The retention of ^{59}Fe and ^{65}Zn by preruminant lambs given milk-substitutes based on either casein or soya-bean-protein isolate

BY R. HILL

The Royal Veterinary College (University of London), Boltons Park, Potters Bar, Herts EN6 1NB

AND D. M. WALKER

Department of Animal Husbandry, University of Sydney, Sydney, New South Wales 2006, Australia

(*Received 27 January 1984 – Accepted 3 December 1984*)

1. Eight newborn lambs were given milk-substitutes based on casein (*n* 4) or a soya-bean-protein isolate (*n* 4). On the morning before a 10 d collection period, the milk-substitutes were supplemented with ^{59}Fe as ferric chloride and ^{65}Zn as zinc chloride. Faeces and urine were collected daily and determinations of ^{59}Fe and ^{65}Zn were made on these separately. During a second 10 d period the dosing procedure and collection were repeated but the diet of each lamb was reversed.

2. Mean retention of ^{59}Fe in lambs given the casein diet was 0·50 (SE 0·12) and in those given the soya-bean-protein diet 0·06 (SE 0·05). There were large variations among animals but the treatment effect was significant at $P < 0.05$. Retention of ^{65}Zn was significantly greater than that of ^{59}Fe for all lambs but the effect of dietary treatment was similar to that for Fe. ^{65}Zn retention values for casein and soya-bean-protein diets were 0·84 (SE 0·06) and 0·52 (SE 0·03) respectively, and the difference was significant at $P < 0.01$.

3. There were only very small amounts of ^{59}Fe and ^{65}Zn in urine, representing 0·002–0·003 of the dose.

The recommmended levels of trace elements in milk-substitutes for calves have been given by Roy (1977). However, these recommendations are based on values obtained with diets containing either casein or the proteins of separated milk as the only source of protein. It is not certain that these levels of trace elements will be sufficient in milk-substitutes containing increasing proportions of other proteins and, in particular, of soya-bean proteins.

While there is strong evidence, based on observations made largely with rats, that the availability of zinc is low in diets containing soya-bean proteins (Davies, 1979), this evidence is not universally accepted (Young & Janghorbani, 1981). In human nutrition it is generally accepted that the availability of iron is greater from diets containing proteins from animal sources than from those containing proteins of plant origin (Davidson *et al.* 1979). In animal nutrition the levels of Fe that are used in milk-substitutes for veal production are of critical importance. They must satisfy the dual role of maintaining a healthy animal and producing a pale-coloured flesh (Bremner & Dalgarno, 1973). It is not known whether the availability of Fe from milk-substitutes is affected by the type of protein used.

In the present experiment, preruminant lambs were given milk-substitutes containing either casein or a soya-bean-protein isolate as the only source of protein. The retention of Fe and Zn from these diets was determined after giving an oral dose of ^{59}Fe and ^{65}Zn.

Table 1. *Compositions of the diets (per kg dry matter (DM))*

Diet...	Casein	Soya-bean protein
Casein* (g)	296	—
Soya-bean-protein isolate† (g)	—	295
DL-Methionine (g)	5·6	10·2
Glucose (g)	247	252
Butter oil (g)	343	358
Glyceryl monostearate (g)	43	45
Minerals (g)	65	40
Crude protein‡ (g)	275	280
Diethyl ether extractives (g)	386	403
Ash (g)	73	56
Nitrogen-free extractives (g) (by difference)	266	261
Gross energy (MJ)	25·8	26·2
Calcium (g)	7·3	7·4
Phosphorus (g)	6·6	6·7
Iron (mg)	30	30
Zinc (mg)	20	20
Milk DM (as fed) (g/l)	195	191

* Casein hydrochloride (mg/kg DM): Fe 10·5, Zn 29·4.
† Pro-fam 90/HS (Grain Processing Corporation, Muscatine, Iowa, USA) containing (mg/kg DM): Fe 75·6, Zn 28·9, phytate-P 5950.
‡ N × 6·25.

MATERIALS AND METHODS

Eight cross-bred male lambs, Dorset Horn ♂ × (Border Leicester ♂ × Merino ♀), aged 2–5 d at the start of the experiment, were used. On the 1st day they were given colostrum, on the 2nd whole milk and subsequently, the experimental diets. They were housed individually in metabolism cages and the management was essentially as described by Walker & Faichney (1964). The lambs were bottle-fed twice daily and, after each feed, were fitted with muzzles to prevent the ingestion of metallic elements from the surfaces of the cages. Urine and faeces were collected separately daily for 10 d following each dose of radioactive Fe and Zn.

The compositions of the diets are given in Table 1. The final concentrations of Fe and Zn were 30 and 20 mg/kg dry matter (DM) respectively. Having allowed for the Fe and Zn contents of the ingredients, the amounts of supplementary Fe were 24·6 and 6· 9 mg/kg DM, and of supplementary Zn were 9·9 and 10·7 mg/kg DM, for the casein and soya-bean-protein diets respectively. The concentrations of other minerals and of vitamins were similar to those given by Walker (1975), with the following exceptions (values expressed as mg/MJ): selenium 0·004, iodine 0·018, copper 0·179, manganese 0·179. The concentration of energy in the milk-substitutes was 5 MJ/kg as fed. The gross energy intake was maintained at 1·46 MJ/kg live weight$^{0·73}$ per d throughout each period, based on the live weight of the lamb at the beginning of the period. All lambs were dosed with 1 ml groundnut-oil solution containing 34·4 mg retinyl acetate and 0· 25 mg ergocalciferol at the start of the experiment.

The lambs were divided into two groups: they were paired according to body-weight and allocated randomly within a pair to diet. Mean body-weights at the start of the experiment were: casein diet 3·61 kg and soya-bean-protein diet 3·67 kg.

The experimental period of 24 d was divided into a preliminary period of 4 d and two 10 d collection periods (periods 1 and 2). During the 4 d preliminary period and the first 6 d of period 1, four lambs were given the casein diet and four the soya-bean-protein diet.

On the 4th day of the preliminary period, that is, on day 0 of period 1, each lamb was given an oral dose of an aqueous solution (10 ml) containing 10 μCi ^{59}Fe as ferric chloride and 10 ml of a similar solution containing 10 μCi ^{65}Zn as zinc chloride, added to a minimum amount of milk-substitute. On the 7th day after this first dose the lambs that had been given the casein diet were given the soya-bean-protein diet, and the diets of the other lambs were also reversed. The oral doses of ^{59}Fe and ^{65}Zn were repeated on the 4th day after the diets were changed, i.e. day 0 of period 2. Six lambs completed the change-over experiment without incident. Two of the lambs that were given the casein diet in period 1, and the soya-bean-protein diet in period 2, would not voluntarily consume all the milk offered during period 2. Their results were excluded from the group means and from the values shown in Fig. 1. The concentrations of ^{59}Fe and ^{65}Zn of faeces and urine were calculated from counts made for standards and samples of faeces and urine at two settings of a gamma counter, corresponding to 1·29 and 1·10 MeV, the former giving a count derived almost entirely from ^{59}Fe and the latter a count from ^{59}Fe and ^{65}Zn, with a greater proportion from ^{65}Zn than from ^{59}Fe. Radioactivity was measured on daily faeces samples after incineration in a muffle furnace at 550° overnight. There was no detectable radioactivity in the fresh urine. However, an estimate of the count of ^{59}Fe and ^{65}Zn in the urine was made on the ash derived from 100 ml of a pooled sample, representative of the whole 10 d collection period for each lamb. Radioactive counts in the faeces and urine were expressed as a proportion of the original dose, subtracted from 1·00 to obtain retention values and these were analysed statistically by analyses of variance. The data for each element were analysed separately.

RESULTS

The mean live weight of the lambs was 5·7 kg during period 1 and 7·7 kg during period 2. The live-weight gains were 51 and 47 g/kg live weight$^{0.73}$ per d for the casein and soya-bean-protein diets respectively.

When allowance was made for the small differences in gross energy intakes between diets the live weight gains were identical at 9·9 g/kg live weight$^{0.73}$ per MJ, and corresponded to a live weight gain of approximately 200 g/lamb per d. The apparent digestibilities of the DM of the diets were 0·970 and 0·958, for the casein and soya-bean-protein diets respectively; they were not significantly different.

The faecal losses of ^{59}Fe and ^{65}Zn are shown on a daily basis in Fig. 1, expressed as a proportion of the oral dose. The values for the lambs that were given the casein diet in period 1 and the soya-bean-protein diet in period 2 are shown in Fig. 1(*a*), and for those given the soya-bean-protein diet in period 1 and the casein diet in period 2 in Fig. 1(*b*). The greatest faecal loss of ^{59}Fe was on the 2nd day after dosing, for both groups of lambs and for both diets. By the 7th day after dosing the amount of ^{59}Fe in the faeces was very small, 0·01 of the dose or less. The greatest faecal loss of ^{65}Zn occurred on the 1st, 2nd or 3rd day after dosing and, as with ^{59}Fe, the amount of ^{65}Zn in the faeces by the 7th day after dosing was very small, 0·01 of the dose or less.

The activity of ^{59}Fe and of ^{65}Zn in the faeces of lambs given the soya-bean-protein diet was greater than that in the faeces of lambs given the casein diet (Fig. 1), whilst the ^{59}Fe activity was greater than that of ^{65}Zn, irrespective of diet.

There was no detectable activity of ^{59}Fe or of ^{65}Zn in fresh urine at any time during the experiment. However, measurable amounts were detected in the ash of pooled samples collected for each 10 d period. The mean values, expressed as a proportion of the dose recovered in the urine, were as follows: for ^{59}Fe (n 6), casein diet 0·0030 (SE 0·0005), soya-bean-protein diet 0·0023 (SE 0·0012); for ^{65}Zn (n 6), casein diet 0·0040 (SE 0·0025),

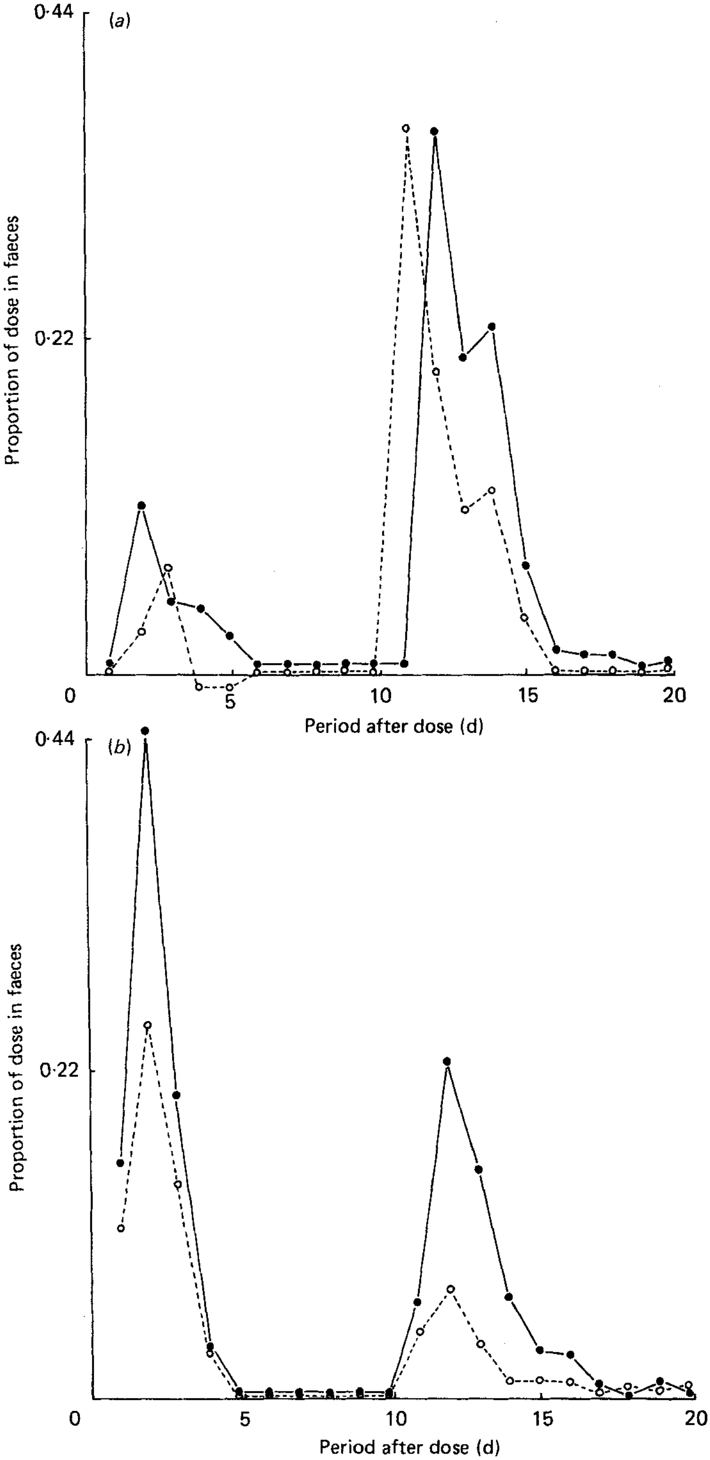

Fig. 1. For legend see opposite.

Table 2. *Retention of ^{59}Fe and ^{65}Zn by preruminant lambs given milk substitutes based on casein or on soya-bean protein*

(Number of lambs in parentheses)

Element	Dietary protein	Proportion of dose retained		
		Period 1 (days 0–10)	Period 2 (days 10–20)	Mean
^{59}Fe	Casein	0·798 (2)	0·347 (4)	0·497 (6)
	Soya bean	0·084 (4)	0·005 (2)	0·058 (6)
				SED 0·144, 4 df
^{65}Zn	Casein	0·918 (2)	0·802 (4)	0·841 (6)
	Soya bean	0·476 (4)	0·619 (2)	0·523 (6)
				SED 0·051, 4 df

soya-bean-protein diet 0·0016 (SE 0·0009). There were no significant differences between diets for either ^{59}Fe or ^{65}Zn.

The amounts of ^{59}Fe and ^{65}Zn retained by the lambs, expressed as a proportion of the dose, and calculated from the total output in the urine and faeces during the 10 d period following the dose, are given in Table 2.

The retention of ^{59}Fe by lambs given the soya-bean-protein diet was significantly lower than that of lambs given the casein diet ($P < 0{\cdot}05$), and the retention of ^{65}Zn by lambs given the soya-bean-protein diet was significantly lower than that of lambs given the casein diet ($P < 0{\cdot}01$). Also, for each lamb in each period the retention of ^{59}Fe was lower than that of ^{65}Zn: the overall retention of ^{59}Fe was 0·278 and that of ^{65}Zn, 0·682.

DISCUSSION

The results described in the present paper showed clearly that when preruminant lambs were fed on a milk-substitute containing isolated soya-bean-protein, they absorbed and retained less Fe and Zn from the diet than when the milk-substitute contained casein as the only source of protein. This observation with lambs is in accord with some, but by no means all, published results, obtained largely with humans and rats. For example, the early work of Bjorn-Rasmussen *et al.* (1973) and of Martinez-Torres & Layrisse (1974), together with the more recent study of Young & Janghorbani (1981) with a variety of soya-bean products, all with humans, gave no indication that the availability of Fe from these products was especially low. However, other recent studies with humans have shown that the presence of soya-bean products in the diet leads to a significant reduction in Fe availability (Cook *et al.* 1981; Morck *et al.* 1981; Schricker *et al.* 1983). There is as yet no satisfactory explanation for these apparently conflicting results.

The literature dealing with the availability of Zn from diets containing soya-bean products, obtained largely with rats, shows a greater measure of agreement than that dealing with Fe absorption and retention. In most cases it is agreed that the presence in the diet of soya-bean meal, or of protein products from the soya bean, leads to a reduction in the

Fig. 1. The daily excretion of ^{59}Fe (——) and ^{65}Zn (– – – –) in faeces of lambs following single doses of ^{59}Fe and ^{65}Zn on days 0 and 10. (*a*) Means of two lambs given the casein diet (period 1, days 0–10) followed by the soya-bean-protein diet (period 2, days 10–20). (*b*) Means of four lambs given the soya-bean-protein diet (period 1, days 0–10) followed by the casein diet (period 2, days 10–20).

availability of Zn when compared with its availability from diets free of these products (O'Dell, 1969; Davies, 1979; Davies & Olpin, 1979; Forbes *et al.* 1979; Meyer *et al.* 1983). However, the experimental results of Young & Janghorbani (1981) are in disagreement with the general view. When young men were fed on diets containing either soya-bean protein or casein the availabilities of Fe and Zn were almost the same, irrespective of the source of dietary protein.

The usual explanation that is given for the reduction in the availability of both Fe and Zn from diets containing soya-bean products, is based on the assumption that these trace elements form insoluble complexes with the phytate present in the soya-bean product (Davies & Olpin, 1979; Schricker *et al.* 1982), and that these complexes are indigestible in the digestive tract of single-stomached species, an explanation that is also given for the low availability of Fe from bread made with 'high-extraction' flour (Widdowson & McCance, 1943). However, Welch & Van Campen (1975) determined the availability of Fe from immature and mature soya beans that contained different levels of phytate, and found no relation between phytate content and reduced Fe availability. From this and later work it is evident that phytate content alone cannot fully explain the reduction in the availabilities of Fe and Zn from soya beans and their products (Walker & Welch, 1982). It is apparent that a number of factors can affect the relation between phytate content and trace element availability. Davies & Flett (1978) have demonstrated the importance of phytase (*EC* 3.1.3.26) activity in the digestive tract, others have pointed to the influence of the calcium concentration in the diet (O'Dell, 1969; Young & Janghorbani, 1981) and to the reduction in availability that is attributable to the formation of indigestible phytate–protein complexes during the processing of soya beans (Erdman & Forbes, 1981). However, the quantitative impact of these factors on trace-element availability is as yet unknown.

In our studies with lambs the concentration of phytate-phosphorus in the soya-bean-protein diet was 1760 mg/kg, and the molar ratio, phytate:Fe plus Zn was 51:1. This value compares with that of 15:1 in the soya-bean-protein diets of Davies & Olpin (1979). These diets were shown to reduce the availability of Zn to such an extent that when rats were fed on them they became Zn deficient. Thus, on balance, it seems likely that the excess of phytate relative to the content of Zn plus Fe was responsible for the reduced availability of these trace elements from the soya-bean-protein diets in our experiment, although it does not explain the failure of Young & Janghorbani (1981) to show a similar effect with young men given diets with an almost identical ratio, phytate:Fe plus Zn.

REFERENCES

Bjorn-Rasmussen, E., Halberg, L. & Walker, R. B. (1973). *American Journal of Clinical Nutrition* **26**, 1313–1319.
Bremner, I. & Dalgarno, A. C. (1973). *British Journal of Nutrition* **30**, 61–76.
Cook, J. D., Morck, T. A. & Lynch, S. A. (1981). *American Journal of Clinical Nutrition* **34**, 2622–2629.
Davidson, S., Passmore, R., Brock, J. F. & Truswell, A. S. (1979). *Human Nutrition and Dietetics*, 7th ed. London: Churchill–Livingstone.
Davies, N. T. (1979). *Proceedings of the Nutrition Society* **38**, 121–128.
Davies, N. T. & Flett, A. A. (1978). *British Journal of Nutrition* **39**, 307–316.
Davies, N. T. & Olpin, S. E. (1979). *British Journal of Nutrition* **41**, 590–603.
Erdman, J. W. & Forbes, R. M. (1981). *Journal of the American Oil Chemists' Society* **58**, 489–493.
Forbes, R. M., Weingartner, K. E., Parker, H. M., Bell, R. R. & Erdman, J. W. (1979). *Journal of Nutrition* **109**, 1652–1660.
Martinez-Torres, C. & Layrisse, M. (1974). *World Review Nutrition and Dietetics* **19**, 51–70.
Meyer, N. R., Stuart, M. A. & Weaver, C. M. (1983). *Journal of Nutrition* **113**, 1255–1264.
Morck, T. A., Lynch, S. R., Skikne, B. S. & Cook, J. D. (1981). *American Journal of Clinical Nutrition* **34**, 2630–2634.
O'Dell, B. L. (1969). *American Journal of Clinical Nutrition* **22**, 1315–1322.
Roy, J. H. B. (1977). *The Composition of Milk Substitute Diets and the Nutrient Requirements of the Pre-ruminant Calf*, p. 41. Dunstable, Beds: Roche Ltd.

Schricker, B. R., Miller, D. D. & Van Campen, D. (1982). *Journal of Nutrition* **112**, 1696–1705.
Schricker, B. R., Miller, D. D. & Van Campen, D. (1983). *Journal of Nutrition* **113**, 996–1001.
Walker, C. D. & Welch, R. M. (1982). *Proceedings of the Fourth Symposium on Trace Element Metabolism in Man and Animals*, Perth, Australia, May 1981, pp. 155–157.
Walker, D. M. (1975). *Australian Journal of Agricultural Research* **26**, 599–614.
Walker, D. M. & Faichney, G. J. (1964). *British Journal of Nutrition* **18**, 187–200.
Welch, R. M. & Van Campen, D. (1975). *Journal of Nutrition* **105**, 253–256.
Widdowson, E. M. & McCance, R. A. (1943). *Lancet* **i**, 588.
Young, V. R. & Janghorbani, M. C. (1981). *Cereal Chemistry* **58**, 12–18.

Printed in Great Britain