The digestibility of amino acids in the small intestine of the sheep

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1. The digestibilities of microbial and food proteins in the small intestine were studied in three sheep fitted with re-entrant cannulas in the proximal duodenum and terminal ileum.

2. The quantities of microbial and food proteins at the small intestine were varied by infusion of a microbial isolate or by dietary manipulation and the balance of amino acids along the small intestine was determined.

3. A mean value of 0·69 for the apparent digestibility and 0·86 for the true digestibility of total amino acids was obtained.

4. From the composition of digesta at the duodenum the daily flows of microbial and food proteins were estimated. Their true digestibilities in the small intestine were calculated by regression and found to be: microbial protein 0·87 and food protein 0·82. The mean endogenous loss of amino acids secreted into the small intestine was estimated to be 13·3 g/d.

Although there has been widespread interest in the synthesis of microbial protein in the stomachs of ruminants there have been few reports on the subsequent utilization of this protein source. In particular, there are few values for the true digestibility of microbial protein in the small intestine, one of the chief factors that determine its nutritional value to the host animal.

Most investigators have used the digestibility of microbial cysteine as a measure of microbial protein digestibility, ^{35}S-labelled rumen bacteria being infused post-ruminally and the recovery of [^{35}S]cysteine in ileal digesta or in faeces measured. Bird (1972) was the first to use ^{35}S for this purpose, estimating total organic non-reducible S which consisted chiefly of methionine and cysteine. Bird (1972) estimated digestibility between the abomasum and the faeces and reported the digestibility in sheep to be 0·74. Bird's (1972) experiment was of long duration which would have given ^{35}S a chance to recycle and the results were reported as apparent digestibilities. Elliott & Little (1977) found the proportion of labelled cysteine absorbed between the duodenum and terminal ileum to be 0·72 in sheep. Using steers, Salter & Smith (1977) found the digestibility to be 0·83 when ^{35}S was the marker to label microbial protein between the duodenum and the ileum.

Other workers have made direct measurements of the apparent digestibility of bacterial amino acids by feeding diets in which microbial protein was the only food protein entering the small intestine. Armstrong *et al.* (1977) reported that the apparent digestibility of total amino acids in the small intestine was 0·70 in twenty sheep given urea as their only nitrogen source. Storm & Ørskov (1979) found the post-ruminal apparent digestibility of total microbial N to be 0·78 in lambs given microbial infusate post-ruminally as their only N source.

In the present study treatments were applied to vary the daily passage of microbial and food proteins into the intestines of sheep. Regression analysis was applied to the flow values obtained to estimate the true digestibilities of microbial and food proteins in the small intestines of three sheep and to calculate the endogenous loss of amino acids from this region of the gut. A preliminary account of the present study has been given by Tas *et al.* (1977).

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Table 1. *Infusion of microbial isolate (g/d) into the proximal duodenum given to sheep nos. 1 and 2 given a constant diet of 400 g concentrates and 400 g chopped hay/d supplying 80·8 g total amino acids/d*

Period of experiment (d)	Infusion (880 ml/d)	Period of experiment (d)
Sheep no. 1		Sheep no. 2
1–6	0·05 M-hydrochloric acid	1–6
7–10	0·05 M-HCl + 33·3 g isolate	7–10 and 20
11–14	0·05 M-HCl + 66·6 g isolate	11–14
15–19	0·05 M-HCl + 100 g isolate	15–19
19–24	0·05 M-HCl	21–26

Table 2. *Diets fed to sheep no. 3*

Dried grass (g/d)	Sugar beet pulp (g/d)	Period of experiment (d)
600	0	1–11
450	150	12–22
300	300	23–32
150	450	33–40

EXPERIMENTAL

Sheep and diets

Three Welsh mountain sheep which had previously been fitted with re-entrant cannulas in the proximal duodenum and terminal ileum, were housed in metabolism cages and fed automatically at 2 h intervals. Water was available to the sheep at all times. Sheep nos. 1 and 2 were given 400 g chopped hay and 400 g concentrates (BOCM; Energy Nuts) daily. These two sheep also received a slow infusion containing graded amounts of dried rumen micro-organisms in accordance with the protocol given in Table 1. Sheep no. 3 was given daily 600 g of a ration of dried grass and sugar beet pulp in the proportions shown in Table 2.

Experimental design

After the sheep had received their basal diets for not less than 7d their re-entrant cannulas were connected to automatic devices (Axford *et al.* 1971) collecting representative samples which were a constant proportion of the digesta passing through the cannulas. Sampling continued on a 24 h basis until the end of the experiment. That daily samples of digesta were freeze-dried and stored for analysis. The food consumed was recorded daily and any uneaten residues were collected and retained for analysis.

Chemical analysis

Analyses were carried out on the daily samples of digesta and on bulked samples of diets and microbial isolate. Dry matter (DM) was determined by drying to constant weight at 105°. Samples for the determination of total nitrogen were digested on a semi-micro scale using the reagents of Siriwardine *et al.* (1966) and the ammonia formed was determined by the method of Fawcett & Scott (1960) adapted to the AutoAnalyzer (Technicon Instrument

Table 3. *Total amino acid infusion (g/d) and flow (g/d) in sheep nos.* 1 *and* 2 *given diets containing* 80·8 *amino acids (g/d)*

Day no.	Amino acids infused	Amino acids passing		Amino acids infused	Amino acids passing	
		Duodenum	Ileum		Duodenum	Ileum
1	0	70·3	33·2	0	88·7	30·3
2	0	80·9	33·5	0	88·1	21·3
3	0	76·7	37·1	0	91·7	23·6
4	0	97·0	29·1	0	100·2	36·6
5	0	91·6	36·3	0	77·9	32·2
6	0	78·0	25·7	0	69·6	35·8
7	14·5	77·7	18·5	14·5	88·4	27·9
8	14·5	118·0	37·0	14·5	99·4	26·3
9	14·5	97·6	29·7	14·5	91·6	33·3
10	14·5	78·5	26·4	14·5	95·8	24·8
11	29·1	84·3	27·5	29·1	111·1	34·5
12	29·1	96·0	34·2	29·1	93·2	30·6
13	29·1	96·9	31·6	29·1	116·8	29·5
14	29·1	105·4	39·4	29·1	111·2	30·8
15	43·7	104·4	28·5	43·7	100·6	44·1
16	43·7	112·6	28·9	43·7	127·5	37·7
17	43·7	107·2	35·1	43·7	121·4	38·9
18	43·7	83·4	25·9	43·7	124·5	40·9
19	43·7	107·1	37·7	43·7	101·3	32·0
20	0	132·6	37·5	29·1	123·6	38·7
21	0	84·5	28·8	0	101·2	26·2
22	0	86·8	31·4	0	92·6	25·7
23	0	67·9	26·6	0	96·4	26·4
24	0	77·2	29·9	0	118·0	27·0
25	0	64·3	26·1	0	111·6	30·9
26				0	112·6	29·4

Co. Ltd, Basingstoke RG2 12YE, Hants). Total amino acids were measured after hydrolysis with 6 M-hydrochloric acid at 108° for 24 h under at atmosphere of N_2 by automated ion-exchange chromatography (Thomas, 1970).

Preparation and infusion of microbial isolate

The microbial isolate was prepared by processing 550 l rumen contents obtained from abatoir-killed sheep. These sheep had been starved overnight before slaughter. The rumen contents were filtered sequentially through a coarse hessian filter, a 200 μ mesh nylon filter and a 50 μ mesh filter. The resultant filtrate was centrifuged twice at 13200 *g* using a Type 1A Sharples Laboratory motor driven open type continuous-flow centrifuge with a 1HY standard clarifier. The material retained in the rotor was freeze-dried and ground in a ball mill. The microbial isolate was stored dry in a dark bottle at room temperature. A total of 2 kg DM was obtained from the original volume of rumen contents.

Infusates were prepared by homogenizing the required quantity of microbial isolate in 600 ml 0·05 M-HCl with a Silverson Homogenizer. The infusate was made up to a final volume of 800 ml with 0·05 M-HCl. The infusate was stirred continuously during infusion and was administered at a normal 1 ml/min via a peristaltic pump to the duodenal digesta reservoir of the sampler.

RESULTS

The microbial isolate contained 95 g N/kg. The amino acid profile (fifteen amino acids) is given in Table 5. The amino acid content calculated from this profile was 494 g/kg.

Table 4. *Total amino acid intake and passage (g/d) at the duodenum and ileum for sheep no. 3 given a diet of variable amounts of sugar beet pulp and dried grass*

Day no.	Amino acid intake	Amino acid flow at duodenum	Amino acid flow at ileum
1	77·4	61·7	15·6
2	77·4	60·7	15·6
3	73·5	60·7	18·1
4	73·5	62·5	15·1
5	77·4	68·2	16·9
6	77·4	55·5	17·2
7	47·0	67·2	20·9
8	46·2	47·9	15·9
9	49·0	42·6	14·7
10	32·2	25·3	12·8
11	25·8	18·1	8·7
12	51·5	25·4	10·3
13	75·6	49·7	12·9
14	38·2	47·8	12·6
15	76·4	56·7	14·2
16	63·9	67·9	12·9
17	84·9	65·6	16·6
18	71·2	88·9	15·3
19	74·3	74·8	15·0
20	84·9	102·0	22·5
21	84·9	105·8	21·6
22	84·9	117·2	28·4
23	71·6	88·9	24·1
24	71·6	108·7	20·1
25	71·6	92·2	16·8
26	71·6	80·4	20·2
27	71·6	73·9	14·8
28	71·6	85·0	14·8
29	71·6	71·5	17·0
30	71·6	57·5	15·7
31	62·8	79·7	16·8
32	62·8	117·0	16·9
33	62·8	117·4	19·1
34	62·8	82·5	19·1
35	62·8	70·9	21·8
36	62·8	77·3	16·5
37	62·8	80·9	19·2
38	62·8	73·8	20·7
39	61·5	56·6	13·4
40	41·4	55·1	14·9

Sheep nos. 1 and 2 consumed their rations completely. These supplied 80·8 g total amino acids/d. Table 3 shows the quantity of total amino acids infused and flowing into the duodenums and from the ileums of these two sheep. The quantity shown flowing at the duodenum is the sum of the rumen-derived amino acid flow and the amino acids infused as microbial protein. The infusion of microbial isolate into the intestines of the sheep depressed the flow of amino acids from their stomachs. This alteration in the basal flow of amino acids at the duodenum prevented the direct calculation of the digestibility of the microbial infusate from the measured flows at the two sampling sites.

The four diets offered to sheep no. 3 supplied 77·4, 84·9, 73·6 and 62·8 g total amino acids/d respectively. The animal had variable appetite and the actual daily intake of total

Table 5. *Amino acid profiles (g/kg amino acid) of microbial isolate and endogenous constituent used in calculation*

Amino acid . . .	Asp	Thr	Ser	Glx	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
Microbial isolate	105·1	59·9	48·9	138·2	58·3	78·9	66·8	26·4	63·2	89·9	49·9	56·2	27·2	79·3	49·6
Pepsinogen*	130·0	75·6	122·0	117·0	55·9	31·9	69·3	13·9	100·0	79·6	80·7	62·0	7·6	28·9	25·8

* Bovine pepsinogen calculated from Chow & Kassell (1968).

amino acids is given in Table 4, which also shows the quantities of total amino acids flowing into the duodenum and ileum of this sheep.

The mean apparent digestibility in the small intestine of total amino acids was calculated for each sheep. The digestibilities were $0{\cdot}65 \pm 0{\cdot}01$, $0{\cdot}69 \pm 0{\cdot}01$ and $0{\cdot}74 \pm 0{\cdot}07$ for sheep nos. 1, 2 and 3 respectively. The true digestibilities of total amino acids in the small intestines of the sheep were calculated by linear regression giving the equation $Y = a + bX$, where Y is the amount absorbed from the small intestine (g/d), X is the input to the small intestine (g/d), a is the constant term and b is the true digestibility coefficient. The constant term a represents the intercept on the Y axis when $X = 0$ or the net quantity of amino acids absorbed from the gut when there is no input. This quantity is negative and is the best estimate of net endogenous loss of amino acids from the small intestine. The equations for the three sheep were:

sheep nos.: 1 $Y = -16{\cdot}2 + 0{\cdot}84X$ r^2 0·92,
2 $Y = -16{\cdot}6 + 0{\cdot}85X$ r^2 0·84,
3 $Y = -8{\cdot}6 + 0{\cdot}88X$ r^2 0·98.

DISCUSSION

While the application of regression analysis to the indirect measurement of metabolic loss has been well documented its use in determining true digestibility has been less widely applied (Mitchell, 1964). The chief objection is that variation in the nature of the diet and particularly in its DM content can cause variation in the rate of excretion of metabolic faecal N (Blaxter & Mitchell, 1948). In these experiments the treatments applied did not alter the DM supplied in the diet nor cause any significant change in the flow of DM at the ileum. Therefore the endogenous or metabolic loss of amino acids from the small intestine into the large intestine can reasonably be expected to be constant and the equation can be applied with endogenous loss represented by a constant term.

The proportion and amount of duodenal total amino acids present as microbial protein was estimated using the method of Evans *et al.* (1975). This method is further described by Offer *et al.* (1978). The amino acid profiles of the microbial isolate and pepsinogen, the endogenous constituent used in the calculation, are listed in Table 5. Table 6 lists the mean profiles of the duodenal digesta for each experimental period. The calculation was carried out on the daily values which are summarized by those means. Application of the method to the daily values for amino acid flow to the duodenum gave the mean partition of amino acids shown in Table 7. The treatments applied to the sheep provided a wide range of daily passage of microbial amino acids to the duodenum as indicated by the standard deviations included in Table 7.

The true digestibilities of dietary, microbial and endogeneous amino acids were estimated from the daily values by multiple regression using the equation:

$$\text{absorbed total amino acids} = c + a * \text{dietary total amino acids} + b * \text{microbial total amino acids} + d * \text{endogenous total amino acids.}$$

The following mean (±SE) values were obtained:

Sheep no.	c	a	b	d
1	−15·7	0·76±0·10	0·89±0·07	0·84±0·43
2	−15·7	0·86±0·09	0·84±0·09	0·78±0·23
3	−8·6	0·88±0·04	0·89±0·02	0·84±0·13

The constant term c represents the net endogenous passage of total amino acids from

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Table 6. *Amino acid profiles (g/kg amino acid) of duodenal digesta of sheep nos.* 1, 2 *and* 3

Amino acid . . .		Asp	Thr	Ser	Glx	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg
Sheep no.	Experimental period no.															
1	1	107·8	55·7	60·0	153·5	56·9	75·7	50·7	34·3	48·8	85·1	54·1	56·8	35·7	74·4	50·1
	2	104·8	55·8	59·1	165·6	57·8	77·2	68·6	35·9	43·8	82·0	53·6	54·5	34·2	74·6	52·1
	3	96·3	61·5	61·0	155·5	57·3	78·4	50·4	33·8	52·9	84·3	53·4	55·0	33·7	77·4	51·8
	4	105·8	55·6	56·4	154·7	57·0	78·7	49·6	35·0	55·3	84·9	52·9	55·9	32·8	76·3	48·6
2	1	101·5	65·6	60·3	143·2	60·9	76·3	50·8	37·9	48·4	86·2	45·3	52·4	43·5	64·9	62·3
	2	101·6	61·8	64·2	153·8	58·7	71·8	45·8	39·2	44·4	85·5	52·6	54·0	36·7	75·0	54·5
	3	106·4	64·6	61·6	150·7	59·3	90·7	45·3	31·0	49·8	85·5	46·8	50·0	30·5	70·2	57·2
	4	94·6	66·0	62·5	153·9	62·2	80·8	47·5	43·1	51·5	88·9	46·6	54·5	31·5	61·4	54·3
3	1	134·9	59·9	60·6	125·3	67·0	98·7	53·1	3·4	45·4	90·5	47·8	58·5	36·4	56·9	61·2
	2	127·0	60·2	58·2	127·9	73·6	101·1	42·1	16·3	43·2	97·9	49·3	61·4	33·0	48·0	60·4
	3	110·6	58·8	57·7	112·4	77·0	101·3	59·2	26·1	48·1	91·7	46·2	57·0	38·1	56·7	58·9
	4	103·7	56·4	55·6	152·6	62·3	90·1	51·8	34·8	46·3	87·6	45·3	56·8	38·2	63·9	53·4

Table 7. *Estimated contribution of dietary, microbial and endogenous amino acids (g/d) to the total amino acids in the duodenal digesta of sheep nos.* 1, 2 *and* 3

(Mean values and standard deviations)

Sheep no.	Period of experiment	Food amino acids		Microbial amino acids		Endogenous amino acids	
		Mean	SD	Mean	SD	Mean	SD
1	24	30·3	9·6	57·5	13·4	3·4	2·3
2	26	45·1	13·2	50·6	14·9	5·3	5·1
3	40	22·9	16·6	44·7	30·7	3·4	4·0

the small intestine. Its value did not differ significantly from the constant term derived by simple regression of the total amino acid digestibility determined previously. The digestibility of the endogenous total amino acid component was the least well defined by the calculation and the standard errors of the digestibility coefficients for this component in the subsequent regression equations were large; however, the contribution of this component was always minor as indicated in Table 7.

The true digestibilities determined for microbial protein in the three sheep were 0·89, 0·84 and 0·89. These values were based on a study of fifteen amino acids, excluding cysteine because of known analytical difficulties encountered with its determination, and agreed with those reported in steers by Salter & Smith (1977) though they are significantly higher than the values for sheep obtained by Elliott & Little (1977). Both these groups derived their values from a study of the digestibility of a single amino acid, ^{35}S-labelled cysteine.

The apparent digestibilities recorded by Bird (1972) over the whole intestine concur with our estimate of 0·69 for the apparent absorption of total amino acids from the small intestine. This latter is also in agreement with the apparent digestibilities reported by Armstrong *et al.* (1977), 0·695 for sheep on urea diets, and 0·708 on hay and concentrate diets. Although Storm & Ørskov (1979) measured digestibility of total N over the whole intestine, their findings of apparent digestibility of 0·78 are consistent with ours when it is considered that by the nature of their experiment endogenous loss would be minimal and the apparent digestibility of N would approach the true digestibility.

Our results show that the microbial protein formed in the rumen of sheep was highly digestible (0·87) in the small intestine.

REFERENCES

Armstrong, D. G., Savage, G. P. & Harrison, D. G. (1977). *Publ. Eur. Ass. Anim Prod.* no. 22 p. 55.
Axford, R. F. E., Evans, R. A. & Offer, N. W. (1971). *Res. vet. Sci.* **12**, 128.
Bird, P. R. (1972). *Aust. J. biol. Sci.* **25**, 195.
Blaxter, K. L. & Mitchell, H. H. (1948). *J. Anim Sci., Camb.* **7**, 351.
Chow, R. B. & Kassell, B. (1968). *J. biol. Chem.* **243**, 1718.
Elliott, R. & Little, D. A. (1977). *Br. J. Nutr.* **37**, 285.
Evans, R. A., Axford, R. F. E. & Offer, N. W. (1975). *Proc. Nutr. Soc.* **34**, 67A.
Fawcett, J. K. & Scott, J. E. (1960). *J. clin. Path.* **13**, 156.
Mitchell, H. H. (1964). *Comparative Nutrition of Man and Domestic Animals*, p. 405. London: Academic Press.
Offer, N. W., Axford, R. F. E. & Evans, R. A. (1978). *Br. J. Nutr.* **40**, 35.
Salter, D. N. & Smith, R. H. (1977). *Br. J. Nutr.* **38**, 207.
Siriwardine, J. A. de S., Thomas, A. J., Evans, R. A. & Axford, R. F. E. (1966). *J. Sci. Fd Agric.* **17**, 456.
Storm, E. & Ørskov, E. R. (1979). *Ann. Rech. Vet.* **10**, 294.
Tas, M. V., Axford, R. F. E. & Evans, R. A. (1977). *Proc. Nutr. Sco.* **36**, 76A.
Thomas, A. J. (1970). *Automation, Mechanisation and Data Handling in Microbiology*, p. 107. London: Academic Press.

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