COMPARATIVE DIGESTIBILITY OF SULPHUR AMINO ACIDS IN RUMEN BACTERIA AND FUNGAL PROTEINS BY SHEEP

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SUMMARY

Mixed rumen bacteria were labelled with ^{35}S sulphate in anaerobic batch culture inoculated with rumen fluid from a sheep fed a lucerne and wheaten hay diet. Amino acid analysis revealed that the label was incorporated into bacterial protein as methionine and cystine. Absorption of these amino acids from the small intestine was measured in sheep prepared with permanent fistulae in the abomasum and terminal ileum, by introducing radioactive labelled bacteria into the abomasum together with Cr-EDTA as the marker for digesta flow. The proportion of ^{35}S-sulphate that was incorporated into bacterial protein which appeared unabsorbed at the terminal ileum averaged 0.23±0.012 (mean±S.E.; n = 3). The absorption of sulphur amino acids from bacterial protein was substantially lower than that obtained for fungal protein using similar procedures.

I. INTRODUCTION

Micro-organisms in the rumen degrade a large proportion of dietary proteins and utilise some of the degradation products in their own protein synthesis (Hungate, 1966). These microbes also utilise non-protein nitrogen (NPN) compounds and can upgrade the dietary proteins of low biological value into microbial proteins of higher biological value (Bergen et al. 1967). Whereas the degradation of the dietary proteins of high biological value is a disadvantage, the utilisation of NPN by the rumen microbes is very advantageous in animal feeding. Therefore it is important to know the digestibility of the protein and amino acids in the microbial population as they constitute most of the protein and amino acids normally absorbed by grazing ruminants (Van der Walt and Meyer 1988). There have been several direct determinations of the digestibility of microbial proteins in ruminants where the digestibility of bacteria was reported to be from 0.39 to 0.85. (Bird 1972; Smith et al. 1975; Elliott and Little 1977; Storm et al. 1983; Salter and Smith 1984; Siddons et al. 1985). Recently we have determined the digestibility of a variety of amino acids from protein of anaerobic rumen fungi and obtained values in excess of 0.90 (Gulati et al. 1989). In this paper we use of the same methods to determine the digestibility of sulphur amino acids (SAA) in mixed rumen bacteria (MRB) and demonstrate that the SAA absorption is lower than for anaerobic fungi.

II. MATERIALS AND METHODS

(a) Chemicals

Sodium [^{35}S]-sulphate (21.1 TBq/mmol) was obtained from Amersham (Australia) Pty Ltd. Other chemicals used were of analytical reagent grade.

(b) Preparation of labelled MRB

MRB were grown in batch culture in a 2 l stirred fermenter containing anaerobic growth medium at 39^{0}C with sucrose (0.5% w/v) as the sole carbon

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source, using the procedures outlined by Gulati et al. (1989), except that no antibiotics were used. The inoculum was 5% (v/v) rumen fluid obtained via a rumen fistula from a sheep that had been fasted for 12 h and maintained on a diet of 800 g/day lucerne and wheaten hay (1:1, w/w). The ^{35}S-sulphate label containing 18.5 MBq was added just prior to inoculation. After growth for 18 h the bacteria were separated from protozoa by centrifuging at 200 g for 2 minutes. The supernatant was further centrifuged at 20,000 g for 20 minutes and the bacterial pellet was washed by centrifugation from distilled water (2 x 100 ml) and subsequently freeze dried.

(c) Animals and experimental procedures

Three adult merino wethers were surgically prepared as described by Hecker (1974) with permanent cannulas in the abomasum near the pylorus and another in the small intestine, 100-150 mm anterior to the ileo-caecal junction. During the experimental period the animals were maintained in metabolism cages in a temperature-controlled (24^{0}C) room on a diet of pelleted lucerne hay and oats (60:40, w/w). Each sheep was fed 800 g/d in eight equally spaced meals to maintain a steady state flow of digesta (Hogan, 1981). MRB, 0.5g dry weight (containing 0.41 MBq/g ^{35}S) were rehydrated overnight (18 h) in 40 ml distilled water at 4^{0}C. Before use, 10 ml Cr-EDTA solution, prepared according to Binnerts et al. (1968), was added to the bacterial suspension and the mixture homogenised using an Ultra-Turrax mixer. The bacterial mixture was quantitatively administered into the abomasum and digesta were sampled at the terminal ileum by procedures previously described (Gulati et al. 1989).

(d) Analytical Methods

The procedures used to determine chromium, radioactivity and amino acids in digesta and bacteria, and the calculation of the amino acids remaining unabsorbed were described by Gulati et al. (1989).

Table 1. Amino acid composition and distribution of ^{35}S in mixed rumen bacteria (MRB)

Amino acid	m moles %	^{35}S-protein* - % Distribution
1/2 Cystine	1.16	80.3
Aspartic acid	10.49	
Methionine	3.84	15.7
Threonine	7.13	
Serine	5.67	
Glutamic acid	12.31	
Proline	3.01	
Glycine	7.89	
Alanine	9.39	
Valine	6.51	
Isoleucine	7.08	
Leucine	6.47	
Tyrosine	4.05	
Phenylalanine	3.57	
Lysine	6.54	
Histidine	1.45	
Arginine	3.45	

* 90.4% of the ^{35}S incorporated into the MRB was in protein.

III. RESULTS AND DISCUSSION

Phase contrast microscopy indicated that the MRB used in this experiment were a normal diverse population and representative of bacterial populations in the rumen. It also revealed that the ciliate protozoa were removed by the procedures used. The amino acid profile and distribution of ^{35}S in the SAA of the MRB are summarized in Table 1. These values for the individual amino acids are similar to those reported for washed rumen bacteria by Williams (1986) except for the SAA which are higher in our study as they were assessed by a more accurate procedure (described previously by Connell et al. 1987). When an hydrolysate of the ^{35}S-labelled MRB was subjected to amino acid analysis by ion-exchange chromatography, 96% of the radioactivity was contained in two peaks corresponding to methionine and 1/2 cystine (Table 1). The proportion of ^{35}S label, which represented cystine and methionine in the MRB protein administered, appearing unabsorbed at the terminal ileum is presented in Table 2.

Table 2. Proportion of ^{35}S in mixed rumen bacteria (MRB) and rumen fungi remaining unabsorbed at the terminal ileum

Sheep	MRB	Fungi*
1	0.210	0.026
2	0.220	0.017
3	0.250	0.019
Mean	0.230**	0.020**
SE	0.012	0.003

* Data from Gulati et al. (1989) using similar procedures, but different sheep infused with a pure culture of Neocallimastix sp. LM1 labelled with ^{35}S sulphide.

** The means are significantly different (P= 0.0046) (t-Test, unequal variance)

The results show that the true digestibility of bacterial SAA was 0.77, substantially lower than that obtained for fungi using similar procedures and conditions. The values reported here for freeze dried MRB are similar to those obtained by Elliott and Little (1977) for cyst(e)ine. In their study the digestibility of freshly infused ^{35}S-labelled rumen microorganisms was 0.72 and it is probable that bacteria would have constituted a major proportion of total protein (Van der Walt and Meyer 1988). Our values are also similar to those obtained by Storm et al. (1983) for the digestion of SAA in rumen microorganisms using lambs nourished by intragastric infusion, being 0.73 and 0.89 for cystine and methionine respectively. Moreover, they are in agreement with studies using ^{15}N-labelled microbial protein where estimates of digestion ranged from 0.72 to 0.79 (Salter and Smith 1984; Siddons et al. 1985).

From these results it would appear that the digestibility of SAA in rumen bacterial protein is less than that of rumen fungi, where values in excess of 0.90 for pure isolates were obtained (Gulati et al. 1988, 1989). Thus enhancing fungal populations in the rumen at the expense of bacteria would be unlikely to cause deleterious effects on the quality of protein available for absorption at the small intestine. This may have important implications in sheep grazing low

quality forages if procedures can be developed to increase the rumen fungal population thereby enhancing the voluntary feed intake and rate of fibre degradation.

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