Urinary excretion of purine derivatives in *Bos indicus* × *Bos taurus* crossbred cattle

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Four experiments were performed to study the kinetics of purine metabolism and urinary excretion in Zebu crossbred cattle. Fasting excretion was established in Expt 1, using eighteen male *Bos indicus* × *Bos taurus* crossbred cattle (261 (SE 9·1) kg body weight), six of each of the following genotypes: 5/8 *Bos indicus*, 1/2 *Bos indicus* and 3/8 *Bos indicus*. No significant differences were observed among genotypes in fasting purine derivative excretion (277·3 (SE 35·43) μmol/metabolic body weight). In a second experiment we measured the xanthine oxidase activity, which was higher in liver than in duodenal mucosa (0·64 and 0·06 (SE 0·12) units/g wet tissue per min respectively; $P > 0.05$) being in plasma 0·60 (SE 0·36) units/l per min. The kinetics of uric acid were measured by intravenous pulse dose of [1,3-^{15}N]uric acid (Expt 3). The cumulative recovery of the isotope in urine was 82 (SE 6·69) %, and uric acid plasma removal, pool size and mean retention time were 0·284 (SE 0·051) per h, 5·45 (SE 0·823) mmol and 3·52 (SE 0·521) h, respectively. Allantoin was removed from plasma at an estimated fractional rate of 0·273 (SE 0·081) per h and mean retention was 3·66 (SE 1·08) h. In Expt 4, the relationship between urinary purine derivative excretion (*Y*; mmol/d) and digestible organic matter intake (*X*, kg/d) was defined by the equation: $Y = 7.69$ (SE 4·2) $+ 5.69$ (SE 1·68) X; *n* 16, SE 1·31, *r* 0·67.

***Bos indicus*: Purine derivatives: Xanthine oxidase**

Microbial protein synthesised in the rumen is the main source of amino acids for the host animal. In tropical conditions, under difficult feeding conditions rumen microbes are actually the only source of digestible protein (Ørskov, 1992). Therefore, the knowledge of microbial protein yield in the rumen is essential to the improvement of dietary formulation and ruminant production. Unfortunately that parameter is extremely difficult to determine because its estimation requires surgery and accurate measurements of both digesta and microbial flow to the duodenum. Urinary excretion of purine derivatives might be an alternative to conventional methods of determining duodenal flow of microbial purine bases (Stangassiner *et al.* 1995). Indeed, that indirect method has been used extensively in sheep (Chen *et al.* 1990; Balcells *et al.* 1991) and European cattle (Verbic *et al.* 1990; Orellana-Boero *et al.* 2001). To use the indirect method, some parameters must be defined in this species: urinary recovery of absorbed purine bases (PB), endogenous excretion of purine derivatives (PD) and xanthine oxidase (XO) activity in intestine, liver and plasma.

It is desirable to extend the application of this predictive procedure to species such as Zebu cattle (or crossbred animals), which are of considerable importance in many tropical (or subtropical) countries; however, the development of new response models or the refinement of existing models has been limited because of the scarcity of information about purine metabolism in these species. In this study we report on the endogenous contribution of PB to total excretion of PD, tissue XO (*EC* 1.2.3.2) activity and renal excretion of PD in three genotypes of Zebu crossbred cattle.

Materials and methods

The care and handling of the animals was conducted as outlined in the guidelines of the 'Comité Etico de Experimentación Animal' of the University of Zaragoza.

Expt 1. Urinary excretion of purine derivatives during fasting

In this experiment, we used eighteen male *Bos indicus* × *Bos taurus* crossbred animals that were distributed in three groups of six animals, with each group characterised by different proportions of *Bos indicus* inheritance: 3/8: L (271 (SE 8·1) kg live weight), 1/2: M (246 (SE 7·7) kg live weight) and 5/8: H (266 (SE 9·8) kg live weight). Animals were housed individually in metabolic cages and provided with feeders and free access to water. Over a period of 21 d, they were fed once a day (08.00 hours) at maintenance levels of *Cynodon dactylon* hay (10·3 % crude protein (AOAC, 1990) and 49 % DM digestibility, by

total faeces collection). After that period feed offered was reduced stepwise within 2 d (60 %, 30 % and 0 %), and thereafter animals were fasted for 5 d. Urine was collected daily in plastic bottles containing 200 ml 1 M-H_2SO_4 during the maintenance (21 d), restricted feeding (4 d) and fasting phases (7 d). The urine was weighed and its density measured using the relationship between weight and volume and, afterwards, urine was sampled (1 %) and diluted with distilled water (adjusted to 1 litre). Two sub-samples (25 ml) of diluted urine were taken and stored at $-20°C$ for later analysis of allantoin (Young & Conway, 1942) and uric acid (TPTZ-Ligand Method, Biogamma Lab., CA, USA). Total PD were calculated as the sum of uric acid and allantoin.

Expt 2. Xanthine oxidase activity in plasma, liver and intestinal tissues

Six male Zebu crossbred cattle (previous nutrition stated unknown), three H (456 (SD 12·3) kg live weight) and three M (485 (SD 16·9) kg live weight) were selected from a local slaughterhouse. Blood samples were taken from the jugular vein prior to slaughter and collected in heparinised 20 ml test tubes, and immediately ultra-centrifuged for 15 min at 3000 **g** and 4°C, and plasma analysed within 4 h after sampling. Samples were taken from the liver, washed in cold 0·15 M-KCl and 1 g was homogenised, and the supernatant fraction was used for enzyme analysis (Chen *et al.* 1996). Intestinal samples were taken from the proximal duodenum (30 cm measured from the pylorus area) and the lumen was washed with a cold solution of 0·15 M-KCl. The samples were frozen immediately (liquid N), and defrosted at 4°C over the subsequent 2 h. Intestinal lumen was washed with 0·05 M-HEPES buffer (pH 7·5) containing 0·25 mM-EDTA and 0·25 mM-phenylmethylsulphonyl fluoride (PMSF). To remove 1 g of intestinal mucosa we applied finger pressure along the portion of intestine and collected the mucosa layer cells at the bottom in 9 ml HEPES–EDTA–PMSF buffer. The extract containing the enzyme XO (*EC* 1.3.2.2.) was purified in the same manner as applied to liver samples, but using HEPES–EDTA–PMSF buffer. Activity of XO was measured as the rate of uric acid production when the xanthine was incubated at 37°C for 60 min with the tissues extracts (Chen *et al.* 1996; IAEA, 1997). A unit of XO was defined as 1 mmol of uric acid produced per min at 37°C in excess of substrate.

Expt 3. Excretion kinetics of ^{15}N-labelled uric acid

Two male *Bos indicus* × *Bos taurus* crossbred cattle (H) that weighed 265 and 269 kg were kept in metabolic cages with free access to drinking water. From 11 d before and until the end of the experiment, animals were fed *Cynodon dactylon* at 60 or 95 % of the previously registered *ad libitum* intake. The tracer ([1,3-^{15}N]uric acid 98 % + ; ISOMED, Madrid, Spain) was diluted in a 100 ml solution (saline solution (9 g NaCl/l)–glycerol, 50:50) and the pH was maintained above 8 to avoid uric acid precipitation. Tracer solution was sterilised (110°C, 20 min) and stored at 4°C until infusion. The tracer was infused through the jugular catheter at pulse doses of 200 mg uric acid (1·19 mmol tracer) and immediately flushed with saline solution to ensure quantitative injection of the tracer. After jugular infusion urine was collected over periods of 6 h on 5 d into vessels containing 200 ml 0·5 M-H_2SO_4 (to obtain a pH < 3) to prevent microbial contamination. Urine was collected directly from the metabolic

cage and weighed before being sub-sampled and stored at $-20°C$. At the same time, jugular-vein samples were collected in heparinised vacutainers. For isotope background determinations urine and blood samples were taken the day before the start of the isotope infusion.

Allantoin and uric acid were determined in urine using the method of Balcells *et al.* (1992a). Isotopic enrichment of allantoin and uric acid in urine, plasma and infusates was determined following the method proposed by Chen *et al.* (1991). Samples were desalted using an ion exchange resin (AG-1-X8 100–200 mesh chloride form) and water eluted samples were derivatised to produce *tert*-butydimethylsilyl derivatives of uric acid and allantoin. MS was performed on an HP-5890 gas chromatograph (model HP-5890) coupled with a quadrupole mass spectrometer (Hewlett Packard, Manchester, UK) operated under electron impact ionisation conditions.

The isotopic enrichment of PD was calculated as $E(\delta) = [(r_{sa} - r_r)/r_r] \times 1000‰$, where r_{sa} is the isotope ratio in the sample and r_r that in the background sample. A two-compartment model was fitted to the data for isotopic enrichment of PD *v.* time. The diagram of the model is shown in Fig. 1, and the differential equations describing each compartment are as follows:

1. First compartment: uric acid

   One dose of tracer at time (t) $= 0$.
   Uric acid is partially excreted directly into the urine and converted into allantoin.

   $$\frac{dEua(t)}{dt} = K_{ua} \times E_{ua}(t)$$

   The uric acid excretion model was the following:

   $$Eua(t) = Eua_0 \times e^{-K_{ua}t}$$

2. Second compartment: allantoin

   Enrichment at initial time is zero.
   Allantoin is formed from uric acid and excreted into urine.

   $$\frac{dEa(t)}{dt} = K_{ua-a} \times Eua(t) - K_a \times Ea(t)$$

   $$\frac{dEa(t)}{dt} = K_{ua-a} \times (Eua_0 \times e^{-K_{ua}t}) - K_a \times Ea(t)$$
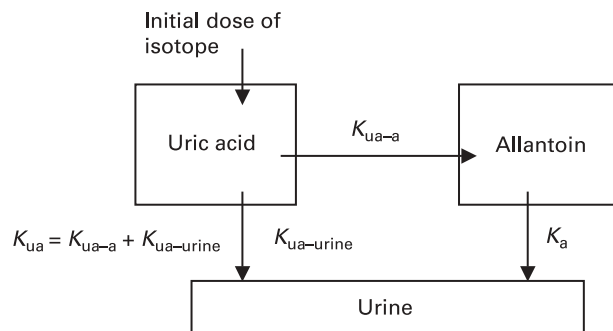

Fig. 1. Diagram of the two-compartment model used to describe the metabolism of purine derivatives in Zebu cattle.

Using Laplace's transform, the final model was:

$$Ea(t) = \frac{(K_{ua-a} \times Eua_0)}{(K_{ua} - K_a)} \times (e^{-K_a t} - e^{-K_{ua} t})$$

where t is the time, and Eua and Ea refer to the enrichment (δ) in the uric acid and allantoin compartments, respectively. Eua_0 was determined using the extrapolation of the enrichment in the uric acid compartment to time zero, and K (K_{ua} or K_a for uric acid or allantoin, respectively) is the rate constant describing the fractional turnover of each compartment. The uric acid compartment size (CS_{ua}) was determined, $CS = Dose/Eua_0$, and excretion rate of labelled uric acid as $F_{ua} = K_{ua} \times CS$. Isotope recovery data were described by a mono-exponential function of the injection time (t, h): $y = b(1 - e^{-kt})$, where y is the cumulative recovery of the isotope, b is the maximum potential recovery of the isotope (%) and k is the fractional rate of the isotope excretion.

Expt 4. Urinary excretion of purine derivatives at different levels of feed intake

Four animals of H (245 (SE 15·9) kg live weight) were penned individually in metabolic cages, provided with feeders and water and fed *Cynodon dactylon* hay (10·3% crude protein, 49% DM digestibility) once daily (08.00 hours). Animals were offered the food at 40 (D-40), 60 (D-60), 80 (D-80) and 95% (D-95) of *ad libitum* intake (109 g DM/kg) in a 4×4 Latin Square design. Each experimental period lasted 21 d, including 11 d for dietary changeover and 10 d for urine and faeces collection. Faeces were collected daily, weighed, sampled (10% of the total per animal) and dried at 60°C to a constant weight. Organic matter from hay and faeces were measured using AOAC (1990). Digestible organic matter intake (DOMI) was calculated as the difference between organic matter intake and faeces content. Urine collection and sampling procedures were the same as those used in Expt 1.

Statistical analyses

In Expt 1, to study the effect of animal and time on the urinary excretion of PD during fasting, data were analysed as a repeated observation on each experimental unit (animal). An orthogonal set of contrast over time, comparing daily means with the mean of the following period, was used to partition the error sum of squares into components associated with each contrast (Rowell & Walter, 1976). In Expt 2, data were analysed by a factorial design using the model:

$$y_{ijk} = \mu + G_i + T_j + GT_{ij} + A_k + \varepsilon_{ijk}$$

where A_k, G_i, T_j, the effect of animal, genotype and tissue (liver and duodenum) and their interaction (GT_{ij}) were contrasted against the residual error term ε_{ijk}. For Expt 3 only means and standard errors are presented. The isotope enrichment *v.* time curves were fitted using WinSAAM (version 3.0.7). In Expt 4, data were analysed as a Latin Square design following the model:

$$y_{ijk} = \mu + D_i + A_j + P_k + \varepsilon_{ijk}$$

where D_i, A_j, P_k: D, dietary (treatment), A, animal and P, period were the main effects that were contrasted against the residual error term (ε). Treatments were regrouped for evaluation using orthogonal contrasts (Steel & Torrie, 1985), identified as C1 (D-40 *v.* rest) and C2 (D-95 *v.* rest). A linear regression model was also fitted to the urinary excretion of PD (y, mmol/d) and digestible organic matter intake (x, kg DOMI/d) data following the previously described procedures.

Results

Urinary excretion of purine derivatives during fasting

Feed restriction caused a rapid decrease in allantoin and PD excretion, which reached a steady basal value in the last 4 d of the fasting period (Fig. 2). That value was considered the endogenous contribution to urinary excretion, assuming that urinary PD coming from intestinal absorption of purines represented only a minor proportion. We did not detect significant differences among Zebu-genotypes in basal or endogenous values, therefore only treatment means are presented in Table 1. Between the fourth and seventh days of the fasting period total PD excretion values levelled off at 277·3 (SE 35·43) µmol/kg metabolic body weight ($W^{0·75}$). The whole urinary PD excretion was composed of allantoin plus uric acid, with the former being the most abundant (89–96% of the total urinary excreted). During fasting, allantoin followed a pattern similar to that of total PD, with significant differences ($P < 0.05$) between maintenance, restriction and fasting periods, regardless of genotype.

Xanthine oxidase activity in plasma, liver and intestinal tissues

Fig. 3 shows the increase in uric acid when xanthine was incubated with different tissue extracts as a measure of their XO activity, and values of enzyme activity are presented in Table 2. We detected significant differences in XO activity between liver (0·64 units/g wet tissue) and intestinal mucosa (0·06 units/g wet tissue), whereas oxidative activity of the enzyme in plasma was 0·60 units/l. Although, as shown in Fig. 3, apparently M animals exhibited lower XO activity in plasma than did H animals, the difference was not statistically significant. These results need to be treated with caution because of the small number of animals used in the analysis.

Excretion kinetics of ^{15}N-labelled uric acid

When the isotope was administered, the animals did not appear to be unduly disturbed. We did not detect significant changes in uric
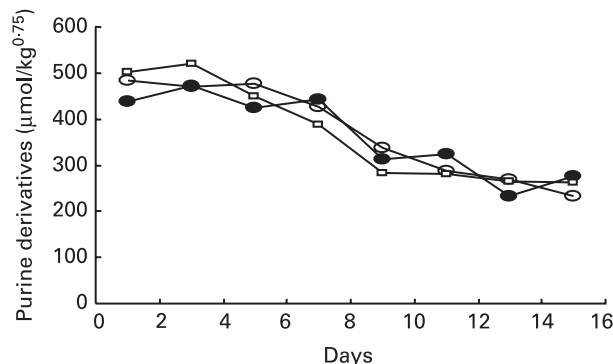

Fig. 2. Urinary excretion of purine derivatives in 5/8 (○), 1/2 (●) and 3/8 (□) *Bos indicus* × *Bos taurus* crossbred animals during maintenance, nutritional restriction and fasting periods.

Table 1. Urinary excretion of purine derivatives (PD; μmol/kg metabolic body weight) in eighteen *Bos indicus* × *Bos taurus* crossbred cattle (three groups of six animals each of 3/8, 1/2 and 5/8 *Bos indicus*, respectively) under progressive feeding restriction, from *ad libitum* feeding to fasting (in Expt 1)†

Periods	Allantoin	Uric acid	Total PD
Feeding (days 1−4)			
Maintenance level	437.3[a]	28.8	499.4[a]
Pre-fasting (days 5−8)			
Mean values for 60/30 % restriction	323.6[b]	28.9	353.6[b]
Fasting (days 12−15)			
Mean values for 4 d	246.9[c]	30.3	277.3[c]
SE	30.18	3.23	29.78
Significant period effect	*	NS	*

[a,b,c]Means values within a column with unlike superscript letters were significantly different; *$P < 0.05$.

† Values are means for the last 4 d of maintenance, and pre-fasting include 30 plus 60 % restriction (days 5−8), whereas the fasting period corresponds to the last 4 d of fasting (days 12−15).

acid kinetics either between animals or intake level; therefore data were pooled.

Following the intravenous injection of [1,3-^{15}N]uric acid, there was a rapid appearance of enriched PD as uric acid, but also as allantoin, in the urine. Six hours after injection, more than 50 % of the isotope was excreted and, 30 h later, no differences were detected between natural and ^{15}N-enrichment in urinary PD (Fig. 4). That said, urinary excretion was monitored for 5 d. With the issue of low sample size in mind, we observed that urinary recovery of the tracer was 78.3 and 88.5 % for the first and second animal, respectively, and the level of intake did not have a significant effect. The cumulative recovery of the isotope following the administration of the tracer is shown in Fig. 5. The data were fitted to a mono-exponential function and the fitted equation $(y = 82.27 \text{ (SE 6.69)}(1 - e^{-(0.17(\text{SE }0.051)t)})$, n 28, R^2 0.77) showed that 82.3 (SE 6.69) % of the uric acid was excreted in urine as PD. We were not able to determine isotope enrichment (^{15}N) of uric acid and allantoin in plasma samples because of their low concentrations. A general approach to the uric acid kinetics in plasma can, however, be estimated by assuming that the enrichment determined in urinary PD reflects that of plasma before clearance by the kidney. In our study, the enrichment in any urine sample accumulated over 6 h was assumed to represent the average enrichment in plasma in the corresponding collection period. In that case, we assume a linear evolution within the urine collection interval, whereas the actual evolution of enrichment seems to be exponential. The assumption of linearity might lead to some inaccuracies in the estimated fractional rate of tracer turnover and is considered to be a potential error in our approach.

After intrajugular injection of labelled uric acid, there was a rapid decay in the enrichment of uric acid N that can be described by the following exponential equation: E (‰) uric acid N = 28 339 (SE 5017) $e^{(-0.284(\text{SE }0.051)t)}$, R 0.947; whereas the enrichment of allantoin N starts from zero and its time course is better fitted to the following function: E (‰) allantoin N = 498 270 (SE 44 844) $e^{(-0.273t(\text{SE }0.081))} - e^{(-0.284t(\text{SE }0.05)1)}$, R 0.931.

The parameters derived from the fitted curves (Fig. 4), including uric acid compartment size (CS), flow rate (F) and turnover time ($1/K$), are presented in Table 3. On average, the uric acid compartment was 5.14 (SE 0.823) mmol, with a disappearance rate of 24.3 (SE 4.02) μmol/min. Based on the fitted equations,

uric acid leaves the plasma pool at a constant rate (K_{ua}) of 0.284 (SE 0.051) per h, which represents the uric acid renal excretion rate ($K_{ua-urine}$, 0.101(SE 0.0302)) plus the oxidation through the allantoin pathway (K_{ua-a}, 0.183 (SE 0.027)). Finally, allantoin was excreted from the plasma pool at a constant rate of 0.2732 (SE 0.081) (K_a).
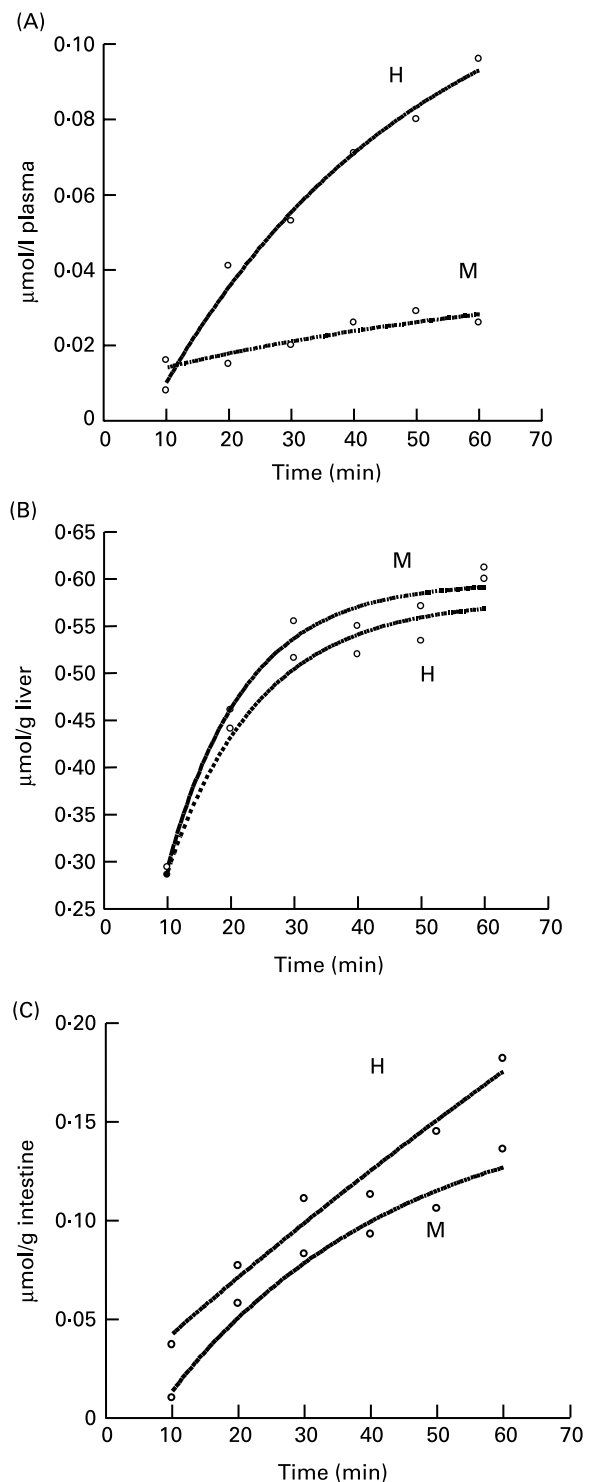

Fig. 3. Production of uric acid when xanthine oxidase was incubated with plasma (A), liver (B) or extract of intestinal mucosa (C) of 1/2 (M) and 5/8 (H) *Bos indicus* × *Bos taurus* crossbred animals.

Table 2. Xanthine oxidase activity in plasma, liver tissue and duodenal mucosa cells in *Bos indicus* × *Bos taurus* crossbred cattle

		Animals				Statistical significance‡			
	Group†	1	2	3	Average	SE	G	T	G × T
Plasma (units/l)	H	0·92	0·77	0·83	0·83	0·48			
	M	0·16	0·33	0·71	0·37	0·26			
Liver (units/g wet tissue)	H	0·29	0·85	0·72	0·62	0·12	NS	**	NS
	M	0·45	0·76	0·76	0·66				
Duodenum (units/g wet tissue)	H	0·09	0·09	0·08	0·09				
	M	0·02	0·02	0·04	0·03				

† Group: H, 5/8 *Bos indicus*; M, 1/2 *Bos indicus*.
‡ Statistical significance of: group (G), tissue (T) and the interaction (G × T); **$P < 0.01$.

Urinary excretion of purine derivatives at different levels of feed intake

PD excretion at different feed intake levels are presented in Table 4. Total urinary excretion of PD (Y, mmol/d) responded significantly ($P < 0.01$) to variations in DOMI (X, kg/d), being the relationship between the variables defined by the following equation:

$$Y = 7.69 (\text{SE } 4.2)$$
$$+ 5.69 \ (\text{SE } 1.68) \ X; \quad n \ 16, \quad \text{SE } 1.31, \quad r \ 0.67 \quad (1)$$

Orthogonal contrasts showed significant differences ($P < 0.05$) in allantoin excretion (mmol/d) when the high (D-90) or low (D-40) DOMI levels were compared against the rest ($P < 0.05$).

Discussion

Purine derivative excretion during fasting and in response to feed intake

Fasting excretion may be considered as a proximate estimate of the endogenous contribution to urinary excretion, although experimental evidence of a complete absence of the duodenal flow of exogenous purine compounds during food restriction does not exist (Balcells *et al.* 1991). Furthermore, a reduction of the energy available, which occurs during fasting, can affect tissue RNA turnover and consequently the irreversible losses derived from that process. In any case, the excretion of

PD responded significantly to the food restriction, which agrees with previous values in cattle (Liang *et al.* 1994) and Zebu (Osuji *et al.* 1996), and reached an average basal value of 277·3 (SE 35·43) μmol/W$^{0.75}$. No differences were observed among different types of cattle/Zebu crossbred animals. Indeed, our results are quite similar to the endogenous values (μmol/W$^{0.75}$) obtained in European (236–385; Verbic *et al.* 1990; Orellana-Boero *et al.* 2001) and in Zebu cattle (172–275, Boran and Kedah-Kelantan varieties; Osuji *et al.* 1996; Liang *et al.* 1999). Osuji *et al.* (1996), however, compared pure Zebu against their crosses and observed a higher value in Boran Bulls (*Bos indicus*) than in their crosses with *Bos taurus* (172 and 108 μmol/W$^{0.75}$, respectively). The excretion of PD decreased during fasting, but it mostly reflects changes in allantoin excretion, given that uric acid showed the opposite trend, apparently increasing with food restriction. Changes in the exogenous supply of purine compounds mostly affect allantoin excretion and it has been previously described (Balcells *et al.* 1991; Giraldéz, 1992), suggesting a preferential oxidation of exogenous compounds. That finding, however, has not been reported in fasted cattle.

Allantoin and PD excretion responded to food restriction and to different levels of feed intake, which supports previous results in Zebu cattle (Liang *et al.* 1994) and other species (Daniels, 1993; Giesecke *et al.* 1993). We used the regression approach to analyse the effect of feed intake, and Equation 1 describes the relationship between digestible organic matter intake and PD excretion. The response appears to be linear, although the fitted intercept
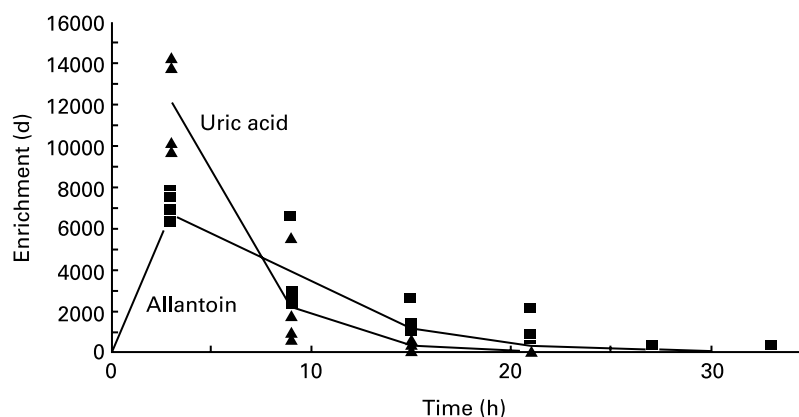

Fig. 4. Time course in the enrichment of urinary allantoin (■) and uric acid (▲) after the intrajugular injection of [1,3-^{15}N] uric acid in two *Bos indicus* × *Bos taurus* crosses.
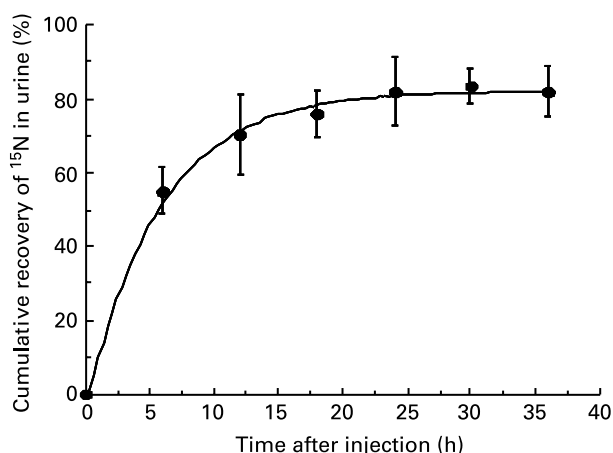
Fig. 5. Cumulative recovery of isotope (^{15}N) in urine after intrajugular injection of [1,3-^{15}N]uric acid in two *Bos indicus* × *Bos taurus* crossbred animals.

(7·69 mmol/d or 0·14 mmol/W$^{0.75}$) was significantly lower than the endogenous values determined using fasted animals (0·28 mmol/W$^{0.75}$). In this sense, it is also difficult to explain why PD excretion in Zebu cattle consuming 0·4, 0·6 and 0·8 of *ad libitum* intake was similar to those levels obtained in fasted animals. If the response model in Zebu cattle was similar to that described in sheep (Chen *et al.* 1990; Balcells *et al.* 1991), urinary PD excretion would only respond to duodenal input over a threshold level where exogenous purines cover endogenous losses. The low XO activity in duodenal mucosa might support some availability of salvageable purine compounds although concentration in Zebu urine samples was negligible (Pimpa *et al.* 2001).

Additionally, the slope of the regression line (5·7 mmol PD/kg DOMI) was lower than the expected values obtained in *Bos indicus* cattle (8·3–11·3 using Kedah Kelatah, and Ongola or Bali varieties; Liang *et al.* 1993; Soejono *et al.* 1999) and European cattle (15·7–25·2; Lindberg & Murphy, 1991; Daniels, 1993; Liang *et al.* 1993). Values reported in the literature suggest that the rate of PD excretion per unit of DOMI is lower in *Bos indicus* than in *Bos taurus*, although both endogenous excretion and urinary recovery of duodenal PB (Pimpa *et al.* 2001) do not appear to support that conclusion.

Table 3. Compartment size, flux rates determined from the fitted curves describing the time course of enrichment of uric acid and allantoin in urine samples after bolus injection of doses of [1,3-^{15}N]uric acid as isotope tracer in *Bos indicus* × *Bos taurus* crossbred cattle

	Uric acid	SE	Allantoin	SE
Excretion (mmol/d)	2·34	0·387	17·00	2·115
E_0 (δ_0)	28339	4984	0	0
K (per h)	0·28	0·051	0·273	0·081
$K_{ua-urine}$	0·10	0·032	–	
K_{ua-a}	0·18	0·027	–	
CS_{ua} (mmol)	5·14	0·823	–	
F_{ua} (μmol/min)	24·3	4·02	–	
$F_{ua-urine}$	8·6	1·57	–	
F_{ua-a}	15·65	2·032	–	
$1/K_{ua}$ (h)	3·5	0·52	3·66	0·623

CS, compartment size; F, flow rate; 1/K, turnover time.

Xanthine oxidase activity

XO is the enzyme responsible for the irreversible oxidation of both intermediate metabolites, xanthine and hypoxanthine, to uric acid. High activities in gut mucosa and liver cause an irreversible oxidation of re-utilisable purine compounds and, therefore, animals are unable to recycle compounds of exogenous origin. Consequently, endogenous losses need to be covered constantly with a *de novo* synthesis and, therefore, in those species that show such enzyme profiles, the relationship between duodenal input and urinary output is linear (Verbic *et al.* 1990; Orellana-Boero *et al.* 2001), given that exogenous purine salvage is restricted to the gastrointestinal tract (Balcells *et al.* 1992*b*).

Plasma activity (units/l) in Zebu crossbred cattle (0·60) was lower than in buffalo and cattle (24·5 and 1·4; Chen *et al.* 1996), but higher than the low value detected in goats (0·001; Belenguer *et al.* 2002) and it was nil in sheep (Chen *et al.* 1996). Liver activity (units/g wet tissue; 0·64) was in the range reported in buffalo and cattle (0·44 and 0·30, respectively; Chen *et al.* 1996), but higher than in sheep (0·08; Chen *et al.* 1996) and goats (0·12; Belenguer *et al.* 2002). However, duodenal activity in Zebu cattle (0·06 units/g wet tissue) was as low as that value in sheep (0·04; Chen *et al.* 1996) and goats (0·0009; Belenguer *et al.* 2002), but lower than buffalo and cattle (0·31 and 0·18, respectively; Chen *et al.* 1996).

The high activity of XO in liver, moderate in plasma, but low in duodenum, might confirm some absorption of purine compounds in salvageable form in these species.

Renal excretion of ^{15}N-labelled uric acid

Uric acid can be removed from the blood in two main ways, clearance by kidney and catabolism (oxidation) through the uricase pathway to allantoin. If disappearance rate is a function of two removal mechanisms, then the rate can be represented by the sum of their components. We used a two-compartment model (Fig. 1) and assumed that labelled uric acid was removed from plasma at a constant rate (K_{ua}) and that would include oxidation to allantoin (K_{ua-a}) and excretion ($K_{ua-urine} = K_{ua} - K_{ua-a}$) from the plasma pool. Initially, allantoin was not enriched in the plasma pool compartment, then the enrichment increases to a maximum and after that it diminishes, depending on excretion of allantoin (K_a), mostly through renal routes, and its appearance by irreversible uric acid oxidation (K_{ua-a}).

Despite differences in purine metabolism among ruminant species (Chen *et al.* 1990), absorbed PB are not fully recovered in urine, and 5–30 % may be excreted through non-renal routes. In our experiment, urinary recovery of the isotope (^{15}N) as purine derivatives (82·3 (SE 6·69) %) was similar to values reported in sheep or cattle, although it is important to note that in buffalo (J. B. Liang *et al.*, personal communication) the urinary recovery of intrajugular infused uric acid was as high as 80 %, although it was only 11 % when administered through the duodenum (Pimpa *et al.* 2001). Obviously, intestinal mucosa plays an essential role in purine metabolism, which might explain differences in the ratio of PD excreted by kg of DOMI between cattle and zebu crosses used in our experiment.

Regarding the incomplete recovery of purine metabolites, several studies have observed a rapid removal of allantoin from the plasma pool, with excretion being directly determined by the

Table 4. Intake of dry matter and digestible organic matter, and urinary excretion of creatinine and purine derivatives (PD) in *Bos indicus* crossbred cattle at different levels of feed intake

	Level of feed intake†				SS‡			
	0·4	0·6	0·8	0·95	SE	D	C1	C2
Intake (kg/d)								
Dry matter	3·50[a]	5·60[ab]	7·38[b]	8·85[b]	0·71	**		
Digestible organic matter	1·35[a]	2·16[b]	2·84[b]	3·41[bc]	0·20	**		
Urinary excretion ((mol/kg metabolic body weight)								
Allantoin	185·2	243·3	260·5	318·6	49·0	NS	*	*
Uric acid	27·0	26·3	27·2	26·7	1·72	NS		
Total PD	201·3	254·5	281·9	354·0	45·2	NS		
Creatinine	533·5	468·3	487·0	431·5	68·2	NS		
Purine/digestible organic matter	170·9[a]	123·8[b]	99·1[b]	100·3[b]	26·6	**		

[abc] Mean values within a column with unlike superscript letters were significantly different, $P < 0.05$.

† Levels of feed intake: 40 (0·4), 60 (0·6), 80 (0·8) and 95 (0·95) % of the *ad libitum* intake; n 4 in each group.

‡ SS, statistical significance of level of intake (D) and orthogonal contrasts, C1 (0·4 *v.* rest) and C2 (0·95 *v.* rest): *$P < 0.05$; **$P < 0.01$.

input of allantoin (Chen *et al.* 1991; Surra, 1994). A small but significant fraction, however, can reversibly exchange with other pools, which can delay its urinary recovery (Khan & Nolan, 2000). However, in relation to allantoin precursors (in cattle, only uric acid), it is possible that they might be more easily excreted by non-renal routes than allantoin, in which case confidence in the PD index may be reduced. In our study, the appearance in urine of nearly 80 % of labelled PD after intravenous injection confirms the fast excretion rate of uric acid. Furthermore, as much as 77 % of the isotope was excreted as allantoin (0·77 (SE 0·01)) 6 h after injection, which indicates a high uricase activity. Unfortunately, we did not assess uricase activity in this work. Previous results showed no uricase activity in cow blood (Chen *et al.* 1990), therefore uric acid must be oxidised to allantoin in the liver. Nevertheless, our results must be considered with caution because of the estimation of plasma disappearance kinetics based on serial urine samples and the small number of animals used in our study. Fig. 1 shows the proposed model for kinetics and excretion of uric acid. It appears that allantoin is rapidly removed from the plasma pool (Surra, 1994) and is turned over approximately once every hour (Khan & Nolan, 2000); therefore, the excretion of allantoin might depend directly on the influx of allantoin (Chen *et al.* 1991). However, when we compare the constant rate of allantoin excretion (K_a) with values reported in the literature, our estimate (0·27 (SE 0·081) per h) is apparently similar to those reported by Khan & Nolan (2000; 0·213 per h) and Chen *et al.* (1991) in plasma (0·29 per h) and urine (0·22 per h), using an extra dose of labelled [14C]allantoin or non-labelled allantoin, respectively. Indeed, when using a flooding dose to monitor urinary recovery of allantoin, it only depends on the capacity of the kidney to remove allantoin from plasma. However, in our studies we tested both the capacity of the kidney to remove allantoin from blood and the capacity of liver uricase to oxidise uric acid to allantoin.

Non-renal losses of allantoin can be estimated by subtracting urinary losses from daily allantoin influx (F_{ua-a}). In our study, non-renal excretion of allantoin accounted for 19·2 %. We compared our values with those reported in sheep, and ours are higher than in Khan & Nolan (2000; 5 % using labelled [14C]allantoin after 100 h urine collection), but similar to those of Chen *et al.* (1991; 28 %) and Surra *et al.* (1997; 19 and 22 % in sheep fed chopped and pelleted hay, respectively), in both cases using overloaded doses of non-labelled allantoin.

Using the model presented in Fig. 1, total uric acid excretion (F_{ua-to}) accounted for 8·63 (SE 2·67) μmol/min, whereas urinary excretion only accounted for 1·70 μmol/min. It means that 20 % of uric acid leaves the plasma pool through the renal way, although this value was characterised by high variability. Renal uric acid losses varied from 35 to 15 % of the total outflow of uric acid, these values being much lower than previous values reported in the existing literature. The high level of non-renal losses of uric acid and the high registered variability may be explained by three reasons: (1) to use extra doses of uric acid through the jugular vein could affect partitioning between renal and non-renal routes; (2) the methodology employed in the present assay could underestimate renal losses in relation to continuous sampling of urine or plasma; and finally (3) the possibility that uric acid exchanges with other pools with a low return rate cannot be detected.

Experimental evidence demonstrates that allantoin and uric acid can leave the plasma pool by different non-renal routes such as saliva (Surra *et al.* 1997), milk (González-Ronquillo *et al.* 2003) and, possibly, through the gut (as suggested by the evidence in humans; Zollner, 1982) and other species (oxypurines in hamster, Berlin & Hawkins, 1968)). Uric acid is rapidly metabolised in the gut by micro-organisms, however, and consequently, cannot be determined by direct analysis (Zollner, 1982; Chen *et al.* 1991). By using intrajugular administration of [14C]uric acid and quantifying radioactivity into the gut, Löffler *et al.* (1982) estimated that 30–50 % of the total excretion of uric acid is through the gut wall.

In conclusion, purine metabolism in Zebu (*Bos indicus*) cattle is similar to that observed in European breeds (*Bos taurus*) in relation to the endogenous (fasting) contribution to urinary excretion and in the urinary recovery of intrajugularly administered uric acid, with the majority of PD excreted through non-renal routes in the form of uric acid. However, in our study, the urinary response, in terms of PD, to increases of DOMI was lower than those values obtained in European cattle, which suggests species-specific differences in the absorption process.

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