The nutritive value of rumen micro-organisms in ruminants

3. The digestion of microbial amino and nucleic acids in, and losses of endogenous nitrogen from, the small intestine of sheep

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1. An experiment was conducted with three sheep maintained entirely by intragastric nutrition to estimate the digestibility of isolated individual constituents and amino acids (AA) of rumen micro-organisms (RMO) in the small intestine.

2. Five levels of RMO were infused into the abomasum. The apparent and true disappearance of the individual components were measured by regression of abomasal input on the passage at the ileum.

3. The true digestibility values of N, AA-N, DNA and RNA were 0·82, 0·85, 0·81 and 0·87, respectively.

4. The digestibility of individual AA varied between 0·80 and 0·88, the only exceptions being diaminopimelic acid (0·37), histidine (0·68) and cystine (0·73), which were significantly lower than the average (0·847).

5. The endogenous components in the ileal fluid in sheep given protein-free infusions, expressed in mg/kg live weight$^{0.75}$ per d, were total N 42 and AA-N 20.

Since the proportion of amino acid N (AA-N) and nucleic acid-N (NA-N) account for approximately 0·80 and 0·15 of the N contained in rumen micro-organisms (RMO) respectively, separate estimates of their true digestibility are required for new systems of protein evaluation (see Agricultural Research Council, 1980) since each fraction has a different nutritive value (Smith *et al.* 1974; Peers, 1977; Razzaque *et al.* 1981). In order to proceed further to state the AA requirement of ruminants precisely it is also necessary to estimate the digestibility of each AA from RMO since this is the main protein source for ruminants.

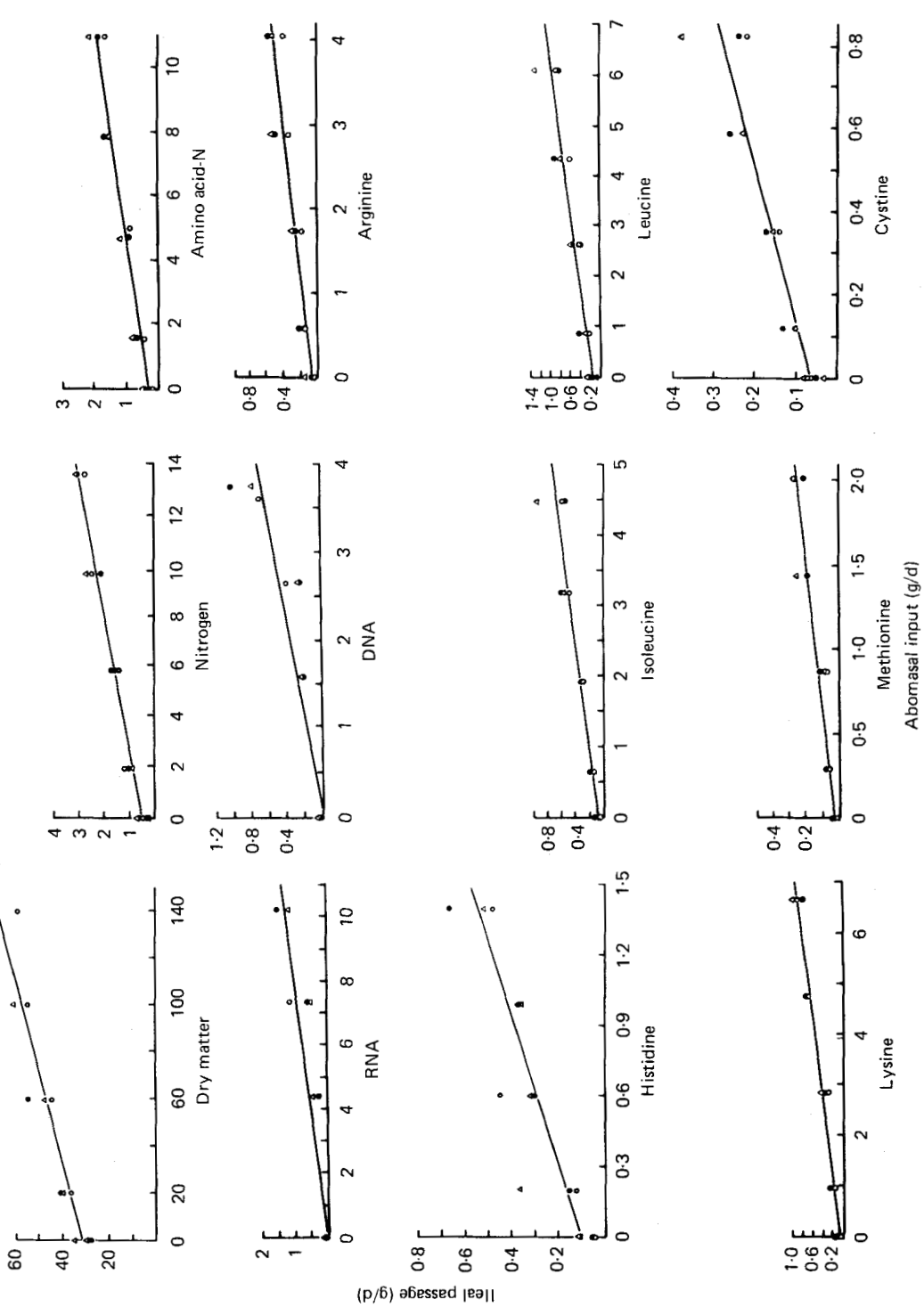
The present paper describes the estimation by regression analysis of the true digestibilities of total N, total AA-N, RNA, DNA and nineteen individual AA of RMO and the endogenous losses of these substances in the small intestine of three sheep sustained entirely by a constant intraruminal infusion of volatile fatty acids (VFA) and minerals and abomasal infusion of five different amounts of RMO. A preliminary account of some of this work has been given previously (Storm & Ørskov, 1982).

MATERIALS AND METHODS

Animals. Three Suffolk × (Finnish Landrace × Dorset Horn) wether lambs, weighing between 27 and 36 kg, were used.

Each animal was surgically prepared with a permanent rumen cannula and an abomasal catheter, as previously described by Ørskov *et al.* (1979). Furthermore, the animals were fitted with a simple T-piece cannula in the distal part of the ileum, 15–20 mm anterior to the ileo-caecal junction. The open end of the ileal cannula was exteriorized midway up on the right flank of the animal and a plastic loop arranged on each side of the cannulas according to Hecker (1974) so that total collection of ileal fluid was possible.

Infusion treatment. After the lambs had been fitted with the cannulas they were kept in individual metabolism cages for 2 weeks before intragastric infusion was commenced. The infusion regimen was then gradually introduced over 10–12 d. Clean drinking water was freely available at all times.



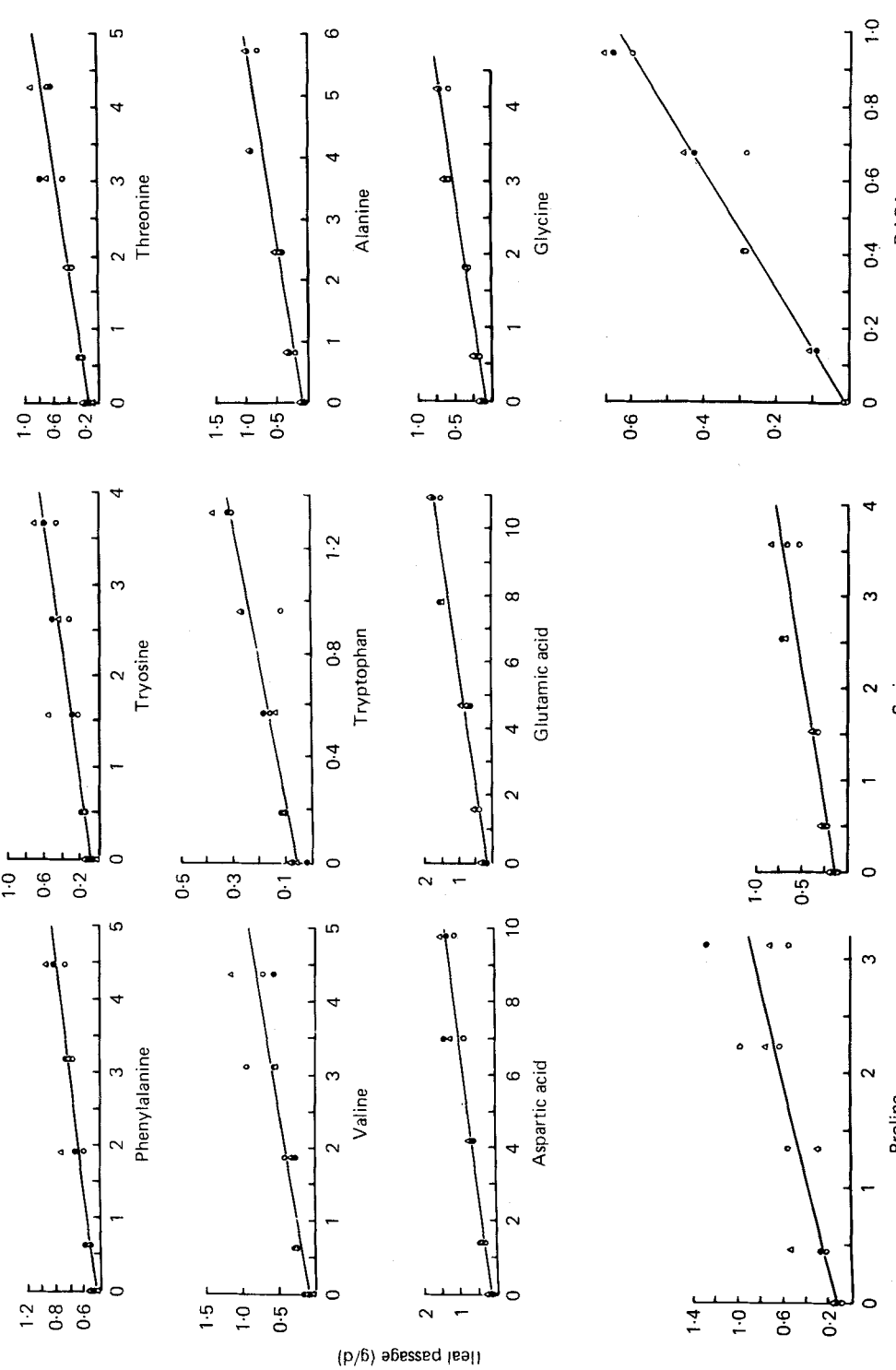


Fig. 1. The regressions of input of constituents of rumen micro-organisms and of the individual amino acids *v.* the ileal output illustrating their true digestibility and the endogenous output. DAPA, diaminopimelic acid.

During the entire experimental period of approximately 10 weeks, the lambs were entirely nourished by continuous intragastric infusion of VFA and minerals intraruminally and various amounts of isolated RMO intra-abomasally (Storm *et al.* 1983). The composition, preparation and administration of the various infusates and other details of the infusion regimen were as previously described (Storm *et al.* 1983).

Experimental treatments. The molar proportions of the VFA mixture used were 65, 25 and 10 for acetic, propionic and butyric acids respectively. The energy level maintained by rumen VFA infusion alone was 540 kJ/kg live weight$^{0.75}$ ($W^{0.75}$) per d, which was estimated to provide 1·25 times the energy requirement for maintenance (Ørskov *et al.* 1979). The freeze-dried RMO were isolated as described by Storm & Ørskov (1983). RMO was infused abomasally as the only N source in amounts of 140, 100, 60, 20 and 0 g freeze-dried material/d, together with the indigestible marker polyethylene glycol 4000 (PEG) at 10 g/kg RMO. Each level of RMO input was maintained for 4 or 5 d with ileal collections taken on four occasions at 3 h intervals on the last day. The ileal digesta was collected into a sample container partly submerged in an insulated bath of iced water and sodium chloride which maintained the temperature of the collected digesta at approximately $-3°$.

Chemical analyses. Analyses of AA, DNA, RNA and PEG were carried out on composite samples of the total ileal material obtained from each animal in each period. Methods of analyses were as described by Storm & Ørskov (1983).

RESULTS

The daily flow of individual N compounds in the ileum at different levels of RMO input was estimated from the concentration of these compounds and that of PEG in the ileal samples.

The output of each compound (y) at the terminal ileum was related to the abomasal input (x) by the expression: $y = bx + a$, so that the slope b represents the truly-indigestible proportion of x and the intercept, a, the endogenous ileal loss (i.e. with protein-free infusion). The linear regressions for all compounds are given in Fig. 1, where the individual values on which the regressions were calculated are plotted. The values for $1 - b$, i.e. the true digestibility of the individual compounds, are given in Table 1, together with the values for the intercept a, standard errors for each and residual standard deviations for the equations.

The average true digestibility of the AA-N was 0·847 and the difference in digestibility between individual AA was generally very small. Only diaminopimelic acid (DAPA) was very low, as expected (Mason & Palmer, 1971; Mason & White, 1971). Of the other AA, only histidine (0·68), cystine (0·73) and proline (0·76) had values substantially less than the average. The remainder were in the range 0·80–0·88. Values for RNA and DNA were also within that range.

DISCUSSION

Digestibility of different fractions of RMO-N

There are relatively few estimates of digestibility of the different fractions of RMO in the small intestine. The digestibility of the total N fraction was estimated by Armstrong & Hutton (1975), Salter & Smith (1977) and Lee & Tasaki (1977) to be 0·65, 0·62 and 0·55 respectively compared with our estimate of 0·82. The digestibility of the AA fraction was estimated by Tas *et al.* (1981) to be 0·87 compared with our estimate of 0·84; however, Tas *et al.* (1981) did not include cystine, tryptophan, proline and DAPA in their estimate. The estimates of digestibility of RNA and DNA of 0·89 and 0·80 respectively by Smith & McAllan (1971) compare well with our respective estimates of 0·87 and 0·81.

Comparisons with values from the literature for digestibility of individual AA are given in Table 2. The apparent digestibilities reported by Sharma *et al.* (1974), Armstrong &

Table 1. *The mean digestibilities* (*1*−b) *and endogenous losses* (a) *of total microbial nitrogen, dry matter* (*DM*) *and twenty-two individual N constituents in the small intestine*

(Values of $1-b$ and a calculated by linear regression of ileal output (y) on abomasal input (x) from $y = bx+a$, together with the residual standard deviation (RSD) and the respective standard errors. The values for the endogenous component have also been calculated as mg/kg $W^{0.75}$)

	1-*b* (mg/kg)		*a* (g/d)			
	Mean	SE	Mean	SE	RSD	*a* (mg/kg $W^{0.75}$)
DM	0·742	0·056	31·667	2·695	1·338	2436
Total N	0·815	0·024	0·545	0·031	0·096	42
AA	0·852	0·013	1·905	0·603	1·581	147
AA-N	0·847	0·012	0·262	0·079	0·422	20
RNA	0·871	0·015	−0·041	0·092	0·218	−3
DNA	0·812	0·028	−0·030	0·064	0·151	−2
NA-N	0·859	0·022	−0·087	0·103	0·180	−5
Arginine	0·890	0·012	0·084	0·028	0·074	6
Histidine	0·683	0·039	0·104	0·032	0·083	8
Isoleucine	0·863	0·014	0·070	0·037	0·096	5
Leucine	0·868	0·014	0·164	0·051	0·136	13
Lysine	0·879	0·008	0·075	0·031	0·086	5
Methionine	0·887	0·008	0·028	0·009	0·025	2
Cystine	0·730	0·030	0·066	0·014	0·037	5
Phenylalanine	0·884	0·013	0·090	0·034	0·087	7
Tyrosine	0·863	0·015	0·090	0·032	0·084	7
Threonine	0·850	0·014	0·129	0·035	0·093	10
Valine	0·836	0·023	0·103	0·059	0·153	8
Tryptophan	0·814	0·020	0·059	0·016	0·042	5
Essential and semi-essential AA	0·845	0·014	1·062	0·336	0·877	82
Alanine	0·847	0·012	0·113	0·038	0·100	9
Aspartic acid	0·879	0·010	0·172	0·056	0·144	13
Glutamic acid	0·858	0·009	0·190	0·055	0·145	15
Glycine	0·856	0·010	0·103	0·024	0·063	8
Proline	0·763	0·039	0·138	0·072	0·189	11
Serine	0·833	0·017	0·130	0·034	0·090	10
Non-essential AA	0·829	0·027	0·846	0·751	1·207	65
Diaminopimelic acid	0·374	0·051	0·001	0·031	0·063	0

AA, amino acid; AA-N, amino acid-N; NA-N, nucleic acid-N.

Hutton (1975) and Lee & Tasaki (1977) were considerably lower than ours. This is to be expected; apparent digestibilities will always be lower than true digestibilities. The high apparent digestibility of cystine observed by Armstrong & Hutton (1975) was not found in our work nor that of Sharma *et al.* (1974). Most other workers (see Table 2) have found the digestibility of methionine to be higher than the digestibility of the other AA. This was not confirmed in our work.

The endogenous loss of N in the small intestine and the truly digested proportions of the various individual N fractions of whole digesta were estimated by regression to zero abomasal input of RMO which assumes that the individual endogenous contributions remain approximately constant (Blaxter & Mitchell, 1948; Mitchell, 1964) regardless of level of RMO input. Tas *et al.* (1981) also used the same method to estimate the endogenous N loss and true digestibility of total AA in the small intestines of sheep fed on conventional diets, while their animals were duodenally infused with various levels of isolated RMO. In

Table 2. *Published estimates for the digestibility in the small intestine of individual amino acids (AA) of rumen micro-organisms*

Source...	Sharma *et al.* (1974)	Armstrong & Hutton (1975)	Lee & Tasaki (1977)	Present study
Animals...	Sheep	Sheep	Goats	Sheep
Arginine	0·72	0·76	0·67	0·89
Histidine	0·62	0·72	0·44	0·68
Isoleucine	0·69	0·63	0·45	0·86
Leucine	0·67	0·57	0·63	0·87
Lysine	0·71	0·70	0·53	0·88
Methionine	0·66	0·88	0·83	0·89
Cystine	0·30	0·92	—	0·73
Phenylalanine	0·66	0·74	0·63	0·88
Tyrosine	0·66	0·72	0·62	0·86
Threonine	0·67	0·73	0·63	0·85
Valine	—	0·71	0·51	0·84
Tryptophan	—	—	—	0·81
Essential and semi-essential AA	—	0·70	0·58	0·85
Alanine	—	0·63	0·59	0·85
Aspartic acid	—	0·75	0·67	0·88
Glutamic acid	—	0·58	0·55	0·86
Glycine	—	0·59	0·52	0·86
Proline	—	0·68	0·36	0·76
Serine	—	0·71	0·55	0·83
Diaminopimelic acid	—	—	—	0·37
Non-essential AA	—	—	0·55	0·83

addition to some difficulties encountered in accurately controlling the daily basal flow of AA from the rumen, which may account for the rather large error associated with the estimates of Tas *et al.* (1981), their mixing of 'ball-milled RMO' with 0·05 M-hydrochloric acid for up to 24 h before infusion may not closely simulate abomasal digestion in the sheep.

While indirect estimations of regression have been attempted, no direct determination of endogenous flow at the terminal ileum has been reported previously, probably because only the technique used here can ensure that there is no complication with flow of microbial N from the rumen. Our value for total endogenous ileal loss of N of 37 mg N/kg $W^{0·75}$ is similar to the value obtained for loss of endogenous faecal N reported by Storm *et al.* (1983) of 36 mg N/kg $W^{0·75}$.

The most important conclusions to be made from this assessment of digestibility of the individual components of RMO appear to be that the mean true digestibility of AA-N is 0·847 in the small intestine and that the digestibility of the different AA varies little, with the exception of DAPA, which is a known constituent of bacterial cell walls. Only cystine and histidine showed values significantly lower than the average.

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