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Effect of soya-bean protein on meat iron solubility and absorption in rats

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1. Soya-bean proteins were used to replace 30 and 50% of the protein from ^{59}Fe-labelled pigeon (*Columba* L.) and chicken meat, and the solubility of the meat ^{59}Fe in vitro and its absorption in vivo in rats in the presence and absence of soya-bean proteins were measured.

2. Replacement of part of the chicken meat by soya-bean proteins reduced ^{59}Fe solubility from chicken meat at all stages during simulated in vitro digestion.

3. ^{59}Fe absorption from ^{59}Fe-labelled chicken meat when given to both Fe-replete and Fe-deficient rats was reduced in the presence of soya-bean proteins but was unaffected by the presence of casein or bovine serum albumin. ^{59}Fe-absorption from pigeon meat in the presence of soya-bean proteins was not reduced to the same extent as that from chicken meat.

4. There was no significant effect of soya-bean proteins on ^{59}Fe-labelled haemoglobin Fe absorption in vivo in Fe-replete rats.

5. Absorption of ^{59}Fe from the isolated haemoproteins from chicken meat was unaffected by soya-bean proteins but ^{59}Fe absorption from the main non-haem-Fe fractions was strongly inhibited, particularly from haemosiderin.

The inhibitory effect of soya-bean protein on meat iron absorption (Cook *et al.* 1981; Hallberg & Rossander, 1982) is a matter of current nutritional concern since Fe deficiency is still a common problem in developing as well as developed countries (Food and Agriculture Organization/World Health Organization, 1970). The mode and mechanism of this interaction between soya-bean protein and meat Fe is still unresolved. The high availability of meat Fe has been ascribed to the presence of haem-Fe (which represents a significant proportion of meat Fe) and is said to be easily transferred across the intestinal mucosa intact (Conrad *et al.* 1967). Recent investigations have, however, thrown doubt on this mechanism of haem Fe absorption, the absorption of haemoglobin (Hb)-Fe being very different from that of haemoproteins in the meat environment (Hazell *et al.* 1978, 1980). Moreover, meat Fe consists not only of haem compounds but both haem- and non-haem-Fe compounds which differ considerably between different meat types and whose absorptions also differ considerably (Hazell, 1982; Bogunjoko *et al.* 1983; Latunde-Dada & Neale, 1986).

In earlier studies the effect of soya-bean protein on either non-haem-Fe absorption alone or on the total Fe absorption from a meal was investigated. In the present studies the effect of soya-bean proteins on the total intrinsically-labelled meat Fe, which includes both haem- and non-haem-Fe, has been investigated using in vitro solubility and in vivo Fe absorption techniques.

MATERIALS AND METHODS

The soya-bean-protein products used were defatted soya-bean flour (DF; British Arkady, Manchester); soya-bean concentrate (SC; FPD/Hypac, Swindon, Wiltshire) and soya-bean isolate (SI; Ralson Purina Co. Ltd, McAuley-Edwards Ltd, Baldock, Herts). Casein was sodium caseinate obtained from the Scottish Milk Marketing Board, Renfrewshire and

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Table 1. *Iron levels of chicken meat and chicken meat–soya-bean protein combinations at two levels of chicken replacement subjected to an in vitro digestion procedure*

Chicken-meat protein replaced by soya-bean protein (%)	Contributions of chicken and soya-bean to the total Fe following in vitro digestion* (μg/l digest)			
	Chicken	DF	SC	SI
0	1220	—	—	—
30	854	1302	922	628
50	610	2170	1330	1050

DF, defatted soya-bean flour; SC, soya-bean concentrate; SI, soya-bean isolate.
* All test combinations had a constant protein content of 0·24 g/l before or after replacement by soya-bean protein.

bovine serum albumin (BSA) was obtained from the Sigma Chemical Co., Poole, Dorset.

The procedure for obtaining the radioactively-labelled chicken and pigeon (*Columbo* L.) meat and Hb was as previously described (Bogunjoko *et al.* 1983; Latunde-Dada & Neale, 1986).

In the in vitro digestion studies a known weight of chicken leg meat, calculated to give a protein content of approximately 20 g/l in 0·1 M-hydrochloric acid, was homogenized for 1 min in an MSE homogenizer with stainless-steel blades. Further samples when part of the meat slurry was replaced by the soya-bean product calculated to replace either 30 or 50% of the total meat protein in the slurry were also prepared and the mixture homogenized. The slurry was adjusted to pH 1·5 with concentrated HCl and the digestion procedure followed as described previously (Latunde-Dada & Neale, 1986). The total Fe levels from chicken alone and from soya bean and chicken in combination at two levels of chicken-protein substitution, as used in the in vitro studies, are shown in Table 1. It is evident that the total Fe levels varied with the level of replacement and the type of soya-bean product used, yet the total protein content was maintained at a constant level.

The in vivo absorption technique was as previously described (Bogunjoko *et al.* 1983; Latunde-Dada & Neale, 1986). Fe-deficient rats were obtained by feeding weanling male Wistar rats with a casein-based low-Fe (7 mg/kg) diet for 4 weeks and fasting overnight. In total, sixty-eight Fe-replete rats and twenty-eight Fe-deficient rats were used, all weighing between 200 and 250 g. Rats were randomly assigned the various test meals with eight rats in the 'meat only' group and four rats with meat protein replaced by either casein, BSA or soya-bean proteins. Blood Hb levels were determined to indicate the degree of anaemia (Boehringer test kit; Boehringer, London). The soya-bean products were also substituted for chicken or pigeon meat or Hb on a protein basis at two levels of 50 and 30% protein replacement. While the total protein content of the test meals was constant (0·65 g/5 ml test dose) except in the case of 50% chicken alone, the Fe levels differed as shown in Table 2.

Statistics

Statistical analysis of in vitro and in vivo results were performed using the Student's t test and P values $< 0{\cdot}05$ were considered to be significant.

Table 2. *Iron levels (mg) present in meals for intragastric dosing using chicken and pigeon (Columba L.) meat alone or in combination with various soya-bean proteins replacing either 30 or 50% of the meat protein*

(Each meal contained the sum of the Fe contents listed for its individual ingredients)

Chicken-meat protein replaced by soya-bean protein (%)	Total meal Fe levels (μg/5 ml)			
	Chicken	DF	SC	SI
0	35·9	—	—	—
30	25·1	38·5	27·2	18·6
50	17·9	64·0	45·3	30·5
Pigeon-meat protein replaced by soya-bean protein (%)	Pigeon			
0	147·6	—	—	—
50	73·8	64·0	45·3	30·5

DF, defatted soya-bean flour; SC, soya-bean concentrate; SI, soya-bean isolate.

Table 3. *Percentage ^{59}Fe solubility from raw chicken meat in combination with soya-bean proteins during simulated in vitro digestion*

(Values are means with their standard errors for four experiments)

Digestion conditions*...	1		2		3		4		5	
Chicken/soya-bean combinations	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
Raw chicken alone	61·0	1·4	34·2	0·5	40·6	1·2	54·8	2·1	61·1	2·0
Chicken protein replacement (%)										
50 DF	43·8	2·6	29·5	1·5	32·5	1·6	37·8	0·8	39·8	0·7
30 DF	55·4	1·4	26·6	1·1	37·1	1·2	45·3	1·9	45·9	0·8
50 SC	45·4	0·5	22·3	0·4	21·1	0·7	24·4	0·9	25·7	0·6
30 SC	51·9	1·3	21·1	0·8	23·4	0·4	27·5	0·5	29·6	1·3
50 SC (plus ascorbic acid)	69·9	3·1	25·6	1·2	28·2	0·9	33·8	1·1	45·5	0·8
50 SI	54·6	1·1	27·0	1·0	47·9	1·3	45·5	1·3	47·9	1·3
30 SI	60·5	0·2	23·8	0·79	47·9	1·1	46·1	1·5	52·7	1·2

DF, defatted soya-bean flour; SC, soya-bean concentrate; SI, soya-bean isolate.

* 1, hydrochloric acid + pepsin (*EC* 3.4.23.1) 1·5 h; 2, HCl + pepsin + sodium bicarbonate (neutralization); 3, 4, 5, HCl + pepsin + $NaHCO_3$ + pancreaticin-bile extract digested for 1, 2 and 4 h respectively.

RESULTS

In vitro digestion of chicken – soya-bean test meals

Table 3 shows the percentage of ^{59}Fe solubility from the intrinsically-labelled chicken meat at the two levels of protein substitution by soya-bean products. Soya-bean proteins significantly ($P < 0·05$) reduced the soluble ^{59}Fe released from the raw chicken meat when 50% of the meat proteins were replaced by soya-bean proteins but the reduction in solubility was not significant after only 30% replacement. The soya-bean concentrate was the most inhibitory while the isolate was least. The addition of ascorbic acid at a molar ratio,

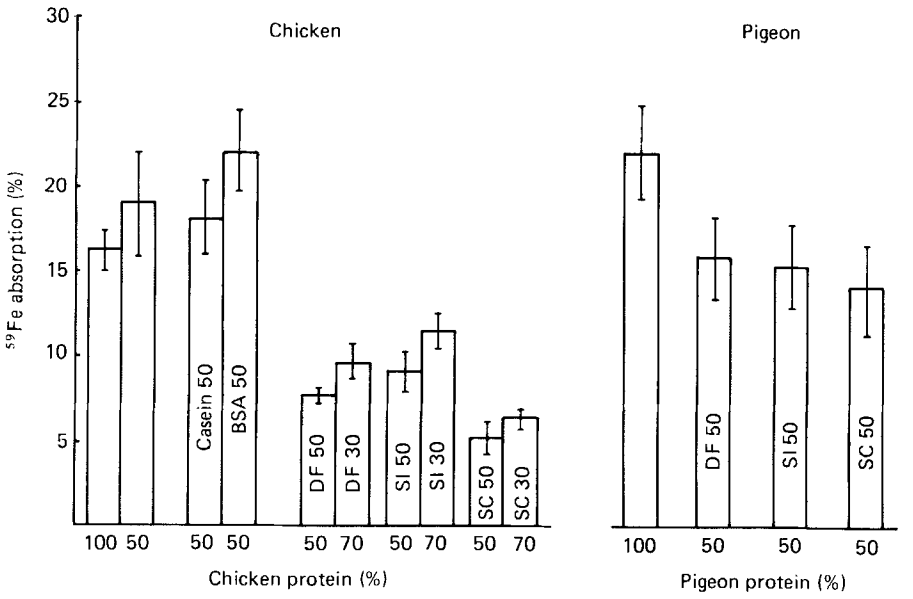

Fig. 1. Percentage absorption of ^{59}Fe from raw chicken and pigeon (*Columba* L.) meat alone or after replacement of 30 or 50% of the meat protein by soya-bean proteins, casein or bovine serum albumin (BSA) in Fe-replete rats. Mean values with their standard errors, represented by vertical bars, for four to eight rats per group. DF, defatted soya-bean flour; SI, soya-bean isolate; SC, soya-bean concentrate.

Fe:ascorbic acid of 1:10 to the soya-bean concentrate–chicken (50:50, w/w) combination significantly increased the soluble ^{59}Fe in the presence of soya-bean concentrate under all conditions of digestion. It is thought that the Fe complexes formed during digestion in the presence of ascorbic acid became more soluble because of both the reducing and chelating properties of ascorbic acid.

In vivo ^{59}Fe absorption by Fe-replete and Fe-deficient rats from raw chicken or pigeon meat partially replaced by soya-bean proteins

When the Fe-replete rats were given the various test meals the ^{59}Fe absorption trend, while considerably lower quantitatively, followed a similar pattern to that of the in vitro ^{59}Fe solubilities and the results are shown in Fig. 1. Higher levels of meat replacement by soya bean gave greater reductions in the absorption of ^{59}Fe from chicken meat.

The presence of soya-bean concentrate was most inhibitory to ^{59}Fe absorption from chicken and pigeon meat. Thus when 50% chicken protein was replaced, ^{59}Fe absorption was reduced by 52·8, 69·1 and 43·1% by the soya-bean flour, concentrate and isolate respectively. In contrast, lower reductions in ^{59}Fe absorption of 27·5, 36·1 and 29·6% respectively for soya-bean flour, concentrate and isolate were obtained when pigeon as opposed to chicken meat was replaced at the same level by the soya-bean protein. That the effect was not due simply to reducing the amount of chicken meat in the test meal by 50% is shown by there being no significant effect on the percentage ^{59}Fe absorption from chicken when fed as either the standard test meal (i.e. 40 μg Fe) or when the protein and Fe level were reduced by half. Replacement of 50% of the chicken protein by casein or BSA had no inhibitory effect on ^{59}Fe absorption and, in fact, a slight enhancement occurred. This shows that the reduced level of chicken and Fe present in the test meal and its substitution by protein from sources other than soya bean (i.e. casein or BSA) could not account for the specific inhibitory effect on meat Fe absorption observed with soya bean alone.

The ^{59}Fe absorption results when the various chicken–soya-bean combinations were given

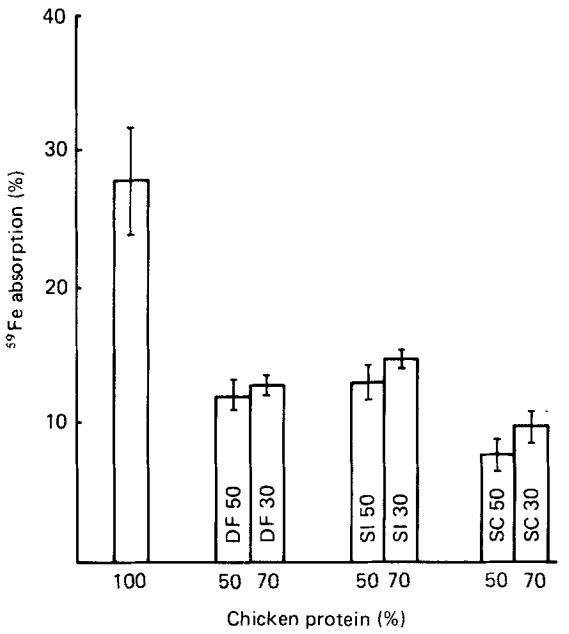

Fig. 2. Percentage absorption of ^{59}Fe from raw chicken meat alone or after replacement of 30 or 50% of the meat protein by soya-bean proteins in Fe-deficient rats. Mean values with their standard errors, represented by vertical bars, for four to eight rats per group.

to Fe-depleted rats (average blood Hb 78·4 g/l) are shown in Fig. 2. Compared with Fe-replete rats, ^{59}Fe absorption was increased when chicken meat alone was given. This agrees with the work of Bogunjoko *et al.* (1983). Replacement of either 30 or 50% of the meat protein by soya-bean proteins reduced ^{59}Fe absorption from chicken meat in a similar manner to that shown for the Fe-replete rats (Fig. 1). In contrast to results with Fe-replete rats, however, there was no significant difference in the reduction in ^{59}Fe absorption from chicken at the two different levels of soya-bean replacement ($P > 0{\cdot}05$).

In vivo absorption of ^{59}Fe-labelled Hb/protein test meals

^{59}Fe absorption from ^{59}Fe-labelled Hb in the presence of BSA and soya-bean proteins is shown in Table 4. Addition of BSA increased ^{59}Fe-labelled Hb absorption but there was no significant effect on Hb-Fe absorption in the presence of an equivalent amount of extra protein from the three soya-bean-protein products. This suggests that Hb and possibly myoglobin in a meat environment would similarly be protected from soya-bean-protein inhibition.

In vivo ^{59}Fe absorption from chicken-meat fractions alone and in combination with soya-bean concentrate

Since Fe absorption from Hb alone was not inhibited by soya-bean protein, the final experiments attempted to identify the chicken-meat fraction(s) which did interact with soya bean to produce the observed inhibition of Fe absorption. Chicken meat was therefore fractionated into the various ^{59}Fe-containing components by the method described previously (Bogunjoko *et al.* 1983). Soya-bean concentrate replaced 50% of the protein from the chicken-meat fractions and the test meals were given intragastrically to Fe-replete rats; ^{59}Fe absorption results are shown in Table 5. The results show that the absorption of ^{59}Fe from the haemoproteins was not significantly affected by soya-bean concentrate

Table 4. *Percentage absorption of ^{59}Fe at 120 min after intragastric dosing of various ^{59}Fe-labelled haemoglobin (Hb)–protein systems in Fe-replete rats*

(Values are means with their standard errors; no. of rats in parentheses)

Hb–protein combination	Protein level (mg/5 ml dose)	Fe level (μg/5 ml dose)	^{59}Fe absorption (%)	
			Mean	SE
Hb alone	4·8	20·0	8·9	0·82 (7)
Hb+200 mg BSA	195	20·0	13·8	2·4 (3)
Hb+DF	195	61·0	8·39	0·64 (4)
Hb+SC	195	53·4	9·0	1·2 (4)
Hb+SI	195	39·5	9·48	0·93 (4)

BSA, bovine serum albumin; DF, defatted soya-bean flour; SC, soya-bean concentrate; SI, soya-bean isolate.

Table 5. *Percentage absorption of ^{59}Fe at 120 min from chicken meat fractions with or without soya-bean concentrate (SC) in Fe-replete rats*

(Values are means with their standard errors for four rats)

Test meal	^{59}Fe absorption (%)				
	Without SC		With SC*		Percentage reduction with SC
	Mean	SE	Mean	SE	
Soluble meat extract	13·4	0·84	9·36	0·98	30·1
Haemoproteins (heat-denatured)	9·3	1·0	9·19	0·92	1·2
Ferritin+low-molecular-weight Fe	9·4	0·73	6·37	1·1	31·9
Haemosiderin (insoluble residue)	7·46	0·47	3·3	0·33	55·8

* 50% chicken meat protein was replaced by SC.

(as before with Hb-Fe) but Fe absorption from the other three fractions was reduced, the greatest inhibition being from the insoluble residue. Thus, while the Fe in the haemoproteins (Hb and myoglobin) was protected from the inhibitory action of soya-bean concentrate, absorption of all the other non-haem chicken Fe compounds was reduced to varying extents by the presence of soya-bean proteins.

DISCUSSION

Replacement of part of the meat protein by soya-bean protein products while keeping total protein levels constant significantly reduced ^{59}Fe absorption from ^{59}Fe-labelled chicken and pigeon meat. This observation extends recent reports of reduced Fe availability due to the presence of soya-bean protein in semi-synthetic meals (Cook *et al.* 1981), cereal/soya-bean blended foods (Morck *et al.* 1981) and in meat products (Cook *et al.* 1981; Hallberg & Rossander, 1982). The magnitude of the soya-bean protein inhibition observed in the present study varied in extent with the type of meat, the amount of meat protein replaced by soya-bean protein and also with the different soya-bean products. In all cases, however, the addition of soya-bean proteins with their high levels of 'cold' Fe considerably diluted the ^{59}Fe specific activity from meat and the effect of this on meat ^{59}Fe absorption is not known. With Fe-replete rats it seems likely that increasing the Fe content of the meal would not correspondingly increase the amount of Fe absorbed. If the meat and soya-bean Fe

were competing equally for absorption, the increased Fe from soya bean would reduce the proportion of the total Fe absorbed and hence reduce the proportion of the radioactivity absorbed also. Such an effect is likely to explain, at least in part, the reduction in ^{59}Fe absorption in the presence of soya bean.

Further work will aim at quantifying the competitive effect of increasing amounts of available Fe on meat ^{59}Fe absorption.

The inhibitory factors in soya bean and the mechanism of this inhibition have been critically discussed by other workers (Cook *et al.* 1981; Hallberg & Rossander, 1982). The latter authors found that the removal of phytate from soya-bean flour did not reduce the inhibitory effect of soya-bean protein on non-haem-Fe absorption from a hamburger meal. It was therefore concluded that the phytate in soya-bean flour could not be implicated in this inhibitory action. The concept of the 'non-haem-Fe pool' in the intestine originating from non-haem-Fe in foods and being freely exchangeable has been widely accepted (Cook *et al.* 1972; Hallberg & Bjorn-Rasmussen, 1972). The non-haem-Fe content in meat which has, until now, not really been considered seriously, would likely react similarly to other sources of non-haem-Fe (primarily of plant origin in the meal) and would then be influenced by similar enhancing and inhibiting agents. The ultimate fate of the haemoproteins during digestion and their subsequent absorption is, however, far from clear. Earlier work has suggested that the majority of the dietary Fe in the form of Hb and myoglobin was absorbed as intact 'haem' and thus protected from enhancers or inhibitors. In the light of this widely-held view, therefore, it is very difficult to interpret the recent studies of Lynch *et al.* (1985) who showed in humans that haem-Fe absorption from meat was stimulated in the presence of soya bean (27–59% rise) but unaffected by other powerful inhibitors of Fe absorption e.g. bran, tea and desferrioxamine.

Other evidence has, however, accumulated over recent years to indicate that the 'haem' moiety in the meat haemoproteins is not refractory and can be digested or degraded to low-molecular-weight non-haem complexes in both in vitro and in vivo studies (Hazell *et al.* 1978, 1980). The resulting non-haem-Fe complexes which are very well absorbed in the duodenum may themselves be protected from inhibitory chelators through their interactions with amino acid and peptide fragments and together may form a separate non-haem-Fe pool but derived from haem-Fe. This so-called 'haem-derived non-haem-Fe pool' may well be distinct from the more well-known 'non-haem-Fe pool' which is derived initially from non-haem-Fe. The availability for absorption of the Fe in this haem-derived non-haem-Fe pool is not known but the studies of Hazell *et al.* (1978) suggest that it is high.

At the present time it is not known what proportion of the haem-Fe in meat is converted to the haem-derived non-haem-Fe pool during normal meat digestion either alone or in association with other foods. If enzymes present in both the stomach and small intestine are responsible for this conversion, then factors which influence stomach emptying and intestinal transit time could have a marked effect on the extent of this conversion; the greater the extent of this conversion, the greater the proportion of the total haem-Fe which will ultimately be absorbed as Fe from the haem-derived non-haem-Fe pool. The observed increase in Fe absorption from meat in Fe-deficient rats (Bogunjoko *et al.* 1983), in which stomach-emptying was markedly delayed, could be explained as being due to an enhanced conversion of haem-Fe to the highly available haem-derived non-haem-Fe which is then very well absorbed, particularly in the Fe-deficient intestine. Clearly, as these studies have shown, the inhibition of meat-Fe absorption by soya-bean is greater with meats of a high non-haem-Fe level (low haem level), for example chicken, compared with that of meat with a low non-haem-Fe level such as pigeon. In addition, studies with Hb and soya bean (Table 4) and with the individual meat fractions and soya bean (Table 5) showed that the predominant effect of soya bean was on the absorption of Fe from the non-haem-Fe fraction

about which little is known. If, as seems likely, soya-bean protein is interacting with meat Fe primarily through its influence on the non-haem-Fe common pool, the question that follows is what factors or substances intrinsic to soya bean are responsible for this inhibition. Evidence of inhibitory factors associated with soya bean include its dietary fibre and phytate content, its poor protein digestibility and different amino acid composition compared with that of meat. Fibre fractions extracted from DF were found to bind Fe in an in vitro model system (G. O. Latunde-Dada and R. J. Neale, unpublished results). In extrapolating this work from animals to humans it would appear that reductions in 'biological availability' of Fe by soya beans as shown in human studies (Cook *et al.* 1981; Hallberg & Rossander, 1982) are operating at the level of absorption and not utilization. The technique used in these studies for measuring absorption, therefore, would seem to be very useful for studying these interactions at a fundamental level.

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REFERENCES

Bogunjoko, F., Neale, R. J. & Ledward, D. A. (1983). *British Journal of Nutrition* **50**, 511–520.
Conrad, M. E., Benjamin, B. J., Williams, H. L. & Foy, A. L. (1967). *Gastroenterology* **53**, 5–10.
Cook, J. D., Layrisse, M., Martinez-Torres, C., Walker, R., Monsen, E. & Finch, C. A. (1972). *Journal of Clinical Investigation* **51**, 805–815.
Cook, J. D., Morck, T. A. & Lynch, S. R. (1981). *American Journal of Clinical Nutrition* **34**, 2622.
Food and Agriculture Organization/World Health Organization (1970). *Requirements of Ascorbic Acid, Vitamin D, Vitamin B_{12}, Folate and Iron. Technical Report Series* no. 452. Rome/Geneva: FAO/WHO.
Hallberg, L. & Bjorn-Rasmussen, E. (1972). *Scandinavian Journal of Haematology* **9**, 193–197.
Hallberg, L. & Rossander, L. (1982). *American Journal of Clinical Nutrition* **36**, 514–520.
Hazell, T. (1982). *Journal of the Science of Food and Agriculture* **33**, 1049–1056.
Hazell, T., Ledward, D. A. & Neale, R. J. (1978). *British Journal of Nutrition* **39**, 631–638.
Hazell, T., Ledward, D. A., Neale, R. J. & Root, I. C. (1980). *Meat Science* **39**, 397–405.
Latunde-Dada, G. O. & Neale, R. J. (1986). *British Journal of Nutrition* **55**, 409–418.
Lynch, S. R., Dassenko, S. A., Morck, T. A., Beard, J. L. & Cook, J. D. (1985). *American Journal of Clinical Nutrition* **41**, 13–20.
Monsen, E. R., Hallberg, L., Layrisse, M., Hegsted, D. M., Cook, J. D., Hertz, W. & Finch, C. A. (1978). *American Journal of Clinical Nutrition* **31**, 134–143.
Morck, T. A., Lynch, S. R., Skikne, B. S. & Cook, J. D. (1981). *American Journal of Clinical Nutrition* **34**, 2630–2634.

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