

Studies on the composition of food

3.* The nutritive value of beef from intensively reared animals

BY J. M. HARRIES,† *Ministry of Agriculture, Fisheries and Food, Great Westminster House, Horseferry Road, London, SW 1,*

A. W. HUBBARD, *Ministry of Technology, Laboratory of the Government Chemist, London, SE 1,*

F. E. ALDER, *ARC Grassland Research Institute, Hurley, Maidenhead, Berks,*

M. KAY, *ARC Rowett Research Institute, Bucksburn, Aberdeen and*

D. R. WILLIAMS,† *ARC Meat Research Institute, Low Temperature Research Station, Cambridge*

(Received 13 March 1967—Accepted 1 September 1967)

1. An attempt has been made to compare the nutritive value of beef from intensively reared animals with that from more extensively reared stock.
2. The difficulties of such a comparison are described and the consequent limitations of this experiment specified.
3. It was found that samples of longissimus dorsi and superficial digital flexor muscles showed no significant differences between intensively reared and extensively reared animals in their content of moisture, intramuscular fat, protein, non-protein nitrogen, iron, thiamine, riboflavine, or nicotinic acid.
4. The longissimus dorsi muscles had more non-protein nitrogen and more nicotinic acid, but less iron and less riboflavine, than the superficial digital flexor muscles.
5. There was less vitamin A and less carotene in samples of liver from intensively reared animals than in comparable samples from extensively reared animals.

The recent trend to rear beef cattle on diets high in barley, the so-called 'barley beef', has led to some concern being expressed about the value of such beef as human food; this paper describes an attempt to compare the nutritive value of beef from intensively reared animals with that of beef from stock reared more conventionally. No previous work on this specific subject has been reported in the scientific literature.

The term 'barley beef' is imprecise. In practice, there is a spectrum ranging from free-grazing animals that receive no supplementary feed to animals reared indoors at a high density under artificial light and fed exclusively on concentrates. Feeding practice with the more intensively reared animals also varies widely. There is no such simple dichotomy between 'free range' and 'intensive' beef as has sometimes been assumed, but it has been estimated that some 15–20%, or about 180000 tons per annum, of home-produced beef is produced by intensive or semi-intensive methods (National Agricultural Advisory Service, internal papers, unpublished).

The nutritional value of beef can be affected by a great many factors: during production by breed, sex, age, degree of finish, and feed, by post-slaughter treatments

* Paper No. 2: *Br. J. Nutr.* (1966), **20**, 747.

† Present address: Meat Research Institute, Agricultural Research Council, Langford, near Bristol, Somerset.

such as freezing, conditioning, and trimming, including the particularly important variable of the part of the animal from which samples are taken, and finally during preparation by factors such as type and time of cooking. No investigator can hope to include all these variables in one experiment, and initial decisions to exclude or standardize them limit the applicability of the results. The factors varying during production are particularly difficult to control. Any comparison of intensively and extensively produced food is inevitably confused by the intrusion of secondary factors. Intensively reared stock are necessarily younger at slaughter than more extensively reared animals, and have necessarily been managed differently. The effect of intensity of production cannot be isolated from these intrusive factors except by including such unusual practices as the keeping of intensively reared stock beyond normal age, or by slaughtering 'free range' stock before they are ready for market. Such treatments produce meat with a lean to fat ratio atypical of market beef. The problem of breed is not as acute as in similar attempts to assess the influence of intensity of production with poultry. Since the most popular breed with 'barley-beef' producers is Friesian, or a Hereford \times Friesian cross, it was decided to limit this experiment to those breeds, in spite of the fact that our results for beef from the more conventionally reared cattle might not be typical of all such beef, in so far as breed may influence nutritive value. In one of the trials, grazing cattle were slaughtered before they would have been considered ready for market under commercial conditions.

A preliminary survey of trials in progress, from which material might be drawn for the purpose of this comparison, revealed only one experiment, being conducted at the Grassland Research Institute (see p. 23), from which directly comparable animals would be available; but it was felt that intensive production covered such a variety of methods that it was advisable to include in the present experiment material from other trials, even when no extensively reared animals were available for comparison. It was hoped to compare results for these animals with published observations on the nutritive value of beef from animals which it could be assumed had been reared extensively. The variety of husbandry methods and feeding practices included in the comparison is shown on pp. 23-25.

It was decided to analyse only raw beef, and to limit the experiment to fresh beef, on the assumption that any effect of chilling, freezing, conditioning, or cooking on nutritive value of beef would not be differential between intensively and extensively reared material.

The water-soluble vitamins are concentrated mainly in the lean, though the fat provides much of the energy value, and it was felt that finish, degree of trim and such factors could be omitted from this experiment, especially since the results from the Meat Research Institute experiment (p. 24) would provide information on the comparative proportion of fat to lean and carcass composition of animals intensively and extensively reared. This decision meant that results for fat content would refer only to intramuscular fat, and that the moisture contents found would relate only to lean meat. Little guidance was available on which parts of the carcasses should be analysed for nutrient content. The dearth of information on the distribution of vitamins in beef carcasses, and on their development with age, coupled with the limited analytical

resources available, led to a decision to analyse only the following specific parts of the animal: (1) the longissimus dorsi muscle from the 11th-12th lumbar vertebrae region (the eye-muscle from the wing-rib); (2) the superficial digital flexor muscle (leg of beef); (3) a piece of liver from the region adjacent to the gall-bladder. Since it was impossible to examine more than a few samples from each animal, it was felt that the three items above would serve to represent, respectively, those cuts normally roasted, those normally stewed, and the offal.

EXPERIMENTAL

The animals

Animals from three separate trials were included in the experiment.

The Grassland Research Institute (Hurley). The animals were all autumn-born Hereford × Friesian steers, 6 months old in April 1964. They were allocated to two trials, one field trial and one yard trial with the object of studying the effect of different levels of concentrate feeding on their live-weight gain. Full results of this experiment are being published elsewhere, and further details have been given by Tayler, Rudman & Chapas (1965). Included in the present experiment were animals of the field and yard treatments shown in Table 1 with the coding used to identify them in the tables of results.

Table 1. *Treatment and slaughter weights of beef cattle from the Grassland Research Institute*

Code	Treatment	No. of animals	Mean live weight at slaughter (kg)	Mean carcass weight (kg)
HC	Grazing, high stocking rate, concentrates, <i>ad lib.</i>	5	399	229
HO	Grazing, high stocking rate, no concentrates	2	279	140
		3	399	225
LC	Grazing, low stocking rate, concentrates <i>ad lib.</i>	4	393	224
LO	Grazing, low stocking rate, no concentrates	2	292	147
		1	396	224
C	Yard feeding, concentrates, <i>ad lib.</i>	4	404	228
GC	Yard feeding, concentrates, and grass <i>ad lib.</i>	4	405	224
GO	Yard feeding, no concentrates, grass, <i>ad lib.</i>	2	268	132

In the field experiment, the cattle were rotationally grazed in separate plots according to treatment. Self-feed hoppers were placed in the plots for cattle in the HC and LC treatments (Table 1). In the yard-feeding experiment, all cattle were fed individually; concentrates were fed from self-feed hoppers to animals in treatments C and GC (Table 1). Grass was cut fresh daily and weighed to give an expected surplus of at least 15% at the end of 24 h. Uneaten grass was weighed each morning and removed before fresh grass was offered.

The concentrates mixture consisted of 85% rolled barley and 15% of a protein supplement, made up of 45% white fish meal, 45% soya-bean meal, 5% molasses, 2.5% sodium chloride, 2.5% dicalcium phosphate with 1 oz per ton cobalt sulphate, 40×10^6 i.u. vitamin A per ton and 8×10^6 i.u. vitamin D per ton.

The cattle from each of the different treatments were slaughtered at similar ages to produce carcasses of two weights as indicated, for the appropriate treatments, in Table 1. The animals from the grazing and grass-feeding treatments at the earlier slaughter would not normally have been considered fit for slaughter commercially. The mean weights at slaughter, and mean carcass weights of the animals included in this experiment are given in Table 1.

The Meat Research Institute (Cambridge). At the time this comparison was planned, an experiment was already in progress at the Meat Research Institute to compare carcass quality and growth rate of traditionally fed and intensively fed groups of three breeds of animal—Friesian, Hereford, and Hereford \times Friesian crosses. Most of the traditionally fed animals had already been disposed of, but samples from three such animals, one Friesian and two Hereford \times Friesian crosses, at 2 years of age were still available for comparison with six intensively fed animals, two Friesians and four Hereford \times Friesian crosses, at 1 year of age. Both groups were reared at the Norfolk Agricultural Station. The calves in the intensive group were housed by breed in pens of four animals. They were fed for the first 10 weeks on milk equivalent at 4 l./calf daily, and hay and proprietary concentrate were offered from arrival, and gradually changed to a farm mixture within a few weeks. The hay was limited to 907 g per four calves and changed to a weaning concentrate farm mixture at 14 weeks. On 6 April the groups for slaughter at 12 months were separated from the rest and were housed by breed in pens of three animals. They continued to be fed on weaning farm mixture *ad lib.*, plus 680 g hay per three animals daily. After 6 weeks the feed was changed to a mixture of 1 part proprietary pellets plus 3 parts rolled barley; hay was continued at the same level. The pellets contained 40×10^6 i.u. vitamin A/ton. After 16 weeks the mixture was changed to 1 part pellets plus 4 parts rolled barley and the animals were kept on this diet until slaughter on 21 and 28 October and 4 November. The cold carcass weights of the intensively reared animals ranged from 224.5 kg to 251.5 kg, with a mean of 238.2 kg; for the conventionally reared animals the range was from 301 kg to 325.7 kg, with a mean of 311.4 kg. A further description of this experiment is in preparation for publication by the Royal Smithfield Club (A. C. Owers, B.M. Scott, R. W. Pomeroy and D. R. Williams).

The Rowett Research Institute (Aberdeen). An experiment was being conducted at the Rowett Research Institute on Hereford \times Friesian steers that had been weaned at 8 weeks on to a high-energy diet in which fish meal was used as the source of supplementary protein (Kay, Preston, MacLeod & Philip, 1966). When the calves weighed 90 kg, they were gradually changed over on to a fattening diet which contained 80% bruised barley and 20% of a protein-mineral-vitamin supplement in which soya-bean meal provided the additional protein. The concentrations of calcium, phosphorus, salt and trace minerals in the diet were adjusted to fall in line with the Agricultural Research Council (1965) recommendations on nutrient requirements of farm animals.

During the calf stage the steers were individually penned on straw; after reaching a weight of 90 kg they were housed on slatted floors in groups of six. They did not receive additional roughage at any stage. The mean slaughter weight of the steers was 360 kg.

Samples from seven of these animals were available for the present experiment. No conventionally reared animals were available for direct comparison.

Analysis of carcass samples

Samples from the Grassland Research Station and the Meat Research Institute were taken to the Laboratory of the Government Chemist immediately each animal was killed. Samples from the Rowett Research Institute were packed with solid carbon dioxide in heat-sealed plastic bags, sent by passenger train from Aberdeen to London, and taken immediately to the same laboratory.

Moisture was determined by drying for 16 h at $100^{\circ} \pm 1^{\circ}$ in an air oven (Association of Official Agricultural Chemists, 1965*a*). Fat was determined on a dried sample by the method given by the Association of Official Agricultural Chemists (1965*b*) using light petroleum (b.p. 40–60°). Total nitrogen was determined by the Kjeldahl method (Association of Official Agricultural Chemists, 1965*c*), and the non-protein nitrogen content by the method of Mezincescu & Szabo (1936) using trichloroacetic acid. Iron was determined as the *o*-phenanthroline complex by the method of Pringle (1946).

All vitamin analyses were made directly on prepared samples without drying. The thiamine content was determined by the method of the Association of Vitamin Chemists (1951) and nicotinic acid and riboflavine by microbiological assays according to the procedures recommended by the Society for Analytical Chemistry: Analytical Methods Committee (1946). Pyridoxine was determined by the method of Atkin, Schultz, Williams & Fry (1943) as modified by Parrish, Loy & Kline (1955). Folic acid was determined by the method of Jones & Morris (1949) using a 72 h incubation period and employing *Streptococcus faecalis* as the test organism. Vitamin B₁₂ was extracted by the method recommended by the Society for Analytical Chemistry: Analytical Methods Committee (1956), and determined by the method of Skeggs, Nepple, Valentik, Huff & Wright (1950). Vitamin A and carotene were determined on the unsaponifiable matter of the extracted fat and separated on a polyethylene glycol chromatographic column, according to the method of Wilkie, Jones & Morris (1959).

RESULTS

Samples of muscle

The longissimus dorsi muscles were found to contain more non-protein nitrogen, less iron, less riboflavine, and more nicotinic acid than the superficial digital flexor muscles. To facilitate comparison between intensively and extensively reared animals, therefore, the results are shown separately for the two muscles in Tables 2 and 3. The treatments included in the experiment at the Grassland Research Institute have been arranged in an order progressing from free range (treatment LO) to intensive (treatment C). The early slaughter of the grass-fed cattle was reflected in the low fat and high moisture contents. There were, in all these trials, no significant differences

Table 2. Mean results for composition of longissimus dorsi muscle of beef cattle

Source of samples	Treatment*	No. of animals	Moisture (%)	Fat (%)	Protein (%)	Non-protein nitrogen (%)	Iron (mg/100 g)	Thiamine (mg/100 g)	Riboflavine (mg/100 g)	Nicotinic acid (mg/100 g)	
Grassland Research Institute	Grazing:										
	LO	3	75.1	1.5	22.0	0.43	1.73	0.07	0.15	7.91	
	HO	5	75.4	1.8	21.6	0.43	1.05	0.06	0.15	7.28	
	LC	4	73.9	2.6	21.7	0.43	1.86	0.06	0.15	7.00	
	HC	5	73.8	3.0	22.3	0.43	1.74	0.07	0.16	6.66	
Meat Research Institute	Yard feeding:										
	GO	2	74.8	1.4	22.0	0.39	1.51	0.06	0.12	7.71	
	GC	4	73.2	3.7	22.1	0.41	1.18	0.06	0.15	7.14	
	C	4	73.8	2.8	22.5	0.41	1.86	0.07	0.14	6.71	
	Intensive	3	71.8	5.2	22.1	0.41	1.82	0.05	0.13	7.36	
Rowett Research Institute	Intensive	6	72.3	3.8	22.1	0.41	1.64	0.07	0.15	6.79	
	Intensive	6	71.6	5.0	21.5	0.39	1.80	0.12	0.14	6.99	

* See Table 1.

Table 3. Mean results for composition of superficial digital flexor muscle of beef cattle

Source of samples	Treatment*	No. of animals	Moisture (%)	Fat (%)	Protein (%)	Non-protein nitrogen (%)	Iron (mg/100 g)	Thiamine (mg/100 g)	Riboflavine (mg/100 g)	Nicotinic acid (mg/100 g)	
Grassland Research Institute	Grazing:										
	LO	3	76.3	1.2	21.8	0.32	2.68	0.07	0.17	4.49	
	HO	5	75.9	2.2	21.3	0.30	3.16	0.07	0.19	4.12	
	LC	4	74.8	2.4	21.5	0.33	2.65	0.08	0.22	4.06	
	HC	5	75.4	2.2	21.5	0.30	2.84	0.07	0.22	4.28	
Meat Research Institute	Yard feeding:										
	GO	2	76.6	1.0	22.0	0.29	2.33	0.05	0.17	4.29	
	GC	4	74.2	2.1	23.2	0.30	2.48	0.06	0.20	4.40	
	C	4	73.4	3.1	21.7	0.31	2.61	0.08	0.18	4.21	
	Intensive	3	72.2	4.5	21.8	0.31	2.89	0.05	0.16	4.04	
Rowett Research Institute	Intensive	6	73.6	3.1	21.2	0.33	2.68	0.08	0.18	5.16	
	Intensive	7	73.2	3.9	20.7	0.29	3.18	0.15	0.18	4.60	

* See Table 1.

between intensively reared and conventionally reared beef in respect of any nutritional characteristic examined, for either the longissimus dorsi or the superficial digital flexor muscles. This was the case both when the analyses of all the carcasses were considered together and also when the results of the experiment at the Grassland Research Institute were considered separately, though in the latter case, there were significant differences between some of the seven separate treatments in the moisture and fat contents of the samples, though not for any other characteristic examined. Moreover, the differences in fat and moisture were as great among the different variants of grazing treatments and also among the different variants of 'yard feeding' treatments as they were in comparisons between the two different types of treatment. As explained on page 22, further information on fat to lean ratios is necessary before the differences in fat and moisture can be evaluated. The thiamine contents of both these muscles from the Rowett Research Institute cattle were significantly higher than those of the same muscles from all other cattle in this experiment.

Table 4 shows the general means and standard errors for the nutrient contents of samples of each of the two muscles in this experiment. It should be noted that the values, given separately for the two muscles, are means for the three trials included and for the different husbandry practices. The standard errors do not indicate the variation normally encountered in the vitamin content of beef, since the evidence so far is that the variation from one tissue to another within one animal is greater than the variation brought about by different husbandry practices, and two muscles are not enough on which to base an assessment of the within-animal variation. It should also be emphasized that the standard errors shown for moisture and fat are necessarily low, since they are based only on determinations made on lean meat.

Samples of liver

The results are given in Table 5. Differences were found between methods of husbandry in respect of vitamin A and carotene, and published values give some indication of the range encountered. Thus Watt & Merrill (1963) state that values for vitamin A vary widely in all kinds of liver, ranging from about 100 to more than 100000 i.u./100 g. McCance & Widdowson (1960) state that the vitamin A content of raw ox liver may vary from 10000 to 40000 i.u./100 g.

All samples of liver from the Rowett Research Institute showed negligible amounts of vitamin A, whereas samples of liver from intensively reared animals from the Meat Research Institute varied from 600 i.u./100 g to 22000 i.u./100 g. The samples from the Meat Research Institute, therefore, were all within the range quoted by Watt & Merrill, though the mean value for intensively reared animals was significantly less than that quoted by them. The mean value for extensively reared animals was not significantly different from that of Watt & Merrill.

The results for fat, protein, iron and thiamine in the samples of liver also differed from those quoted by McCance & Widdowson, whose results relate to a mixed sample of liver from different species. There were no differences in these nutrients attributable to husbandry practice.

Table 4. General means and, in parentheses, standard errors for nutrients in muscle of beef cattle

Muscle	Moisture (%)	Fat (%)	Protein (%)	Non-protein nitrogen (%)	Iron (mg/100g)	Thiamine (mg/100g)	Riboflavin (mg/100g)	Nicotinic acid (mg/100g)
Longissimus dorsi	73.4 (0.2)	3.2 (0.2)	22.0 (0.10)	0.41 (0.004)	1.69 (0.06)	0.07 (0.004)	0.15 (0.003)	7.09 (0.17)
Superficial digital flexor	74.4 (0.2)	2.8 (0.2)	21.6 (0.15)	0.31 (0.004)	2.82 (0.09)	0.08 (0.006)	0.19 (0.004)	4.42 (0.12)

Table 5. Mean results for contents of nutrients in liver of beef cattle

Source of samples	Treatment	No. of animals	Moisture (%)	Fat (%)	Protein (%)	Non-protein nitrogen (%)	Iron (mg/100g)	Thiamine (mg/100g)	Riboflavin (mg/100g)	Nicotinic acid (mg/100g)	Pyridoxine (mg/100g)	Vitamin B ₁₂ (µg/100g)	Folic acid (µg/100g)	Vitamin A (i.u./100g)	Carotene (ppm)
Meat Research Institute	Intensive	6	69.4	3.7	21.2	0.36	5.0	0.15	2.71	13.1	0.81	93	148	13 000	< 0.5
Rowett Research Institute	Intensive	6	70.8	3.1	21.2	0.33	5.8	0.19	2.64	13.1	1.11	58	143	100	< 0.5
Meat Research Institute	Extensive	2	71.0	1.3	19.5	0.37	3.6	0.17	2.68	13.0	0.76	105	148	37 000	9.8
McCance & Widdowson (1960)	—	—	73.3*	8.1*	16.5*	—	13.9*	0.30	3.0	13.0	0.7	50	300	20 000	—
Watt & Merrill (1963)	—	—	69.7	3.8	19.9	—	6.5	0.25	3.26	13.6	—	—	—	43 900	—

* Values obtained on a mixed sample of raw liver. Values for the vitamins refer to raw ox liver.

DISCUSSION

Muscle

Though in the literature there is no previous report of a specific comparison between the vitamin content of beef from intensively and extensively reared animals, there is a considerable amount of information about the influence of various production practices. Of some relevance to our results are those of Cover & Smith (1956) who found no differences in the thiamine content of samples of longissimus dorsi muscles either from animals receiving different proportions of concentrates in their diets, or from animals of different rates of growth and degrees of finish. Meyer, Thomas & Buckley (1960) found no significant differences in the thiamine, riboflavine or nicotinic acid content of muscles from animals finished on grain and finished on grass.

The findings of the present experiment are therefore consistent with both these sets of results. The results of Meyer *et al.* (1960) are also similar to our own in that they found significant differences between the two muscles they examined (longissimus dorsi and semimembranosus) in thiamine and riboflavine content. They found no significant differences in nicotinic acid content between the two muscles.

The conclusions to be drawn from our experiment are necessarily limited. Before a comprehensive comparison of the nutritive value of beef from intensively and extensively reared animals can be made, information about the relative proportions of fat to lean in the whole carcass is necessary, and further knowledge is required about the distribution of the vitamins in the different muscles of a beef animal. The small amount of evidence available suggests that husbandry practice is not an important factor in determining the nutritive value of lean beef. Nevertheless, the difficulties described in the introduction, of making a true comparison between the intensively and extensively produced beef, limit the findings of the experiment to the particular practices in the three trials included. Within this limitation and for the limited number of muscles examined, raw fresh beef showed no differences in nutritive value between animals reared intensively and conventionally. No explanation is offered for the higher thiamine values found in muscles from the animals reared at the Rowett Research Institute.

Liver

There is strong evidence that the livers of the intensively reared animals were deficient in vitamin A. The diet used at the Rowett Research Institute contained 6×10^6 i.u. vitamin A/ton. Repeated assays for vitamin A in the supplement indicated that the stability of the vitamin was poor and considerable deterioration was taking place. This led to dietary concentrations of the vitamin which were far below those considered satisfactory for adequate liver storage.

Quarterman & Mills (1964) showed that the liver storage of vitamin A, by cattle fattened on an all-concentrate diet containing 4×10^6 i.u./ton, was 1430 i.u./100 g fresh weight compared with levels of 25600 i.u./100 g for traditionally fattened cattle. A subsequent report by Quarterman (1966) suggests that a diet containing 6×10^6 i.u./ton promotes a liver storage of 25400 i.u./100 g fresh weight. This rate of vitamin inclusion is approximately three times the requirement suggested by the Agricultural

Research Council (1965). Topps, Elliott, Johnson & Reed (1966) reported concentrations of 26000 i.u. vitamin A/100 g for steers receiving a diet containing 4×10^6 i.u./ton. This liver concentration may seem high but, as appreciable amounts of vitamin A were found in the livers of steers receiving no vitamin A in their diet, it may be assumed that the animals' liver reserves were high before the experiment began.

Two explanations have been put forward for the higher requirement of vitamin A by steers fattened on a high-energy diet and both have been supported by some experimental evidence. Klatte, Mitchell & Little (1964) have reported a 34% destruction of vitamin A when incubated in rumen fluids of pH 6.2–6.8. The rumen pH in intensively reared cattle tends to be below 6.0, and the destruction is likely to be greater. Church, Pope & MacVicar (1956) and Roberts & Philips (1963) have shown an increase in the requirement of cattle associated with an increase in growth rate. In any event, the cattle from the Rowett experiment had an estimated daily intake of approximately 28000 i.u. vitamin A rather than the intended 47000 i.u.

The differences between the values for fat shown in Table 5 and the value given by McCance & Widdowson (1960) may well be due to the fact that the latter was obtained for a mixed sample of raw liver from different species, whereas our results relate to a specific part of ox liver. The values given by Watt & Merrill (1963) for protein, iron, thiamine and folic acid are closer to our findings, as also are those for protein and iron given by Kizlaitis, Steinfeld & Siedler (1962), who obtained their samples either from a local market or a meat packing station. However, it is clear that the method of husbandry affected only the vitamin A content.

REFERENCES

- Agricultural Research Council (1965). *The Nutrient Requirements of Farm Livestock*. No. 2. Ruminants. London: Agricultural Research Council.
- Association of Official Agricultural Chemists (1965*a*). *Methods of Analysis*, 10th ed. p. 346, para. 23:003. Washington, DC: Association of Official Agricultural Chemists.
- Association of Official Agricultural Chemists (1965*b*). *Methods of Analysis*, 10th ed. p. 346, para. 23:005. Washington, DC: Association of Official Agricultural Chemists.
- Association of Official Agricultural Chemists (1965*c*). *Methods of Analysis*, 10th ed. p. 346, para. 23:009. Washington, DC: Association of Official Agricultural Chemists.
- Association of Vitamin Chemists, Inc. (1951). *Methods of Vitamin Assay*, 2nd ed. New York: Interscience Publishers Inc.
- Atkins, L., Schultz, A. S., Williams, W. L. & Fry, C. N. (1943). *Ind. Engng Chem. analyt. Edn* **15**, 141.
- Church, D. C., Pope, L. S. & MacVicar, R. (1956). *J. Anim. Sci.* **15**, 1078.
- Cover, S. & Smith, W. H. J. (1956). *J. Anim. Sci.* **15**, 902.
- Jones, A. & Morris, S. (1949). *Analyst* **74**, 29.
- Kay, M., Preston, T. R., MacLeod, N. A. & Philip, E. B. (1966). *Anim. Prod.* **8**, 43.
- Kizlaitis, L., Steinfeld, M. I. & Siedler, A. J. (1962). *J. Fd Sc.* **27**, 459.
- Klatte, F. J., Mitchell, G. E. & Little, C. O. (1964). *J. agric. Fd Chem.* **12**, 420.
- McCance, R. A. & Widdowson, E. M. (1960). *Spec. Rep. Ser. med. Res. Coun.* no. 297.
- Meyer, B., Thomas, J. & Buckley, R. (1960). *Fd Technol., Champaign* **14**, 190.
- Mezincescu, M. D. & Szabo, F. (1936). *J. biol. Chem.* **115**, 131.
- Parrish, W. P., Loy, H. W. Jr, & Kline, O. L. (1955). *J. Ass. off. agric. Chem.* **38**, 506.
- Pringle, W. J. S. (1946). *Analyst* **71**, 490.
- Quarterman, J. (1966). *Vet. Rec.* **78**, 855.
- Quarterman, J. & Mills, C. F. (1964). *Proc. Nutr. Soc.* **23**, x.
- Roberts, W. K. & Philips, G. D. (1963). *Can. J. Anim. Sci.* **43**, 31.
- Skeggs, H. R., Nepple, H. M., Valentik, K. A., Huff, J. W. & Wright, L. D. (1950). *J. biol. Chem.* **184**, 211.

- Society for Analytical Chemistry: Analytical Methods Committee (1946). *Analyst* **71**, 397.
- Society for Analytical Chemistry: Analytical Methods Committee (1956). *Analyst* **81**, 132.
- Taylor, J. C., Rudman, J. E. & Chapas, L. C. (1965). *Intern. Rep. Grassl. Res. Inst.* no. 17.
- Topps, J. H., Elliott, R. C., Johnson, P. D. & Reed, W. D. C. (1966). *Proc. Nutr. Soc.* **25**, xxxiv.
- Watt, B. K. & Merrill, A. L. (1963). *Composition of Foods. Handbook* no. 8. Washington DC: USDA Agricultural Research Service.
- Wilkie, J. B., Jones, S. W. & Morris, W. W. Jr (1959). *J. Ass. off. agric. Chem.* **42**, 422.