Influence of peptides and amino acids on fermentation rate and *de novo* synthesis of amino acids by mixed micro-organisms from the sheep rumen

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The influence of different N sources on fermentation rate and *de novo* amino acid synthesis by rumen micro-organisms was investigated *in vitro* using rumen fluid taken from four sheep receiving a mixed diet comprising (g/kg DM): grass hay 500, barley 299·5, molasses 100, fish meal 91, minerals and vitamins 9·5. Pancreatic casein hydrolysate (P; comprising mainly peptides with some free amino acids; 10 g/l), free amino acids (AA; casein acid hydrolysate + added cysteine and tryptophan; 10 g/l), or a mixture of L-proline, glycine, L-valine and L-threonine (M; 0·83 g/l each) were added to diluted (1 : 3, v/v), strained rumen fluid along with $^{15}NH_4Cl$ (A; 1·33 g/l) and 6·7 g/l of a mixture of starch, cellobiose and xylose (1 : 1 : 1, by weight). P and AA, but not M, stimulated net gas production after 4 and 8 h incubation ($P < 0·05$) in comparison with A alone. P increased microbial-protein synthesis ($P < 0·05$) compared with the other treatments. All of the microbial-N formed after 10 h was synthesized *de novo* from $^{15}NH_3$ in treatment A, and the addition of pre-formed amino acids decreased the proportion to 0·37, 0·55, and 0·86 for P, AA, and M respectively. *De novo* synthesis of amino acids (0·29, 0·42 and 0·69 respectively) was lower than cell-N. Enrichment of alanine, glutamate and aspartate was slightly higher than that of other amino acids, while enrichment in proline was much lower, such that 0·83–0·95 of all proline incorporated into particulate matter was derived from pre-formed proline. Glycine, methionine, lysine, valine and threonine tended to be less enriched than other amino acids. The form in which the amino acids were supplied, as P or AA, had little influence on the pattern of *de novo* synthesis. When the concentration of peptides was decreased, the proportion of microbial-N formed from NH_3 increased, so that at an initial concentration of 1 g peptides/l, similar to the highest reported ruminal peptide concentrations, 0·68 of cell-N was formed from NH_3. Decreasing the NH_3 concentration at 1·0 g peptides/l caused proportionate decreases in the fraction of cell-N derived from NH_3, from 0·81 at 0·53 g NH_3-N/l to 0·40 at 0·19 g NH_3-N/l. It was concluded that different individual amino acids are synthesized *de novo* to different extents by mixed rumen micro-organisms when pre-formed amino acids are present, and that the source of N used for synthesis of cell-N and amino acids depends on the respective concentrations of the different N sources available; however, supplementing only with amino acids whose synthesis is lowest when pre-formed amino acids are present does not stimulate fermentation or microbial growth.

Amino acid: Ammonia: Protein synthesis: Rumen: Sheep

Ammonia has an important role in providing N for protein synthesis by rumen micro-organisms. Estimates of the contribution of NH_3 *v.* preformed amino acids to protein synthesis by the mixed rumen population have been highly variable. ^{15}N studies using $^{15}NH_3$ or [^{15}N]urea (which rapidly releases NH_3) infused into the rumen or added as a single dose indicated values of microbial-N derived from NH_3 that ranged from 18 to 100 % (Pilgrim *et al.* 1970; Al-Rabbat *et al.* 1971; Mathison & Milligan, 1971; Nolan, 1975; Nolan *et al.* 1976; Salter *et al.* 1979). Incorporation of NH_3 was greater in the bacterial fraction (50–78 %) than in protozoa (31–64 %) (Pilgrim *et al.* 1970; Mathison & Milligan, 1971). The composition of the diet affects the proportion of microbial protein formed *de novo*: lucerne hay gave a lower proportional uptake than wheat hay (Pilgrim *et al.* 1970), and grass hay gave a lower uptake than barley (Mathison & Milligan, 1971). The dietary factors responsible for these differences between feeds are the availability of readily fermentable energy (Ben-Ghedalia *et al.* 1978) and the presence of peptides and amino acids (Salter *et al.* 1979).

Peptides and amino acids stimulate the growth of rumen bacteria (Cotta & Russell, 1982; Chen *et al.* 1987*b*; Cruz

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Soto *et al*. 1993). Indeed, there may be different responses to peptides compared with amino acids (Argyle & Baldwin, 1989) depending on the microbial population present (Armstead & Ling, 1993; Ling & Armstead, 1995). The efficiency of utilization of peptides and amino acids is very low, however (Cotta & Russell, 1982), and it would be advantageous to identify whether specific amino acids or groups of amino acids limit the growth of the mixed rumen microbial population. Maeng & Baldwin (1976), Maeng *et al*. (1976) and Argyle & Baldwin (1979) found that only complete mixtures of amino acids gave maximum responses, yet it is clear from other studies that different amino acids are formed *de novo* to differing extents (Salter *et al*. 1979). The present study was undertaken to investigate how *de novo* synthesis of microbial protein and individual amino acids by mixed rumen micro-organisms varied with different N sources and at different concentrations of NH_3 and peptides, and how the rate of fermentation was affected under these circumstances.

Methods

Animals and diets

Four rumen-fistulated adult sheep received a mixed diet (g/kg): hay 500, barley 299·5, molasses 100, fishmeal 91, minerals and vitamins 9·5, fed in equal meals of 500 g at 08.00 and 16.00 hours. Rumen samples used for measuring gas production and $^{15}NH_3$ incorporation were taken before feeding in the morning from each sheep in order to decrease peptides and amino acids in the inoculum to a minimum. The rumen fluid was kept warm and was strained through linen cloth before use.

Incubations with rumen fluid in vitro

Gas production. Incubations were carried out using the methods described by Menke & Steingass (1988). Strained rumen fluid was added under CO_2 to a buffer and minerals solution (1 : 2, v/v) at 39°. The buffer and minerals solution contained (ml/l): trace elements solution 0·12, buffer 237, minerals solution 237, 1 ml/l resazurin 1·22, reducing solution 49·5. The trace elements solution contained (g/l): $CaCl_2.2H_2O$ 132, $MnCl_2.4H_2O$ 100, $CoCl_2.6H_2O$ 10, $FeCl_2.6H_2O$ 8. The buffer consisted of (g/l): $NaHCO_3$ 35, $(NH_4)HCO_3$ 4. The minerals solution contained (g/l): Na_2HPO_4 5·7, KH_2PO_4 6·2, $MgSO_4.7H_2O$ 0·6. The reducing solution contained: 5·76 g/l $Na_2S.7H_2O$ and 40·4 ml 1 M-NaOH. Samples of diluted rumen fluid (30 ml) were then added to 100 ml glass syringes containing energy and N sources, the syringes were incubated at 39° and gas volume was measured hourly. The energy source was 200 mg of a mixture of soluble starch, cellobiose and xylose (1 : 1 : 1, by weight). Four different N sources were used. The added N sources were NH_4Cl (40 mg), NH_4Cl + Trypticase (pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA; 40 mg and 300 mg respectively), NH_4Cl + casein acid hydrolysate (Oxoid, Basingstoke, Hants., UK, containing added 1·4 g L-cysteine and 8·68 g L-tryptophan per 992 g casein acid hydrolysate; 40 mg and 300 mg respectively), or a mixture of glycine, L-proline, L-valine and L-threonine (25 mg each).

^{15}N incorporation. The influence of peptides and amino acids on $^{15}NH_3$ incorporation was investigated using the same samples of rumen fluid in smaller-scale incubations in tubes fitted with Bunsen valves and the same additions of energy and N sources, except that the NH_4Cl added to the fermentation mixture was replaced with $^{15}NH_4Cl$ (99·6 atom % ^{15}N; Europa Scientific, Europa House, Ches., UK). The strained rumen fluid was diluted in the same way as before and the mixtures were incubated under CO_2 at 39°. Samples (5 ml) were removed into 5 ml 100 g/l TCA, then were stored at 4°.

A further set of incubations was carried out in which samples of rumen fluid from the same sheep were supplemented with different concentrations of Trypticase, or with less NH_4Cl while adding 1 g Trypticase/l. Incubations and sampling were carried out in the same way as before.

^{15}N and N analyses

Pellets from TCA-treated samples were obtained by centrifuging at 28 000 ***g*** for 15 min and washing once with 50 g/l TCA. The pellets were freeze-dried, then resuspended in 10 ml 0·5 M-NaOH and heated at 100° for 30 min. ^{15}N enrichment was measured by isotope ratio mass spectrometry as described by Barrie & Workman (1984). N was also measured by a Kjeldahl procedure (Davidson *et al*. 1970). Freeze-dried pellets were prepared for analysis of ^{15}N enrichment by hydrolysing in 6 M-HCl at 115° overnight. Samples were evaporated at 70°, then resuspended in 2 ml 0·1 M-HCl. The samples were centrifuged again and the supernatant fractions were applied to a Bio-Rad AG 50W-X8 column (Bio-Rad Laboratories Ltd, Hemel Hempstead, Herts., UK) (1·0 ml). The column was washed twice with 2 ml water, then the amino acids were eluted with 2 ml 2 M-NH_3 followed by 1 ml water. The eluted liquid was freeze-dried and stored at −20°. Tertiary butyldimethysilyl derivatives of the amino acids were prepared and the ^{15}N enrichment in individual amino acids was determined by GC–mass spectrometry as described by Calder & Smith (1988). NH_3 was measured by the phenol–hypochlorite method adapted from Whitehead *et al*. (1967). ^{15}N enrichment in NH_3 was determined by incorporating $^{15}NH_3$ into norvaline using glutamate dehydrogenase (*EC* 1.4.1.2) and 2-oxopentanoate as described by Nieto *et al*. (1996). The proportion of microbial-N derived from NH_3 was calculated as:

$$2(p_tP_t - p_0P_0)/(a_t + a_0)(P_t - P_0),$$

where a_t is the ^{15}N enrichment of NH_3 at time t (atom %), P_t is the particulate N concentration at time t (g N/l), and p_t is the ^{15}N enrichment of particulate N at time t (atom %). Enrichment in individual amino acids was calculated in the same way.

Statistical analysis

Values used to calculate the proportion of particulate N derived from NH_3 are given in Table 1 together with the relevant standard deviations. Differences between treatments were compared by an ANOVA table using the Genstat

computer program (Genstat 5 Committee, 1987, Genstat 5 User's Manual, Oxford University Press, Oxford, Oxon., UK).

Results

Mixed rumen micro-organisms were incubated anaerobically for 10 h with a mixture of soluble carbohydrates in the presence of $^{15}NH_4Cl$ and different added N sources. Microbial-protein synthesis continued throughout the 10 h period, as did NH_3 production in the Trypticase and amino acids treatments (Fig. 1(a) and (b)). Microbial protein increased more with peptides than with the other treatments, while NH_3 production from peptides and amino acids was similar, and higher than from the four amino acids treatment.

The proportion of microbial-N derived from NH_3 during microbial growth was calculated using the average enrichment of ^{15}N in NH_3 over the incubation period and the increase in particulate N over the same period. NH_3 production from Trypticase and amino acids resulted in the ^{15}N enrichment in NH_3 falling substantially (Table 1). The proportion of microbial-N derived from NH_3 was 0·37

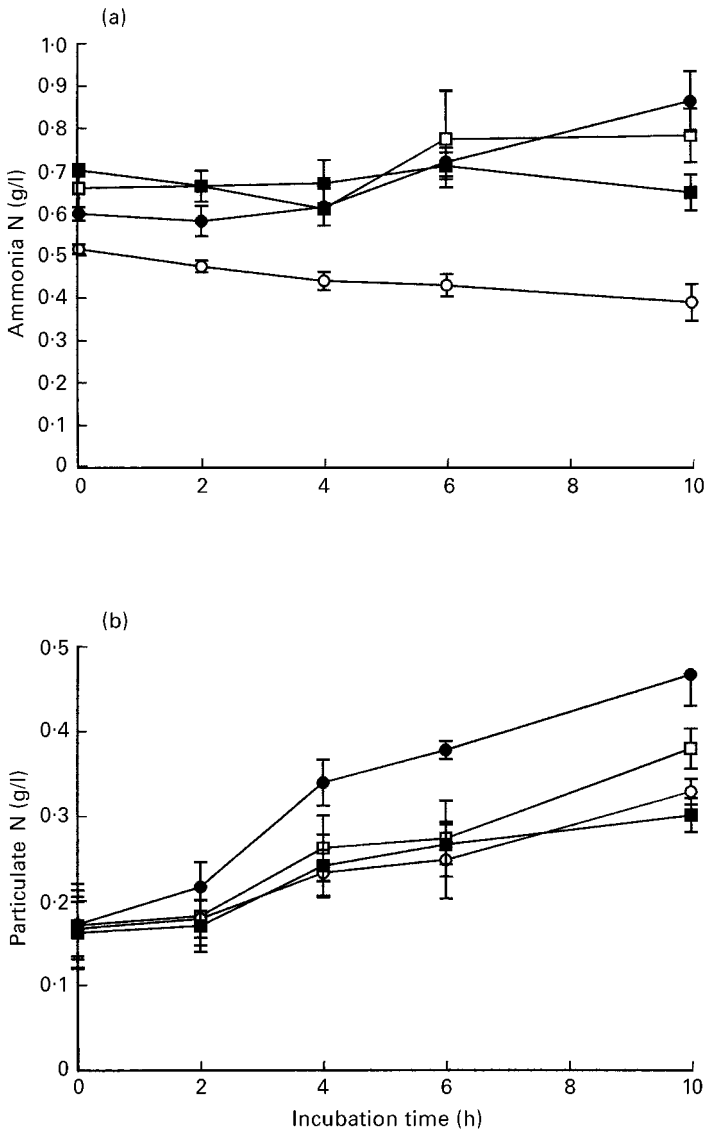

Fig. 1. (a) Ammonia and (b) particulate nitrogen concentrations during the fermentation of a mixture of soluble starch, cellobiose and xylose by mixed micro-organisms from sheep rumen fluid *in vitro* with different sources of nitrogen. Additions were: 1·33 g NH_4Cl/l (○); 10 g Trypticase + 1·33 g NH_4Cl/l (●); 10 g amino acids + 1·33 g NH_4Cl/l (□); 0·83 g glycine + 0·83 g L-proline + 0·83 g L-valine + 0·83 g L-threonine + 1·33 g NH_4Cl/l (■). Values represent mean values for duplicate incubations of samples of rumen fluid from four sheep, with standard deviations represented by vertical bars. Trypticase, pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA. Amino acids, casein acid hydrolysate (Oxoid, Basingstoke, Hants., UK) + 1·4 g L-cysteine and 8·68 g L-tryptophan per 992 g casein acid hydrolysate.

Table 1. Influence of different nitrogen sources on [^{15}N]ammonia incorporation by mixed micro-organisms from sheep rumen fluid fermenting a mixture of soluble starch, cellobiose and xylose (Mean values and standard deviations for duplicate incubations of samples of rumen fluid from four sheep)

N source	Concentration (g/l)	Incubation time (h)	Particulate protein-N (g N/l)		^{15}N enrichment of particulate N (Atom %)		NH_3 concentration (g N/l)		^{15}N enrichment in NH_3 (Atom %)		Proportion of particulate N derived from NH_3	
			Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
NH_4Cl	1·33	0	0·17	0·04	0·4	0·0	0·51	0·01	64·0	0·6		
		10	0·33	0·02	30·0	5·2	0·39	0·05	57·2	3·4	1·00	0·08
Trypticase* + NH_4Cl	10 + 1·33	0	0·17	0·05	0·4	0·0	0·60	0·02	61·1	0·7		
		10	0·47	0·04	12·2	1·7	0·86	0·08	38·1	2·7	0·37	0·02
Amino acids† + NH_4Cl	10 + 1·33	0	0·17	0·06	0·4	0·0	0·63	0·02	60·2	0·6		
		10	0·38	0·03	14·4	1·5	0·78	0·07	37·0	0·3	0·55	0·12
Gly+Pro+Val+Thr+ NH_4Cl	0·83 of each amino acid + 1·33 NH_4Cl	0	0·16	0·05	0·4	0·0	0·70	0·02	63·3	1·1		
		10	0·30	0·02	22·1	2·8	0·65	0·05	49·7	4·3	0·86	0·23

* Pancreatic casein hydrolysate: BBL, Becton Dickinson, Cockeysville, MD, USA.
† Casein acid hydrolysate (Oxoid, Basingstoke, Hants., UK) with 1·4 g L-cysteine and 8·68 g L-tryptophan added per 992 g casein acid hydrolysate.

with peptides, 0·55 with amino acids, and 0·86 with the four amino acids.

Samples taken at 10 h were analysed for ^{15}N enrichment in individual amino acids (Table 2). With the NH_4Cl treatment, where almost all particulate N was formed *de novo*, the enrichment in all amino acids was high, although the enrichment in proline and lysine was slightly lower than the others, indicating some incorporation of pre-formed amino acids, presumably present in the inoculum. The presence of Trypticase or amino acids decreased *de novo* synthesis. While incorporation of ^{15}N into all amino acids was decreased, the effect of Trypticase was greatest with proline, followed by methionine, valine, lysine, and threonine. Free amino acids decreased the *de novo* synthesis of amino acids to a lesser extent than Trypticase, but the pattern of enrichment in different amino acids was similar, except for lysine and threonine which seemed to be incorporated from Trypticase more effectively than from free amino acids. The most enriched amino acids were glutamate, followed by alanine and aspartate.

When the mixture of proline, valine, threonine and glycine was added to the incubations, *de novo* synthesis of these amino acids fell markedly. *De novo* synthesis of all other amino acids except lysine fell as well, notably in leucine and isoleucine (Table 2). The proportion of total amino acids formed *de novo* (Table 2) was lower than that of total particulate N (Table 1) in all incubations.

A low rate of gas production occurred in the absence of any addition to the diluted rumen fluid, due to endogenous metabolism, and when Trypticase and amino acids were added to strained rumen fluid in the absence of added energy source, stimulation of gas production occurred via the fermentation of the amino acids to CO_2. At each sampling time and for each N source addition, the net gas production from added carbohydrate (Table 3) was calculated by subtracting gas production in the absence of energy source from total gas production in the presence of energy source. The rate of fermentation of the mixed carbohydrates was increased significantly ($P < 0·05$) by Trypticase and amino acids at 4 and 8 h. The four amino acids treatment did not increase fermentation rate above that found with NH_4Cl (Table 3).

Similar incubations were carried out in order to determine the influence of altering the concentration of peptides on the incorporation of $^{15}NH_3$ (Table 4). At 1 g/l Trypticase (equivalent to 0·125 g N/l), the amount of cell-N derived from NH_3 was 0·68. This value varied above and below 1 g/l Trypticase roughly according to NH_3 : total soluble N in the medium. The concentration of peptides remaining at the end of the 8 h incubation period was not measured, but the extent of peptide breakdown can be assessed by the amounts of NH_3 released and microbial-N synthesized. This calculation indicated that less than half of the Trypticase was broken down after 8 h incubation at an initial concentration of 5 g/l. At lower concentrations, all of the Trypticase would be expected to be incorporated or broken down to NH_3 or incorporated into microbial cells after 8 h (Table 4).

A similar experiment was carried out in which the concentration of NH_3 was varied, while maintaining an initial concentration of 1 g Trypticase/l (Table 5). The fraction of cell-N derived from NH_3 fell as the concentration decreased, again roughly in proportion to NH_3 : total soluble

Table 2. Influence of different nitrogen sources on [^{15}N]ammonia incorporation into individual amino acids by mixed micro-organisms from sheep rumen fluid fermenting a mixture of soluble starch, cellobiose and xylose

(Mean values for duplicate 10 h incubations of rumen fluid from four sheep)

	Proportion of new amino acid-N formed which was derived from $^{15}NH_3$					
N source† . . .	NH_4Cl	Trypticase‡	Amino acids§	Gly+Val+Pro+Thr	SED	Statistical significance of difference between means (ANOVA).: *P*
Ala	1·06	0·50	0·68	1·04	0·074	***
Gly	0·93	0·29	0·39	0·31	0·032	***
Val	0·97	0·22	0·31	0·49	0·039	***
Leu	0·90	0·27	0·33	0·69	0·045	***
Ile	0·90	0·27	0·37	0·68	0·042	***
Pro	0·79	0·06	0·09	0·20	0·020	***
Ser	0·92	0·33	0·47	0·77	0·046	***
Thr	0·92	0·24	0·38	0·50	0·034	***
Met	0·92	0·16	0·24	0·84	0·052	***
Phe	0·87	0·34	0·41	0·80	0·046	***
Asp	0·89	0·40	0·63	0·84	0·051	***
Glu	0·92	0·42	0·66	0·88	0·056	***
Lys	0·74	0·23	0·42	0·79	0·043	***
Tyr	0·90	0·32	0·43	0·84	0·050	***
Mean	0·90	0·29	0·42	0·69	0·042	***

*** $P < 0·001$.
† For N concentration in each N source, see Table 1.
‡ Pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA.
§ Casein acid hydrolysate (Oxoid, Basingstoke, Hants., UK) with 1·4 g L-cysteine and 8·68 g L-tryptophan added per 992 g casein acid hydrolysate.

N in the medium. Particulate-N production fell at the highest NH_3 concentration (0·35 g NH_3-N/l), but because no sugar estimations were done it is not clear if this was caused by decreased growth yield or growth rate.

Discussion

The present experiments were undertaken to determine the influence of pre-formed amino acids on the *de novo* synthesis of different amino acids and the influence of the nature of these amino acids (whether free or in the form of peptides) and their concentration on *de novo* synthesis of cell-N and amino acids. Batch incubations were used, supplemented with a mixture of soluble starch, cellobiose and xylose. Thus the micro-organisms whose growth would be supported most would be those fermenting non-structural carbohydrates (Russell *et al*. 1992). Many of the calculations are based on average values obtained from 8 or 10 h incubations. Although some error is introduced by this approximation, such as when initial peptide concentrations were low and peptides would have been depleted during the incubation (Table 4), the patterns of NH_3 concentration and microbial-protein synthesis (Fig. 1) suggest that the fermentation was continuous over this period and the assumption is not greatly in error in most cases.

It is well known that rumen micro-organisms, as a population, have no absolute requirement for amino acids (Virtanen, 1966; Salter *et al*. 1979). The present data, from incubations with no added amino acids, provide confirmation of these findings. Most rumen bacteria have simple N requirements and can synthesize the majority of their amino acids from NH_3, via glutamate dehydrogenase (*EC* 1.4.1.2) or alanine dehydrogenase (*EC* 1.4.1.1) (Wallace *et al*. 1997), particularly if C skeletons are available (Allison & Bryant, 1963; Allison, 1969, 1970). Even those bacteria requiring some amino acids can probably, in the mixed population, scavenge amino acids released by the breakdown of protein by other species.

The extent to which rumen micro-organisms actually use their capability for *de novo* amino acid synthesis is of more relevance to practical feeding conditions. The results presented here demonstrate that the proportion of cell-N formed from NH_3 is not fixed, but varies approximately according to NH_3-N : total N available for growth. Thus, because this ratio varies between diets and throughout the day on any single diet if animals are meal-fed (Chalmers & Synge, 1954; Chen *et al*. 1987*a*,*b*; Broderick & Wallace, 1988; Wallace & McKain, 1990; Williams & Cockburn,

Table 3. Influence of nitrogen source on fermentation rate of mixed rumen micro-organisms *in vitro*†

(Mean values for duplicate incubations of rumen fluid from four sheep)

	Net gas production (ml)		
Incubation time . . .	4 h	8 h	12 h
Treatment			
NH_4Cl	15·9	43·4	57·0
Trypticase‡	19·4	47·0	59·5
Amino acids§	17·6	46·7	59·4
Gly + Pro + Val + Thr	15·1	41·2	57·9
SED	1·35	1·70	2·14
Statistical significance of difference between means (ANOVA): *P*	*	*	

* $P < 0·05$.
† For N concentrations in each N source, see Table 1.
‡ Pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA.
§ Casein acid hydrolysate (Oxoid, Basingstoke, Hants., UK) with 1·4 g L-cysteine and 8·68 g L-tryptophan added per 992 g casein acid hydrolysate.

Table 4. Influence of different concentrations of Trypticase† on [^{15}N]ammonia incorporation by mixed micro-organisms from sheep rumen fluid fermenting a mixture of soluble starch, cellobiose and xylose

(Mean values for triplicate 8 h incubations of rumen fluid from three sheep)

	Concentration of Trypticase (g N/l)							Statistical significance of difference between means (ANOVA) : *P*
	0	0·013	0·063	0·125	0·625	1·25‡	SED	
Proportion of cell-N derived from $^{15}NH_3$	1·14	0·96	0·80	0·68	0·53	0·37	0·066	***
NH_3 concentration (g N/l)								
Initial	0·536	0·562	0·560	0·570	0·572	0·599	0·012	
Final	0·429	0·448	0·487	0·543	0·683	0·862	0·019	***
Mean NH_3-N : total soluble N	1·00	0·97	0·89	0·81	0·50	0·37		
NH_3 produced (g N/l)	–0·107	–0·114	–0·073	–0·027	0·111	0·262	0·015	***
Particulate N produced (g/l)	0·125	0·146	0·165	0·170	0·169	0·295	0·019	
N formed from sources other than NH_3 (g/l)	0·018	0·032	0·092	0·143	0·280	0·557	0·013	***

*** $P < 0·001$.
† Pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA.
‡ Data reproduced from Table 1 (duplicate 10 h incubations of rumen fluid from four sheep).

Table 5. Influence of different concentrations of ammonia on [^{15}N]ammonia incorporation by mixed micro-organisms from sheep rumen fluid fermenting a mixture of soluble starch, cellobiose and xylose and 1 g Trypticase/l†

(Mean values for triplicate 8 h incubations of rumen fluid from four sheep)

	Concentration of NH_3 added (g N/l)					Statistical significance of difference between means (ANOVA) : *P*
	0·014	0·070	0·210	0·350	SED	
Proportion of cell-N derived from $^{15}NH_3$	0·40	0·52	0·72	0·81	0·062	***
NH_3 concentration (g N/l)						
Initial	0·191	0·247	0·381	0·533	0·010	***
Final	0·142	0·190	0·330	0·483	0·008	***
Mean NH_3-N : total soluble N	0·56	0·63	0·73	0·80		
Particulate N produced (g/l)	0·120	0·131	0·124	0·125	0·005	

*** $P < 0·001$.
† Pancreatic casein hydrolysate; BBL, Becton Dickinson, Cockeysville, MD, USA.

1991), the proportion of cell-N formed from NH_3 will also vary widely. This factor alone may account for the wide variation in values found in *in vivo* studies (see p. 307). The constant proportion of 0·34 assumed by Russell *et al*. (1992) when both peptides and NH_3 are available (based on *in vitro* mixed cultures with casein and mixed soluble carbohydrates carried out by Russell *et al*. 1983) may have to be varied to account for different diets. A wider range of conditions will have to be investigated to determine the precise way in which variation occurs.

Another issue is whether preformed amino acids are beneficial to the rate of fermentation or to growth yield. These questions have been dealt with in detail elsewhere (Maeng & Baldwin, 1976; Maeng *et al*. 1976; Argyle & Baldwin, 1979; Russell *et al*. 1983, 1992; Cruz Soto *et al*. 1993; Chikunya *et al*. 1996). Here, peptides and amino acids supported an increased fermentation rate (Table 3) and growth yield (Tables 1 and 4), consistent with proposed effects of peptides on non-cellulolytic rumen bacteria (Russell *et al*. 1992; Chikunya *et al*. 1996).

The incorporation of NH_3 into amino acids was lower than incorporation into total cell-N, presumably reflecting the different precursors of nucleic acids and peptidoglycan biosynthesis compared with protein. All amino acids and peptides treatments caused similar differences between the proportions of total cell-N and amino acid-N derived from NH_3. The amino acids were enriched to very different extents when pre-formed amino acids were available. Glutamate and alanine, followed by aspartate, were always synthesized to a greater extent than other amino acids and their synthesis was suppressed least by the addition of Trypticase or amino acids, reflecting the role of these amino acids in the early reactions of NH_3 assimilation (Chalupa *et al*. 1970; Erfle *et al*. 1977; Blake *et al*. 1983; Wallace *et al*. 1997). Exactly how the NH_3 becomes incorporated in the first instance remains unclear, however. Amides were not measured here, but in view of the evident importance of amides in the initial phase of NH_3 trapping (Salter *et al*. 1979), part of the regulation may occur at the level of amide synthesis. The best known microbial enzyme system for NH_3 assimilation via amide-N is the glutamine synthetase (*EC* 6.3.1.2)–glutamate synthase (*EC* 1.4.7.1) (GS-GOGAT) couple (Brown *et al.* 1974). GOGAT has been demonstrated to be present in rumen micro-organisms, but only under conditions of low NH_3 concentrations (Erfle *et al*. 1977).

The synthesis of glutamate and alanine decreased by 28–54 % in the presence of Trypticase and amino acids, while *de novo* synthesis of some amino acids was inhibited by more than 80 %. Proline synthesis was particularly subject to suppression, and glycine, valine, leucine and threonine synthesis were also suppressed more than others. Why

proline biosynthesis should be so sensitive to pre-formed proline when other amino acids were affected less is not clear. ^{15}N abundance was also low in proline in the experiments of Salter *et al*. (1979), although not to the same extent as found here.

The suppression of *de novo* synthesis of different amino acids followed a fairly similar pattern with the different additions. It was therefore considered possible that proline could be most stimulatory to the mixed fermentation, so proline was one of the four amino acids added to the mixed fermentation in order to determine if adding the amino acids which rumen micro-organisms tend to avoid synthesizing could alter the overall incorporation pattern of $^{15}NH_3$ or change the rate of fermentation. The four amino acids did not stimulate the rate of fermentation. The *de novo* synthesis of the four amino acids was, not unexpectedly, greatly decreased. The finding that an incomplete mixture of amino acids fails to support growth in the same way as a complete mixture is similar to the findings of others (Maeng *et al*. 1976; Argyle & Baldwin, 1979). It would be possible to add other individual amino acids to determine their influence on fermentation rate (e.g. lysine is an example of an amino acid which is synthesized less than others when pre-formed amino acids are available) but the indications from the other studies are that a response is improbable.

Implications

The finding that NH_3 and peptide uptake by rumen micro-organisms is only loosely regulated is surprising, particularly in view of the evident regulation of synthesis of some individual amino acids, notably proline. A similar pattern was found in pure cultures of non-cellulolytic rumen bacteria (Atasoglu *et al*. 1998). One might have expected a more closely regulated switch mechanism than actually occurs, where the N source used for growth appears to depend to a large extent on the relative quantities of each that are available. Future research should investigate the implications of these findings for growth rates and yields, and nutritional strategies should recognize that, for example, the provision of non-protein-N as a supplement may suppress the incorporation of amino acids from true protein in the feed.

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