

## Degradation and outflow of amino acids from the rumen of sheep

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(Received 8 March 1988 – Accepted 14 October 1988)

1. In hay-fed, cannulated sheep the apparent degradation in and outflow from the rumen were determined for graded doses of mixtures of the amino acids lysine, threonine and methionine, administered intraruminally and using polyethylene glycol (PEG) as a liquid marker. The doses ranged between 2.5 and 15 g for each amino acid in the mixtures.
2. Relative rate of apparent degradation in the first 4 h was highest for lysine, and lowest for methionine. The apparent degradation in 24 h was highest for lysine and lowest for threonine. Conversely the fraction flowing out of the rumen in intact form in 24 h was highest for threonine and lowest for lysine. Rates of apparent degradation as well as outflow were dose-dependent.
3. The validity of the estimated outflow of amino acids from the rumen was corroborated by measurements of concentrations of the amino acids in duodenal contents and in blood plasma which were also dose-dependent.
4. It was concluded that part of the requirement for the essential amino acids threonine and methionine may be met, even when these amino acids are delivered in unprotected form, given as a feed supplement.

As all dietary constituents fed to sheep are first subjected to degradation in the rumen and the main limiting nutrients for wool growth are the essential amino acids, it is surprising that there is very little information on the degradabilities *in vivo* of the individual essential amino acids.

Most of the early work on amino acid degradation has been done *in vitro* with rumen fluid from cattle (Lewis & Emery, 1962; Skoch *et al.* 1975). The few studies done *in vivo* have produced conflicting information on the relative degradation rates of the important amino acids (Chalupa, 1976). For example the studies of Bird & Moir (1972), Chalupa (1976), Emery (1971), Langar *et al.* (1973), suggest that methionine is degraded relatively slowly in the rumen, while the work of Doyle & Adams (1980), Doyle & Moir (1979) and Reis *et al.* (1978), suggests that rapid degradation occurs.

There have been few studies of the effects of dose rate of methionine on subsequent passage of methionine to the duodenum, plasma and wool follicle. Doyle & Bird (1975) gave varying dietary supplements of methionine to sheep, but each group of sheep fed at a particular level contained different animals. Wool growth was measured, but flow of methionine to the intestines was not assessed.

Doyle & Adams (1980) found that plasma methionine concentrations did not increase until more than 27 g methionine were infused intraruminally. However, the degradation rate in the rumen was not measured. There has been some European work done with methionine supplementation, but the experiments have been based on only one dose rate of methionine (e.g. Champredon *et al.* 1976) or have not monitored the flow of methionine to the intestines (e.g. Vrubcheva, 1984).

Methionine appears to be degraded to a greater extent when given alone rather than in combination with other amino acids (Chalupa, 1976). There have been no studies done in

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vivo to determine the optimum combination of amino acids required to achieve the desired flow of undegraded dietary amino acids and microbial protein to the intestines.

The requirements of the sheep for individual amino acids have been studied more extensively (see Buttery & Foulds, 1985). These studies have normally involved post-ruminal infusions and hence the modifying influence of the rumen has not been determined (e.g. Brooks *et al.* 1973, Tao *et al.* 1974, Downes *et al.* 1976, Strath & Shelford, 1978, Mesbah & Asplund, 1984). There is little information on the optimum ratio of absorbed amino acids for wool growth.

The experiments reported here provide information on the degradation and outflow of methionine, lysine and threonine when given as intrarumen injections.

## METHODS

### *Experimental procedure*

Rates in vivo of amino acid metabolism were determined in three sheep with rumen and T-shaped duodenal cannulas. The sheep weighed an average of 87.5 (SE 3.5) kg. They were fed on 1180 g hay, containing their estimated maintenance energy requirements, once daily, in the morning. Hay of the same batch was used throughout the whole experimental period. Based on the values given in Norwegian feed tables for the type of hay used, the daily ration contained 1 kg dry matter, 8.81 MJ metabolizable energy, 500 g nitrogen-free extract, 77 g ash and 86 g digestible crude protein. Assuming the rumen-degradable N (RDN) fraction to be 80%, the daily supply of RDN would be 11.0 g which corresponds closely to the amount recommended by the Agricultural Research Council (1980) at this energy intake. Thus the fermentation of the amino acid doses administered were apparently not limited by the intake of either energy or RDN.

Sheep no. 1 died 2 weeks after Expt 5.

The amino acid mixtures used were prepared in rumen fluid taken from hay-fed sheep, and to the rumen fluid was added 2 g dioctylsulphosuccinate/l. On each of the 4 d before an experiment, and on the experimental day (day 5), the amino acid mixture (Table 1) was administered via the rumen cannula into each sheep at the time of feeding. On the experimental day, the animals were also given 15 g polyethylene glycol-8000 (PEG) simultaneously via the same route. Rumen and duodenal samples, and blood obtained by venipuncture from the jugular vein, were taken at hourly intervals for 2 h, then every 2 h for 8 h.

Heparinized blood samples were centrifuged at 10000 g for 10 min. Plasma, rumen fluid and duodenal fluid samples were deproteinized with 150 g trichloroacetic acid/l and centrifuged, and the resultant supernatant fractions stored at  $-10^{\circ}$  before analysis. Amino acid concentrations were determined by high-performance liquid chromatography, using the methods described by Bidlingmeyer *et al.* (1984). PEG was assayed according to the procedure of Smith (1959).

### *Mathematical procedures*

Rumen fluid pool size and flow rates were calculated from PEG kinetics by fitting a least squares regression to the plot of log PEG concentration *v.* time. Steady state conditions were assumed. Under the conditions of the experiments, non-steady state could not be excluded. Fluid intake might have varied during the sampling periods, although water was available continuously, and the feed intake only represented about 120 g water. Fluctuations in rumen volume after feeding would change amino acid concentrations which in turn would affect the estimation of degradation rates. However, the  $r^2$  of the ln PEG *v.* time regressions were high (over 0.8), suggesting limited fluctuations in rumen fluid volume and flow.

Table 1. *Experimental amino acid mixtures (g/d)*

Expt no.	Sampling date	Methionine	Lysine	Threonine
1	1/1/1986	—	—	—
2	14/1/1986	5	—	—
3	22/1/1986	5	5	5
4	29/1/1986	10	10	10
5	3/2/1986	15	15	15
6	26/2/1986	5	5	5
7	5/3/1986	2.5	2.5	2.5
8	10/3/1986	15	15	15

A microcomputer spreadsheet template using Lotus 123 (Lotus Development Corporation, 1986, Cambridge, Mass., USA) was written to calculate the following.

Amino acid outflow ( $\mu\text{mol}/\text{interval}$ ) = average amino acid concentration during the interval ( $\mu\text{mol}/\text{l}$ )  $\times$  fluid flow rate ( $\text{l}/\text{min}$ )  $\times$  interval ( $\text{min}$ ).

Amino acid degradation ( $\mu\text{mol}/\text{interval}$ ) = (start concentration ( $\mu\text{mol}/\text{l}$ ) – end concentration ( $\mu\text{mol}/\text{l}$ ))  $\times$  pool size ( $\text{l}$ ) – outflow ( $\mu\text{mol}$ ).

Amino acid concentration at time zero ( $t_0$ ) = amino dose ( $\mu\text{mol}$ )/pool size ( $\text{l}$ ).

The outflows and degradations of amino acids during the 24 h following the doses of amino acids were calculated by summing the outflows and degradations calculated for each interval between samplings during this period.

The initial degradation rates of each amino acid were estimated after inspection of the cumulative degradation curves (see Fig. 1). The lysine degradation rates were calculated from the 0–60 min interval. The methionine and threonine degradation rates were calculated from the 0–60, 0–120, 60–120 or 60–240 min intervals. The rates were calculated from the earliest interval possible with positive, linear degradation rates. The 0–60 min interval was therefore not used for these two amino acids when initial negative degradation values were recorded. Negative values occurred when the amino acid concentrations at 60 min were higher than expected. Poor mixing of the dose or a low estimate of pool size would cause these negative degradation values (see Discussion).

Velocity ( $V_{\text{max}}$ ) and Michaelis-Menten constant ( $K_M$ ) values were calculated by fitting the degradation rates from 60–120 min to the equation  $Y = a + bX$ , where  $Y$  is  $1/\text{degradation rate}$  ( $\mu\text{mol}/\text{min}$ ),  $X$  is  $1/\text{substrate concentration at 60 min}$  ( $\text{mmol}$ ),  $a$  is  $1/V_{\text{max}}$  and  $a/b$  is  $-1/K_M$ . Thus  $V_{\text{max}}$  and  $K_M$  were determined from the Lineweaver Burk plot of degradation rates (Fig. 2).

Differences in mean values were compared by  $t$  tests, assuming independent samples.

## RESULTS

Values for the average size of the rumen fluid pool and the liquid flow rate are given in Table 2, together with the effect of the largest doses of amino acid mixtures on these variables. Fluid turnover time was longer in sheep given the largest doses ( $P < 0.01$ ).

The mean cumulative apparent degradation of each amino acid for each dose rate is shown in Fig. 1. The means were calculated by estimating rates within sheep and then calculating the mean and standard deviations.

The calculated rates of apparent degradation ( $\text{mmol}/\text{h}$ ) are given in Table 3.  $V_{\text{max}}$  and  $K_M$  values are presented in Table 4.  $V_{\text{max}}$  was converted to  $\text{mmol}/\text{h}$  by dividing the estimate from Fig. 2 by pool size (9.6 litres); these estimates have large confidence intervals as they are derived from values some distance from the mean substrate concentrations. The 95%

Table 2. *Pool size, turnover time and flow rate of rumen fluid in hay-fed sheep given intrarumen infusions of graded doses of amino acid mixtures\**

(Mean values and standard deviations for animals of body-weight 87.5 (SE 3.5) kg)

	n	Pool size (l)		Turnover time (h)		Flow rate (l/h)	
		Mean	SD	Mean	SD	Mean	SD
Overall means	18	9.63	1.085	21.8	4.09	0.45	0.071
Means for low doses of amino acids (2.5–5 g)	10	9.49	1.309	20.3	3.40	0.47	0.063
Means for high doses of amino acids (10–15 g)	8	9.80	0.674	23.7	4.07	0.42	0.070

\* For details of procedure, see p. 398.

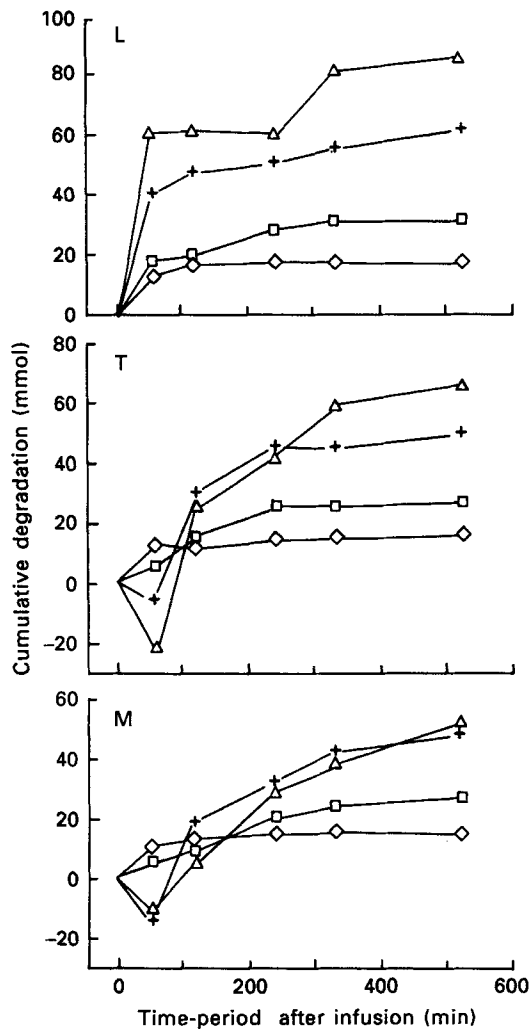


Table 3. Apparent degradation rates (mmol/h and percentage of dose) of amino acids in the rumen of sheep given intrarumen infusions of graded doses of amino acid mixtures\*

(Mean values and standard deviations for three sheep in Expts 2-5 and for two sheep in Expts 6-8)

Expt no.	Dose of each amino acid†	Rate of apparent degradation											
		Lysine				Threonine				Methionine			
		mmol/h		% / d		mmol/h		% / d		mmol/h		% / d	
Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
2	5	—	—	—	—	—	—	—	—	9.1	1.46	86	2.6
3	5	17.0	1.79	91	0.7	6.4	0.52	57	8.3	8.3	1.51	83	1.4
4	10	56.8	15.10	89	3.5	19.9	3.80	62	18.9	15.2	2.38	79	5.2
5	15	65.5	8.91	86	1.3	21.4	4.70	40	15.8	20.6	0.89	70	6.1
7	2.5	15.0	2.30	93	0.9	6.7	0.29	76	1.6	6.5	0.04	88	3.2
6	5	19.1	2.89	94	1.2	13.0	2.26	82	2.8	8.9	1.36	86	4.6
8	15	36.4	5.94	85	1.0	46.4	5.40	55	8.0	26.9	9.63	82	0.4

\* For details of procedure, see p. 398.

† In each experiment, except Expt 2, the mixture consisted of lysine, threonine and methionine.

Table 4. Kinetics of amino acid degradation in the rumen of hay-fed sheep given intrarumen infusions of graded doses of amino acid mixtures\* compared with findings of Chalupa (1976) using cows

Amino acid	Present investigation		Chalupa (1976) (in vitro)	
	$V_{max}$ (mmol/l per h)	$K_M$ (mmol/l)	$V_{max}$ (mmol/l per h)	$K_M$ (mmol/l)
Lysine	10.4	15.9	0.32	0.59
Threonine	13.9	39.5	0.50	0.81
Methionine	4.2	10.4	0.09	0.29

$V_{max}$ , velocity;  $K_M$ , Michaelis-Menten constant.

\* For details of procedures, see p. 398.

confidence intervals for  $1/V_{max}$  (min/ $\mu$ mol) were  $0.0015 \pm 0.00507$ ,  $0.0006 \pm 0.00503$  and  $0.0004 \pm 0.00532$  for methionine, lysine and threonine respectively. The 95% confidence intervals for  $-1/K_M$  (l/mmol) were  $-0.10 \pm 0.321$ ,  $-0.06 \pm 0.340$  and  $-0.03 \pm 0.208$  for methionine, lysine and threonine respectively.

Fig. 3 shows the mean dose-dependent concentrations of lysine, threonine and methionine in rumen fluid, duodenal contents and plasma on day 5, following dosage of the amino acid mixtures in the morning for five consecutive days. As appears from the values, even the pre-dosing concentrations on day 5 were related to the doses administered on the

Fig. 1. Cumulative apparent degradation v. time-period after infusion of amino acids in the rumen as a function of dose infused intraruminally. Average of all sheep in all experiments. In all experiments mixtures of equal doses of lysine (L), threonine (T) and methionine (M) were given by intrarumen administration at feeding time in the morning for five consecutive days. Samples collected on day 5 (for details of procedures, see p. 398).  $\diamond$ — $\diamond$ , 2.5 g LTM;  $\square$ — $\square$ , 5 g LTM; +—+, 10 g LTM;  $\triangle$ — $\triangle$ , 15 g LTM.

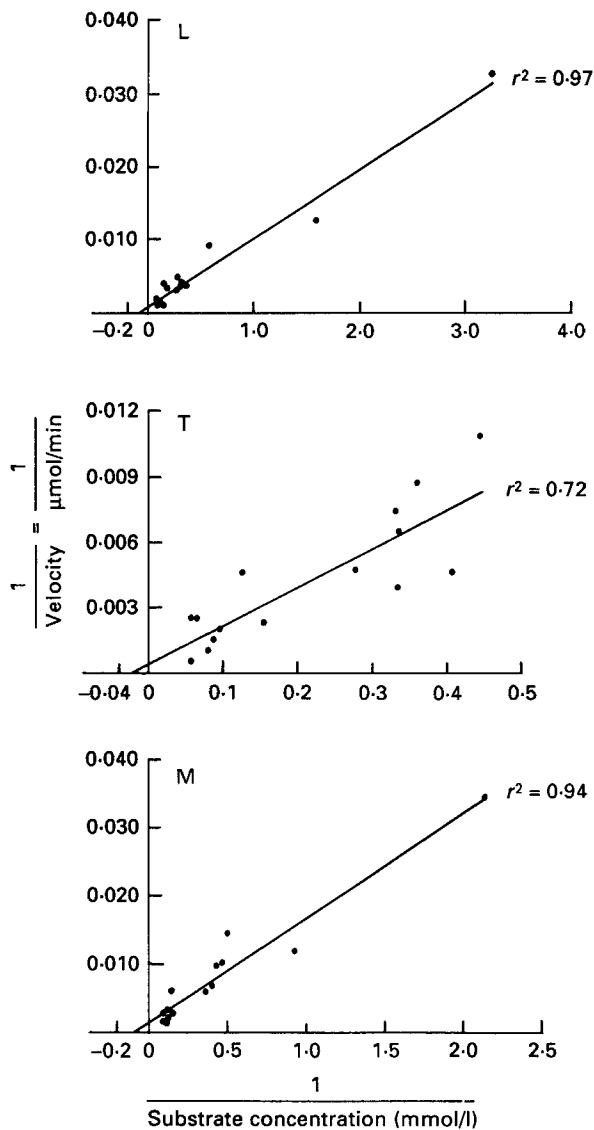


Fig. 2. Lineweaver-Burk plots of rates of apparent degradation of lysine (L), threonine (T) and methionine (M) as a function of substrate concentration in vivo. Substrate concentrations were calculated on the basis of rumen fluid volume estimated by use of polyethylene glycol as the liquid marker in each experiment (for details of procedures, see p. 398).

four preceding days. This applied to all three fluids examined. The absolute values differed, depending *inter alia* on the kinetic variables (Tables 3 and 4).

The estimated amounts of each amino acid flowing intact out of the rumen during the first 24 h after dosing are given in Table 5. The values in Tables 3 and 5 taken together account for total disposal of the offered dose, except for threonine in Expts 5 and 8. In this case, following the initial rapid rates of degradation, a high residual concentration of threonine remained in the rumen 24 h after dosing.

Table 6 shows the influence of intrarumen injections of mixtures of lysine, threonine and methionine on the concentration of these and some other amino acids in plasma.

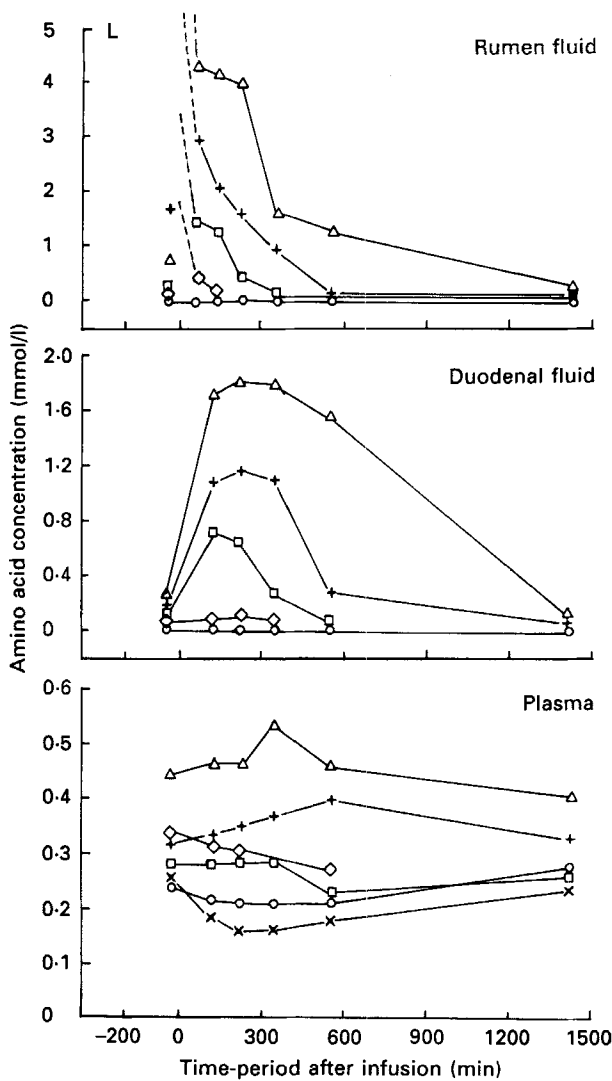


Fig. 3(a).

Fig. 3. Concentration profiles for (a) lysine (L), (b) threonine (T) and (c) methionine (M) in rumen fluid, duodenal fluid and plasma as a function of dose size. In each experiment except one ( $\times$ — $\times$ ), mixtures of equal doses of the three amino acids were given by intrarumen administration at feeding time in the morning for five consecutive days. Samples collected on day 5 (for details of procedures, see p. 398).  $\circ$ — $\circ$ , Control values;  $\diamond$ — $\diamond$ , 2.5 g LTM;  $\times$ — $\times$ , 5 g M;  $\square$ — $\square$ , 5 g LTM; +—+, 10 g LTM;  $\triangle$ — $\triangle$ , 15 g LTM.

#### DISCUSSION

When considering the curves illustrating the cumulative apparent degradation of the three amino acids in the rumen (Fig. 1), some differences between the amino acids were noted. Thus, for lysine the apparent degradation was positive at all dose rates. As for methionine and threonine, the two highest dose rates (10 and 15 g) apparently caused an initial negative degradation, for which no final explanation has been found. The analytical reliability is high. The most likely errors would be overestimation of pool size, changing pool sizes,

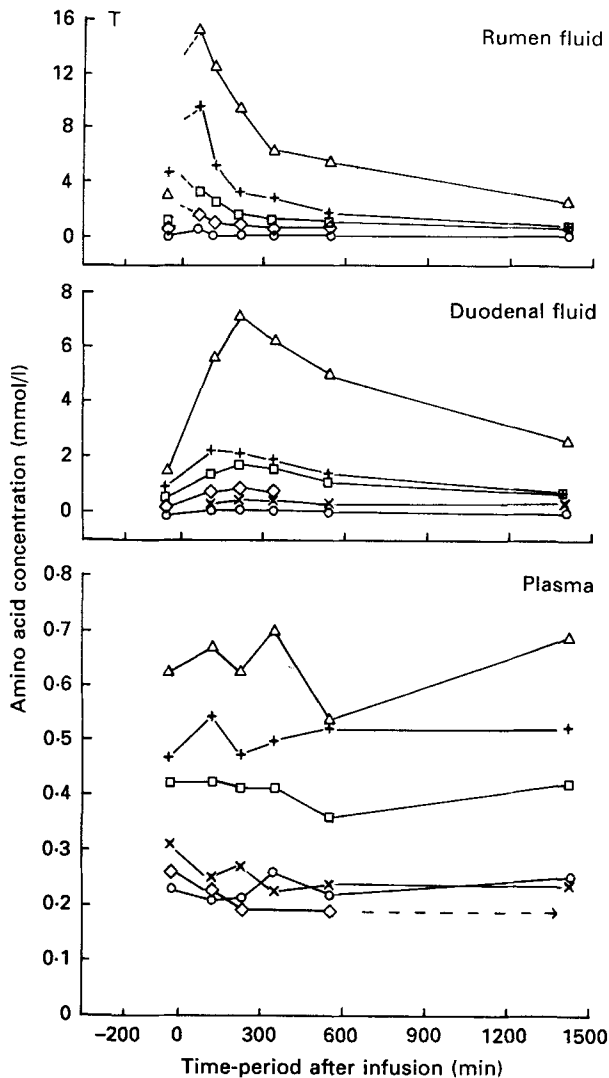


Fig. 3(b).

overestimation of flow rate and poor mixing of large doses of amino acids. Probably the explanation is to be found in the differences in solubility between amino acids. Lysine is extremely water-soluble, whereas methionine and threonine take longer to 'spread' through the rumen fluid. In accordance with this, the concentrations of methionine and threonine were high in the first sample taken at 60 min. Alternatively the initial degradation rate of lysine may also have been underestimated. The uncertainty with respect to the true concentrations at time zero was reduced by calculating degradation rates during the period 60–240 min after administration of the amino acids.

The relative rates of degradation were in general agreement with the findings of Chalupa (1976).

The  $V_{\max}$  values obtained in the present study are much higher than those reported by Chalupa (1976) and Skoch *et al.* (1975). This raises doubts about the validity of extending



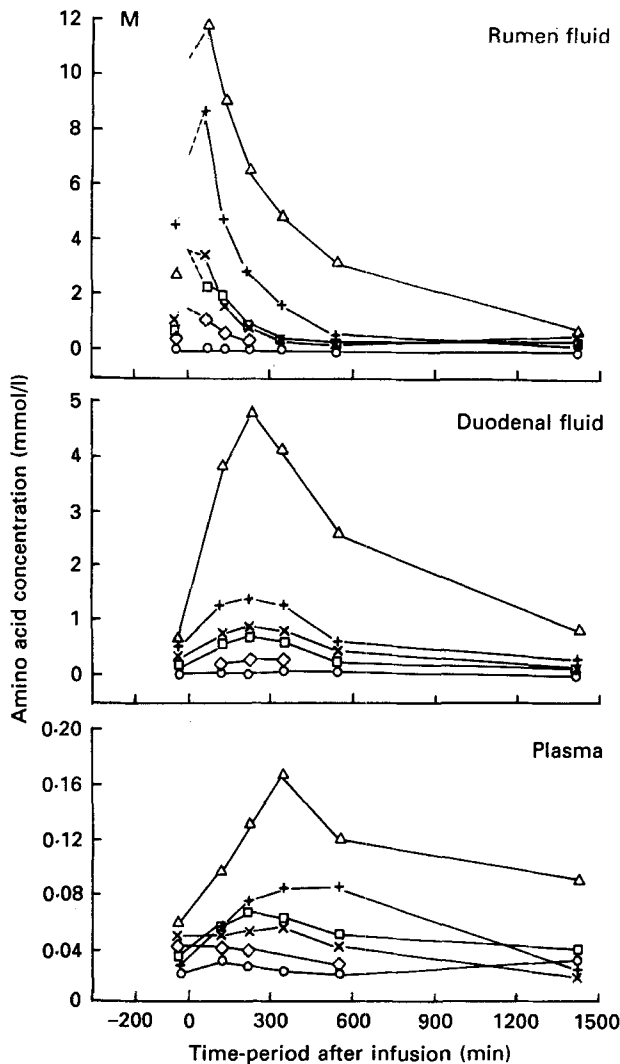


Fig. 3(c).

the previously published *in vitro* values to the *in vivo* situation. The degradation rates (mmol/l per h) obtained *in vitro* by Chalupa (1976) were much lower than the rates estimated in the present study. For example, at a concentration of 1.7 mmol/l (2.5 g amino acid in rumen), Chalupa (1976) recorded degradation rates *in vitro* of 0.08 and 0.22 mmol/l per h for methionine and lysine respectively, whereas 0.66 and 1.55 mmol/l per h were the respective rates obtained in the present study *in vivo*. The  $K_M$  values found by us (1.5–4.7 g/l) are well in excess of normal levels of these amino acids in rumen fluid. Accordingly, degradation of amino acids in the rumen on ordinary diets will be well below 50% of maximal potential velocity. Therefore degradation rates would be expected to increase substantially when large amounts of amino acids are given.

Unlike the observations made by Chalupa (1976), our results showed a rapid decrease in apparent degradation 1–2 h after administration of the amino acids as concentrations decreased (see Fig. 1).

Table 5. *Outflow of amino acids from the rumen of hay-fed sheep given intrarumen infusions of graded doses of amino acid mixtures\**

(Mean values and standard deviations for three sheep in Expts 3-5 and for two sheep in Expts 6-8)

Expt no.	Dose (g) of each amino acid	Outflow during first 24 h†											
		Lysine				Threonine				Methionine			
		mmol		%		mmol		%		mmol		%	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
3	5	3.1	0.37	9.0	1.09	14.0	1.49	33.5	3.54	5.6	0.20	16.6	0.59
4	10	7.4	2.17	10.9	3.18	27.0	9.58	32.2	11.42	14.0	3.53	21.0	5.29
5	15	13.4	1.14	13.1	1.11	50.0	9.73	39.7	7.73	28.4	4.59	28.5	4.61
7	2.5	0.7	0.23	3.9	1.32	4.8	0.21	22.6	1.01	1.7	0.52	10.3	3.12
6	5	1.9	0.07	5.6	0.21	7.0	0.56	16.7	1.33	3.3	0.47	9.7	1.39
8	15	14.6	0.42	14.2	0.41	40.8	4.76	32.4	3.78	17.2	0.38	17.1	0.38

\* For details of procedure, see p. 398. In each experiment the mixture of amino acids consisted of lysine, threonine and methionine.

† Given both in mmol and in percentage of dose of each amino acid.

Table 6. *Effect of intrarumen administration of lysine (L), threonine (T) and methionine (M) on plasma amino acid concentrations of hay-fed sheep*

(Mean values and standard deviations of peak values ( $\mu\text{mol/l}$ ) for three sheep, except for 2.5 g when only two sheep were used, in control experiments (dose 0) and changes in peak values relative to these after graded amounts of amino acid mixtures had been administered)

Dose*... Amino acids in plasma	Control 0		LTM 2.5		LTM 5.0		LTM 10.0		LTM 15.0		M 5.0	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Lysine	278	18.6	69	25.5	56	57.2	116	57.4	261	167.3	-34	18.6
Threonine	262	59.9	-39	7.9	262	47.7	284	165.6	592	226.4	52	92.1
Methionine	37	1.3	6	0.5	31	8.2	56	20.4	146	80.8	24	8.5
Glycine	1992	279.1	-636	207.3	-630	129.0	-760	297.0	-1121	83.5	NV	
Arginine	310	32.3	-65	35.1	-36	14.4	-72	40.8	-96	42.0	16	18.7
Tyrosine	79	1.5	-3	11.8	-4	14.3	-18	8.1	-17	8.7	7	5.3
Valine	401	32.5	-80	15.0	-78	61.3	-198	47.0	-210	31.0	31	18.4

NV, no value.

\* Dose (g) of each amino acid in the mixture.

The degradation of methionine appeared slightly more rapid (+9%) and more complete (+4%) when given alone than when given together with the two other amino acids. The differences in methionine degradation rates were not significant and were smaller than those found by Chalupa (1976) (+86%), but similar to those of Skoch *et al.* (1975) (+10%).

It should be pointed out that fractions of the administered lysine, threonine and methionine probably were incorporated directly into microbial protein. Thus intraruminal degradation may have been slightly overestimated by our method. If this is the case, not only did the animals get the benefit of the fractional outflow of free amino acids, but also of the fractions of the same amino acids carried by microbial protein into the intestine.

The validity of the estimated outflow of intact amino acids from the rumen (Table 5) was strengthened by the demonstrable dose-dependent increase in the concentrations of the same amino acids in duodenal fluid (Fig. 3).

For cows fed on graded amounts of urea, Broderick *et al.* (1981) found 7, 16 and 12% of the requirements for methionine, lysine and threonine could be covered by the passage of rumen free-amino-acids. The requirements of these amino acids in sheep have been estimated by various authors as follows: methionine (g/d): 2.7 for 32 kg animals (Strath & Shelford, 1978), 3.2 for 87 kg animals (Armstrong & Annison, 1973), 5.9 for 87 kg animals (Reis *et al.* 1978); lysine (g/d): 6.8 for 45 kg animals (Brooks *et al.* 1973); threonine (g/d): 9.5 for 87 kg animals (Armstrong & Annison, 1973).

It has become conventional to assume that dietary amino acids are extensively degraded in the rumen, such that efforts to balance particular amino acid needs of ruminants would require protection of dietary amino acids from rumen degradation. The present results suggest that important proportions of tissue amino acid needs might possibly be met by giving amino acids in unprotected form. Thus at a dose level of 15 g amino acid, amounts of 2.0, 5.6 and 4.3 g lysine, threonine and methionine respectively left the rumen in an intact form, representing approximately 30, 60 and 70% of the daily requirements respectively. When 5 g amino acid were administered, the peak concentration of amino acid in duodenal fluid was increased approximately tenfold relative to the controls (Fig. 3).

Following intrarumen administration of the three amino acids at dose rates of 5, 10 and 15 g/d for 5 d, the plasma levels of these acids increased rapidly in a dose-dependent manner (Fig. 3 and Table 6). At a 5 g/d dose rate, peak plasma amino acid concentrations increased by 23, 64 and 109% compared with control levels for lysine, threonine and methionine respectively. As regards methionine, Doyle & Adams (1980), using a continuous intrarumen infusion technique, did not observe any increase in plasma levels above 36  $\mu\text{mol/l}$  until the daily dose was increased to 24 g. The discrepancy between the results of Doyle & Adams (1980) and the present findings is probably due to the different modes of administration. Continuous infusion undoubtedly facilitates degradation much more than when single doses are administered. Also, contrary to Reis *et al.* (1978), who did not find an increase in plasma methionine when single doses of 10 g methionine were given orally, significant increases were observed in our study even at a dose level as low as 5 g.

The fractional outflow from the rumen in 24 h was much higher for threonine than for the two other amino acids studied. Threonine appeared to be degraded very rapidly for 1–2 h, but had the lowest degradation over the 24 h period. The reason for this is not known.

Intrarumen amino acid injections seemed to cause a reduction in the concentration of glycine, valine and arginine in plasma (Table 6). Arginine is involved in urea formation. Its plasma level was reduced when lysine and threonine were given. This was not found by Nimrick *et al.* (1970). Brooks *et al.* (1973) reported a reduction in the levels of arginine as well as valine in plasma when lysine was abomasally infused in amounts of up to 4 g/d, but an increase in arginine and valine occurred when doses up to 8 g/d were given. Following methionine administration, a reduction in plasma glycine level has previously been reported by Strath & Shelford (1978) and Tao *et al.* (1974). Glycine is involved in several reactions in which methionine also participates (formation of creatine, cysteine and thereby also glutathione). Administration of methionine may stimulate the biosynthesis of these compounds.

In conclusion our results show that rumen microbes of hay-fed sheep have a high capacity for degradation of unprotected amino acids. Nevertheless, even when given in moderate doses, substantial amounts of the amino acids studied flow out of the rumen in an intact form. Economic comparisons of feeding unprotected *v.* protected amino acids

should be done on the basis of the cost of providing amino acids to the tissues and the resulting production response. Finally, previous findings of differences between amino acids in rates of apparent degradation were substantiated.

The technical assistance of Dieneke Grønset was greatly appreciated.

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