Rumen digestion and urinary excretion of purine derivatives in response to urea supplementation of sodium-treated straw fed to sheep

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The present study examined the effect of urea-N supplementation of a N-deficient diet on digestion and metabolism in the rumen. Five Rasa Aragonesa ewes, each fitted with a rumen cannula, were offered alkali-treated barley straw *ad lib.* alone or supplemented continuously via the cannula with four levels of urea-N (3, 6, 9 and 12 g/d). Rumen NH_3 concentrations increased in response to urea infusion (6–128 mg/l; $P < 0.001$). At the highest level of rumen NH_3 concentration there was a significant increase, compared with the unsupplemented treatment, in dry matter (DM) intake (846–1206 g/d; $P < 0.001$) and apparent digestibility of DM (0·38–0·43), organic matter (0·38–0·45) and neutral-detergent fibre (0·41–0·49; $P < 0.01$). Rumen outflow rates of particulate matter and potential DM disappearances, assessed using nylon bags, were not affected by the experimental treatments, although fractional rate of DM disappearance increased significantly with increasing levels of urea infusion (2·4–4·6 per h). Urinary excretion of total purine derivatives increased with N supplementation, although the response was exclusively due to an increase in allantoin excretion (26·9–66·4 mg/kg live weight $(W)^{0.75}$ per d; $P < 0.001$). Xanthine, hypoxanthine and uric acid excretion rates were constant, averaging 1·8 (SE 0·17); 5·4 (SE 0·21) and 7·2 (SE 0·36) mg/kg $W^{0.75}$ per d respectively. The maintenance of a minimum rumen NH_3 concentration (approximately 50 mg/l) was necessary to avoid significant reductions in DM intake and fermentation rate. Higher levels, however, may further increase microbial N flow at the duodenum, as suggested by the response in urinary allantoin excretion over the range of rumen NH_3 concentrations.

Rumen ammonia: Purine excretion: Sheep

N availability is frequently a major constraint on rumen digestion of straws and poor-quality roughages, and the concentration of rumen NH_3, which constitutes the main N source for bacterial growth, may be used as a suitable index to monitor the requirements for dietary N supplementation.

However, conflicting results have been reported concerning the optimal rumen NH_3 concentrations. The concentrations required to achieve the maximum rate of fermentation (Mehrez *et al.* 1977; Erdman *et al.* 1986) are usually higher than those reported as adequate for maximal microbial synthesis, in both *in vitro* (Satter & Slyter, 1974) and *in vivo* experiments (Slyter *et al.* 1979; Kang-Meznarich & Broderick, 1981). Although these discrepancies may well be explained by the fermentability of the substrate (Erdman *et al.* 1986), a differential response as a result of an uncoupled fermentation cannot be excluded (Harrison & McAllan, 1979; Song & Kennelly, 1990).

The *in vivo* measurement of microbial synthesis has been hindered by technical difficulties in the use of post-ruminally cannulated animals and attendant errors in measurements of digesta flow and the microbial components. Urinary purine excretion, which has been

shown to be correlated with the amount of duodenal nucleic acids (Antoniewicz *et al.* 1980; Sibanda *et al.* 1982; Giesecke *et al.* 1984; Fujihara *et al.* 1987), may provide a simple and non-invasive method of estimating microbial protein flow at the duodenum, assuming that dietary nucleic acids are extensively degraded in the rumen (McAllan & Smith, 1973). Urinary allantoin excretion has been shown to be responsive to rumen N supply (Elliott & Topps, 1963; Laurent & Vignon, 1979) and, recently, a correlation equation relating urinary purine excretion to duodenal flow of purines has been established using both intragastrically-infused (Chen *et al.* 1990*a*) and conventionally-fed sheep (Balcells *et al.* 1991). From these studies it is apparent that allantoin excretion could be used to determine the response of microbial yield to changes in rumen-degradable N supply.

In the present experiment the effect of urea supplementation of a N-deficient diet on the urinary excretion of purine derivatives was studied. In addition, *in sacco* degradability and particulate matter flow determinations were carried out in order to elucidate the effect of degradable N supply on rumen metabolism. A brief account of these results has been previously reported (Guada *et al.* 1990).

MATERIAL AND METHODS

Animals and treatments

Five Rasa Aragonesa ewes, averaging 44·5 (SE 2·32) kg live weight (W) and fitted with rumen cannulas, were maintained in metabolism cages during the experimental periods and individually penned during the adaptation periods, under continuous lighting and with free access to water.

A basal diet of NaOH-treated barley straw (50 g NaOH/kg dry matter (DM)), ground (3 mm screen) and pelleted, was given *ad lib.* once daily (09.30 hours) allowing a refusal margin of 0·10. The average composition of weekly samples was (g/kg); DM 912 (SE 0·9), organic matter (OM) 917 (SE 1·3), crude protein (N × 6·25; CP) 34·5 (SE 0·8), crude fibre (CF) 419 (SE 1·5), neutral-detergent fibre (NDF) 769 (SE 4·9), acid-detergent fibre (ADF) 494 (SE 1·7), permanganate lignin (PL) 104 (SE 1·4).

Five experimental treatments, including a basal diet (0) and four levels of urea-N supplementation (3, 6, 9 and 12 g/d), were supplied in a 5 × 5 Latin Square design with the following order: 0, 3, 6, 9, 12 (sheep no. 1) 3, 6, 9, 12, 0 (sheep no. 2) 6, 9, 12, 0, 3 (sheep no. 3) 9, 12, 0, 3, 6 (sheep no. 4), 12, 0, 3, 6, 9 (sheep no. 5). The urea and a mineral supplement consisting of; S 0·6, 1, 1·2, 1·4 and 1·7 g for the five experimental treatments respectively, plus P 3·3 g, Ca 4·5 g, Mg 1 g, Cu 2·5 mg and Co 0·3 mg were diluted daily in 1 litre distilled water and infused via the cannula into the rumen at a continuous flow rate of 0·93 ml/min for 18 h, starting immediately after feeding.

Experimental procedures

Each experimental treatment was maintained for twenty-two consecutive days, comprising 14 d of adaptation and 8 d of experimental period. At 48 h before the experimental period the animals were placed in metabolism crates and faeces (days 1–7) and urine (days 2–5) collected daily. On day 3 of each period 10 g Cr-labelled straw (10·58 mg Cr/kg DM) were given via the cannula immediately after feeding, and faeces collected and sampled (50 g approximately) at 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 40, 48, 60, 72, 92 and 120 h after dosing. The labelling was achieved using the method of Udén *et al.* (1980) by treating the straw with 157 g sodium dichromate/kg DM at 105° for 24 h. After reduction with ascorbic acid, the Cr complex was washed and dried at 60°.

From day 4 to day 7 the kinetics of DM disappearance were estimated by incubating 3 g milled straw (3 mm screen) in nylon bags (1500–1700 pores/cm^2), suspended in the rumen

for 3, 6, 12, 24, 48 and 72 h. After withdrawal, the bags were thoroughly rinsed in a washing machine and dried in a forced-draught oven for 48 h at 60°. An additional set of five bags was immersed in stirred cold water for 30 min to determine DM disappearance at zero time. DM disappearance was estimated from the weight loss during incubation.

On the last day of each experimental period (day 8) rumen fluid samples were taken by means of a manual pump at 1, 2, 4, 6, 8, 10, 15 and 24 h after feeding.

Sample preparation and chemical analysis

Daily composite samples of feed (5% of daily intake) and the whole individual feed refusals were bulked (days 1–7), dried (60°, 48 h), weighed, ground (1 mm screen) and stored at room temperature. Daily collected faeces were dried (60°, 48 h), weighed and bulked on individual bases until the end of the collection period when a sample was ground (1 mm screen) and stored at room temperature. During the measurement of outflow rate (days 3–5) faecal samples were dried and the weight added to the non-sampled bulked material in order to obtained the daily faecal excretion.

DM was determined by drying at 105° to constant weight, and OM by ashing at 500° for 8 h. NDF, ADF and PL were determined according to Goering & Van Soest (1970), and N content by the Kjeldahl procedure using Se as a catalyst. Cr concentration in faecal samples was measured by atomic absorption spectrophotometry after wet digestion according to Williams *et al.* (1962).

Urine was collected daily under toluene (40 ml), which was removed by decanting. Urine weight and specific gravity were recorded daily and four subsamples (2% of total) stored immediately at −20°. Those for NH_3 and total N analyses were previously acidified to pH < 2 with HCl and H_2SO_4 respectively. Samples of rumen fluid were filtered through a metallic strainer (1 mm pore) to remove coarse particles. After recording the pH, the samples were acidified with HCl (0·2 M) and stored at −20° for NH_3 analysis.

Urinary allantoin was measured using the Rimini-Schryver reaction (Young & Conway, 1942). Uric acid, hypoxanthine and xanthine were analysed by reverse-phase HPLC using a Hypersil ODS C-18 (4·0 × 250 mm) column (Balcells *et al.* 1991) and urea was determined using the diacetylmonoxine reaction (Technicon Instruments Co. Inc., 1972). Ammonia concentration in urine and rumen fluid samples was determined by direct distillation with $Na_2B_4O_7$.

Calculations and statistical analysis

The relationship between DM disappearance from nylon bags (y) and incubation time (x) was described by the model proposed by Ørskov & McDonald (1979):

$$y = a' + b'(1 - e^{-ct}),$$

where the constants a',b' and c are the last square estimates of the rapidly soluble fraction, the degradable fraction and the fractional rate of degradation respectively. However, since washing losses (a) were always higher than the estimated rapidly soluble fraction (a') the McDonald (1981) modification was used in order to calculate a lag time (t_0) estimation:

$$t_0 = 1/c \ln [b'/(a' + b' - a)].$$

Total mean retention time (TMRT), transit time (TT) and the slow (k_1) and rapid (k_2) rates of passage were estimated from the Cr-dilution curve in faeces by the model proposed by Grovum & Williams (1973). The degradation kinetics constants (a',b' and c) were combined with those of rate of passage to calculate the effective degradability (ED) as proposed by McDonald (1981):

$$\mathrm{ED} = a + [b'c/(c + k_1)] \exp\{-(c + k_1)t_0\}.$$

Values were examined by analysis of variance as a Latin Square design assuming no carry-over effects. Orthogonal polynomials were used to describe the response to urea supplementation in the diet. The least significant difference ($P < 0.05$) was used to compare treatments means (Steel & Torrie, 1960). Responses in allantoin excretion, degradation rate and rumen NH_3 concentration were studied by regression analyses and the iterative procedure described by Carriedo *et al.* (1978) for a linear fit with break points.

RESULTS

The animals remained in good health throughout the experiment. However, one animal in one treatment failed to maintain feed intake during the rumen sampling day and the corresponding observations were estimated as missing values. W changed throughout the experiment in response to the experimental treatments and, therefore, all variables were expressed in relation to W at the beginning of the experiment. Daily W losses averaging 35 (SE 4·3) and 18 (SE 6·2) g/d were recorded on the control treatment and the lowest level of supplementation respectively, while W gains of 6 (SE 3·4), 15 (SE 4·6) and 25 (SE 13·5) g/d were observed with the successive increments in urea supply.

Intake and digestibility

Average DM and OM intakes and apparent digestibility coefficients of DM, OM and NDF are given in Table 1. Voluntary intake and digestibility increased significantly in response to the first level of N supplementation, but no further increases were achieved by increasing the urea-N supply above 6·0 g/d. In all cases the quadratic component of the responses was significant ($P < 0.05$).

Rumen variables

The profiles of NH_3 concentration through the day are shown in Fig. 1 and the daily mean values together with the mean estimate of passage and degradation constants are given in Table 2. Urea infusion maintained a constant NH_3 concentration through the day, although individual variation in rumen NH_3 concentration increased with the level of N supplementation. Mean values increased from 6 to 128 mg/l in response to the increased level of urea infusion. Rumen pH was also very stable throughout the sampling periods and showed a small but statistically significant decrease with increasing levels of urea supplementation. Outflow rates of solid digesta from the rumen (k_1) were apparently higher on the N-supplemented diets but the differences did not reach statistical significance, probably due to the low precision of these estimates (coefficient of variation 26%). TT was significantly higher on the control treatment compared with other treatments and the TMRT also tended to be higher. Potential DM degradability ($a+b$) of the straw was not significantly modified by the experimental treatments, averaging 65 (SE 0·45)%. However, the lag time decreased and the fractional rate of degradation increased in response to the first level of N supplementation. This effect was reflected in a gradual increase in the effective degradability which levelled off at the N supplementation level that elicited the maximum response in DM intake. The quadratic component of the response showed statistical significance ($P < 0.05$).

Urinary purine excretion and nitrogen losses

Urinary excretion of purine derivatives and other N compounds is shown in Table 3. Urea-N and NH_3-N excretion increased with N supplementation but, whereas NH_3-N levelled off at a N supply of 6·0 g/d, urea-N excretion continued to rise at an increasing rate in response

Table 1. *Dry matter (DM), digestible organic matter (DOM) intake and neutral-detergent fibre (NDF) apparent digestibility of NaOH-treated straw in sheep supplemented by continuous infusion of urea*†

(Mean values for five sheep)

Level of urea-N supplementation (g/d)...	0	3	6	9	12	SEM	Statistical significance of treatment effects: Lin	Quad
Intake (g/d)								
DM	846	1054	1189	1237	1206	64·7	***	*
DOM	294	423	495	504	497	31·6	**	*
Apparent digestibility								
DM	0·36	0·42	0·43	0·43	0·43	0·010	**	*
DOM	0·38	0·43	0·44	0·45	0·45	0·012	**	*
NDF	0·41	0·47	0·49	0·49	0·49	0·012	**	*

Lin, Quad, linear and quadratic effects of urea-N supplementation respectively.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.
† For details of procedures, see pp. 722–723.

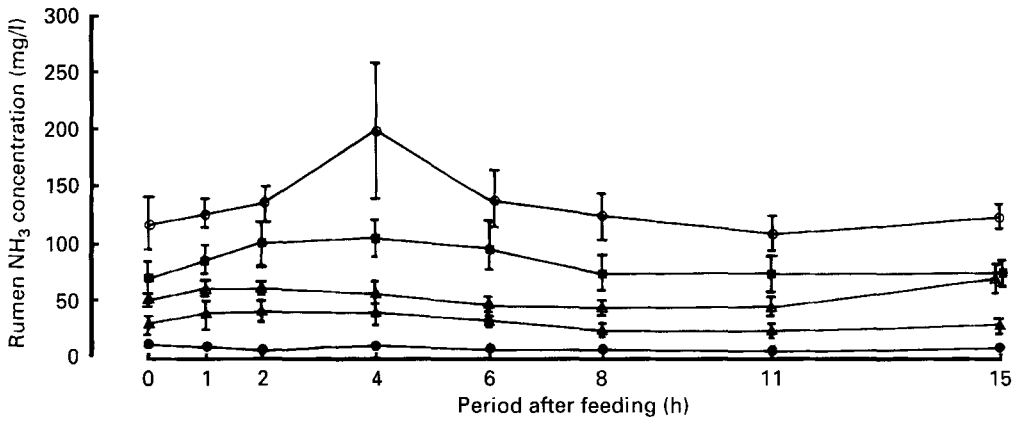

Fig. 1. Rumen NH_3 concentration (mg/l) in sheep (1, 2, 4, 6, 8, 11 and 15 h after feeding) fed on alkali-treated straw (●) and supplemented with 3 (▲), 6 (△), 9 (■) and 12 (○) g urea-N/d. Values are means and standard deviations represented by vertical bars. For details of procedures, see pp. 722–723.

to further levels of supplementation. Urinary recovery of incremental N supply increased from 5 and 40% at the lowest and intermediate levels of supplementation to reach 100% at the highest levels of N supplementation. On the other hand, creatinine excretion was not affected by treatment and averaged 32·5 (SE 2·5) mg/kg $W^{0·75}$ per d.

Excretion of total purine derivatives was significantly affected by the treatments imposed, but the response was exclusively due to the increase in allantoin excretion. Xanthine, hypoxanthine and uric acid excretion rates were remarkably constant, averaging 1·8 (SE 0·17); 5·4 (SE 0·21) and 7·2 (SE 0·36) mg/kg $W^{0·75}$ per d, respectively. Consequently, the relative proportion of the allantoin increased from 0·68 to 0·83 with incremental levels of urea-N supplementation. The increase in allantoin excretion (y; mg/kg $W^{0·75}$ per d) was

Table 2. *Rumen NH_3 concentration, pH and kinetics and passage and digestion in sheep fed on NaOH-treated straw supplemented by continuous infusion of urea*‡

(Mean values for five sheep)

Level of urea-N supplementation (g/d)...	0	3	6	9	12	SEM	Statistical significance of treatment effects: Lin	Quad
Rumen NH_3 (mg/l)	5·6	29·7	49·1	82·6	128·3	3·17	***	NS
pH	6·88	6·80	6·60	6·71	6·70	0·031	***	NS
Passage kinetics								
k_1 (%/h)	3·24	4·64	4·10	4·14	4·38	0·541	NS	NS
k_2 (%/h)	9·32	9·86	10·02	11·45	11·80	1·077	NS	NS
TT (h)	17·3	15·7	13·1	12·0	14·4	1·13	*	NS
TMRT (h)	59·6	48·7	49·7	47·8	48·3	3·04	†	NS
Digestion kinetics								
$(a+b)$ (%)	65·7	63·4	63·6	63·1	68·8	0·52	NS	NS
c (%/h)	2·1	4·4	5·4	5·6	4·6	0·59	**	*
t_0 (h)	3·1	2·4	2·3	2·1	2·1	0·54	*	NS
ED (%)	28·2	33·0	37·0	38·5	36·5	1·63	**	*

Lin, Quad, linear and quadratic effects of urea-N supplementation respectively; k_1, k_2, slow and fast rates of passage; TT, transit time; TMRT, total mean retention time; $a+b$, potential degradability; c, fractional rate of degradation; t_0, zero time; ED, effective degradability; NS; not significant.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, † $P < 0·1$.

‡ For details of procedures, see pp. 722–723.

Table 3. *Daily urinary excretion of N compounds and purine derivatives (mg/kg live weight$^{0·75}$ per d) and allantoin excretion (g): digestible organic matter intake (DOMI; kg) in sheep fed on NaOH-treated straw supplemented by continuous infusion of urea*‡

(Mean values for five sheep)

Level of urea-N supplementation (g/d)...	0	3	6	9	12	SEM	Statistical significance of treatment effects: Lin	Quad
N								
Total	74	114	221	310	486	23·0	**	†
Urea	6·3	14·2	82·2	144	307	34·5	***	†
NH_3	4·5	9·5	27·1	31·7	28·9	15·02	*	NS
Purine derivatives								
Allantoin	26·9	44·1	58·3	57·4	66·4	3·40	***	†
Uric acid	6·3	7·6	7·1	7·4	7·4	0·81	NS	NS
Hypoxanthine	4·9	5·6	5·1	5·8	5·2	0·46	NS	NS
Allantoin:DOMI	1·60	1·81	2·13	2·02	2·31	0·147	*	NS

Lin, Quad, linear and quadratic effects of urea-N supplementation respectively; NS; not significant.

* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$, † $P < 0·1$.

‡ For details of procedures, see pp. 722–723.

linearly related to digestible OM (DOM) intake (x; g/kg $W^{0.75}$ per d), the relationship between both variables being defined by the following equation:

$$y = 2{\cdot}03\,(\text{SE}\ 0{\cdot}265)\,x - 1{\cdot}58\,(\text{SE}\ 6{\cdot}491)\,(r\ 0{\cdot}848, \text{residual SD (RSD)}\ 6{\cdot}36).$$

DISCUSSION

Voluntary intake

DM intake was moderately high, even on the basal diet, in agreement with previous observations on voluntary intake of alkali-treated pelleted straw (Saxena *et al.* 1971; Xande & Dermarquilly, 1983; Deryche *et al.* 1985), and probably pelleting avoided the off-feed problems reported in other experiments (Neutze *et al.* 1986). A high level of DM intake could involve a risk of osmotic effects (Na^+) and rumen alkalosis (OH^-) in view of the high NaOH content of the alkali-treated straw (50 g/kg DM); however, Na intake never reached toxic levels (2·2–2·4 g/kg $W^{0.75}$, according to Ali *et al.* 1977) and rumen pH was always below 7·0.

DM and OM digestibilities were low compared with the values reported for either unsupplemented (Xande & Demarquilly, 1983) or urea-supplemented NaOH-treated pelleted straw (Coombe *et al.* 1979; Deryche *et al.* 1985; Wanapat *et al.* 1985). This low digestibility was coincident with a fast outflow rate of particles from the rumen, probably as a consequence of the fineness of grinding (3 mm) (Fadlalla & Kay, 1987) in combination with the alkali treatment (Coombe *et al.* 1979).

Response in DM intake to the level of N supplementation followed a similar pattern to that described by Neutze *et al.* (1986) and Ørskov & Grubb (1978) with NaOH-treated wheat and barley straw respectively, and Milne *et al.* (1979) with freeze-stored heather (*calluna vulgaris*), confirming the sensitiveness of voluntary intake to impaired rumen fermentation.

Rumen variables

The continuous infusion of urea-N into the rumen elicited a steady NH_3 concentration which ensured a constant N availability at each level of supplementation. Rumen NH_3-N concentration with the basal diet was insufficient to meet microbial requirements (Satter & Slyter, 1974) and this was reflected in a longer lag time (t_0) and a depressed rate of degradation (c) in comparison with the supplemented diets.

Fig. 2 shows the relationship between the rate of DM disappearance from the rumen and the concentration of rumen NH_3. In order to assess the NH_3 concentration which maximizes the rate of DM disappearance from the rumen, the values were analysed as two linear models defined by two straight lines ($y = bx + a$ for $x < x_s$ and $y = c$ for $x > x_s$). The resulting equations were:

$$\text{for } x < x_s \quad y = 0{\cdot}093\,x + 1{\cdot}27,$$
$$\text{for } x > x_s \quad y = 5{\cdot}6,$$

where y is the rate of DM disappearance from the rumen (per h), x is rumen NH_3 concentration (mg/l) and x_s is an estimate of the optimum rumen NH_3 concentration obtained by inspection (46 mg/l). The concentration at which this was achieved was the same level of urea-N supply (6 g/d) that promoted the maximum response in voluntary intake and digestibility, and it was within the range of NH_3 concentrations described by Alvarez *et al.* (1984) and Milne *et al.* (1979) to maximize the fermentation rate of NaOH-treated mature hay and the voluntary intake of heather respectively. Ørskov (1982), however, suggested a lower NH_3 concentration (20 mg/l) as the optimum to obtain the maximum rate of DM disappearance from nylon bags with NaOH-treated barley straw, and much higher NH_3-N values have been reported for concentrate feeds (Mehrez *et al.* 1977; Erdman *et al.* 1986).

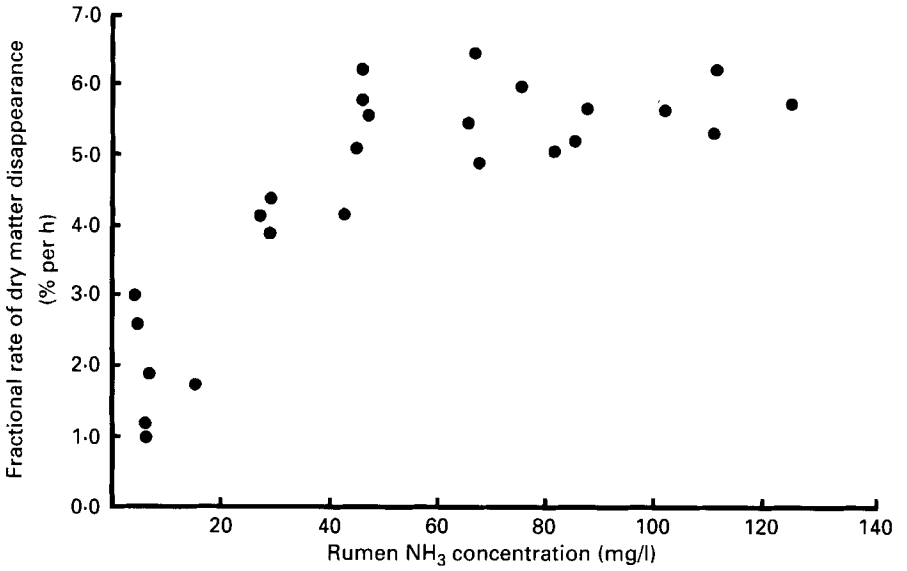

Fig. 2. Relationship between rumen NH_3 concentration (mg/l) and fractional rate of dry matter disappearance obtained in sheep fed on alkali-treated straw and supplemented with 3, 6, 9 and 12 g urea-N/d. For details of procedures, see pp. 722–723.

The optimum rate of DM disappearance was comparable to those values previously reported for NaOH-treated pelleted straw (Coombe *et al.* 1979; Wanapat *et al.* 1986; Adebowale *et al.* 1989) and it was significantly higher than that recorded on the unsupplemented diet.

Urinary excretion of purine derivatives

Ruminants are able to compensate for dietary N deficiencies by decreasing urinary N losses and recycling blood urea-N to the rumen (Neutze *et al.* 1986). However, in the present experiment the reduction in urinary excretion of urea and NH_3, recorded with the unsupplemented diet, was not sufficient to maintain rumen NH_3 concentration at the level required for maximal microbial growth (Satter & Slyter, 1974), and this was reflected in a lowered excretion of urinary purine derivatives. In agreement with our previous observations (Balcells *et al.* 1991), urinary excretion of xanthine, hypoxanthine and uric acid was constant, whereas allantoin excretion responded significantly to the experimental treatments. Variations in the proportion of urinary purine derivatives have also been reported by Chen *et al.* (1990*a*) and Lindberg (1991), although these authors also showed a progressive increase in the excretion of allantoin precursors with duodenal purine supply. In both reports young animals (lambs and goat kids respectively) were used and much higher levels of purine supply were tested, and this may explain the different responses observed. Levels of allantoin excretion obtained in our experiment varied within the range of values reported by Laurent & Vignon (1979) and Maloiy *et al.* (1970) for unsupplemented (14–34 mg/kg $W^{0·75}$) or barley-supplemented straw (61–95 mg/kg $W^{0·75}$) respectively. However, the values were consistently higher than those estimated from the equations developed by Dewhurst & Webster (1988), based on NaOH-treated straw diets supplemented with variable amounts of cellulose and starch.

The lowest allantoin excretion recorded for the basal diet (26·9 (SE 2·32) mg/kg $W^{0·75}$) was higher than the endogenous losses reported by Chen *et al.* (1990*b*), Giesecke *et al.*

Table 4. *Microbial nitrogen supply to the duodenum estimated from the urinary excretion of allantoin (g/d) and expressed as absolute amount (g/d) or in relation to the intake of organic matter apparently digested in the whole tract (g/kg DOMI) or in the rumen (g/kg RDOMI) of sheep as assessed from the* in sacco *dry matter effective degradability*

(Mean values for five sheep)

Level of urea-N supplementation (g/d)...	0	3	6	9	12	SEM	Statistical significance of treatment effects: Lin	Quad
Allantoin (g/d)	3·55	5·67	7·92	7·85	9·68	0·540	***	NS
Microbial N:								
g/kg DOMI	11·9	13·5	15·9	15·6	19·6	1·05	*	NS
g/kg RDOMI	12·8	16·9	18·2	17·4	23·3	1·22	*	NS

Lin, Quad, linear and quadratic effects of urea-N supplementation respectively; DOMI, digestible organic matter intake; RDOMI, rumen DOMI; NS, not significant.
* $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

(1984) and Balcells *et al.* (1991) and exceeded the threshold level (21 mg/kg $W^{0·75}$) required to maintain a constant urinary recovery of the duodenal purine input (Balcells *et al.* 1991) once the endogenous losses have been met. Endogenous purine excretion is constant and shown to be independent of the feeding level (Lindberg & Jacobsson, 1990), and in view of the negligible contribution of the undegraded straw to the absorbed purines (purine content 3·3 mmol/kg DM) the response in allantoin excretion may be confidently attributed to the duodenal flow of microbial purines. Table 4 shows the microbial N flow to the duodenum with the basal diet and at the four level of N supplementation, estimated from a response model previously developed (Balcells *et al.* 1991). The calculations assumed no contribution of undegraded dietary purines to the duodenal flow and that nucleic acids make up 0·152 of the microbial N (Storm & Ørskov, 1983) and contain 0·15 g N and 1·10 mmol purines/g (Balcells *et al.* 1991). The assessed values are similar to those reported by Neutze *et al.* (1986) from ^{15}N labelling with urea-supplemented, NaOH-treated straw-fed sheep (6·8, 9·6 and 10·7 g/d).

There was a significant ($P < 0·01$) relationship between allantoin excretion (y; mg/kg $W^{0·75}$ per d) and rumen NH_3 concentration (x; mg/l) described by the equation;

$$y = 24·74\,(\text{SE}\ 7·889) + 0·761\,(\text{SE}\ 0·175)x - 0·034\,(\text{SE}\ 0·0124)x^2 \quad (r\ 0·91, \text{RSD}\ 7·732),$$

and illustrated in Fig. 3. This equation, based on the relatively narrow range of minimal NH_3 concentrations found in this experiment, shows that the NH_3 concentration required to attain the maximum response (114 mg/l) seems to be higher than the critical concentration necessary to maximize the rate of degradation. Hume *et al.* (1970) also found that higher NH_3 concentrations (133 mg/l) than those required to attain the maximal microbial population in the rumen (88 mg/l) were able to promote an increased duodenal flow of microbial protein. Slyter *et al.* (1979) observed an increase in N retention in steers in response to NH_3 levels in excess to those estimated (46 mg/l) as adequate to elicit the maximum rumen concentration of volatile fatty acids and tungstic acid-precipitable N, although systematic errors in the N balance technique were invoked to justify this response.

The response of urinary allantoin in the present study was due, in part, to the increased

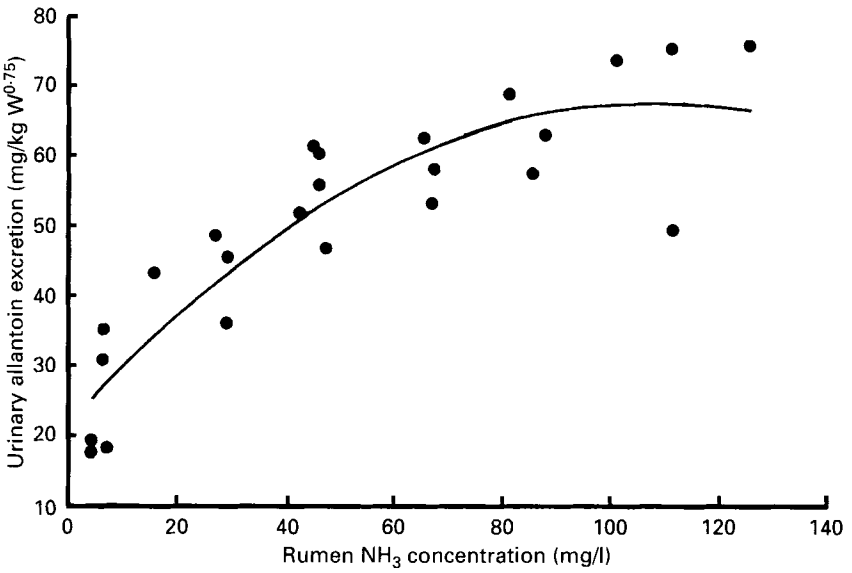

Fig. 3. Relationship between urinary allantoin excretion and rumen NH_3 concentration obtained in sheep fed on alkali-treated straw and supplemented with 3, 6, 9 and 12 g urea-N/d. Each point represents an individual measurement and the fitted regression line is: $y = 24{\cdot}74 + 0{\cdot}76x - 0{\cdot}0335x^2$; r^2 0·82, residual SD 7·73.

DOM intake (Table 1). Nevertheless, when expressed per unit DOM intake, both allantoin excretion (Table 3) and the estimated microbial N flow (Table 4) increased linearly with rumen NH_3 concentration. Microbial N flow was also expressed per unit OM apparently digested in the rumen (Table 4), as assessed from the effective degradability of DM, assuming the relationship between OM and DM digestibilities in the whole tract applies to the rumen. Whatever the basis for the calculations a significant increase ($P < 0{\cdot}05$) was always evident, reflecting an effect of rumen NH_3 concentration on microbial synthesis efficiency. Neutze *et al.* (1986) observed that an increased proportion of DOM was apparently digested in the rumen as a result of urea supplementation of alkali-treated straw. Assuming that the effective degradability is an acceptable estimate of the rumen digestibility, it was assessed that, in the present experiment, the proportion of DOM apparently digested in the rumen increased from 0·77 on the basal diet to 0·79, 0·85, 0·87 and 0·86 with the successive levels of N supplementation, which would help to explain the previously described response in microbial yield. However, the fact that an increase was still evident when the microbial yield was expressed per unit DOM apparently digested in the rumen (Table 4) seems to preclude this explanation.

The good agreement between the microbial yield estimates when fermentation was not constrained by N availability (from 15·9 to 19·6 g N/kg DOM) and those determined using ^{15}N (17·2–19·0 g N/kg DOM) in similar feeding conditions (Neutze *et al.* 1986) indicates that allantoin excretion can provide reliable estimates of microbial yield.

In conclusion, with alkali-treated straw diets the maintenance of a minimum rumen NH_3 concentration close to 50 mg/l is necessary to avoid reductions in DM intake and fermentation rate, but higher NH_3 levels up to 110 mg/l may further increase the microbial N flow at the duodenum, as suggested by the response in allantoin excretion through the overall range of NH_3 concentrations. Nevertheless, the highest proportion of this increase was due to enhanced DOM intake promoted by an adequate rate of fermentation.

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