Short Communication

Excretion of endogenous and exogenous purine derivatives in sheep: effect of increased concentrate intake

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The present study examined the endogenous urinary excretion of purine derivatives (PD; allantoin, uric acid and xanthine plus hypoxanthine) in fed animals. Four Rasa Aragonesa ewes fitted with simple cannulas in the rumen and proximal duodenum were used. Animals were given a lucerne (*Medicago sativa*) hay diet, as sole feed (A) or supplemented, respectively, with 220 (B), 400 (C), and 550 (D) g rolled barley grain/d following a 4 × 4 random factorial design. Duodenal flow of purine bases (PB) was determined by the dual-phase marker system. ^{15}N was infused continuously into the rumen to label exogenous or microbial PB. Duodenal PB flow and urinary excretion of PD increased with digestible organic matter intake showing a constant recovery of duodenal PB. The isotope dilution of PD in urine samples confirmed the presence of an endogenous fraction, originating from tissues, that increased from 115·2 (SE 5·84) μmol/kg $W^{0.75}$ for the basal diet to 304·2 (SE 7·6) μmol/kg $W^{0.75}$ at the highest level of duodenal PB.

Purines: Feed intake: Sheep

The fact that urinary excretion of purine derivatives (PD; allantoin, uric acid, xanthine and hypoxanthine) in ruminants may be used as a predictive index of rumen microbial synthesis has stimulated considerable scientific interest in the underlying mechanisms involved. The principle is that microbial purine bases (PB) absorbed into the duodenum are extensively degraded and eliminated in urine. However, this is masked by the presence of an endogenous fraction, originating from the nucleic acid turnover of the animal. Current methods, proposed to quantify the urinary endogenous excretion of PD, are based on the elimination of the duodenal flow of PB and in all existing reports it is assumed that endogenous losses are equivalent to the basal excretion when there is no exogenous PB input to the small intestine (Giesecke *et al.* 1984; Chen *et al.* 1990; Balcells *et al.* 1991). However, experimental evidence for this assumption does not exist and it is probable that changes in size and/or turnover rate of body tissue nucleic acids affect such excretion.

In the present study, the origin of urinary purine compounds was estimated in sheep receiving different supplies of labelled purine bases of microbial origin through increasing the level of concentrate and digestible organic matter (DOM) intake. Part of this work has been recently published (Pérez *et al.* 1996) with regard to the efficiency of urinary excretion of PD as an index of microbial rumen production, against measurements based on duodenal flow estimates.

Materials and methods

Experimental

Four sheep (Rasa Aragonesa breed, 37·4 (SE 3·16) kg, each fitted with a rumen cannula and a T-shape duodenal cannula were used. Animals were fed continuously on lucerne (*Medicago sativa*) hay (550 g/d) given as a sole diet (diet A) or supplemented, respectively, with 220 (diet B), 400 (diet C) or 550 (diet D) g rolled barley grain/d following a 4 × 4 complete crossover design. Ytterbium acetate and Co-EDTA were used as flow markers and [^{15}N]ammonium sulfate (25 mg ^{15}N/d (10 atoms % ^{15}N, Isotech Inc., OH, USA)) as a microbial marker infused continuously into the rumen.

Urine was collected by means of a urethral catheter (Foley 18 CH 6·0 mm, balloon 10 ml) in buckets containing 100 ml 1 M-H_2SO_4 to keep pH < 2. Collection commenced just after the initiation of isotope infusion (0 h) and was

Abbreviations: ape, atom percent excess; DOM, digestible organic matter; PB, purine bases; PD, purine derivatives.

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sampled at 24, 36, 48, 60 and 72 h. After finishing urine collection duodenal digesta samples (150–200 ml) were taken at 6 h intervals over 48 h. A complete description of the experimental protocol was presented in Pérez *et al.* (1996).

Extraction of purine bases and purine derivatives

PB in duodenal digesta and non-allantoin PD (uric acid plus xanthine and hypoxanthine) in urine were extracted by specific precipitation with Ag ions as a Ag complex (Aharoni & Tagari, 1991). The purity of the precipitate was tested by HPLC (Balcells *et al.* 1992) by screening the effluent from 205 to 320 nm.

Urinary allantoin was extracted by ion-exchange chromatography after its conversion to allantoic acid by alkali hydrolysis. The extraction procedure was as follows: 2 ml urine was mixed with 0·5 ml 0·5 M-NaOH and heated in a boiling water bath for 20 min. After cooling in tap water the pH of the solution was adjusted to 9 and injected onto an ion-exchange column (120 × 20 mm; Amberlite IRA-400, Sigma Co., MA, USA) that had previously been conditioned (2 h) with boric acid buffer (0·05 M-boric acid adjusted to pH 9 with NaOH). The column was washed with the same buffer for 2 h and then treated with K_2HPO_4/KH_2PO_4 (0·05 M, pH 6) for 45 min after which the allantoic acid was eluted using 1 M-NaCl. Chromatography was carried out at room temperature and at a flow rate of 0·6 ml/min. Allantoic acid eluted after 25 min of starting saline elution and its presence was colorimetrically detected in 0·5 ml subsamples (Young & Conway, 1942). Allantoic acid fractions were mixed (6 or 8 ml), freeze dried and stored until ^{15}N analysis. Purity of the eluted sampled was tested by re-diluting samples of the freeze-dried material, injecting the resultant solution on the HPLC system (Balcells *et al.* 1992) and screening for the presence of interfering compounds at wavelengths of 205–320 nm.

Calculations and theoretical aspects

The time necessary to obtain equilibrium of labelled purine compounds through the digestive and metabolic pathways was estimated from the time course of the urinary allantoin-^{15}N enrichment after the beginning of the isotope infusion of two sheep in the first period (diet A and diet C). Steady state was assumed to be achieved when ^{15}N abundance in urinary allantoin reached the maximum (plateau) values of a monoexponential graphical plot (^{15}N abundance *v.* time). The standard monoexponential function was: $Y = a + b\ (1 - e^{-kt})$ and the adjustment of the function to the experimental data:

animal 14: $Y = 0{\cdot}3702 + 0{\cdot}077\ (1 - e^{-0{\cdot}040\,t})$;

R^2 0·96; $(a + b) = 0{\cdot}4479$; f (66 h) = 0·4425;

animal 53: $Y = 0{\cdot}3712 + 0{\cdot}079\ (1 - e^{-0{\cdot}052\,t})$;

R^2 0·98; $(a + b) = 0{\cdot}4506$; f (66 h) = 0·4468;

where Y is the ^{15}N abundance (%), a is the basal ^{15}N abundance at time 0, $(a+b)$ is the asymptotic or plateau value and t is time after commencement of the intraruminal isotope infusion. The proportion of urinary purine derivatives coming from tissue nucleic acids was calculated at steady state conditions (f (66 h)) as follows:

endogenous excretion of PD (%) = 1 − (urinary PD-^{15}N atom % excess (ape) (allantoin or allantoin precursors)/duodenal PB-^{15}N ape) × 100.

^{15}N ape values for urinary PD and duodenal PB were obtained from the background (0·3663 atom %) corrected enrichments. Statistical treatment of the results was described by Pérez *et al.* (1996).

Results and Discussion

Labelling microbial-N by continuous infusion of ^{15}N may constitute a source of naturally-labelled PB suitable to be used for describing purine metabolism in conventionally-fed animals. However, the utilization of this methodology involves a low ^{15}N enrichment of the purine pools. HPLC methodology (Balcells *et al.* 1992) allows an acceptable determination of allantoin and oxypurine concentrations in urine samples, but attempts to isolate the individual compounds did not give consistent isotope ^{15}N abundances, even when preparative columns were used. We failed to purify allantoin as such using several methodologies, but it was obtained after transformation to allantoic acid by alkali hydrolysis followed by separation on an anion exchange column as described previously.

HPLC determination of the resolubilized PD after precipitation with Ag ions confirmed the complete precipitation of hypoxanthine and xanthine and the partial precipitation of uric acid (50–70 %). Considering that hypoxanthine and xanthine constituted the main proportion (> 60 %) of urinary non-allantoin PD, it was assumed that ^{15}N enrichments of precipitated PD were representative of such compounds.

In agreement with previous non-isotopic studies (Chen *et al.* 1990; Balcells *et al.* 1991) allantoin contributed to the majority of the urinary excretion of absorbed labelled PB. However, ^{15}N abundance of urinary non-allantoin PD confirms the participation of such compounds in the urinary excretion of exogenous PB and their capability to pass through the liver to some extent without suffering oxidation to allantoin. Similar results have been previously described in rats (Sonoda & Tatibana, 1978), and also in adult sheep, and preruminant lambs, after receiving an oral dose of labelled PB (Razzaque *et al.* 1981). Direct evidence also has been provided in previous reports from Giesecke *et al.* (1984) and Chen *et al.* (1990) who showed an increase in urinary excretion of those catabolites (hypoxanthine and uric acid respectively) with increased duodenal PB input. Although differences did not reach statistical significance it is worth noting that ^{15}N enrichment (ape) was lower in allantoin than in its precursors. These differences are probably attributable to the differential inflows from endogenous and exogenous sources.

As mentioned earlier, isotopic abundance in allantoin samples increased rapidly during the first 36 h, but more slowly afterwards, and values apparently reached a steady-state by 66 h. The predicted asymptote from the equation

Table 1. Mean values for duodenal flow and urinary output of purine compounds (mmol/d) and their ^{15}N enrichment (atom % excess), together with the urinary contribution of endogenous purine compounds obtained in sheep fed on lucerne hay supplemented with different levels of barley grain*

Ground barley (g/d)...	0	220	400	550		Significance of effects	
Lucerne hay (g/d)...	550	550	550	550	SE	Lin	Quad
Duodenal flow of purine bases (mmol/d)	7·47	12·13	18·20	13·50	0·675	***	***
Urinary excretion of purine derivatives (mmol/d)	5·88	8·68	13·47	10·59	0·261	***	***
^{15}N enrichment† (atom % excess)							
Chymus-purine bases	1·087	1·037	1·047	0·697	0·0057	**	*
Urine							
Allantoin	0·737	0·507	0·647	0·447	0·0043	NS	NS
Precursors‡	0·807	0·637	0·617	0·537	0·0055	*	NS
Endogenous contribution to urinary excretion (μmol/$W^{0·75}$)							
Allantoin	94·2	242·5	270·1	195·2	16·65	***	***
Precursors	21·0	40·7	33·9	25·1	6·07	NS	NS

Lin, linear; Quad, quadratic.
* A complete description of this experiment is given by Pérez *et al.* (1996).
† ^{15}N enrichment over a natural abundance of 0·3636 atoms %.
‡ Allantoin precursors: hypoxanthine + xanthine + uric acid.

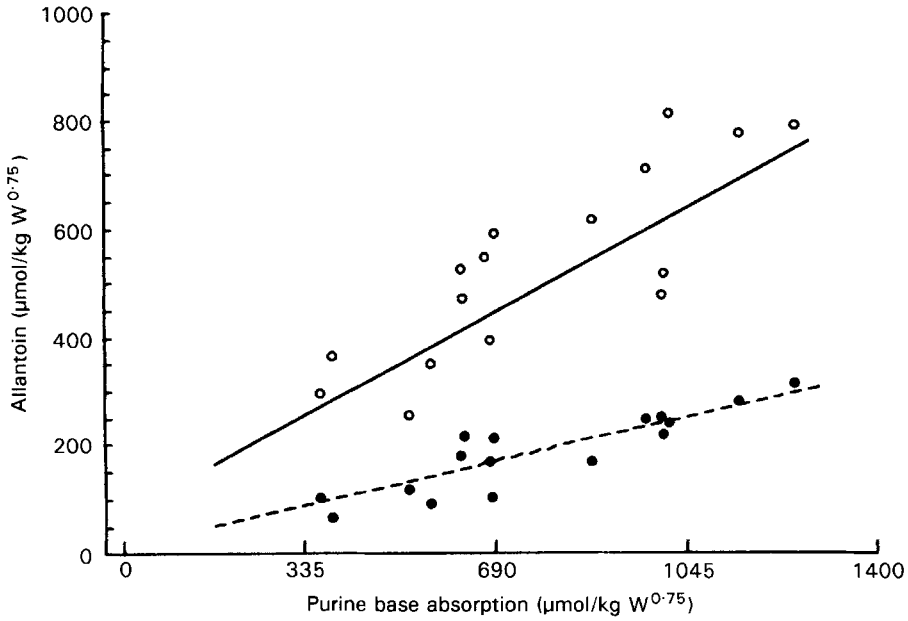

Fig. 1. Urinary excretion (μmol/kg $W^{0·75}$) of total allantoin (○) and allantoin of endogenous origin (●) in relation to duodenal purine bases supply in four sheep given lucerne hay with different levels of barley supplementation. (——) Plotted from the equation $y = 102·9 + 0·56x$; n 16; R 0·83; RSD 1·86, which was derived from the observed individual values. (- - -) Plotted from $y = 24·87 + 0·23x$; n 16; R 0·8; RSD 0·58 and represents the estimated endogenous contribution to the urinary allantoin.

did not differ significantly from the observed abundance at 66 h. The urinary ^{15}N enrichment time course after the beginning of isotope infusion was consistent with data which suggest that 48 h is needed to obtain a homogeneous distribution of the isotope within the rumen ecosystem (Broderick & Merchen, 1992) and that 24 h elapses before urinary excretion responses are obtained following duodenal PD absorption (Balcells *et al.* 1991). In this way, we used the allantoin-^{15}N ape obtained at 66 h to estimate the origin of the urinary PD, minimizing a likely pitfall due to the return of labelled-PB previously incorporated into tissue nucleic acids.

The isotopic enrichment of duodenal PB decreased ($P < 0·01$) with the experimental treatment and it was reflected in a decrease in the isotopic enrichment of allantoin and its precursors, with the later differences attaining statistical significance ($P < 0·05$, Table 1). Considering that body purine rings are only partially degraded, ^{15}N enrichment (ape) of urinary purine compounds can provide some information about the origin of PD excreted

in urine. Dilution of the isotope in urinary PD *v.* duodenal PB confirmed the presence of an endogenous fraction in urinary PD. The magnitude of the PD excretion coming from endogenous sources (94·2 μmol/kg $W^{0.75}$) when animals were fed on the basal diet was in the range described in non-isotopic studies (72–150 μmol/kg $W^{0.75}$; Giesecke *et al.* 1984; Chen *et al.* 1990; Balcells *et al.* 1991). However, the endogenous contribution to the urinary excretion seems to increase with duodenal PB (Fig. 1). Such an increase with PB and/or energy supply may be related to an increase in the body turnover rate of nucleic acid and with associated changes in *de novo* synthesis. These processes will also increase isotopic exchange within the body and add an extra complication to the metabolic interpretation.

It is known that food supply affects cellular metabolism, protein synthesis and breakdown, and probably nucleic acid turnover given the close relationship between protein and nucleic acid metabolism (Guernesey & Edelman, 1983). On the other hand, several workers (see Chen *et al.* 1990) have argued that the contribution of the *de novo* synthesis pathway decreases or is completely inhibited when exogenous PB input increases.

From these results it is suggested that urinary excretion of endogenous PD, obtained by isotopic measurements, probably reflects changes in the whole-body nucleic acid turnover as a result of changes in energy or PB supply.

Acknowledgements

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