CSIRO PUBLISHING
www.publish.csiro.au/journals/ajar *Australian Journal of Agricultural Research*, 2006, **57**, 173–177

A comparison of the excretion rate of endogenous purine derivatives in the urine of *Bos indicus* and *Bos taurus* steers

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Abstract. Estimates of microbial crude protein (MCP) production by ruminants, using a method based on the excretion of purine derivatives in urine, require an estimate of the excretion of endogenous purine derivatives (PD) by the animal. Current methods allocate a single value to all cattle. An experiment was carried out to compare the endogenous PD excretion in *Bos taurus* and high-content *B. indicus* (hereafter, *B. indicus*) cattle. Five Holstein–Friesian (*B. taurus*) and 5 Brahman (>75% *B. indicus*) steers (mean liveweight 326 ± 3.0 kg) were used in a fasting study. Steers were fed a low-quality buffel grass (*Cenchrus ciliaris*; 59.4 g crude protein/kg dry matter) hay at estimated maintenance requirements for 19 days, after which hay intake was incrementally reduced for 2 days and the steers were fasted for 7 days. The excretion of PD in urine was measured daily for the last 6 days of the fasting period and the mean represented the daily endogenous PD excretion. Excretion of endogenous PD in the urine of *B. indicus* steers was less than half that of the *B. taurus* steers (190 μmol/kg $W^{0.75}$.day *v*. 414 μmol/kg $W^{0.75}$.day; combined s.e. 37.2 μmol/kg $W^{0.75}$.day; $P < 0.001$). It was concluded that the use of a single value for endogenous PD excretion is inappropriate for use in MCP estimations and that subspecies-specific values would improve precision.

Additional keywords: rumen microbial protein, cattle.

Introduction

The method involving measurement of the excretion of purine derivatives (PD) in urine, for estimating microbial crude protein (MCP) flow to the small intestines in ruminants, is based on the principle that the urinary PD originate largely from the degradation of absorbed microbial nucleic acids (McAllan and Smith 1973; Verbic *et al*. 1990; Chen and Gomes 1995). However, PD in the urine also originate from tissue nucleic acid turnover and these endogenous PD need to be accounted for in the determination of the MCP production by this technique.

In the equation used widely for calculating MCP flow in cattle, which was developed by Verbic *et al*. (1990) and revised by Chen and Gomes (1995), a constant value for endogenous excretion of PD in the urine of cattle is assumed, *viz*. 385 μmol/kg $W^{0.75}$.day. This value was derived using *Bos taurus* cattle but several studies have shown that pure-bred and high-content *B. indicus* cattle may have lower rates of excretion of endogenous PD (Osuji *et al*. 1996; Liang *et al*. 1999; Pimpa *et al*. 2001; Ojeda *et al*. 2005). However, the comparison of cattle subspecies in this regard has been confounded by the technique used to estimate endogenous PD excretion. Most experiments with *B. taurus* cattle have used the technique of total nourishment of animals by intragastric infusion (Fujihara *et al*. 1987; Chen *et al*. 1990; Verbic *et al*. 1990), whereas those with *B. indicus* cattle have used a technique involving the prolonged fasting of animals with associated measurement of urinary PD excretion (Osuji *et al*. 1996; Liang *et al*. 1999; Ojeda *et al*. 2005).

In our experiment the endogenous excretion of PD in the urine was compared between *B. taurus* and high-content *B. indicus* (hereafter *B. indicus*) steers using the fasting technique. A brief report that gives some aspects of these data has appeared elsewhere (Bowen *et al*. 2003).

Materials and methods

Animals and management

Five Holstein–Friesian (*B. taurus*) and 5 Brahman (>75% *B. indicus*) steers, 16 months of age with initial average liveweights of 323 ± 3.8 kg and 329 ± 4.7 kg, respectively, were used. The steers were treated with moxidectin (Cydectin, Fort Dodge Australia Pty Ltd) to control internal and external parasites at the commencement of the experiment. The experiment was run at the Mt Cotton Research Unit of the University of Queensland and was approved by the University of Queensland Animal Ethics Committee.

10.1071/AR05182 0004-9409/06/020173

The experiment consisted of a 14-day preliminary feeding period in individual, concrete-floor pens and a 14-day sampling period in metabolism crates. Steers were allocated to pens and crates in a completely randomised layout. The steers were fed a low-quality tropical-grass hay (buffel grass, *Cenchrus ciliaris*). Intake was fixed at a level equivalent to metabolisable energy (ME) maintenance requirements (calculated according to SCA (1990); mean: 41.8 MJ/steer.day) during the preliminary feeding period and for the first 5 days of the sampling period. Over Days 6, 7, and 8 of the sampling period the feed intake of the steers was reduced to 60%, 30%, and 0% of ME maintenance requirements, respectively, with fasting continuing for a total of 7 days. The steers had free access to drinking water at all times.

Experimental procedures

For each steer, hay intake was recorded over the first 7 days, and daily faecal output over the duration of the sampling period. Organic matter digestibility (OMD) of the diet was determined for Days 1–5. During the sampling period, total urine was collected daily into bins containing a sufficient quantity of 20% sulfuric acid so that the final pH of urine was kept below 3. Urine was weighed and a 10% daily subsample was kept and bulked for each steer over Days 1–5. These samples were stored frozen (−20°C) and later thawed, mixed thoroughly, subsampled, and diluted 1 in 10 with 0.1 M ammonium phosphate ($NH_4H_2PO_4$) buffer. The buffered subsamples were frozen prior to analysis for PD concentration. Over the final 9 days of the sampling period the urine for each steer was not bulked but was subsampled and diluted, as described above, daily. The steers were weighed at the beginning and end of the sampling period.

Analytical procedures

Hay samples, 1-mm ground, were analysed for total nitrogen (N) concentration by a combustion method (Sweeney 1989) using an ELEMENTAR RapidN combustion analyser (ELEMENTAR ANALYSENSYSTEME GmbH, Germany). Ash-free neutral detergent fibre (NDFom) and ash-free acid detergent fibre (ADFom) were determined by the methods of Van Soest and Wine (1967) and Van Soest (1963), respectively, adapted for the Fibretec 2021 Fibrecap system developed by FOSS TECATOR (FOSS TECATOR 2002*a*, 2002*b*).

Acidified and buffered urine samples were thawed, filtered with 0.2-µm Alltech cellulose nitrate or acetate membrane filter followed by a C18, 300-mg filter, and analysed for PD concentration using a high-performance liquid chromatograph according to the method outlined by Balcells *et al*. (1992).

Calculations

The endogenous production of PD was determined as the total PD (sum of allantoin, uric acid, hypoxanthine, and xanthine) excretion by steers over Days 9–14 of the sampling period (see Statistical analyses). The flow of MCP to the intestines of steers for the first 5 days of the sampling period was calculated using the equations proposed by Chen and Gomes (1995). Calculations were made using their published value for endogenous PD excretion (385 µmol/kg $W^{0.75}$.day) and also by using the value derived in our experiment during the 9–14-day sampling period, for each subspecies of cattle.

Statistical analyses

The statistical package, Genstat for Windows, 6th edn (Genstat Committee 2000), was used for all statistical analyses. All values are given as mean ± s.e. unless otherwise indicated. An unpaired *t*-test was used to compare the 2 subspecies of cattle for the variables OMD, PD excretion, MCP flow, and efficiency of microbial protein synthesis (EMPS) during the first 5 days of the sampling period.

PD excretion during the fasting period, Days 9–14, was analysed using a linear mixed model fitted using restricted maximum likelihood (REML). The full model included the fixed effects time and subspecies, and their interaction, with the residual variance, steer.time, as the random effect. Time had 6 levels, the Days 9–14, and subspecies had 2 levels, *B. taurus* and *B. indicus*. Day 8 was not included in the analysis since large PD excretion values for several steers on Day 8 indicated that these steers had not yet reached their endogenous PD level. To account for possible correlation over time within steers, the covariance structure of the residual variance was investigated. Likelihood ratio tests were used to compare competing random models for the residual variance, resulting in the selection of an unstructured covariance model. Significance of fixed effects was then assessed using Wald tests. Since the interaction between subspecies and time was not significant ($P = 0.07$) it was removed from the model. For significant fixed effects, approximate pair-wise comparisons of means were made at $P = 0.05$.

Results

The buffel grass hay fed to steers during the first 7 days of the sampling period had a composition of organic matter (OM), NDFom, ADFom, and crude protein (CP) of 895, 729, 441, and 59.4 g/kg dry matter (DM), respectively. The OMD averaged 580 ± 4.3 g/kg and was not significantly different ($P = 0.72$) between subspecies of cattle.

The faecal DM output declined rapidly over Days 7 and 8 of the sampling period and then more slowly over the last 6 days of the 7-day fasting period (Fig. 1). Faecal output had decreased to an average of 132 ± 28.4 g DM/day by Day 7 of fasting. *B. taurus* and *B. indicus* steers had similar liveweight loss over the 14-day sampling period, with liveweights declining by 48 ± 3.4 kg and 44 ± 2.7 kg, respectively.

Mean excretion of PD during the first 5 days of the sampling period, when the steers were fed

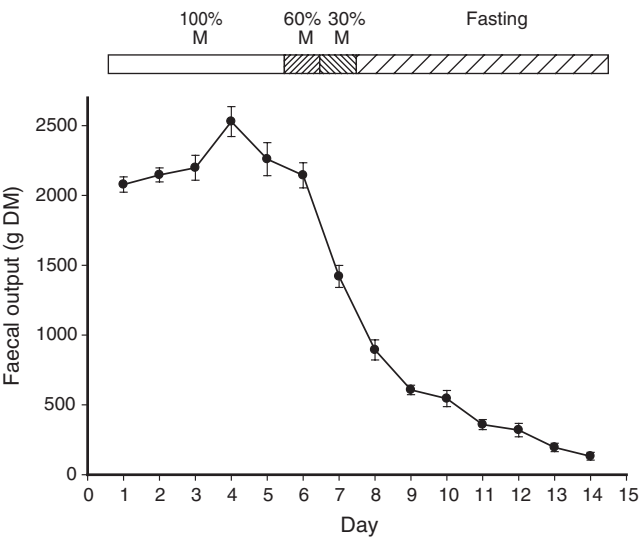

Fig. 1. Daily faecal output by steers during the sampling period [values are the mean of all steers from both subspecies ± s.e.; the intake by steers is shown in the bar graph as a percentage of estimated ME maintenance requirements (M)].

at maintenance, averaged 1059.5 ± 136.04 and 840.8 ± 77.52 µmol/kg $W^{0.75}$.day for *B. taurus* and *B. indicus* steers, respectively, with the difference not being significant ($P = 0.20$). During the period of reducing intake (Days 6 and 7) the excretion of PD for both groups of steers first increased sharply, then declined sharply by Day 9, remaining relatively constant to the end of the fasting period (Fig. 2). For Days 9–14, when steers were fasted, mean excretion of PD (assumed to be endogenous excretion) for *B. taurus* steers was more than double that for their *B. indicus* counterparts (414.0 *v.* 189.8 (combined s.e. 37.2) µmol/kg $W^{0.75}$.day; $P < 0.001$). These values were considered to represent endogenous PD excretion, assuming that urinary PD coming from intestinal absorption of purines represented only a minor proportion. There was no significant difference in PD excretion between each of Days 9–12 or Day 14. However, PD excretion for Day 13 was significantly lower ($P < 0.05$) than that for all other days except Day 12. Allantoin was the major PD present in the urine and changes in its concentration were the major influence on the decrease in PD excretion with fasting (Fig. 3). Negligible amounts of hypoxanthine and xanthine were detected in the urine of either subspecies group. The mean excretion of individual PD over the first 5 days of sampling when steers were fed at maintenance was: 869.9 ± 71.31, 80.2 ± 11.72, 0 ± 0, and 0.1 ± 0.07 µmol/kg $W^{0.75}$.day for allantoin, uric acid, hypoxanthine, and xanthine, respectively.

Discussion

This experiment has provided the first true comparison of *B. taurus* and *B. indicus* cattle in terms of their endogenous excretion of PD and indicates a 2-fold difference between subspecies in this parameter, although large standard errors were recorded. This indicates that the use of a single value for endogenous PD excretion (e.g. 385 µmol/kg $W^{0.75}$.day), for cattle of different subspecies, is inappropriate.

The endogenous excretion of PD we have measured for Holstein–Friesian steers, using the fasting technique, falls within the large range of values reported by other researchers for *B. taurus* cattle using a number of other techniques (mean of all values: 466 ± 36.0 µmol/kg $W^{0.75}$.day). These included the intragastric infusion method (428–514 µmol/kg $W^{0.75}$.day; Fujihara *et al*. 1987; Chen *et al*. 1990; Verbic *et al*. 1990), the rumen emptying technique (560 µmol/kg $W^{0.75}$.day; Giesecke *et al*. 1993), the extrapolation of infusion/excretion regression relationships to zero purine input (531 µmol/kg $W^{0.75}$.day; Beckers and Thewis 1994), and the isotopic labelling of exogenous purine bases (236 and 512 µmol/kg $W^{0.75}$.day; Orellana Boero *et al*. 2001; Gonzalez-Ronquillo *et al*. 2003). It is also similar to the value used by Chen and Gomes (1995; 385 µmol/kg $W^{0.75}$.day), which was based on the mean of all measurements made in the laboratory of Verbic *et al*. (1990) with adjustment downwards of 0.22 for the proportion of exogenous purines salvaged. This latter value was also estimated by Verbic *et al*. (1990) from the abomasal infusion of microbial extract (*Pruteen*), and interpreted as utilisation of exogenous purines in the intestinal mucosa. Unfortunately, there appear to be no published values for endogenous PD excretion in *B. taurus* cattle, which have been obtained using the fasting technique.

Similarly, the value measured in the present experiment for Brahman steers falls within the large range of values for

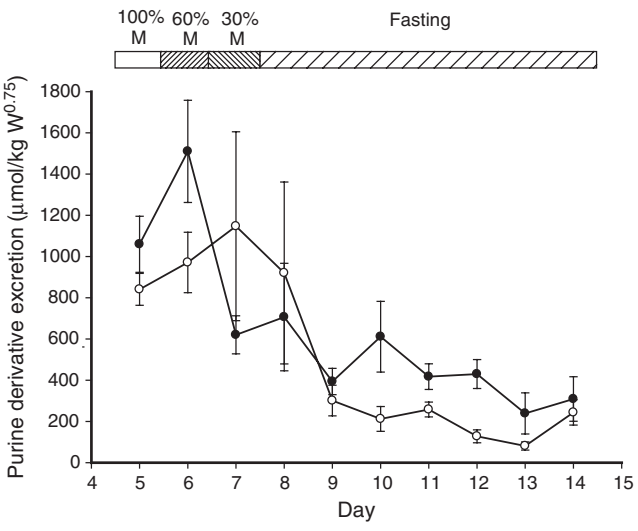

Fig. 2. Daily excretion of purine derivatives in urine of *B. taurus* (●) and *B. indicus* (○) steers during the sampling period [mean ± s.e.; the intake by steers is shown in the bar graph as a percentage of estimated ME maintenance requirements (M)].

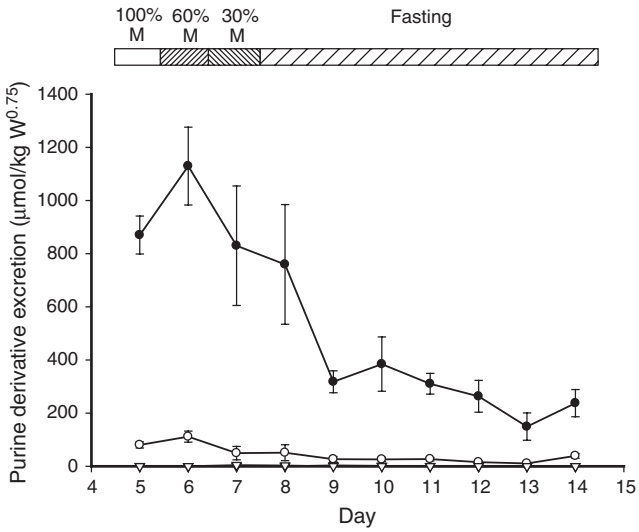

Fig. 3. Daily excretion of allantoin (●), uric acid (○), xanthine (▼) and hypoxanthine (▽) in urine over the sampling period [values are the mean of all steers from both subspecies ± s.e.; the intake by steers is shown in the bar graph as a percentage of estimated ME maintenance requirements (M)].

endogenous PD excretion for *B. indicus*-content cattle (mean of all values: 219 ± 28.2 μmol/kg $W^{0.75}$.day). Published values for *B. indicus*-content cattle included 108 and 172 (Osuji *et al*. 1996), 275 (Liang *et al*. 1999), and 277 μmol/kg $W^{0.75}$.day (Ojeda *et al*. 2005) using the fasting method, and 147 μmol/kg $W^{0.75}$.day (Pimpa *et al*. 2001) using the excretion curve after purine infusion.

However, an important consideration is the effect that the proportion of *B. indicus* content in crossbred cattle has upon endogenous PD excretion. In various experiments studying *B. indicus* × *B. taurus* cattle, the endogenous PD excretion for cattle with lower *B. indicus* content, for instance 3/8 and 1/2 *B. indicus* content (Osuji *et al*. 1996; Ojeda *et al*. 2005), was found to be in the same range as for purebred *B. indicus* cattle. This suggests that the endogenous PD excretion for *B. indicus* × *B. taurus* cattle is more closely aligned to the *B. indicus* than the *B. taurus* subspecies.

The validity of the subspecies comparison in the present experiment was supported by recordings of similar values for OMD and liveweight loss, and similar patterns of both faecal DM output and urinary PD excretion for the 2 subspecies over the sampling period. This indicated that subspecies differences in factors such as diet digestibility, or rate of liveweight loss with fasting, did not confound the between-subspecies comparison of endogenous PD excretion when the steers were fasted.

It is significant that the fasting technique generally gave similar results to those reported using other methods despite the possibility that nutritional restriction may alter metabolic activity or the rate of degradation of tissue nucleic acids, compared with fully fed animals. There has also been speculation (Osuji *et al*. 1996; Belenguer *et al*. 2002) that the duodenal flow of microbe-derived purine bases may not be completely discontinued during the period of fasting and thus may contribute to urinary PD excretion, which is otherwise assumed to be solely endogenous in origin. Although no direct evidence was obtained in this experiment to show that this was not the case, faecal output declined rapidly with food restriction to reach negligible values by Day 7 of fasting. This indicated that the majority of residual material in the reticulo-rumen had been voided and therefore would have made negligible contribution to the total urinary PD output.

Table 1 summarises the estimates of MCP flow and EMPS in cattle, calculated using either the endogenous value for PD excretion proposed by Chen and Gomes (1995), i.e. 385 μmol/kg $W^{0.75}$.day, or those values determined in our study. The application of the endogenous PD excretion values measured in the present experiment brought the estimates of MCP flow and EMPS much closer together for the 2 subspecies of cattle than occurred when the constant proposed by Chen and Gomes (1995) was used. Using the latter to calculate MCP flow suggests that *B. indicus* cattle have a lower, although not significantly so in this study, MCP flow and EMPS than *B. taurus* cattle consuming the same forage. By contrast, the results of our study suggest that MCP production and EMPS are not significantly different for *B. taurus* and *B. indicus* cattle consuming a low-quality tropical forage.

Table 1. Microbial crude protein (MCP) flow and efficiency of MCP synthesis (EMPS) estimated using the values for endogenous purine derivative excretion proposed by Chen and Gomes (1995) for all cattle (385 μmol/kg $W^{0.75}$.day) or those determined in our experiment for individual cattle subspecies

Results are presented as mean ± s.e.; DOMI, digestible organic matter intake

	B. taurus	*B. indicus*	*P*
	Chen and Gomes (1995)		
MCP flow (g/day)	274 ± 55.3	189 ± 34.4	0.23
EMPS (g MCP/kg DOMI)	98 ± 20.1	78 ± 12.7	0.42
	This study		
MCP flow (g/day)	262 ± 55.3	269 ± 35.2	0.91
EMPS (g MCP/kg DOMI)	94 ± 20.1	112 ± 12.4	0.47

The reason for the low endogenous PD excretion in *B. indicus* cattle is unclear. The lower values for endogenous PD excretion in *B. indicus* cattle, compared with *B. taurus* cattle, could result from a lower xanthine oxidase activity in the former. A high activity of xanthine oxidase prevents salvage of a significant proportion of endogenous PD by causing the diversion of hypoxanthine from the salvage cycle to form xanthine, and then uric acid and allantoin (Chen *et al*. 1990), neither of which can be incorporated into tissue nucleic acids. Ojeda *et al.* (2005) made measurements of xanthine oxidase activity in *B. indicus* cattle and found that, compared with reports for *B. taurus* cattle (Chen *et al*. 1990), cattle with 1/2 and 5/8 *B. indicus* content had lower activity of xanthine oxidase in the plasma and duodenum but similar activity in the liver. However, if *B. indicus* cattle do have a lower overall xanthine oxidase activity than *B. taurus* cattle, higher concentrations of xanthine and hypoxanthine would be expected in the urine, as occurs with sheep and goats which have low xanthine oxidase in most tissues and none in the blood (Chen *et al*. 1990; Belenguer *et al*. 2002). On the contrary, in the present experiment and in others using *B. indicus*-content cattle (Osuji *et al*. 1996; Liang *et al*. 1999; Pimpa *et al*. 2001; Ojeda *et al*. 2005), allantoin has been found to be the major component of total urinary PD, with negligible amounts of xanthine and hypoxanthine, similar to that found for *B. taurus* cattle.

An alternative hypothesis for the low endogenous PD excretion of *B. indicus* cattle has been offered by Osuji *et al*. (1996). They suggested that as part of the evolution of *B. indicus* cattle to conserve N they have an enhanced ability to dispose of purine metabolites via non-renal routes, e.g. by increased recycling to the rumen. However, the validity of this theory is challenged by the results of other researchers who found that the recovery of supplied exogenous purine

bases in the urine was 0.82 for 5/8 *B. indicus*-content cattle (Ojeda *et al*. 2005) and 0.85 for Kedah–Kelantan (pure bred *B. indicus*) cattle (Pimpa *et al*. 2001), and thus similar to that found for European cattle (0.85, Verbic *et al*. 1990; 0.86, Vagnoni *et al*. 1997; and 0.84, Orellana Boero *et al*. 2001).

Conclusions

Whilst the current experiment used only small group sizes to represent the 2 subspecies, the large differences in mean endogenous PD excretion rate between them, supported by previous trends, indicate the inappropriateness of using a single value for all cattle and suggest improved precision in estimating MCP production if between-genotype differences are recognised. Furthermore, our findings suggest that previously published estimates of MCP production for *B. indicus* cattle need to be re-evaluated. The large between-animal differences related to our measurements highlight the risks associated with use of the method for comparing individual animals and indicate its use be directed mainly to group comparisons.

Acknowledgments

This study was mainly funded by the Department of Primary Industries and Fisheries, Queensland, which provided post-graduate support for M. K. Bowen. M. K. Bowen was also in receipt of an Australian Government Postgraduate Research Award. The analysis of purine derivatives by M. Nielsen and the technical assistance of 'Mt Cotton' staff are appreciated, as well as veterinary supervision of the cattle by J. Gibbs.

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Manuscript received 25 May 2005, accepted 10 October 2005