INFLUENCE OF AMINO ACID SUPPLEMENTS TO A STRAW-MAIZE-BASED UREA DIET ON DUODENAL DIGESTA FLOW AND DIGESTION IN SHEEP

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Summary

Amino acid (AA) substituted diets had no influence on rumen levels of total volatile fatty acids (VFA), ammonia and α-amino-N, but tended to increase molar proportions of isovalerate and counts of total viable AA utilizing and cellulolytic bacteria in the rumen as compared with the control urea diet. The AA diets did not affect daily flow to the duodenum of dry matter (DM), organic mater (OM) and acid detergent fibre (ADF), and rumen digestibility of these nutrients. However, the AA diets, in particular the 10 essential AA (EAA) diet improved total digestibility of DM, OM and ADF by decreasing faecal output of these fractions. Although N flow to the duodenum and N retention were not affected with the dietary treatments, duodenal bacterial flow appeared to increase by the AA diets when it was estimated by means of 2,6-diaminopimelic acid (DAP) and nucleic acid-purine bases (PB) as markers. The results suggest that AA supplements to a urea diet could improve feed utilization by stimulating microbial activity and proliferation in the rumen but an increased microbial activity per se is not necessarily associated with improvement of feed conversion.
(**Key Words**: Amino Acid Supplements, Duodenal Flow, Microbial Efficiency, Digestion, Sheep)

Introduction

In ruminant nutrition rumen microbes play an important role because their amount and composition affect rumen fermentation rate, which in turn relates to feed intake of the animal, and the microbes leaving from the rumen serve as a protein source for the host animal. Accordingly an attempt to increase microbial activity and yield with appropriate supplements will be useful for the improvement of animal performance, particularly for animals receiving poor quality roughages and non-protein nitrogen (N) such as urea.

Of these supplements amino acid (AA) supplements have been extensively investigated *in vitro* and *in vivo*. Additions of methionine to media containing urea as sole N source stimulate microbial protein synthesis of strained rumen fluid from goats (Takahashi et al., 1974b). Methionine added to a urea diet alters AA composition of rumen microbes and thereby increases duodenal concentrations of AA and N retention in goats (Lee and Tasaki, 1977a,b). Likewise methionine supplement to a urea diet improves rumen fermentation rate and dry matter (DM) digestibility by affecting particle dilution rate of digesta in beef cows (Clark and Petersen, 1988). Branched-chain AAs or their fatty acids, singly or in combination, also improve *in vitro* digestibility of barley straw (Mir et al., 1986).

Moreover, several AA mixtures under *in vitro* conditions are found to be effective in stimulating growth yields of rumen microbes from cows (Maeng et al., 1976; Maeng and Baldwin, 1976; Argyle and Baldwin, 1989), goats (Takahashi et al., 1974a,b) and sheep (Fujimaki et al., 1989). These AA mixtures are a 10 essential AA mixture (10 EAA), an 8 nonessential AA mixture (8 NEAA), and an 18 AA (10 EAA plus 8 NEAA) mixture. Additionally, a 3 AA (leucine, methionine and histidine: LMH) mixture was as potent as the 10 EAA mixture (Fujimaki et al., 1992). In contrast to a single AA supplement, there seems no information how these AA mixtures exert influence upon rumen microbes, duodenal digesta, N metabolism, fibre digestion and animal performance when the mixtures were supplemented to urea diets. Because of a great positive effect of AA mixtures on microbial growth yields *in vitro*, it is of interest to assess their *in vivo* effects and verify a generally accepted concept that increased microbial yields will improve feed utilization in the ruminant.

We report here such a quantitative appraisal

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by the analyses of ruminal and duodenal contents and of excreta when AA mixtures were supplemented to a rice straw maize starch based urea diet in sheep.

Materials and Methods

Animals, diets and experimental design

Four adult cross breed (Corriedale × Suffolk) sheep, weighing 34.8 ± 1.7 kg on the average, were used and each animal was fitted with simple rumen and duodenal (T-shaped) cannulas. The sheep were assigned randomly to a 4 × 4 latin square design, kept in metabolism crates and were given the diets (table 1), each diet for 14 d.

The composition of experimental diets is shown in table 1. The diets were approximately isoenergetic and isonitrogenous. The urea diet (control) contained 3% urea (30 g/kg of the air dried diet) as a principal N source, a part (7.5 g/kg) of which being replaced with an equal amount of AA-N in the AA supplemented diets. The 10 EAA diet was added a mixture containing arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine in an equal molar proportion. The 18 AA diet was made with a mixture consisting of equal molar alanine, aspartic acid, cysteine, glutamic acid, glycine, proline, serine, tyrosine and 10 EAA described above. Similarly, the LMH diet was prepared by adding an equal molar mixture of leucine, methionine and histidine equivalent to the N concentration of 10 EAA or 18 AA mixture.

TABLE 1. COMPOSITION OF EXPERIMENTAL DIETS

Diet	Urea	10 EAA	18 AA	LMH
Ingredients (g/kg air dried matter)				
Rice straw	400	400	400	400
Maize starch	300	285	282	286
Molasses	200	200	200	200
Urea	30	22.5	22.5	22.5
Amino acid mixture[1]		23.0	25.9	21.8
Mineral mixture[2]	70	70	70	70
Vitamin mixture[3]	trace	trace	trace	trace
Chemical composition (g/kg dry matter, DM)				
Nitrogen	21.1	21.6	21.4	21.4
Acid detergent fibre	231	231	228	232
ME (MJ/kg DM)[4]	10.3	10.2	10.1	10.2

[1] 10 EAA: Arg. HCl, His, Ile, Leu, Lys. HCl, Met, Phe, Thr, Trp and Val; 18 AA: 10 EAA plus Ala, Asp, Cys, Glu, Gly, Pro, Ser and Tyr; LMH: Leu, Met and His.

[2] The mixture contained (g/kg): $CaHPO_4 \cdot 2H_2O$ 494.3, K_2CO_3 252.1, NaCl 59.5, $FeSO_4 \cdot 7H_2O$ 13.3, $Na_2B_4O_7 \cdot 10H_2O$ 2.87, $ZnSO_4$ 1.10, $MnSO_4 \cdot 5H_2O$ 1.27 and $MgSO_4 \cdot 7H_2O$ 175.0, and (mg/kg): $CuCO_3Cu(OH)_2 \cdot H_2O$ 338, KI 253, MoO_3 6.33, and $CoCl_2 \cdot 6H_2O$ 4.65.

[3] Vitamin A, D and E.

[4] Calculated from energy values for rice straw, maize starch and molasses.

The energetic increase due to AA substitutions were corrected to be nearly isoenergetic by reducing the same amount of maize starch as that of added AA from the AA diets. The increases in sulphur contents in AA diets were also corrected by removing $MgSO_4$ equal to sulphur of AA from the diets and the resultant decreases in Mg were complemented with the addition of $MgCl_2 \cdot 6H_2O$. All the constituents of diets except rice straw were mixed well, dried and ground with a mill into mashed meal for the animals.

For the first 5 d each sheep received a daily ration (456 g mash and 360 g rice straw, air dried matter basis) in two equal feedings at 09:00 and 18:00 hours with free access to water. From day 6 to day 14 the animal received the daily ration being divided into 8 equal portions at 3 h intervals. During each diet feeding period

of 14 d, 8 d (day 1 to day 8) were allotted for adaptation to diet, 3 d (day 9 to day 11) for total faecal and urine collection, and 2 d for duodenal samples (day 12 to day 13) and for rumen samples (day 11 and day 14). From day 6 to day 14 a polyethylene glycol solution (PEG; molecular weight 4,000, 15 g/l) and Cr-mordanted fibre (Cr-NDF) were given to sheep through the rumen cannule to determine liquid and particulated digesta flow to the duodenum as described by Faichney (1975). The PEG solution was infused with the aid of an infusion pump (Cole-Parmer, Model No. 7568-00) at the rate of 500 ml/d and Cr-NDF, 8 g/d, was given every 3 h at the time of feeding. Cr-NDF was prepared from NDF fractionated from the rice straw of diets (Van Soest and Wine, 1967) and $Na_2Cr_2O_7$ according to the procedure of Uden et al. (1980).

Samples and preparations

Faeces collected for 3 d were sprayed with a 5% HCl solution (w/w), pooled, weighed, dried at 60℃ for 48 h and ground for chemical analyses. Three d urine samples were collected into a bottle containing 20 ml of 10% H_2SO_4 solution (w/w) through 2 layers of surgical gauze, mixed well and measured the volume. A part of these samples was transferred to another bottle and stored frozen at −20℃ until assayed for N.

Strained rumen fluid taken at day 11 was used for protozoal count and to enumerate total viable, AA utilizing and cellulolytic bacteria. Fifty ml of the rumen fluid collected at day 14 was stored frozen for the determination of volatile fatty acids (VFA), ammonia-N and α-amino-N. Using another 800 ml of the rumen fluid bacterial fraction was prepared by differential centrifugation at 4℃ as described before (Fujimaki et al., 1989) and lyophilized to determine 2,6-diaminopimelic acid (DAP) and nucleic acid-purine bases (PB) contents as microbial markers.

Duodenal samples, taken at every feeding time for 2 d, 50 ml each, were frozen immediately and pooled after the completion of sampling. Forty ml of the duodenal samples was centrifuged at 21,000 × g for 10 min, the resultant supernatant was subjected to analyses of NH_3-N and α-amino-N. Half of the duodenal sample (400 ml) was dried at 60℃ for 48 h and ground for the analyses of dry matter (DM), organic matter (OM), N and acid detergent fibre (ADF). The residual sample (360 ml) was lyophilized and used for DAP and PB determinations.

Analytical procedures

DM, OM and N in excreta and duodenal samples were determined by the usual method for grains (A.O.A.C., 1975). The procedure for ADF determination followed that of Van Soest and Wine (1967). Determinations of Cr and PEG were carried out using the technique described by Kosaka (1971) and Smith and McAllan (1971), respectively. Assuming that the recovery of markers is complete, duodenal flow (g DM/d) of particulate or liquid fraction is estimated by dividing the daily amount of marker intake (g) by the marker concentration in the duodenal sample (g/g DM) (Faichney, 1975). Likewise duodenal flow of microbes was calculated by using concentrations of DAP, PB and N in duodenal samples and rumen bacteria. DAP determination followed that of Czerkawski (1974). PB were determined by the method of Ushida et al. (1986) using yeast RNA as the standard.

Concentrations of NH_3-N, α-amino-N and VFA in rumen and duodenal samples, and rumen protozoal counts were determined as described elsewhere (Kobayashi et al., 1990). Rumen bacteria were enumerated in the anaerobic roll tubes with the following media: a complete carbohydrate agar (CCA) medium for total viable bacteria (Caldwell and Bryant, 1966), a selective medium with cellulose broth as the single energy source for cellulolytic bacteria (Hungate, 1957) and a CCA medium in which trypticase and $(NH_4)_2SO_4$ were replaced by an isonitrogenous 18 AA mixture for AA utilizing bacteria.

Results were expressed as means with their standard errors (SE). The influence of treatments (T), animals (A) and feeding periods (P) was evaluated using analysis of variance (ANOVA) for 4 × 4 latin square design (Snedecor and Cochran, 1967). When ANOVA indicated significance, differences between dietary treatments were analyzed by Students t-test.

Results

Effects of AA diets on rumen characteristics

Concentrations of rumen VFA, NH_3-N and α-amino-N are shown in table 2. Total VFA concentrations were slightly higher in the AA

diets than in the urea diet, but the differences among diets were not significant. Molar proportions of acetate, propionate and butyrate were also not significantly altered by the dietary treatment. Isovalerate proportion, however, increased in the AA diets, the difference between the urea and LMH diets being significant ($p < 0.05$). Neither NH_3-N nor α-amino-N levels were significantly affected by the dietary treatments, irrespective of differences among animals and periods (table 2).

Table 3 shows counts of total viable, AA utilizing, and cellulolytic bacteria, and protozoa in the rumen fluid samples. Total viable bacterial counts tended to increase with AA substitutions except 18 AA, without any statistical significance. In contrast, AA utilizing bacteria increased in all the AA diets, and a significant increase was observed in the 10 EAA diet as compared to the urea diet. Cellulolytic bacteria seemed to increase in the 18 AA and LMH diets, though the increases were not significant (table 3). The rumen samples consisted of a great number of Entodiniinae protozoa and a small number of protozoa clas-

TABLE 2. CONCENTRATIONS OF VOLATILE FATTY ACIDS (VFA), AMMONIA (NH_3) AND α-AMINO-NITROGEN (N) IN RUMEN FLUID OF SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Total VFA (mmol/l)	236	6.1	279	0.9	268	1.1	246	2.2	NS	NS	NS
Acetate (%)	66.3	2.2	64.7	2.4	65.0	1.4	63.1	1.8	NS	NS	*
Propionate (%)	26.7	2.1	27.1	2.8	28.2	2.1	28.2	2.4	NS	NS	**
Butyrate (%)	6.4	0.5	6.3	0.3	6.1	0.9	6.1	0.3	NS	NS	NS
Isovalerate (%)	0.6	0.2[a]	1.5	0.5[ab]	0.7	0.2[ab]	2.3	0.4[b]	*	NS	NS
NH_3-N (mg/l)	287	40	262	45	241	21	240	37	NS	*	**
α-amino-N (mg/l)	121	10	111	10	120	11	169	30	NS	NS	*

[1] Analysis of variance for 4 × 4 latin square design. T: treatments; A: animals; P: periods; NS: not significant.

* $p < 0.05$, ** $p < 0.01$.

[a,b] Means in the same row without common letter differ ($p < 0.05$).

TABLE 3. COUNTS OF BACTERIA AND PROTOZOA IN RUMEN FLUID OF SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Bacteria (× 10^6/ml)											
Total viable	359	110	491	85	310	70	490	152	NS	NS	*
AA utilizing	165	48[a]	312	36[b]	209	74[ab]	380	172[ab]	*	NS	NS
Cellulolytic	2.68	1.14	2.37	0.19	7.62	5.08	10.05	6.33	NS	NS	*
Protozoa (× 10^3/ml)											
Total	143	52	151	25	132	18	140	27	NS	NS	NS
Composition (%)											
Entodiniinae	99.5		99.4		99.4		99.8				
Diplodiniinae	0		0.3		0.3		0.1				
Isotrichidae	0.5		0.3		0.3		0.1				

[1] Analysis of variance for 4 × 4 latin square design. T: treatments; A: animals; P: periods; NS: not significant.

* $p < 0.05$.

[a,b] Means in the same row without common letter differ ($p < 0.05$).

sified as Diplodiniinae and Isotrichidae. Diplodiniinae appeared only in the AA diets but total counts of protozoa and their principal compositions were not affected with AA substitutions for urea (table 3).

DM, OM, ADF and N flow to the duodenum and digestibility

Daily DM flow to the duodenum and DM digestion are shown in table 4. Neither the flow rate nor the rumen digestibility was affected by dietary treatments. However, there appeared a decreasing tendency of faecal output in the AA diets, and the total digestibility of DM was improved, the improvement in the 10 EAA diet being significant ($p < 0.05$) (table 4). OM flow and digestive properties were similar to those of DM: the increased total digestibility in the 10 EAA diet was significant in comparison with the urea diet ($p < 0.05$) (table 5). Likewise, ADF flow and rumen digestion were not affected by AA supplementation, but ADF total digestion tended to increase along with decrease in ADF faecal output in the AA diets, particularly in the 10 EAA diet ($p < 0.05$) (table 6).

The flow and digestion of N are shown in table 7. There were no differences in total N flow to the duodenum among diets. NH_3-N and α-amino-N flow were slightly lower in the AA diets without any statistical significance. The dietary treatments also had no influence on faecal N output, total N digestion, urinary N output and N retention.

TABLE 4. DRY MATTER (DM) INTAKE, DUODENAL FLOW AND DIGESTION IN SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Intake (g/d)	719		727		718		716				
Duodenal flow (g/d)	421	9	412	13	423	16	432	5	NS	*	NS
Rumen digestibility (%)	41.5	1.3	42.5	1.8	41.0	2.2	39.6	0.7	NS	*	*
Faecal output (g/d)	274	9	250	11	254	24	268	8	NS	NS	*
Total digestibility (%)	60.2	1.3^{a}	65.9	1.1^{b}	64.7	3.4^{ab}	62.6	1.2^{ab}	*	NS	*

[1] Analysis of variance for 4 × 4 latin square design. T: treatments; A: animals; P: periods; NS: not significant.

* $p < 0.05$.

[a,b] Means in the same row without common letter differ ($p < 0.05$).

TABLE 5. ORGANIC MATTER (OM) INTAKE, DUODENAL FLOW AND DIGESTION IN SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Intake (g/d)	606		610		601		602				
Duodenal flow (g/d)	297	6	295	7	303	9	309	4	NS	*	*
Rumen digestibility (%)	51.0	1.1	51.7	1.2	49.6	1.5	48.7	0.6	NS	*	*
Faecal output (g/d)	203	4	186	5	187	18	200	7	NS	NS	*
Total digestibility (%)	66.5	0.7^{a}	69.5	0.7^{b}	68.9	3.0^{ab}	66.8	1.2^{ab}	*	NS	*

[1] Analysis of variance for 4 × 4 latin square design.

T: treatments; A: animals; P: periods; NS: not significant.

* $p < 0.05$.

[a,b] Means in the same row without common letter differ ($p < 0.05$).

TABLE 6. ACID DETERGENT FIBRE (ADF) INTAKE, DUODENAL FLOW AND DIGESTION IN SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Intake (g/d)	166		166		166		166				
Duodenal flow (g/d)	104	2	107	5	108	6	106	2	NS	NS	NS
Rumen digestibility (%)	37.2	1.0	35.4	2.9	35.3	3.6	36.3	1.0	NS	NS	NS
Faecal output (g/d)	104	3[a]	88	4[b]	92	10[ab]	97	5[ab]	*	NS	**
Total digestibility (%)	37.4	1.8[a]	46.6	2.7[b]	44.5	6.1[ab]	41.4	3.2[ab]	*	NS	**

[1] *Analysis of variance for 4 × 4 latin square design.*
T: treatments; A: animals; P: periods; NS: not significant.
* $p < 0.05$, ** $p < 0.01$.
[a,b] Means in the same row without common letter differ ($p < 0.05$).

TABLE 7. NITROGEN (N) INTAKE, DUODENAL FLOW, DIGESTION AND RETENTION IN SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
Intake (g/d)	15.2		15.5		15.6		15.3				
Duodenal flow (g/d)											
Total-N	13.5	0.5	13.2	0.5	13.2	0.6	14.0	0.2	NS	*	*
NH_3-N	3.6	0.4	2.9	0.3	2.8	0.2	2.7	0.3	NS	*	**
α-amino-N	1.0	0.3	0.9	0.1	0.9	0.1	0.8	0.1	NS	NS	*
Faecal output (g/d)	5.3	0.1	5.5	0.2	5.4	0.7	5.8	0.3	NS	NS	*
Total digestibility (%)	65.0	0.8	64.4	1.3	65.3	5.5	62.1	1.5	NS	NS	NS
Urinary output (g/d)	6.1	0.6	5.9	0.5	6.0	0.3	5.7	0.5	NS	**	NS
Retention (g/d)	3.9	0.6	4.1	0.5	4.2	0.9	3.9	0.7	NS	*	NS

[1] *Analysis of variance for 4 × 4 latin square design*
T: treatments; A: animals; P: periods; NS: not significant.
* $p < 0.05$. ** $p < 0.01$.

Bacterial flow and growth yield efficiency

The analyses of rumen samples revealed that concentrations of N, DAP, and PB in rumen bacteria were not altered with the dietary treatments (data not shown).

The evaluation based on two microbial markers is shown in table 8. Bacterial DM flow to the duodenum, calculated by means of DAP, was higher in the AA diets, particularly in the LMH diet than in the control urea diet though the difference from control did not attain to significance ($p = 0.09$). Accordingly, bacterial growth yield efficiency (g DM/kg OM apparently digested) in the LMH diet elevated by 44% above the control ($p < 0.05$). Effects of diets on bacterial N flow and N efficiency (g N/kg OM apparently digested) were virtually identical with those observed in DM flow and efficiency (table 8). When these microbial parameters were calculated by using PB marker, a similar evaluation was obtained, though PB marker tended to give a smaller difference between diets than DAP marker did (table 8).

Discussion

The present study demonstrated that the LMH diet increased ruminal isovalerate proportion

TABLE 8. BACTERIAL FLOW AND EFFICIENCY CALCULATED BY 2,6 DIAMINOPIMELIC ACID (DAP) OR NUCLEIC ACID PURINE BASES (PB) AS THE MARKER IN SHEEP ON VARIOUS DIETS

	Diet								Statistical significance[1]		
	Urea		10 EAA		18 AA		LMH				
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	T	A	P
DAP-based calculation											
Duodenal flow:											
DM (g/d)	103	6	105	6	119	7	142	17	NS	NS	NS
N (g/d)	8.8	0.5	8.8	0.7	10.1	0.3	12.2	1.5	NS	NS	NS
Efficiency:											
(g DM/kg OM)[2]	33.6	2.3^{a}	33.1	1.6^{a}	40.4	3.5^{ab}	48.5	4.3^{b}	*	NS	NS
(g N/kg OM)	2.8	0.2	2.8	0.2	3.4	0.2	4.2	0.5	NS	NS	NS
DAP flow (mg/d)	589	77	659	72	649	51	714	63	NS	NS	*
PB-based calculation											
Duodenal flow:											
DM (g/d)	84	7	77	5	80	10	92	5	NS	NS	*
N (g/d)	7.1	0.5^{ab}	6.5	0.2^{a}	6.8	0.6^{ab}	7.9	0.4^{b}	*	NS	NS
Efficiency:											
(g DM/kg OM)	27.6	3.0^{ab}	24.6	2.1^{a}	27.4	4.1^{ab}	31.3	1.5	*	NS	*
(g N/kg OM)	2.3	0.2	2.1	0.1	2.3	0.3	2.7	0.1	NS	NS	NS
PB flow (g/d)	6.1	0.5^{ab}	6.0	0.1^{a}	6.3	0.5^{ab}	7.2	0.3^{b}	*	NS	NS

[1] Analysis of variance for 4 × 4 latin square design.
T: treatments; A animals; P: periods; NS: not significant.
* $p < 0.05$. DM: dry matter; N: nitrogen; OM: organic matter.
[2] Efficiency in terms of bacterial DM or N/kg apparently digested OM.
[a,b] Means in the same row without common letter differ ($p < 0.05$).

without influencing concentrations of other metabolites, and cellulolytic bacteria as well as AA utilizing bacteria tended to increase with the AA diets. The increase in isovalerate may be attributed to the added branched-chain AA, amounts of which should be enough to increase isovalerate in rumen fluid in the LMH diet, but not in the diets of 18 AA or 10 EAA (table 2). In addition, a tendency toward increasing cellulolytic bacteria by AA supplements (table 3) was consistent with our previous findings (Fujimaki et al., 1989) and others (Bryant and Doetsch, 1955; Miura et al., 1980) where branched-chain AAs or their branched-chain fatty acids are found to be essential nutrients for proliferation of cellulolytic bacteria.

The present study confirmed that AA supplemented diets could improve total digestibility of DM, OM and ADF coupling with decreasing faecal output of these nutrients without a notable influence on rumen digestion (tables 4-6). This suggests that the AA diets improve postruminal digestion of DM, particularly that of ADF, *presumably by affecting microbial activity* and population in the lower gut. Support for this view comes from the following findings: With a range of diets, between 5% to 30% of cellulose that escaped rumen digestion is digested in the large intestine (Ulyatt et al., 1975). Counts of cellulolytic bacteria of 10^{5}-10^{9}/g are found in cecal contents of sheep (Ulyatt et al., 1975; Lewis and Dehority, 1985) and thus cecal digesta have potent cellulolytic activity comparable to or above rumen digesta. The microbial population of the large intestine can be modified with constituents of digesta entering the intestine (Mann and Orskov, 1973). The quantity of ADF digested in the large intestine increases when concentrate was added to the diet of sheep (DeGregorio et al., 1982; Lewis and Dehority, 1985).

ADF digestion in the lower tracts ranging from 5.1% to 11.2% in our AA diets was consistent with the estimates described above, but in the urea diet the amount of ADF digested was extremely small (table 6). Hence, it seems likely that the duodenal digesta of sheep on AA

diets were qualitatively altered to be favourable for microbial fermentation of cellulose, because there were no quantitative differences in the digesta among the diets. A possible alteration due to AA supplements is a change in AA composition of digesta, as evidenced that methionine supplement to a urea diet improves nutritive value of microbial proteins synthesized in the rumen and thereby affects on AA concentrations of intestinal digesta and on AA absorption from the intestine in goats (Lee and Tasaki, 1977b). An alternative possibility is that the AA supplement stimulated DM or ADF digestion in the rumen in such a degree as to produce small molecular fragments which were still evaluated as DM or ADF at the duodenum. These possibilities however remain to be elucidated.

Cellulolytic bacteria isolated from ileal and cecal contents such as *Butyrivibrio fibrisolvens* and *Ruminococcus flavefaciens* are shown to be closely related to rumen strains of these species so that bacteria in the hindgut may originate from the rumen (Lewis and Dehority, 1985). However, it is unlikely in the present study that cellulolytic bacteria which proliferated with AA diets in the rumen and escaped from abomasum destruction related to the increased ADF digestion in the large intestine, because the improvement was observed only in the 10EAA in which both rumen cellulolytic bacteria and duodenal microbial flow did not increase (tables 3 and 8).

In contrast to improved digestibility of DM, OM or ADF, the AA diets had no influence on N digestibility and N retention (table 7). Lee and Tasaki (1977a) have observed that a methionine supplemented urea diet has no influence on N digestibility but increases N retention in comparison with the control diet containing urea as sole N source and lacking sulphur in goats. It is probable that the disagreement between their result and ours about N retention is due to the difference in control urea diet, because our urea diet contained inorganic sulphur instead of sulphur containing AA. Clark and Petersen (1988) have reported that heifer weight gains are improved when methionine was added to urea to a point equal to that of heifers fed a soybean meal-based supplement. However, Scott et al. (1972) have failed to get a positive effect on N utilization when methionine was supplemented to a good quality soybean diet. Effects of rumen-protected AA on dairy cows are also conflicting (Guillaume et al., 1991), suggesting that effects of AA supplement on N economy are variable depending on diets, animals and feeding conditions.

In the present study, it should be noted that bacterial flow to the duodenum as well as bacterial counts in the rumen was greater in sheep on the LMH diet than in the control or the 10 EAA diet, the differences between these diets being nearly significant (table 8). The AA mixture of LMH proved to be as potent as that of 10 EAA in stimulating rumen microbial growth *in vitro* (Fujimaki et al., 1992) so that it is not surprising that the LMH diet enhanced microbial proliferation in the rumen and then increased microbial flow to the lower tracts. However, increases in microbial amount and efficiency were not necessarily associated with improved digestibility of nutrients and N retention: the 10 EAA diet rather than the LMH diet was more effective in the improvement of digestibility of DM, OM and ADF. These results suggest that even under poor nutritional conditions, increased rumen microbial activity and yield per se would not improve feed utilization and hence it should be careful to apply *in vitro* results obtained so far to *in vivo* conditions. Further studies are needed to find a suitable AA supplement that increases feed conversion and animal performance when animals were reared with urea and poor quality carbohydrate resources.

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