IRON-INDUCED HYPOCUPROSIS

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SUMMARY

Eight yearling cattle were dosed regularly for seven months with 30 mg Fe/kg liveweight/day as ferric hydroxide (Phase 1) and then for a further four months with the same amount of iron as ferrous carbonate (Phase 2). The cattle were grazed together as one herd with eight matched control animals on pasture of adequate copper and low molybdenum status. In Phase 1 liver and blood copper, caeruloplasmin and amine oxidase levels were all very markedly depressed by the iron treatment. In Phase 2 there was a slight recovery in liver copper and a greater recovery in blood copper in the treated animals. These increases were associated with a lower level of iron in the pastures being grazed. The iron treatments did not affect liveweight gain or general health despite a reduction in liver copper to 7 ppm and blood copper to 0.33 mg/l in the treated animals at the end of Phase 1.

INTRODUCTION

A higher-than-normal level of iron, either in the soils, the pasture plants, or the animals themselves is frequently associated with hypocuprosis in grazing stock. Thus greensands (hydrated silicates of potassium and iron), ferruginous sandstones and coastal shell sands (Bennetts, 1935; Bennetts and Beck, 1942) containing up to 5 000 ppm Fe (Underwood, 1971) have all been associated with hypocuprosis. Innes and Shearer (1940) recorded unusually high iron levels (*ca.* 1200 ppm) in pasture in areas of Derbyshire where swayback was prevalent. Hartley *et al.* (1959), reported pasture iron values of up to 1800 ppm and, concurrently, liver copper values as low as 6.5 ppm.

A high tissue iron level in animals suffering from one or other of the syndromes preventable by copper therapy is frequently recorded, and is sometimes sufficiently extensive and severe to be diagnosed as haemosiderosis (Sjollema, 1938; Bennetts *et al.*, 1941; Bennetts and Beck, 1942; Blakemore and Venn, 1950; Schulz *et al.*, 1951; Hofstra, 1953).

The explanation given for elevated tissue iron in such cases is that copper deficiency prevents the mobilization of iron from its repository sites in spleen, liver and duodenal mucosa (see Sourkes,

1970) so that it tends to accumulate. The possibility that an elevated iron intake may be responsible for copper depletion has not been widely recognized.

Very high daily iron intakes (*ca.* 190 mg Fe/kg liveweight) by dairy cows from pasture irrigated with bore water containing 17 ppm Fe resulted in hypocuprosis-like symptoms—scouring, loss of weight and coat condition, and production decline (Coup and Campbell, 1964). These symptoms were rapid in onset when iron intake increased and it seemed unlikely that copper depletion could have taken place. However, Dick (1954a) produced "steely wool" in sheep on a high molybdenum and sulphate diet within seven days of applying the treatment, although liver and blood copper levels had not by then been depleted. He suggested that a physiological deficiency of copper may have been induced.

Other evidence for the implication of iron in hypocuprosis comes from the work of Dick (1954b) who fed ferrous sulphide extensively (but not exclusively) in trials to confirm the effect of inorganic sulphate in reducing liver copper storage. Abdellatif (1968) has also recorded reduced liver copper accumulation in copper-supplemented sheep fed iron salts, but without apparent alteration in blood copper levels.

The objective of the work reported here was to study the long-term effects of a moderately elevated iron intake upon tissue copper in otherwise normal grazing stock.

EXPERIMENTAL

Sixteen Jersey and Friesian × Jersey 6- to 8-month-old weaners were divided into two groups of 8, by restricted randomization. The groups were balanced for liver copper, liveweight and breed. For the first seven months of the experiment (Phase 1) one group was dosed daily with 30 mg Fe/kg liveweight as ferric hydroxide, prepared by the method of Coup and Campbell (1964). The control group was dosed with a similar volume of tap water. Sunday drenching of the stock was avoided by giving a total weekly dose of iron to each animal in six equal daily aliquots. Pasture samples were collected weekly for copper, molybdenum, iron, sulphate-sulphur and crude protein determination. Blood samples were taken fortnightly for copper, haemoglobin and packed cell volume. Towards the end of the first phase of the trial, additional blood samples were taken for the estimation of caeruloplasmin and plasma amine oxidase. Liver biopsy samples were taken monthly for copper and iron determinations. On six occasions, pasture DM intake by the stock was estimated from the difference between pre-

and post-grazing pasture samples, and from these intakes the dosed iron was estimated to be equivalent to an additional 1400 ppm in the feed DM.

The second phase of the experiment was consecutive with the first and ran from November 29, 1973 to March 27, 1974. The major difference from Phase 1 was that laboratory grade ferrous carbonate (saccharated) replaced ferric hydroxide as the source of iron. Pasture sampling continued regularly throughout this phase, but blood and liver samples were only taken immediately prior to slaughter and *post mortem* on March 27 and 28, 1974. Details of the analytical procedures can be found elsewhere (Campbell *et al.*, 1974).

RESULTS

Pasture Constituents

Table 1 shows average values and standard errors for the pasture constituents. Copper was adequate throughout; molybdenum was low. Sulphate-sulphur levels were not high and crude protein was within normal limits for ryegrass and white clover pastures. Iron levels were extremely variable in Phase 1. They ranged from 111 to 3850 ppm, with the highest values being found in winter, presumably due to soil contamination. Pasture iron was much lower and less variable during Phase 2. It ranged from 54 to 218 ppm.

TABLE 1: PASTURE ANALYSES

	Phase 1 (n = 23)		*Phase 2 (n = 6)*	
	Mean	*±S.E.*	*Mean*	*±S.E.*
Copper (ppm DM)	10.7	0.32	10.9	0.81
Molybdenum (ppm DM)	1.5	0.12	0.93	0.23
Iron (ppm DM)	581.0	163.00	128.0	24.04
SO_4-sulphur (% DM)	0.126	0.007	0.275	0.088
Crude protein (% DM)	21.4	0.72	17.4	0.86

Liver Copper

Figure 1 shows changes in liver copper with time, and average levels of pasture iron between the liver biopsy dates.

Liver copper values declined in both groups, but more swiftly in the iron-treated animals. The difference which opened up between the groups in the first month continued and was increased

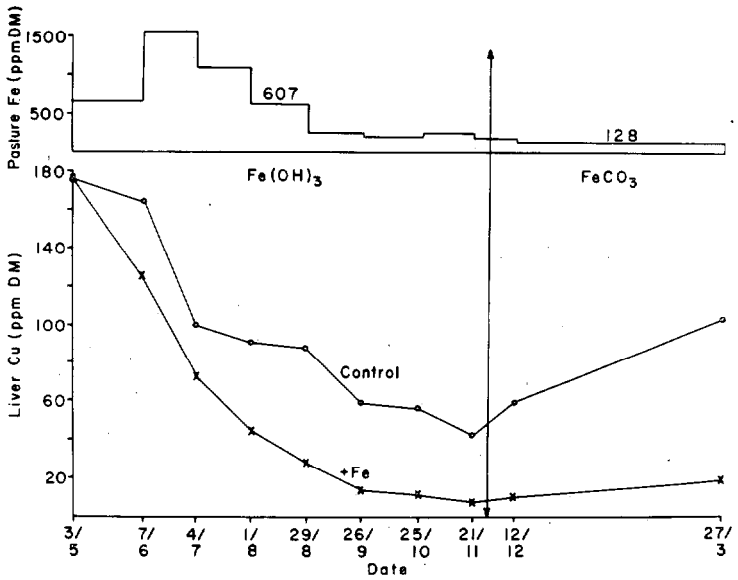

FIG. 1: *Mean liver copper values (ppm DM), at biopsy dates and final slaughter, of iron-dosed and control yearlings. Mean pasture iron values (ppm DM) between biopsy dates. (All differences in liver copper values were significant, or highly significant except that on 4/7/73.)*

with time. The treated group eventually reached 7 ppm of Cu in the liver compared with a minimum of 42 ppm attained by the controls. It is noteworthy that the most rapid fall in liver copper in the control animals was associated with the highest levels of pasture iron, during the second month. That the treated animals did not decline more rapidly in this month may well be due to the operation of those physiological mechanisms which are known to protect animals against iron overload (Kolb, 1963).

The correlation of mean pasture iron level between biopsy dates with the corresponding average daily change in liver copper in the control animals was significant ($r = -0.75$, $P < 0.05$), but it may be unwise to place too strict a causal construction upon this statistic.

The rapid rise of liver copper in the control animals in Phase 2 cannot easily be attributed to the 0.2 ppm increase in pasture copper or the 0.6 ppm decline in average pasture molybdenum, but it was associated with low levels of pasture iron. The much slower rate of liver copper accumulation in the treated than in

the control animals in Phase 2 suggests that the dosing with $FeCO_3$ was powerfully depressing accumulation. Whether the fact that these iron-treated animals increased slightly in mean liver copper value can be attributed to a lesser effectiveness of iron as $FeCO_3$ than as $Fe(OH)_3$ or to the lower total iron intake remains uncertain.

Blood Copper

Blood copper changes, illustrated in Fig. 2, show a pattern rather different from the changes in liver copper. There was no early, dramatic decline in the values for the treated animals. Only when mean liver copper had fallen to about 40 ppm (a value not attained by the control group) did blood copper start to decline seriously. During Phase 2, blood copper rose more rapidly in treated than in control stock, but the difference between them remained significant at slaughter. The blood copper in the control animals would be unlikely to rise beyond a normal physiological maximum on feed containing about 11 ppm copper.

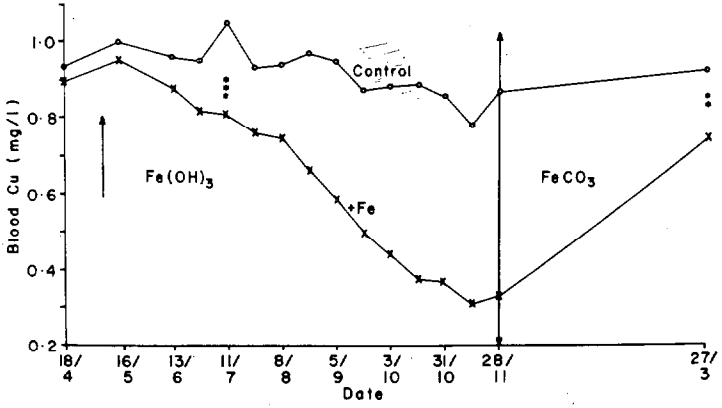

FIG. 2: *Mean blood copper values (mg/l) in iron-dosed and control yearlings. (All differences were significant or highly significant from 11/7/73 to 27/3/74.)*

Enzyme Changes

The copper-containing enzymes caeruloplasmin (the source of physiologically active copper) and plasma amine oxidase (necessary for elastin formation) were estimated on two occasions

towards the end of Phase 1 and again at the beginning and end of Phase 2. Relevant values are set out in Table 2. Both enzymes were very markedly depressed by the iron treatment at the end of Phase 1 but had substantially recovered at slaughter, in line with the general improvement in blood copper values at this time.

TABLE 2: ENZYME LEVELS IN Fe-DOSED AND CONTROL STOCK
(Arbitrary optical density units)

Date	*Caeruloplasmin*			*Amine Oxidase*		
	Fe-Dosed	*Control*	*Sig. of Diff.*	*Fe-Dosed*	*Control*	*Sig. of Diff.*
[$Fe(OH)_3$]						
14/11	0.40	3.82	***[1]	2.50	7.43	***
28/11	1.71	4.23	*[1]	3.53	6.51	***
[$FeCO_3$]						
13/12	0.37	3.30	**[1]	4.03	6.31	***
27/3	2.86	4.79	†	6.65	7.96	n.s.

† = $P < 0.01$.
[1]Analysed by Kolimogorov-Smirnov test because of high proportion of zeros in data from Fe-dosed animals.

Liver Iron

Liver iron values were very variable. Those for the control group averaged about 380 ppm and after one month's dosing the iron-treated group attained a plateau some 125 to 200 ppm higher, a difference which was significant at most samplings.

Haematology, Liveweight and Health

Packed cell volume and haemoglobin values were indistinguishable between groups throughout, and liveweight gains averaged 0.5 kg per day for both groups, nor was there any evidence of clinical disease in the dosed stock.

At the end of Phase 2, analyses were made of liver zinc and manganese, blood zinc, selenium and plasma phosphorus, and spleen copper, zinc, manganese and iron. Only spleen iron differed ($P < 0.001$) between groups, being higher in the iron-treated group.

DISCUSSION

Many researchers investigating the roles and interactions of molybdenum and sulphate in conditioned hypocuprosis have postulated the operation of some as yet unknown factor or factors.

Clearly, elevated iron intake may be one such very potent factor.

Three major sources of elevated iron intake can be identified, excluding the special case of pasture contamination from industrial activity.

(1) High levels of iron are found in ground waters in some areas and stock water drawn from bores and wells may be seriously contaminated. The highest level recorded locally is 370 ppm from a bore in Northland (C. P. Croft, pers. comm.). But iron-contaminated drinking water is probably only a small contributor to hypocuprosis. For example, a 360 kg dairy cow drinking 32 l of water per day, containing 50 ppm Fe, would ingest only 1.6 g Fe from this source, which is only about 4.5 mg Fe/kg liveweight.

(2) Because of the reduction of ferric iron to the ferrous state in waterlogged soils (Islam and Elahi, 1954), pasture plants growing in such situations may take up excessive iron within their tissues (Jones, 1972). A very positive relationship between soil waterlogging and hypocuprosis has been documented by Hartmans (1962) and although this author now considers (pers. comm.) that the association depends upon the level of inorganic sulphate in lush, high protein pasture growing in these situations, the iron status of these pastures has not been examined. A more general association of hypocuprosis with waterlogging can be observed locally; sometimes, as in the Hauraki Plains, combined with low pasture copper, but also, as in the Ruawai area, where pasture copper is adequate.

(3) Healy (1967) has highlighted the extent of soil intake by stock, especially when grazing pressures are high. He has also observed qualitative differences between soil types in the extractability of their mineral elements by ruminal liquor (Healy, 1970). Of interest to this discussion is his finding that the iron in Hauraki clay loam is more soluble in rumen liquor than the iron in any of the other soils studied, and it retained its solubility in duodenal liquor—at the major site of iron absorption—much better than the iron of other soils.

It is from the pasture, and soil contamination of pasture that the major intake of excess iron probably arises. In the present experiment a pasture iron level of 3850 ppm was recorded, and the Plant Tissue Analysis Laboratory of N.Z. Ministry of Agriculture and Fisheries discards as probably being soil contaminated

many samples having more than 1000 ppm Fe. A further source of contamination arises when pastures are flooded by iron-charged ground water. A. C. Harkness (pers. comm.) has recorded 3600 ppm Fe on clover leaves bearing a brownish deposit following inundation.

The absence of clinical effects in the present work supports the belief of Poole and Walshe (1970) and Smith and Coup (1973) that what are currently considered to be deficient levels may be sufficient, at least in the short term. But at lower levels of pasture copper clinical disease may be manifested.

The clear incrimination of iron as yet another potential factor in hypocuprosis may help to resolve some of the conflicts among the experimental evidence (Allcroft, 1963), but will doubtless introduce new complexities. Furthermore, this work shows that iron is an element with a wider potential for causing detrimental biochemical effects than was previously realized—a factor of unsuspected importance in the soil-pasture-animal grazing complex.

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