ESTIMATION OF RUMEN MICROBIAL PROTEIN PRODUCTION FROM URINARY PURINE DERIVATIVES IN ZEBU CATTLE AND WATER BUFFALO

J.B. LIANG, O. PIMPA, N. ABDULLAH, Z.A. JELAN
Department of Animal Science,
Universiti Putra Malaysia,
Serdang,
Malaysia

J.V. NOLAN
Division of Animal Science,
University of New England,
Armidale, New South Wales,
Australia

Abstract

ESTIMATION OF RUMEN MICROBIAL PROTEIN PRODUCTION FROM URINARY PURINE DERIVATIVES IN ZEBU CATTLE AND WATER BUFFALO.

Two experiments were conducted in order to develop equations for predicting rumen microbial protein production for indigenous Kedah-Kelantan (KK) cattle and swamp buffaloes in Malaysia, using urinary purine derivatives (PD) excretion rates. Endogenous PD excretion rates determined by a fasting procedure for KK cattle and swamp buffalo were 275 and 370 μmol/kg $W^{0.75}$/day, respectively. Urinary PD excretion rate per kg digestible organic matter intake (DOMI) for KK cattle was higher than that for swamp buffalo, reconfirming the earlier findings. Glomerular filtration rate, allantoin and uric acid tubular load and PD re-absorption rate for swamp buffalo were generally higher than those for KK cattle. However, due to the large variations among animals within species, these parameters were not significantly different between species. Nevertheless, the higher PD re-absorption in swamp buffalo provides support for the earlier postulation that the lower urinary PD excretion rate of swamp buffalo was due to their higher recycling of plasma PD as compared to KK cattle. Labelled 8-^{14}C uric acid was used to estimate the ratio of renal to non-renal PD excretion. The recovery rates of the radioactive tracer via the renal route for both species were much lower than values reported previously for unlabelled PD for European cattle.

1. INTRODUCTION

Urinary purine derivatives (PD) excretion rate is widely used to predict rumen microbial protein production in ruminant livestock. Prediction equations have been developed based on European cattle [1] and sheep [2]. There is evidence to suggest that the urinary PD excretion rates of Zebu cattle and water buffaloes (*Bubalus bubalis*) differ from those of the European cattle [3, 4]. Therefore, it is pertinent to develop species specific models for the prediction of microbial protein yield in Zebu cattle and water buffalo.

Two experiments were conducted to develop prediction models for the Malaysian Kedah-Kelantan (KK) cattle (*Bos indicus*) and swamp buffaloes (*Bubalus bubalis*). The objective of the first experiment was to estimate the rate of endogenous PD excretion in the two species. The objectives of the second experiment were to: (i) measure the response of PD excretion to digestible organic matter intake (DOMI) and (ii) measure the proportion of plasma PD excreted in the urine. The present paper reports some results from both experiments.

2. MATERIALS AND METHODS

2.1. **Experiment I** Fasting trial

The experiments were conducted according to procedures described in the laboratory manual [5]. In the first experiment, 6 male KK cattle of 12-14 months age and 6 swamp buffaloes of similar sex and age were used. The average body weight of cattle and buffaloes were 108 ± 9.0 and 141 ± 17.7 kg, respectively. The experimental diet consisted of 40% oil palm frond and 60% concentrate pellets, with an energy value of 8.1 MJ ME and 123 g of CP/kg DM. The animals were fed 1% DM of body weight daily for 2 weeks. The amount of feed offered was then reduced stepwise within 2 days (0.5 and 0.25% body weight, respectively) before fasting commenced. The animals were fasted for a total of six days. Urine from each individual animal was collected daily, over a total period of 10 days, including the 6 days of fasting. One blood sample per animal was taken each morning at about 0900 hours during the fasting period. Urine and blood samples were processed and stored according to the procedures described in the laboratory manual [5], for further analysis.

2.2. **Experiment II** Feeding trial

The second experiment was conducted two months later using 4 animals per species drawn from the 6 animals used in the above fasting trial. Prior to the actual trial, the animals were fed individually at *ad libitum* for 1 week to determine the "lowest level of intake" for each species to ensure that all animals allocated later for the highest intake level (L4) were able to consume all the feed offered to them. A double 4 × 4 Latin Square (one for each species) was used for the experiment. The experiment consisted of four 21-day feeding periods and four feeding levels (see Table I - calculation based on 40, 60, 80 and 95% of the "lowest level of intake" of each species determined earlier). During the last 10 days of each period (test period), total urine and faeces excreted were collected daily. On the third day of each test period, 2 cattle and 2 buffaloes (animals that were allocated to treatments L1 and L3) were given a single intravenous administration of 8-^{14}C uric acid (Amersham Life Science – code CFQ9786). Blood samples were collected as in the fasting trial, once before injection of the tracer to determine the background activity, followed by hourly sampling for the next 5 hours and thereafter, at a longer interval, until 96 h post injection.

TABLE I. TREATMENT LEVELS FOR FEEDING TRIAL

Treatment	Buffalo		Cattle	
	Oil palm frond	Concentrate	Oil palm frond	Concentrate
	(kg DM/day)			
Level 1	0.85	1.44	0.84	1.26
Level 2	1.27	1.91	1.12	1.68
Level 3	1.49	2.09	1.40	2.10
Level 4	1.91	2.86	1.68	2.52

Urine and blood samples were processed and stored in a manner similar to that in the fasting trial. Faecal samples were also processed and stored for further analysis according to procedure described earlier [5].

3. MEASUREMENTS

Faeces and feed were analyzed for DM and OM to enable the calculation of DM and OM digestibilities. The urine was analyzed for total N, creatinine and purine derivatives (allantoin, uric acid, xanthine and hypoxanthine) by HPLC following the procedure of Balcells *et al* [6]. Blood plasma samples were also analyzed for creatinine and PD in a manner similar to the urine samples. Urinary PD excretion rates were regressed against their respective digestible organic matter intake (DOMI) using linear regression expressed as a deviation from the endogenous PD excretion determined in Experiment I.

4. RESULTS AND DISCUSSION

4.1. Experiment I Fasting trial

4.1.1. Endogenous PD excretion

The daily PD excretion rates of KK cattle and swamp buffalo recorded during pre-fasting and fasting periods are shown in Figure 1. The average daily endogenous PD excretion rates (Table II) for the last five days of fasting for KK cattle was significantly lower ($P < 0.05$) than that for swamp buffalo (275 and 370 μmol/kg $W^{0.75}$/day, respectively) but both values were slightly higher than those reported earlier [3, 4]. As in previous studies, allantoin remained to be the principal urinary PD; 82.9 and 87.3% of total PD, respectively for KK cattle and swamp buffalo. The uric acid content in the urine samples of swamp buffalo was significantly higher than that of KK cattle (15.5 vs 9.6% of total PD).

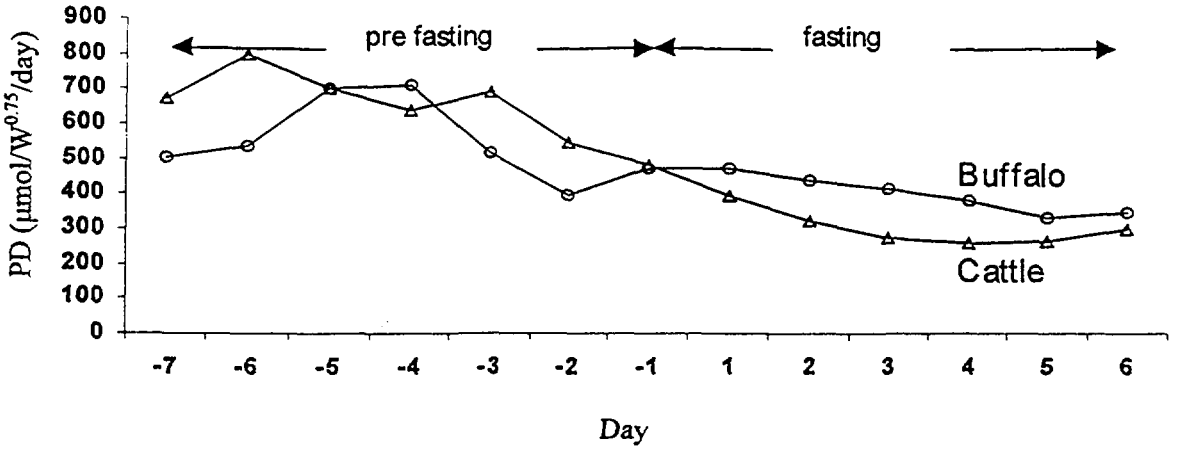

FIG.1. Excretion of urinary PD in buffalo and cattle during pre-fasting and fasting periods.

4.1.2. Glomerular filtration rate (GFR) and PD re-absorption rate

The GFR, tubular load and creatinine excretion and PD re-absorption rates during pre-fasting and fasting are shown in Table III. GFR of swamp buffalo and KK cattle were 334 and 266 L/day, respectively, during pre-fasting. The values decreased to about half during fasting (178 and 95 L/day, respectively). During both periods, the re-absorption of total PD was higher for swamp buffalo than for KK cattle, but the differences were not statistically significant.

TABLE II. DAILY URINARY EXCRETION OF PD IN SWAMP BUFFALO AND KK CATTLE DURING PRE-FASTING AND FASTING

Parameter	Buffalo	Cattle	SED	Significance
	(μmol/kg $W^{0.75}$/day)			
Pre-fasting				
Allantoin	507.3	608.8	58.51	NS
Uric acid	75.1[a]	54.6[b]	8.04	*
Hypoxanthine	6.1 [a]	12.2 [b]	2.71	*
Xanthine	21.0	26.3	2.45	NS
Total PD	609.5	701.9	65.25	NS
Fasting				
Allantoin	307.7 [a]	240.7 [b]	29.79	*
Uric acid	56.4 [a]	26.0 [b]	5.14	**
Hypoxanthine	1.6	2.5	1.87	NS
Xanthine	4.4	2.5	1.87	NS
Total PD	370.0 [a]	274.8 [b]	30.08	**

SED, Standard Error of Difference
NS, Not significant
*, Means with different superscripts within rows are significantly different (P <0.05)
**, Means with different superscripts within rows are significantly different (P <0.01)

TABLE III. DAILY GLOMERULAR FILTRATION RATE, CREATININE EXCRETION IN URINE AND PLASMA, TUBULAR LOAD AND EXCRETION AND RE-ABSORPTION OF PD DURING PRE-FASTING AND FASTING

Parameter	Buffalo	Cattle	SED	Significance
Pre-fasting				
GFR (L/d)	333.5	265.8	70.2	NS
Urine creatinine (mmol)	27.18	18.32	4.616	NS
Plasma creatinine (mmol/L)	0.084	0.081	0.012	NS
Allantoin tubular load (mmol)	79.8	56.4	21.0	NS
Uric acid tubular load (mmol)	7.8	5.5	2.3	NS
Allantoin re-absorption (mmol)	58.4	46.0	18.1	NS
Uric acid re-absorption (mmol)	5.1	3.7	2.3	NS
Total PD re-absorption (mmol)	66.6	51.3	20.7	NS
Fasting				
GFR (L/d)	178 [a]	95 [b]	23.7	*
Urine creatinine (mmol)	21.34	15.18	3.607	NS
Plasma creatinine (mmol/L)	0.141	0.177	0.017	NS
Allantoin tubular load (mmol)	44	30	7.5	NS
Uric acid tubular load (mmol)	17 [a]	11 [b]	2.6	*
Allantoin re-absorption (mmol)	29	21	6.7	NS
Uric acid re-absorption (mmol)	15	11	2.6	NS
Total PD re-absorption (mmol)	44	32	8.8	NS

SED, Standard Error of Difference
NS, Not significant
*, Means with different superscripts within rows are significantly different (P <0.05)

4.2. **Experiment II** Feeding trial

4.2.1. PD excretion rate

The relationships between urinary PD excretion rates and DOMI for the two species are shown in Figure 2. Allantoin was found to be the principal PD in the urinary samples of both species (Table IV). The results of the present study reconfirmed the earlier reports that urinary PD excretion rate per kg DOMI for cattle is higher than that for buffalo [3, 4]. However, the present values were higher than those previously reported for the same two species, by the previous workers.

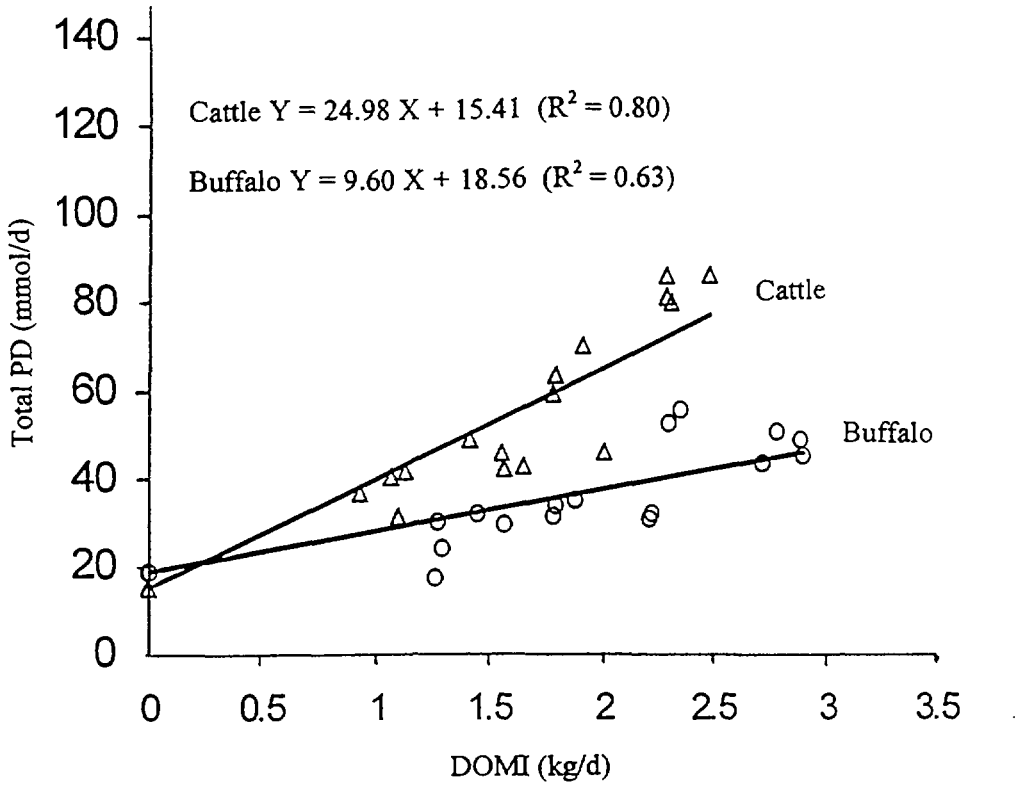

FIG.2. Urinary PD of KK cattle and buffalo as a function of DOMI.

4.2.2. Glomerular filtration rate (GFR)

Generally, GFR increased with increasing feeding level in both species. The GFR recorded in this experiment were 3 to 8 times higher than that recorded in the fasting trial. Irrespective of species, total PD re-absorption increased with increasing level of feeding. GFR values of swamp buffalo were generally higher than KK cattle. However, because of the large variation among animals within species, the differences between the two species were not statistically significant (Table V). Higher GFR, allantoin and uric acid tubular load and PD re-absorption recorded for the buffalo lend support to the earlier postulation that the lower urinary PD excretion rate of buffalo was due to its higher recycling of plasma PD as compared to cattle [4].

TABLE IV. MEAN BODY WEIGHT AND DAILY PD AND CREATININE EXCRETION IN KK CATTLE AND SWAMP BUFFALO UNDER DIFFERENT FEEDING LEVELS

Feeding level	L1	L2	L3	L4	SED	Significance
Cattle						
Body weight (kg)	147	147	155	158	7.40	NS
Allantoin (μmol/kgW$^{0.75}$)	670^{c}	877^{bc}	1252^{b}	2113^{a}	206.4	**
Uric acid (μmol/kgW$^{0.75}$)	116^{b}	142^{ab}	118^{ab}	239^{a}	37.1	*
Hypoxanthine (μmol/kgW$^{0.75}$)	10.2	4.9	16.9	24.0	7.19	NS
Xanthine (μmol/kgW$^{0.75}$)	56.3	26.5	38.7	55.4	19.7	NS
Total PD (μmol/kgW$^{0.75}$)	852.5^{c}	1050^{bc}	1480^{b}	2431^{a}	200.0	**
Creatinine (mmol/kgW$^{0.75}$)	1.08^{b}	1.60^{ab}	1.27^{b}	2.18^{a}	0.29	*
Buffalo						
Body weight (kg)	184	188	193	197	7.12	NS
Allantoin (μmol/kgW$^{0.75}$)	416^{a}	417^{a}	774^{b}	1550^{bc}	256.4	**
Uric acid (μmol/kgW$^{0.75}$)	105	119	106	173	30.88	NS
Hypoxanthine (μmol/kgW$^{0.75}$)	2.9	10.5	6.9	12.3	8.67	NS
Xanthine (μmol/kgW$^{0.75}$)	16.6	31.6	44.2	26.8	16.3	NS
Total PD (μmol/kgW$^{0.75}$)	540.1^{a}	609.0^{a}	931.0^{ab}	1762.1^{abc}	266.8	**
Creatinine (mmol/kgW$^{0.75}$)	1.42	1.45	1.83	1.98	0.65	NS

SED, Standard Error of Difference
NS, Not significant
*, Means with different superscripts within rows are significantly different ($P < 0.05$)
**, Means with different superscripts within rows are significantly different ($P < 0.01$)

4.2.3. Proportion of plasma PD excreted in urine

Measurements of the proportion of plasma PD excreted in urine could provide an explanation of the discrepancy in the urinary PD excretion rates between cattle and buffalo reported earlier [3, 4]. In this experiment, ^{14}C-uric-acid was injected intravenously and used to estimate the recovery of PD by the renal route. The urinary recovery of unlabelled PD has been found to be about 85% for European cattle [7] but there is no similar estimation for buffaloes. Recoveries of PD with the labelled uric acid were lower; 41 and 35% for KK cattle and swamp buffaloes, respectively. When analyzing the urine, the total radioactivity was separated into that associated with PD and other labelled components using the "C_1" and "C_2" separations [5]. Total recovery of radioactivity was about 72 and 69% for swamp buffaloes and KK cattle, respectively. The compounds containing radioactivity were not identified but are most likely to be degradation products of allantoin such as urea (any urinary urea that was degraded could have given rise to labelled bicarbonate but this would not have been retained after the urine was mixed with H_2SO_4 that was used as a preservative). Assuming that the non-PD radioactivity in urine was present as urea, this urea is likely to have been produced by the degradation of allantoin and it should, therefore, be considered to be part of the urinary PD excretion.

TABLE V. EFFECT OF FEEDING LEVEL ON DAILY GLOMERULAR FILTRATION RATE, TUBULAR LOAD AND RE-ABSORPTION OF PD IN BUFFALO AND KK CATTLE

Parameter	L1	L2	L3	L4	SED	Significance
Cattle						
GFR (L/d)	389	380	556	709	115	NS
Allantoin tubular load (mmol)	45[a]	45[a]	67[a]	114[b]	156	**
Uric acid tubular load (mmol)	32	23	35	47	12	NS
Allantoin re-absorption (mmol)	11	10	16	24	5	NS
Uric acid re-absorption (mmol)	22	15	28	38	10	NS
Total PD re-absorption (mmol)	33[a]	26 [a]	44 [a]	64[b]	8	**
Buffalo						
GFR (L/d)	411[a]	421[a]	722[b]	850 [b]	81	**
Allantoin tubular load (mmol)	29	53	56	94	23	NS
Uric acid tubular load (mmol)	28	44	41	83	24	NS
Allantoin re-absorption (mmol)	12	41	31	63	21	NS
Uric acid re-absorption (mmol)	22	47	37	92	27	NS
Total PD re-absorption (mmol)	35	89	68	155	41	NS

SED, Standard Error of Difference
NS, Not significant
**, Means with different superscripts within rows are significantly different (P <0.01)

ACKNOWLEDGEMENTS

This project was jointly financed by the Ministry of Science, Technology and Environment, Malaysia and the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna, Austria, under Research Contract 9052/RB.

REFERENCES

[1] CHEN, X.B., HOVELL, F.D.DeB., ORSKOV, E.R., BROWN, D.S., Excretion of purine derivatives by ruminants: effect of exogenous nucleic acid supply on purine derivative excretion by sheep, Br. J. Nutr. **63** (1990) 131-142.

[2] VERBIC, J., CHEN, X.B., MACLEOD, N.A., ORSKOV, E.R., Excretion of purine derivatives by ruminants: effect of microbial nucleic acid infusion on purine derivative excretion by steers, J. Agri. Sci., Cambridge **114** (1990) 243-248.

[3] VERCOE, J.E., Urinary allantoin excretion and digestibility dry-matter intake in cattle and buffalo, J. Agric. Sci. **86** (1976) 613-615.

[4] LIANG, J.B., MATSUMOTO, M., YOUNG, B.A., Purine derivative excretion and ruminal microbial yield in Malaysian cattle and swamp buffalo, Anim. Feed Sci. Technol. **47** (1994) 189-199.

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

[6] BALCELLS, J., GUADA, J.A., PEIRO, J.M., PARKER, D.S., Simultaneous determination of allantoin and oxypurines in biological fluids by High-performance liquid chromatography, J. Chromatography **575** (1992) 153-157.

[7] CHEN, X.B., . HOVELL, F.D.DeB, ORSKOV, E.R., Excretion of purine derivatives by ruminants: recycling of allantoin into the rumen via saliva and its fate in the gut, Br. J. Nutr. **63** (1990) 197-205.