among the supplemented groups but G 1 recorded significantly (P<0.05) lower index values than G 4 and 5. Lower or higher PDC index values observed in respective groups was mainly due to corresponding lower or higher creatinine excretion coupled with the metabolic body weight of the animals in the concerned groups of sheep. When sheep were fed under *ad lib*. conditions, PD in spot urine may provide a practical indicator of microbial protein supply status (Chen *et al*. 1995b). The PDC index in spot urine of sheep fed *ad lib*. was subject to little diurnal variations and was highly correlated with the daily output of PD.

The estimated microbial purine absorption, microbial nitrogen supply (MN) was significantly (P<0.03) lower in G

Table 3. Microbial purine absorption (MPA), microbial nitrogen supply (MNS), efficiency of microbial N supply (EMNS), purine nitrogen index (PNI) and nitrogen conversion efficiency (NCE) in sheep fed different RDN levels on finger millet strew based diet.

	G1	2	3	4	5	SEM
Body						
MPA (mmol/d) *	4.636[a]	7.533[b]	6.782[ab]	7.619[b]	8.161[b]	0.40
MNS (g/d)*	3.370[a]	5.418[b]	4.931[ab]	5.539[b]	5.934[b]	0.29
MNS (g/kg DOMI)	13.611	16.870	14.449	15.687	15.548	0.76
EMNS(g/kg DOMR)	20.94	25.954	22.231	24.133	23.921	1.17
PNI **	-	0.103[b]	0.099[b]	0.069[a]	0.062[a]	0.006
NCE *	-	1.08[b]	0.72[a]	0.63[a]	0.56[a]	0.064

Values with different superscripts within a row differ significantly *(P<0.05), ** (P<0.01).

1 than G 2, 4 and 5, however, it was comparable among the supplemented groups, indicating that optimum RDN from the soybean extraction would have been available for microbial fermentation, thereby providing matching nitrogen energy requirement to rumen microbes, which would have provided desired rumen environment for increased microbial protein synthesis in G 2. The MN supply when expressed as MNS/kg DOMI or MNS/kg DOMR (EMNS), it remained high in G 2, and around 25% more MN is synthesized in G 2, when compared to unsupplemented group. However MNS/kg DOMI and EMNS recorded were not significantly different among different experimental groups. The lower digestibility of nutrients coupled with lower intakes in G1 and comparable intakes and digesitbilities among the RDN supplemented groups would have resulted in nonsignificant differences in MN supply when expressed as MNS/kg DOMI or DOMR.

EMNS ranged from 21.94 to 25.95 g N/kg DOMR as against the value, 32 g N/kg DOMR adopted by the ARC (1984). Our values in the present study are in the range of reported values, i.e. 14 to 49 mg/kg DOMR (AFRC 1992) and 18.1 to 46.7 g N/kg DOMR Chen *et al*. (1995b). The purine bases absorption in gut showed that the RDN content supplied from soybean extraction had a strong impact on microbial protein (MP) yield and, consequently on the MP supply to the host animal. The MN yield (g/d) obtained in this study is corroborated well with the reported values (2.7 to 9.12 MN g/d) of Mupangwa *et al*. (2000) and Chikagwa-Malunga *et al*. (2009) in sheep.

Very limited literature is available with regards to PNI and NCE of ruminant diets. Comparable PNI values were observed between G 2 and 3, but higher (P<0.01) than G 4 and 5, which were at par. However, NCE values were significantly (P<0.01) higher in G 2, when compared to G 3, 4 and 5. It is suggested that PNI can potentially be used as an indictor of the efficiency with which degradable dietary nitrogen is converted to microbial protein in the rumen (Chen *et al*. 1999). If a diet or a dietary regimen has high nitrogen conversion efficiency (NCE) proportionally more rumen degradable nitrogen is converted to microbial protein and less nitrogen is excreted in urine resulting in a high PNI. However, in the present study, higher PNI and NCE values in G 2 observed indicated that 16.12 g of RDN/kg DOM may be adequate for optimum microbial protein synthesis in sheep fed on finger millet straw based diets. Therefore, our results revealed that, the PDC index, PNI and NCE measured in spot urine of sheep fed *ad lib.* could be used under farm conditions as a practical indicator of microbial protein supply in sheep. Further it is indicated that 16.12 g RDN/kg DOM from soybean extraction may be adequate for maximum microbial protein synthesis in the rumen on finger millet straw based diets in sheep.

REFERENCES

AFRC. 1992. Agricultural Food and Research Council. *Energy and Protein Requirements of Ruminants*. CAB international, Wallingford.

AOAC. 1995. *Official Methods of Analysis*. 16th edn, Vol.1. Association of Official Analytical Chemist. Washington, DC, USA.

ARC. 1984. *The Nutrient Requirements of Ruminant Livestock*. Agricultural research council. Suppl 1. Wallingford, CAB International.

Chandrasekharaiah M., Sampath K T, Prakash C and Praveen U S. 2002. Effect of supplementation of different concentrate ingredients on in vitro NDF digestibility of finger millet straw. *Animal Nutrition and Feed Technology* **2** (2): 169–76.

Chandrasekharaiah M, Sampath K T, Praveen U S and Prakash C. 2003. Improving the digestibility of finger millet straw by strategic supplementation through locally available concentrate ingredients and green fodders/top feeds. *Indian Journal of Animal Sciences* **73** (10): 1184 –86.

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. Occasional publication, Rowett Research Institute, University of Aberdeen, UK.

Chen X B, Chen Y K, Franklin M F, Orskov E R and Shand W J. 1992b. The effect of feed intake and body weight on purine derivative excretion and microbial protein supply in sheep. *Journal of Animal Science* **70**: 1534–42.

Chen X B, DeB F D, Hovell, Orskov E R and Brown D S. 1990. Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivatives excretion by sheep. *British Journal of Nutrition* **63**: 131.

Chen X B, Grubic G, Orskov E R and Osuji P. 1992a. Effect of feeding frequency on diurmal variation in plasma and urinary

purine derivatives in steers. *Animal Production* **55**: 185–91.

Chen X B, Kyle D J, Orskov E R and Hovell F D. 1991. Renal clearance of plasma allantoin in sheep. *Experimental Physiology* **76**: 59–65.

Chen X B, Mejia A T, Kyle D J and Orskov E R. 1995b. Evaluation of the use of the purine derivative: creatinine ratio in spot urine and plasma samples in ruminants: Studies in sheep. *Journal of Agricultural Science* (*Cambridge*) **125**: 137–43.

Chen X B, Subba D B, Orskov E R and Jayasuriya M C N. 1999. Purine nitrogen index, potentially a new parameter for rapid feed evaluation in ruminants. I*AEA-IECDOC* **109**: 97–110.

Chen X B, Susmel P, Stefanon B and Orskov. 1995a. On the use of purine derivatives in spot urine, plasma and milk samples as indicators of microbial protein supply in sheep and cattle. *Proceedings of the 7th Internatinal symposium on protein metabolism and nutrition*. Vale de santarem, Portugal.

Chikagwa- Malunga S K, Adesogan A T, Szabo N J, Littell R C, Phatak S C, Kim S C, Arriola K G, Huisden C M, Dean D B and Krueger N A. 2009. Nutritional characterization of Mucuna pruiens. 3. Effect of replacing soybean meal with Mucuna on intake, digestibility, N balance and microbial protein synthesis in sheep. *Animal Feed Science and Technology* **148**: 107–23.

Goering H K and Van Soest P J. 1970. *Forage Fibre Analysis.* Handbook No.379. ARS USDA, Washington. DC.

IAEA. 2002. Report of final research Co-ordination committee meeting,Vietnam.

ICAR. 1985. *Nutrient Requirements of Livestock and Poultry*. Indian Council of Agricultural Research, New Delhi.

Lindberg J E and Jacobsson K G. 1990. Nitrogen and purine metabolism at varying energy and protein supplies in sheep sustained on intragastric infusion. *British Journal of Nutrition* **64**: 359–70.

Mehrez A Z and Orskov E R. 1977. A study of the artificial bag technique for determining the digestibility of feeds in the rumen. *Journal of Agricultural Science, Cambridge* **88**: 645–50.

Miller E L. 1980. Vicia faba 3/4 Feeding Value, Processing and Viruses. *Protein Value of Feedstuffs for Ruminants*. (Ed.) Bond D A. Martinus. Nijhoff, The Hague.

Mupangwa J F, Ngongoni N T, Topps J H, Acamovic T, Hamudikuwanda H, Nalovu L R. 2000. Dry matter intake, apparent digestibility and excretion of purine derivatives in sheep fed tropical legume key. *Small Ruminant Research* **36**: 261–68.

NRC. 1985. *Nutrient Requirements of Sheep*. 6th edn (Revised). National Academy press, Washington, DC.

Resines J E, Arin M J and Diez M T. 1992. Determination of creatinine and purine derivatives in ruminants urine by reversal phase high performance liquid chromatography. *Journal of Chromatography* **607**: 199–202.

Snedecor G W and Cochran W G. 1989. *Statistical Methods*. 8th edn. Oxford and IBH publishing Co., Kolkatta.