URINARY EXCRETION OF PURINE DERIVATIVES AS AN INDEX OF MICROBIAL PROTEIN SUPPLY IN CROSS-BRED (*Bos indicus* × *Bos taurus*) CATTLE IN TROPICAL ENVIRONMENT

A. OJEDA, O. PARRA
Instituto de Producción Animal, Facultad de Agronomía,
Universidad Central de Venezuela,
Maracay,
Venezuela

Abstract

URINARY EXCRETION OF PURINE DERIVATIVES AS AN INDEX OF MICROBIAL PROTEIN SUPPLY IN CROSS-BRED (*Bos indicus* × *Bos taurus*) CATTLE IN TROPICAL ENVIRONMENT.

Four experiments were carried out to establish a response model between urinary excretion of purine derivatives (PD) and microbial production in *Bos indicus* × *Bos taurus* cross-bred cattle: LZ, MZ and HZ (3/8, 1/2 and 5/8 *Bos indicus*, respectively). The fasting PD excretion was considered as endogenous excretion and amounted to 268 (± 85.1), 294 (± 128.1) and 269 (± 68.4) μmol/kg $W^{0.75}$ for LZ, MZ and HZ, respectively. Urinary recovery of absorbed purine bases (PB) was calculated as the urinary recovery of a single dose of intrajugular infused uric acid (1,3-^{15}N). In HZ crossbred cattle 83% (± 20.3)of infused uric acid was recovered in the urinary PD. The relationship between duodenal purine absorption (X, mmol/d) and urinary PD excretion (Y, mmol/d) was defined in HZ crossbred cattle as $Y = 0.83\ X + 0.269W^{0.75}$ (± 85.1), assuming that the endogenous contribution was constant and independent of the exogenous PB supply. The activity of xanthine oxidase (EC 1.2.3.2.) was determined in HZ and MZ and was found to be higher in the liver (0.62 and 0.66 units/g, respectively) than in intestinal mucosa (0.09 and 0.03 units/g, respectively), whereas xanthine oxidase activity was practically absent in plasma of both cross breeds. The ratio PB:total N was determined in microbial extracts taken from rumen fluid of cows fed Bermuda grass *(Cynodon dactylon)* as the sole diet or supplemented (ratio of 80:20, grass:supplement) with gluten feed, soybean hulls or *Gliricidia* species and were found to range from 1.52-1.62 μmol PB/mg N.

1. INTRODUCTION

Urinary excretion of purine derivatives (PD: i.e. allantoin, uric acid, hypoxanthine and xanthine) has been used as an index to estimate rumen microbial synthesis in sheep, goats and cattle [1-5]. This is a non-invasive method that only requires urine collection and so is suitable for use under farm conditions to monitor the status of microbial protein supply to the animal. Published methods refer only to the above cited species and it would be desirable to extend them to other species or breeds of economic significance in tropical areas. However, probable species differences in the metabolism of purine bases may prevent the direct application of the existing model [6].

The aim of this study was to obtain a response model to predict microbial protein supply in *Bos indicus* × *Bos taurus* crossbred cattle on the basis of: (i) the endogenous contribution of PD to urinary excretion, (ii) the relationship between duodenal absorption and urinary excretion of purine derivatives and (iii) tissue profile of xanthine oxidase (EC 1.2.3.2.) as a key enzyme of purine base metabolism [7]. In the present trails, changes in the concentration of purine bases in microbial N as induced by diet, were also studied.

2. MATERIAL AND METHODS

2.1. Experiment I Urinary excretion of PD during fasting

2.1.1. Animals and diets

Eighteen male, crossbred (*Bos taurus* × *Bos indicus*) cattle of approximately 2 years of age were used in this experiment. The crossbred cattle were either 3/8 *Bos indicus* (LZ) (271 ± 8.1 kg LW); 1/2 *Bos indicus* (MZ) (246 ± 7.7 kg LW) or 5/8 *Bos indicus* (HZ) (366 ± 19.8 kg LW). Six animals from each category were housed individually in metabolism cages and were fed hay (*Cynodon dactylon*) *ad libitum,* for 15 days (adaptation period). Thereafter, feed intake was reduced every 2 days and feed was offered at 60, 30, and 0%, of the previously registered *ad libitum* intake (restriction period), and then fasted for 7 days (fasting period).

2.1.2. Sample collection

On day 7 of the adaptation period urine was collected into buckets containing 200 ml 1M-H_2SO_4 in order to maintain the pH of urine below 3. The daily collection of urine was weighed, density measured and 1% of total daily excretion was diluted to 1 L with distilled water. On day 14, blood samples were taken from the tail vein every 8 h. Thereafter, blood samples were taken every 24 h until the end of the fasting period. Blood samples (15 ml) were centrifuged at 1 500 rpm for 15 min and plasma was frozen for subsequent analyses.

2.2. Experiment II Urinary excretion of PD and isotope recovery at different levels of feed intake

2.2.1. Animals and diets

Four crossbred cattle (HZ) with a mean live weight of 328 ± 11.7 kg were used. Animals were individually housed and randomly allocated to four dietary treatments in a 4x4 Latin Square design. Bermuda grass hay (*Cynodon dactylon*) was given at four levels: 95 (D95), 80 (D80), 60 (D60) or 40% (D40) of *ad libitum* (120 g DM/kg $W^{0.75}$) intake.

2.2.2. Experimental procedure and sample collection

Each experimental period lasted for 21 days, allowing 11 days for dietary change over and 10 days for experimental measurements. The following schedule was employed: Urine and faeces were collected from day 12 to 21 and isotope infusion was carried out from day 18 to 21. Faeces was collected daily, homogenised and sampled (10% of total excretion) and stored at 4°C until analysis. The urine was collected daily in buckets containing an acidic solution (as in Experiment I) and weighed. Specific gravity was recorded and urine was sampled and stored. On day 18, 200 mg of labelled uric acid (1,3-^{15}N 98%+; Isomed, Madrid, Spain) were diluted with 100 ml of 50% (v/v) glycerol/saline solution and made alkaline to pH 8 with 2M NaOH solution to obtain complete solubilization of the uric acid. The uric acid solution was autoclaved and slowly infused through the jugular vein to two animals on each of the treatments D40 and D95. After isotope infusion, urine was sampled every 6 h during 4 days, weighed, specific gravity recorded and 2 sub-samples (2%) stored at -20°C. Blood samples were also taken every 12 h as described in Experiment I.

2.3. Experiment III Xanthine oxidase activity in plasma, liver and intestinal tissue

Samples of plasma, liver and intestinal tissues were taken from six animals (3, HZ and 3, MZ cross-breds) at a local slaughter house for measuring xanthine oxidase activity.

Blood samples were collected into heparinized tubes and centrifuged at 3 000 × *g* for 15 min. Plasma samples were analysed within the same day. The procedure for collection, processing and extraction of liver and intestinal samples were as described in IAEA-TECDOC-945 [8]. Activity of xanthine oxidase was measured as the rate of uric acid production when xanthine was incubated with tissue extracts [8].

2.4. **Experiment IV** Microbial composition of rumen samples

Four, five year old zebu crossbred cows (MZ) (348 ± 48.5 kg), each fitted with a rumen cannula (12 cm Ø) were used in this study. Animals were randomly allocated to four dietary treatments in a 4 x 4 Latin Square design. Treatments consisted of four experimental diets: Bermuda grass hay, given as the sole diet (C, 100%) or at 80% and supplemented with either 20% gluten feed (GF), soybean hulls (SH) or dry *Gliricidia* foliage (*Gliricidia sepium*, G). Animals were housed in individual pens and were fed *ad libitum* (120 g/kg $W^{0.75}$/d) twice a day at 8.00 and 16.00 h, for 2 weeks. On the last day rumen contents were sampled.

The whole rumen contents (500 ml) were squeezed through surgical gauze to remove solid material. Liquid associated bacteria were isolated from the filtrate by centrifuging at 500 × *g* for 5 min, followed by two consecutive centrifugations at 20 000 × *g* for 20 min at 4°C. The microbial extracts were freeze dried for subsequent analysis.

2.5. **Analytical procedure**

Dry matter was determined by drying the samples to constant weight at 105°C and organic matter by ashing at 550°C for 8h. NDF and ADF contents were determined by the procedures of Goering and Van Soest [9]. The total nitrogen content was determined by the Kjeldahl method. Allantoin in acidified urine was determined by colorimetry [10]. Adenine and guanine (PB) in samples of rumen bacteria (15 mg) were determined either by HPLC, after acid hydrolysis with 2 ml 2N-perchloric acid at 100°C for 1h and the addition of 0.20 µmol of allopurinol and immediate neutralisation with 4.5 M KOH [11] or by silver ion precipitation following Zinn and Owens [12]. Creatinine concentration was measured using picric acid [13]. Uric acid was measured by the bound uricase method [8]. The isotope enrichment of ^{15}N was determined using gas Chromatography/Mass Spectrometry following Chen *et al.* [14].

3. RESULTS AND DISCUSSION

3.1. **Urinary excretion of PD during fasting**

The mean daily excretion of urinary PD (allantoin, uric acid and total PD) are presented in Table I. The effect of fasting on PD excretion is shown in Figure 1. Restriction of feed lead to a rapid decrease in urinary excretion of allantoin and total PD, reaching a stable basal value after about 5 d. In the present paper this value was considered as the endogenous contribution to urinary excretion, assuming that duodenal flow of purine bases would represent only a minor fraction.

PD excretion during fasting from day 12 to 14 averaged 266 ± 75, 274 ± 28.7 and 268 ± 42.4 µmol/kg $W^{0.75}$ for HZ, MZ and LZ breed types, respectively. There were no differences

between the breed types. Fasting PD excretion showed a range between 160.5 and 394.2 μmol/kg $W^{0.75}$ and the CV of daily measurements were 17.3, 15.8 and 17.2% for LZ, MZ and HZ, respectively. Creatinine excretion was independent of the animal type and was 384 ± 56.1 μmol/kg $W^{0.75}$.

TABLE I. DAILY URINARY EXCRETION OF PD (μmol/kg $W^{0.75}$) IN THREE DIFFERENT TYPES OF ZEBU × EUROPEAN CROSS-BRED CATTLE (LZ, MZ, AND HZ), PRIOR TO FASTING (PF), UNDER RESTRICTED FEEDING (R) AND DURING FASTING (F)

	Breed type		
	LZ ± sd	MZ ± sd	HZ ± sd
Allantoin			
PF	450.9 ± 170.4	408.9 ± 125.1	452.1 ± 140.7
R	285.3 ± 95.2	339.2 ± 115.2	346.5 ± 166.0
F	236.5 ± 67.7	259.8 ± 127.7	244.5 ± 88.5
Uric acid			
PF	30.2 ± 4.3	31.4 ± 3.0	24.8 ± 1.5
R	30.4 ± 2.5	31.2 ± 1.9	25.1 ± 2.0
F	32.3 ± 2.6	33.9 ± 3.0	24.8 ± 1.5
Total PD			
PF	481.1 ± 171.9	440.4 ± 125.9	476.9 ± 141.2
R	315.7 ± 95.0	370.4 ± 115.7	371.6 ± 167.5
F	268.8 ± 68.4	293.7 ± 128.1	269.4 ± 85.1

Basal excretion of PD during fasting was higher than values reported for sheep (136 to 202 μmol/kg $W^{0.75}$) [1, 15-17] and goats (195 μmol/kg $W^{0.75}$) [3], and lower than values reported for cattle (455 to 609 μmol/kg $W^{0.75}$) [18-20]. Osuji *et al.* [21] reported much lower values of 172 μmol/kg $W^{0.75}$ and 108 μmol/kg $W^{0.75}$ for zebu and zebu cross-bred cattle, respectively. In swamp buffalo, Liang *et al* [22] found a similar PD excretion level during fasting, for both allantoin (228 μmol/kg $W^{0.75}$) and uric acid (152.6 μmol/kg $W^{0.75}$) with a total PD excretion during fasting of 380 μmol/kg $W^{0.75}$. It is not possible to determine whether the differences are due to genetical or to methodological effects but lower PD excretion could be related to a differential capability of N re-utilization depending on the species [6] or breed type.

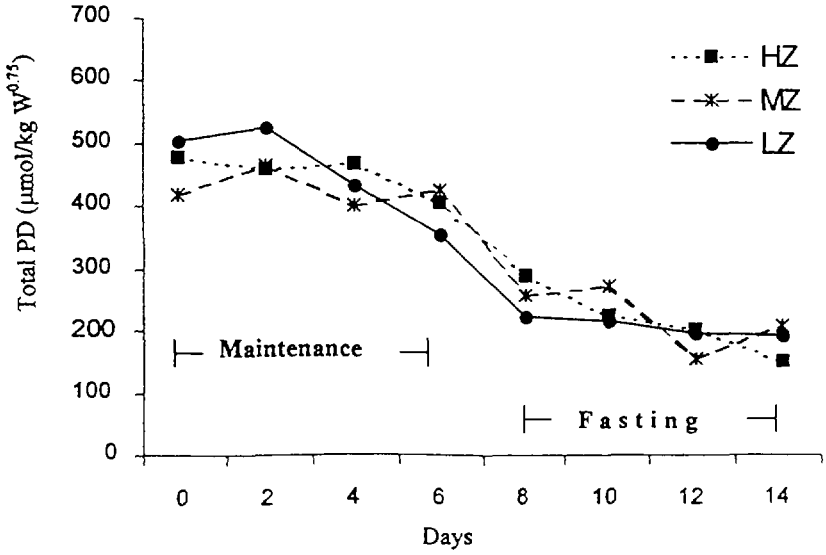

FIG. 1. Daily urinary excretion of PD in cross-bred Bos indicus x Bos taurus cattle (LZ, MZ and HZ) during feed restriction and fasting.

3.2. Urinary excretion of PD at different levels of feed intake

The daily urinary excretion of PD at different levels of intake are presented in Table II. As in Experiment I, allantoin and uric acid accounted for all urinary PD. The PD excretion responded significantly to digestible organic matter intake (DOMI). This increase is mainly explained by the significant response in alloantoin excretion, although intermediate metabolites such as uric acid showed a lower but significant increase. When urinary excretion of PD (mmol/d) was plotted against DOMI, the following equation was obtained.

$$Y = 7.69 (\pm 4.2) + 5.69 (\pm 1.68) X$$

(where n = 16, RSD = 5.22 and r = 0.67)

Although in all cases, values were characterised for high residual variations (CV= 18%), the slope of the regression indicates that the rate of PD excretion per unit of DOMI (5.67 ± 1.68 mmol/kg DOMI) was much lower than values reported for *Bos taurus* (18.5mmol/kg DOMI) [20] or buffalo (8.3 mmol/kg DOMI) [22]. Individual variation adjustment procedure together with the use of different experimental conditions may explain partially the variation registered, yet results seem to suggest that *Bos indicus* shows a lower response to intake of digestible organic matter in terms of urinary allantoin excretion.

TABLE II. INTAKE (kg/d) OF DRY MATTER (DM), ORGANIC MATTER (OM) AND DIGESTIBLE ORGANIC MATTER (DOM) AND URINARY EXCRETION OF CREATININE AND PD (μmol/kg $W^{0.75}$) IN ZEBU CATTLE AT DIFFERENT LEVELS OF FEED INTAKE

	Level feed intake (%)					
	40	60	80	95	SEM	Significance
Intake						
DM	3.50	5.60	7.38	8.85	2.08	**
OM	2.84	4.74	6.26	7.51	1.82	**
DOM	1.35	2.16	2.84	3.41	0.80	**
PD excretion						
Allantoin	15.8	17.0	20.2	20.6	3.01	**
Uric acid	1.95	2.02	2.10	2.12	0.18	NS
Total PD	17.9	19.1	22.3	22.7	2.78	*
Creatinine	533.5	468.3	487.0	431.5	68.2	NS

Statistical significance of the effect of diet (D)
NS, Not significant; * , P <0.05, ** , P <0.01

3.3. Isotope recovery

The relationship of the response model between PD excretion and purine absorption has been defined by infusing known amounts of nucleic acid through the abomasum or duodenum and monitoring the excretion of PD in urine. In cattle, this relationship has been described as a linear model Y= a + bX (where Y = urinary PD and X = duodenal PB [2, 23], "a" the intercept representing the endogenous contribution and "b" the proportion of plasma PD excreted in

urine and representing the incremental recovery in urine of absorbed PB. Such a parameter could be estimated by direct recovery of labelled-PD as uric acid when it is administered directly through the jugular vein [8]. In Table III, the amount of labelled uric acid infused and PD (allantoin plus uric acid) recovered together with isotope recovery in urine are presented.

TABLE III. RECOVERY AS URINARY PD (ALLANTOIN PLUS URIC ACID) OF LABELLED URIC ACID (1,3-^{15}N) INFUSED THROUGH THE JUGULAR VEIN IN TWO CROSSBRED ZEBU CATTLE (HZ) FED DIFFERENT LEVELS OF BERMUDA GRASS (*Cynodon dactylon*)

Animal	^{15}N Uric acid infused (μmol/animal)	^{15}N Urinary recovery (μmol/animal)	Recovery (%)
1	1.07	0.961	88
2	1.05	0.822	78
Mean ± SEM	1.06 ± 0.1	0.891 ± 0.2	83 ± 20.6

The concentration of ^{15}N-uric acid in plasma decreased exponentially due to its excretion in urine as well as by oxidation to allantoin. Therefore, 36 h after the injection of the labelled material no enrichment was detected in the urine samples. With the reservation that the number of animals was small, the results showed that 84% of plasma ^{15}N-uric acid was excreted in urine. The authors are unaware of data in zebu cattle, but those corresponding to *Bos taurus* seem to be in a similar range. Thus, Verbic *et al* [2] found a recovery of 77% and Beckers and Thewis [23] reported a recovery of 74%. Lower values have also been reported by McAllan *et al* [24] in *Bos taurus* cattle with 65 and 40%, where intraduodinally infused adenine and guanine were recovered as urinary metabolites. Although the recovery ratio agrees well with the proportion of renal and non-renal partitioning proposed by Chen *et al* [16] (84 and 16% respectively), such recovery levels do not explain the low response in PD excretion to DOMI obtained in this species. It is necessary to emphasise that when comparing both methodological approaches the isotope recovery trial does not take into account digestion, absorption and metabolism of duodenal nucleic acid.

3.4. Xanthine Oxidase activity in plasma, and liver and intestinal tissues

Figure 2 (A, B and C) shows the increase in uric acid when xanthine was incubated with different tissue extracts as a measure of their xanthine oxidase (XO) activity. Estimated values of XO activity (units/min/g tissue) in plasma and intestine are presented in Table IV. The XO activity was much higher in the liver than in the intestine (0.62 and 0.66 vs 0.09 and 0.03 for HZ and MZ, respectively) whereas plasma showed only traces of XO activity. The differences between tissues confirmed previous results in sheep [5], cattle [16], buffalo [6] and rabbits [25]. However zebu cattle, *Bos taurus* cattle and sheep showed much higher values in the liver than in the gut, while in buffalo and rabbit there was very little activity in the liver. When data were analysed individually for each tissue, HZ showed a consistently higher XO activity in the gut than in MZ ($P < 0.05$) while data from liver and plasma were more variable.

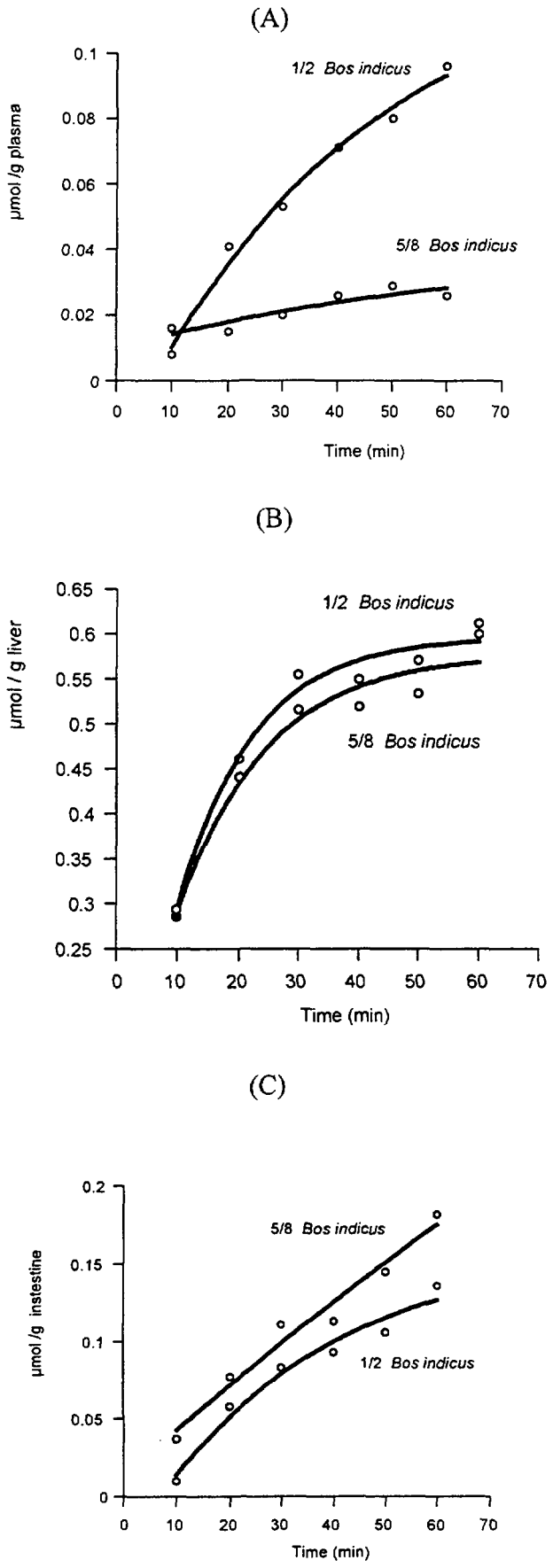

FIG.2. Production of uric acid when xanthine was incubated with plasma (A) liver (B) or extract of intestinal mucosa (C) of MZ (1/2) and HZ (5/8) Bos indicus × Bos taurus crossbred cattle.

TABLE IV. ACTIVITY OF XANTHINE OXIDASE (EC 1.2.3.2) IN PLASMA, LIVER AND INTESTINAL MUCOSA OF MZ AND HZ *BOS INDICUS* × *BOS TAURUS* CROSSBRED CATTLE.

Tissue	Breed type	Animals			Mean	SD
		1	2	3		
Plasma	HZ	0.92	0.77	0.83	0.83	0.075
(unit/L)	MZ	3.16	8.33	7.71	6.37	2.82
Liver	HZ	0.29	0.85	0.72	0.62	0.29
(unit/g wet tissue)	MZ	0.45	0.76	0.76	0.66	0.17
Intestine	HZ	0.09	0.09	0.08	0.09	0.006
(unit/g wet tissue)	MZ	0.02	0.02	0.04	0.03	0.092

3.5. Composition of microbial extracts

Table V shows the composition (N, mg/g DM; PB, μmol/g DM and PB/N ratio) of microbial samples extracted from the liquid fraction of rumen fluid. PB was analysed by both colorimetry [12] and by HPLC methods [11]. In general, there was a tendency for colorimetric methods to overestimate PB values, but differences between colorimetric and HPLC methods were not statistically significant. Such overestimation could be explained by the non-specificity of the calorimetric methods.

TABLE V. COMPOSITION OF MICROORGANISMS ISOLATED FROM THE LIQUID FRACTION OF RUMEN CONTENTS IN ZEBU CROSSBRED CATTLE FED *AD LIBITUM* BERMUDA GRASS GIVEN AS THE SOLE DIET (C, 100%), OR AT 80% AND SUPPLEMENTED EITHER WITH 20% OF GLUTEN FEED, SOYA BEAN HULLS OR DRY GLIRICIDIA FOLIAGE (*Gliricidia sepium*).

Diet	N (g/kg)	Purine Bases	
		μmol/mg N (mgPB-N/100 mg N)	
		HPLC [11]	Zinn & Owens [12]
Hay (*Cynodon* spp)	8.25 ± 1.57	1.56 (11.0)	1.77 (12.4)
Gluten feed	7.69 ± 1.38	1.61 (11.3)	1.93 (13.5)
Soybean hulls	7.81 ± 0.79	1.52 (10.6)	1.80 (12.6)
Gliricidia foliage	7.29 ± 1.33	1.55 (10.8)	1.46 (10.2)
RSD 1	-	3.46	0.18
RSD 2	-	2.33	0.21

Type of diet affected PB content (μmol/g DM) and microbial extracts taken from animals fed hay or gluten feed, showed lower values than those taken from animals fed soybean hulls or Glyricidia ($P < 0.05$). When values were expressed as μmol/mg N there was no difference among extracts.

REFERENCES

[1] BALCELLS J., GUADA, J., CASTRILLO, C., SURRA, J., Urinary excretion of allantoin precursors by sheep after different rates of purine infusion into the duodenum, J. Agric. Sci., Cambridge **116** (1991) 309-317.

[2] VERBIC, J., CHEN, X., MACLEOS, N., ØRSKOV, E., Effect of microbial nucleic acid infusion on purine derivatives excretion by steers, J. Agric. Sci., Cambridge **114** (1990) 243-248.

[3] LINDBERG, J., Excretion of purines in the urine of sheep in relation to duodenal flow of microbial protein, Swedish J. Agric. Research **19** (1989) 45-52.

[4] DEWHURST, R., WEBSTER, A., Effects of diet, level of intake, sodium bicarbonate and monensin in urinary allantoin excretion in sheep, Br. J. Nutr. **67** (1992) 345-353.

[5] CHEN, X., KYLE, D., ØRSKOV, E., HOVELL, F., Renal clearance of plasma allantoin in sheep, Experimental Physiology **76** (1991) 59-65.

[6] CHEN, X., SAMARAWEERA, L., KILE, D., ØRSKOV, E., ABEYGUNAWARDENE, H., Urinary excretion of purine derivatives and tissue xanthine oxidase activity in buffaloes, with special reference to differences between buffaloes and *Bos taurus*, Br. J. Nutr. **75** (1996) 397-407.

[7] ZOLLNER, N., Purine and pyrimidine metabolism, Proceedings of the Nutrition Society **41** (1982) 329-342.

[8] INTERNATIONAL ATOMIC ENERGY AGENCY, Estimation of rumen microbial protein production from purine derivatives in urine, IAEA-TECDOC-945, Vienna (1997).

[9] GOERING, H., VAN SOEST, P., Forage fiber analysis, Agric. Research Service, U.S. Department of Agriculture. Handbooks N° 21 (9970) 31 p.

[10] YOUNG, E., CONWAY, C., On the estimation of allantoin by the Rimini-Schryver reaction, J. Bio. Chem. **142** (1942) 839-852.

[11] MARTÍN ORÚE, S., BALCELLS, J., GUADA, J., CASTRILLO, C., Endogenous purine and pyrimidine derivative excretion in pregnant sows, Br. J. Nutr. **73** (1995) 375-385.

[12] ZINN, R., OWENS, F., A rapid procedure for purine measurement and its use for estimating net ruminal protein synthesis, Can. J. Anim. Sci. **66** (1986) 157-166.

[13] TAUSSKY, H., Standard Methods of Clinical Chemistry, Academic Press, New York. (1996) 235.

[14] CHEN, X., CALDER, A., PRASITKUSOL, P., KILE, P., JAYASURIYA, M. C. N., Determination of ^{15}N isotopic enrichment and concentrations of allantoin and uric acid in urine by gas chromatography/mass spectrometry, J. Mass Spectrometry **33** (1998) 130-137.

[15] ANTONIEWICZ, A., HEINEMANN, W., HANKS, E., The effects of changes in the intestinal flow of nucleic acids on allantoin excretion in the urine of sheep, J. Agri. Sci., Cambridge **98** (1983) 221-223.

[16] CHEN, X., ØRSKOV, E., HOVELL, F., Excretion of purine derivatives by ruminants: endogenous excretion, differences between cattle and sheep, Br. J. Nutr. **63** (1990) 121-129.

[17] GIESECKE, D., SATNGASSINGER, M., TIEMEYER, W., Nucleic acid digestion and urinary purine metabolites in sheep nourished by intragastric infusion, Can. J. Anim. Sci. **64** (1984) 144-145.

[18] BLAXTER, K., WOOD, W., The nutrition of the young Ayrshire calf 1. The endogenous nitrogen and basal energy metabolism of the calf, Br. J. Nutr. **5** (1951) 11-25.

[19] FUJIHARA, T., ØRSKOV, E., REEDS, E., KYLE, D., The effect of protein infusion on urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition, J. Agric. Sci., Cambridge **109** (1987) 7-12.
[20] GIESECKE, D., BALSLIEMKE, J., SUDEKUM, S., SATNGASSINGER, M., Plasma level, clearance and renal excretion of endogenous and ruminal purines in the bovine, J. Anim. Phy. Anim. Nutr. **70** (1993) 180-189.
[21] OSUJI, P., NSAHILAI, I., KHALIL, H., Effect of fasting on the urinary excretion of nitrogen and purine derivatives by zebu (*Bos indicus*) and crossbred (*Bos indicus x Bos taurus*) cattle, J. Applied Anim. Research **10** (1996) 39-47.
[22] LIANG, J., MATSUMOTO, M., YOUNG, B., Purine derivative excretion and ruminal microbial yield in Malaysian cattle and swamp buffalo, Anim. Feed Sci. Technol. **47** (1994) 189-199.
[23] BECKERS, Y., THEWIS, A., Excretion of purine derivatives in urine of Belgian Blue bulls following duodenal infusion of purines from Torula yeast, Proc. Society of Nutritional Physiology **3** 1994) 235.
[24] McALLAN, A., The degradation of nucleic acids and the removal of breakdown products from the small intestines of steers, Br. J. Nutr. **44** (1980) 99-112.
[25] BALCELLS, J., GANUZA, J., PÉREZ, F., MARÍN-ORÚE, S., GONZÁLEZ RONQUILLO, M., Urinary excretion of purine derivatives as an index of microbial-nitrogen intake in growing rabbits, Br. J. Nutr. **79** (1998) (In press).