# Nitrogen and purine metabolism at varying energy and protein supplies in sheep sustained on intragastric infusion

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**Wether sheep were fitted with rumen fistulas and polyethylene tubes to the abomasum and were given all nutrients by intragastric infusion. In Expt 1 volatile fatty acids (VFA) were given at 340, 450 and 630 kJ gross energy (GE)/kg metabolic weight ($W^{0.75}$) and protein at 0, 150, 300, 600, 900 and 1500 mg nitrogen/kg $W^{0.75}$. In Expt 2 VFA were infused at 450 kJ GE/kg $W^{0.75}$ and protein at 0 and 300 mg N/kg $W^{0.75}$. At all levels of energy intake in Expt 1 the N retention was significantly ($P < 0.01$) related to N intake. The basal N requirement was estimated to be 281 mg (SE 21·8) N/kg $W^{0.75}$ at 340 kJ VFA/kg $W^{0.75}$, 226 (SE 21·8) mg N/kg $W^{0.75}$ at 450 kJ VFA/kg $W^{0.75}$ and 207 (SE 19·4) mg N/kg $W^{0.75}$ at 630 kJ VFA/kg $W^{0.75}$. Plasma urea concentrations varied markedly in relation to protein intake and to energy supply. On the other hand plasma ammonia, glucose, insulin and creatinine concentrations, and also urinary excretion of purine derivatives and creatinine were not significantly affected by the treatments imposed. It was concluded that the urinary excretion of purine derivatives in ruminants was largely unaffected by moderate changes in energy intake and by large changes in protein intake.**

**Nitrogen metabolism: Purine derivatives: Protein and energy intake: Sheep**

In most mammals purines are metabolized in a series of reactions to form hypoxanthine, xanthine, uric acid and allantoin (Watts, 1980). The endogenous excretion of purine derivatives (PD) in the urine of young goat kids was distributed between allantoin (0·54–0·76), uric acid (0·13–0·34) and hypoxanthine (0·07–0·13), while xanthine was less than 0·018 (Lindberg, 1989).

The microbial nucleic acids appear to be quantitatively most important for urinary PD excretion (Rys *et al.* 1975). In addition there is also a varying contribution to the nucleic acid pool from the feed (McAllan, 1982) and from endogenous metabolism (Lindberg, 1989). The use of urinary excretion of PD to quantify the intestinal microbial flow in ruminants calls for a better knowledge of the importance of both these nucleic acid sources.

In recent studies on the endogenous excretion of PD in steers, changes in protein supply had little effect on the urinary excretion of PD (Fujihara *et al.* 1987). Also in young growing goat kids the excretion of PD in the urine was only marginally affected by large changes in either protein or energy supply (Lindberg, 1989).

The aim of the present work was to obtain further information on the effect of changing nutrient supply, in a ruminant, on the endogenous urinary excretion of PD. In order to have total control of both energy and protein supply, the sheep used in the present study were maintained entirely on intragastric infusion of nutrients.

## MATERIAL AND METHODS

### *Animals*

In Expt 1 a total of four wether sheep of the Swedish Landrace breed were used. They were about 1 year old at the start of the experiment and weighed 38 (SD 3·5) kg. In Expt 2 four

wether sheep of the Swedish Landrace breed were used. They were 5–6 years old and weighed 80 (SD 4·2) kg. The sheep were fitted with rumen fistulas and polyethylene tubes to the abomasum (Ørskov *et al.* 1979*b*).

### *Nutrition*

The sheep were gradually adapted to intragastric infusion of all nutrients needed according to Ørskov *et al.* (1979*b*). In Expt 1 volatile fatty acids (VFA) were given at the following three levels of intake: 340, 450 and 630 kJ gross energy (GE)/kg metabolic live weight ($W^{0.75}$), with relative molar percentages of acetate, propionate and butyrate kept at 65:25:10. Casein was used as the protein source and was infused at 0, 150, 300, 600, 900 and 1500 mg nitrogen/kg $W^{0.75}$. In Expt 2, VFA were infused at a level of 450 kJ GE/kg $W^{0.75}$ with molar proportions as in Expt 1. Casein was infused at 0 and 300 mg N/kg $W^{0.75}$ for one collection period each.

### *Experimental design*

In Expt 1 animals were allocated to the three energy levels according to a change-over design. Within each energy level protein was given in the order 300, 600, 900, 1500, 150 and 0 mg N/kg $W^{0.75}$. The adaptation period to each change in protein supply was 3 d followed by collection of faeces and urine for 3 d. Blood samples were taken from the jugular vein on the last day in each collection period. In Expt 2 adaptation and collection periods were as in Expt 1; only urine was collected.

### *Collection of faeces and urine*

The sheep were kept in metabolism crates for the collection of faeces and urine. Faeces were frozen and urine was collected in 10% sulphuric acid to keep the pH below 3. Urine samples were stored at 4° until analysed.

### *Chemical analysis*

Total N in faeces and urine was analysed after hydrolysis using an AutoAnalyzer according to the method of Technicon Instruments Inc. (1977*a*). Ammonia was determined according to the method of Technicon Instruments Inc. (1977*a*) and urea with diazetylmonoxin according to the method of Technicon Instruments Inc. (1972). Plasma glucose was assayed using a glucose oxidase (*EC* 1.1.3.4) kit (Merck; God-Pap method 3395). Plasma insulin was assayed by insulin radio-immunoassay (Pharmacia). Allantoin was determined by the Rimini–Schryver reaction (Young & Conway, 1942) adapted to the AutoAnalyzer (Lindberg & Jansson, 1989). Uric acid, hypoxanthine and xanthine were analysed by reversed-phase high-performance liquid chromatography (HPLC) using a Spherisorb ODS (5 $\mu$m) $C_{18}$ column (Lindberg *et al.* 1989); thymin was used as an internal standard. Creatinine was analysed using picric acid (Technicon Instruments Inc., 1977*b*).

### *Statistical analysis*

In Expt 1 analysis of variance using a change-over model was used to examine the effect of energy levels. A split-plot analysis of variance was used to examine the effect of protein levels with energy as main plot and protein as subplot within animal. Analysis of covariance was used to evaluate the effect of protein intake on N retention and of plasma urea

Table 1. *Expt 1. Urinary excretion (mg nitrogen/kg metabolic live weight ($W^{0.75}$)) of total N and urea N, and retention of N (mg N/kg $W^{0.75}$) in sheep maintained on intragastric infusion of energy (kJ/kg $W^{0.75}$) and protein (mg N/kg $W^{0.75}$) at different levels**

(Means with their standard errors†)

| Energy infused from | | Level of protein infusion | | Urinary excretion | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Total N | | Urea N | | Retention of N | |
| VFA | Protein | | *n* | Mean | SE | Mean | SE | Mean | SE |
| 340 | 0 | 0 | 2 | 255 | 39 | 149 | 32 | −274 | 39 |
| | 24 | 150 | 3 | 261 | | 154 | | −156 | |
| | 47 | 300 | 3 | 348 | | 209 | | −58 | |
| | 94 | 600 | 3 | 446 | | 298 | | 123 | |
| | 141 | 900 | 3 | 571 | | 421 | | 306 | |
| | 235 | 1500 | 3 | 1074 | | 878 | | 397 | |
| 450 | 0 | 0 | 2 | 214 | | 109 | | −226 | |
| | 24 | 150 | 3 | 291 | | 141 | | −115 | |
| | 47 | 300 | 3 | 282 | | 180 | | −5 | |
| | 94 | 600 | 3 | 373 | | 243 | | 211 | |
| | 141 | 900 | 3 | 510 | | 364 | | 362 | |
| | 235 | 1500 | 3 | 801 | | 624 | | 680 | |
| 630 | 0 | 0 | 3 | 208 | | 78 | | −219 | |
| | 24 | 150 | 3 | 233 | | 64 | | −106 | |
| | 47 | 300 | 3 | 276 | | 114 | | 7 | |
| | 94 | 600 | 3 | 387 | | 152 | | 221 | |
| | 141 | 900 | 3 | 510 | | 257 | | 354 | |
| | 235 | 1500 | 3 | 675 | | 445 | | 807 | |

VFA, volatile fatty acids.

* For details of infusions, see p. 360.

† Standard errors between energy levels at the same protein level with 3 df.

concentration on urinary urea excretion. In Expt 2 the effect of treatment was *t* tested (Dunn & Clark, 1974).

All calculations were made using the GLM-procedure (SAS, 1982).

## RESULTS

### *Expt* 1

*General.* For the most part there were no problems in slowly changing from the forage diet to a totally intragastric feeding regimen. With a few exceptions collections were carried out for three sheep per treatment. One animal had to be taken out of Expt 1 after the first period due to problems in maintaining rumen pH and osmolality within an acceptable range (pH 6·0–7·0; 250–350 mosmol/kg). Data from this animal were excluded from all calculations.

*Faecal N excretion.* Faecal N excretion was very variable during the experiment with no obvious relation to the treatments imposed. On average the animals excreted 28 (SE 3·64) mg faecal N/kg $W^{0.75}$. Regression for digested N *v.* N intake gave an estimated endogenous faecal loss of 30 (SE 7·0) mg N/kg $W^{0.75}$ and the true digestibility of N was estimated to be 100 (SD 0·8)%.

*N retention.* At all levels of energy intake N retention was significantly ($P < 0.01$) related to N intake. The amount of N retained was significantly affected ($P < 0.05$) by the level of energy intake (Table 1, Fig. 1). The relationship between N intake ($x$) and N retention ($y$)

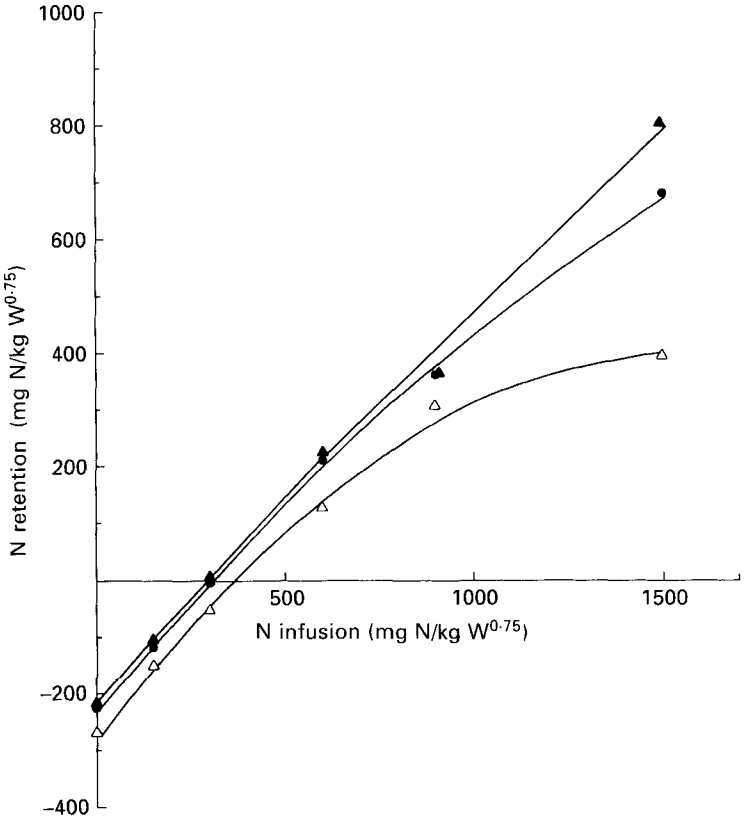


Fig. 1. Expt 1. Nitrogen retention (mg N/kg metabolic live weight ($W^{0.75}$)) in relation to N intake (mg N/kg $W^{0.75}$) in sheep infused with 340 (△), 450 (●) and 630 (▲) kJ energy from volatile fatty acids. For details of procedures, see p. 360.

(mg N/kg $W^{0.75}$) was significantly different ($P < 0.01$) at all levels of energy intake. The regressions were:

VFA infusion, 340 kJ/kg $W^{0.75}$:

$$y = -281\ (\text{SE}\ 21.8) + 0.87\ (\text{SE}\ 0.074)\,x - 0.0003x^2;\ n\ 17;\ R^2\ 0.98.$$

VFA infusion, 450 kJ/kg $W^{0.75}$:

$$y = -226\ (\text{SE}\ 21.8) + 0.77\ (\text{SE}\ 0.074)\,x - 0.0001x^2;\ n\ 17;\ R^2\ 0.98.$$

VFA infusion, 630 kJ/kg $W^{0.75}$:

$$y = -207\ (\text{SE}\ 19.4) + 0.63\ (\text{SE}\ 0.072)\,x;\ n\ 18;\ R^2\ 0.98.$$

The quadratic component was significant ($P < 0.05$) at energy infusion levels of 340 and 450 kJ VFA energy/kg $W^{0.75}$.

Zero N intake gave estimates of the basal N requirement of 281 mg N/kg $W^{0.75}$ at 340 kJ VFA energy/kg $W^{0.75}$, 226 mg N/kg $W^{0.75}$ at 450 kJ VFA energy/kg $W^{0.75}$ and 207 mg N/kg $W^{0.75}$ at 630 kJ VFA energy/kg $W^{0.75}$.

*Urinary excretion of total N and urea N.* As shown in Table 1 there was a reduction in both total N ($P < 0.01$) and urea N ($P < 0.001$) excretions with decreasing N intakes. With decreasing total N excretion there was a concomitant decline in the proportions of urea N in the urine. The proportion of urea in the urine was significantly ($P < 0.05$) higher at 340 kJ/kg $W^{0.75}$ than at 630 kJ/kg $W^{0.75}$, with 450 kJ/kg $W^{0.75}$ being intermediate.

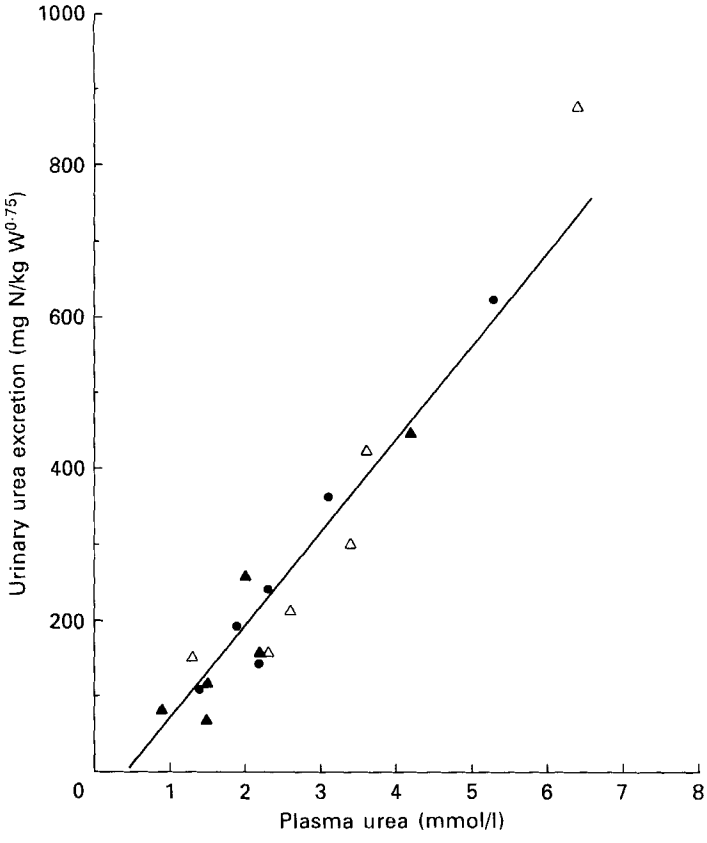


Fig. 2. Expt 1. The relationship between plasma urea concentration (mmol/l) and the loss of urea nitrogen (mg N/kg metabolic live weight ($W^{0.75}$)) in the urine of sheep infused with 340 (△), 450 (●) and 630 (▲) kJ energy from volatile fatty acids. $y = -56.9$ (SE 26.2) + 123.4 (SE 8.3) $x$; $n$ 48; $R^2$ 0.87.

*Plasma concentration of urea, ammonia, glucose, insulin and creatinine.* While plasma ammonia concentrations remained unaffected by the treatments imposed, the plasma urea concentrations were significantly affected by the protein infused ($P < 0.001$) and the energy given ($P < 0.05$). There was a steady increase in plasma urea concentrations with increasing levels of protein infused and at all energy levels. Higher energy intake invariably exposed lower plasma urea concentrations (Table 2). The relationship ($P < 0.01$) between plasma urea concentration ($x$, mmol/l) and the loss of urea N ($y$, mg N/kg $W^{0.75}$) was not significantly affected by energy levels and all the data were pooled in one regression:

$$y = -56.9\,(\text{SE } 26.2) + 123.4\,(\text{SE } 8.3)\,x;\ n\,48;\ R^2\,0.87.$$

The intercept was significantly different from zero. As shown in Fig. 2 there was a very close relationship between plasma urea concentrations and the urinary excretion of urea N in the present experiment.

Intragastric infusion is clearly not a normal practice. Also the range in N supply was outside what would be given under normal conditions. Plasma glucose, insulin and creatinine concentrations were therefore measured to allow comparison with values from animals fed normally. Plasma glucose and insulin concentrations did not show any consistent pattern relating to the treatments (Table 2) and were not significantly affected by

Table 2. *Expt 1. Plasma concentrations of urea, ammonia, glucose (mmol/l), insulin (μU/ml) and creatinine (μmol/l) in sheep maintained on intragastric infusion of energy (kJ/kg metabolic live weight ($W^{0.75}$)) and protein (mg nitrogen/kg $W^{0.75}$) at different levels**

(Means with their standard errors†)

| Energy infused from | | Level of protein infusion | $n$ | Plasma concentration | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Urea | | Ammonia | | Glucose | | Insulin | | Creatinine | |
| VFA | Protein | | | Mean | SE | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 340 | 0 | 0 | 2 | 1·3 | 0·69 | 0·28 | 0·044 | 3·5 | 0·38 | 3·3 | 3·31 | 69 | 5·98 |
| | 24 | 150 | 3 | 2·3 | | 0·29 | | 3·5 | | 2·7 | | 79 | |
| | 47 | 300 | 3 | 2·6 | | 0·36 | | 3·7 | | 4·8 | | 68 | |
| | 94 | 600 | 3 | 3·4 | | 0·29 | | 3·4 | | 8·2 | | 73 | |
| | 141 | 900 | 2 | 3·6 | | 0·28 | | 3·5 | | 4·8 | | 64 | |
| | 235 | 1500 | 3 | 6·4 | | 0·34 | | 3·6 | | 4·4 | | 67 | |
| 450 | 0 | 0 | 2 | 1·4 | | 0·31 | | 3·7 | | 5·1 | | 84 | |
| | 24 | 150 | 3 | 2·2 | | 0·31 | | 3·6 | | 4·3 | | 83 | |
| | 47 | 300 | 3 | 1·9 | | 0·30 | | 3·8 | | 3·8 | | 72 | |
| | 94 | 600 | 3 | 2·3 | | 0·30 | | 3·8 | | 8·0 | | 68 | |
| | 141 | 900 | 2 | 3·1 | | 0·28 | | 3·7 | | 9·0 | | 58 | |
| | 235 | 1500 | 3 | 5·3 | | 0·31 | | 3·9 | | 9·5 | | 67 | |
| 630 | 0 | 0 | 3 | 0·9 | | 0·33 | | 4·9 | | 12·1 | | 64 | |
| | 24 | 150 | 3 | 1·5 | | 0·29 | | 3·9 | | 9·0 | | 71 | |
| | 47 | 300 | 3 | 1·5 | | 0·31 | | 4·2 | | 7·7 | | 75 | |
| | 94 | 600 | 2 | 2·2 | | 0·29 | | 3·5 | | 5·3 | | 66 | |
| | 141 | 900 | 3 | 2·0 | | 0·30 | | 3·6 | | 6·7 | | 55 | |
| | 235 | 1500 | 3 | 4·2 | | 0·29 | | 3·8 | | 10·0 | | 61 | |

VFA, volatile fatty acids.

* For details of infusions, see p. 360.

† Standard errors between energy levels at the same protein level with 3 df.

Table 3. *Expt* 1. *Urinary excretion (mg nitrogen/kg metabolic live weight ($W^{0.75}$)) of allantoin N, uric acid N, hypoxanthine N and creatinine N in sheep maintained on intragastric infusion of energy (kJ/kg $W^{0.75}$) and protein (mg N/kg $W^{0.75}$) at different levels**

(Means with their standard errors†)

| Energy infused from | | Level of protein infusion | | Urinary excretion | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Allantoin N | | Uric acid N | | Hypoxanthine N | | Creatinine N | |
| VFA | Protein | | *n* | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| 340 | 0 | 0 | 2 | 5·1 | 0·82 | 4·2 | 1·02 | 1·5 | 0·41 | 27·0 | 2·51 |
| | 24 | 150 | 3 | 5·0 | | 3·3 | | 1·1 | | 17·1 | |
| | 47 | 300 | 3 | 4·6 | | 4·2 | | 1·3 | | 22·0 | |
| | 94 | 600 | 3 | 4·4 | | 4·4 | | 0·8 | | 21·9 | |
| | 141 | 900 | 3 | 5·4 | | 3·9 | | 1·0 | | 21·4 | |
| | 235 | 1500 | 3 | 5·5 | | 4·5 | | 1·2 | | 20·3 | |
| 450 | 0 | 0 | 2 | 3·9 | | 3·0 | | 0·7 | | 20·5 | |
| | 24 | 150 | 3 | 6·1 | | 5·0 | | 1·1 | | 17·7 | |
| | 47 | 300 | 3 | 4·1 | | 3·2 | | 0·6 | | 19·9 | |
| | 94 | 600 | 3 | 4·2 | | 3·7 | | 0·5 | | 20·1 | |
| | 141 | 900 | 3 | 5·1 | | 4·0 | | 0·7 | | 17·5 | |
| | 235 | 1500 | 3 | 5·8 | | 5·4 | | 1·2 | | 19·1 | |
| 630 | 0 | 0 | 3 | 4·5 | | 3·0 | | 0·7 | | 20·1 | |
| | 24 | 150 | 3 | 5·4 | | 2·9 | | 0·7 | | 18·1 | |
| | 47 | 300 | 3 | 3·8 | | 3·9 | | 0·5 | | 20·4 | |
| | 94 | 600 | 3 | 3·8 | | 3·2 | | 0·6 | | 19·1 | |
| | 141 | 900 | 3 | 5·3 | | 4·4 | | 1·2 | | 20·4 | |
| | 235 | 1500 | 3 | 6·1 | | 5·4 | | 1·4 | | 21·3 | |

VFA, volatile fatty acids.

* For details of infusions, see p. 360.

† Standard error between energy levels at the same protein level with 3 df.

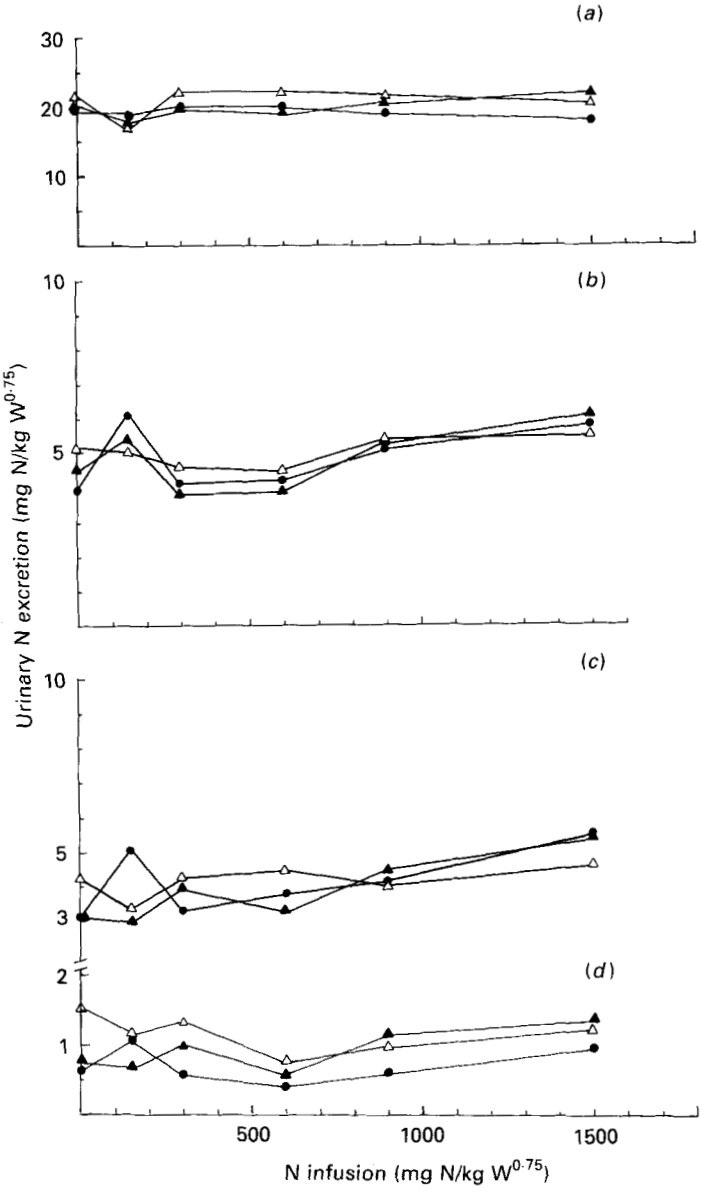


Fig. 3. Expt 1. Urinary excretion of (*a*) creatinine N, (*b*) allantoin N, (*c*) uric acid N and (*d*) hypoxanthine N in sheep maintained on intragastric infusion: (△), 340 kJ volatile fatty acid (VFA) energy/kg metabolic live weight ($W^{0.75}$); (●), 450 kJ VFA energy/kg $W^{0.75}$; (▲), 630 kJ VFA energy/kg $W^{0.75}$.

the treatments imposed. At 630 kJ VFA energy/kg $W^{0.75}$ there were indications of a simultaneous increase in both plasma glucose and plasma insulin at 0, 150 and 300 mg N/kg $W^{0.75}$. Plasma creatinine values were in the range 55–82 $\mu$mol/l, which is within the normal range, with no obvious relation to the treatments used (Table 2).

*Urinary excretion of PD and creatinine.* The urinary excretion of the PD allantoin, uric acid and hypoxanthine was not significantly affected by the treatments imposed (Table 3; Fig. 3). On average the urinary excretions of allantoin N, uric acid N and hypoxanthine N (mg/kg $W^{0.75}$) were respectively: 5·04 (SD 0·77), 4·15 (SD 1·00) and 1·17 (SD 0·6) at

340 kJ VFA energy/kg $W^{0.75}$; 4·97 (SD 1·21), 4·13 (SD 0·97) and 0·84 (SD 0·46) at 450 kJ VFA energy/kg $W^{0.75}$; and 4·82 (SD 0·82), 3·81 (SD 1·32) and 0·85 (SD 0·65) at 630 kJ VFA energy/kg $W^{0.75}$. No xanthine was found in the urine.

At zero N infusion, allantoin N, uric acid N and hypoxanthine N made up, on average across energy intakes, 0·018 (SD 0·0010), 0·014 (SD 0·0015) and 0·004 (SD 0·0017) respectively, of total N in the urine. Of the total purine-derived N recovered in the urine, allantoin N constituted on average 0·49 (SD 0·018), uric acid N 0·41 (SD 0·011) and hypoxanthine N 0·10 (SD 0·019).

Creatinine N excretion was not significantly affected by the treatments imposed (Table 3) and was on average 21·2 (SD 4·00), 19·0 (SD 2·32) and 19·9 (SD 2·46) mg N/kg $W^{0.75}$ at 340, 450 and 630 kJ VFA energy/kg $W^{0.75}$ infusion respectively.

### *Expt 2*

*N, allantoin and creatine excretion.* At zero N infusion the endogenous urinary N loss was 216 (SD 26·8) mg N/kg $W^{0.75}$. The urinary allantoin excretion was on average 3·7 (SD 0·29) mg N/kg $W^{0.75}$ at zero N infusion and 4·2 (SD 0·14) mg N/kg $W^{0.75}$ when 300 mg N/kg $W^{0.75}$ were infused. This difference was significant at $P < 0.05$.

Creatinine excretion was not significantly different and was on average 18·2 (SD 2·85) mg N/kg $W^{0.75}$ at zero N infusion and 17·0 (SD 2·40) mg N/kg $W^{0.75}$ when 300 mg N/kg $W^{0.75}$ were infused.

## DISCUSSION

### *Energy and N intakes and utilization*

At each given energy intake, N retention will be closely related to N intakes (Balch, 1967). As shown in Fig. 1 the increase in N retention declined gradually in Expt 1 as N intake increased above 600 mg N/kg $W^{0.75}$ at the lowest level of VFA energy infusion (340 kJ/kg $W^{0.75}$). However, with energy intakes of 450 and 630 kJ VFA energy/kg $W^{0.75}$ an indication of a reduced N retention was seen only at the highest level (1500 mg N/kg $W^{0.75}$) of N infusion. These findings are in agreement with previous results obtained in animals on intragastric infusion of nutrients (Ørskov & MacLeod, 1982; Storm *et al.* 1983).

In sheep maintained on intragastric infusion, zero energy balance can be expected when 450 kJ VFA energy/kg $W^{0.75}$ are given (Ørskov *et al.* 1979*a*). Despite infusion of 340 kJ VFA energy/kg $W^{0.75}$, N retention was positive above an N intake of 368 mg N/kg $W^{0.75}$. At zero N balance total energy intake was only 398 kJ GE/kg $W^{0.75}$, which was well below expected energy needs. Similar observations have previously been made in sheep (Hovell *et al.* 1983*b*), goats (Lindberg, 1989) and cattle (Ørskov *et al.* 1983). It was shown by Lindberg (1989) that in young growing goat kids with a high protein requirement, N accretion has a priority over fat deposition. The findings of Fattet *et al.* (1984) also showed that, in sheep, N can be retained despite substantial weight loss. It is possible that part of the positive N retention seen at the low energy intake could be due to an overestimation in the N balance trials because of errors in the procedure. It appears unlikely, however, that this could account fully for the positive N retention obtained at the low (340 kg VFA energy/kg $W^{0.75}$) energy intake.

In Expt 1 the faecal N excretion was on average 28 (SE 3·64) mg/kg $W^{0.75}$ which was comparable with values obtained in cows (24–25 mg N/kg $W^{0.75}$; Ørskov & MacLeod, 1982; Ørskov *et al.* 1983) and steers (32 mg N/kg $W^{0.75}$; Ørskov *et al.* 1983) sustained on intragastric infusion. The casein infused was found to be completely digestible which was in agreement with findings of Hovell *et al.* (1983*b*). It appears likely that the faecal N excreted was equivalent to the endogenous faecal loss.

At N infusions from 600 to 1400 mg N/kg $W^{0.75}$ on average 0·57 and 0·63 kg/kg were retained at VFA energy infusions of 450 and 630 kJ/kg $W^{0.75}$ respectively. In young growing goat kids (Lindberg, 1989), given milk N at the same level of intake, on average 0·57 kg/kg were retained. When ony 340 kJ VFA energy/kg $W^{0.75}$ were infused the N utilization for retention was 0·57 kg/kg between 400 and 600 mg N/kg $W^{0.75}$, but declined thereafter as N intakes increased.

When no protein was infused the basal urinary N loss was 255 mg N/kg $W^{0.75}$ at 340 kJ VFA energy/kg $W^{0.75}$ and 214 and 208 mg N/kg $W^{0.75}$ at 450 and 630 kJ VFA energy/kg $W^{0.75}$ respectively in Expt 1. In Expt 2 the basal urinary N loss was 216 mg N/kg $W^{0.75}$ at 450 kJ VFA energy/kg $W^{0.75}$. Although not significant there was an indication that the basal N excretion was increased when energy intake was reduced below maintenance. It has been shown that in both dry cows and steers (Ørskov & MacLeod, 1982) total N excretion is increased substantially when energy intakes as VFA are reduced.

The basal N requirements estimated from Expt 1 (281 mg N/kg $W^{0.75}$ at 340 kJ VFA energy/kg $W^{0.75}$, 226 mg N/kg $W^{0.75}$ at 450 kJ VFA energy/kg $W^{0.75}$ and 207 mg N/kg $W^{0.75}$ at 630 kJ VFA energy/kg $W^{0.75}$) were markedly lower than values obtained in cows, steer and lambs sustained on intragastric infusion of nutrients (Ørskov & MacLeod, 1982; Hovell *et al.* 1983*a*; Ørskov *et al.* 1983; Storm *et al.* 1983). A possible explanation for this difference could be related to both sex and age. The sheep used in Expt 1 were about 1 year old and were castrated at about 6 months of age. The sheep used in previous studies (Hovell *et al.* 1983*a*; Storm *et al.* 1983) were referred to as lambs (without specifying age) and the males were not castrated.

*Urinary excretion of urea*

With decreasing N infusion in Expt 1 the proportion of urea N in the urine also decreased. Similar observations have been made in goat kids fed on decreasing N intakes in liquid diets (Lindberg, 1989) and also in cows and steers maintained on intragastric infusion (Ørskov & MacLeod, 1982). The proportion of urea N was highest at the low energy intake (340 kJ VFA energy/kg $W^{0.75}$) and decreased in order of energy supply. As shown in Fig. 4 this change was accompanied by a similar change in the analysed component N as a proportion of total urinary N. This was also found in goat kids fed on liquid diets with decreasing N content (Lindberg, 1989). No explanation for this can be given at the present.

As shown in Fig. 2 plasma urea concentrations appear to be a good indicator of urea N loss through the urine in these animals. This also indirectly suggests that a high proportion of urea N in the urine (which occurs at high N intakes) is indicative also of high urea recycling in the body. In contrast to normally fed ruminants these animals have no urea drain (as the rumen and caecum is in a normally fed ruminant) and consequently have to excrete the urea in the urine.

*Urinary excretion of PD and creatinine*

The results from Expt 1 (Table 3 and Fig. 3) suggest that the endogenous excretion of allantoin, uric acid and hypoxanthine was unaffected by moderate changes in energy supply and dramatic changes in protein supply. It was reported by Lindberg (1989) that in young growing goats kids only marginal changes in the endogenous excretion of PD occur despite large changes in protein supply. Also, in steers maintained on intragastric infusion of nutrients (Fujihara *et al.* 1987), the endogenous excretion of total PD was unaffected by the level of N infused. In Expt 2, on the other hand, there was a significant increase in allantoin excretion when N infusion was reduced from 300 mg N/kg $W^{0.75}$ to zero N infusion. The increase in allantoin excretion (approximately 15%) was, however, modest in view of the drastic reduction in N supply. Giesecke *et al.* (1984) reported a reduction in PD excretion

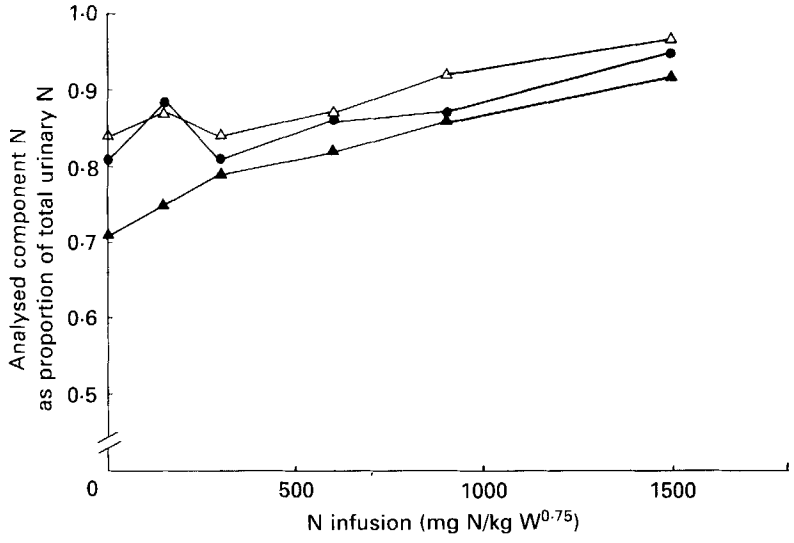


Fig. 4. Expt 1. Analysed component nitrogen (allantoin N, uric acid N, hypoxanthine N, creatinine N) in the urine, expressed as proportions of total urinary N, in sheep maintained on intragastric infusion: (△), 340 kJ volatile fatty acid (VFA) energy/kg metabolic live weight ($W^{0.75}$); (●), 450 kJ VFA energy/kg $W^{0.75}$; (▲), 630 kJ VFA energy/kg $W^{0.75}$.

when energy intake was increased from 0·25 to 2·0 times the energy maintenance in sheep maintained on intragastric infusion.

Total PD excretion (9·8 mg N/kg $W^{0.75}$) was of the same magnitude as those found in sheep (Fujihara *et al.* 1987) sustained on intragastric infusion. However, with a value for the proportion of allantoin of 0·49 (Expt 1), the urinary excretion of allantoin N was considerably lower than that reported previously for sheep (Walker & Faichney, 1964; Walker, 1967; Sibanda, 1982) and for goat kids (Lindberg, 1989).

The between-animal variation in PD excretion was markedly higher in the present study compared with previous experiments with liquid-fed goat kids (Lindberg, 1989). On average the coefficient of variation (CV) in Expt 1 was 20·7% for allantoin-N excretion, 29·0% for uric acid excretion and 58·3% for hypoxanthine excretion. No simple explanation can be given for this observation.

In agreement with previous studies on cattle (Ørskov & MacLeod, 1982) and goats (Lindberg, 1985, 1989) the creatinine excretion remained fairly constant irrespective of treatments applied. As also shown for PD, the between-animal variation in creatinine excretion was higher (CV 12·0–18·6%) in the present study compared with previous reports (Lindberg, 1985, 1989).

As discussed previously the urinary excretion of PD in ruminants is related to the flow of microbial nucleic acids, nucleic acids from the feed and to the metabolism of endogenous nucleic acids (McAllan, 1982; Lindberg, 1985). The findings presented here, together with those of Fujihara *et al.* (1987) and Lindberg (1989), give strong support to the opinion that, in ruminants, the microbial nucleic acids are quantitatively the most important source for urinary PD excretion (Rys *et al.* 1975) with only a smaller contribution from endogenous metabolism. The uncertainty about the contribution from feed nucleic acids to the urinary excretion of PD catabolites in a normally fed animal should, however, be kept in mind (McAllan, 1982).

The present study gives further support to the validity of using urinary excretion of PD to quantify the intestinal flow of microbial protein in ruminants.

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