Iron metabolism in the veal calf

2.* Iron requirements and the effect of copper supplementation

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1. The iron requirements of eighteen Ayrshire bull calves reared on fat-supplemented skim milk for 14 weeks from *c.* 16 d of age have been studied. There was a highly significant relationship between dietary Fe intake (10, 40 and 100 mg/kg dry diet) and blood haemoglobin concentration, packed cell volume and plasma Fe concentration.

2. A microcytic normochromic anaemia developed in the calves given a diet containing 10 mg Fe/kg diet, but not in the other calves.

3. Plasma Fe concentrations increased to > 5 μg Fe/ml in calves receiving 100 mg Fe/kg diet but there were no effects of Fe treatment on plasma Fe-binding capacity, which rose from 4·9 to 8·4 μg Fe/ml during the experiment. Saturation of plasma transferrin was only 3% in the Fe-deficient calves.

4. There were some significant effects of Fe treatment on tissue concentrations of Fe and cytochrome *c*.

5. Dietary supplementation with 5 mg copper/kg dry diet had little effect on the growth or haematological status of the calves, although there were significant Cu × Fe interactions for mean corpuscular haemoglobin and mean corpuscular volume. There was no evidence of Cu deficiency in the calves, as measured by blood and liver Cu concentrations and by ceruloplasmin and cytochrome oxidase activities.

In a previous paper (Bremner & Dalgarno, 1973) the haematological status of veal calves maintained on fat-supplemented skim milk was described. The effects of dietary supplementation with various forms of iron were investigated and differences in Fe availability were found. As the calves were found to be marginally copper-deficient the effects of dietary Cu supplementation were briefly studied. The present experiment was designed to assess more fully the Fe and Cu requirements of veal calves for the maintenance of normal blood indices.

EXPERIMENTAL

Diets and supplements

The diet, which consisted of a spray-dried emulsion of skim milk and fat, reconstituted with deionized water at 37°, has been described earlier (Bremner & Dalgarno, 1973). The Fe and Cu contents of the ration were 10·8 and 0·47 mg/kg milk powder respectively. The concentration of solids in the reconstituted milk was increased by about 12 g/l at weekly intervals over the first 5 weeks from 118 to 165 g/l and was maintained thereafter at 177 g/l. The calves were fed twice a day, from plastic buckets. The volume of milk given per feed was increased at weekly intervals by about 0·6 l, from 2·4 to 8·4 l over the first 12 weeks and was maintained at this level thereafter.

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The Fe and Cu supplements were added to the milk in the form of aqueous solutions of $FeSO_4$ and $CuSO_4$ in the appropriate amounts.

Experimental design

The experiment was of a randomized block design and consisted of six treatments in each of three blocks of Ayrshire bull calves. The treatments, arranged in 3 × 2 factorial design, were:

Treatment	Supplement (mg/kg dry feed)	
	Fe	Cu
1	0	0
2	30	0
3	90	0
4	0	5
5	30	5
6	90	5

The calves were transferred to respiration chambers for 3 d in the course of the experiment, generally at some time during weeks 9–12, for determinations of basal metabolic rates (the results from this study will be reported in a separate publication). They were starved during this time and their food intake was restricted for a short time afterwards. All surviving calves were slaughtered after they had been on experiment for 14 weeks.

Calves

Details of the calves, which were on average 16 d old at the start of the experiment, and their care were as described previously (Bremner & Dalgarno, 1973). During the experiment three calves died of ruminal or abomasal bloat (in treatment groups 2, 3 and 4) and one of heart failure (treatment 1). In a fifth calf which died, there was dystrophic degeneration of muscle together with extensive pinpoint haemorrhage of the abomasal mucosa (treatment 1). One calf died after 8 weeks on experiment (treatment 2) and the tissues from this animal were not used for any analyses. Tissues from the other calves, which died during weeks 10–13, were used for all analyses except total Fe as, with this one exception, the results did not reveal any consistent differences from those for the calves slaughtered normally.

Analytical methods

Plasma Fe and total Fe-binding capacity (TIBC) measurements were made by the automated method of Young & Hicks (1965). Fe concentrations in freeze-dried tissues were determined on acid digests by the same method. Total non-haem Fe, ferritin and haemosiderin in liver and spleen were measured by the non-chromatographic methods of Drysdale & Ramsay (1965). Cytochrome *c* was measured by the method of Rosenthal & Drabkin (1943).

Blood and tissue Cu concentrations were determined by atomic absorption spectroscopy. Plasma ceruloplasmin was measured by the method of Houchin (1958), using

the standardization method of Rice (1962). Cytochrome oxidase activity was measured by the method of Mills & Dalgarno (1970).

Statistical analysis of results was by standard analysis of variance techniques. Both mean untransformed and log values have been listed in the tables when there was evidence of heterogeneous variability. Only the latter have been used for significance tests.

RESULTS

Live-weight gain and food conversion ratio. The effects of dietary supplementation with Fe and Cu on live-weight gain and food conversion ratio are shown in Table 1. Although no significant differences were detected between the various treatments, there was a trend for weight gains to increase with dietary Fe intake. Dietary Cu supplementation tended to decrease weight gains from an average of 1·04 to 0·93 kg/d.

Haematological measurements. The mean values of some of the blood indices at intervals during the experiment are given in Table 2. No values have been given for calves after 12 weeks on experiment because the number of surviving calves was insufficient to permit statistical analysis. There was no consistent evidence that the addition of the Cu supplement in treatments 4–6 influenced these indices and the results have been quoted solely on the basis of Fe intake. Relationships between Fe intake and both haemoglobin concentration and packed cell volumes were noted after 5 weeks ($P < 0{\cdot}05$). These persisted throughout the remainder of the experiment and their significance was greatest ($P < 0{\cdot}001$) during weeks 9–11.

One calf receiving 100 mg total Fe/kg diet and no supplementary Cu behaved abnormally throughout the experiment. It was apparently incapable of utilizing much of the dietary Fe as its blood haemoglobin concentrations fell as low as 73 g/l. There were other effects also. Post-mortem examination revealed that this animal, which died of bloat, had extensive disease of the coronary arteries; this will be discussed later. Results for this animal have not been included in any of the statistical analyses.

In the later stages of the experiment the haemoglobin concentrations and packed cell volumes were significantly lower when the calves received a total of 10 mg Fe/kg diet than when 40 mg Fe/kg diet were given, whereas increasing the Fe intake from 40 to 100 mg/kg diet did not cause such marked differences (Fig. 1). The changes in haemoglobin concentrations and packed cell volumes were greatest in the early stages of the experiment. Calves receiving 40 mg Fe/kg diet had their lowest haemoglobin concentrations after 7 weeks (97 g/l), whereas in the calves receiving 100 mg Fe/kg diet there was a transient increase at week 6 followed by a gradual fall to around 110 g/l. The average daily falls in haemoglobin concentrations over the 12-week period were 0·745, 0·217 and 0·275 g/l (approximate SE of differences between means = 0·251) for calves receiving 10, 40 and 100 mg Fe/kg diet respectively.

There were no significant effects of treatment on either red cell or white cell counts, although there was a downward trend in the former over the experimental period. Plasma Fe concentrations were dependent on Fe but not on Cu intake (Table 2). Within 4 weeks a relationship between dietary Fe concentration and plasma Fe

Table 1. *Effects of dietary iron and copper supplementation on the performance of calves*

(Mean values for three calves/treatment)

Treatment no.	Dietary concentration (mg/kg dry feed) Fe	Cu	Initial wt (kg)	Wt after 6 weeks (kg)	Wt after 12 weeks (kg)	Wt after 14 weeks (kg)	Food conversion ratio (kg dry food intake : kg wt gain) (0–12 weeks)
1	10	0·5	36·0	80·2	118*	155†	1·60*
2	40	0·5	32·7	67·0	120*	134*	1·42*
3*	100	0·5	40·7	87·3	146	168	1·36
4	10	5·5	36·2	73·7	104	119	1·76
5	40	5·5	36·8	75·8	114	135	1·72
6	100	5·5	36·5	79·2	125	146	1·51
SE of differences between means	—	—	2·6	5·6	21	32	0·23

* Based on two calves/treatment.
† Based on one surviving calf.

Table 2. *Effects of dietary iron supplementation on haematological status of calves*

(Mean values for six calves/treatment)

Week	Treatment no.	Dietary Fe concentration (mg/kg dry feed)	Haemoglobin concentration (g/l)	PCV (%)	RCC (10^{-6}/mm^3)	WCC (10^{-6}/mm^3)	TIBC (μg/ml)	Plasma Fe concentration		Transferrin saturation	
								μg/ml	$\log_{10}$(μg/ml)	%	$\log_{10}$(%)
0	1+4	10	127	40·2	10·1	12·6	5·15	1·12	0·0251	23·4	1·3286
	2+5	40	130	41·3	9·3	9·8	4·63	0·88	−0·0903	20·0	1·2580
	3+6†	100	135	42·5	10·2	11·8	5·05	1·11	0·0260	22·5	1·3237
	SE of differences between means		18·1	5·7	1·4	3·7	0·96	—	0·1602	—	0·1873
6	1+4	10	81	24·8	6·9	7·6	9·27	0·71	−0·3804	7·6	0·6536
	2+5	40	103	31·4	8·7	8·4	8·00	0·80	−0·1313	11·2	0·9786
	3+6†	100	136	35·6	7·7	8·7	7·86	4·96	0·6477	63·0	1·7546
	SE of differences between means		13·1	4·66	1·6	2·2	1·27	—	0·2174	—	0·2390
	Significance of treatment effects		***	*	—	—	—	—	***	—	***
12	1+4†	10	65	20·3	5·6	8·7	8·65	0·45	−0·6034	5·5	0·4663
	2+5†	40	107	33·1	8·0	8·5	8·45	0·78	−0·1682	9·1	0·9083
	3+6†	100	111	32·6	7·2	9·8	8·24	3·23	0·4738	39·7	1·5576
	SE of differences between means	—	14·5	4·0	0·66	3·5	1·30	—	0·2986	—	0·2933
	Significance of treatment effects	—	**	**	—	—	—	—	**	—	***

PCV, packed cell volume; RCC, erythrocyte count; WCC, leucocyte count; TIBC, total Fe-binding capacity.
* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$.
† Only five calves/treatment.

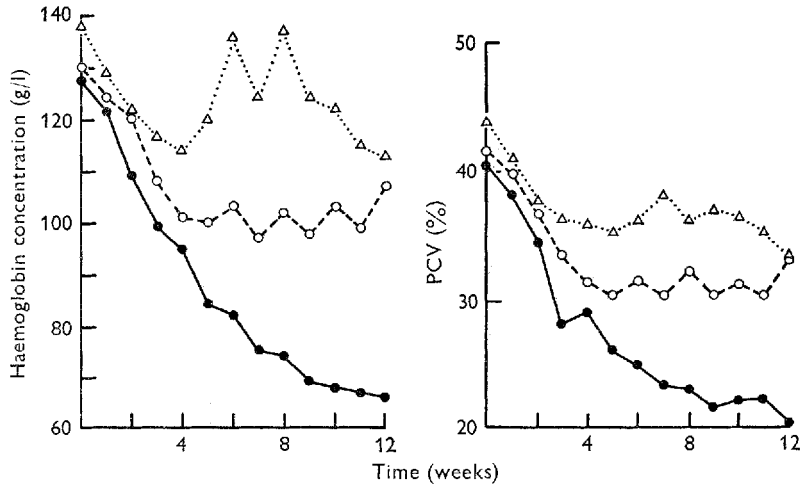

Fig. 1. Effect of iron treatment on haemoglobin concentration and packed cell volume (PCV) in calves receiving diets containing 10 (●), 40 (○) and 100 (△) mg Fe/kg.

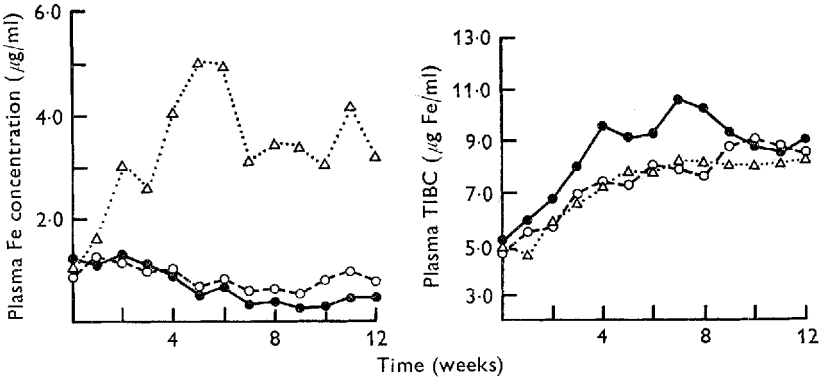

Fig. 2. Effect of iron treatment on plasma Fe concentration and total Fe-binding capacity (TIBC) in calves receiving diets containing 10 (●), 40 (○) and 100 (△) mg Fe/kg.

concentration was evident ($P < 0{\cdot}05$). This persisted throughout, but was most significant ($P < 0{\cdot}01$ and $< 0{\cdot}001$) in the middle of the experiment. From Fig. 2 it can be seen that, after a small increase over the 1st week, there was a downward trend in plasma Fe concentrations for calves receiving 10 or 40 mg Fe/kg diet, with a tendency for plasma Fe concentrations to increase in later weeks when 40 mg Fe/kg diet were given. Plasma Fe concentrations for calves given 100 mg Fe/kg diet rose over the first 5 weeks to > 5 μg/ml (for some individual calves concentrations > 6 μg/ml were recorded) but fell later to around 3·0 μg Fe/ml. It is of interest to compare these plasma Fe concentrations with those for the calf receiving 100 mg Fe/kg diet, which had disease of the coronary arteries. Initially the plasma Fe concentrations were high in this calf (2·5 μg/ml) but they fell rapidly and from week 6 onwards were about 0·25 μg Fe/ml, that is, about 8% of the concentration in other calves with the same Fe intake.

TIBC in the plasma increased from about 5 μg Fe/ml at the start of the experiment to about 9 μg Fe/ml from week 6 onwards (Table 2 and Fig. 2). There was no effect of Cu supplementation and (with the exception of week 4, $P < 0.05$) no statistically significant effect of Fe on TIBC, although there was a trend towards a higher TIBC in calves receiving 10 mg Fe/kg diet. The degree of saturation of the plasma transferrin was highly significantly dependent on Fe intake (Table 2). In calves receiving 10 and 40 mg Fe/kg diet there was a gradual decrease from 22% saturation to 3 and 6% respectively after 9 weeks. In calves receiving 100 mg Fe/kg diet, the degree of saturation increased initially to 65% (week 5) and then decreased to about 40% in subsequent weeks.

Calculation of mean cell volumes (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) revealed considerable week-to-week variations. However, when the average values of these measurements over the 9–12 week period were compared, linear relationships between Fe intake and MCV ($P < 0.001$), MCH ($P < 0.001$) and MCHC ($P < 0.01$) were found with, in addition, Cu × Fe interactions for MCV ($P < 0.05$) and MCH ($P < 0.05$) (Table 3). The anaemia of the unsupplemented calves was microcytic, especially for the calves given supplementary Cu but, conversely, mean cell volumes were greatest when maximum supplements of Fe and Cu were given. Although the hypochromicity of the cells was increased with low Fe intake, the MCHC was not sufficiently low (312 g/l) for the anaemia to be classified as hypochromic.

There were no statistically significant effects of treatment on the weights and, with the exception of liver, dry-matter (DM) contents of tissues. The DM content of liver was greatest when the diet contained 40 mg Fe/kg (Table 4). The trend in most tissues was for the Fe concentration to be related to the dietary Fe concentration but, as a result of the high mortality rate of the calves and the inclusion of results for slaughtered animals only, the statistical analysis is necessarily imprecise. In the calves given supplementary Cu, tissue Fe concentrations about doubled when the dietary Fe intake was increased from 10 to 100 mg/kg diet. There was some indication that Fe concentrations were also influenced by supplementary Cu, especially in spleen, where it caused a 40–50% reduction.

About 30% of the total Fe in the livers was present in the form of non-haem Fe, compared with about 12% in the spleen. Although no statistically significant effects were noted, there was a strong tendency for concentrations of non-haem Fe in the liver to be lower in calves given Cu (Table 5). There was a significant effect of dietary Fe on total non-haem Fe, ferritin and haemosiderin in spleen, where about 24–38% of the non-haem Fe was present as ferritin, compared with 8–16% in liver. The remainder of the non-haem Fe was mainly haemosiderin, although the fact that the sum of ferritin and haemosiderin Fe frequently exceeded the estimated total non-haem Fe suggests that the separation procedures did not always differentiate clearly between these Fe compounds.

Blood Cu and ceruloplasmin concentrations were not affected by either Fe or Cu treatment (Table 6). There was a significant decrease in blood Cu concentration with all treatments over the experimental period, from a mean of 1·28 to 1·05 μg/ml (SE =

Table 3. *Effects of dietary iron and copper supplementation on haematological status of calves*

(Mean values for three calves/treatment)

Treatment no.	Dietary concentration (mg/kg dry feed) Fe	Cu	Week 0 MCV (μm^3)	MCH (pg)	MCHC (g/l)	Week 6 MCV (μm^3)	MCH (pg)	MCHC (g/l)	Weeks 9–12 MCV (μm^3)	MCH (pg)	MCHC (g/l)
1	10	0·5	41·3	13·2	319	36·3	12·4	344	35·7	11·1	312
2	40	0·5	43·8	13·6	312	36·2	11·6	319	41·1†	13·5†	327†
3†	100	0·5	44·2	14·1	320	46·2	19·0	413	41·6	13·8	334
4	10	5·5	38·7	12·1	314	36·7	11·6	317	29·4	9·2	311
5	40	5·5	44·4	14·0	316	36·9	12·5	339	40·4	13·0	322
6	100	5·5	41·2	12·8	312	44·8	15·7	349	47·6	16·0	335
SE of differences between means	—	—	3·7	1·2	10	2·9	1·3	29	3·3	1·3	9
Significance of treatment effects Fe	—	—	—	—	—	**	***	*	***	***	**
Cu × Fe	—	—	—	—	—	—	—	—	*	*	—

MCV, mean cell volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration.
* Significant at $P < 0·05$. ** Significant at $P < 0·01$. *** Significant at $P < 0·001$.
† Based on two calves/treatment.

Table 4. *Effects of dietary iron and copper supplementation on tissue Fe concentrations of calves*

Treatment no.	Dietary concentration (mg/kg dry feed)		No. of surviving calves	Liver DM content (g/kg)	Fe concentration (μg/g DM)				
	Fe	Cu			Liver	Spleen	Kidney	Heart	Muscle
1	10	0·5	1	247	125	850	120	234	31
2	40	0·5	2	275	83·5	936	100	185	25
3	100	0·5	2	270	103	1191	171	234	41
4	10	5·5	2	266	57	433	65	133	19
5	40	5·5	3	278	88	568	121	183	22
6	100	5·5	3	270	108	699	151	227	42
SE of differences between means	—	—	—	7·2	21	279	63	23	3·7
Significance of treatment effects	—	—		Fe quadratic (**)	—	Cu (*)	—	Cu (*) Fe (*)	Cu (almost *) Fe (***) Fe quadratic(*)

DM, dry matter.
* Significant at $P < 0{\cdot}05$. ** Significant at $P < 0{\cdot}01$. *** Significant at $P < 0{\cdot}001$.

Table 5. *Effects of iron and copper supplementation on concentrations of non-haem iron in liver and spleen of calves*

(Mean values for three calves/treatment)

Treatment no.	Dietary concentration (mg/kg dry feed)		Liver				Spleen			
			Concentration (μg/g DM)			% of non-haem Fe present as ferritin	Concentration (μg/g DM)			% of non-haem Fe present as ferritin
	Fe	Cu	Non-haem Fe	Ferritin Fe	Haemosiderin Fe		Non-haem Fe	Ferritin Fe	Haemosiderin Fe	
1	10	0·5	37·8	5·0	33·1	15·7	54·2	15·4	41·0	28·2
2†	40	0·5	30·4	2·5	30·3	8·9	63·0	23·9	48·6	35·3
3†	100	0·5	48·8	6·8	42·9	13·8	212·2	51·4	177·1	24·3
4	10	5·5	22·9	1·9	20·2	8·2	50·1	16·7	42·4	34·6
5	40	5·5	23·5	2·8	18·5	11·9	52·0	19·9	50·8	38·2
6	100	5·5	28·1	4·9	22·6	16·0	122·3	34·9	109·1	29·7
SE of differences between means	—	—	12·4	2·2	13·8	5·8	64·8	15·8	58·1	6·1
Significance of Fe effect	—	—	—	—	—	—	*	*	*	—

DM, dry matter.
* Significant at $P < 0{\cdot}05$.
† Based on two calves/treatment.

Table 6. *Effect of dietary iron and copper supplementation on Cu status of calves*

(Mean values for three calves/treatment)

Treatment no.	Dietary concentration (mg/kg dry feed) Fe	Cu	Blood Cu† (μg/ml)	Cerulo-plasmin† (iu/ml)	Liver Cu (μg/g DM)	Liver Cu $\log_{10}$ (μg/g DM)	Spleen Cu (μg/g DM)	Kidney Cu (μg/g DM)	Heart Cu (μg/g DM)	Muscle Cu (μg/g DM)
1	10	0·5	1·17	18·8	68·5	1·8137	5·2	21·0	12·9	2·57
2‡	40	0·5	1·04	15·4	54·4	1·5762	4·0	21·0	17·8	2·68
3‡	100	0·5	1·00	28·8	63·8	1·8914	3·7	22·8	17·6	2·59
4	10	5·5	1·07	19·2	562	2·7369	5·9	20·3	17·1	3·18
5	40	5·5	1·09	28·6	556	2·7440	4·4	21·3	17·8	2·17
6	100	5·5	0·99	20·2	586	2·7666	4·7	20·7	19·4	2·85
SE of differences between means	—	—	0·14	7·5	—	0·1026	0·6	2·5	2·1	0·32
Significance of treatment effects	—	—	—	—	—	Cu (***)	—	—	—	Cu × Fe quadratic (**)
						Fe (*)			Fe (*)	Fe quadratic (*)

DM, dry matter.
* Significant at $P < 0·05$.
** Significant at $P < 0·01$.
† At 12 weeks.
‡ Based on two calves/treatment.

Table 7. *Effect of dietary iron and copper supplementation on tissue cytochrome* c *concentrations and cytochrome oxidase activities in calves*

(Mean values for three calves/treatment)

Treatment no.	Dietary concentration (mg/kg dry feed)		Cytochrome *c* concentration (μg/g wet weight)		Cytochrome oxidase activity (μmol cytochrome *c* oxidized/min per g wet wt)	
	Fe	Cu	Heart	Kidney	Heart	Liver
1	10	0·5	123	23·7	49·2	7·1
2†	40	0·5	142	29·8	35·4	7·6
3†	100	0·5	178	28·6	44·5	7·8
4	10	5·5	99	25·5	41·1	8·6
5	40	5·5	122	30·2	30·8	7·6
6	100	5·5	185	31·4	35·8	7·6
SE of differences between means	—	—	26	6·1	9·5	2·2
Significance of treatment effects	—	—	Fe (**)	—	—	—

** Significant at $P < 0.01$.
† Based on two calves/treatment.

0·055). In only one instance was the blood Cu concentration < 0·8 μg/ml. The main effect of the supplementary Cu was to increase liver Cu concentrations to > 550 μg/g DM (Table 6). These tended to be also dependent on Fe intake in calves given no Cu supplement, with lowest mean Cu concentrations occurring when the total dietary Fe concentration was 40 mg/kg. There were no significant effects of dietary Cu on Cu concentrations in kidney, heart or spleen. In spleen the Cu concentration decreased as Fe intake increased ($P < 0·05$), whereas in heart they were directly related ($P < 0·05$).

The activity of cytochrome oxidase in both liver and heart was determined in order to obtain more information on the relative Cu status of the calves. There were no significant effects of either Cu or Fe treatment (Table 7). Concentrations of cytochrome *c* in heart were significantly dependent on Fe intake ($P < 0·01$) but in kidney, although a similar trend was evident, the differences were not significant.

DISCUSSION

Eeckhout, Casteels & Buysse (1969) have reported that the performance of veal calves is related to their dietary Fe intake. The failure to observe significant treatment effects in either this or the previous experiment (Bremner & Dalgarno, 1973) can perhaps be attributed to the large variations in the Fe status of individual calves at birth and the small number of animals in each replicate. However, there was a tendency for Fe supplementation to improve, and Cu supplementation to reduce, weight gain and food conversion ratio. The inferior performance of the calves in this, compared with the previous, experiment was partly a consequence of the starvation period involved in the determination of basal metabolic rate. Average daily weight gains over the first 12 weeks were 1·01 kg/d compared with 1·14 kg/d.

A dietary intake of 100 mg Fe/kg was found to be insufficient to prevent a decrease in blood haemoglobin concentrations and in packed cell volumes during the experiment. The final haemoglobin concentrations were, however, similar to those reported for adult cattle (110–120 g/l; Underwood, 1971). There was little difference between the final haemoglobin concentrations attained when the dietary Fe concentration was 40 or 100 mg/kg, although the initial decrease in haemoglobin concentration was greater with the diet containing 40 mg/kg. At no time were the mean haemoglobin concentrations in these calves indicative of a state of anaemia. The calves receiving 10 mg Fe/kg diet became progressively more anaemic as the experiment continued, although the average rate of decrease in haemoglobin concentration (0·74 g/l per d) was less than that reported previously (0·82 g/l per d) (Bremner & Dalgarno, 1973). In the calves given 40 mg/kg diet, the rates of decrease were 0·22 and 0·30 g haemoglobin/l per d in this and the previous experiment respectively. It seems probable that these differences between the two experiments reflect the decreased growth rates and also the greater initial haemoglobin concentrations in the present experiment (130 g/l) compared with the previous one (108 g/l).

The changes in packed cell volume were generally similar to those in haemoglobin concentration, and consequently there was little change in MCHC throughout the experiment. The occurrence of a relationship between MCV and dietary Fe intake

agrees with the known microcytic character of Fe-deficiency anaemia in calves (Blaxter, Sharman & MacDonald, 1957). It is not clear how important is the somewhat inconsistent Cu × Fe interaction in which a low Cu intake resulted in the cell volumes being greater than normal when the Fe intake was low and smaller when Fe intake was high. This conflicts with the report that Cu deficiency in cattle causes a macrocytic anaemia (Cunningham, 1946).

The plasma Fe concentrations recorded when milk containing 100 mg Fe/kg was given to the calves were remarkably high (being > 4 μg/ml at weeks 4–6) compared with the concentrations of around 1·4 μg/ml reported in Australia for milk-fed calves of similar age which had access to dry feed with an Fe content of 300–450 mg/kg (K. C. Bremner, 1966). These higher concentrations may reflect the greater availability of Fe when it is in soluble form, although that does not explain why they were not attained until some weeks after the start of the experiment. It is of interest that, unlike the situation normally encountered in Fe-deficiency anaemia, there was no effect of Fe supplementation on TIBC. The persistence of TIBC values from week 8 onwards of > 8 μg Fe/ml again conflicts with the findings of K. C. Bremner (1966), who reported values of only 4·1 μg Fe/ml in 3-month-old calves, although there was a transient increase to 6·5–8·4 μg Fe/ml in the 1st month after birth. It has been suggested that TIBC is regulated by tissue oxygen balance and is related to blood haemoglobin concentration (Morgan, 1962) but no such relationship was observed here.

The only tissues in which there was a significant dependence of Fe concentration on dietary Fe treatment were muscle, heart and (non-haem Fe only) spleen. Concentrations of Fe in most organs, and especially in spleen, were higher than those reported previously (Bremner & Dalgarno, 1973). The concentrations of non-haem Fe in liver were similar to those recorded previously for veal calves (Charpentier, 1966) but much less than those recorded by K. C. Bremner & Ronalds (1965) for conventionally reared calves of similar age. Their suggestion that concentrations of non-haem Fe < 20 μg/g fresh liver (i.e. < 70–80 μg/g DM) are indicative of Fe deficiency is not supported by our results. It may be, however, that there are differences in Fe metabolism between milk-fed and conventionally reared calves. The distribution of non-haem Fe between ferritin and haemosiderin in liver and spleen was similar to that found in young rats before weaning (Leslie & Kaldor, 1971).

In addition to the development of anaemia, Fe deficiency has been shown to cause several, well-defined tissue changes in rats (Beutler, 1964) and pigs (Gubler, Cartwright & Wintrobe, 1957) but not so far in calves. Dallman (1969) has demonstrated that the reduction in tissue concentrations of haem protein in rats is an early manifestation of Fe depletion, especially during the early period of rapid growth. It is of interest, therefore, that in this experiment cytochrome *c* concentrations in heart were dependent on Fe intake, as were myoglobin concentrations in skeletal muscle (MacDougall, Bremner and Dalgarno, to be published). Furthermore, the differences in tissue concentrations of haem protein between animals receiving 40 and 100 mg Fe/kg diet were greater than the differences in final blood haemoglobin concentrations, although these differences in haemoglobin concentration were not so small in the earlier stages of the experiment.

Dietary supplementation with Cu tended to reduce weight gains and had a significant effect on spleen Fe concentrations. The reduction in liver non-haem Fe, although not statistically significant, was similar to a decrease of 34% reported in the previous experiment (Bremner & Dalgarno, 1973) and attributed to an increase in ceruloplasmin and therefore ferroxidase activity. However, ceruloplasmin concentrations were unaffected by Cu treatment in this experiment and it must be presumed that the concentrations of Fe on Cu treatment were reduced by some other mechanism.

Liver Cu reserves in cattle diminish during the grazing season and are replenished when the cattle are on winter rations (Hartmans & Bosman, 1970) and foetal Cu reserves in other ruminants are dependent on maternal Cu status (Suttle & Field, 1968). The calves in the previous experiment were born in the autumn and might therefore have had smaller Cu reserves than those in this experiment which were born in the spring. It is noteworthy, therefore, that the Cu status of all but one of the calves in the present experiment was satisfactory, whereas in the previous experiment (Bremner & Dalgarno, 1973) there was in many instances evidence of Cu deficiency. Liver Cu concentrations in calves given no supplementary Cu in this experiment averaged 60 μg/g DM, compared with 25 μg/g DM in the previous experiment. In neither experiment were blood Cu and ceruloplasmin concentrations or cytochrome oxidase activities abnormally low.

The high liver Cu concentrations ($>$ 550 μg/g DM) in the supplemented calves again illustrate the extremely high affinity of that organ for Cu in young calves. Over 50% of the dietary Cu was retained in the liver, which is much greater than Cu retention reported in other species. C. F. Mills (personal communication) has recorded liver retention of between only 3 and 7% in lambs maintained on different rations. It may be that the high absorption of Cu is related to the milk-feeding of the calves.

Sourkes, Lloyd & Birnbaum (1968) reported that there was an inverse relationship between dietary Fe intake and liver Cu concentration in rats, and Marston, Allen & Swaby (1971) found the same relationship in heart when the diet contained adequate Cu although, in one experiment only, there was a direct relationship when the diet was Cu-deficient. Different relationships between Fe intake and Cu concentrations were found in this experiment in liver (Cu $\times$ Fe quadratic), heart (direct), spleen (inverse) and muscle (Cu $\times$ Fe quadratic). These cannot be explained at present but similar trends in spleen were observed previously for comparable dietary treatments.

A common sign of Cu deficiency in avian species is the occurrence of aortic rupture, which results from a failure of elastin formation associated with reduction in the activity of the Cu-enzyme amine oxidase (Bird, Savage & O'Dell, 1966). It is of some interest, therefore, that the only calf which showed signs of Cu deficiency also showed signs of severe arteritis of larger coronary vessels of the heart, with thickening of endothelium and fragmentation of elastic membranes. There is no direct evidence, however, that these changes were connected with the animal's Cu-deficient state, and it may be relevant that there were disturbances of Fe metabolism also.

In conclusion, it has been confirmed that a microcytic normochromic anaemia develops in Ayrshire veal calves given no supplementary Fe. A diet containing 40 mg

Fe/kg prevented the anaemia but did not eliminate the initial reduction in blood haemoglobin concentrations. An intake of 100 mg/kg diet had little further effect on the haematological status of the calves, especially when they were near to slaughter weight, although there were effects on plasma Fe, heart cytochrome *c* and muscle Fe concentrations. Although there was some Cu × Fe interaction, this did not seriously affect the haematological status of the calves. It seems that the Fe and Cu reserves of the calf at birth may be of some importance in determining its requirement for these elements when it is reared for veal production.

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