The nutritive value of silages
Digestion of organic matter, gross energy and carbohydrate constituents in the rumen and intestines of sheep receiving diets of grass silage or grass silage and barley

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1. Two experiments were conducted to study the digestion of organic matter, gross energy and carbohydrate constituents in the rumen, small intestine and caecum and colon of sheep given grass silage diets. Three silages made from perennial ryegrass (*Lolium perenne*) with formic acid as an additive were used. One was made from first-harvest grass in the spring and the others from regrowth grass cut from a single sward in either early autumn or late autumn. Expt 1 involved a comparison between the spring silage given alone or supplemented with barley (silage: barley, 4:1 dry matter (DM) basis). Expt 2 involved a comparison between the early-cut and late-cut autumn silages.

2. In Expt 1, supplementation of the silage with barley resulted in a non-significant ($P > 0.05$) reduction in the proportion of digestible energy (DE) and digestible organic matter digested in the rumen and an increase in the proportions digested in the small intestine. There were also pronounced effects of barley on ruminal cellulolysis and the proportion of digestible cellulose broken down in the rumen was reduced ($P < 0.05$) from 0.90 to 0.77. There was an increased passage of α-linked glucose polymers to the duodenum but even with the supplemented diet 0.91 of the dietary polymers were digested in the rumen. The molar proportion of propionic acid in the rumen tended to be reduced and there were increases in the proportions of butyric acid ($P < 0.01$) and acetic acid.

3. In Expt 2, the digestibility of organic matter, gross energy and cellulose in the early-cut silage was higher ($P < 0.01$) than in the late-cut silage but there were no significant ($P < 0.05$) differences between silages in the sites of digestion of these constituents. However, the molar proportion of acetic acid in the rumen was higher ($P < 0.01$) and the molar proportion of propionic acid was lower ($P < 0.01$) with the late-cut silage than with the early-cut silage.

4. The results are discussed in relation to the voluntary intake and utilization of high-digestibility silages.

In calorimetric studies of energy metabolism in sheep Kelly & Thomas (1978) examined the utilization of the energy of three perennial ryegrass (*Lolium perenne*) silages prepared using formic acid as an additive. One silage was made from first harvest grass in the spring and the others were from regrowth cut either early or late in the autumn. Each silage was given as a sole food and the spring and late-cut autumn silages were also given supplemented with barley.

The experiments reported here were to study the sites of digestion of organic matter, gross energy and carbohydrates in sheep given silage diets similar to some of those used by Kelly and Thomas (1978). The object was to provide a fuller understanding of the effects of barley supplementation and of the maturity of the grass ensiled on the digestion processes, and to investigate whether differences between silages in the efficiency of utilization of metabolizable energy for fattening (k_f), observed in the earlier experiments, could be related to the pattern of digestion of the silages.

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MATERIALS AND METHODS

Animals and their management

Expt 1. Six Finnish Landrace × Dorset Horn wether sheep were used. They were 2 or 3 years old and weighed 50–60 kg. Each had a rumen cannula and simple cannulas in the proximal duodenum (approximately 30 mm posterior to the pylorus) and terminal ileum (approximately 230 mm anterior to the ileocaecal junction). The animals were held in metabolism cages in a small animal house. Food was given in two equal meals each day at 09.30 hours and 16.00 hours and at these times the animals were also given, via the rumen cannula, 3 g paper impregnated with chromic oxide. Water and mineralized-salt blocks were freely available.

Expt 2. A total of seven Finnish Landrace × Dorset Horn wether sheep were used. They were 3 years old and weighed 50–60 kg. Each had a rumen cannula and simple cannulas in the proximal duodenum and terminal ileum as in Expt 1. Feeding and management of the animals was as in Expt 1.

Foods

The preparation of the silages was as described by Kelly & Thomas (1978). Briefly, the silage for Expt 1 was from grass cut in mid-May 1974, wilted overnight, harvested with a precision-chop forage harvester (New Holland Ltd, Aylesbury Bucks.) and ensiled with the addition of a solution of formic acid (850 g formic acid/l at a rate of 2·3 l/t fresh grass) in a 300 t wedge silo. The silage used was from the same wedge as that for the silage used in Expt 1 of Kelly & Thomas (1978) although the batch of silage described here was taken later in the winter and thus from a different part of the silo. The silages used in Expt 2 were from batches of grass cut on 6 August and 17 September 1975 from the same sward as that used for the autumn silages described by Kelly & Thomas (1978). The grass for each silage was harvested with a single-chop forage harvester (A. Kidd, Devizes, Wiltshire) and ensiled directly into 3 t polyethylene-bag silos with the addition of formic acid at a rate of 2·3 l/t.

After the silages were removed from the silos their handling and storage at -5° was as described by Kelly & Thomas (1978).

Experimental designs and procedures

Expt 1. The experiment was conducted as a replicated comparison of two dietary treatments consisting of silage given alone or the same amount of silage supplemented with rolled barley to provide a silage:barley value of 4:1 (on a dry matter basis). Six animals were allocated at random three to each of two treatment sequences, either silage followed by silage plus barley or the reverse. For each animal food intake was adjusted before the first period of the experiment to a level designed to ensure complete consumption of the diets throughout the experiment. Mean amounts of the diets offered were (on a dry matter basis) 644 g silage/d and 644 g silage plus 151 g barley/d.

Expt 2. This experiment was designed to compare the digestion of the two autumn silages prepared from the same grass sward cut at two stages of maturity. Because of limitations for cold storage of the silages after their removal from the silos, determinations with the silages were carried out in two sequential periods, the early-cut silage was given first followed by the late-cut silage. Each silage was given, at a level intended to provide approximately 700 g dry matter/d, to five sheep. It was intended that the same five animals should be used in the first and second periods of the experiment. However, in the second period two of the sheep selected refused varying but sometimes significant amounts of the late-cut silage and were replaced by two additional animals.

In Expt 1 and Expt 2 experimental periods consisted of a 14 d controlled feeding period followed by a 7 d period when faeces and urine (Expt 1 only) were collected and a 7 d period during which samples of ileal, then duodenal and then ruminal digesta were taken, each being sampled during a separate 24 h period, each period being separated by at least 24 h from the next sampling period. Duodenal and ileal samples (approximately 60 and 30 g respectively) were taken at intervals of 2 h throughout a 24 h period and each pooled to provide a sample for analysis. Samples of rumen fluid were taken at 09.30 hours (before feeding) and at 10.30, 11.30, 13.30 and 15.30 hours.

Analytical methods

Methods of analysis were as described by Kelly & Thomas (1978) and Chamberlain *et al.* (1976).

Calculations and statistical analysis

The amounts of constituents passing to the duodenum and ileum were calculated using Cr_2O_3 as an indigestible marker (MacRae & Armstrong, 1969). Apparent digestion occurring before the duodenal cannula has been referred to as digestion in the rumen, that occurring between the duodenal and ileal cannulas as digestion in the small intestine and that between the ileal cannula and the faeces as digestion in the caecum and colon.

The results were analysed using a paired t test (Expt 1) or by analysis of variance.

RESULTS

The composition of the diets

The chemical composition of the dietary ingredients are shown in Table 1. All three silages were well preserved with low pH values and low contents of butyric acid. The late-cut autumn silage had a lower content of nitrogen and a higher content of cellulose than did the early-cut silage.

The digestion of organic matter and gross energy

The amounts of organic matter and gross energy ingested, at the proximal duodenum, at the terminal ileum and in the faeces are shown in Tables 2 and 3.

The supplementation of the spring silage with barley increased the intake of organic matter and energy and there were consequent increases in the passage of organic matter to the intestine and to the faeces. However, the digestibilities of organic matter and energy were significantly ($P < 0.001$ and $P < 0.01$) higher for the supplemented diet. There were no significant ($P < 0.05$) differences between the diets in the importance of the rumen, small intestine and caecum and colon as sites of digestion but supplementation of the silage with barley tended to reduce the proportion of digestion in the rumen and increase the proportion in the lower gut, mainly in the small intestine.

The intakes of organic matter and gross energy with the late-cut autumn silage were slightly higher than with the early-cut autumn silage but the differences were small. The digestibility of organic matter and energy was significantly ($P < 0.001$) lower in the late-cut silage than in the early-cut material and associated with this there were significant differences between the silages in the passage of organic matter and gross energy to the duodenum and ileum. There were no significant ($P < 0.05$) differences between the silages in the proportions of digestible organic matter or energy disappearing in the rumen, small intestine or caecum and colon. However, for organic matter there was a tendency for a lower proportion to be digested in the rumen and a higher proportion in the small intestine with the early-cut silage than with the late-cut silage. This trend was less apparent in the digestion of gross energy.

Table 1. *The chemical composition of the dietary ingredients*

	Expt 1		Expt 2	
	Spring silage	Barley	Early-cut autumn silage	Late-cut autumn silage
Dry matter (g/kg)*	274	837	232	188
Ash (g/kg DM)	78	22	101	91
Gross energy (MJ/kg DM)	18·19	18·13	19·98	19·49
Cellulose (g/kg DM)	275	64	239	298
Water-soluble carbohydrates (g/kg DM)	63	—	82	73
α-linked glucose polymers (g/kg DM)	3·8	725·0	—	—
Total nitrogen (g/kg DM)	23·4	16·0	31·9	22·1
pH	3·70	—	4·02	4·0
Lactic acid (g/kg DM)	121	—	61	73
Acetic acid (g/kg DM)	11	—	16	19
Butyric acid (g/kg DM)	6	—	0·4	0·3

* Values for silage obtained by the method of Dewar & McDonald (1961).

Table 2. *Mean quantities of organic matter present in the food, entering and leaving the small intestine and in the faeces of sheep given diets of spring silage and of spring silage and barley (Expt 1) and diets of early-cut autumn silage and of late-cut autumn silage (Expt 2) and values for the proportion of digestible organic matter 'disappearing' before and in the small intestine and in the caecum and colon*

(Mean values with their standard errors; no. of animals/treatment in parentheses)

	Expt 1 (6)			Expt 2 (5)		
	Spring silage	Spring silage and barley	SEM	Early-cut autumn silage	Late-cut autumn silage	SEM
Organic matter (g/24 h)						
In food†	590	740	—	616	648	—
At proximal duodenum	317	401	10·9***	250	288	12·8
At terminal ileum	217	257	8·1**	151	210	5·8***
In faeces	178	200	4·1**	110	164	5·9***
Apparent digestibility of organic matter	0·697	0·731	0·005***	0·827	0·746	0·008***
Disappearance of digestible organic matter						
Before small intestine	0·655	0·626	0·020	0·722	0·746	0·030
In small intestine	0·246	0·269	0·009	0·196	0·160	0·031
In caecum and colon	0·099	0·105	0·013	0·082	0·095	0·014

Statistical significance of difference between treatments (see p. 483): ** $P < 0·01$, *** $P < 0·001$.
† Includes chromic oxide paper.

The digestion of carbohydrate constituents

The digestion of water-soluble carbohydrates and α-linked glucose polymers were examined only in Expt 1. The silage in the diets provided on average 40·3 g water-soluble carbohydrates/d and the duodenal passage of the water-soluble carbohydrates was 1·2 and 1·3 g/d (SEM 0·12) with the silage and the silage and barley diets respectively. The intake (g/d) of α-linked glucose polymers by animals receiving silage alone was low, 2·4, but for those receiving barley the intake was 112·2. Corresponding passages of α-linked glucose polymers (g/d) were 3·1 and 10·7 (SEM 0·83; $P < 0·001$) at the duodenum, 1·2 and 1·8 (SEM 0·59) at the ileum and 0·4 and 0·4 (SEM 0·04) in the faeces.

Table 3. *Mean quantities of gross energy present in the food, entering and leaving the small intestine and in the faeces of sheep given diets of spring silage and of spring silage and barley (Expt 1) and diets of early-cut autumn silage and of late-cut autumn silage (Expt 2) and values for the proportion of digestible energy 'disappearing' before and in the small intestine and in the caecum and colon*

(Mean values with their standard errors; no. of animals/treatment in parentheses)

	Expt 1 (6)			Expt 2 (5)		
	Spring silage	Spring silage and barley	SEM	Early-cut autumn silage	Late-cut autumn silage	SEM
Gross energy (MJ/24 h)						
In food†	11·63	14·41	—	13·65	13·87	—
At proximal duodenum	6·79	8·46	0·26**	6·24	7·12	0·35
At terminal ileum	4·95	5·63	0·23	3·87	4·99	0·16**
In faeces	4·00	4·43	0·12*	2·83	3·77	0·13**
Apparent digestibility of gross energy	0·655	0·694	0·008**	0·793	0·729	0·009**
Disappearance of digestible energy						
Before small intestine	0·625	0·594	0·026	0·683	0·668	0·030
In small intestine	0·241	0·286	0·015	0·221	0·211	0·043
In caecum and colon	0·133	0·121	0·025	0·096	0·121	0·018

Statistical significance of difference between treatments (see p. 483): * $P < 0.05$, ** $P < 0.01$.
† Includes chromic oxide paper.

The results for the digestion of cellulose are given in Table 4. Supplementation of the spring silage with barley led to a small increase in cellulose intake but there was a disproportionate and significant ($P < 0.05$) increase in the passage of cellulose to the duodenum, ileum and faeces. The digestibility of cellulose was significantly ($P < 0.01$) reduced. For the diet of silage alone the proportion of the apparently-digested cellulose disappearing in the rumen was approximately 0·90 but this was reduced to 0·77 by supplementation of the silage with barley. Most of the compensatory increase in cellulose digestion was in the small intestine.

For both the early-cut and late-cut autumn silages the digestibility of cellulose was high although there was a significant ($P < 0.001$) reduction in digestibility associated with the maturity of the grass ensiled. For both silages a very high proportion, 0·98, of the apparently digested cellulose disappeared in the rumen.

Fermentation in the rumen

There were postprandial changes in rumen pH and in the concentrations of total and individual short-chain fatty acids in the rumen but the changes were similar for all dietary treatments and the results have been summarized in Table 5 as mean values. With the diets containing silage alone the rumen pH was high, 6·85–7·06 units. Supplementation of the spring silage with barley reduced the pH but the effect was not significant ($P > 0.05$). Supplementation also resulted in an increase in the concentration of total short-chain fatty acids in the rumen and an associated increase ($P < 0.01$) in the proportion of butyric acid. The compensatory changes in other acids were an increase in the proportion of acetic acid and a reduction in the proportion of propionic acid.

There were significant ($P < 0.05$) differences in the composition of the mixture of short-chain fatty acids in the rumen between the early-cut and late-cut autumn silages and for the late-cut material the proportion of acetic acid was especially high.

Table 4. *Mean quantities of cellulose present in the food entering and leaving the small intestine and in the faeces of sheep given diets of spring silage and of spring silage and barley (Expt 1) and diets of early-cut autumn silage and of late-cut autumn silage (Expt 2) and values for the proportion of digestible cellulose 'disappearing' before and in the small intestine and in the caecum and colon*

(Mean values with their standard errors; no. of animals/treatment in parentheses)

	Expt 1 (6)			Expt 2 (5)		
	Spring silage	Spring silage and barley	SEM	Early-cut autumn silage	Late-cut autumn silage	SEM
Cellulose (g/24 h)						
In food†	179	189	—	166	215	—
At proximal duodenum	64	89	3·4***	26	47	2·1***
At terminal ileum	57	69	3·4*	26	49	2·5***
In faeces	51	61	1·3***	23	43	1·6***
Apparent digestibility of cellulose	0·715	0·682	0·007**	0·859	0·799	0·007***
Disappearance of digestible cellulose						
Before small intestine	0·898	0·773	0·023**	0·981	0·981	0·015
In small intestine	0·051	0·162	0·033*	0·001	−0·014	0·010
In caecum and colon	0·051	0·064	0·022	0·018	0·032	0·014

Statistical significance of difference between treatments (see p. 483): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

† Includes chromic oxide paper.

Table 5. *The pH and concentrations of total and individual short-chain fatty acids (VFA) in the rumen of sheep given diets of spring silage and of spring silage and barley (Expt 1) and diets of early-cut autumn silage and of late-cut autumn silage (Expt 2)*

(Mean values with their standard errors; no. of animals/treatment in parentheses)

	Expt 1 (6)			Expt 2 (5)		
	Spring silage	Spring silage and barley	SEM	Early-cut autumn silage	Late-cut autumn silage	SEM
pH	6·85	6·62	0·09	7·06	6·91	0·06
Total VFA (mmol/l)	83·5	90·8	3·4	62.3	68·3	6·4
Individual fatty acids (mmol/mol total VFA)						
Acetic acid	589	600	6	637	707	13**
Propionic acid	266	250	10	202	163	8**
Isobutyric acid	10	10	1	20	12	1**
Butyric acid	109	118	2**	91	74	9
Isovaleric acid	12	13	1	31	31	3
Valeric acid	14	14	9	18	14	1*

Statistical significance of difference between treatments (see p. 483): * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

DISCUSSION

Within the limitations discussed by Kelly & Thomas (1978) the silages used in these experiments were typical of two types of high-quality formic acid silages. The autumn silages were direct-cut and harvested with a single-chop forage harvester (giving silage with low dry matter and high water-soluble carbohydrate contents and a long chop-length) whilst the spring silage was wilted and harvested with a precision-chop machine (giving silage with relatively high dry matter and low water-soluble carbohydrate contents and a short chop-length). Although the silages used here were not identical to those of Kelly & Thomas (1978) they were from the same grasses and prepared in the same way and in most respects the differences in composition between corresponding silages were small, being confined mainly to the concentrations of the fermentation end-products and to the gross energy contents. It is probably safe to assume that the sites of digestion of the silages used in the earlier studies were similar to those reported here.

The results of Expt 2 confirm the findings of Kelly & Thomas (1978) that the digestibility of organic matter, gross energy and cellulose was significantly ($P < 0.05$) reduced with the increasing maturity of the grass ensiled. However, there were no significant ($P < 0.05$) differences between the early-cut and late-cut silages in the relative importance of the rumen, small intestines and caecum and colon as sites of digestion. For gross energy, approximately 0·67–0·68 of that apparently digested 'disappeared' in the rumen and corresponding values for the small intestine and caecum and colon were 0·21–0·22 and 0·10–0·12 (Table 3). The results for the digestion of organic matter (Table 2) were broadly similar to those for energy but a rather larger proportion of the organic matter digested disappeared in the rumen. These results are in close agreement with those of Beever *et al.* (1977) obtained with a direct-cut, single-chopped, silage prepared without an additive but contrast with those of Beever *et al.* (1971) obtained with a similar 'no-additive' silage. The latter workers reported a smaller proportion, 0·57, of the apparently-digested energy 'disappearing' in the rumen and a larger proportion, 0·34, disappearing in the small intestine than was observed here. Also a smaller proportion of the digestible cellulose was broken down in the rumen, 0·87 of that apparently digested as compared with 0·98 here and 0·96 reported by Beever *et al.* (1977).

Supplementation of the spring silage with barley in Expt 1 resulted in a significant ($P < 0.01$) reduction in the digestibility of cellulose and in the proportion of digestible cellulose 'disappearing' in the rumen (Table 4). There was an associated reduction in the ruminal breakdown of digestible organic matter and DE which was compensated for mainly by an increased digestion in the small intestine (Tables 2 and 3). Part of this increase was associated with the passage of α-linked glucose polymers from the rumen, although it should be stressed that the amounts were small, 10·7 g/d. Part was also due to the digestion of cellulose (Table 4) presumably through microbial fermentation in the terminal part of the ileum where there is an active microbial population (Maki & Picard, 1965) and significant fermentation activity (Boyne *et al.* 1956). Digestion of cellulose in the small intestine has been observed with other diets (Thomson *et al.* 1972) and can apparently be significant where a combination of dietary conditions leads to the passage of relatively unlignified, highly-digestible, cellulose to the lower gut. The marked reduction in cellulose digestion in the rumen in response to the supplementation of the diet with barley is worthy of note since it represents a larger associative effect than observed with mixtures of hay and cereals (Macrae & Armstrong, 1969; Chamberlain & Thomas, 1979). With high-quality silages the ruminal digestion of cellulose is apparently sensitive to the presence of starch in the diet and this has considerable significance. The evidence suggests that the voluntary intake of high-quality silages is controlled through a 'rumen-fill' mechanism (Thomas *et al.* 1976; Farhan &

Thomas, 1978) and a reduction in the rate of cellulose digestion will reduce the rate of rumen emptying. In a number of experiments with highly digestible silages barley supplements have been shown to produce especially marked reductions in the voluntary intake of silage (Castle & Watson, 1975, 1976).

The present experiments do not allow a statistical comparison between the spring silage and the autumn silages but the results from Expt 1 and Expt 2 suggest that the proportion of digestible organic matter and gross energy 'disappearing' in the rumen with the spring silage were lower than with the autumn silages. The reason for this is uncertain but it could relate to the differences between the spring and autumn silages in physical form. Precision chopping of grass for ensilage may enhance the passage of small, partially digested, silage particles from the rumen. Consistent with this the digestibility of cellulose in the spring silage tended to be low (cf. the late-cut autumn silage) as was the proportion of digestible cellulose broken down in the rumen.

Kelly & Thomas (1978) found that for an early-cut autumn silage similar to that used here ME:DE was 0·855 and k_f was 53% whilst for a corresponding late-cut autumn silage the energy lost as methane was greater, ME:DE was 0·831 and k_f was 44%. The present results indicate that these differences were in no way related to differences between the silages in sites of digestion of energy or carbohydrate constituents. However, the difference in ME:DE is partly explained by the difference between the silages in the pattern of rumen fermentation (Table 5). Stoichiometric considerations (Hungate, 1966) dictate that the high acetate fermentation with the late-cut silage would lead to a greater production of methane/mol VFA produced than would the low acetate fermentation observed with the early-cut silage. The high acetate proportion with the late-cut silage may also be seen as consistent with the view that k_f is reduced with diets for which a large proportion of DE is absorbed as acetate (Blaxter, 1967).

The rumen fermentation pattern and the sites of digestion of energy for spring silage differed from the autumn silages. There was a lower proportion of acetic acid and a higher proportion of propionic acid in the rumen, a lower proportion of DE 'disappearing' in the rumen and a higher proportion of DE 'disappearing' in the small intestine. Assuming that a reduced proportion of acetate in the rumen and an increased uptake of substrates from the small intestine would tend towards a high k_f (Blaxter, 1967) then the spring silage could be expected to have had a higher k_f than the autumn silages but in reality the k_f of the spring silage was especially low (Kelly & Thomas, 1978).

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REFERENCES

Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock No.* 2, *Ruminants.* London: Agricultural Research Council.
Beever, D. E., Thomson, D. J. Cammell, S. B. & Harrison, D. G. (1977). *J. agric. Sci., Camb.* **88**, 61.
Beever, D. E., Thomson, D. J., Pfeffer, E. & Armstrong, D. G. (1971). *Br. J. Nutr.* **26**, 123.
Blaxter, K. L. (1967). *The Energy Metabolism of Ruminants*, p. 217. London: Hutchinson.
Boyne, A. W., Campbell, R. M., Davidson, J. & Cuthbertson, D. P. (1956). *Br. J. Nutr.* **10**, 325.
Castle, M. E. & Watson, J. N. (1975). *J. Br. Grassld Soc.* **30**, 217.
Castle, M. E. & Watson, J. N. (1976). *J. Br. Grassld Soc.* **31**, 191.
Chamberlain, D. G. & Thomas, P. C. (1979). *J. Sci. Fd Agric.* **30**, 677.
Chamberlain, D. G., Thomas, P. C. & Wilson, A. G. (1976). *J. Sci. Fd Agric.* **27**, 231.
Dewar, W. A. & McDonald, P. (1961). *J. Sci. Fd Agric.* **12**, 790.

Farhan, S. M. A. & Thomas, P. C. (1978). *J. Br. Grassld Soc.* **33**, 151.
Hungate, R. E. (1966). *The Rumen and its Microbes*, p. 269. New York: Academic Press.
Kelly, N. C. & Thomas, P. C. (1978). *Br. J. Nutr.* **40**, 205.
MacRae, J. C. & Armstrong, D. G. (1969). *Br. J. Nutr.* **23**, 377.
Maki, L. R. & Picard, K. (1965). *J. Bact.* **89**, 1244.
Thomas, P. C., Kelly, N. C. & Wait, M. K. (1976). *J. Br. Grassld Soc.* **31**, 19.
Thomson, D. J., Beever, D. E., Coelho da Silva, J. F. & Armstrong, D. G. (1972). *Br. J. Nutr.* **28**, 31.

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