Journal of Agricultural Science (2003), **140**, 101–105. © 2003 *Cambridge University Press*
DOI: 10.1017/S0021859602002873 *Printed in the United Kingdom*

The effect of rumen protozoa on the urinary excretion of purine derivatives in goats

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(*Revised MS received 14 November 2002*)

SUMMARY

Urinary purine derivative (PD) excretion was estimated to examine the effect of rumen protozoa on total PD excretion in goats fed hay and a concentrate diet. The effect of increasing protozoa number in the rumen on nitrogen (N) balance and urinary PD excretion was determined after inoculation. Protozoa increased slowly until 4 days after inoculation, and on the 5th day after inoculation rapidly, finally (10 days) reaching $4{\cdot}1 \times 10^5$/ml of rumen contents similar to that before defaunation. Urinary N excretion showed a small (non-significant) decrease. Urinary PD excretion did not change until the 7th day, and then the level decreased on the 8th day after faunation presumably due to the effect of increased protozoa in the rumen. The mean urinary total PD excretion significantly ($P<0{\cdot}05$) decreased in the defaunated group compared with that in the faunated group. Comparable changes were not seen in plasma PD level of faunated and defaunated groups.

INTRODUCTION

Rumen bacteria, protozoa and fungi are important protein sources for their ruminant hosts. The amount of these microbes produced in the rumen is closely related to the degradation and fermentation of ingested feed in ruminants (Takenaka *et al*. 1991; Nakagawa *et al*. 1992). The estimation of microbial proliferation in the rumen will help to understand the nutritional status of ruminants.

In ruminant animals, most nucleic acid reaching the lower digestive tract is microbial, because that in the feed is completely degraded in the rumen (McAllan 1982). Topps & Elliott (1965) reported a close relationship between nucleic acids in the rumen and purine derivatives (PD) excreted in urine. Nucleic acids synthesized in the rumen are mostly absorbed from the digestive tract and metabolized, suggesting that urinary PD could be useful as an index to estimate the microbial proliferation in the rumen (Smith & McAllan 1970). Using an intragastric infusion technique (Ørskov *et al*. 1979), it has been reported that there is a positive correlation between the bacterial proteins infused in the abomasum and total PD excretion via urine in cattle and sheep (Fujihara *et al*. 1987; Chen *et al*. 1990). Thus urinary PD may be useful for estimating the amount of microbial protein (MP) synthesized in the rumen.

The bacteria (10^9–10^{11}/ml) and protozoa (10^5–10^6/ml) constitute the greater part of rumen microbes. However, their rate of passage to the lower digestive tract is not the same; the flow rate of protozoa seems to be slower than that of bacteria (Weller & Pilgrim 1974). There is little information on the effect of protozoa on supply of MP and urinary PD excretion in ruminants (Matsumoto *et al*. 1991).

In the present study, urinary PD excretion and the plasma PD concentration were measured in defaunated goats and after inoculation with protozoa. Part of this work has been briefly described by Fujihara *et al*. (1994).

MATERIAL AND METHODS

Animals and diet

Three castrated male Japanese Saanen goats (10–12-month-old, and average body weight: BW, $30{\cdot}2 \pm 4{\cdot}5$ kg) were used. They were bottle-fed pre-weaning, and therefore accepted bottle-feeding in the experiment. They were kept in metabolism crates throughout the experiment. They received a diet consisting of lucerne hay cubes, barley straw, rolled barley and wheat bran (Table 1) $1{\cdot}2\times$ maintenance for TDN (429 g TDN/30 kg BW) and protein (42 g DCP/30 kg BW) requirement respectively (NRC 1981). Half of

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Table 1. *Chemical composition of milk replacer, and crude protein content of ingredients in the experimental diet*

	g/kg DM
Milk replacer	
Crude protein	240
Crude fat	180
Crude fibre	10
Crude ash	100
NFE*	457
Calcium	8
Phosphorus	5
Experimental diet (g/kg)	
Lucerne hay cube	168·2
Barley straw	19·3
Rolled barley	137·8
Wheat bran	177·8

* Nitrogen-free extract.

the daily ration was given at 09.00 and the other at 17.00 h. Fresh water and salt licks containing trace elements were freely available.

Experimental procedure

Defaunation of the animals was accomplished by bottle-feeding milk replacer (MR) for 2 weeks, followed by feeding on a mixed diet (9:1 to 0:10 DM of MR and solid diet) for 10 days (see Fig. 1). The animals were then fed on a solid diet alone for 1 week. After confirming they were protozoa-free in the rumen contents taken through a stomach tube, urine and jugular blood samples were collected just before the morning feed for 5 consecutive days.

To faunate the animals after inoculation, 100 protozoa (*Holotrichida* and *Oligotrichina*, 1:19) collected from a sheep fed on hay and concentrate diet were inoculated into each animal immediately after sampling of urine and jugular blood in the experiment with faunated animals. The samples of urine and jugular blood were collected just before the morning feed for 10 days after inoculation with protozoa.

Sampling and analytical procedure

Urine was collected with 100–150 ml of 10% H_2SO_4 solution to adjust pH value below 3·0 to prevent disappearance of PD in urine (Fujihara *et al.* 1991), and stored at −20 °C until analysis. Blood was collected by sampling of 10 ml from the jugular vein, and plasma was stored in a deep freezer (−40 °C) until analysis.

Nitrogen (N) in feed and urine was analysed by the Kjeldahl method, and PD in urine and plasma was analysed by the methods of Young & Conway (1942) and Fujihara *et al.* (1987). The protozoa in ruminal

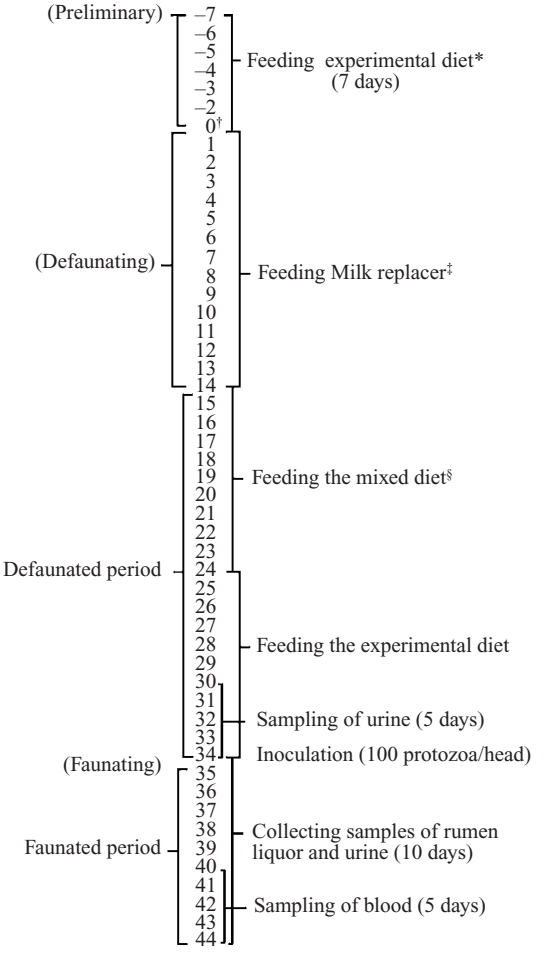

Fig. 1. Experimental schedule. * Experimental diet (see Table 1) with milk replacer (1st/1·5:8·5 – 2nd/3·0:7·0 – 3rd/4·5:5·5 – 4th/6·0:4·0 – 5th/7·5:2·5 – 6th/9·0:1·0 – 7th/10:0). † Days. ‡ Milk replacer (see Table 1). § Milk replacer and experimental diet (1st/9:1 – 2nd/8:2 – 3rd/7:3 – 4th/6:4 – 5th/5:5 – 6th/4:6 – 7th/3:7 – 8th/2:8 – 9th/1:1 – 10th/0:10).

contents were counted using a Thoma–Zeiss blood cell counter.

Tests for significance of differences between the two groups (faunated and defaunated groups) were undertaken by *t*-test.

RESULTS AND DISCUSSION

Increase of protozoa

The inoculation consisted of mixed species of protozoa (Holotrichs and Oligotrichs). Figure 2*a* shows the increase in numbers that occurred after inoculation. Although Itabashi *et al.* (1984) have reported that Holotrich and Oligotrich protozoa ferment different substrates, there was no attempt to identify the

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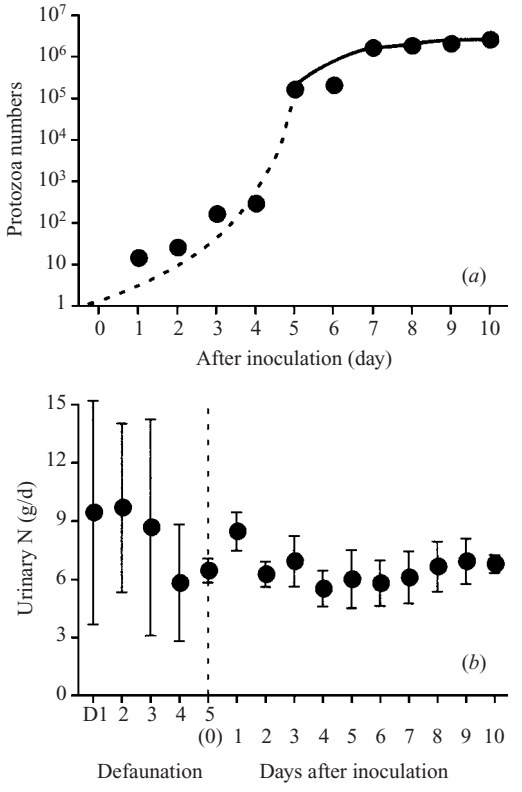

Fig. 2. Increase of protozoa (*a*) and urinary nitrogen excretion (*b*) after inoculation. (Average values during defaunated period.)

protozoa present in the inoculates used in the current study. The values for protozoa in the first 4 days were too low to be determined in the counter. However, visual inspection of drops of rumen fluid suggested they were initially 0–10, rising to around 200–400 by day 5. There was clearly a massive increase around day 5 (to about 10^5/ml) with only a small further increase over the next 5 days (10^5–10^6/ml).

Urinary N excretion

Figure 2*b* shows daily excretion of urinary N with the lapse of time during the 10 days after inoculation. The mean excretion in the 5-day defaunated period was 8·67 ± 4·24 g N/day and 6–10 days after inoculation 6·45 ± 1·05 g N/day – lower, but not significantly so. Protozoa are up to 10^5-fold lower in number than bacteria, but about 10^3-fold larger. Thus in the rumen the total biomass of the two may be fairly similar. However, there are indications that protozoa are retained in the rumen to a greater extent than bacteria (Czerkawski 1987). If the decrease in urinary N is real, it may reflect a decrease in availability of MP because protozoan predation has increased the protozoan mass at the expense of bacterial mass (Koenig *et al*. 2000).

Table 2. *Urinary PD excretion of goats in defaunated and faunated periods*

	Allantoin	Uric acid*	Total PD
	(μmol/d/kgBW$^{0·75}$)		
Defaunated	878·9^{a} ± 53·7†	202·3 ± 15·7	1081·2^{a} ± 69·2
Faunated	585·3^{b} ± 34·4‡	188·2 ± 8·0	773·5^{b} ± 41·1

* Uric acid, xanthine plus hypoxanthine.
† Mean ± S.E. of three goats.
‡ Mean ± S.E. of three goats during 3 days (8th–10th day) after inoculation.
Values in the same column with different superscripts differ significantly ($P < 0·01$).

Effect of protozoa on urinary PD excretion

Table 2 shows urinary PD excretion of goats during the defaunated period. The mean value was 1081·2 μmol/d/kgBW$^{0·75}$. Matsumoto *et al*. (1991) reported daily allantoin excretion into urine of 2292 ± 242 mg/day or 994·87 μmol/day in defaunated goats. This figure is higher than that obtained in the present study (allantoin: 878·9 ± 53·7 μmol/day). Although not significant, the difference could be due to the feeding regimes, because the concentrate used in the former study contained 10 g urea/kg as a N source.

Figure 3*a* shows urinary PD excretion during the 10 days after inoculation and the average value during the defaunated period, which was used as reference. Urinary excretion of uric acid (which included xanthine and hypoxanthine) varied little regardless of the presence of protozoa in the rumen. In ruminants, there is little variation in xanthine oxidase activity, while uricase activity depends on the amount of uric acid available. Urinary PD excretion is, therefore, mainly altered by a change in allantoin excretion. Urinary PD excretion tended to decrease on the 8th day after inoculation. As shown in Fig. 2*a*, protozoa markedly increased in the rumen 4–5 days after inoculation, but there was no clear decrease in urinary PD excretion.

Figure 3*b* shows the ratio of total PD, allantoin and uric acid (also containing hypoxanthine and xanthine) during the inoculation and defaunated periods. It is clear that the changes in urinary PD excretion after inoculation were mainly due to changes in allantoin excretion, although uric acid with xanthine and hypoxanthine was higher (more than 100 %) than that in the defaunated period. At 8–10 days after inoculation, urinary PD excretion was about 82 % of that in the defaunated period, and this was consistent with the notion that protozoa contributed to the decrease in urinary PD excretion.

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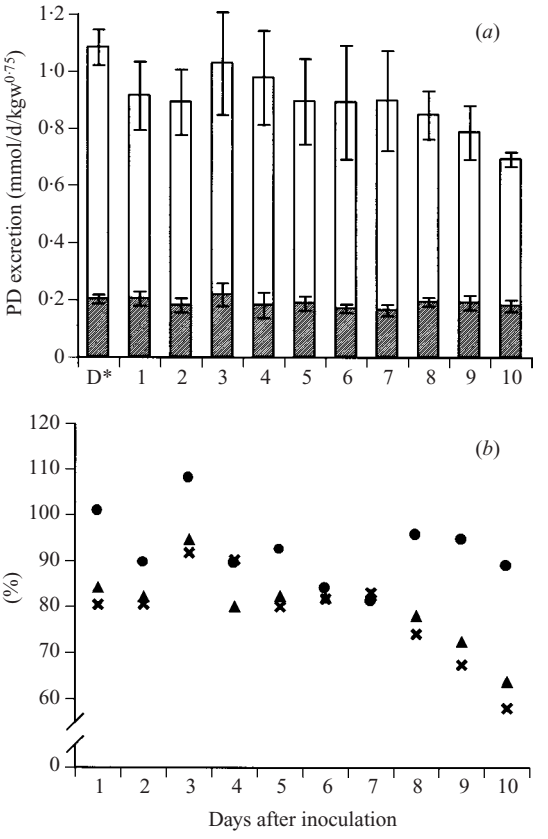

Fig. 3. Urinary PD excretion (*a*) and the ratio of PD to the value in defaunated period (*b*) after inoculation. (*a*) Allantoin (□) and uric acid (including xanthine and hypoxanthine) (▨); (*b*) Total PD (●), allantoin (×) and uric acid (including xanthine and hypoxanthine) (▲). (Average values during defaunated period.)

Table 2 shows the mean urinary PD excretions in both the defaunated and faunated periods. The PD value in the defaunated period was significantly ($P<0·01$) greater than that in the faunated period. This is consistent with the notion that bacteria decreased with an increase of protozoa in the rumen after inoculation.

Effect of protozoa on plasma PD level

As shown in Table 3, the mean concentration of PD in blood plasma was 40·87±4·89 μmol/l. This value was lower than that of about 50 μmol/l reported by Chen *et al.* (1997) using sheep nourished by intragastric infusion. In the current study, dietary N supply was 870 mg N/kgBW$^{0·75}$, which is much higher than the 280 mg N/kgBW$^{0·75}$ reported by Chen *et al.* (1997). The discrepancy in plasma PD level in the two experiments might be due to differences in N intake and/or in feeding regime (purine-free or ordinary feed).

Table 3. *Plasma PD levels of goats in defaunated and faunated periods*

	Allantoin	Uric acid*	Total PD
	(μmol/l)		
Defaunated	36·39±4·77†	4·51±0·77	40·87±4·89
Faunated	36·39±6·37‡	5·83±0·34	42·22±6·64

* Uric acid, xanthine plus hypoxanthine.
† Mean±S.E. of three goats.
‡ Mean±S.E. of three goats during 3 days (8th–10th day) after inoculation.

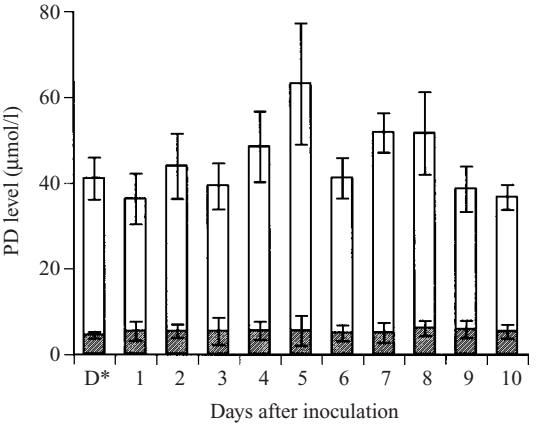

Fig. 4. Plasma PD concentration after inoculation. Allantoin (□), uric acid (including xanthine and hypoxanthine) (▨). (Average values during defaunated period.)

As indicated in Fig. 4, there was an increase in plasma PD level about 4–5 days after inoculation, but this was not sustained. According to Chen *et al.* (1997), there is a close relationship between the amount of purine nucleoside infused into the abomasum and plasma PD level in sheep nourished by intragastric infusion. In the present experiment, plasma PD level did not accurately reflect changes in absorbed purine during the 10 days after inoculation. This might have been due to the time of blood collection, as sampling was done just before the morning feed but at 3–4 h after the morning feed in the study of Chen *et al.* (1997).

From the results obtained in the present study, it is probable that protozoa affect urinary PD excretion in ruminants, but the effect needs further clarification.

The authors are very grateful to Ms N. Oka for her helpful assistance during the course of the experiment, and to Dr T. Tokuda for her technical assistance on the manuscript.

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