The effect in sheep of physical form on the sites of digestion of a dried lucerne diet

2.* Sites of nitrogen digestion

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1. The effect of altering the physical form of a regrowth crop of dried lucerne (*Medicago sativa* L. var. *Chartainvilliers*) on the site of nitrogen digestion was studied with sheep fitted with a rumen cannula and re-entrant cannulas at the proximal duodenum and the terminal ileum. Chopped, cobbed, and ground and pelleted diets were prepared by processing the same high-temperature dried crop and given twice daily to sheep at a level of intake of 910 g dry matter/24 h.

2. The chopped diet had a slightly lower content of total N and amino acid N than the other forms but the amino acid composition was similar and there was no difference between the diets in the apparent digestibility of total N.

3. For all three physical forms the amounts of total N reaching the small intestine were greater than those ingested. The disappearance of apparently digested N in the caecum and colon of the sheep was significantly higher with the chopped form of the diet than with the cobbed and ground, pelleted forms ($P < 0{\cdot}01$).

4. Of the individual amino acids, the greatest (and significant) increases compared with the food at the proximal duodenum were found for methionine, lysine, tryptophan and cysteine/cystine, and there were significant differences between diets for methionine, tryptophan, valine and cysteine/cystine. The amounts of isoleucine were also significantly increased at the proximal duodenum. Significant losses at the duodenum occurred for aspartic acid (all diets) and for phenylalanine and proline (two diets).

5. The amounts of tryptophan, methionine, cysteine/cystine and serine absorbed from the small intestine, relative to amounts ingested, were significantly greater on the chopped and pelleted diets than on the cobbed diet. Differences between the chopped and pelleted diets in relative amounts absorbed from the small intestine were observed for tryptophan ($P < 0{\cdot}01$) and for cysteine/cystine ($P < 0{\cdot}05$).

6. Apparent digestibilities of total amino acid N within the small intestine (based on amounts entering the small intestine) were 71·5, 66·2 and 69·8 % for the chopped, cobbed and pelleted diets respectively.

7. Mean values for apparent digestibility of individual amino acids within the small intestine ranged from 47 % for histidine to 80 % for methionine. Of the eighteen amino acids examined, values for apparent digestibility in the small intestine of thirteen of them were lowest on the cobbed diet; the effect was particularly noticeable for histidine. Despite this variation, there was a very significant positive correlation between the amounts of individual amino acids reaching the small intestine and absorbed from it with all three diets ($n = 54$; $r = 0{\cdot}990$; $P < 0{\cdot}001$); the same was true for each individual diet.

8. Significantly lower amounts of nucleic acid N entered the small intestine of sheep given the pelleted diet ($P < 0{\cdot}01$). From the results for nucleic acid N it was calculated that on the chopped and cobbed diets 44–46 % of the total N entering the small intestine was of microbial origin; on the pelleted diet the value was 32 %.

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In the previous paper of this series (Thomson, Beever, Coelho da Silva & Armstrong, 1972) sites of organic matter, energy and carbohydrate digestion in sheep fed on a dried lucerne (*Medicago sativa* L. var. *Chartainvilliers*) in each of three physical forms, chopped, cobbed and pelleted, have been determined. In this paper the results pertaining to the nitrogen fractions of the diets are given.

Studies of the effect of pelleting on the apparent digestibility of total N in a grass and a lucerne hay have been reported by Demarquilly & Journet (1967). They found that the effect of pelleting in reducing the apparent digestibility was greater in the grass than in the lucerne. Nix, Anthony & Cope (1965) reported that pelleting of dried lucerne did not significantly depress the apparent digestibility of total N.

Hogan (1965) studied the flow of total N at the duodenum of sheep fed on fresh lucerne and Hogan & Weston (1967) studied the flow of total N through the abomasum of sheep receiving a lucerne hay given chopped or pelleted. Clarke, Ellinger & Phillipson (1966) and Unsworth (1970) have reported the movements not only of total N but also of individual amino acids through the digestive tract of sheep given hay or hay–concentrate diets. In this paper the mean amounts of total N, ammonia-N and nucleic acid N (NA-N) and of eighteen amino acids ingested, entering and leaving the small intestine and excreted in the faeces of three sheep given the lucerne diets are reported.

EXPERIMENTAL

Details of the diets and the amounts offered, of the sheep used and their management, and of the methods of collection of faecal and digesta samples have been given by Thomson *et al.* (1972).

Preparation of samples for analysis

For the determination of total N and of individual amino acids, feed samples were ground in a Christy and Norris hammer-mill through a 1 mm sieve; for the measurement of ammonia-N and NA-N a known weight of distilled water was added to a given weight of ground feed sample, the whole homogenized in a Kenwood mixer (Kenwood Manufacturing Ltd, Havant, Hampshire) and the wet sample then treated as for the digesta samples. Wet faeces were mixed with five times their weight of water, homogenized and treated as for digesta samples for all determinations.

After removal from the deep-freeze the digesta samples were kept overnight at room temperature to thaw out slowly; they were then mixed thoroughly and portions were taken for analysis using a wide-mouth pipette. Wet samples were used for the determinations of total N, ammonia-N and NA-N; freeze-dried samples were used for the determination of individual amino acids.

Analysis of samples

Total N was determined by the Kjeldahl procedure using selenium–copper sulphate catalyst. Ammonia-N was determined by the Conway microdiffusion technique (Conway & Byrne, 1933). For the determination of fifteen individual amino acids, other than cysteine/cystine, methionine and tryptophan, samples were hydrolysed with 6 M-HCl (twice glass-distilled) in sealed Pyrex tubes for 24 h at 110°; the ratio of total

N to acid varied from 1:2000 to 1:4000. After hydrolysis the acid was removed on a rotary-evaporator under reduced pressure at 60° and the residue dissolved in citrate buffer, pH 2·0. Individual amino acids were determined by column chromatography on a Technicon TSM 1 AutoAnalyzer (Technicon Instruments Co. Ltd, Basingstoke, Hants). The values for valine, leucine and isoleucine were adjusted for incomplete release in a 24 h hydrolysis period by the use of appropriate correction factors, determined previously for each category of sample analysed (feed, duodenal, ileal and faecal) by hydrolysing samples for periods of 24, 48, 72 and 96 h.

For the determination of cysteine/cystine and of methionine the samples were first treated with performic acid before normal hydrolysis with 6 M-HCl, according to the method described by Moore (1963). For the determination of tryptophan the sample was hydrolysed with barium hydroxide and tryptophan was measured calorimetrically using *p*-dimethylaminobenzaldehyde reagent (Miller, 1967).

Nucleic acids were determined by the method of McAllan & Smith (1969).

RESULTS

Total N

Table 1 gives the total N content and its fractionation in the three diets used, together with the amounts of individual amino acids present. Although all three forms were prepared from the same harvested lucerne, the chopped form had a slightly lower content of total N and amino acid N (AA-N). The AA-N accounted for 70% of the total N present and the values for individual amino acids, expressed as g/16 g N, indicated little variation in the composition of the protein. For the individual amino acids the values (g/16 g N) were within the range given by Harvey (1956), with the exception of glutamic acid, tyrosine and phenylalanine, which were higher and methionine which was lower.

Table 2 shows the intake of total N and its flow through the digestive tract. Apparent digestibility of total N was not significantly affected by physical form.

The amounts of total N reaching the small intestine were greater than those ingested with all three physical forms of the diet; the increases were 30, 14 and 25% for the chopped, cobbed and pelleted diets respectively.

Within the small intestine there were no significant differences due to diet in the disappearance of N (chopped 17·8, cobbed 19·1 and pelleted 20·3 g/24 h) or percentage disappearance of apparently digested N. The disappearance of apparently digested N (AD-N) in the caecum and colon of the sheep was significantly higher ($P < 0{\cdot}01$) with the chopped form of the diet than with the cobbed and pelleted diets, which were also different ($P < 0{\cdot}01$).

Ammonia- and urea-N

The quantities of ammonia-N flowing through the digestive tract were small; for the chopped, cobbed and pelleted diets respectively amounts (g/24 h) ingested were 0·13, 0·15 and 0·13, amounts at the proximal duodenum 1·29, 1·88 and 1·44, amounts at the terminal ileum 1·26, 1·54 and 1·17; amounts excreted in the faeces were 0·23, 0·31 and 0·25. The only significant difference was in an increased amount of such N reaching

Table 1. *Fractionation of total nitrogen and contents of individual amino acids present in the three forms of dried lucerne*

(All results are expressed on a percentage dry-matter basis with the exception of those in parentheses which are expressed as g/16 g total N)

	Chopped lucerne	Cobbed lucerne	Pelleted lucerne
Total N	2·55	2·93	2·85
Ammonia-N	0·014	0·016	0·014
Amino acid N	1·78	2·02	1·98
RNA-N	0·50	0·50	0·50
DNA-N	0·21	0·21	0·21
Amino acids			
Threonine	0·76 (4·77)	0·87 (4·75)	0·86 (4·81)
Valine	0·82 (5·15)	0·98 (5·35)	0·93 (5·20)
Isoleucine	0·75 (4·70)	0·86 (4·70)	0·76 (4·27)
Leucine	1·20 (7·53)	1·33 (7·26)	1·25 (7·02)
Phenylalanine	0·96 (6·02)	1·08 (5·90)	1·09 (6·10)
Histidine	0·29 (1·82)	0·34 (1·86)	0·31 (1·74)
Lysine	0·58 (3·64)	0·67 (3·66)	0·69 (3·88)
Arginine	0·67 (4·20)	0·78 (4·26)	0·77 (4·32)
Tryptophan	0·16 (1·00)	0·21 (1·15)	0·20 (1·12)
Methionine	0·10 (0·63)	0·12 (0·66)	0·09 (0·51)
Aspartic acid	2·02 (12·68)	2·22 (12·14)	2·23 (12·51)
Serine	0·66 (4·14)	0·73 (4·00)	0·67 (3·76)
Glutamic acid	1·55 (9·73)	1·76 (9·60)	1·76 (9·90)
Proline	0·82 (5·15)	0·89 (4·86)	0·87 (4·88)
Glycine	0·76 (4·77)	0·87 (4·75)	0·86 (4·84)
Alanine	0·80 (5·02)	0·92 (5·02)	0·88 (4·96)
Tyrosine	0·59 (3·70)	0·68 (3·70)	0·58 (3·26)
Cysteine/cystine	0·09 (0·56)	0·10 (0·55)	0·12 (0·67)

RNA-N, ribonucleic acid N, calculated on the assumption that RNA contains 14% of total N (McAllan & Smith, 1969).

DNA-N, deoxyribonucleic acid N, calculated on the assumption that DNA contains 14·8% of total N (McAllan & Smith, 1969).

Table 2. *Mean quantities of total nitrogen present in the feed, entering and leaving the small intestine and in the faeces of three sheep given chopped, cobbed and pelleted lucerne*

(The values for digesta at the duodenum and ileum have been adjusted for 100% recovery of chromic oxide. Mean values for the disappearance of apparently digested N before and in the small intestine and in the caecum and colon are also given)

	Chopped	Cobbed	Pelleted	SEM
Total N (g/24 h)				
In food	23·28	26·58	25·76	—
At proximal duodenum	30·28	30·22	32·15	—
At terminal ileum	12·45	11·08	11·82	—
In faeces	6·72	7·32	7·43	—
Apparent digestibility of N (%)	71·1	72·5	71·1	0·98
Disappearance of apparently digested N (%)				
Before small intestine	−42·4	−19·0	−35·0	5·73
In small intestine	107·8	99·4	111·0	5·51
In caecum and colon	34·6	19·6	24·0	0·65

Table 3. *Mean amounts of amino acid nitrogen (g/24 h) present in the feed, entering and leaving the small intestine and in the faeces of three sheep given chopped, cobbed and pelleted lucerne. Also included are mean values relative to the amounts ingested for amino acid N reaching the small intestine, disappearing in the small intestine and in the caecum and colon*

(The values for digesta at the duodenum and ileum have been adjusted for 100 % recovery of chromic oxide)

	Chopped	Cobbed	Pelleted	SEM
In food (g/24 h)				
TAA-N	16·26	18·39	17·94	—
EAA-N	8·33	9·56	9·44	—
NEAA-N	7·93	8·83	8·50	—
At proximal duodenum (g/24 h)				
TAA-N	18·88	19·61	20·31	0·282
EAA-N	10·67	11·03	11·19	0·163
NEAA-N	8·21	8·58	9·12	0·307
At terminal ileum (g/24 h)				
TAA-N	5·39	6·63	6·13	0·349
EAA-N	2·88	3·72	3·66	0·244
NEAA-N	2·51	2·91	2·47	0·149
In faeces (g/24 h)				
TAA-N	3·37	3·58	3·59	0·345
EAA-N	1·88	1·94	1·95	0·059
NEAA-N	1·49	1·64	1·64	0·174
Amount at proximal duodenum (% of amount ingested)				
TAA-N	116·2	106·6	113·2	4·43
EAA-N	128·1	115·4	118·6	5·60
NEAA-N	103·6	97·1	107·3	3·65
Amount disappearing in the small intestine (% of amount ingested)				
TAA-N	83·0	70·6	83·8	5·78
EAA-N	93·6	76·5	88·8	6·88
NEAA-N	72·0	64·2	78·2	4·95
Amount disappearing in the caecum and colon (% of amount ingested)				
TAA-N	12·4	16·5	9·4	2·92
EAA-N	12·0	18·6	9·1	2·92
NEAA-N	12·8	14·3	9·8	3·22

TAA-N, total amino acid N, calculated as the sum of the N contents of the individual amino acids determined.

EAA-N, essential amino acid N, the assumption is made that threonine, valine, isoleucine, leucine, phenylalanine, histidine, lysine, arginine, tryptophan and methionine are essential (see p. 48).

NEAA-N, non-essential amino acid N, this fraction comprises aspartic acid, serine, glutamic acid, proline, glycine, alanine, tyrosine and cysteine/cystine.

the small intestine on the cobbed diet (cobbed *v.* chopped, $P < 0{\cdot}001$; cobbed *v.* pelleted, $P < 0{\cdot}05$). Expressed as a percentage of the total N present, ammonia-N comprised 0·5 % in the feed, from 4·3 to 6·2 % in duodenal samples, from 9·9 to 13·9 % in ileal samples and from 3·4 to 4·3 % in faecal samples (see Table 7). The value in the feed was lower than that reported by Ferguson & Terry (1954); the values for duodenal digesta are in agreement with those of Hogan (1965) and of Unsworth (1970).

Only very small amounts of urea N were found in the digesta entering the small intestine (mean values ranged from 16 to 28 mg urea N/24 h) and no urea-N was detected in digesta leaving the small intestine.

Total amino acid N

Mean values for total amino acid N (TAA-N), essential amino acid N (EAA-N) and non-essential amino acid N (NEAA-N) ingested, entering and leaving the small intestine and excreted in the faeces are presented in Table 3. It should be noted that, in the absence of relevant information for the ruminant animal, the amino acids grouped as essential in this paper are those required by the growing rat (Mitchell, 1964) with the addition of arginine. The studies of Black, Kleiber, Smith & Stewart (1957), of Downes (1961) and of Schingoethe, Hageman & Larson (1967) provide some evidence that these amino acids are essential for growth and milk production in the ruminant animal.

TAA-N values, derived by summation of the N contents of the individual amino acids measured, are not exactly comparable with α-amino N values reported by Clarke *et al.* (1966) or by Unsworth (1970) for one or both of two reasons. Firstly, TAA-N values include some non α-amino N groups and, secondly, some amino acids occurring in certain samples have not been determined (i.e. α, ε-diaminopimelic, α-aminoisobutyric or 2-aminoethylphosphonic acid).

There were no significant differences between diets in the amounts of AA-N passing through the various parts of the digestive tract (see Table 3). The amounts of TAA-N which disappeared in the small intestine, expressed as percentages of the amounts entering the small intestine, were calculated to be 71·5, 66·2 and 69·8 for the chopped, cobbed and pelleted diets respectively. In Table 3 the amounts of AA-N entering the small intestine and disappearing from it or from the caecum and colon have also been expressed as percentages of the amounts ingested. It can be seen that the relative amounts of EAA-N reaching the proximal duodenum were considerably greater than those of NEAA-N; furthermore, the relative amounts of EAA-N disappearing in the small intestine were appreciably higher than those of NEAA-N.

Individual amino acids

Fig. 1 shows graphically the relative amounts (intake = 100) of each essential amino acid entering and leaving the small intestine and excreted in the faeces. The amounts of each essential amino acid reaching the small intestine, and disappearing from it and from the caecum and colon, relative to the amounts ingested are given in Table 4. From Fig. 1 it can be seen that the greatest increases at the proximal duodenum were for methionine (cobbed, $P < 0.05$; chopped and pelleted, $P < 0.001$), for lysine (all diets, $P < 0.001$) and for tryptophan (all diets, $P < 0.001$); isoleucine also increased (chopped and cobbed, $P < 0.01$; pelleted, $P < 0.001$). Only with phenylalanine on the cobbed and pelleted diets were the amounts entering the small intestine less than the amounts ingested ($P < 0.05$).

Significant differences between diets in the relative amounts reaching the small intestine were noted only for valine, tryptophan and methionine; significantly more

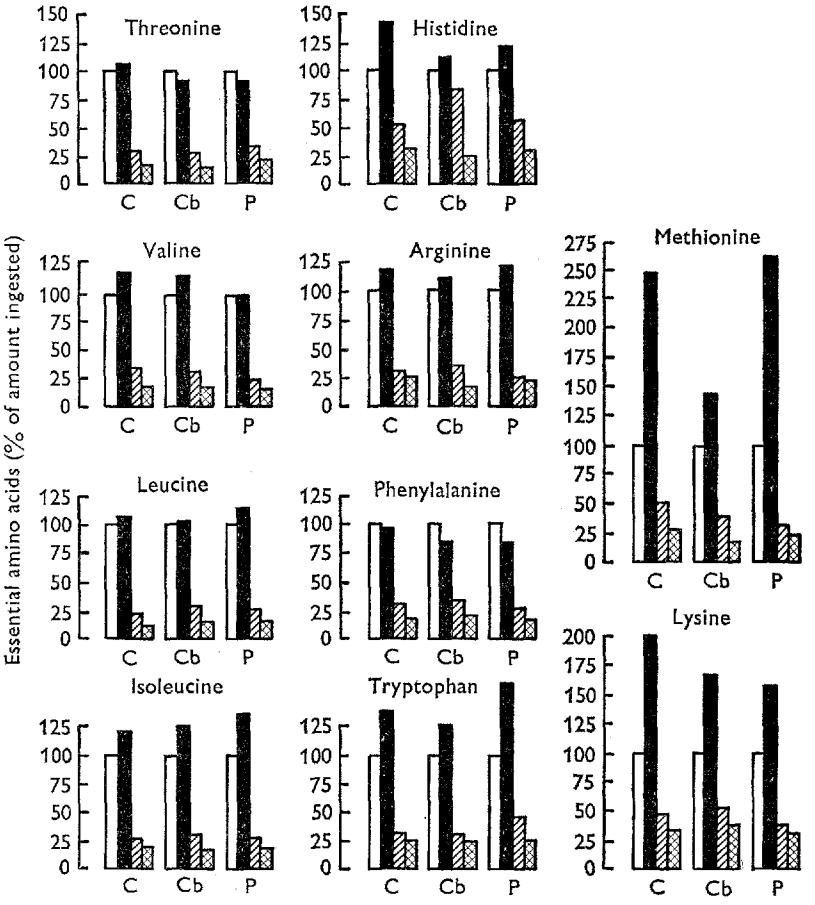

Fig. 1. Mean amounts of each amino acid assumed to be essential entering and leaving the small intestine, and in the faeces relative to the amounts ingested (intake = 100) for sheep given chopped (C), cobbed (Cb) and pelleted (P) lucerne. The quantities entering and leaving the small intestine have been adjusted for 100% recovery of chromic oxide. □, in diet; ■, at proximal duodenum; ▨, at terminal ileum; ▩, in faeces.

tryptophan and methionine entered the small intestine on the chopped and pelleted diets than on the cobbed diet (for tryptophan, pelleted *v.* chopped or cobbed $P < 0{\cdot}01$ and chopped *v.* cobbed $P < 0{\cdot}05$; for methionine, chopped *v.* cobbed $P < 0{\cdot}05$ and pelleted *v.* cobbed $P < 0{\cdot}01$). For valine the relative amounts entering the small intestine were significantly higher on the chopped and cobbed feeds ($P < 0{\cdot}05$).

The relative amounts of tryptophan and methionine disappearing within the small intestine were significantly higher with the chopped and pelleted diets (for tryptophan, pelleted *v.* chopped or cobbed $P < 0{\cdot}01$ and chopped *v.* cobbed $P < 0{\cdot}05$; for methionine, chopped *v.* cobbed $P < 0{\cdot}05$ and pelleted *v.* cobbed $P < 0{\cdot}01$).

The apparent digestibilities of each essential amino acid in the small intestine (calculated as the amount disappearing in the small intestine as a percentage of the amount entering) are given in parentheses in Table 4. In general, the between-diet agreement in the apparent digestibilities of any one amino acid was quite good, although with

Table 4. *Mean amounts of individual essential amino acids (mmol/100 mmol of amino acid in feed) entering the small intestine and disappearing therein and also in the caecum and colon of three sheep given chopped, cobbed and pelleted lucerne*

(The values in parentheses refer to the disappearance in the small intestine expressed as a percentage of the amount entering the small intestine)

Amino acid	Physical form of dried lucerne diet	Entering the small intestine	Disappearing in the small intestine	Disappearing in the caecum and colon
Threonine	Chopped	105·8	75·5 (71·3)	12·9
	Cobbed	91·6	61·4 (67·1)	13·7
	Pelleted	91·4	65·1 (71·2)	9·6
	SEM	5·96	6·90	2·14
Valine	Chopped	122·4	83·6 (68·2)	19·1
	Cobbed	116·2	82·6 (71·1)	14·1
	Pelleted	99·4	75·2 (75·7)	7·4
	SEM	2·29	2·83	2·47
Isoleucine	Chopped	119·6	88·1 (73·6)	12·6
	Cobbed	124·8	95·8 (76·7)	9·7
	Pelleted	135·2	106·6 (78·7)	7·2
	SEM	4·97	5·80	3·04
Leucine	Chopped	106·6	83·2 (78·1)	9·0
	Cobbed	101·5	72·9 (71·9)	13·4
	Pelleted	112·9	87·9 (77·9)	9·7
	SEM	5·94	6·20	6·83
Phenylalanine	Chopped	96·8	65·5 (67·6)	12·4
	Cobbed	84·2	54·9 (65·2)	11·6
	Pelleted	83·4	55·8 (66·9)	10·7
	SEM	4·53	5·35	2·48
Histidine	Chopped	141·9	88·6 (62·5)	21·5
	Cobbed	112·4	29·8 (26·4)	27·1
	Pelleted	121·2	63·8 (52·5)	57·8
	SEM	15·50	24·27	11·80
Lysine	Chopped	202·8	157·3 (77·5)	13·0
	Cobbed	167·6	114·1 (68·1)	16·6
	Pelleted	157·3	121·4 (77·1)	6·0
	SEM	9·09	10·24	3·02
Arginine	Chopped	124·5	94·3 (75·7)	5·6
	Cobbed	112·0	77·1 (68·9)	19·1
	Pelleted	120·7	97·1 (80·3)	4·2
	SEM	8·07	9·26	3·88
Tryptophan	Chopped	138·0	106·1 (77·1)	6·3
	Cobbed	127·7	95·4 (74·8)	7·2
	Pelleted	164·5	118·6 (72·0)	19·4
	SEM	1·77	3·03	2·67
Methionine	Chopped	248·1	195·8 (78·9)	24·3
	Cobbed	144·9	105·1 (72·4)	19·9
	Pelleted	261·2	229·1 (87·5)	7·3
	SEM	16·64	14·35	4·86

seven of the ten essential amino acids the values on the cobbed diet were lower than those on the chopped or pelleted diet. The value for histidine on the cobbed diet (26·4 % ± 14·73 SE) was very markedly lower than that for the other two forms: chopped 62·5 ± 9·94 and pelleted 52·5 % ± 8·14. Methionine had the highest apparent digestibility (mean value, all diets, 79·8 %) followed by isoleucine (mean value 76·3 %);

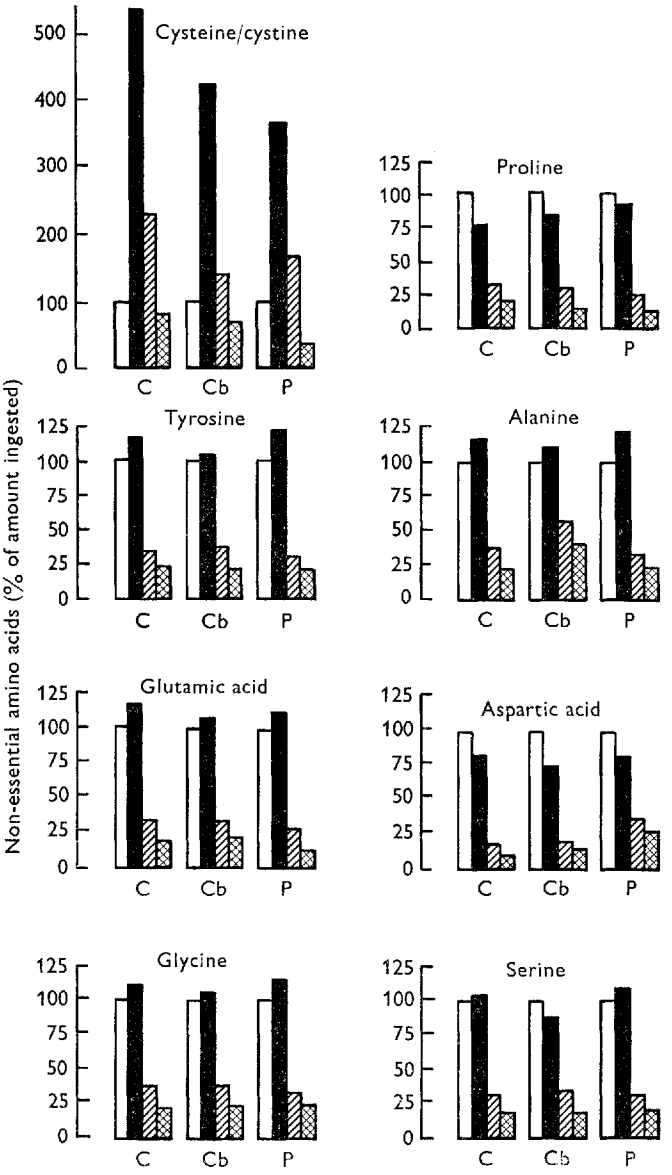

Fig. 2. Mean amounts of each amino acid assumed to be non-essential entering and leaving the small intestine, and in the faeces relative to the amount ingested (intake = 100) for sheep given chopped (C), cobbed (Cb) and pelleted (P) lucerne. The quantities entering and leaving the small intestine have been adjusted for 100% recovery of chromic oxide. □, in diet; ■, at proximal duodenum; ▨, at terminal ileum; ▩, in faeces.

low values were found for histidine (mean value 47·1%) and for phenylalanine (mean value 66·6%).

Fig. 2 shows graphically the relative amounts (intake = 100) of each non-essential amino acid entering and leaving the small intestine and excreted in the faeces. The amounts of each non-essential amino acid reaching the small intestine, and disappear-

Table 5. *Mean amounts of individual non-essential amino acids (mmol/100 mmol of amino acid in feed) entering the small intestine and disappearing therein and also in the caecum and colon of three sheep given chopped, cobbed and pelleted lucerne*

(The values in parentheses refer to the disappearance in the small intestine expressed as a percentage of the amount entering the small intestine)

Amino acid	Physical form of dried lucerne diet	Entering the small intestine	Disappearing in the small intestine	Disappearing in the caecum and colon
Aspartic acid	Chopped	83·8	63·2 (75·4)	7·5
	Cobbed	76·2	53·4 (70·0)	9·2
	Pelleted	80·8	61·0 (75·5)	6·6
	SEM	2·62	3·42	1·83
Serine	Chopped	102·3	71·6 (70·0)	11·6
	Cobbed	86·6	50·4 (58·2)	18·5
	Pelleted	109·2	78·1 (71·5)	11·3
	SEM	3·39	5·44	3·33
Glutamic acid	Chopped	117·2	84·8 (77·3)	13·9
	Cobbed	107·6	74·4 (69·1)	9·5
	Pelleted	111·6	83·7 (74·9)	14·7
	SEM	3·66	5·14	3·32
Proline	Chopped	75·9	44·0 (57·9)	14·2
	Cobbed	83·5	52·6 (63·0)	15·8
	Pelleted	92·5	65·7 (71·0)	9·1
	SEM	4·29	6·30	3·30
Glycine	Chopped	111·6	75·1 (67·3)	14·2
	Cobbed	105·2	68·4 (65·0)	14·0
	Pelleted	115·1	82·0 (71·2)	9·2
	SEM	5·12	6·17	5·00
Alanine	Chopped	116·2	80·1 (69·0)	14·1
	Cobbed	111·2	73·3 (65·9)	15·7
	Pelleted	123·3	90·0 (73·0)	9·6
	SEM	7·15	8·80	4·85
Tyrosine	Chopped	117·4	84·7 (72·2)	10·4
	Cobbed	104·3	67·6 (64·8)	15·8
	Pelleted	122·5	93·9 (76·8)	8·5
	SEM	7·50	5·92	1·81
Cysteine/cystine	Chopped	522·2	299·0 (57·1)	147·5
	Cobbed	416·7	278·1 (66·6)	68·4
	Pelleted	361·4	197·7 (54·7)	128·0
	SEM	23·77	15·49	20·08

ing from it and from the caecum and colon, relative to the amount ingested are given in Table 5. It can be seen from Fig. 2 that there were marked increases in the amounts of cysteine/cystine ($P < 0{\cdot}001$) reaching the proximal duodenum with all three diets. With aspartic acid, losses occurred on all diets ($P < 0{\cdot}001$). The same was true for proline but only with the chopped and cobbed diets were the losses significant ($P < 0{\cdot}01$).

With the cobbed diet significantly less serine reached the small intestine and less was apparently absorbed from it than with the other two diets ($P < 0{\cdot}05$). Significant differences were also observed in the relative amounts of cysteine/cystine either reaching the small intestine (chopped *v.* cobbed $P < 0{\cdot}05$ and chopped *v.* pelleted

Table 6. *Mean quantities of nucleic acid nitrogen* (g/24 *h*) *present in the feed, entering and leaving the small intestine and in the faeces of three sheep given chopped, cobbed and pelleted lucerne*

(The values for digesta at the duodenum and ileum have been adjusted for 100% recovery of chromic oxide)

	Chopped	Cobbed	Pelleted	SEM
In food				
RNA-N	0·64	0·64	0·63	—
DNA-N	0·29	0·29	0·29	—
Total	0·93	0·93	0·92	—
At proximal duodenum				
RNA-N	1·82	1·72	1·36	0·069
DNA-N	0·46	0·43	0·30	0·022
Total	2·28	2·15	1·66	0·072
At terminal ileum				
RNA-N	0·42	0·33	0·23	0·017
DNA-N	0·11	0·09	0·07	0·006
Total	0·53	0·42	0·30	0·021
In faeces:				
RNA-N	0·30	0·32	0·29	0·015
DNA-N	0·06	0·06	0·07	0·007
Total	0·36	0·38	0·36	0·020

RNA-N, ribonucleic acid N, calculated on the assumption that RNA contains 14% of total N (McAllan & Smith, 1969).
DNA-N, deoxyribonucleic acid N, calculated on the assumption that DNA contains 14·8% of total N (McAllan & Smith, 1969).

$P < 0{\cdot}01$) or apparently absorbed from it (chopped or cobbed *v.* pelleted $P < 0{\cdot}05$ and chopped *v.* cobbed $P < 0{\cdot}01$).

Again it can be seen from the apparent digestibilities of individual amino acids in the small intestine (Table 5) that for six of the eight non-essential amino acids, the apparent digestibility was lower on the cobbed than on the chopped or pelleted diet. On the basis of the mean for all three diets, cysteine/cystine had the lowest apparent digestibility (mean value 59·5%) and aspartic acid had the highest (mean value 73·6%). Notwithstanding the differences between amino acids, both essential and non-essential, in their apparent digestibilities in the small intestine, the general observation can be made that amounts disappearing in the small intestine were closely related to the amounts entering. This is clear from the very significant positive correlations observed for each of the diets between the amounts of individual amino acids entering the small intestine and disappearing within it (for chopped $r = 0{\cdot}991$, for cobbed $r = 0{\cdot}991$ and for pelleted $r = 0{\cdot}996$; $P < 0{\cdot}001$; $n = 18$). Combining the values for all three diets gave a correlation coefficient $r = 0{\cdot}990$ ($P < 0{\cdot}001$; $n = 54$).

NA-N

Mean NA-N values are shown in Table 6. The amounts of ribonucleic acid N (RNA-N) and of deoxyribonucleic acid N (DNA-N) present at the duodenum showed no significant difference between the chopped and cobbed form but the values for the pelleted diet were significantly lower than the mean ($P < 0{\cdot}05$); the same was true for total NA-N ($P < 0{\cdot}01$). The amounts of NA-N leaving the small intestine were also

Table 7. *Contribution of the various nitrogen fractions analysed to total N in feed, duodenal, ileal and faecal samples from three sheep given chopped, cobbed and pelleted lucerne (expressed as percentages of the total N present)*

	Chopped	Cobbed	Pelleted	SEM
In food sample				
TAA-N	69·9	69·3	69·6	—
NA-N	4·0	3·5	3·6	—
Ammonia-N	0·5	0·5	0·5	—
Total	74·4	73·3	73·7	—
In duodenal sample				
TAA-N	62·3	64·7	63·3	1·16
NA-N	7·5	7·0	5·3	0·33
Ammonia-N	4·3	6·2	4·5	0·35
Total	74·1	77·9	73·1	—
In ileal sample				
TAA-N	43·3	59·8	44·6	3·21
NA-N	4·2	3·8	2·6	0·18
Ammonia-N	10·1	13·9	9·9	2·35
Total	57·6	77·5	57·1	—
In faecal sample				
TAA-N	50·4	49·0	48·3	5·11
NA-N	5·4	5·1	4·9	0·33
Ammonia-N	3·5	4·3	3·4	0·64
Total	59·3	58·4	56·6	—

TAA-N, total amino acid N; NA-N, nucleic acid N.

significantly different (chopped *v.* cobbed $P < 0{\cdot}05$; chopped *v.* pelleted $P < 0{\cdot}01$ and cobbed *v.* pelleted $P < 0{\cdot}05$). From 77 to 82% of the NA-N entering the small intestine disappeared within it.

Summation of N fractions

Table 7 shows the amounts of the total N accounted for in the various fractions analysed. The values for the diets indicate that about 74% of the total N was accounted for: comparable values for duodenal samples ranged from 73 to 78%. These percentages were lower with ileal and faecal samples, varying from 57 to 59%, except for the ileal sample from sheep given the cobbed diet when a mean value of 77·5% of the total N was recovered. This relatively high recovery was due to the high percentage of TAA-N found in this sample (cobbed *v.* chopped or pelleted $P < 0{\cdot}05$). It would appear that the protein entering the small intestine was less available when the lucerne was cobbed than when it was chopped or pelleted. Some evidence that this might be a characteristic of processing by cobbing or wafering is indicated from recent findings for red clover diets (Beever, Thomson & Harrison, 1971).

DISCUSSION

Summation of N fractions

Summation of the N fractions analysed accounted for only 71–74% of the total N in the lucerne diets and therefore considerable amounts of non-protein N (NP-N), such as amines, amides, betaines, nitrates, must have been present. Ferguson & Terry (1954) reported that amino N values varied from 52 to 83% of the total N in forage samples;

in the present study the values for AA-N were 69–70% of the total N. The values reported for duodenal samples (62–65% of the total N) agree well with the amino N values published by Hogan (1965) and by Clarke *et al.* (1966). The decline in proportion of total N present as AA-N in duodenal digesta is in part associated with increased contents of NA-N and ammonia-N. In part it is also likely to be due to the presence of considerable quantities of amino sugars (*N*-acetylglucosamine, *N*-acetylmuramic acid) which are components of the bacterial cell wall (Ghuysen, 1968). The relatively large amounts of N in ileal and faecal samples not accounted for in these studies may well be associated with the increasing preponderance of bacterial cell-wall debris and, in addition, the presence of amino sugars such as *N*-acetylneuraminic acid (Kent, 1967) in the glycoproteins of mucus secreted within the small and large intestines. Certainly the amino acid traces of the hydrolysates of ileal and faecal samples, and to a lesser extent those of duodenal samples, showed a number of unknown peaks. Furthermore, Smith, McAllan & Hill (1969) pointed out the likely presence in ileal contents at least of partly digested nucleic acids; these would not be measured by the analytical procedure used for the determination of nucleic acids.

Feed, duodenal and faecal N relationships

That physical form of the diet did not affect the apparent digestibility of total N is in agreement with the findings of Rodrigue & Allen (1960), Minson (1967), Demarquilly & Journet (1967), Hogan & Weston (1967) and Stone, Trimberger & Tro (1966).

Hogan & Weston (1970) reported that, when dried forages containing more than approximately 4% of total N in the digestible organic matter were given to sheep equipped with a cannula into either the abomasum or proximal duodenum, the amount of N other than ammonia entering the small intestine was less than the N intake. In the present experiment dietary ratio of N to digestible organic matter varied from 4·6 to 5·3% and therefore a net loss of N before the duodenum would have been expected. A gain was measured for all three diets (N other than ammonia at duodenum as percentage of the total N intake was 125% for the chopped, 110% for the cobbed and 115% for the pelleted diet). The present results also differ from those of Gray, Pilgrim & Weller (1958), who reported losses amounting to an equivalent of 52% of the ingested N occurring in the stomach when sheep were fed on a chopped lucerne hay (23 g N intake/24 h; 2·9% of N in dry matter). To what extent the temperature and rate of drying of the forage might have affected the solubility of the protein and therefore be responsible for the discrepancy between the present results and those of earlier workers is not known. The forage used in the present experiment was dried in equipment with an exhaust temperature of 120°; the forage diets of Hogan & Weston (1970) were dried at a low temperature.

Kay (1969) has drawn attention to the very considerable losses of AD-N that can occur in the caecum and colon and the present studies substantiate this. Certainly the considerable disappearances of digestible cellulose and of digestible hemicellulose that were found on these diets (Thomson *et al.* 1972) indicate a considerable secondary fermentation. What significance can be attached to the disappearance of N in this region of the digestive tract is not clear without further study.

Amounts of amino acids reaching the small intestine

If the assumption is made that the daily outflow of gastric juice yields 2 g N/24 h (Phillipson, 1964) and that all the N is present as pepsin, it can be calculated from the amino acid composition of pepsin (Spector, 1956) that the contribution of amino acids from gastric juice represents between 9 and 17% of each of the amino acids entering the small intestine in the present experiment, with the exception of arginine, lysine and histidine, where the contribution is negligible, and serine in which gastric secretions may contribute about 26% (there was no information for alanine). With these figures and assumptions in mind, it is clear that the gains before the duodenum in the amounts of four amino acids, namely methionine, lysine, tryptophan and cysteine/cystine, could not be accounted for solely by the contribution of gastric secretion. The microbial fermentation has markedly enhanced the amounts of these amino acids entering the small intestine. Microbial activity has resulted in a considerable net loss of phenylalanine, proline and aspartic, partly masked by a contribution from gastric secretion. The above conclusions are dependent not only on the validity of the gastric juice contribution but also on the assumption that amino acids are not absorbed in any considerable amount before the small intestine. That amino acids can be absorbed from the rumen has been demonstrated by Demaux, Le Bars, Molle, Rerat & Simonnet (1961), McLaren (1964) and Cook, Brown & Davis (1965), but the quantity is likely to be small.

The increased amounts of methionine, lysine, tryptophan and cysteine/cystine can be partly explained by the results shown in Fig. 3, in which the mean amino acid contents of the lucerne used in this experiment have been plotted against the mean values for twenty-two species of rumen bacteria given by Purser & Buechler (1966). It is apparent that for cysteine/cystine and for methionine the concentrations in the bacteria are at least three times higher than in lucerne, and for tryptophan and lysine the concentrations are approximately twice as high. Fig. 3 also shows that the concentrations of phenylalanine, proline and aspartic acid, for which there was a net loss between mouth and duodenum, are appreciably less in bacteria than in the lucerne. It has also been suggested that loss of proline before the small intestine (Clarke *et al.* 1966) is associated with its participation in a Stickland-type reaction with subsequent formation of δ-amino valeric acid (El-Shazly, 1952).

Fate of amino acids in the small intestine

The very high positive correlation between amounts of individual amino acids reaching the small intestine and absorbed from it has been noted. Whether or not microbial fermentation may thus have increased the amounts of the essential amino acids, lysine, methionine and tryptophan available to the animal depends not only on the magnitude of the increase but on their relative availabilities in the intact food protein and in the microbial protein. For methionine and for lysine the amounts absorbed from the small intestine relative to the amounts ingested were 177 and 131% respectively. Thus the intervention of microbial fermentation has enhanced the supply of these two essential amino acids to the host animal (gastric secretion appears to

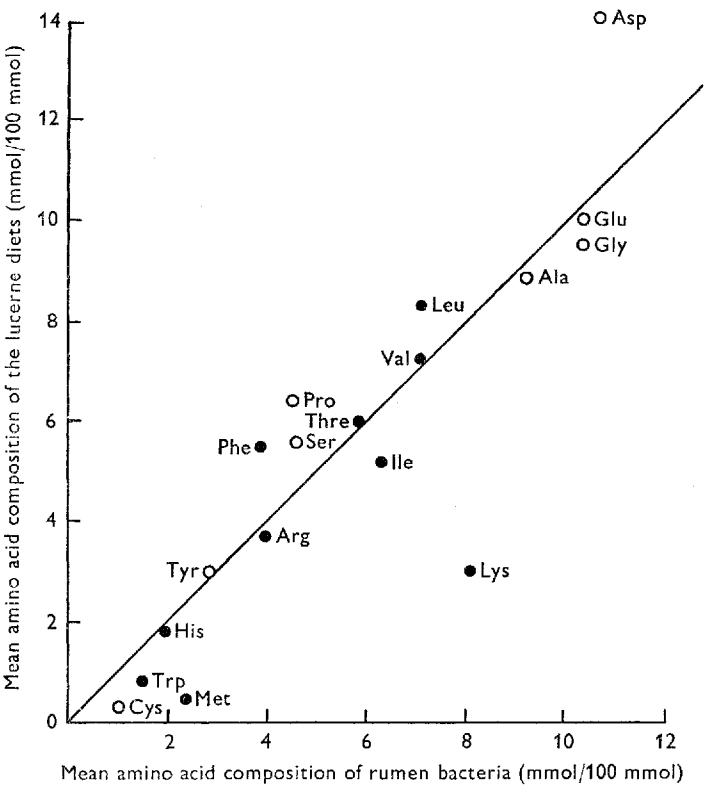

Fig. 3. Amino acid composition of the lucerne diets compared with that of rumen bacteria of sheep given by Purser & Buechler (1966). The value for tryptophan is from Abdo, King & Engel (1964) and relates to microbial protein. ●, essential amino acids; ○, non-essential amino acids.

contribute little to lysine entering the small intestine). For tryptophan the comparable value was 107%; when allowance is made for the contribution of gastric secretion and the possibility of a higher availability of tryptophan in the lucerne protein than that found in microbial protein, microbial fermentation may have resulted in only limited enhancement of the supply of this amino acid to the host animal. For cysteine/cystine there is no doubt that microbial fermentation had markedly increased the amount absorbed from the small intestine (amount absorbed relative to the amount ingested, mean value 258%).

In Fig. 4 mean values for the amino acid composition of duodenal and ileal digesta are compared. With the exception of histidine, phenylalanine and threonine, the mean concentrations of individual essential amino acids at the terminal ileum were lower than those at the proximal duodenum and, apart from aspartic and glutamic acids, the reverse is true for the non-essential amino acids. Apart from the results for histidine, phenylalanine, aspartic acid and tyrosine these relationships agree with those reported by Purser (1970) from an examination of the results of Clarke *et al.* (1966). Purser (1970) suggested that there was some preferential absorption of the essential amino acids other than threonine from the small intestine. It is noteworthy, however, that

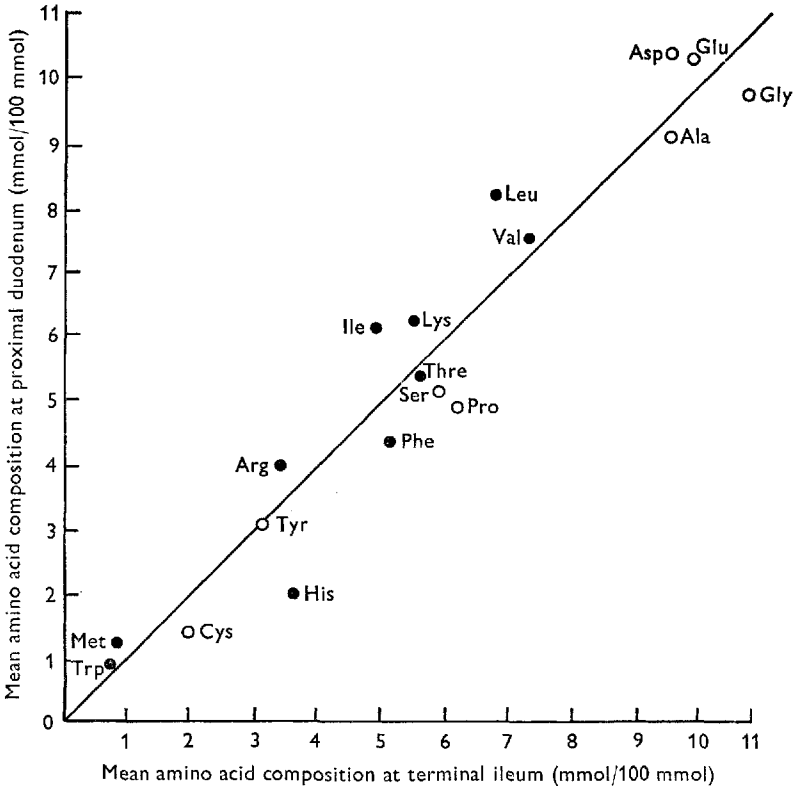

Fig. 4. Amino acid composition of digesta at the proximal duodenum compared with that at the terminal ileum of sheep given chopped, cobbed and pelleted lucerne. ●, essential amino acids; ○, non-essential amino acids (mean values for the three lucerne diets).

mucins, which are continuously secreted in the small intestine, have high concentrations of threonine, proline and alanine, and low concentrations of leucine, tyrosine, phenylalanine and lysine (see Clarke *et al.* 1966); thus the apparent preferential absorption of some of the essential amino acids may, to some extent, be an artifact.

Estimates of microbial N

From relationships involving the amounts of organic matter disappearing before the small intestine and the amounts of NA-N present in duodenal digesta, it is possible to estimate the contribution of microbial N to total N entering the small intestine. Use of the relationship of Hogan & Weston (1970) (based on measurements of α,ϵ-diaminopimelic acid, an amino acid specific to bacteria), namely 3·7 g bacterial N formed per 100 g organic matter disappearing in the stomach, together with the mean amounts of organic matter disappearing before the small intestine of the sheep given the three lucerne diets (see Thomson *et al.* 1972), allows the amounts of bacterial N entering the small intestine to be calculated. Using these values and Smith & McAllan's (1970*a*) estimate of 19% for the NA-N content of bacterial N, the contribution of bacterial NA-N to total NA-N at the duodenum can be derived. Assuming that all the NA-N at the duodenum is of microbial origin, then the difference between total NA-N and

Table 8. *Estimates of the microbial nitrogen contribution to total N entering the small intestine of three sheep given chopped, cobbed or pelleted lucerne (expressed as percentages of the total N at the proximal duodenum)*

	Chopped	Cobbed	Pelleted
Bacterial N	29·1	26·5	19·3
Protozoal N	16·8	17·5	12·4
Microbial N	45·9	44·0	31·7

bacterial NA-N will be that contributed by the protozoa. Smith & McAllan (1970*a*) have estimated that the NA-N content of total protozoal N is 12 %. The contribution of protozoal N to total N in the digesta at the duodenum can be calculated using this estimate. The results of these calculations for the three lucerne diets are shown in Table 8.

On the basis of the estimates shown in Table 8, it would appear that, on the chopped and cobbed diets, some 44–46 % of the total N at the duodenum was of microbial origin, the value falling to 32 % on the pelleted diet. On all three diets the contribution of bacterial N to microbial N ranged from 60–63 %.

While there is evidence that NA-N is related to microbial protein synthesis (Price, 1951–2; Ellis & Pfander, 1965; see also Smith, 1969), the validity of the calculations described above also rests on the assumption that NA-N present at the duodenum is only of microbial origin. There is no evidence as to the correctness of this assumption for, although it has been shown that nucleic acid added to the rumen is degraded rapidly (Smith & McAllan, 1970*b*), nucleic acid of feed origin if trapped within unfermented feed particles may escape fermentation in the rumen.

Amino acid requirements

Nimrick, Hatfield, Kaminski & Owens (1970*a*, *b*) have provided evidence that the limiting order of essential amino acids for growing lambs given urea as the sole source of N is methionine, lysine and threonine. In preliminary studies, Wakeling, Lewis & Annison (1970) have given a requirement for methionine for a 50 kg sheep and a value for lysine which they consider to be in excess of requirement. Wakeling (1970) has also determined a value for threonine. The requirements are expressed in terms of amounts of amino acid entering the small intestine. They were corrected to yield requirements in terms of amounts absorbed from the small intestine by multiplying them by the appropriate mean values for apparent digestibility of individual amino acids found in the present study (see Table 4), namely for methionine 79·8 %, for threonine 69·8 % and for lysine 74·2 %. The measured uptakes (mean values) of the three amino acids from the small intestine were then expressed as percentages of these corrected requirements for each of the three diets; the values are shown in Table 9.

On the basis of the requirements used, it would appear from Table 9 that on the chopped and pelleted diets none of the three amino acids was limiting, but that on the cobbed diet methionine appeared to be so. However, it must be noted that cystine partially spares methionine and the uptake of cysteine/cystine was very appreciable on all three diets. To what extent methionine uptake would be limiting on the cobbed diet

Table 9. *Uptakes of methionine, threonine and lysine by three sheep given chopped, cobbed and pelleted lucerne, as percentages of the requirements given by Wakeling* (1970) *and Wakeling* et al. (1970), *corrected for incomplete absorption from the small intestine*

	Methionine	Threonine	Lysine
Chopped	109	138	226
Cobbed	66	128	187
Pelleted	112	134	204

is thus open to question. Certainly the values in Table 9 suggest that threonine would be limiting before lysine on lucerne diets. Wakeling (1970) calculated the requirements of a 50 kg sheep for other essential amino acids, except tryptophan and arginine but including tyrosine and cystine, based upon values for the pig. With one exception, uptakes from the small intestine measured in the present study were in excess of these requirements by amounts greater than the range of values shown for threonine in Table 9. The exception was histidine on the cobbed diet (uptake equivalent to 67% of the calculated requirement). It must be emphasized that the above-mentioned requirements were not related to level of energy intake and that, in view of the present very limited knowledge of amino acid requirements for ruminants, the above conclusions must be treated with caution.

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