# The effect in sheep of physical form and stage of growth on the sites of digestion of a dried grass

## 2.* Sites of nitrogen digestion

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1. The effect of grinding and pelleting S 24 perennial rye-grass (*Lolium perenne* L.), harvested at two stages of maturity and artificially dried, on the sites of digestion of nitrogenous constituents was studied with sheep equipped with a fistula into the rumen and re-entrant cannulas at the proximal duodenum and terminal ileum. Chopped and pelleted grasses at both stages of maturity were each given at 900 g dry matter (DM)/24 h to each of five sheep; the two forms of the early-cut grass were also given each to four of the five sheep at 1400 g DM/24 h.

2. The pelleting process significantly depressed apparent digestibility of total nitrogen in both grasses ($P < 0.05$). For the early-cut grass neither physical form nor level of feeding had any significant effect on N retention; for the medium-cut grass N retention was significantly higher ($P < 0.05$) when the pelleted form was given. N retention was significantly lower on the medium-cut grass ($P < 0.05$) than on the early-cut grass.

3. For individual amino acids the greatest relative increases (intake = 100) at the proximal duodenum were found for methionine and cysteine/cystine ($P < 0.001$) on all six diets, together with tryptophan ($P$ at least $< 0.01$) and lysine ($P$ at least $< 0.05$) on five of the diets, and including histidine on the early-cut diets ($P$ at least $< 0.01$). At the low level of feeding, increase in stage of growth at cutting significantly depressed the relative amounts of phenylalanine, lysine, serine ($P < 0.05$), histidine, aspartic acid and tyrosine ($P < 0.01$) entering the small intestine, and increased those of tryptophan ($P < 0.05$). Pelleting of the early-cut grass significantly increased the amounts of methionine entering the small intestine compared with feeding it chopped ($P < 0.05$); the same was true for phenylalanine at the high level of feeding only.

4. The relative amounts of threonine, isoleucine, leucine and arginine disappearing in the small intestine were not significantly affected by physical form, stage of growth or in the case of the early-cut grass, by level of feeding. At the low level of feeding increase in maturity at cutting significantly lowered uptake of histidine ($P < 0.01$) and phenylalanine ($P < 0.05$) from the small intestine. With tryptophan, uptake from the small intestine was greater ($P < 0.01$) and for lysine was lower ($P < 0.05$) for the medium-cut than for the early-cut material but only with the chopped form. Pelleting of the medium-cut grass significantly lowered relative uptake of tryptophan ($P < 0.05$) and increased that of lysine ($P < 0.05$) compared with the chopped form. Pelleting of the early-cut grass significantly increased the relative amounts of methionine disappearing in the small intestine ($P < 0.05$) at both levels of intake; the same was true for phenylalanine at the higher level of feeding only.

5. The apparent digestibilities in the small intestine of total amino acid N in chopped grass given at the low level were 78.7 and 70.1% with early- and medium-cut grass respectively. With pelleted rations given in low levels the digestibilities were 75.1 and 72.6% with early- and medium-cut grass. When the early-cut grass was given at high level of intake, the digestibilities were 74.9 and 67.4% with chopped and pelleted rations respectively.

6. Despite differences in the apparent digestibilities of individual amino acids in the small intestine and between-diet differences for a given amino acid a high positive correlation between

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the amounts of individual amino acids entering the small intestine and absorbed from it was found for all the values ($n = 108$; $r = 0.982$, $P < 0.001$); the same was true for each individual diet.

7. The amount of nucleic acid N entering the small intestine was significantly lower ($P < 0.05$) when the early cut diet was given pelleted at the high level. From the results for nucleic acid N, the amount of total N entering the small intestine which was of microbial origin was calculated to vary from 62 to 71 % on the chopped diets and from 48 to 63 % on the pelleted diets; the lower values relate to the high level of feeding.

Studies of the effect of grinding and pelleting on the apparent digestibility of total nitrogen in hays or dried grasses have been reported by Blaxter & Graham (1956), Rodrigue & Allen (1960), Haenlein & Holdren (1965), Stone, Trimberger & Tro (1966), Demarquilly & Journet (1967) and Minson (1967). Beever, Thomson, Pfeffer & Armstrong (1969) have reported flows of total N through the proximal duodenum and terminal ileum of sheep fed on fresh (frozen) and dried rye-grass. Flows of total N through the abomasum of sheep fed on fresh and dried rye-grass have also been reported by Hogan (1965) and Weston & Hogan (1968). In a previous paper (Coelho da Silva, Seeley, Thomson, Beever & Armstrong, 1972) the sites of digestion of total N and of other nitrogenous constituents of a dried lucerne offered in three physical forms have been reported. There appear to be no studies, on dried grasses, of the effect of physical form, level of dry-matter (DM) intake or stage of growth at harvest on the sites of digestion of total N and individual nitrogenous fractions.

Beever, Coelho da Silva, Prescott & Armstrong (1972) studied the sites of digestion of organic matter, energy and carbohydrates contained in an early and later cut of dried S 24 perennial rye-grass (*Lolium perenne* L.) when offered to sheep either chopped or after grinding and pelleting; the early-cut material was given at two levels of DM intake. In this paper, the results pertaining to the sites of digestion of various nitrogenous fractions obtained in the same experiment are reported.

## EXPERIMENTAL

Details concerning the experimental design, diets and sheep have been given by Beever *et al.* (1972) and the management of animals and of the collection procedures were given by Beever, Thomson, Pfeffer & Armstrong (1971). Urine was collected in a glass bottle placed below each cage and containing 100 ml 5 M-HCl. A daily sample equivalent to 10 % by weight of the urine excreted was stored at 4° and used to prepare a composite sample for the 7 d collection period. This sample was stored in a deep freeze until required for analysis. The preparation of the samples for determination of the nitrogenous constituents and the chemical procedures used were as described by Coelho da Silva *et al.* (1972). At the low level of DM intake (900 g DM/24 h) each of the four diets were offered to five sheep; due to the accidental loss of one sheep only four of the five animals received each of the two forms of the early-cut material at the high level of intake (1400 g DM/24 h). Accordingly, in the statistical analysis of the results a missing value was computed for each of the two diets given at the high level of DM intake. The interaction between factors was statistically significant in almost all analyses; so results are discussed as comparisons between six treatments, and not in terms of main effects of factors.

Table 1. *Fractionation of total nitrogen and amounts of individual amino acids in the dried grasses given to the sheep*

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

| Stage of growth ... | Early cut | | Medium cut | |
|---|---|---|---|---|
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted |
| Total N | 3·18 | 3·05 | 3·15 | 3·03 |
| Ammonia-N | 0·020 | 0·018 | 0·027 | 0·024 |
| Amino acid N | 2·33 | 2·22 | 2·43 | 2·43 |
| RNA-N | 0·130 | 0·130 | 0·128 | 0·128 |
| DNA-N | 0·035 | 0·035 | 0·039 | 0·039 |
| Amino acids: | | | | |
| Threonine | 0·92 (4·63) | 0·85 (4·46) | 0·82 (4·17) | 0·82 (4·33) |
| Valine | 1·24 (6·20) | 1·13 (5·93) | 1·03 (5·24) | 1·03 (5·44) |
| Isoleucine | 0·88 (4·43) | 0·88 (4·62) | 0·84 (4·27) | 0·84 (4·43) |
| Leucine | 1·49 (7·50) | 1·47 (7·72) | 1·31 (6·66) | 1·31 (6·92) |
| Phenylalanine | 0·78 (3·92) | 0·71 (3·73) | 1·02 (5·19) | 1·02 (5·38) |
| Histidine | 0·48 (2·42) | 0·46 (2·41) | 0·87 (4·42) | 0·88 (4·65) |
| Lysine | 0·80 (4·00) | 0·73 (3·83) | 0·88 (4·47) | 0·88 (4·64) |
| Arginine | 0·86 (4·33) | 0·81 (4·25) | 1·00 (5·08) | 1·00 (5·28) |
| Tryptophan | 0·26 (1·31) | 0·24 (1·26) | 0·18 (0·90) | 0·17 (0·87) |
| Methionine | 0·24 (1·21) | 0·14 (0·73) | 0·20 (1·02) | 0·20 (1·06) |
| Aspartic acid | 1·93 (9·55) | 1·83 (9·61) | 2·42 (12·30) | 2·42 (12·76) |
| Serine | 0·76 (3·83) | 0·70 (3·67) | 0·84 (4·27) | 0·84 (4·43) |
| Glutamic acid | 2·45 (12·38) | 2·37 (12·44) | 2·22 (11·29) | 2·22 (11·72) |
| Proline | 1·21 (6·10) | 1·18 (6·19) | 0·99 (5·03) | 0·99 (5·23) |
| Glycine | 0·98 (4·93) | 0·99 (5·20) | 0·87 (4·42) | 0·87 (4·59) |
| Alanine | 1·35 (6·80) | 1·33 (6·98) | 1·26 (6·41) | 1·26 (6·65) |
| Tyrosine | 0·66 (3·32) | 0·64 (3·36) | 0·74 (3·76) | 0·74 (3·91) |
| Cysteine/cystine | 0·11 (0·55) | 0·12 (0·63) | 0·11 (0·56) | 0·10 (0·53) |

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).

## RESULTS

### *Total N*

The contents of total N and of individual nitrogenous fractions in the grasses are given in Table 1. The content of N tended to be slightly lower in both pelleted diets than in the chopped diets. Although the medium-cut grass was harvested 6 weeks later than the early-cut, there was no difference in their total N content and little in that of nucleic acid N or of total amino acid N (TAA-N). The TAA-N accounted for about 73% of the total N present in the early-cut material and 78% in the medium-cut material. The most noticeable differences between the two grasses in their amino acid compositions were the higher values observed for histidine and, to a lesser extent, for phenylalanine and aspartic acid in the medium-cut material. The results reported for individual amino acids in the dried grasses (g/16 g N) are in general agreement with those listed by Harvey (1956) for grass hay and for perennial rye-grass, with the exception of the values for methionine and cysteine/cystine which are lower than those of Harvey.

Table 2. *Mean quantities of total nitrogen present in the food, entering and leaving the small intestine and in the faeces of sheep fed on dried grass and their mean N retentions. At the low level of intake each diet was given to five sheep; at the high level to four sheep*

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

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| Total N (g/24 h): | | | | | | | |
| In food | 28·69 | 28·74 | 26·92 | 26·64 | 45·01 | 43·46 | — |
| At proximal duodenum | 30·30 | 34·07 | 29·45 | 29·79 | 51·79 | 50·29 | 1·16 |
| At terminal ileum | 8·89 | 9·69 | 11·21 | 10·40 | 12·94 | 17·46 | 0·28 |
| In faeces | 6·71 | 7·48 | 6·63 | 7·34 | 10·62 | 12·93 | 0·22 |
| Apparent digestibility (%) | 76·6 | 74·0 | 75·4 | 72·5 | 76·4 | 70·4 | 0·63 |
| N retention (% of total N ingested) | 22·3 | 18·6 | 4·3 | 10·4 | 24·8 | 22·3 | 1·84 |
| Disappearance of apparently digested N (%): | | | | | | | |
| Before small intestine | −7·3 | −25·1 | −12·5 | −16·4 | −20·6 | −23·1 | 4·88 |
| In small intestine | 97·4 | 114·8 | 89·9 | 100·5 | 113·7 | 108·3 | 4·98 |
| In caecum and colon | 9·9 | 10·3 | 22·6 | 15·9 | 6·9 | 14·8 | 1·65 |

DM, dry matter.

* Means for amounts reaching the small intestine and beyond include a missing value.

† In this and subsequent tables, the SE of a mean for a high level of DM intake is 1·12 times the value quoted, and the SE of the difference between one of these means and any mean for a low level is 1·52 times the value quoted.

Mean values for the intakes of total N, flows of N through the digestive tract and N retentions are given in Table 2. The apparent digestibility of total N was significantly affected ($P < 0·05$) by the pelleting process but not by the stage of growth when the grasses were offered at the low level of feeding. At the high level of feeding (determinations on early-cut grass only) the pelleting process significantly depressed the apparent digestibility of total N ($P < 0·05$). For the early-cut grass neither physical form, nor level of feeding, significantly affected N retention; for the medium-cut grass physical form did affect N retention ($P < 0·05$). At the low level of intake N retention was significantly lower on the medium-cut grass ($P < 0·05$). Although the gains in N before the small intestine, expressed as percentages of the apparently digested N (ADN), tended to be higher for the pelleted diets than for the corresponding chopped forms, the differences were only significant ($P < 0·05$) for the early-cut grass at the low level of intake. At the low level of feeding the relative amounts of ADN disappearing in the small intestine tended to be higher for the pelleted diets than for the corresponding chopped forms, but again the differences were only significant ($P < 0·05$) for the early-cut grass at the low level of intake. At the low level of feeding the mean amounts of ADN disappearing in the caecum and colon were significantly higher for the medium-cut diets (chopped, $P < 0·01$; pelleted, $P < 0·05$). At the high level of feeding, pelleting significantly increased the disappearance of ADN in the caecum and colon ($P < 0·05$).

Table 3. *Mean quantities of ammonia-nitrogen (g/24 h) present in the food, entering and leaving the small intestine and in the faeces of sheep given dried grass. At the low level of intake each diet was given to five sheep; at the high level to four sheep*

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

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| In food | 0·182 | 0·166 | 0·229 | 0·213 | 0·286 | 0·250 | — |
| At proximal duodenum | 1·97 | 1·66 | 2·45 | 1·81 | 2·82 | 1·73 | 0·12 |
| At terminal ileum | 0·71 | 0·98 | 1·87 | 1·32 | 1·09 | 1·90 | 0·08 |
| In faeces | 0·19 | 0·42 | 0·27 | 0·22 | 0·42 | 0·69 | 0·03 |

DM, dry matter.
* Means for amounts reaching the proximal duodenum and beyond include a missing value.
† For explanation see footnote to Table 2.

### *Ammonia- and urea-N*

Table 3 shows the results for flows of ammonia-N through the digestive tract. The amounts of ammonia-N entering the small intestine tended to be greater with the chopped than with the pelleted diets but only for the early-cut material at the higher level of feeding and the medium-cut grass were the differences statistically significant ($P < 0·05$). Expressed as percentages of the total N present, ammonia-N comprised from 0·6 to 0·9% in the food, from 3·8 to 8·4% in duodenal samples, from 8·4 to 16·7% in ileal samples and from 2·9 to 5·6% in faecal samples. Duodenal values were within the range reported for fresh forage diets by Hogan (1965) and for dried lucerne diets by Coelho da Silva *et al.* (1972). The ranges given for ileal and faecal samples were similar to values determined for dried lucerne diets (see Coelho da Silva *et al.* 1972). Only very small amounts of urea-N were present in the digesta samples (mean values ranged from 3 to 20 mg/24 h in duodenal digesta and from 3 to 5 mg/24h in ileal digesta).

### *Total amino acid N*

Table 4 shows mean values for 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. It should be noted, firstly, that TAA-N values are not exactly comparable with $\alpha$-amino-N values and, secondly, in the absence of reliable information as to which of the amino acids are essential for the various classes of ruminant livestock, the amino acids classified as essential are those required for maximum growth in the rat (see Coelho da Silva *et al.* 1972).

The amounts of TAA-N reaching the proximal duodenum were not affected by physical form or stage of growth of the grasses nor did stage of growth affect either the amounts of TAA-N reaching the terminal ileum or those excreted in the faeces. For the early-cut grass the amounts of TAA-N reaching the terminal ileum were greater ($P < 0·05$) on the pelleted food but only at the high level; the amounts excreted in the

Table 4. *Mean amounts of amino acid nitrogen present in the food, entering and leaving the small intestine and excreted in the faeces of sheep fed on dried grass diets. At the low level of intake each diet was given to five sheep; at the high level to four. Also included are mean values relative to the amounts ingested and their standard errors 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)

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| In food (g/24 h): | | | | | | | |
| TAA-N | 21·04 | 20·95 | 20·77 | 21·33 | 33·01 | 31·67 | — |
| EAA-N | 10·74 | 10·49 | 11·12 | 11·42 | 16·86 | 15·86 | — |
| NEAA-N | 10·30 | 10·46 | 9·65 | 9·91 | 16·15 | 15·81 | — |
| At proximal duodenum (g/24 h): | | | | | | | |
| TAA-N | 19·47 | 20·23 | 17·70 | 19·26 | 32·54 | 29·95 | 1·00 |
| EAA-N | 11·00 | 11·42 | 10·14 | 11·04 | 18·04 | 16·95 | 0·59 |
| NEAA-N | 8·47 | 8·81 | 7·56 | 8·22 | 14·50 | 13·00 | 0·45 |
| At terminal ileum (g/24 h): | | | | | | | |
| TAA-N | 4·13 | 5·06 | 5·30 | 5·28 | 8·14 | 9·75 | 0·42 |
| EAA-N | 2·08 | 2·61 | 2·57 | 2·82 | 4·14 | 5·00 | 0·21 |
| NEAA-N | 2·05 | 2·45 | 2·73 | 2·46 | 4·00 | 4·75 | 0·21 |
| In faeces (g/24 h): | | | | | | | |
| TAA-N | 3·38 | 4·43 | 3·87 | 4·09 | 6·13 | 7·11 | 0·24 |
| EAA-N | 1·70 | 2·33 | 2·13 | 2·26 | 3·18 | 3·72 | 0·14 |
| NEAA-N | 1·68 | 2·10 | 1·74 | 1·83 | 2·95 | 3·39 | 0·19 |
| Amounts at proximal duodenum (% of amount ingested): | | | | | | | |
| TAA-N | 92·5 | 96·5 | 85·2 | 90·3 | 98·8 | 94·8 | 4·3 |
| EAA-N | 102·4 | 110·8 | 91·2 | 96·6 | 107·3 | 107·1 | 4·6 |
| NEAA-N | 82·2 | 82·3 | 78·3 | 83·0 | 90·0 | 82·4 | 4·2 |
| Amounts disappearing in the small intestine (% of amount ingested): | | | | | | | |
| TAA-N | 72·9 | 72·4 | 59·7 | 65·6 | 74·0 | 63·9 | 4·0 |
| EAA-N | 83·0 | 85·9 | 67·1 | 72·0 | 82·5 | 75·4 | 4·4 |
| NEAA-N | 62·3 | 58·9 | 51·8 | 58·2 | 65·2 | 52·3 | 3·8 |
| Amounts disappearing in the caecum and colon (% of amount ingested): | | | | | | | |
| TAA-N | 3·6 | 3·0 | 6·9 | 5·6 | 6·1 | 8·3 | 1·9 |
| EAA-N | 3·5 | 2·6 | 5·6 | 4·9 | 5·7 | 8·1 | 1·8 |
| NEAA-N | 3·6 | 3·3 | 8·4 | 6·3 | 6·5 | 8·5 | 2·0 |

DM, dry matter.

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. 361).

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

* Means for amounts reaching the proximal duodenum and beyond include a missing value.

† For explanation see footnote to Table 2.

faeces on the pelleted material were significantly higher ($P < 0{\cdot}01$) at both feeding levels.

Physical form did not significantly affect the amount of EAA-N reaching the duodenum (medium-cut and high-level of early-cut; $P < 0{\cdot}05$), but it significantly affected the amounts leaving the terminal ileum (high level of early-cut material; $P < 0{\cdot}05$) and excreted in the faeces (both levels of early-cut material; $P < 0{\cdot}05$). Stage of maturity significantly affected amounts of EAA-N reaching the small intestine and excreted in the faeces but only for the chopped grass ($P < 0{\cdot}05$).

With the early-cut material fed at the high level, physical form significantly affected amounts of NEAA-N entering and leaving the small intestine ($P < 0{\cdot}05$). With the grass given chopped, stage of maturity had a significant affect on the amount of NEAA-N leaving the small intestine ($P < 0{\cdot}05$).

From the values in Table 4 it can be calculated that the amounts of TAA-N which disappeared in the small intestine, expressed as percentages of the amounts entering it, were for the chopped and pelleted diets respectively: early-cut at the low level of intake, 78·7 and 75·1%; medium-cut at the low level of intake, 70·1 and 72·6%; early cut at the high level of intake, 74·9 and 67·4%.

In Table 4 the amounts of TAA-N, EAA-N and NEAA-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 either entering the small intestine or disappearing from it were considerably greater than comparable values for the NEAA-N. The relative amounts of EAA-N either reaching the proximal duodenum or disappearing from the small intestine were significantly higher when sheep received the early-cut diets than when the medium-cut diets were given ($P < 0{\cdot}05$).

### *Individual amino acids*

The amounts of each amino acid assumed to be essential entering the small intestine, disappearing from it and from the caecum and colon relative to the amounts ingested are given in Table 5. From Table 5 it can be seen that the amounts of methionine entering the small intestine significantly exceeded the intake with all the diets ($P < 0{\cdot}001$); the same was true for lysine on five of the six diets ($P$ at least $< 0{\cdot}05$), the exception being the medium-cut grass when given chopped. The amounts of tryptophan entering the small intestine were significantly increased compared to the amounts ingested on five of the six diets ($P$ at least $< 0{\cdot}05$), the exception relating to the early-cut chopped grass given at the high level. With histidine the amounts entering the small intestine exceeded the amounts ingested with the early-cut grasses ($P$ at least $< 0{\cdot}01$); with the medium-cut grasses the amounts entering the small intestine were less than those ingested. Significant losses of phenylalanine occurred on the medium-cut grass ($P < 0{\cdot}001$).

The relative amounts of threonine, isoleucine, leucine and arginine either entering the small intestine or disappearing from it were not significantly affected by physical form, stage of growth or, with the early-cut grass, by level of feeding. At the low level of feeding, increase in maturity of grass significantly depressed the relative amount of

Table 5. *Mean amounts of individual amino acids assumed to be essential (mmol/*100 *mmol amino acid in food) entering the small intestine (A) and disappearing there (B) and also in the caecum and colon (C) of sheep fed on dried grass diets. At the low level of intake each diet was given to five sheep; at the high level to four sheep. The values in parentheses are the amounts disappearing in the small intestine expressed as a percentage of the amount entering the small intestine*

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| Threonine: | | | | | | | |
| A | 85·2 | 82·6 | 93·1 | 92·3 | 98·6 | 85·1 | 5·2 |
| B | 61·2 | 61·0 | 68·1 | 67·5 | 73·2 | 57·4 | 5·2 |
| | (71·8) | (73·8) | (73·2) | (73·2) | (74·6) | (67·8) | |
| C | 3·2 | 2·4 | 7·1 | 5·2 | 7·8 | 5·2 | 2·4 |
| Valine: | | | | | | | |
| A | 79·3 | 96·4 | 100·3 | 119·9 | 99·1 | 91·0 | 3·1 |
| B | 64·3 | 74·8 | 74·5 | 92·3 | 77·1 | 63·0 | 3·4 |
| | (81·1) | (77·5) | (74·2) | (77·0) | (77·7) | (69·1) | |
| C | 2·4 | 4·2 | 9·2 | 7·0 | 6·8 | 8·5 | 2·1 |
| Isoleucine: | | | | | | | |
| A | 92·4 | 96·2 | 96·7 | 108·9 | 109·5 | 71·8 | 6·5 |
| B | 72·7 | 74·3 | 73·9 | 83·8 | 86·8 | 64·7 | 6·0 |
| | (78·7) | (77·2) | (76·3) | (77·1) | (79·5) | (70·6) | |
| C | 4·0 | 2·3 | 3·7 | 2·9 | 3·2 | 3·8 | 2·2 |
| Leucine: | | | | | | | |
| A | 83·5 | 86·5 | 83·5 | 80·6 | 94·2 | 85·2 | 3·8 |
| B | 68·9 | 67·3 | 60·7 | 56·3 | 75·3 | 60·3 | 4·2 |
| | (82·5) | (77·8) | (72·7) | (69·9) | (80·6) | (70·6) | |
| C | 0·9 | 4·3 | 7·0 | 6·6 | 4·8 | 7·2 | 2·0 |
| Phenylalanine: | | | | | | | |
| A | 106·1 | 117·7 | 74·1 | 70·1 | 64·2 | 116·3 | 4·0 |
| B | 84·3 | 78·8 | 39·6 | 36·3 | 32·3 | 65·3 | 4·0 |
| | (79·5) | (67·0) | (53·4) | (52·0) | (51·6) | (56·8) | |
| C | 1·2 | 7·9 | 18·6 | 16·6 | 10·7 | 23·1 | 3·4 |
| Histidine: | | | | | | | |
| A | 149·1 | 169·7 | 79·7 | 83·5 | 159·8 | 153·4 | 10·8 |
| B | 115·8 | 137·5 | 54·5 | 62·5 | 117·6 | 110·9 | 11·5 |
| | (77·6) | (81·1) | (68·6) | (74·9) | (72·8) | (71·6) | |
| C | 16·3 | 5·2 | 4·7 | 4·4 | 19·5 | 20·7 | 3·6 |
| Lysine: | | | | | | | |
| A | 129·7 | 139·4 | 102·2 | 124·4 | 139·7 | 132·1 | 8·2 |
| B | 100·6 | 103·2 | 68·5 | 92·2 | 105·9 | 84·2 | 7·8 |
| | (77·7) | (74·0) | (67·0) | (74·2) | (75·9) | (63·6) | |
| C | 6·5 | 1·3 | 7·5 | 2·5 | 2·2 | 6·7 | 3·6 |
| Arginine: | | | | | | | |
| A | 91·9 | 97·4 | 85·2 | 86·7 | 89·5 | 97·3 | 5·8 |
| B | 80·2 | 77·6 | 68·3 | 67·5 | 72·8 | 75·3 | 5·6 |
| | (87·2) | (79·7) | (80·1) | (77·8) | (82·2) | (78·1) | |
| C | −1·1 | 0·1 | −0·6 | 2·3 | 2·0 | 3·6 | 1·4 |
| Tryptophan: | | | | | | | |
| A | 138·7 | 135·6 | 192·0 | 159·0 | 85·4 | 139·9 | 7·0 |
| B | 114·3 | 116·2 | 154·1 | 118·7 | 70·2 | 107·7 | 7·1 |
| | (82·4) | (85·6) | (80·4) | (74·6) | (82·3) | (76·7) | |
| C | 4·8 | 2·1 | 13·0 | 10·7 | −3·4 | 3·2 | 2·2 |
| Methionine: | | | | | | | |
| A | 123·3 | 209·3 | 169·0 | 173·8 | 139·1 | 196·2 | 5·0 |
| B | 109·8 | 176·5 | 144·1 | 149·8 | 120·3 | 152·6 | 5·9 |
| | (89·0) | (84·4) | (85·4) | (86·3) | (86·9) | (78·3) | |
| C | −2·6 | 19·3 | 1·8 | 1·0 | −6·2 | −7·6 | 3·5 |

DM, dry matter.
* Means include a missing value.
† For explanation see footnote to Table 2.

phenylalanine and lysine ($P < 0.05$) and of histidine ($P < 0.01$) entering the small intestine. Pelleting of the early-cut grass significantly increased the amounts of methionine entering the small intestine at either level of intake ($P < 0.05$) compared with the amounts on the chopped diet; at the high level of feeding only this was also true for phenylalanine ($P < 0.05$). For tryptophan, increases in the relative amounts reaching the proximal duodenum were greater for the medium-cut than for the early-cut diets (chopped, $P < 0.01$; pelleted, $P < 0.05$). With reference to the relative amounts of phenylalanine and histidine disappearing in the small intestine, the comments made above concerning the relative amounts reaching the proximal duodenum apply equally well. Concerning tryptophan, the relative amount disappearing in the small intestine was significantly greater ($P < 0.01$) for the medium-cut than for the early-cut grass when given in the chopped form; for the medium-cut material it was also higher on the chopped than on the pelleted form ($P < 0.05$). With lysine the relative amount disappearing in the small intestine was significantly less ($P < 0.05$) for the medium-cut than for the early-cut grass when given in the chopped form; for the medium-cut material it was also lower on the chopped than on the pelleted form ($P < 0.05$). For methionine, pelleting of the early-cut material resulted in a significant increase in the relative amounts of the amino acid disappearing in the small intestine at either level of intake. With the chopped form the relative amount of methionine disappearing in the small intestine was greater on the medium-cut material ($P < 0.01$); with the pelleted form the reverse was true ($P < 0.05$).

The apparent digestibility of each essential amino acid in the small intestine is given in parentheses in Table 5. It can be seen that, in general, the values decreased with increasing level of DM intake for the early-cut grass and that the effect was greater with the pelleted diet. Methionine showed the highest apparent digestibility in the small intestine (mean value for all diets 84.5%), followed by arginine (mean value 80.7%) and tryptophan (mean value 80.2%); a low value was found for phenylalanine (mean value for all diets 58.6%).

The amounts of each non-essential amino acid reaching the small intestine, disappearing from it and from the caecum and colon relative to the amount ingested are given in Table 6 from which it can be seen that only with cysteine/cystine were the amounts entering the small intestine significantly greater than the intakes from all the diets ($P < 0.001$). Small gains at the duodenum were observed for tyrosine with all the early-cut diets and these were significant ($P$ at least $< 0.05$) except for the pelleted diet at the high level of feeding. Consistent losses in the relative amounts entering the small intestine were found for alanine, proline and aspartic acid ($P$ at least $< 0.01$), and for glutamic acid ($P$ at least $< 0.05$) on all diets. Serine showed losses for all diets but they were significant only on the medium-cut grass ($P$ at least $< 0.01$).

There were no significant between-diet differences in the relative amounts of glutamic acid or alanine reaching the small intestine but for glycine there was a significant stage of maturity effect although only with the pelleted form ($P < 0.05$). The relative amounts of aspartic acid, tyrosine ($P < 0.01$) and of serine ($P < 0.05$) entering the small intestine were significantly greater with the early-cut compared with the medium-cut diets; the opposite was true for proline of which significantly

Table 6. *Mean amounts of individual amino acids assumed to be non-essential (mmol/100 mmol of amino acid in food) entering the small intestine (A) and disappearing there (B) and also in the caecum and colon (C) of sheep fed on dried grass diets. At the low level of intake each diet was given to five sheep; at the high level to four sheep. The values in parentheses are the amounts disappearing in the small intestine expressed as a percentage of the amount entering the small intestine*

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| Aspartic acid: | | | | | | | |
| A | 77·3 | 81·4 | 65·2 | 67·0 | 87·1 | 80·8 | 3·8 |
| B | 61·5 | 62·6 | 47·9 | 51·1 | 66·0 | 55·9 | 3·6 |
| | (79·5) | (76·9) | (73·5) | (76·3) | (75·8) | (69·2) | |
| C | 2·2 | 1·0 | 4·5 | 2·2 | 4·8 | 5·3 | 1·6 |
| Serine: | | | | | | | |
| A | 90·6 | 92·3 | 75·2 | 82·1 | 94·5 | 90·0 | 4·6 |
| B | 70·4 | 68·2 | 53·9 | 61·6 | 67·8 | 58·4 | 4·7 |
| | (77·7) | (73·8) | (71·6) | (75·1) | (72·1) | (65·4) | |
| C | 3·8 | 4·1 | 5·4 | 2·6 | 9·7 | 8·5 | 2·4 |
| Glutamic acid: | | | | | | | |
| A | 71·8 | 71·6 | 79·2 | 84·1 | 81·4 | 76·6 | 4·4 |
| B | 54·3 | 51·1 | 51·4 | 58·6 | 59·5 | 50·1 | 4·0 |
| | (75·5) | (71·4) | (64·9) | (69·6) | (72·6) | (64·9) | |
| C | 4·7 | 3·6 | 10·9 | 7·9 | 6·8 | 7·9 | 2·0 |
| Proline: | | | | | | | |
| A | 63·5 | 69·7 | 76·9 | 81·9 | 72·5 | 64·7 | 4·0 |
| B | 50·0 | 53·7 | 53·6 | 60·8 | 52·6 | 45·0 | 4·1 |
| | (78·7) | (77·0) | (69·8) | (74·2) | (72·3) | (69·4) | |
| C | 3·3 | 2·6 | 6·5 | 4·4 | 7·2 | 3·8 | 2·4 |
| Glycine: | | | | | | | |
| A | 85·8 | 80·0 | 92·6 | 96·2 | 104·7 | 88·1 | 5·3 |
| B | 64·8 | 55·4 | 59·5 | 63·6 | 76·0 | 54·5 | 4·6 |
| | (75·6) | (69·2) | (64·2) | (66·1) | (72·8) | (62·0) | |
| C | 3·4 | 3·1 | 12·9 | 9·6 | 8·5 | 9·8 | 2·4 |
| Alanine: | | | | | | | |
| A | 76·1 | 78·1 | 74·8 | 80·1 | 78·7 | 72·5 | 4·2 |
| B | 56·6 | 54·1 | 43·5 | 51·5 | 56·3 | 39·9 | 3·8 |
| | (74·5) | (69·3) | (58·1) | (64·2) | (71·5) | (55·0) | |
| C | 4·3 | 4·6 | 14·6 | 9·9 | 4·9 | 11·1 | 2·2 |
| Tyrosine: | | | | | | | |
| A | 114·6 | 124·6 | 84·5 | 95·7 | 119·8 | 106·2 | 6·6 |
| B | 91·5 | 100·6 | 57·1 | 71·2 | 93·1 | 72·9 | 7·1 |
| | (79·7) | (80·6) | (67·4) | (74·4) | (77·4) | (68·4) | |
| C | 2·5 | −2·1 | 3·3 | 1·3 | 0·5 | −1·0 | 2·8 |
| Cysteine/cystine: | | | | | | | |
| A | 560·7 | 328·8 | 258·2 | 282·7 | 410·3 | 423·4 | 16·3 |
| B | 320·0 | 82·4 | 137·2 | 136·6 | 199·0 | 202·0 | 21·4 |
| | (57·1) | (24·9) | (53·1) | (48·7) | (48·7) | (48·1) | |
| C | 4·4 | 52·1 | −63·6 | 54·4 | 23·2 | 121·1 | 14·3 |

DM, dry matter.
* Means include a missing value.
† For explanation see footnote to Table 2.

greater relative amounts entered the small intestine on the medium-cut diets ($P < 0.05$). For cysteine/cystine greater relative amounts entered the small intestine on the early-cut diet compared with the medium-cut diets although only when the material was chopped was the difference statistically significant ($P < 0.05$); at the low level of intake of the early-cut material, pelleting significantly reduced the amount of cysteine/cystine reaching the small intestine ($P < 0.01$).

With reference to relative amounts disappearing from the small intestine, there were no significant between-diet effects for glutamic acid or proline. With serine and alanine there was a significantly greater disappearance with the early-cut compared to medium-cut material but only with the chopped form was the difference significant ($P < 0.05$). When given at the low level, pelleting of the early-cut material significantly reduced the relative disappearance of alanine and of glycine ($P < 0.05$). For aspartic acid, tyrosine and cysteine/cystine comments relating to the relative amounts entering the small intestine also apply to the amounts disappearing from it.

Again, when the early-cut diet was given pelleted at the high level of feeding it can be seen that in most instances the apparent digestibility of each non-essential amino acid in the small intestine (values in parentheses, Table 6) tended to be lower. Exceptionally low was the value for cysteine/cystine on the early-cut pelleted diet at the low level of feeding (24·9 %) and, indeed, on the basis of a mean value for all the diets, this amino acid had the lowest apparent digestibility in the small intestine (47·2 %). Aspartic acid and tyrosine had the highest apparent digestibilities in the small intestine (mean values 74·6 and 74·2 % respectively). Despite differences in the apparent digestibilities of individual amino acids (both essential and non-essential) in the small intestine and between-diet differences for any one amino acid, a high positive correlation was consistently noted between the amounts of amino acids entering the small intestine and absorbed from it. The relevant correlation coefficients, one for each of the six grasses, ranged from +0·965 to +0·996 (all $P < 0.001$); that for all diets ($n = 108$) was +0·982 ($P < 0.001$). Similar high correlations have been noted in lucerne diets given to sheep (Coelho da Silva *et al.* 1972).

### *Nucleic acid N*

Mean nucleic acid N values are shown in Table 7. The amounts of nucleic acid N entering the small intestine were from 2·5 to 3·5 times greater than those present in the food. When the chopped, as compared to the pelleted, diets were given, consistently more nucleic acid N entered the small intestine, but the only difference that was significant was for the early-cut diet when given at the high level ($P < 0.05$). This last-mentioned difference was due to the ribonucleic acid N (RNA-N); the results for deoxyribonucleic acid N (DNA-N) showed no significant difference. From Table 7 it can be calculated that from 85 to 93 % of the nucleic acid N entering the small intestine disappeared within that organ, the values tending to be higher with the chopped diets. Comparable values for ruminating calves fed on a variety of diets have been reported to be 80 % (Smith, McAllan & Hill, 1969); for sheep given dried lucerne diets, values ranged from 77 to 82 % (Coelho da Silva *et al.* 1972). From results presented in Table 7 it can be calculated that the losses of nucleic acid N in the caecum

Table 7. *Mean quantities (g/24 h) of nucleic acid nitrogen in the food, entering and leaving the small intestine and in the faeces of sheep fed on dried grass diets. At the low level of intake each diet was given to five sheep; at the high level to four*

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

| Level of DM intake ... | Low | | | | High* | | |
|---|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted | SEM† |
| In food: | | | | | | | |
| RNA-N | 1·17 | 1·23 | 1·10 | 1·13 | 1·84 | 1·85 | — |
| DNA-N | 0·32 | 0·33 | 0·34 | 0·35 | 0·50 | 0·50 | — |
| At proximal duodenum: | | | | | | | |
| RNA-N | 3·36 | 3·04 | 3·07 | 2·83 | 5·05 | 3·29 | 0·18 |
| DNA-N | 0·97 | 0·92 | 1·08 | 0·95 | 1·41 | 1·52 | 0·09 |
| At terminal ileum: | | | | | | | |
| RNA-N | 0·31 | 0·29 | 0·35 | 0·33 | 0·43 | 0·50 | 0·02 |
| DNA-N | 0·13 | 0·16 | 0·17 | 0·17 | 0·10 | 0·23 | 0·02 |
| In faeces: | | | | | | | |
| RNA-N | 0·29 | 0·30 | 0·34 | 0·36 | 0·40 | 0·46 | 0·02 |
| DNA-N | 0·05 | 0·06 | 0·07 | 0·07 | 0·07 | 0·06 | 0·004 |

DM, dry matter.

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).

* Means for the amounts reaching the proximal duodenum and beyond include a missing value.

† For explanation see footnote to Table 2.

and colon varied from 10 to 30% of the amounts entering this part of the digestive tract.

*Summation of N fractions*

With the food samples some 79–86% of the total N has been accounted for, TAA-N alone accounting for 73–80% of the total N. The values for nucleic acid N are slightly lower than the 6% value reported by Smith & McAllan (1970) for grass samples. In duodenal samples the total amount of N recovered varied from 73 to 85%, the lower recoveries being associated with the early-cut pelleted diets (73 and 76%). In the ileal samples the recoveries of total N in the fractions analysed were lower, especially at the low levels of intake, partly because of the significantly lower percentages of TAA-N in these samples ($P < 0{\cdot}01$). In the faeces the recoveries of total N were even lower than those at the terminal ileum (values ranged from 59 to 70%). Coelho da Silva *et al.* (1972) found comparable low recoveries in studies on sheep given dried lucerne diets and have commented on the likely cause of them.

## DISCUSSION

Even though the early cut of grass was harvested 6 weeks before the medium cut, on the basis of their contents of total N and ADN it might be considered that the two dried grasses were very similar. However, that there were appreciable differences in their composition, particularly with reference to contents of structural carbohydrates and as sources of digestible organic matter (DOM) for the animal, is evident from the results of Beever *et al.* (1972). Furthermore, studies on the potential of these grasses to promote carcass gains in lambs (Roux, Prescott & Armstrong, 1970) indicate a considerable superiority of the early-cut material in terms of nutritive value per unit DM. Additional confirmation of the existence of a difference between the two grasses can be seen from the values for N retention obtained in the present study (Table 2). On equal intakes of DM (900 g/24 h) and closely similar intakes of ADN, the daily N balances were significantly higher on the early-cut grass diets.

In the present experiment, physical form did affect apparent digestibility of total N in the two grasses when offered at the level of 900 g DM/24 h; the even greater depression noted when the early-cut grass given at the higher level of intake (1400 g DM/24 h) was pelleted is in keeping with the findings of Haenlein & Holdren (1965), who reported that apparent digestibility of total N was affected to a greater extent by level of intake than by physical form.

The intakes of dietary N as g/100 g DOM for the six diets used in the present experiment ranged from 4·2 to 4·7. According to the relationship given for dried forages by Hogan & Weston (1970), such ratios might have been expected to induce small net losses in the amount of N other than ammonia (NA-N) entering the small intestine. Calculation from values in Tables 2 and 3 show that in one grass (early-cut chopped, low level of intake) there was a small net loss, in another (medium-cut chopped, low level) no change, and in the remaining four diets increases ranging from 5 to 13% in NA-N at the proximal duodenum (in terms of the total N ingested).

In assessing the contribution of microbial fermentation in the reticulo-rumen to the net gains or losses in individual amino acids occurring between mouth and pylorus, account should be taken of the possible absorption of amino acids from the rumen and also the contribution made by gastric secretion. No quantitative information is available on the first-mentioned but amounts are likely to be small. An estimate of the possible contribution of gastric secretion to individual amino acids reaching the proximal duodenum has been made (see Coelho da Silva *et al.* 1972) and on this basis it can be said that, with two exceptions, four amino acids, namely cysteine/cystine, methionine, lysine and tryptophan, showed marked or appreciable gains as a result of microbial fermentation. These findings are in agreement with those reported for a dried lucerne diet (Coelho da Silva *et al.* 1972). The two exceptions are lysine on the medium-cut grass given chopped and tryptophan on the early-cut chopped diet given at the high level. Proline, aspartic and glutamic acids, alanine and leucine are examples of amino acids which clearly suffered a net loss in the reticulo-rumen through the intervention of microbial fermentation. The same is probably true for isoleucine, threonine, serine and glycine when the masking effect of input from gastric secretion is removed. To what extent the

Table 8. *Estimates of the microbial nitrogen contribution to total N entering the small intestine of sheep fed on dried grass diets. The values are expressed as percentages of the total N at the proximal duodenum*

| Level of DM intake ... | Low | | | | High | |
|---|---|---|---|---|---|---|
| Stage of growth ... | Early cut | | Medium cut | | Early cut | |
| Physical form ... | Chopped | Pelleted | Chopped | Pelleted | Chopped | Pelleted |
| Bacterial N | 55·4 | 42·3 | 43·5 | 42·3 | 47·5 | 42·6 |
| Protozoal N | 31·4 | 29·9 | 48·6 | 38·9 | 28·8 | 12·3 |
| Non-microbial N | 13·2 | 27·8 | 7·9 | 18·8 | 13·7 | 45·1 |

DM, dry matter.

apparent gains of histidine on the early-cut grass and apparent losses on the medium-cut grass are related to the very different dietary levels of this amino acid requires further study. The histidine content of the medium-cut grass was virtually twice that of the early-cut (see Table 1). Similar differences in the phenylalanine content of the grasses may also be the cause of the apparent difference in effect of microbial fermentation on the early-cut compared with the medium-cut when these grasses were given at the low level.

An explanation for some of these findings lies in the different amino acid composition of the grass protein on the one hand and rumen bacterial protein (Purser & Buechler, 1966) or rumen microbial protein (Abdo, King & Engel, 1964) on the other. Thus cysteine/cystine, methionine, lysine and tryptophan occur in greater amounts in bacterial or in microbial protein than in the grass protein whereas the reverse is true for proline, alanine, serine and glutamic acid.

From the amounts of organic matter disappearing before the small intestine and the amounts of nucleic acid N entering that organ, Coelho da Silva *et al.* (1972) estimated the contribution of microbial N to total N flow at the duodenum. Similar calculations have been made from the results reported in this paper and the estimates are shown in Table 8.

From these estimates it would appear that pelleting appreciably reduced the contribution of microbial N to total N entering the proximal duodenum. Furthermore, considering the values for the early-cut grass only, it can be seen that the contribution fell with increasing level of intake and that the fall was particularly marked for the pelleted food. Compared with the proteins in the dried lucernes used by Coelho da Silva *et al.* (1972), it would seem that the proteins in these dried grasses were much more susceptible to microbial fermentation.

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## REFERENCES

Abdo, K. M., King, K. W. & Engel, R. W. (1964). *J. Anim. Sci.* **23**, 734.
Beever, D. E., Coelho da Silva, J. F., Prescott, J. H. D. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 347.
Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1969). *Proc. Nutr. Soc.* **28**, 26A.
Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1971). *Br. J. Nutr.* **26**, 123.
Blaxter, K. L. & Graham, N. McC. (1956). *J. agric. Sci., Camb.* **47**, 207.
Coelho da Silva, J. F., Seeley, R. C., Thomson, D. J., Beever, D. E. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 43.
Demarquilly, C. & Journet, M. (1967). *Annls Zootech.* **16**, 123.
Haenlein, G. F. W. & Holdren, R. D. (1965). *J. Anim. Sci.* **24**, 810.
Harvey, D. (1956). *Tech. Commun. Commonw. agric. Bur.* no. 19.
Hogan, J. P. (1965). *Aust. J. agric. Res.* **16**, 179.
Hogan, J. P. & Weston, R. H. (1970). In *Physiology of Digestion and Metabolism in the Ruminant* p. 474 [A. T. Phillipson, editor]. Newcastle upon Tyne: Oriel Press.
McAllan, A. B. & Smith, R. H. (1969). *Br. J. Nutr.* **23**, 671.
Minson, D. J. (1967). *Br. J. Nutr.* **21**, 587.
Purser, D. B. & Buechler, S. M. (1966). *J. Dairy Sci.* **49**, 81.
Rodrigue, C. B. & Allen, N. N. (1960). *Can. J. Anim. Sci.* **40**, 23.
Roux, H. F., Prescott, J. H. D. & Armstrong, D. G. (1970). *Anim. Prod.* **12**, 370.
Smith, R. H. & McAllan, A. B. (1970). *Br. J. Nutr.* **24**, 545.
Smith, R. H., McAllan, A. B. & Hill, W. B. (1969). *Proc. Nutr. Soc.* **28**, 28A.
Stone, J. B., Trimberger, G. W. & Tro, B. V. (1966). *Bull. Cornell Univ. agric. Exp. Stn* no. 1010.
Weston, R. H. & Hogan, J. P. (1968). *Aust. J. agric. Res.* **19**, 963.

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