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Copper, iron, manganese and zinc concentrations in the carcases of lambs and calves and the relationship to trace element requirements for growth

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1. The minced carcases of twenty-seven lambs, ranging from 18 to 69 kg in live weight, and twenty-five calves (30–90 kg) were analysed for copper, iron, manganese and zinc. The lambs were weaned whereas the calves were reared exclusively on milk.

2. Mean concentrations of Fe, Mn and Zn for groups of lamb carcases fell within the ranges 52·6–75·1, 0·7–1·2 and 20·8–25·6 mg/kg fresh carcase weight respectively. The concentrations of Fe and Mn decreased while that of Zn increased slightly with age at slaughter. The concentrations of Fe, Mn and Zn in calves were close to those in lambs.

3. For both species, the concentration of Cu in the carcase varied erratically: variation in hepatic Cu storage was implicated. In an additional study of ten full-term foetuses from Cu-depleted or Cu-supplemented ewes, a dietary Cu supplement (10 mg/kg dry matter (DM)) increased foetal Cu status 10-fold, due largely to an increase in foetal liver Cu.

4. The mean retentions of trace elements in the lamb carcases (% intake) were approximately: Cu 2·0, Fe 1·3, Mn 0·08, Zn 4·0. The corresponding values for the milk-fed calves were all probably much higher (Cu 23, Fe 43·7, Mn 4·9, Zn 34·0) but Cu intake was not accurately measured.

5. After allowing for tissue storage of Fe and Mn, values of 55, 0·85 and 24 mg/kg carcase gain were taken to represent the approximate net growth requirements of lambs for Fe, Mn and Zn respectively: the corresponding value for Cu was probably $<$ 1·0 mg/kg. Values for calves were similar to those for lambs.

6. It was concluded that the total net requirements of ruminants for Fe and Zn should be considered in terms of daily intakes of the metals rather than dietary concentrations because of the relatively large and constant contribution of the growth component to the total requirement.

Attempts to define the trace element requirements of growing ruminants in feeding trials have frequently given conflicting results. For example, Mills *et al.* (1967) estimated that the growth requirement of lambs for zinc was no greater than 7 mg/kg dry matter (DM) whereas Ott *et al.* (1965) concluded that it was between 18 and 33 mg/kg DM. Such inconsistencies may reflect differences in experimental conditions in addition to true differences in requirement. An alternative approach is to derive requirements by means of a factorial model of the type used by the Agricultural Research Council (1965). In a factorial approach, the maintenance (M) and growth (G) components of the net requirement are summated and divided by a coefficient of absorption to give a daily gross or dietary requirement. The growth component is the amount of an element accumulating in each unit gain in empty-body-weight. Unfortunately there are no published values for trace element concentrations in the lamb carcase and those recently published for calves are inconsistent (Kirchgessner & Neesse, 1976; Williams, 1978). One possible source of error is that carcase concentrations of a trace element reflect the accumulation of dietary excesses in certain storage organs. Wiegand & Kirchgessner (1977) have suggested that the storage component should always be ignored in deriving net requirements for growth. The object of this paper is, therefore, to present values for the concentrations of four elements, copper, iron, manganese and zinc, in the carcases of lambs and calves given known intakes of these elements and to assess the validity and nutritional implications of the net growth requirements obtainable from this basic information.

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Table 1. *Trace element concentrations (mg/kg* DM*) in the diets of lambs and calves from a series of carcase analysis studies*

Expt no.*	Diet†	Copper	Iron	Manganese	Zinc	Food intake (kg/d)
1	Ruminant A	9·6	280	34·6	44·3	1·85
2	Ruminant A	8·2	244	53·3	66·0	1·98
3	Milk-substitute	1·15‡	91	14·2	45·9	0·69
Kirchgessner & Neesse (1976)	Milk-substitute	1·75	—	0·75	24·0	1·8

DM, dry matter.

* For details, see p. 90.

† For details, see p. 90.

‡ Inclusion of 100 g dried microbial cells/kg in the diet M_{10} (see p. 90) affected only the Cu concentration which was increased to 1·65 mg/kg DM.

MATERIALS AND METHODS

Animals, diets and management

Expts 1 and 2. Lamb carcases were obtained from two experiments designed to examine the effects of sub-clinical worm infections on the health and productivity of cross-bred lambs. Expt 1 used female lambs from the initial slaughter (CI) and uninfected groups fed *ad lib.* (ALC) from a study by Sykes & Coop (1977). Expt 2 used male and female lambs from the corresponding groups (CI and ALC) from another experiment (A. R. Sykes and R. L. Coop, unpublished results). The CI groups were 4 months old and the ALC groups were given a complete ruminant diet (ruminant A; Wainman *et al.* 1970) for 98 (Expt 1) or 280 (Expt 2) d. Concentrations of trace elements in the diets and average food intakes are given in Table 1. The conditions for both experiments were similar to those described by Sykes & Coop (1976).

Expt 3. Calf carcases were obtained from an experiment designed to examine effects of substituting 100 g dried microbial cells (DMC)/kg and whey for dried skimmed milk in a milk-substitute fed to Ayrshire calves between 2 and 65 d of age at five levels of intake (Hinks, 1977). The hot-water supply used to prepare the milk was sampled after the experiment to assess the possible contributions of trace elements in the water to daily intakes. Three carcases from a CI group, twelve from a control group (Mo) and ten from a group given DMC (M_{10}) were analysed.

Expt 4. Three groups of three Scottish Blackface ewes were given a basal semi-purified diet of low Cu content (Suttle & Field, 1968) throughout pregnancy: one group was given a Cu supplement, 10 mg/kg DM, another was given a molybdenum supplement, 25 mg/kg DM, while the third was given no supplements. Foetuses were removed from the ewes by caesarean section 140 d after tupping. In all, ten foetuses weighing (mean ± SE) 3·9 ± 0·2 kg were obtained. The Mo supplement did not affect the Cu status of the foetus and two groups, one containing four foetuses from Cu-supplemented ewes and the other six from unsupplemented ewes, were formed. The gut plus contents were discarded and the carcase and liver retained for Cu and Zn analyses.

Analytical methods

Both the lambs in Expts 1 and 2 and the calves in Expt 3 were stunned by captive bolt and bled out. The blood was collected and mixed with either the whole carcase (Expts 1 and 2) or included in the offal fraction (Expt 3). Carcases were minced using the equipment described by Smith and Sykes (1974). Duplicate (Expts 2 and 3) or quadruplicate (Expt 1) samples of

Table 2. *Mean trace element concentrations (mg/kg fresh weight) and fresh weights (kg) of minced carcases of lambs (Expts* 1 *and* 2) *and calves (Expt* 3) *from three experiments*

(Mean values with their standard errors of differences)

		No of ani-	Carcase fresh wt (kg)		Copper		Iron		Manganese		Zinc	
Expt no.	Group*	mals	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	CI	8	19·6	0·5	2·9	0·35	75·1	3·4	1·21	0·11	20·8	1·05
	ALC	8	34·6		2·5		58·7		0·77		23·7	
2	CI	6	20·8	4·4	1·0	0·17	61·0	5·8	0·96	0·11	25·4	0·98
	ALC	6	61·1		1·9		52·6		0·89		25·6	
3	CI	3	32·3	0·7†	2·7	0·15†	51·7	11·7†	0·55	0·06†	22·7	1·31†
	Mo	12	53·1	7·8	3·7	0·40	66·2	4·5	0·92	0·06	25·4	1·27
	M_{10}	10	57·1		3·6		60·3		0·88		26·1	

* For details, see p. 90.
† Standard error of mean.

freeze-dried mince, 1 g, were dry ashed at 450 ° for 24 h. The foetal carcases were minced (Hobart F4522 mincer; Hobart Ltd, 73 Dykehead Street, Glasgow) and 500 g samples were dissolved in concentrated nitric acid, following the procedure of Field & Suttle (1966) but on a much smaller scale. Samples of diet, liver and carcase solutions were digested with a mixture of HNO_3 and perchloric acid and taken to dryness (Thompson & Blanchflower, 1971). The ashes and digests were dissolved in warm hydrochloric acid (50 g/l) and the concentrations of Cu, Fe, Mn and Zn determined by atomic absorption spectrophotometry using standards prepared in a similar medium.

Statistical analysis

The analyses of results from Expts 1 and 2 were those for randomized block designs. Covariance analysis was used to assess the effect of feed intake on carcase composition in Expt 3.

RESULTS

Trace element concentrations in the fresh carcase

The mean concentrations of Cu, Fe, Mn and Zn in the fresh minced carcases in Expts 1, 2 and 3, are given in Table 2. There were large differences between the concentrations of the four elements, that of Fe being 60 times greater than that of Mn. Values were similar in calves and lambs and the variation between groups of animals was least for Zn. Concentrations of Fe and Mn in the fresh carcase were significantly less in the ALC than in the CI group in Expt 1 ($P < 0·01$) but the effect was less evident in the lambs from Expt 2 and opposite in the calves. Cu concentrations varied more than those of other elements: they increased by 90 and 37% during Expts 2 and 3 and decreased by 14% during Expt 1.

Trace element concentrations in the dry carcase

The results for the dry carcase are given in Table 3. The effects and trends evident in the fresh carcase were generally more marked in the dry carcase, the major difference being for Zn in the lamb carcases which decreased as carcase weight increased. The fat content of the carcase increased with carcase weight in both species and conversion of trace element

Table 3. *Mean trace element concentrations, dry matter* (DM) *and fat contents of minced carcases of lambs* (*Expts* 1 *and* 2) *and calves* (*Expt* 3) *from three experiments*

(Mean values with their standard errors of differences)

Expt no.	Group*	No. of animals	Carcase dry wt (g/kg)		Carcase fat (g/kg DM)		Copper		Iron		Manganese		Zinc	
									(mg/kg DM)					
			Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
	CI	8	310		304		9·3		241		3·9		66·9	
1				0·1		18		0·75		8·5		0·19		1·8
	ALC	8	428		541		5·9		137		1·8		55·4	
	CI	6	337		362		2·9		183		2·9		76·0	
2				0·1		27		0·27		14·8		0·25		2·6
	ALC	6	512		672		3·6		102		1·7		50·0	
	CI	3	163	2·5†	43	4†	9·3	0·6†	176	43·1†	1·9	0·26†	76·4	3·3†
3	Mo	12	176		201		11·6		206		2·9		79·7	
				7·5		35		0·93		12·3		0·19		4·0
	M_{10}	10	188		221		10·6		178		2·6		77·2	

* For details, see p. 90.
† Standard error of means.

concentrations to a fat-free DM basis reduced the magnitude of the changes associated with advancing maturity of the carcase.

There were no significant differences in trace element concentration between the sexes in Expt 2 or between dietary treatments and levels of food intake in Expt 3, except for Fe in the calf carcase which decreased when DMC was included in the diet.

Trace element content of calf carcase fractions

There were marked differences in the way in which the four elements were distributed between the offal and meat + bone fractions of the calf carcase: the mean values (± SE) for all treatments for Cu, Fe, Mn and Zn were respectively, 3·0 ± 0·1, 225 ± 2, 2·9 ± 0·3 and 92 ± 2·5 mg/kg DM for meat + bone and 20·8 ± 0·9, 151 ± 19, 2·2 ± 0·2, and 62 ± 2·0 for offal. The offal fraction containing the liver, thus contained far more Cu but less of the other elements than the meat + bone fraction. The mean dry weights of the two fractions were 7·5 and 9·6 kg and the fat contents 157 and 152 g/kg DM for offal and meat + bone respectively. The only element to be affected by plane of nutrition was Zn: Zn concentrations increased in offal and decreased in meat + bone as food intake increased.

Hepatic Cu and Zn accumulation

In Expt 4 the addition of a Cu supplement to the diet of ewes during pregnancy increased the mean (± SE) Cu concentration in the foetal carcase at 140 d from 0·81 ± 0·11 to 2·6 ± 0·4 mg/kg fresh weight and the mean (± SE) total Cu content from 2·09 ± 0·29 to 7·83 ± 2·33 mg. The increase was due almost entirely to an increase in the mean (± SE) Cu content of the liver from 0·38 ± 0·10 to 5·33 ± 1·83 mg. The Zn content of the carcase was unaffected by the treatments. The mean Zn concentration was 27·3 ± 1·1 mg/kg fresh carcase weight and the mean (± SE) Zn content 74·7 ± 5·3 mg, the liver providing 12·3% of the total foetal Zn.

Table 4. *Retention (% intake) of ingested trace elements in the lamb and calf carcases as determined by comparative-slaughter technique*

Expt. no.*	No. of animals	Copper		Iron		Manganese		Zinc	
		Mean	SE	Mean	SE	Mean	SE	Mean	SE
1	8	1·7	0·35	1·0	0·17	0·04	0·048	5·1	0·32
2	6	2·4	0·27	1·6	0·10	0·12	0·01	3·0	0·10
3	22	23·1†	1·9	43·7	4·5	4·9	0·4	34·0	2·9
Kirchgessner & Neesse (1976)		21		—		20		41·5	

* See Tables 1, 2 and p. 90.

† The value for Cu retention is only an approximate one because of the uncertain intake of Cu from water.

Table 5. *Concentrations of copper, iron, manganese and zinc in the fresh carcase (mg/kg) of calves recorded in previous experiments*

Source	Body-wt (kg)	Copper	Iron	Manganese	Zinc
Kirchgessner & Neesse (1976)	57·9	3·4	—	1·1	38·1
	98·5	2·1	—	0·8	23·1
	154·7	1·7	—	0·6	24·8
Williams (1978)	69	8·4	95	1·3	48·0

Trace element retentions

Retention of a trace element in the carcase was calculated by the conventional comparative-slaughter technique, i.e. as the average change in carcase content, and expressed as a percentage of the total intake of that element throughout the experiments (Table 4). The milk-fed calves appeared to retain all elements far more efficiently than the weaned lambs and the retention of Mn by both species was apparently far lower than that of other elements. The water used to reconstitute the calf feed probably provided a significant amount of Cu, approximately 10 mg/kg DM intake, a small amount of Zn (approximately 1·2 mg/kg DM intake) and negligible amounts of Fe and Mn. These amounts were included in the calculation of percentage retentions.

DISCUSSION

The mean trace element concentrations presented for calf carcases (Table 2) agree fairly well with those published by Kirchgessner & Neesse (1976) but are consistently lower than those published by Williams (1978; Table 5). The differences may reflect analytical errors, including the contamination of samples during mincing, or the variable accumulation of body stores of an element in animals on diverse nutritional regimens. The analytical methods used in the present study were compared with those of other laboratories during the study and were found to be in good agreement (Consultative Committee for Development of Spectrochemical Work, unpublished results). Although the extent of contamination at mincing cannot be fully assessed, the low coefficients of variation obtained for the concentrations of all elements, except Cu, in the carcases of the ALC groups in Expts 1 and 2 (Table 2) suggests that intermittent contamination during mincing did not seriously affect the values for Fe, Mn or Zn.

The extent to which accumulation of tissue stores is likely to have influenced trace element concentrations in the whole carcase varies from element to element. At one extreme there is Zn which is not stored to any great extent and at the other there is Cu which is readily stored in the tissues (see Underwood, 1977). The relative consistency of Zn concentrations within the present study can be explained in terms of the poor storage capacity for the element but the inconsistency with the results of Williams (1978) cannot be explained in such terms. The value presented for Zn in the dry calf carcase (92 mg/kg DM) falls between those found in the principal carcase constituents, muscle and bone, by Miller *et al.* (1968; 129 and 79 mg/kg DM respectively): similarly, that for total Zn in foetal lambs (74·7 mg) is close to the value of 80·7 found in twin lambs by Williams *et al.* (1978). It would, therefore, appear that the over-all mean value from Table 2, 24 mg/kg, represents a good estimate of the net growth requirement of ruminants for this element.

The results presented for Cu are wholly unsatisfactory as a basis for predicting Cu requirements for growth. Cu retention was found to exceed dietary Cu intake in the calves until the Cu derived from water was retrospectively brought into the calculations. In Expt 4 the marked influence of Cu nutrition during pregnancy on body Cu content of lambs at birth was demonstrated. The eightfold range in Cu concentrations found in the calf carcases by various workers (Tables 2 and 5) undoubtedly reflects the varying contribution of hepatic Cu stores in the different experiments. The net growth requirement for Cu must, therefore, either be derived from minced carcases which exclude the liver or from animals which have at no time received more dietary Cu than they require. Even the lowest value (1 mg Cu/kg; Group CI, Expt 2) recorded for lambs in the present study may be an over-estimate of the net growth requirement; the true value may be closer to that of 0·45 mg/kg estimated by Suttle (1978) for calves of marginally-adequate Cu status.

The introduction of errors due to the accumulation of Fe and Mn in tissue stores is more difficult to evaluate. Calves can be born with substantial tissue reserves of Fe (Charpentier, 1966) which are subsequently redistributed and they can store a dietary excess of Mn in the first few weeks of life (Howes & Dyer, 1971). In later life most species develop homoeostatic mechanisms which effectively control the accumulation of Fe and Mn in their tissues (Underwood, 1977). The results presented in this paper are consistent with these general findings. The high whole-body concentrations of Fe and Mn in the CI lambs from Expt 1 (Table 2) probably reflect the presence of initial tissue reserves of these elements and they are not, therefore, suitable for the derivation of nutritional requirements for growth. The fact that whole-body concentrations of Fe and Mn fell during Expt 1 despite the provision of more dietary Fe and Mn than the animals were thought to require (Agricultural Research Council, 1965) suggests that the accumulation of stores of Fe and Mn during the Expts 1 and 2 is unlikely to have occurred and that the mean values for ALC groups of lambs (55·5 mg Fe and 0·85 mg Mn/kg carcase gain) may be close to the net growth requirements of lambs for these elements.

The large variation within the small CI group of calves (Table 2) makes it impossible to assess the significance of the apparent increase in whole-body Fe and Mn concentrations during Expt 3 in terms of changes in tissue reserves. The diet did not contain excessive amounts of either element (Table 1): indeed the Fe concentration (91 mg/kg DM) might be regarded as marginally inadequate for normal myoglobin formation in calves in view of the results of MacDougall *et al.* (1973). The Fe and Mn concentrations found in the ALC groups of calves are approximately 30% lower than those reported by Williams (1978), which received a whole-milk diet supplemented with unspecified amounts of Fe and Mn. Using values for the Fe content of the major body Fe pools, Blaxter *et al.* (1957) estimated that the calf's Fe requirement for 1 kg carcase gain was 50–60 mg. This estimate agrees closely with the whole-body Fe concentration presented in Table 2. It is therefore suggested that the mean values of 63 mg Fe and 0·90 mg Mn/kg carcase weight for groups Mo and M_{10} (Table 2) indicate at least the approximate order of the calves, net requirements of Fe and Mn for growth.

The common practice of stating trace element requirements in terms of dietary concentrations (Agricultural Research Council, 1965) leads to the provision of greater intakes as an animal grows since food intake is related to body-weight. The contribution of G to the total net requirement will, however, remain constant for a given growth rate and it follows that unless M is large relative to G, the total net requirement will rise less rapidly than food intake as the animal grows. The limited information available on values for M suggests that for Zn and Fe they are small relative to G. A value of 0·053 mg/kg body-weight can be calculated for M_{Zn} in pregnant heifers from the results of Hansard *et al.* (1968) and one of 0·015 for M_{Mn} in cows from the results of Sansom *et al.* (1972). No values are available for M_{Fe} in ruminants but the ability to excrete Fe from the body is regarded to be limited (Underwood, 1977) and in man the value has been reported to be 0·014 mg/kg body-weight (Hallberg *et al.* 1974). These values would give total M requirements of 0·70, 2·65 and 0·75 mg/d for Fe, Zn and Mn in a 50 kg calf which contrast with the G values, estimated in the present paper of 63, 24 and 0·9 mg/d respectively, required for 1 kg carcase gain/d. It would, therefore, appear that the dietary concentrations of Fe and Zn required by young calves and lambs decrease markedly with age and might, therefore, be more appropriately defined in terms of daily intakes which remain relatively constant at a given growth rate. Support for this suggestion comes from the recovery in haemoglobin values shown by milk-fed calves given diets of fixed Fe concentration low enough to cause the rapid initial development of anaemia (Bremner & Dalgarno, 1973; Bremner *et al.* 1976; Van Hellemond & Sprietsma, 1977). Furthermore, the relatively low dietary Zn concentration required by weaned lambs in Mills *et al.*'s (1967) experiment may be partly due to the relatively high live weight of the lambs (40 kg) when their requirement was estimated.

A corollary to the argument for decreases with age in Fe and Zn requirements/kg body-weight is that the retentions of Fe and Zn/kg body-weight will also decrease, provided that the efficiency of absorption remains constant. This arises because deposition of the elements in growing tissues accounts for a progressively smaller proportion of the amount absorbed.

Dietary requirements are also affected by the efficiency of trace element absorption and there was indirect evidence that this may differ for preruminant and ruminant animals. The retentions of trace elements by milk-fed calves in the present study and that of Kirchgessner & Neesse (1976) were much higher than those found in the ruminant lambs (Table 4). While this could partly reflect adaptation to lower intakes of the elements (Table 1), it also probably reflects a general increase in the efficiency of trace element absorption in the preruminant animal. The absorptions of Cu (Suttle, 1975), Zn (Miller & Cragle, 1965) and Fe (Kay *et al.* 1978) have each been found to be high when the element had not passed through a functional rumen. Changes in the efficiency of trace element absorption will obviously disturb the relationship between age and dietary requirements for trace elements.

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