ESTIMATING RUMEN MICROBIAL PROTEIN SUPPLY FOR INDIGENOUS RUMINANTS USING NUCLEAR AND PURINE EXCRETION TECHNIQUES IN INDONESIA

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Abstract

ESTIMATING RUMEN MICROBIAL PROTEIN SUPPLY FOR INDIGENOUS RUMINANTS USING NUCLEAR AND PURINE EXCRETION TECHNIQUES IN INDONESIA.

The microbial protein supply to ruminants can be estimated based on the amount of purine derivatives (PD) excreted in the urine. Four experiments were conducted to evaluate the PD excretion method for Bali and Ongole cattle. In the first experiment, six male, two year old Bali cattle (*Bos Sondaicus*) and six Ongole cattle (*Bos Indicus*) of similar sex and age, were used to quantify the endogenous contribution to total PD excretion in the urine. In the second experiment, four cattle from each breed were used to examine the response of PD excretion to feed intake. ^{14}C-uric acid was injected in one single dose to define the partitioning ratio of renal:non-renal losses of plasma PD. The third experiment was conducted to examine the ratio of purine N:total N in mixed rumen microbial population. The fourth experiment measured the enzyme activities of blood, liver and intestinal tissues concerned with PD metabolism.

The results of the first experiment showed that endogenous PD excretion was 145 ± 42.0 and 132 ± 20.0 μmol/kg $W^{0.75}$/d, for Bali and Ongole cattle, respectively. The second experiment indicated that the proportion of plasma PD excreted in the urine of Bali and Ongole cattle was 0.78 and 0.77 respectively. Hence, the prediction of purine absorbed based on PD excretion can be stated as $Y = 0.78\ X + 0.145\ W^{0.75}$ and $Y = 0.77\ X + 0.132\ W^{0.75}$ for Bali and Ongole cattle, respectively. The third experiment showed that there were no differences in the ratio of purine N:total N in mixed rumen microbes of Bali and Ongole cattle (17% vs 18%). The last experiment, showed that intestinal xanthine oxidase activity of Bali cattle was lower than that of Ongole cattle (0.001 vs 0.015 μmol uric acid produced/min/g tissue) but xanthine oxidase activity in the blood and liver of Bali cattle was higher than that of Ongole cattle (3.48 vs 1.34 μmol/min/L plasma and 0.191 vs 0.131 μmol/min/g liver tissue). Thus, there was no difference in PD excretion between these two breeds. Liver uricase in Bali and Ongole cattle was 1.46 and 1.17 nmol/min/g tissue, where as no activity was detected in the intestinal tissue and blood.

1. INTRODUCTION

The major constraint to improving animal production in tropical countries is under-nutrition due to inadequate or fluctuating nutrient supply. Therefore, the strategy for improving production has been to maximize the efficiency of utilization of the available feed resources.

The utilization of roughages as a feed resource for ruminant livestock depends mainly on the efficiency of its microbial fermentation in the fore stomach. Moreover, in such diets rumen microbes constitute the main source of digestible protein to the host animal.

The efficiency of rumen fermentation and rumen microbial protein output can be easily estimated from the amount of purine derivatives (PD) excreted in the urine. This method has been developed with European breeds of cattle [1] and sheep [2], although experimental evidence suggest significant differences in purine metabolism amongst ruminant species [3].

The objective of the present study was to investigate whether Bali cattle (*Bos sondaicus)* and Ongole cattle (*Bos indicus)* have PD excretion patterns different to European breeds, and if so to establish response models between duodenal flow and renal excretion of PD for these two breeds. In order to facilitate this, four experiments were conducted to determine (i) the endogenous contribution of PD to urinary excretion, (ii) the activity of xanthine oxidase (XO) of liver, blood and intestinal tissue as a key enzyme in purine metabolism and (iii) the specific relationship between uptake and renal excretion of purine compounds.

2. MATERIALS AND METHODS

2.1. Experiment I Estimation of endogenous PD excretion (Fasting trial)

2.1.1. Animals and diets

Six, two year old male Bali cattle and six male Ongole cattle were used in this experiment. The feed consisted of King grass (a *Pennisetum* hybrid) harvested after 42 days of planting.

2.1.2. Experimental procedure and sample collection

All animals were kept in individual pens and fed *ad libitum* for one week and weighed before moving to the metabolism cages. *Ad libitum* feeding was continued for one week and urine samples were collected daily. On the last day, two blood samples were taken at 0800 and 1500 h. After one week of urine collection, the feed was reduced gradually within two days to 60 and 30%, followed by fasting for 6 days. During the fasting period, urine collection was continued and the blood samples were taken every two days. Urine and blood samples were processed and stored for subsequent analysis according to procedures described in the IAEA TECDOC [4].

2.2. Experiment II The response of purine excretion to feed intake and measurement of the proportion of plasma purine derivatives excreted in the urine

2.2.1. Animals and diets

Four animals from each breed used in the fasting trial (Experiment I) were used in this experiment. All animals were fed twice daily at 0800 and 1500 h with King grass as in Experiment I.

2.2.2. Experimental procedure and sample collection

During the preliminary period, all animals were fed at *ad libitum* level of intake for over a week to determine the lowest intake amongst the animals of the same breed. This level of intake was defined as the "voluntary intake" for that breed. During the experimental period four animals of each breed were fed at four fixed levels namely 95, 80, 60 and 40% of "voluntary intake". The treatments were allocated according to a 4x4 Latin Square design. All animals were kept in individual metabolism cages. During each feeding period, feed samples were obtained daily and made into composite samples.

Each feeding period lasted for 3 weeks. Urine and faeces were collected during the last 10 days (collection period) of each feeding period. On the third day of each collection period each animal was given a single dose of 8-^{14}C uric acid (280 μCi/animal in 45 ml solution)

intravenously via a jugular catheter. The tracer administration was performed in the morning just before feeding. Blood sampling was carried out using the jugular catheter at 1, 2, 3, 4, 6, 7, 8, 14, 20, and 26 h after tracer administration. Blank blood samples were taken just before tracer administration to determine the background activity.

Urine, faeces and blood samples collected were processed and stored for further analysis according to procedure described earlier [4].

2.3. Experiment III The measurement of the ratio of purine nitrogen to microbial nitrogen in mixed rumen microorganisms

2.3.1. Animals and feeding

Three year old, male Bali and Ongole cattle which were used in Experiments I and II, were used as donors of rumen fluid. The body weight of animals were recorded. Animals were kept in individual pens and were fed King grass *ad libitum*. The grass was offered twice a day at 0800 and 1500 h. Composite samples of feed were kept for nutrient analyses.

2.3.2. Sample collection

Rumen fluid samples were collected from each animal at 3-6 h after feeding in the morning. The samples were transferred into a warm vacuum flask and taken to the laboratory for processing.

Processing of rumen fluid samples for the preparation of microbial matter for purine analysis was carried out according to the procedure described in the IAEA-TECDOC [4].

2.4. Experiment IV The measurement of xanthine oxidase and uricase activity in plasma, liver and intestinal tissue

2.4.1. Sample collection and processing

The intestinal mucosa and liver samples were taken from 2 year old, male Bali and Ongole cattle. The materials were obtained from a slaughter house in Yogyakarta for Ongole cattle and Denpasar for Bali cattle, respectively.

2.4.2. Preparation of tissue extracts

Blood samples were taken from the same animals used in the previous experiments (Experiment I and Experiment II), into four, 10 ml heparinised tubes and were centrifuged for 10 min at 2 500 *g*, at 4°C. The plasma obtained was transferred into the vials and used for assaying enzyme activity within 2 h.

About 100 g of liver tissue were taken from the slaughtered animals and the material was transferred to the laboratory in a polythene bag stored in ice. The samples were washed in cold 0.15 M KCl solution, blotted dry and frozen immediately until analyses.

The first 30 cm segment of the small intestine was obtained and the lumen washed with cold 0.15 M KCl solution and then with 0.05 M HEPES buffer (pH 7.5) containing 0.25 mM EDTA and 0.25 mM. PMSF. The segment of the intestine was cut length wise, opened flat and the mucosal cells were isolated by scrapping them with a spatula. The mucosal cell samples were weighed and 1 g was homogenized in 9 ml of the HEPES-EDTA-PMSF buffer. The extract was centrifuged at 40 000 *g* for 30 min at 4°C. The supernatant was dialysed for 24 h against the HEPES-EDTA-PMSF buffer. The contents of the dialysis tubing were centrifuged at 40 000 *g* for 30 min at 4°C and the supernatant was stored at 4°C.

Further preparation of tissue extracts for measuring xanthine oxidase (XO) and uricase enzyme activity was as described in the IAEA-TECDOC [4].

2.5. Measurements

Feed and faeces samples from all four experiments were analyzed for DM and nitrogen according to standard procedure. Blood and urine samples were analyzed for allantoin, uric acid and ^{14}C activity according to procedure described in the IAEA TECDOC [4]. Creatinine analysis was carried out according to procedure described by Hawk *et al.* [5]. Total urinary nitrogen was determined by the Kjeldahl method.

The XO activity in plasma and extracts of intestinal mucosa and liver samples was measured as the rate of uric acid production when xanthine was incubated with plasma or tissue extracts. The activity of uricase was measured as the rate of uric acid disappearance when uric acid was incubated with plasma or tissue extracts. Uric acid production or uric acid disappearance was measured by spectrophotometer, where OD was read at 292 nm [4].

3. RESULTS AND DISCUSSION

3.1. Experiment I

3.1.1. Estimation of endogenous PD excretion

The daily excretion of urinary PD, creatinine and total nitrogen in Bali and Ongole cattle are presented in Table I. The mean excretion of total PD was 460 (± 231) and 541 (± 75.6) μmol/kg $W^{0.75}$/d respectively, for Bali and Ongole cattle fed *ad libitum* and the difference was not significant. The relative proportion of urinary allantoin and uric acid in the PD of Bali and Ongole cattle during *ad libitum* feeding were 0.86 and 0.14 and 0.85 and 0.15, respectively (Figure 1).

TABLE I. URINARY PD, CREATININE AND NITROGEN EXCRETION IN BALI AND ONGOLE CATTLE DURING FASTING AND WHEN FED *AD LIBITUM*

	Breed of Cattle		SE	Significance
	Bali	Ongole		
Ad libitum (μmol/kg $W^{0.75}$/d)				
Allantoin	395.1 ± 205.1	461.7 ± 62.9	64.11	NS
Uric acid	65.1 ± 26.2	78.9 ± 12.7	8.40	NS
Purine derivatives	460.3 ± 231.3	540.6 ± 75.6	69.02	NS
Creatinine	861.8 ± 17.0	720.0 ± 49.0	51.66	NS
Nitrogen *	715.0 ± 72.0	735.0 ± 51.0	25.43	NS
Fasting (μmol/kg $W^{0.75}$/d)				
Allantoin	111.9 ± 35.0	101.6 ± 12.5	11.36	NS
Uric acid	33.5 ± 8.4	30.7 ± 9.2	3.84	NS
Purine derivatives	145.4 ± 42.0	132.3 ± 20.0	13.85	NS
Creatinine	828.5 ± 179.6	639.1 ± 99.7	60.82	**
Nitrogen*	348.5 ± 79.4	360.1 ± 102.5	45.67	NS

* mg/kg $W^{0.75}$/d

NS, Not significant; **, P <0.01

When feed allowance was reduced from *ad libitum* (100%) to 60, 30 and 0% (fasting), PD excretion decreased rapidly as seen in Figure 2.

The endogenous PD excretion of Bali cattle, based on the last 3 days of fasting was 145 μmol/kg $W^{0.75}$/d, while for Ongole cattle it was 132 μmol/kg $W^{0.75}$/d. Xanthine and hypoxanthine were not detectable in urine samples, thus PD included only allantoin and uric acid. The proportional contribution of both compounds to total PD during fasting were respectively, 0.77 and 0.23 for Bali cattle and 0.82 and 0.18 for Ongole cattle (Figure 1). Values in both breeds were similar to those reported by Chen *et al.* [3] for European breeds.

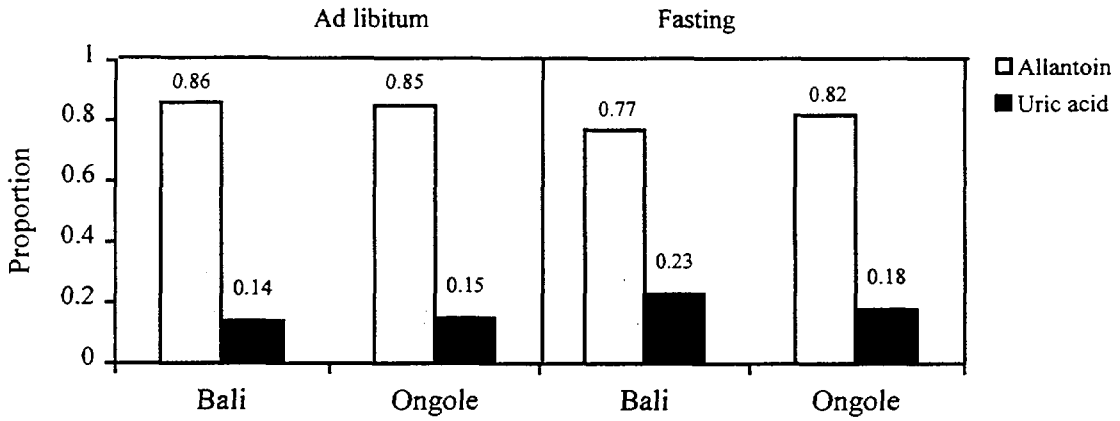

FIG.1. The proportion of allantoin and uric acid in PD in Bali and Ongole cattle during fasting and ad libitum feeding.

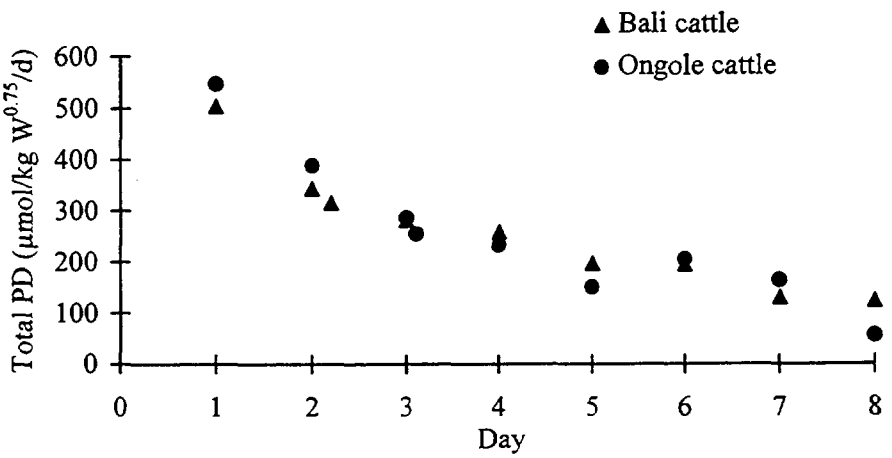

FIG.2. PD excretion in the urine of Bali and Ongole cattle during fasting period.

There was no difference in urinary nitrogen excretion of Bali and Ongole cattle fed, either at *ad libitum* intake or during fasting (Table I). Endogenous nitrogen excretion was 715 and 735 mg/kg $W^{0.75}$/d for Bali and Ongole cattle, respectively at *ad libitum* feeding while they were 348 and 360 mg/kg $W^{0.75}$/d, respectively during fasting. The proportion of allantoin to total nitrogen excretion decreased from 8.7 to 5.1% in Bali and from 9.9 to 4.5% in Ongole cattle. The proportion of uric acid to total nitrogen excretion was not influenced by the treatments. It represented 1.5 and 1.6% in Bali and 1.8 and 1.4% in Ongole cattle for *ad libitum* feeding and fasting, respectively. The results are in the range of 2.2-22.8% and 0.60-1.81%, for proportion of allantoin:total N and uric acid:total N excretion respectively, as reported by Bristow *et al* [6].

Urinary creatinine excretion in Bali cattle was higher ($P < 0.01$) than that of Ongole cattle during the fasting period (829 vs 639 μmol/kg $W^{0.75}$/d), while the opposite trend was true during the feeding period.

3.1.2. Glomerular filtration rate (GFR)

The plasma PD, GFR, tubular load and reabsorption of PD are shown in Table II. Plasma allantoin concentration was affected by the dietary treatment. It was significantly lower ($P < 0.01$) both in Bali and Ongole cattle during fasting compared to *ad libitum* feeding (99 and 72 μmol/L vs 146 and 174 μmol/L). There was no difference in plasma PD concentration between Bali and Ongole cattle when fed *ad libitum* but plasma allantoin and PD concentration in Bali cattle were significantly higher ($P < 0.01$) than those of Ongole cattle during the fasting period. The uric acid concentration was not influenced by the dietary treatment.

The GFR in Bali cattle was higher ($P < 0.01$) than that of Ongole cattle (1265 vs 948 L/d) when they were being fed *ad libitum*. It decreased almost 50% in both cases as a result of fasting (650 vs 538 L/d) but with no significant difference between the breeds. No differences were observed in tubular load of allantoin, uric acid and PD between Bali and Ongole cattle during *ad libitum* feeding but during fasting Bali cattle showed a significantly ($P < 0.05$) higher rate of PD tubular load compared to Ongole cattle (81.9 vs. 50.3 μmol/d).

The PD reabsorption during both fasting and *ad libitum* feeding periods tended to be higher in Bali cattle (though not significantly) than in Ongole cattle (86.5 vs 81.6% and 86.5 vs 80.3%, for Bali and Ongole cattle, respectively).

3.2. Experiment II

3.2.1. Response of PD excretion to feed intake

Urinary PD excretion in Bali and Ongole cattle fed at different levels of intake are shown in Table III. The daily excretion of allantoin and uric acid in both breeds showed a positive response to the level of intake. For Ongole cattle PD excretion was correlated to digestible organic matter intake (DOMI) according to the following equation (Figure 3).

$$Y = 11.30\,X + 8.89\ (R^2 = 0.74;\ n = 16;\ P < 0.01)$$

For Bali cattle PD excretion was correlated to DOMI according to the following equation but the correlation coefficient was not significant.

$$Y = 10.04\,X + 8.36\ (R^2 = 0.14;\ n = 16;\ NS)$$

TABLE II. GFR, TABULAR LOAD AND REABSORPTION OF PD IN BALI AND ONGOLE CATTLE DURING FASTING AND *AD LIBITUM* FEEDING

	Breed of Cattle		SE	Significance
	Bali	Ongole		
Ad libitum				
Plasma (μmol/L)				
Allantoin	146.3 ± 23.3	173.5 ± 23.8	10.5	NS
Uric acid	23.1 ± 4.5	24.8 ± 6.7	2.6	NS
Purine derivatives	169.4 ± 26.7	198.2 ± 24.4	11.6	NS
Creatinine	43.8 ± 10.4	50.1 ± 3.7	3.2	NS
Urine creatinine (mmol/d)	54.7 ± 11.4	47.3 ± 3.6	3.5	NS
GFR (L/d)	1265 ± 149	948 ± 111	53.8	**
GFR ($L/W^{0.75}/d$)	19.9	14.5	0.9	***
Tabular load (mmol/d)				
Allantoin	182.9 ± 23.4	164.9 ± 31.9	12.5	NS
Uric acid	28.7 ± 2.8	23.4 ± 6.9	2.4	NS
Purine derivatives	211.6 ± 24.7	188.4 ± 33.6	13.2	NS
Reabsorption (mmol/d)				
Allantoin	158.1 ± 20.5	134.7 ± 34.2	12.6	NS
Uric acid	24.6 ± 3.0	18.3 ± 7.2	2.5	NS
Purine derivatives	182.7 ± 21.6	152.9 ± 36.0	13.3	NS
Reabsorption (%)				
Allantoin	86.6 ± 5.5	80.7 ± 5.3	2.2	NS
Uric acid	85.7 ± 5.2	76.3 ± 6.2	2.5	NS
Purine derivatives	86.5 ± 5.3	80.3 ± 5.1	2.3	NS
Fasting				
Plasma (μmol/L)				
Allantoin	98.7 ± 13.0	71.7 ± 11.7	5.5	**
Uric acid	26.9 ± 6.9	23.2 ± 2.6	2.4	NS
Purine derivatives	125.6 ± 14.9	94.9 ± 12.3	6.1	**
Creatinine	74.6 ± 14.1	71.3 ± 10.5	5.1	NS
Urine creatinine (mmol/d)	46.9 ± 9.8	37.9 ± 6.6	3.5	NS
GFR (L/d)	650.9 ± 2.0	538.3 ± 95.0	64.5	NS
GFR ($L/W^{0.75}/d$)	11.5 ± 3.7	9.1 ± 1.4	1.1	NS
Tabular load (mmol/d)				
Allantoin	65.5 ± 22.3	37.8 ± 3.8	7.2	NS
Uric acid	16.5 ± 3.2	12.5 ± 2.7	1.3	NS
Purine derivatives	81.9 ± 23.4	50.3 ± 5.7	9.3	*
Reabsorption (mmol/d)				
Allantoin	57.4 ± 20.3	30.6 ± 4.7	6.6	NS
Uric acid	13.9 ± 3.6	10.6 ± 2.7	1.4	NS
Purine derivatives	71.3 ± 21.1	41.2 ± 6.2	6.9	NS
Reabsorption (%)				
Allantoin	83.0 ± 3.8	79.8 ± 5.5	2.1	NS
Uric acid	53.5 ± 1.9	83.7 ± 3.6	1.3	NS
Purine derivatives	86.5 ± 3.4	81.6 ± 3.9	1.6	NS

NS, not significant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$

TABLE III. URINARY PD AND CREATININE EXCRETION IN BALI AND ONGOLE CATTLE FED AT DIFFERENT LEVELS OF INTAKE

	Level of feed intake (%)				SE	Significance.
	95	80	60	45		
Bali cattle (mmol/d)						
Allantoin	23.0 ± 13.2	17.7 ± 8.8	15.6 ± 4.7	12.3 ± 3.3	1.9	*
Uric acid	4.7 ± 2.1	3.9 ± 1.4	3.0 ± 0.9	2.3 ± 0.4	0.4	*
Purine derivatives	27.7 ± 15.2	21.7 ± 10.3	18.6 ± 5.7	14.7 ± 3.7	2.3	*
Ongole cattle (mmol/d)						
Allantoin	25.7 ± 2.8	23.1 ± 3.2	16.5 ± 3.7	14.7 ± 2.1	0.9	**
Uric acid	4.5 ± 0.6	4.0 ± 1.1	2.9 ± 0.3	2.2 ± 0.5	0.3	*
Purine derivatives	30.3 ± 3.2	27.2 ± 2.9	19.4 ± 3.9	16.9 ± 2.3	0.9	**

*, P <0.05; **, P <0.01

The extrapolated endogenous PD excretion for Ongole cattle was close to endogenous PD excretion obtained from the fasting trial (8.89 vs 9.10 mmol/d). However, for Bali cattle using all four animals gave a poor and non-significant correlation between PD excretion and DOMI, presumably because of the high variability amongst the animals. It appeared that two animals had low PD excretion rates while the other two had high rates of excretion. Separating them into two groups gave better correlations (Figure 3). The extrapolated endogenous PD excretion for animals with high excretion rates was similar to the endogenous PD excretion obtained from the fasting trial (10.7 vs 10.6 mmol/d) but was far from close for the animals with lower excretion rates (0.10 vs 10.62 mmol/d).

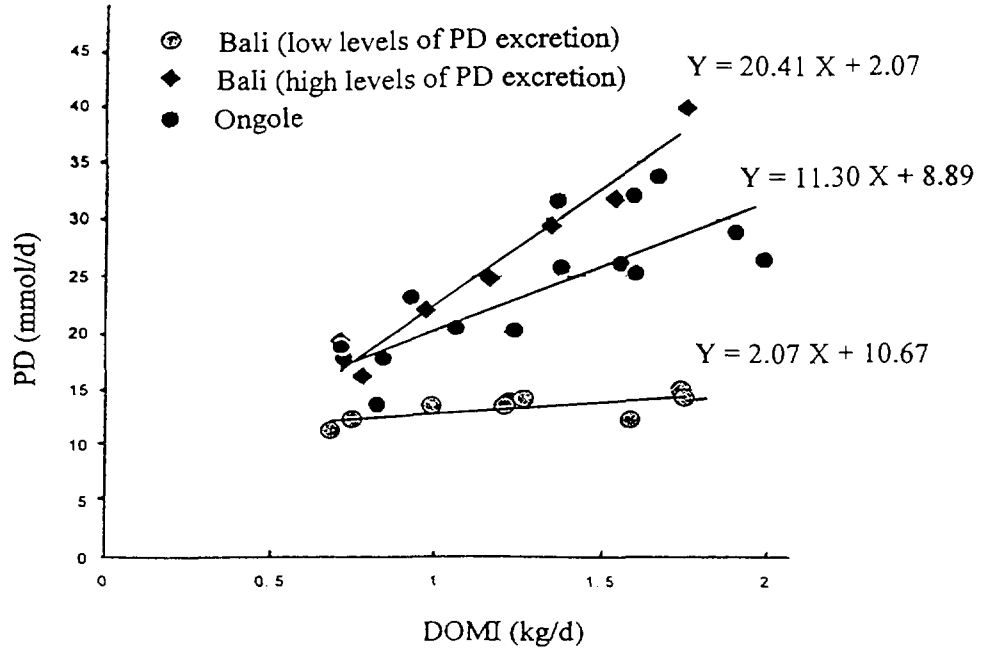

FIG.3. Relationship between PD excretion and digestible organic matter intake (DOMI) in Bali and Ongole cattle.

There were no differences in allantoin, uric acid and PD excretion between the breeds. The plasma PD of Bali cattle tended to be lower than that of Ongole cattle, but the GFR was not different between the two breeds. This can be explained by the data of PD reabsorption (Table II) which was higher in Bali than in Ongole cattle (77.6% vs 75.39%), since there were no effects on urine PD excretion between the breeds.

3.2.2. Glomerular filtration rate

Plasma PD, creatinine, GFR, tubular load and reabsorption of PD are given in Tables IV and V. Plasma allantoin, uric acid and PD of both breeds were not significantly affected by the level feed intake.

The GFR of Bali cattle was not affected by the level of feed intake but in Ongole cattle level of feed intake had a significant effect on the GFR ($P < 0.01$). However, when the values were expressed on the basis of metabolic body weight, the GFR of Bali cattle decreased significantly ($P < 0.01$) with decreasing level of feed intake, while that of Ongole cattle was not affected.

TABLE IV. GFR, TABULAR LOAD AND REABSORPTION OF PD IN BALI AND ONGOLE CATTLE

	Breed of Cattle		SE	Significance
	Bali	Ongole		
Plasma (μmol/L)				
Allantoin	91.5	109.6	8.8	**
Uric acid	27.3	29.3	3.7	NS
Purine derivatives	117.7	136.1	9.9	NS
Creatinine	92.0	89.4	4.9	NS
Urine creatinine (mmol/d)	68.9	60.6	7.1	NS
GFR (L/d)	758.5	701.1	58.4	NS
GFR (L/ $W^{0.75}$/d)	10.9	9.5	0.9	*
Tabular load (mmol/d)				
Allantoin	70.8	70.6	10.1	NS
Uric acid	20.5	20.3	3.0	NS
Purine derivatives	91.4	95.2	12.0	NS
Reabsorption (mol/d)				
Allantoin	53.4	54.8	8.0	NS
Uric acid	16.9	16.9	2.9	NS
Purine derivatives	70.4	71.7	9.5	NS
Reabsorption (%)				
Allantoin	75.4	72.7	1.9	NS
Uric acid	82.9	81.5	2.4	NS
Purine derivatives	77.7	75.3	2.5	NS

NS, not significant; *, $P < 0.05$; **, $P < 0.01$

3.2.3. Digestibility of the feed

Dry matter and organic matter digestibilities of King grass given to Bali and Ongole cattle are given in Table VI. The digestibilities were not affected by level of feed intake and were not different between the breeds, presumably because feed intake was below energy maintenance.

TABLE V. GFR, TABULAR LOAD AND PD REABSORPTION IN BALI AND ONGOLE CATTLE FED AT DIFFERENT LEVELS OF INTAKE

	Level of feed intake				SE	Significance
	95%	80%	60%	40%		
Bali cattle						
Plasma (μmol/L)						
Allantoin	110.2	85.1	88.8	81.8	7.0	NS
Uric acid	28.3	28.8	27.1	25.3	1.5	NS
Purine derivatives	138.5	117.5	108.9	110.4	6.9	NS
Creatinine	87.9	88.7	88.7	102.7	3.7	NS
Urine creatinine (mmol/d)	76.8	70.2	64.8	63.7	2.6	*
GFR (L/d)	891.0	792.5	717.5	633.0	58.3	NS
GFR ($L/W^{0.75}/d$)	12.7	11.2	10.4	9.1	0.9	**
Tabular load (mmol/d)						
Allantoin	101.2	70.9	58.5	51.6	9.4	*
Uric acid	24.4	22.6	19.3	15.8	1.8	NS
Purine derivatives	125.6	94.7	77.8	67.4	10.8	*
Reabsorption (mmol/d)						
Allantoin	78.2	53.1	42.9	39.3	7.7	*
Uric acid	19.7	18.7	16.2	13.4	1.5	NS
Purine derivatives	97.9	71.8	59.2	52.7	8.4	*
Reabsorption (%)						
Allantoin	78.3	75.9	72.5	74.9	1.8	NS
Uric acid	80.9	81.7	84.4	84.6	0.6	*
Purine derivatives	79.1	77.8	75.6	78.1	1.1	NS
Ongole cattle						
Plasma (mmol/L)						
Allantoin	123.0	104.5	109.6	101.2	10.2	NS
Uric acid	23.2	29.2	31.3	33.3	4.6	NS
Purine derivatives	135.2	133.6	140.9	134.5	12.2	NS
Creatinine	81.8	85.0	92.2	98.2	3.3	*
Urine creatinine (mmol/d)	61.0	70.9	54.7	56.0	2.6	*
GFR (L/d)	759.0	835.2	602.8	607.5	36.9	**
GFR ($L/W^{0.75}/d$)	10.3	11.4	8.3	8.2	1.0	NS
Tabular load (mmol/d)						
Allantoin	91.8	86.3	65.2	56.0	4.9	**
Uric acid	17.4	24.7	19.4	19.9	2.9	NS
Purine derivatives	109.3	110.9	84.6	75.9	5.7	**
Reabsorption (mmol/d)						
Allantoin	66.1	63.2	48.7	41.3	2.8	*
Uric acid	12.9	20.6	16.5	17.7	4.7	NS
Purine derivatives	79.0	83.8	65.2	58.9	5.6	NS
Reabsorption (%)						
Allantoin	71.5	72.6	73.5	73.2	2.1	NS
Uric acid	73.4	80.7	83.3	88.5	3.1	NS
Purine derivatives	71.9	75.2	76.3	77.7	1.5	NS

NS, not significant; *, $P < 0.05$; **, $P < 0.01$

TABLE VI. NUTRIENT DIGESTIBILITY OF KING GRASS FED TO BALI AND ONGOLE CATTLE AT DIFFERENT LEVELS OF INTAKE

	Level of feed intake (%)				SE	Significance
	95	80	60	40		
Bali cattle						
DM digestibility (%)	58.7	58.4	60.3	59.7	1.9	NS
OM digestibility (%)	62.0	62.1	64.2	64.3	2.0	NS
DOMI (kg/d)	1.7	1.4	1.1	0.7	0.02	**
Ongole cattle						
DM digestibility (%)	60.0	58.0	57.8	60.7	2.6	NS
OM digestibility (%)	63.2	61.9	61.8	65.1	2.2	NS
DOMI (kg/d)	1.8	1.5	1.1	0.8	0.3	**

NS, not significant; **, P <0.01

3.2.4. Nitrogen balance and ^{14}C uric acid excretion

Nitrogen excretion of Bali and Ongole cattle are shown in Table VII. Faecal and urine nitrogen were significantly affected by the level of feed intake (P <0.01), but the nitrogen excretion was not affected by the breed type. The nitrogen balance decreased when the intake of digested organic matter was reduced. The regression between nitrogen excretion (Y) and DOMI (X) were Y = - 19.27 + 2.88 X and Y = - 21.36 + 6.72 X respectively, for Bali and Ongole cattle. The extrapolated endogenous nitrogen excretion of Bali and Ongole cattle were -19 and -21 g/d respectively. Those values were higher than the nitrogen excretion during the fasting period (-46 and -45 g/d, respectively for Bali and Ongole cattle). This was probably due to higher nitrogen catabolism during fasting compared to the level of protein used for production of glucose precursors.

TABLE VII. THE EFFECT OF LEVEL OF FEED INTAKE ON NITROGEN BALANCE OF BALI AND ONGOLE CATTLE

	Level of feed intake (%)				SE	Significance
	95	80	60	40		
Bali cattle (g/d)						
Nitrogen intake	47.3	39.8	29.8	19.9	0.8	***
Fecal nitrogen	18.4	16.0	9.9	6.7	0.8	***
Urine nitrogen	34.8	30.1	28.8	23.1	1.8	**
Nitrogen balance	-5.9	-6.1	-8.9	-9.9	1.8	NS
Ongole cattle (g/d)						
Nitrogen intake	45.5	41.7	31.4	20.8	0.7	***
Fecal nitrogen	17.7	16.2	11.2	9.1	1.3	***
Urine nitrogen	33.9	30.9	29.5	25.0	1.2	**
Nitrogen balance	-2.2	-5.5	-9.3	-13.3	2.5	NS

NS, not significant; **, P <0.01; ***, P <0.001

The concentration of ^{14}C uric acid in the plasma of both breeds, decreased exponentially with time. After 24 h, tracer disappearance from the plasma of Bali and Ongole cattle fed at the level of 95 and 60% of voluntary intake were not different. On the other hand, tracer recovery in the urine of cattle fed at the level of 95% voluntary intake was higher than cattle fed at the level of 60% (85.6 vs 74.4% and 84.7 vs 55.8% for Bali and Ongole cattle, respectively) (Table VIII).

Based on these results it can be presumed that when animals were fed at a lower level of intake, PD excretion via renal route decreased while excretion via non-renal routes increased. The ratio PD excretion via renal:non-renal routes in Bali and Ongole cattle fed at 90% level intake were 85.5:14.4 and 84.7:15.3, respectively, while when the animals were fed at 60% level of intake, the ratio was found to be 74.4:15.6 and 55.8:44.2, respectively.

TABLE VIII. RECOVERY OF ^{14}C URIC ACID IN THE URINE OF CATTLE RECEIVING TWO LEVELS OF FEED INTAKE

Recovery ^{14}C (%)	Bali		Ongole	
	Level of intake (%)			
	95	60	95	60
Total ^{14}C	85.6 ± 10.4	74.4 ± 12.4	84.7 ± 8.9	55.8 ± 29.9
^{14}C Purine Derivatives	77.8	66.0	76.6	50.2
^{14}C as other compounds	7.8	8.2	8.1	5.7

The presumption that PD in the plasma can be excreted via renal and non-renal routes is supported by results reported by Chen *et al.* [7]. They stated that concentration of allantoin and uric acid in sheep plasma was 52 and 6 μmol/L while in saliva it was 120 and 16 μmol/L. They also stated that the presence of allantoin and uric acid in sheep saliva demonstrated that purine derivatives in the plasma can be recycled via salivary secretion to the rumen. Chen *et al.* [8] also stated that the proportion of absorbed exogenous purine excreted as derivatives in the urine was 0.84.

3.3. Experiment III

Nitrogen and nucleic acid content in rumen microbes are listed in Table IX. The table shows that the amount of microbial matter was 1.021 and 0.77 g/L for Bali and Ongole cattle, respectively. The nitrogen content of Ongole rumen microbes was slightly higher than that of Bali cattle (6.3 vs 5.9% DM).

The RNA content of the microbes from the two breeds was very similar (19.2 for Bali cattle and 20.3 for Ongole cattle), but appear to be higher than values reported by other workers [9]. This could be due to errors associated with RNA determination. Not withstanding this discrepancy, based on the nitrogen content in microbes and nitrogen in RNA, it could be calculated that the ratio of purine N:microbial N in mixed rumen microbes was 0.42 ± 0.06 for Bali cattle and 0.43 ± 0.04 for Ongole cattle, respectively. The ratio obtained can be used to calculate microbial nitrogen production based on the amount of PD excretion.

The ratio of N-RNA:N-total microbes of Bali cattle were not significantly different from that of the Ongole cattle.

Based on the assumption that purine base content was equal to pyrimidine base, it can be stated that the ratio of purine N:total N was about 0.21 and 0.22 for Bali and Ongole cattle, respectively. The values were higher than the purineN:total N in the microbes (0.116) as reported by Chen *et al.* [8]. This could be partly due to the above mentioned errors associated with the purine N determination in the microbes.

3.3.1. Xanthine oxidase activity

Figures 4, 5 and 6 show the uric acid production from plasma, liver and intestinal tissue extracts in Bali and Ongole cattle as an index of XO activity. The rate of uric acid produced by the enzyme was 1.74 μmol/min and 0.67 μmol/min for Bali and Ongole cattle, respectively. These values gave an activity of 3.48 and 1.34 μmol/min/L for plasma in Bali and Ongole cattle. The xanthine oxidase activity in plasma of Ongole cattle was nearly the same as that reported by Chen *et al.* [3], (1.13 μmol/min/L) while xanthine oxidase activity in the plasma of Bali cattle was higher.

TABLE IX. NITROGEN AND RNA CONTENT OF RUMEN MICROBES IN BALI AND ONGOLE CATTLE

Breed	Microbe (g/L)	N-microbe	RNA (% DM)	N-RNA	Ratio of N-RNA:N-Microbe
Bali	1.02 (± 0.12)	5.90 (± 0.08)	19.20 (± 0.34)	2.49 (± 0.34)	0.42 (± 0.06)
Ongole	0.77 (± 0.20)	6.30 (± 0.47)	20.82 (± 2.80)	2.71 (± 0.41)	0.43 (± 0.04)

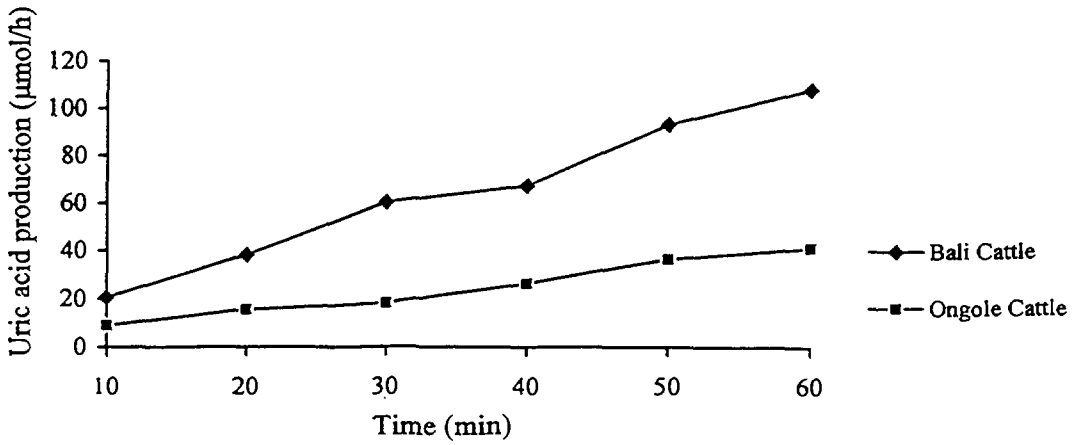

FIG.4. Xanthine oxidase activity in the plasma of Bali and Ongole cattle.

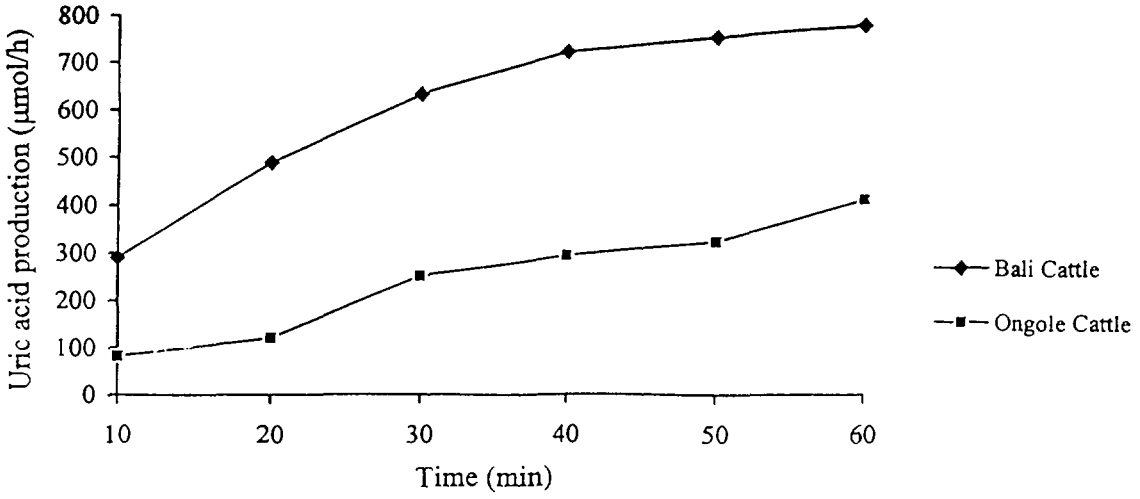

FIG.5. Xanthine oxidase activity in the liver of Bali and Ongole cattle.

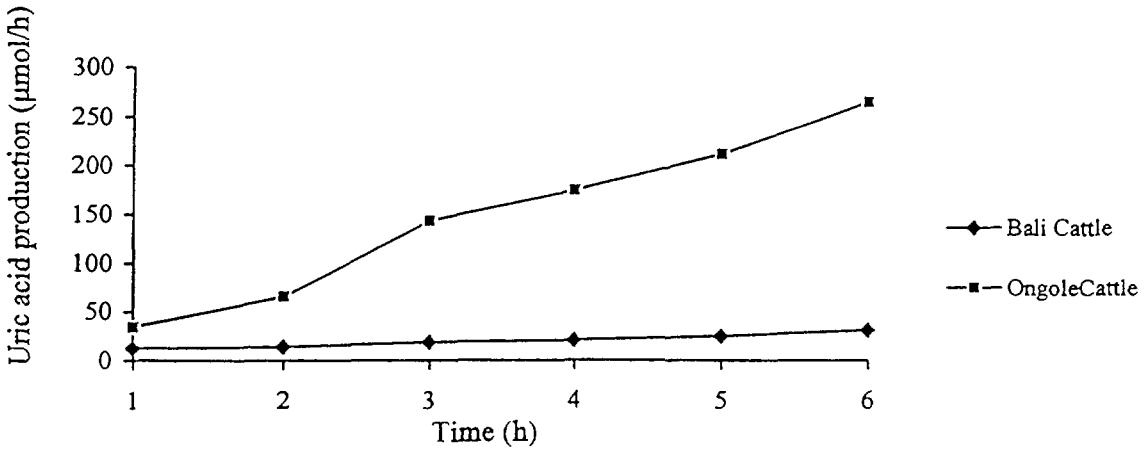

FIG.6. Xantine oxidase activity in the intestinal mucosa of Bali and Ongole cattle.

The liver XO activity in Bali and Ongole cattle was 0.191 and 0.131 µmol/min/g tissue, respectively, while the activity of the intestinal mucosa was 0.001 and 0.015 µmol/min/g tissue. The XO activity in intestinal mucosa of Ongole cattle was in the range described by Chen *et al* [3] in European cattle. It appears that XO activity converts most of the absorbed purines into uric acid and therefore became unavailable for tissue nucleic acid synthesis. In Bali cattle, however, the activity of xanthine oxidase was low and therefore absorbed purines could have entered the liver unchanged and became available for salvage. The low XO activity in gut mucosa of Bali cattle would have been compensated by much higher liver and blood enzyme activity resulting in similar PD excretion in the two species.

3.3.2. Uricase

There was a slight activity of uricase in plasma of both breeds (Figure 7). These results were in agreement with the results reported by Chen *et al.* [3]. They stated that there was no metabolism of uric acid in cow or pig plasma, indicating the absence of uricase. They showed that in sheep plasma, there was slight uricase activity.

It seems that there was no uricase activity in the intestinal mucosa of both Bali and Ongole cattle. The rate of uric acid degradation by liver uricase of Bali and Ongole cattle was 0.035 and 0.028 μmol/h per reaction mixture. This is equivalent to an activity of 1.46 and 1.17 nmol/min per g tissue.

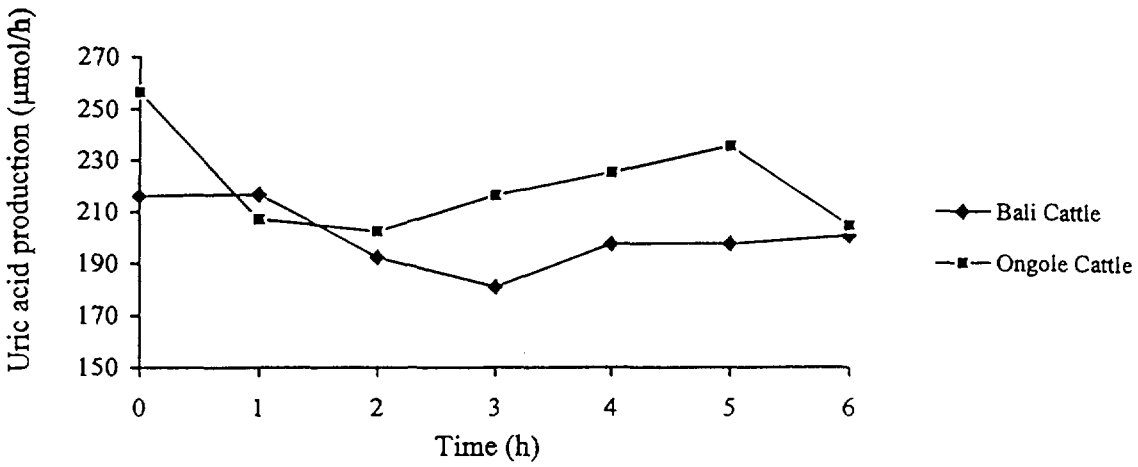

FIG.7. Uricase activity in the plasma of Bali and Ongole cattle.

4. CONCLUSIONS

These experiments showed that the endogenous PD excretion in both Bali and Ongole cattle were lower than endogenous PD excretion reported by Chen *et al.* [1], therefore the equation between PD excretion and PD absorbed which has been postulated by Chen *et al.* [8] should be adjusted. The endogenous PD excretion for Bali cattle was 145 μmol/kg $W^{0.75}$/d while for Ongole cattle it was 132 μmol/kg $W^{0.75}$/d.

These experiments demonstrated that not all of the PD in the blood is excreted in the urine and therefore the PD excretion via non-renal routes should be taken into account when PD excretion is used to predict microbial protein syntheses in the rumen. The proportion of plasma PD excreted in the urine of Bali and Ongole cattle fed 90% level of intake was 0.78 and 0.77 respectively. At 60% level of feeding the proportion of pasma PD excreted was 0.66 and 0.50 for Bali and Ongole cattle respectively.

The prediction of purine absorbed based on PD excretion can be stated as: $Y = 0.78 X + 0.145 W^{0.75}$ and $Y = 0.77X + 0.132 W^{0.75}$ for Bali and Ongole cattle respectively. The coefficient of 0.21 and 0.215 found for the ratio of purine N:total N in mixed rumen microbes of Bali and Ongole cattle, could be used for calculating microbial N production.

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