PLASMA AMINO ACID PATTERNS IN SHEEP RECEIVING ABOMASAL INFUSIONS OF METHIONINE AND CYSTINE

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Abstract

Plasma amino acid patterns were studied in sheep receiving varying amounts of DL- or L-methionine (0·6–10·0 g/day) or L-cystine (2·0 and 8·0 g/day) as abomasal supplements or DL-methionine (10 g/day) as a dietary supplement.

Total amino acids in plasma decreased when small amounts (0·6–2·5 g/day) of methionine were infused, but increased substantially with the larger amounts of methionine (4·9–10·0 g/day). However, when methionine and taurine were excluded, the remaining amino acids decreased with all amounts of methionine. Cystine supplementation caused smaller changes in total amino acids.

Methionine infusions significantly increased the concentration of methionine, cystine, taurine, and cystathionine in plasma. Cystine infusions increased the concention of the latter three compounds, but not that of methionine. Infusion of small amounts of methionine (0·6–2·5 g/day) produced only small increases in plasma methionine; with the infusion of larger amounts plasma methionine increased rapidly. The relationship between plasma methionine and amount of methionine infused was described by two straight lines which intersected at about 3·3 g/day of methionine. With an infusion of 10 g/day the mean plasma methionine concentration was 235 μmoles/100 ml, which represented 64% of the total plasma amino acids. In contrast, an equimolar amount of cystine (8 g/day) produced only a small increase in plasma cystine, and had no effect on plasma methionine.

Infusions of both methionine and cystine caused considerable reductions in the plasma concentrations of the branched-chain amino acids (valine, leucine, and isoleucine), and of serine and glycine.

Dietary supplements of methionine (10 g/day) caused no change in the total concentration of amino acids in plasma nor in the concentration of any individual amino acid.

I. Introduction

Abomasal or parenteral administration of sulphur-containing amino acids (*S*-amino acids) has a substantial influence on the rate of wool growth (see Reis *et al.* 1973). Whereas supplements of 1–2 g/day cyst(e)ine or methionine markedly increase wool growth rate, the administration of larger amounts of methionine (6–10 g/day), but not of cystine, has an adverse effect on the rate of wool growth (Reis 1967; Reis *et al.* 1973). These effects may be mediated by changes in the supply of amino acids to the wool follicles, as the synthesis of wool keratin is presumed to proceed from free amino acids (Downes 1961; Mercer 1961; De Bersaques and Rothman 1962).

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The addition of excessive amounts of methionine to the diet of rats and humans changes the plasma amino acid profile markedly and results in very high levels of methionine in plasma (Klavins 1965; Daniel and Waisman 1968; Snyderman *et al.* 1968). Two preliminary reports (Schelling 1970; Wakeling *et al.* 1970) indicated that, when DL- or L-methionine was infused into the abomasum or duodenum of sheep, there was a substantial increase in plasma methionine above a certain level of infusion. Reis and Tunks (1971) compared the effects of abomasal infusions of 2 and 10 g/day DL-methionine on the plasma amino acid profile; very high levels of plasma methionine were obtained with the higher infusion rate.

In the present experiments, changes in plasma amino acid patterns in response to supplements of *S*-amino acids were studied to determine whether these changes could be related to the effects of *S*-amino acids on wool growth (Reis 1967; Reis *et al.* 1973). Plasma samples were obtained from sheep in two of the experiments reported by Reis *et al.* (1973), in which abomasal supplements of DL- and L-methionine and L-cystine were given primarily to study the effects on wool growth, and from sheep receiving dietary supplements of DL-methionine.

II. Experimental

(*a*) *Sheep and Diet*

Mature Merino wethers, each fitted with an abomasal cannula, were kept in metabolism cages in a room maintained at 23 ± 3°C. The daily ration was 800 g of a mixture of equal parts of chopped wheaten and lucerne hays, fed either as a loose mix (expts. 1 and 3) or ground and pelleted (expt. 2). The sheep were fed once daily at about 10 a.m. (expts. 1 and 3) or eight times daily (100 g every 3 hr; expt. 2). Drinking water was available *ad libitum*.

(*b*) *Plan of Experiments*

Amino acids were administered as either abomasal infusions or dietary supplements in three experiments. Complete details of the infusion experiments, and of the procedures for administration of amino acids, are given by Reis *et al.* (1973). Blood samples were collected from the jugular vein as indicated in each experiment.

Experiment 1.—[See Reis *et al.* (1973), expt. 1]. Four sheep were given four dose levels of L-methionine (0·62, 1·23, 2·46, and 4·92 g/day) by continuous infusion into the abomasum in successive 14-day periods. Blood samples were taken 4–5 hr after feeding, during a control period and on the ninth day of infusion periods 2, 3, and 4. Thus, blood samples were only available for three sheep receiving each amount of methionine.

Experiment 2.—[See Reis *et al.* (1973), expt. 3]. Two sheep received L-cystine (2·0 and 8·0 g/day) and two sheep received DL-methionine (2·5 and 10·0 g/day) as continuous abomasal infusions during two periods, each of 20 days. Six blood samples were taken from each sheep, at about 2 p.m as follows: two pre-experimental, and two on the fifteenth and eighteenth day of each infusion period. Note that with one sheep (No. 9057) the infusion of 10 g/day methionine was stopped after 10 days due to feed refusals and an infusion of 6 g/day was given for the last 7 days of the 20-day period. Blood samples were taken on the fourth and fifth days of the 6-g infusion.

Experiment 3.—Two sheep were given 10 g/day DL-methionine added to the diet. Blood samples were taken from each sheep 4–5 hr after feeding during an initial control period and on the ninth and eleventh day of methionine supplementation.

(*c*) *Amino Acid Analysis*

The collection of blood and the preparation of plasma for amino acid analysis was carried out as described by Hogan *et al.* (1968). A sample corresponding to 1·0 ml plasma was analysed with a Technicon amino acid analyser (Technicon Co., New York), using a 145 by 0·6 cm column with a Technicon type A resin, and a gradient elution system based on that of Efron (1966), modified to give adequate resolution in 16 hr. With this system tryptophan was not determined and proline did not separate consistently. Amino acid concentrations were calculated by reference to norleucine, used as an internal standard. Methionine concentrations include methionine sulphoxide and sulphone, when present.

TABLE 1

EFFECT OF ABOMASAL INFUSION OF METHIONINE ON THE CONCENTRATION OF AMINO ACIDS IN PLASMA

Plasma samples are from experiment 1. Samples were taken from three sheep on one occasion during the infusion of each supplement, and from four sheep on two occasions when no supplement was given. Each value is thus a mean of eight determinations, or an adjusted mean of three determinations

Amino acid	Plasma amino acid concentrations (μmoles/100 ml) for L-methionine supplementation (g/day) of:					Significance of infusion effects
	Nil	0·62	1·23	2·46	4·92	
Glycine	42·9	38·1	38·6	30·9	32·3	n.s.†
Serine	17·8	13·0	10·6	10·4	9·1	**
Alanine	14·1	15·7	15·3	13·8	12·8	n.s.
Ornithine	7·4	6·0	5·4	5·6	6·6	n.s.
Glutamic acid	6·3	6·0	6·4	6·7	5·5	n.s.
Aspartic acid	3·6	2·9	2·8	4·0	4·4	n.s.
Tyrosine	3·1	5·2	4·2	5·8	5·5	n.s.
Threonine	10·6	11·0	11·9	9·5	8·7	n.s.
Valine	11·8	10·2	8·3	8·3	7·5	**
Leucine	5·0	4·1	3·2	3·4	2·4	**
Isoleucine	4·5	4·2	3·5	3·6	2·6	*
Lysine	7·2	6·8	5·8	5·8	5·4	n.s.
Histidine	4·6	5·3	5·8	6·8	6·1	**
Arginine	8·6	9·2	10·2	9·0	9·5	n.s.
Phenylalanine	2·6	2·7	3·2	3·9	3·4	**
Cystine (half)	1·6	2·4	3·1	3·7	3·8	**
Methionine	0·6	1·7	0·5	5·1	38·9	**
Taurine	2·2	3·0	3·8	9·1	20·5	**
Cystathionine	Nil	Nil	0·1	0·8	3·7	**
Total amino acids	155	145	143	146	189	*
Total amino acids‡	152	140	139	131	126	n.s.

* $P < 0·05$. ** $P < 0·01$. † n.s.,not significant at 5% level.

‡ Excluding methionine, taurine, and cystathionine.

Disulphide-bound cystine in plasma (Downes *et al.* 1965) was estimated as the difference between total plasma cyst(e)ine and free cyst(e)ine as measured in the amino acid analytical system described above; results were expressed as half-cystine residues. Total plasma cyst(e)ine was measured by a procedure based on the method of Gaitonde (1967), using dithiothreitol (Cleland 1964) to convert free and disulphide-bound cystine to cysteine.

(d) Statistical Analysis

In the methionine infusion experiments the experimental design was non-orthogonal. Adjusted mean values were calculated where appropriate and analysis of variance was used to assess the significance of the changes in plasma amino acid concentration during infusion of methionine and cystine.

III. Results

The total concentration of amino acids in plasma was significantly altered by methionine infusion; it was decreased with small amounts of methionine but was increased by amounts of methionine above 2·5 g/day (Tables 1 and 2). However, the increases were due largely to substantial increases in methionine, and to a lesser extent taurine. When methionine, taurine, and cystathionine were excluded, the remaining amino acids were significantly decreased in experiment 2 by all levels of methionine infusion; in experiment 1, although there appeared to be a small decrease in these amino acids related to the amount of methionine infused, the effect was not significant. The total concentration of amino acids in plasma was also significantly altered by cystine infusion (Table 2). The effects were smaller than those observed with methionine.

Table 2

EFFECT OF ABOMASAL INFUSION OF METHIONINE AND CYSTINE ON THE CONCENTRATION OF AMINO ACIDS IN PLASMA

Plasma samples are from experiment 2. Samples were taken from two sheep on two separate occasions, except with 6 and 10 g methionine, when only one sheep was sampled on two occasions. Each value is thus a mean of four determinations, or an adjusted mean of two determinations

Amino acid	Plasma amino acid concn. (μmoles/100 ml) for L-cystine supplementation (g/day) of:			Significance of infusion effects	Plasma amino acid concn. (μmoles/100 ml) for DL-methionine supplementation (g/day) of:				Significance of infusion effects
	Nil	2·0	8·0		Nil	2·5	6·0	10·0	
Glycine	50·0	35.1	37·5	**	58·7	29·6	32·8	22·7	**
Serine	18·9	11.9	11·3	**	21·8	8·8	8·7	9·8	**
Alanine	23·3	25·0	24·6	n.s.	17·6	16·4	21·1	10·8	**
Ornithine	7·6	5·0	4·9	*	7·3	4·2	4·7	4·2	**
Glutamic acid	4·8	5·2	5·3	n.s.	4·7	5·0	3·9	2·9	n.s.†
Aspartic acid	3·4	2·9	2·6	**	3·6	2·1	1·7	4·4	*
Tyrosine	5·5	7·0	7·6	**	6·2	6·6	4·9	5·2	*
Threonine	33·2	29·7	29·0	n.s.	32·2	23·8	19·1	24·7	**
Valine	17·4	12·1	11·6	**	17·5	10·8	11·0	8·9	**
Leucine	8·6	6·6	5·6	**	8·6	5·6	5·8	3·0	**
Isoleucine	7·6	6·4	5·9	*	6·7	5·4	4·0	2·3	**
Lysine	10·2	9·5	8·0	*	10·3	7·2	8·5	6·4	**
Histidine	5·6	6·3	6·9	n.s.	5·8	6·2	7·4	6·3	**
Arginine	9·8	9·8	8·3	n.s.	9·2	8·8	9·8	6·7	n.s.
Phenylalanine	3·6	4·4	5·6	**	4·0	4·4	4·5	3·3	*
Cystine (half)	2·2	5·2	9·2	**	1·3	3·9	3·2	5·1	**
Methionine	2·8	3·9	3·5	n.s.	2·7	9·8	145·2	233·9	**
Taurine	1·2	10·4	30·1	**	0·2	9·5	12·8	8·5	**
Cystathionine	Trace	0·5	2·3	**	Trace	1·7	1·8	2·0	**
Total amino acids	216	197	220	*	219	170	311	371	**
Total amino acids‡	212	182	184	*	216	149	151	127	**

* $P < 0·05$. ** $P < 0·01$. † n.s., not significant at 5% level.

‡ Excluding methionine, taurine, and cystathionine.

When individual plasma amino acids are considered, the most notable effects of methionine and cystine infusions were on the *S*-amino acids themselves and on their metabolites, cysathionine and taurine. Increasing the amount of methionine infused into the abomasum resulted in the appearance of cystathionine in plasma, together with substantial increases in plasma taurine and small increases in plasma cystine (Tables 1 and 2). Infusion of amounts of methionine up to 2·5 g/day produced only small increases in plasma methionine; the infusion of larger amounts of methionine caused considerable increases in plasma methionine (Tables 1 and 2). With an infusion of 10 g/day methionine (experiment 2) the mean plasma methionine concentration of 235 μmoles/100 ml represented 64% of the total plasma amino acids.

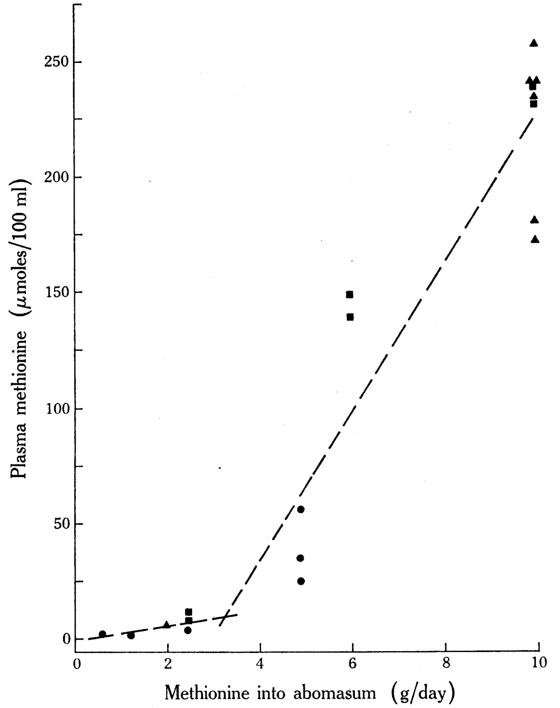

Fig. 1.—Effect of abomasal infusion of methionine on the concentration of methionine in plasma. The data are derived from experiments 1 (●) and 2 (■), and from Reis and Tunks (1971) (▲). Individual plasma methionine values are plotted for the three highest levels of methionine infusion. At the lower levels of infusion (0·6–2·5 g/day) the range of values was too small for individual values to be plotted; the points represent three values (expt. 1), two values (expt. 2), and six values (Reis and Tunks 1971). The two dotted lines were fitted by two-piece regression analysis.

The relationship between plasma methionine and the amount of methionine infused into the abomasum is shown in Figure 1; data from experiments 1 and 2, and from Reis and Tunks (1971), are presented in the figure. Initially there was only a slight increase in plasma methionine concentration with increasing amounts of methionine infused, followed by a steep rise with larger amounts infused. The relationship between plasma methionine and amount of methionine infused was described by two straight lines (fitted by two-piece regression analysis) which intersected at about 3·3 g/day of methionine infused, with 95% confidence limits of 1·9 and 4·7 g/day.

In contrast to the effects of an infusion of 10 g/day methionine, an equimolar amount of cystine (8 g/day) produced only a small increase in plasma cystine, and no effect on methionine (Table 2). Cystine infusions had similar effects to methionine infusions on plasma cystathionine, and plasma taurine was increased to a greater extent than with methionine infusions (Table 2).

The concentrations of many other amino acids in plasma were also significantly altered by the methionine and cystine infusions (Tables 1 and 2). However, the results of the statistical analysis should be treated with caution, because of the limited number of samples analysed and the different responses obtained with some amino acids in experiments 1 and 2. Cystine and methionine infusions both produced substantial decreases in the concentration of two groups of amino acids in plasma, the branched-chain amino acids (valine, leucine, and isoleucine) and the non-essential amino acids (serine and glycine). Although in experiment 1 (Table 1) the two lower amounts of methionine had little effect on plasma glycine and the overall effect of the infusion just failed to reach significance at the 5% level, a decrease in the concentration of glycine in plasma does appear to be a consistent effect of *S*-amino acid supplementation. Changes in the concentration of other amino acids in plasma were either small, inconsistent, or not significant; more extensive data would be needed to establish any effects of methionine or cystine supplementation.

When DL-methionine was given as a dietary supplement (10 g/day, experiment 3), the results were in marked contrast to those observed with the abomasal infusion of the same amount of methionine. There was no change in the total concentration of amino acids in plasma nor in any individual amino acid, when compared with the basal values (Table 3).

TABLE 3

EFFECT OF DIETARY SUPPLEMENTS OF METHIONINE ON THE CONCENTRATION OF AMINO ACIDS IN PLASMA

Plasma samples are from experiment 3. Samples were taken from two sheep on two separate occasions. Each value is thus a mean of four determinations

Amino acid	Plasma amino acid concn. (μmoles/100 ml) for DL-methionine supplementation (g/day) of: Nil	10	Amino acid	Plasma amino acid concn. (μmoles/100 ml) for DL-methionine supplementation (g/day) of: Nil	10
Glycine	41·9	43·1	Isoleucine	6·3	5·1
Serine	17·5	16·7	Lysine	7·2	9·6
Alanine	16·9	15·6	Histidine	5·7	5·5
Ornithine	8·7	8·8	Arginine	8·0	9·3
Glutamic acid	5·2	5·0	Phenylalanine	3·8	4·0
Aspartic acid	3·4	3·7	Cystine (half)	1·6	1·5
Tyrosine	6·2	4·7	Methionine	2·0	1·2
Threonine	26·8	24·4	Taurine	0·7	0·6
Valine	12·5	12·8	Cystathionine	Trace	Trace
Leucine	7·6	6·9			
Total amino acids				182	178
Total amino acids†				179	177

† Excluding methionine, taurine, and cystathionine.

In experiment 2, total plasma cystine (free plus disulphide-bound) was measured and disulphide-bound cystine was obtained by difference. The main effect of L-cystine infusions was to increase free cystine, although disulphide-bound cystine may have increased slightly with an infusion of 8 g/day (Table 4). Infusion of 2·5 g/day DL-methionine had a similar effect to an equimolar amount of cystine (2·0 g/day), but when larger amounts of methionine were given disulphide-bound cystine was reduced (Table 4).

TABLE 4

EFFECT OF ABOMASAL SUPPLEMENTS OF CYSTINE AND METHIONINE ON FREE AND DISULPHIDE-BOUND CYSTINE IN PLASMA

Plasma samples are from experiment 2. Samples were taken on two separate occasions for each treatment. Each value is thus a mean of two determinations

Sheep No.	Abomasal infusion (g/day of amino acid)		Plasma cystine* (μmoles/100 ml)		
			Free	Total	Disulphide-bound†
5842	Nil		1·4	7·6	6·2
	L-Cystine	2·0	3·6	9·6	6·0
	L-Cystine	8·0	6·0	13·2	7·2
9055	Nil		2·3	9·3	7·0
	L-Cystine	2·0	5·0	12·4	7·4
	L-Cystine	8·0	9·8	17·8	8·0
6003	Nil		1·4	7·6	6·2
	DL-Methionine	2·5	3·5	9·6	6·1
	DL-Methionine	10·0	3·8	6·4	2·6
9057	Nil		0·6	7·9	7·3
	DL-Methionine	2·5	2·6	8·6	6·0
	DL-Methionine	6·0	1·6	6·6	5·0

* Half cystine. † Obtained by difference between total and free cystine.

IV. DISCUSSION

The effects of the infusion of *S*-amino acids on plasma amino acids will be discussed in relation to the effects on wool growth reported in a previous paper (Reis *et al.* 1973). These effects should be considered in relation to an estimated supply from the basal ration of 1·0–1·5 g/day *S*-amino acids (cyst(e)ine + methionine) available for absorption from the intestines (Reis 1967).

It is apparent that the abomasal infusion of either methionine, which can be irreversibly converted to cyst(e)ine (Meister 1965), or of cystine can substantially alter the pattern of free amino acids available to the wool follicles via the blood supply. The effects are greatest when large amounts of methionine are given. Infusion of 10 g/day methionine results in very high concentrations of methionine in plasma and also has an adverse effect on wool growth rate (Reis 1967; Reis *et al.* 1973). In contrast, small amounts of methionine (0·6–2·5 g/day) that stimulate wool growth (Reis 1967; Reis *et al.* 1973) have little effect on plasma methionine concentration. Thus, the inhibitory effects on wool growth obtained with infusions of large amounts of methionine are related to the accumulation of methionine. High concentrations of methionine in plasma could affect the transport of various amino acids, as suggested by Reis (1967), and hence inhibit wool growth.

In contrast to methionine, the infusion of large amounts (8 g/day) of cystine does not result in high concentrations of cystine or methionine in plasma nor does it reduce wool growth rate (Reis *et al.* 1973). Thus, it appears that the sheep is able to remove large amounts of cystine from the circulation quite rapidly; the increase in plasma taurine during cystine infusion indicates breakdown of cystine. The measurements of disulphide-bound cystine do not indicate any increased storage of cystine in this form when large amounts of cystine are infused. The high concentrations of methionine and the low levels of taurine in plasma when large amounts of methionine are infused indicate that the sheep is unable to convert a large influx of methionine to cysteine, which could then be readily removed. This lack of conversion may be attributed to a reduced availability of serine (indicated by lower plasma values) and a decrease in cystathionine synthase activity. These effects have been noted in rats receiving large amounts of methionine (Daniel and Waisman 1969; Girard-Globa *et al.* 1972).

The considerable decreases in the concentration of serine and glycine when large amounts of methionine are given may be related to the need for these amino acids to alleviate the effects of excess methionine (Benevenga and Harper 1967, 1970; Adkins *et al.* 1968; Girard-Globa *et al.* 1972), and the requirement for serine in the conversion of methionine to cysteine via cystathionine. However, no explanation can be given of why cystine infusions also reduced the concentration of glycine and serine in plasma.

The reduction in total plasma amino acids (excluding methionine) with methionine infusions confirms the observations of Reis and Tunks (1971), who pointed out that there may be a number of explanations for this effect. The reduction in the concentration of some essential amino acids in plasma (valine, leucine, and isoleucine) with infusions of either methionine or cystine is consistent with the expected effects of supplying a limiting amino acid, and may indicate that the utilization of these amino acids for the synthesis of wool or tissue proteins was stimulated by supplying methionine or cystine. Because wool proteins contain very little methionine (Crewther *et al.* 1965), methionine may stimulate wool growth mainly by supplying cyst(e)ine, and either amino acid may be regarded as limiting for wool growth. With large amounts of methionine infused into the abomasum, although utilization of amino acids for wool growth, as discussed above, would be depressed, the utilization of amino acids for body weight gain would be stimulated (Reis 1967). Thus, it is not unreasonable to postulate the increased utilization of some essential amino acids due to infusion of large amounts of methionine, even though wool growth is depressed.

It has been proposed that the requirement for a limiting amino acid can be determined from the inflexion point of a curve relating the plasma concentration of the amino acid to the dietary supply. This technique has been applied to rats (Morrison *et al.* 1961; McLaughlan and Illman 1967; Stockland *et al.* 1970), chickens (Zimmerman and Scott 1965), pigs (Mitchell *et al.* 1968; Keith *et al.* 1972), and sheep (Wakeling *et al.* 1970). In the latter study the supply of the amino acid to the duodenum replaced the dietary supply. However, this procedure may not be a valid one for assessing the amount of methionine required for maximum stimulation of wool growth. From Figure 1, the amount of extra methionine required at the abomasum was estimated as 3·3 g/day, with 95% confidence limits of 1·9 and 4·7 g/day. Although a precise estimate of the methionine requirement for wool growth cannot be made from the data available, this estimate is above the amount of supplementary methionine (1·0–2·0 g/day) required for maximum stimulation of wool growth on this diet, when the wool growth responses to methionine infusions were measured (Reis 1967; Reis *et al.* 1973).

There were differences between experiments in the total concentration of amino acids in plasma during the control period; in particular there were large differences in the concentration of threonine. However, within an experiment the variation was small for all amino acids, even though within- and between-sheep differences were combined. The differences between experiments may be related to the feeding regime (once daily in experiments 1 and 3; eight times daily in experiment 2). However, Theurer *et al.* (1966) reported only small diurnal variations in the concentration of most amino acids in plasma from sheep fed once daily, and Leibholz (1965) found larger but transient increases in plasma amino acids for 1–3 hours following feeding. Sampling at 4–5 hours after feeding with once daily feeding was therefore chosen to minimize variability within an experiment.

The failure of dietary supplements of 10 g/day methionine to alter plasma amino acids, in contrast with the effects of abomasal infusions, indicates that very little extra methionine was absorbed intact from the gastrointestinal tract, and provides indirect evidence for substantial degradation of methionine by rumen microorganisms. Even if the dietary supplements of 10 g/day methionine resulted in the absorption of only 1–2 g/day methionine, some changes in plasma amino acid patterns (e.g. total amino acids, branched-chain amino acids, serine, and glycine) should have been evident. This result is in accord with the general failure of methionine as a dietary supplement for wool growth (see Reis *et al.* 1973), and with the evidence of Downes *et al.* (1970) for the poor utilization of dietary supplements of [^{35}S] methionine. The results do not support the suggestion of Bird (1972) and Bird and Moir (1972) that the degradation of methionine in the rumen may be sufficiently slow for dietary supplements to stimulate wool growth.

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