

# The rate of free amino acid disappearance from the rumen content

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(Received 30 August 1993; accepted 21 February 1994)

## ABSTRACT

A mixture of free amino acids was incubated *in vitro* with rumen liquor or administered as either a single dose or continuous infusion into the rumen of sheep fitted with a simple rumen cannula and reentrant duodenal cannula. The rate of disappearance of amino acids from the incubated liquor was slow and varied for individual amino acids. The most rapidly disappearing amino acid was cysteine. Glycine, alanine, histidine and valine were decomposed more slowly reaching a level of degradation not exceeding 70% of their initial concentration after 24 h of incubation. Phenylalanine was not degraded during 24 h incubation.

*In vivo*, amino acids rapidly disappeared from the rumen at varied rates and only a small proportion of the amino acids introduced into the rumen reached the duodenum upon continuous infusion (3.4%) or single injection (7.4%). Most resistant to decomposition were thyrosine, cysteine and phenylalanine, while proline, methionine, asparagine, serine and glutamine were most readily decomposed.

**KEY WORDS:** sheep, rumen, duodenum, amino acid, deamination

## INTRODUCTION

It is generally believed that free amino acids released in the rumen from feed protein are rapidly deaminated by bacterial deaminase. Such a conclusion is based on the low free amino acid concentration in rumen liquor (Wright and Hungate, 1967; Nugent and Mangan, 1981; Wallace and Cotta, 1988).

Lewis (1956) reported that the normal level of  $\alpha$ -amino nitrogen was in the range from 0.1 to 1.4 mg/100 ml liquor in sheep fed on hay with casein, hay with dried grass or hay alone, but a value of 4.9 mg/100 ml was found in the rumen of young bulls fed a urea/molasses diet (Kowalczyk et al., 1970; Ramirez and Kowalczyk, 1971). Some authors have reported that the rate of amino acid disappearance from the rumen liquor was relatively slow (Lewis and Emery, 1962; Emery, 1971; Isaacs and Owens, 1971).

The aim of the present study was to measure the rate of disappearance of individual free amino acids from rumen liquor *in vitro* and *in vivo*.

## MATERIAL AND METHODS

*Experiment in vitro*

Rumen liquor was obtained from 3 adult sheep fitted with rumen cannulas and fed a diet consisting of 80% meadow hay and 20% ground barley with a vitamin-mineral mixture. Portions of rumen liquor were filtered through a cheese cloth and pooled to obtain a homogeneous medium for incubation. Portions of 100 ml were placed in incubation vessels and incubated alone or with a mixture of free amino acids. A mixture of 4  $\mu$ mol of each amino acid per ml of rumen liquor and hydrochloric acid or enzymatically hydrolyzed casein (30-40 mg/ml) were used as the source of free amino acids. Free amino acids were also added into the rumen liquor and boiled under a reflux column. Unsterilized rumen liquor with no addition of amino acids was also incubated as a control sample.

The medium was saturated with  $\text{CO}_2$  and the pH adjusted to 6.9 with  $\text{Na}_2\text{CO}_3$ . Incubations were carried out at 39°C with continuous bubbling of  $\text{CO}_2$ . When the proper temperature, pH and  $\text{CO}_2$  saturation were attained, the respective aliquots of amino acids were pipetted into the incubation vessels. Five millilitre samples were taken at 0, 0.5, 1, 2, 4, 6, 8, 12 and 24 h of incubation and transferred to test tubes containing appropriate amount of sulphosalicylic acid solution so as to obtain a final concentration of 3% in order to precipitate the protein and stop the enzymatic decomposition of amino acids. The test tubes were left overnight at 4°C, centrifuged and free amino acids concentrations were determined in the supernatant.

*Experiment in vivo*

The experiment was carried out with 8 sheep of 36 kg body weight fitted with simple cannulas into the rumen and reentrant cannulas into the duodenum. The animals were fed at 8.00 and 20.00 h with 400 g meadow hay and 100 g ground barley with a vitamin-mineral mixture. Solutions of 96 g hydrolyzed or unhydrolyzed casein were infused either continuously for 8 h or as a single injection into the rumen at the beginning of the morning feeding. All of the sheep were submitted to each treatment.

The samples of rumen liquor for measuring pH and amino acid concentration were withdrawn at 0, 5 and 12 h after the infusion had started or the single injection had been administered. The amount of digesta entering the duodenum was measured for 12 h by the total collection method. Five per cent equivalents of digesta were taken for analysis at 2, 4, 8 and 12 h.

Samples of the rumen liquor and duodenal digesta were spinned at 40,000 g and the supernatant stored in deep freeze until analyzed for total nitrogen

content with the Kjeldahl method and free amino acids using a T-339 Automatic Amino Acids Analyzer.

## RESULTS

### *Experiment in vitro*

The amount of free amino acids in control rumen liquor with no addition of amino acids was negligible since it was below the detection limit of the method used. The recovery of amino acids from boiled rumen liquor after 24 h incubation was about 100%. The pattern of *in vitro* disappearance of amino acids from the rumen liquor shown in Figure 1 as an average values was different for individual amino acids. The most rapidly disappearing amino acid was cysteine. Glycine, alanine, histidine and valine were decomposed more slowly, reaching a level of degradation not exceeding 70% of their initial amount after 24 h of incubation. Phenylalanine was not decomposed but, surprisingly, its amount even increased during incubation.

### *Experiment in vivo*

The pattern of ammonia concentration in the rumen liquor differed among treatments. Its concentration was similar in all groups before introduction of amino acids or casein into the rumen, but increased systematically during 12 h continuous infusion, more than when unhydrolyzed casein was given. After a single injection of amino acids or casein, the ammonia level increased about 5-fold with amino acids, and 3.5-fold with unhydrolyzed casein at 5 h post administration. Thereafter it decreased, with the lowest values found in the group receiving hydrolyzed casein (Table 1).

The pH of the rumen liquor was similar in all groups of animals at time 0, reaching average values of 6.71 and 6.25 at 5 h; after 12 h of continuous infusion the pH increased to about 6.16 and reached 6.53 after a single injection was given (Table 1).

The amount of crude protein entering the duodenum during 12 h digesta collection was less when hydrolyzed casein was given with continuous or single method than when unhydrolyzed casein was introduced and also less when hydrolyzed or unhydrolyzed casein was given by continuous method than when single injection was applied (Table 1).

The concentration of individual amino acids in the rumen liquor (Table 2) was very low in all of the animals at time 0 and after 5 and 12 h in animals given a single injection of hydrolyzed or unhydrolyzed casein or continuous infusion of unhydrolyzed casein. The exception was phenylalanine whose concentration was

TABLE 1  
Ammonia concentration, pH values in the rumen liquor and crude protein amount entering duodenum after administering of casein into the rumen

Treatment	Continuous of casein			Single injection of casein	
	Sampling time, h			Hydrolyzed	Unhydrolyzed
	Ammonia concentration, mg/100 ml				
0	20.2	22.7	21.5	20.5	
5	42.7	35.7	106.0	77.2	
12	62.1	56.7	51.2	57.8	
	pH - values				
0	6.78	6.64	6.68	6.74	
5	6.23	6.24	6.26	6.26	
12	6.14	6.18	6.51	6.56	
	Amount of crude protein entering duodenum, g				
0-12	40.4	47.4	51.2	60.8	

TABLE 2  
Free amino acid concentration in the rumen liquor after introducing into the rumen hydrolyzed or unhydrolyzed casein as continuous or as a single injection, mg/100 ml

Treatment	Continuous infusion of casein						Single injection of casein					
	Hydrolyzed			Unhydrolyzed			Hydrolyzed			Unhydrolyzed		
Amino acid	0 h	5 h	12 h	0 h	5 h	12 h	0 h	5 h	12 h	0 h	5 h	12 h
Cys	0.7	3.4	3.9	1.3	4.1	5.4	1.0	1.2	1.2	1.0	11.1	1.4
Asp	0.4	19.4	37.6	0.5	0.8	1.1	0.7	0.8	0.8	0.7	2.0	0.7
Thr	0.0	19.3	40.0	0.0	0.3	0.3	0.0	1.0	0.1	0.6	0.3	0.1
Ser	0.0	22.1	29.4	0.0	0.3	0.6	0.0	0.2	0.2	0.1	0.8	0.1
Glu	1.0	121.9	156.6	1.0	1.4	2.1	0.8	2.3	1.9	1.4	2.3	1.8
Pro	0.0	56.5	115.5	0.0	0.4	0.1	0.0	0.3	0.2	0.0	0.2	0.1
Gly	0.1	7.7	13.0	0.1	1.2	0.3	0.1	0.5	0.2	0.2	0.2	0.1
Ala	0.2	17.5	23.5	0.1	0.3	0.7	0.1	2.0	0.5	0.2	0.5	0.3
Val	0.0	30.3	50.0	0.1	1.0	0.5	0.1	6.9	0.2	0.2	0.5	1.0
Met	0.0	8.1	14.1	0.0	0.1	0.1	0.0	1.8	0.0	0.0	0.2	0.1
Ile	0.0	15.8	27.2	0.0	0.1	0.1	0.1	1.1	0.0	0.0	0.1	0.0
Leu	0.0	24.3	37.9	0.0	0.5	0.5	0.0	1.1	0.0	0.0	0.1	0.0
Tyr	0.0	8.1	5.9	0.1	1.3	1.5	0.0	0.2	0.5	0.3	2.9	0.1
Phe	0.2	21.2	32.9	0.2	2.8	3.4	0.0	72.3	0.4	0.1	20.7	0.2
His	1.0	16.7	26.2	0.4	1.5	1.9	0.4	4.3	1.0	0.6	1.6	0.5
Lys	0.3	40.0	59.6	0.3	0.9	1.1	0.3	8.4	0.4	0.3	0.9	0.4
Arg	0.0	6.5	18.8	0.0	0.0	0.2	0.0	0.2	0.0	0.0	0.0	0.0
Total AA	3.9	438.8	688.1	4.1	17.0	19.9	3.7	104.6	7.6	5.7	44.4	6.9

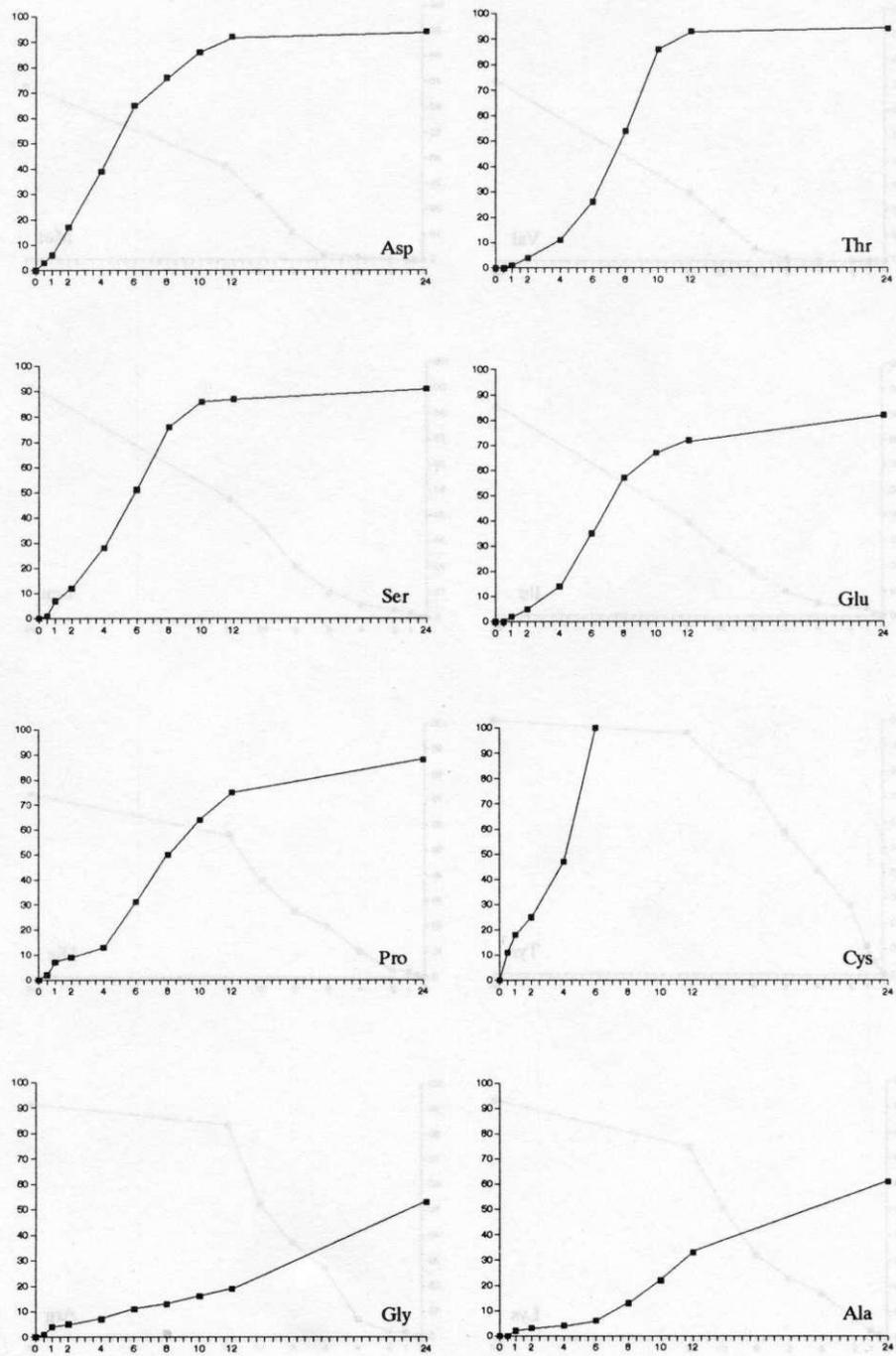


Fig. 1. Disappearance of free amino acids from the rumen liquor *in vitro*, % of initial amount

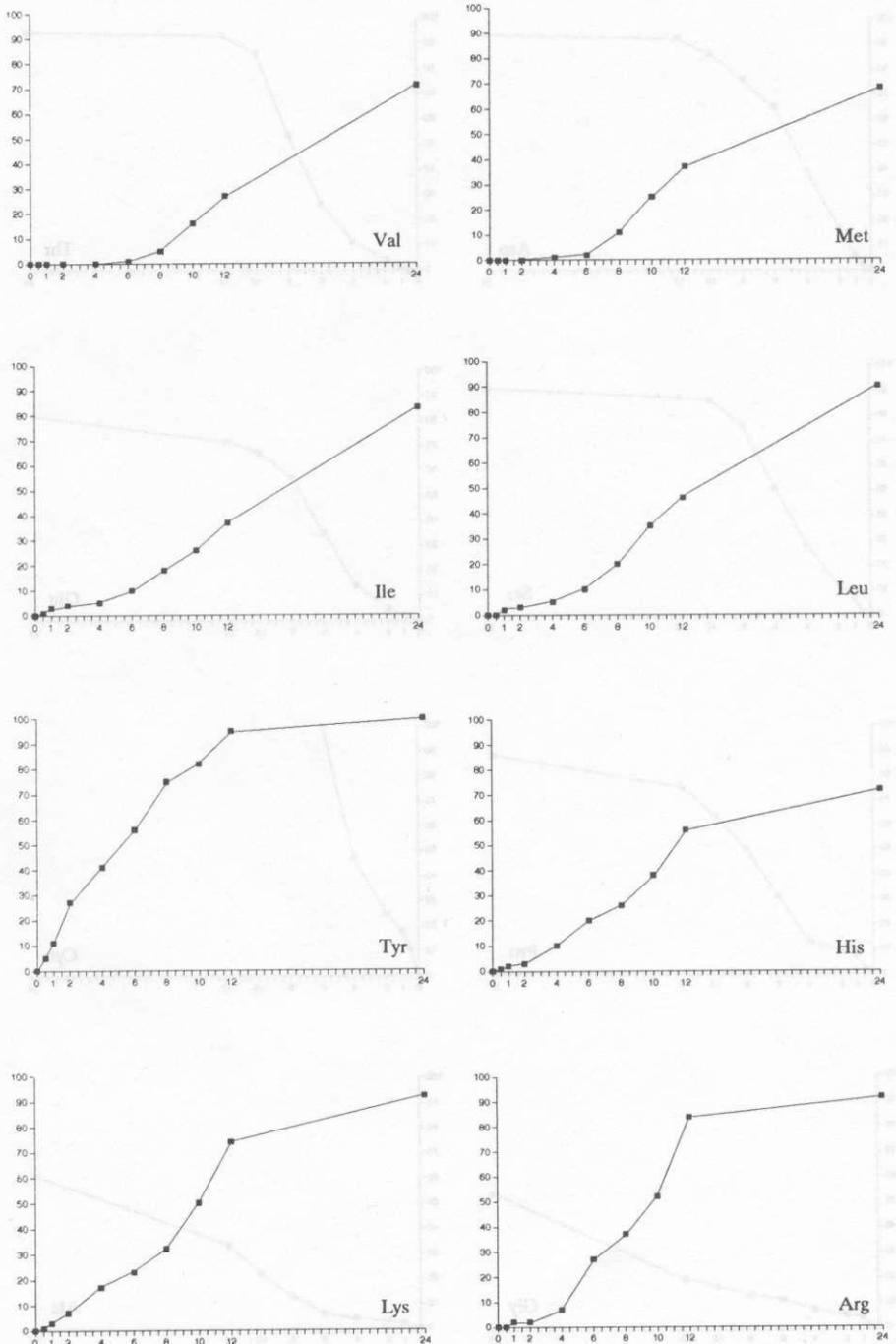


Fig. 1. Disappearance of free amino acids from the rumen liquor *in vitro*, % of initial amount

72.3 and 20.7 mg/100 ml in animals given single injections of hydrolyzed or unhydrolyzed casein, respectively.

The level of amino acids in the rumen liquor during continuous infusion of hydrolyzed casein increased significantly with time, reaching a total value of 438.8 and 688.1 mg/100 ml after 5 and 12 h, respectively.

The amounts of free amino acids administered to the rumen with hydrolyzed casein in a single dose or continuous infusion were equal (Table 3). The content of free amino acids in unhydrolyzed casein was very low and their amount introduced with casein into the rumen could be neglected.

More free amino acids were found in digesta entering the duodenum during the 12 h period of collection in sheep given a single injection of hydrolyzed casein than during continuous infusion, reaching proportions of 6.8 and 3.5 per cent of the total free amino acids introduced into the rumen, respectively. However, the recovery of individual amino acids introduced into the rumen from the duodenum differed markedly ranging from 1 to 20 per cent on continuous infusion and from 0 to 33 per cent on single injection. The highest recovery was for proline, asparagine, arginine, serine, methionine and glutamine. The concentration of free amino acids in the duodenal digesta after a single injection was highest in the first two hours and then decreased with time. The amount of free amino acids entering the duodenum during continuous infusion was low at the beginning of infusion and increased with time (Table 3).

The amount of free amino acids in digesta entering the duodenum of sheep receiving unhydrolyzed casein was markedly lower than in sheep receiving hydrolyzed casein, particularly after single injection: 45 and 24 per cent for continuous and single treatment, respectively (Table 3). Changes in the amount of free amino acids with time after introducing unhydrolyzed casein into the rumen were less pronounced than after hydrolyzed casein.

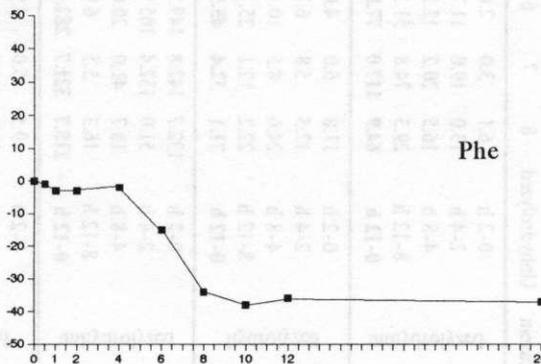


Fig. 1. Disappearance of free amino acids from the rumen liquor *in vitro*, % of initial amount

TABLE 3

The amount of free amino acids introduced into the rumen with hydrolyzed or unhydrolyzed casein and their amount entering duodenum during digesta collection time, mg

Treatment	Amino acid	Asp	Thr	Ser	Glu	Pro	Cys	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	Total	
Introduced free amino acid with casein	Hydrolyzed	6574	3757	4770	21810	12591	446	1646	2828	4422	1660	2436	4503	686	2538	3142	7478	2975	84262	
	Unhydrolyzed	8	7	6	14	0	9	2	18	14	3	6	17	16	9	10	20	7	166	
Single injection of casein	unhydrolyzed	0-2 h	6.1	3.0	2.6	16.5	0.0	11.6	8.1	7.9	9.9	1.3	1.3	4.3	9.5	30.8	30.5	8.6	0.5	152.5
		2-4 h	13.0	19.0	11.7	87.7	0.0	15.8	10.5	21.3	32.6	6.8	12.3	23.3	20.5	61.5	56.8	34.3	5.0	432.1
		4-8 h	16.5	20.2	12.2	126.8	46.3	26.0	13.9	38.8	83.8	9.8	25.3	41.1	44.5	76.3	71.5	54.9	12.3	720.2
		8-12 h	29.3	74.8	51.3	436.0	59.9	36.9	30.9	53.8	161.4	23.3	61.9	93.4	60.4	153.6	132.2	140.2	13.4	1612.7
		0-12 h	<b>64.9</b>	<b>117.0</b>	<b>77.8</b>	<b>667.0</b>	<b>106.2</b>	<b>90.3</b>	<b>63.4</b>	<b>121.8</b>	<b>287.7</b>	<b>41.2</b>	<b>100.8</b>	<b>162.1</b>	<b>134.9</b>	<b>322.2</b>	<b>291.0</b>	<b>238.0</b>	<b>31.2</b>	<b>2917.5</b>
	hydrolyzed	0-2 h	11.8	6.0	4.0	8.7	0.0	11.8	4.9	6.7	6.5	0.8	2.7	8.1	10.6	22.4	36.4	5.8	0.5	147.7
		2-4 h	12.5	5.8	6.3	13.7	0.0	24.5	6.4	10.5	8.2	2.6	4.8	13.3	29.7	52.9	42.1	11.6	4.9	249.8
		4-8 h	24.6	8.5	10.1	38.8	0.0	50.0	9.4	16.6	12.5	2.9	7.6	14.0	65.8	56.5	80.0	30.1	7.3	434.7
		8-12 h	22.2	12.1	25.1	34.7	0.0	46.3	13.0	17.8	16.3	3.1	9.6	15.6	69.5	54.1	73.0	49.1	7.7	469.2
		0-12 h	<b>71.1</b>	<b>32.4</b>	<b>45.5</b>	<b>95.9</b>	<b>0.0</b>	<b>132.6</b>	<b>33.7</b>	<b>51.6</b>	<b>43.5</b>	<b>9.4</b>	<b>24.7</b>	<b>51.0</b>	<b>175.6</b>	<b>185.9</b>	<b>231.5</b>	<b>96.6</b>	<b>20.4</b>	<b>1301.4</b>
Continuous infusion of casein	unhydrolyzed	0-2 h	132.7	142.8	149.2	677.3	0.0	24.5	55.6	101.7	190.7	50.9	99.4	169.3	93.3	99.3	143.3	303.1	62.7	2495.4
		2-4 h	51.0	132.4	105.9	524.7	0.0	17.3	40.5	85.4	189.0	40.0	85.5	129.0	56.2	211.2	108.6	248.4	26.7	2051.8
		4-8 h	18.7	41.0	20.0	143.1	0.0	23.6	18.8	25.4	73.1	6.6	22.4	37.7	37.4	227.7	80.1	102.3	7.7	885.4
		8-12 h	16.3	5.5	6.9	51.3	0.0	21.2	13.1	8.4	11.4	0.8	4.5	14.9	36.2	18.9	62.2	17.4	7.6	296.6
		0-12 h	<b>218.7</b>	<b>321.7</b>	<b>282.0</b>	<b>1396.4</b>	<b>0.0</b>	<b>86.6</b>	<b>128.0</b>	<b>220.9</b>	<b>463.8</b>	<b>98.3</b>	<b>211.6</b>	<b>350.9</b>	<b>223.1</b>	<b>557.1</b>	<b>394.2</b>	<b>671.2</b>	<b>104.7</b>	<b>5729.2</b>
	hydrolyzed	0-2 h	9.5	5.0	4.9	12.1	0.0	38.8	4.6	9.3	7.4	1.1	3.3	11.5	27.1	32.1	37.6	11.5	1.3	217.1
		2-4 h	23.2	10.6	10.6	18.1	0.0	91.3	8.5	13.6	18.6	5.1	8.7	37.3	60.1	45.9	74.3	35.0	2.6	463.5
		4-8 h	16.4	13.1	14.4	23.9	0.0	42.7	10.7	26.7	23.4	5.6	15.0	36.6	53.8	2.5	56.8	16.9	6.4	364.9
		8-12 h	22.5	10.3	11.4	35.4	0.0	43.6	14.7	17.4	13.8	3.8	8.2	22.8	51.7	0.0	79.2	18.7	2.4	355.9
		0-12 h	<b>71.6</b>	<b>39.0</b>	<b>41.3</b>	<b>89.5</b>	<b>0.0</b>	<b>216.4</b>	<b>38.5</b>	<b>67.0</b>	<b>63.2</b>	<b>15.6</b>	<b>35.2</b>	<b>108.2</b>	<b>192.7</b>	<b>80.5</b>	<b>247.9</b>	<b>82.1</b>	<b>12.7</b>	<b>1401.4</b>

## DISCUSSION

El-Shazly (1952) was one of the first who reported that degradation of protein by rumen microorganisms resulted in decomposition of amino acids. Sirotnak et al. (1953) investigated the metabolism of amino acids by a mixed suspensions of rumen microorganisms. Of twenty two amino acids tested, aspartic and glutamic acids, serine, arginine, cysteine and cystine were found to be attacked at optimum pH 6.9. Lewis and Emery (1962) divided individual amino acids into 3 groups with regard to their relative rate of deamination during incubation. Serine, cysteine, threonine and arginine were attacked most completely, followed by glutamic acid, phenylalanine, lysine and cystine and those attacked least were tryptophan, methionine, alanine, valine, isoleucine, histidine, glycine and proline. The D or L forms of serine and tryptophan were decomposed at the same relative rates, whereas D isomers of aspartic acid, lysine, threonine and phenylalanine were not attacked. Portugal and Sutherland (1966) showed that  $^{14}\text{C}$  labelled glutamic acid was rapidly decomposed and only a non significant percentage of it was incorporated into bacterial protein.

Isaacs and Owens (1971) demonstrated that aspartic acid, glutamate and arginine were extensively decomposed – up to 90%, phenolic amino acids were reduced under the same conditions by 50% but valine, leucine, isoleucine, methionine, alanine and glycine appeared to be relatively stable toward microbial action. Chalmers and Huges (1969) observed that glycine produced no increase in ruminal ammonia. Prins et al. (1979) reported that glutamate, proline, aspartate, serine and alanine were rapidly deaminated followed by arginine, phenylalanine, leucine, threonine, glycine, isoleucine which were decomposed more slowly and methionine, lysine and histidine were relatively most resistant to microbial attack. One of the reason for slow degradation of free amino acids in the rumen liquor could be that anaerobic bacteria preferentially take up peptides instead of amino acids or are even unable to utilize free amino acids directly (Prins, 1977; Wallace and Cotta, 1988).

From the present *in vitro* experiment it can be seen that all amino acids were relatively slowly decomposed and that disappearance was complete after 6 to 24, or even more, hours of incubation with a characteristic lag time for the start of decomposition (Figure 1). The reason for this lag is unsure; a possible explanation is that the conditions of incubation should be adapted to *in vitro* activity after introducing amino acids into the medium.

The rates and extent of disappearance of free amino acids from incubates, as reported by various authors were changeable in different experiments and difficult to compare and interpret. The reasons for this are thought to lie in the different experimental conditions, proportion and concentration of individual amino acids, type of donor animals and their feeding regimen, rumen microflora

compartment, incubation procedure etc. (Prins et al., 1979; Wallace and Cota, 1988). On analyzing the slow rate of disappearance of amino acids found in our experiment and the results of the *in vitro* experiments cited above, one could conclude that, of the free amino acids introduced into the rumen with the ration, a substantial proportion of them could avoid deamination in the rumen, be transported with digesta into the duodenum and be absorbed from the small intestine. However, that was not confirmed in the *in vivo* experiment.

The amount of total protein reaching the duodenum in the experiment *in vivo* was less than administered into the rumen, pointing to intensive decomposition of amino acids and absorption of nitrogen from the rumen. This effect was well pronounced when hydrolyzed casein was introduced or continuous infusion applied.

The proportion of free amino acids reaching the duodenum with digesta was calculated from the results given in Table 3; it amounted to only about 6.8% on single injection and to about 3.4% on continuous infusion of free amino acids introduced into the rumen. This clearly demonstrates that major part of the free amino acids present in the rumen were decomposed there and only a small proportion of them reached the duodenum. Free amino acids were also found in duodenal digesta of sheep given unhydrolyzed casein, but in relatively small amounts. They could have derived from protein hydrolysis in the abomasum.

The rate of disappearance of individual amino acids from the rumen varied. Tyrosine, cysteine and phenylalanine were found to be the most resistant to rumen degradation and about 28, 20 and 17%, respectively, of the amount administered to the rumen was recovered in the duodenum. Only 11% of histidine did not undergo decomposition, 9% valine and about 6% isoleucine, lysine, alanine, glycine, leucine and threonine; from 5.3 to 0.4% glutamine, serine, asparagine, methionine and proline was recovered in duodenal digesta. These results are convincing evidence that in order to avoid decomposition of amino acids introduced into the rumen effective protection from rumen degradation is essential.

## CONCLUSIONS

The rate of disappearance of free amino acids incubated with rumen liquor *in vitro* or administered into the rumen was different for individual amino acids.

Amino acids disappeared in the rumen *in vivo* faster than when incubated with rumen liquor *in vitro*. Only a small proportion of amino acids administered into the rumen reached the duodenum on continuous infusion (3.4%) or after a single injection (7.4%). To cover the deficiency in the ration of a limiting amino acid for

a ruminant, an amino acid efficiently protected from rumen degradation should be used.

Most resistant to decomposition in the rumen were: tyrosine, cysteine and phenylalanine, the least: glutamine, serine, asparagine, methionine and proline.

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## STRESZCZENIE

**Tempo rozkładu wolnych aminokwasów w treści żwacza owiec**

Mieszaninę wolnych aminokwasów inkubowano *in vitro* z płynem żwaczowym lub podawano do żwacza owiec z przetoką do żwacza i mostkową przetoką dwunastnicy jednorazowo bądź metodą infuzji ciągłej. Tempo rozkładu aminokwasów w warunkach *in vitro* było stosunkowo powolne i różne dla poszczególnych aminokwasów. Najszybciej rozkładana była cysteina. Glicyna, alanina, histydyna i walina były rozkładane najwolniej osiągając poziom degradacji nie przewyższający 70% początkowego stężenia po 24 godz. inkubacji, a fenyloalanina nie uległa rozkładowi.

Aminokwasy podane do żwacza w doświadczeniu *in vivo* znikaly ze żwacza stosunkowo szybko i zaledwie 3.4% podanej ich ilości przechodziło do dwunastnicy po podaniu metodą infuzji ciągłej, a 7.4% po jednorazowym podaniu do żwacza. Najwolniej rozkładane były w żwaczu treonina, cysteina i fenyloalanina, a najszybciej prolina, metionina, asparagina, seryna i glutamina.