Br. J. Nutr. (1983), **49**, 77

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

The influence of dietary iron and molybdenum on copper metabolism in calves

BY W. R. HUMPHRIES, M. PHILLIPPO, B. W. YOUNG AND I. BREMNER
Rowett Research Institute, Bucksburn, Aberdeen AB2 9SB, Scotland

(*Received 22 March 1982 – Accepted 8 July 1982*)

1. Twenty heifer calves were allocated to four groups and maintained for 32 weeks on a diet based mainly on barley and straw and containing 4 mg copper/kg. The diet was supplemented with 0 or 800 mg iron/kg and 0 or 5 mg molybdenum/kg.

2. Liver and plasma Cu concentrations, erythrocyte superoxide dismutase (*EC* 1.15.1.1) and plasma caeruloplasmin (*EC* 1.16.3.1) activities decreased greatly and rapidly in all calves given the Fe or Mo supplements or both. Levels indicative of severe Cu deficiency were attained within 16 weeks. There were no significant differences in values in animals given Fe, Mo or Fe plus Mo.

3. Clinical signs of Cu deficiency developed after 20 weeks in the calves given the Mo supplement. Growth rates were reduced, skeletal lesions developed and hair texture and colour were affected. No such effects were observed in the calves given only the Fe supplement.

4. Plasma and liver Fe concentrations increased in calves given the Fe supplement but were not greatly affected by Mo, even when the calves were severely Cu-deficient.

5. The significance of the effects of Fe and Mo on Cu metabolism are discussed with special regard to the influence of soil ingestion on Cu availability and to the frequent lack of correlation between the Cu status of animals and their clinical condition.

The development of copper deficiency in ruminant animals can usually be attributed to the presence of dietary factors which reduce the availability of Cu (see Bremner & Davies, 1980). Molybdenum and sulphur are probably the most important of these but metals such as zinc and iron can also affect Cu utilization, at least under experimental conditions. Little is known, however, of the quantitative aspects of the interactions between Cu and its dietary antagonists and their practical significance is often a matter of conjecture. A further complication is that there is some doubt as to whether the clinical lesions which develop in conditioned Cu deficiencies are necessarily identical to those found in simple Cu deficiency.

For Fe, the poor performance of dairy cattle in some areas of New Zealand was attributed to hypocuprosis induced by the high Fe content of water supplies (Coup & Campbell, 1964; Campbell *et al.* 1974). This view was supported by the reduced liver Cu concentrations in cattle and sheep given diets with 1500–3200 mg Fe/kg (Standish *et al.* 1969, 1971; Campbell *et al.* 1974; Grun *et al.* 1978). However, associated increases in S intake and reductions in food intake may have contributed to some of these results. Perhaps because of this, and the excessive amounts of Fe used, little attention has been paid subsequently to the importance of Fe in the occurrence of Cu deficiency under field conditions.

Our interest in the effects of Fe on Cu metabolism was stimulated by the findings that silages in North-east Scotland often contain 2000–4000 mg Fe/kg dry matter (DM) and that cattle given these silages frequently become hypocupraemic. Even though much of this Fe is derived from soil contamination of the silage, the work of Healy (1972) indicates that a significant proportion of the Fe would be released within the alimentary tract.

The aim of this investigation was, therefore, to determine the effect of Fe, at a level thought to be similar to that of the 'available' Fe in silage, on the Cu status of calves and to compare any clinical lesions which developed with those induced by Mo. A secondary

aim was to establish whether high dietary Fe synergized the adverse effect of Mo on Cu utilization by enhancing the excessive hepatic accumulation of Fe often noted in Cu-deficient animals and precipitating the onset of liver dysfunction and growth failure (Mills, 1980). The results show that Fe greatly reduced the retention of Cu but, in contrast to animals given Mo, did not induce clinical signs of Cu deficiency. There was no major synergistic action of Mo and Fe on any aspect of Cu metabolism.

A preliminary report of the results has been published (Humphries *et al.* 1981).

EXPERIMENTAL

Animals and diets

Sixteen Hereford–Friesian and four Aberdeen Angus heifers, aged approximately 3 weeks, were maintained for 1–3 months on a commercial fat-supplemented milk substitute ration containing < 1 mg Cu/kg DM (Volac Ltd, Wendy, Royston, Herts.) to deplete their liver Cu reserves. The calves were then weaned onto a ration based on ground barley and barley straw (Table 1) containing 4 mg Cu, 0·1 mg Mo, 100 mg Fe and 2·8 g S/kg DM. This diet was offered for 3 months, until liver Cu concentrations were reduced to approximately 100 mg/kg DM. Calves were then allocated in a randomized block design on the basis of breed and liver Cu to four treatment groups (Con, Fe, Mo and FeMo). These were given *ad lib.* the diet described in Table 1 but with the addition of saccharated ferrous carbonate or ammonium molybdate (BDH Chemicals, Poole, Dorset) or both to provide an additional 0 or 800 mg Fe/kg and 0 or 5 mg Mo/kg. The animals were kept in individual pens in a 'trace-element-free' environment and were given deionized water to drink. The experiment was terminated after 32 weeks. Animals were weighed and food intakes recorded weekly. Blood and liver biopsy samples were collected at 2- and 4-week intervals respectively.

Analytical methods

Cu and Zn concentrations were measured by atomic absorption spectroscopy after digestion of solid samples with nitric acid:perchloric acid:sulphuric acid (4:1:0·5, by vol.) or after precipitation of blood or plasma proteins with 3 vols trichloroacetic acid (100 g/l). Plasma Fe and unsaturated Fe-binding capacity were measured with ferrozine as described by Ruutu (1975). Tissue Fe was measured by the method of Kennedy (1927). Mo in plasma and in diets was determined by the methods of Quin & Woods (1979) and Bingley (1959) respectively. Dietary S was measured using X-ray fluorescence spectrometry.

Plasma caeruloplasmin (*EC* 1.16.3.1) activities were measured by the method of Smith & Wright (1974). Superoxide dismutase (SOD) (*EC* 1.15.1.1) activities were determined by the method of Beauchamp & Fridovich (1971), using bovine erythrocyte SOD as standard (Miles Laboratories, Slough).

Statistical analysis of results was carried out by analysis of variance, unless otherwise stated.

RESULTS

Growth and clinical condition of calves

None of the dietary treatments affected the growth of the calves until weeks 20–24, when significant reductions ($P < 0·05$) in the weight of both groups of Mo-treated calves (Mo and FeMo) became evident (Table 2, Fig. 1). This inhibitory effect of Mo on growth was even more pronounced during the last 8 weeks of the experiment, when the weight gain of the Mo-treated calves was only 72% of that in the calves in groups Con and Fe. Dietary supplementation with Fe did not influence growth at either level of Mo intake.

The inhibitory effect of Mo on growth during weeks 24–32 could be partly attributed to reductions in food intake, since this was only 87% of that in the calves given no Mo

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

Table 1. *Composition of diet*† *(g/kg)*

Ground barley	678·4	$KHCO_3$	10·24
Ground barley straw	200	$CaHPO_4.2H_2O$	9·7
White fish-meal	50	Na_2SO_4	5·83
Molasses	20	$CaCO_3$	4·25
Urea	10	NaCl	0·99
Arachis oil	10	$MgSO_4.7H_2O$	0·51

† The diet was also supplemented with: $CoSO_4$ and KI to provide 0·1 mg cobalt and 0·8 mg iodine/kg diet, retinol (1·5 mg/kg), cholecalciferol (25 μg/kg) and α-tocopherol (20 mg/kg).

Table 2. *Effect of iron and molybdenum on growth, food intake and bone conformation of calves*

Treatment group ...	Con	Fe	Mo	FeMo	SE of	Significance of treatment effects		
Mo supplement (mg/kg) ...	0	0	5	5	difference			
Fe supplement (mg/kg) ...	0	800	0	800	between means	Fe	Mo	Fe × Mo
Wt. (kg) at week								
0	144	142	130	125	10	NS	NS	NS
24	266	289	252	259	20	NS	*	NS
32	342	376	316	310	23	NS	*	NS
Wt. gain (kg/d) during weeks 24–32	0·84	0·97	0·70	0·61	0·09	NS	**	NS
Food intake (kg/d) during weeks 24–32	7·1	7·8	7·0	6·0	0·5	NS	*	*
Food conversion ratio during weeks 24–32 (kg intake/kg wt gain)	8·9	8·1	10·0	10·5	1·0	NS	*	NS
End-width (mm) of metacarpal†	72·4	74·8	78·4	77·6	1·83	NS	*	
End-width (mm) of metatarsal†	71·4	74·8	75·0	76·1	1·42	*	*	

NS, not significant.
* $P < 0.05$, ** $P < 0.01$.
† End-widths adjusted to shaft widths of 47 and 42 mm for metacarpals and metatarsals respectively, assuming that there is an increase of 1·4 mm in end-width per unit increase in shaft width.

(Table 2). However food conversion efficiencies were also affected by Mo, as the calves in groups Mo and FeMo consumed 10·3 kg food/kg live-weight gain during weeks 24–32 compared with 8·5 kg in the other groups (Table 2). Dietary supplementation with Fe had no effect on food conversion efficiency although it did slightly reduce food intakes during weeks 24–32 by the calves given the Mo-containing diets.

The clinical appearance of the calves was also adversely influenced by Mo, whereas treatment with Fe had only minor effects. Calves in groups Mo and FeMo showed loss of hair pigment and changes in hair texture after approximately 20 weeks. Skeletal changes also became evident in these calves at this time. An index of these changes was obtained in the Hereford–Friesian calves by measurement of the end and shaft widths of the metacarpals and metatarsals after 32 weeks. There were significant increases in end widths in both bones in the Mo-treated calves ($P < 0.05$) but only in the metacarpals in the Fe-treated calves (Table 2). The Mo-treated animals also developed the 'stilted gait' characteristic of Cu deficiency and had difficulty in walking. These symptoms resembled those described by Mills *et al.* (1976) and Smith *et al.* (1975).

None of the calves developed diarrhoea during the experiment.

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

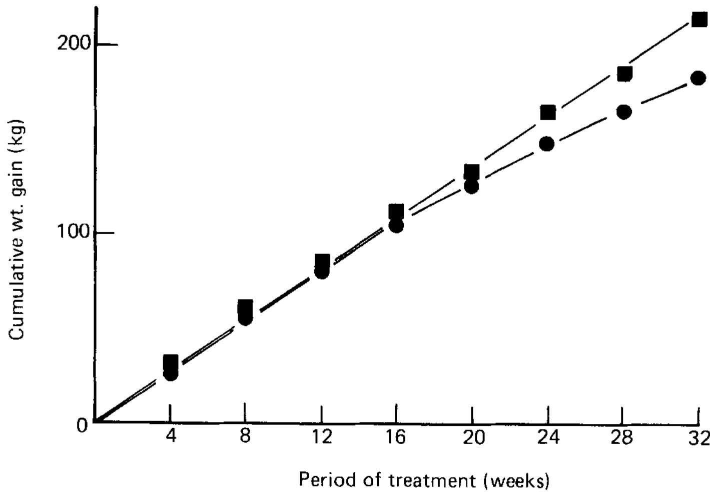

Fig. 1. Cumulative weight gain in calves given diets with no molybdenum (groups Con and Fe, ■) or with molybdenum (groups Mo and FeMo, ●).

Table 3. *Effect of iron and molybdenum on liver copper and superoxide dismutase (EC 1.15.1.1, SOD) activities in calves*

Treatment group ...		Con	Fe	Mo	FeMo		Significance of treatment effects		
Mo supplement (mg/kg) ...		0	0	5	5	SE of difference between means			
Fe supplement (mg/kg) ...	Week of treatment	0	800	0	800		Fe	Mo	Fe × Mo
Liver Cu (mg/kg DM)†	0	110·0	94·5	104·8	104·4				
	8	72·2	19·2	28·3	19·4				
	16	61·9	6·5	3·9	5·8				
	24	49·7	3·9	2·3	3·2				
	32	72·0	3·6	2·4	2·3				
ln Liver Cu (mg/kg DM)	0	4·703	4·549	4·652	4·648	0·128			
	8	4·280	2·956	3·342	2·964	0·242	***	*	***
	32	4·277	1·284	0·859	0·818	0·378	***	***	***
Liver SOD‡ (μg/mg protein)	16	8·26	2·69	3·84	5·97	1·77	NS	NS	*
	24	2·70	1·74	0·96	2·71	0·61	NS	NS	**
	32	3·76	1·29	1·16	1·02	0·32	***	***	***

DM, dry matter.
NS, not significant.
* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.
† Calculated as antilogarithm of mean ln values.
‡ Measured on liver cytosol, obtained after centrifugation of liver homogenates in water at 100000 *g* for 1 h.

Cu status of calves

Dietary supplementation with Fe or Mo or both caused major changes in the Cu status of the animals, as assessed by tissue or blood Cu concentrations and by the activities of Cu-dependent enzymes (Tables 3 and 4). Thus liver Cu concentrations rapidly decreased in all calves in groups Fe, Mo and FeMo (Fig. 2, Table 3). After 8 weeks, concentrations in these groups were less than 30 mg/kg DM, a threshold level frequently used to diagnose

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

Table 4. *Effect of iron and molybdenum on copper concentrations and on caeruloplasmin (EC1.16.3.1) and superoxide dismutase (EC1.15.1.1, SOD) activities in blood*

Treatment group ... Mo supplement (mg/kg) ... Fe supplement (mg/kg) ...	Week of treatment	Con 0 0	Fe 0 800	Mo 5 0	FeMo 5 800	SE of difference between means	Significance of treatment effects: Fe	Mo	Fe × Mo
Plasma Cu (μg/ml)	0	0·78	0·75	0·78	0·82	0·08			
	8	0·70	0·61	0·65	0·54	0·06	*	NS	NS
	16	0·81	0·26	0·19	0·25	0·09	**	***	***
	24	0·80	0·14	0·15	0·16	0·06	***	***	***
	32	1·00	0·21	0·15	0·14	0·06	***	***	***
Plasma caeruloplasmin†	0	15·8	16·0	15·8	14·8	0·4			
	8	15·8	13·2	12·8	12·2	0·2	NS	NS	NS
	16	19·4	4·6	2·8	3·4	0·3	**	**	**
	24	17·4	1·1	1·6	1·6	1·4	***	***	***
	32	21·4	2·3	1·4	2·0	1·3	***	***	***
Erythrocyte Cu (μg/ml)	16	0·97	0·57	0·62	0·50	0·10	**	*	NS
	24	0·85	0·46	0·30	0·34	0·13	NS	**	*
	32	0·94	0·36	0·31	0·30	0·14	*	**	*
Blood SOD (μg/ml)	16	263	148	130	133	35	*	*	*
	24	221	82	83	117	31	*	*	**
	28	222	130	101	66	40	*	**	NS
	32	151	93	74	107	20	NS	NS	**

NS, not significant.
$*P < 0.05$, $** P < 0.01$, $*** P < 0.001$.
† Activities are given as change in absorbance × 10^3 at 525 nm/0·5 ml plasma per min.

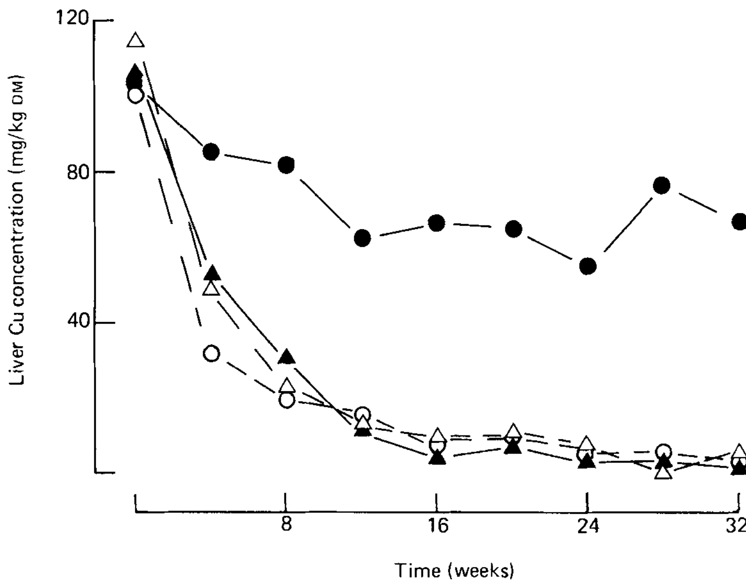

Fig. 2. Effects of dietary supplementation with iron (○), molybdenum (▲) or Fe and Mo (△) on liver copper concentrations (mg/kg dry matter (DM)) in calves. (●), Concentrations in the control calves.

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

Cu deficiency (Roberts, 1976). At 24 weeks, by which time Mo had reduced the rate of growth, liver Cu had declined to as little as 3 mg/kg DM. However, liver Cu fell to equally low concentrations in group Fe, in which growth was unaffected. Significant Fe × Mo interactions occurred throughout the experiment but there were no indications that Fe and Mo exerted additive effects on the rate of liver Cu depletion.

The activity of liver cytosolic (Cu, Zn)–SOD was reduced in all calves given the Fe- and Mo-containing diets but there were no consistent differences between groups Fe, Mo and FeMo (Table 3). Significant decreases in enzyme activities took longer to develop and when expressed as a proportion of initial values were much less than the decreases in total liver Cu content.

Plasma Cu concentrations in the calves in groups Fe, Mo and FeMo were reduced to only 50% of the values in the unsupplemented calves (group Con) after 12 weeks and minimum concentrations of 0·15–0·20 mg/l were attained after 20 weeks (Table 4). There were, however, no significant differences in plasma Cu between the three supplemented groups. Caeruloplasmin activities decreased similarly and were virtually undetectable in all Mo- and Fe-treated calves after 20 weeks (Table 4).

Erythrocyte SOD and Cu concentrations decreased by approximately 50% in the calves in groups Fe, Mo and FeMo at 16 weeks, when these assays were first carried out, and further decreases occurred in the 16–32 week period (Table 4). There were no significant differences between the three groups of supplemented calves.

Iron status of calves

Dietary supplementation with Fe caused significant but variable increases in plasma and liver Fe concentrations ($P < 0·001$) (Figs. 3 and 4). The greatest plasma Fe concentrations occurred in groups Fe and FeMo during weeks 4–12, when concentrations were approximately 3·5 mg/l, compared with approximately 2 mg/l in the calves given no Fe supplements. There were also transient but slight effects of dietary Mo on plasma Fe concentrations which were significantly decreased in both groups of Mo-treated calves during weeks 24–28 ($P < 0·05$).

Total iron-binding capacity (TIBC) of the plasma was inversely related to Fe intake and values in groups Fe and FeMo were less than those in the other groups from week 20 onwards ($P < 0·01$). Concentrations at week 20 in groups Con, Fe, Mo and FeMo were 5·62, 4·94, 6·06 and 4·98 mg Fe/l respectively (SE of difference between means 0·39).

The degree of saturation of plasma transferrin was greatly increased by Fe supplementation during weeks 4–12, when values in groups Fe and FeMo were approximately 80% and approximately double those in the other groups ($P < 0·01$) (Table 5). Slight effects of Mo on transferrin saturation also occurred during weeks 24–48 ($P < 0·05$), average values in groups Mo and FeMo being only 34% compared with 45% in groups Con and Fe (Table 5).

Liver Fe concentrations in both groups of Fe-supplemented calves were two to three times greater than those in the other calves at 8 weeks (Fig. 4). Concentrations declined for a short period thereafter but then increased steadily to aproximately 600 mg/kg DM from week 20 onwards. Liver Fe concentrations in the calves in groups Con and Mo varied during the experiment but never increased above 300 mg/kg DM, even in the calves in group Mo showing signs of Cu deficiency.

The haematological status of the calves was not affected by any treatment. Blood haemoglobin concentrations and packed cell volumes at 32 weeks are given in Table 5.

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

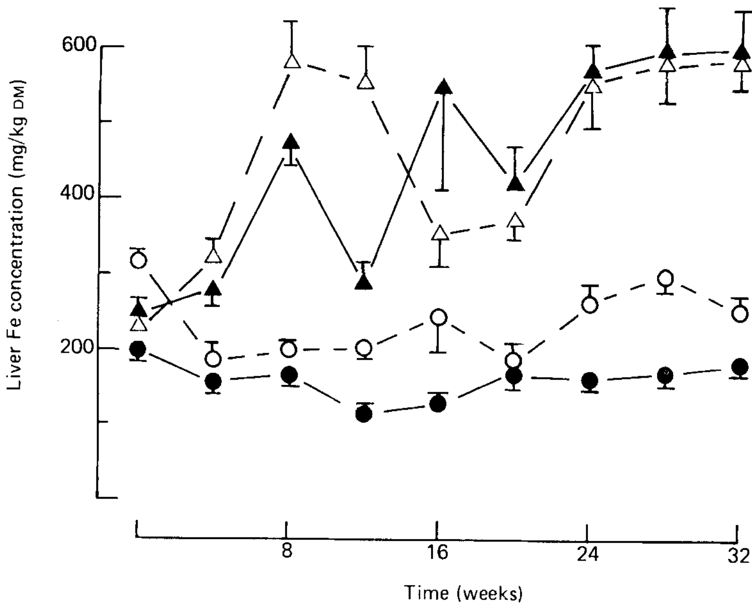

Fig. 3. Effects of dietary supplementation with iron (▲), molybdenum (○) or Fe and Mo (△) on liver Fe concentrations (mg/kg dry matter (DM)) in calves. (●), Concentrations in the control calves. The SEMS are indicated by the vertical bars.

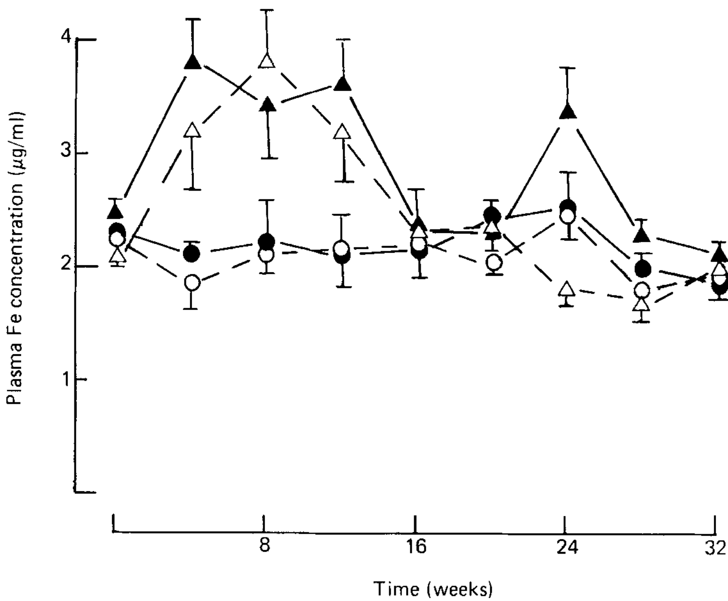

Fig. 4. Effects of dietary supplementation with iron (▲), molybdenum (○) or Fe and Mo (△) on plasma Fe concentrations (μg/ml) in calves. (●), Concentrations in the control calves. The SEMS are indicated by the vertical bars.

Table 5. *Effects of iron and molybdenum on Fe status of calves*

Treatment group ...	Week of treatment	Con	Fe	Mo	FeMo	SE of difference between means	Significance of treatment effects		
Mo supplement (mg/kg) ...		0	0	5	5				
Fe supplement (mg/kg) ...		0	800	0	800		Fe	Mo	Fe × Mo
Blood haemoglobin concentration (g/l)	32	156	131	140	133	11			
Packed cell volume	32	0·408	0·364	0·372	0·370	0·022			
Saturation of plasma	8	0·383	0·619	0·408	0·783	0·086	***	NS	NS
transferrin	24	0·424	0·490	0·357	0·331	0·038	NS	**	NS

NS, not significant.
** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$.

Mo and P

Plasma Mo concentrations in calves in groups Con and Fe were only 0·01–0·02 mg/l, which was close to the limit of detection for the analytical technique used. As expected, concentrations were greater in the calves given the Mo supplement, and were approximately 0·1–0·2 mg/l throughout the 32-week experimental period. Dietary supplementation with Fe also increased plasma Mo concentrations during weeks 4–12 in the calves given Mo ($P < 0{\cdot}01$). For example, the mean (± SEM) concentrations in groups Mo and FeMo at week 8 were 0·081 ± 0·011 and 0·182 ± 0·017 mg/l respectively.

There were no significant effects of Fe or Mo supplementation on P metabolism. Plasma P concentrations after 20 weeks were 92·1, 89·5, 96·2 and 91·7 mg/l (SE of difference between means 3·7) in groups Con, Fe, Mo and FeMo respectively.

DISCUSSION

These results clearly indicate that increased dietary Fe intakes can have a marked inhibitory effect on the utilization of dietary Cu and are in good agreement with the findings of Standish *et al.* (1969) and of Campbell *et al.* (1974). It is significant that the addition of only 800 mg Fe/kg diet was sufficient to affect the Cu status of the calves, since this Fe concentration is within the range found in a variety of animal feedstuffs (Agricultural Research Council, 1976). Indeed, recent findings indicate that a dietary supplement of only 250 mg Fe/kg diet is sufficient to reduce hepatic Cu reserves of calves (I. Bremner, M. Phillippo, W. R. Humphries & B. W. Young, unpublished results). The effects of Fe on the utilization of Cu by cattle may, therefore, be of much greater practical importance than has hitherto been recognized.

It is possible, for example, that the inhibitory effect of soil ingestion on Cu availability to ruminants (Suttle *et al.* 1975) could be at least partly attributable to an increase in their Fe intake. Concentrations of Fe in soils are frequently approximately 20000 mg/kg and a significant proportion of the Fe can be liberated on incubation of soil with rumen contents (Healy, 1972). If soil ingestion accounted for 10% of an animal's DM intake, during grazing or on silage feeding, and if only 25% of this Fe were released in the alimentary tract, the level of 'available' Fe would be equivalent to 500 mg/kg, which would be sufficient to affect considerably the utilization of dietary Cu.

The addition of 800 mg Fe/kg diet had the same effect on all indices of Cu status of the calves as did 5 mg Mo/kg, a concentration of Mo which has previously been shown to decrease the utilization of Cu by cattle and sheep (Suttle, 1975; Mills *et al.* 1977) and to induce Cu deficiency under practical conditions (Smith *et al.* 1975). Thus liver, plasma and

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

erythrocyte Cu content and erythrocyte SOD activity were identical in the calves given Fe, Mo or Fe plus Mo. However, there were important differences in the clinical condition of the calves, since only those given Mo in the diet showed characteristic signs of Cu deficiency (Mills *et al.* 1976) such as impaired growth, reduced food conversion efficiency, skeletal abnormalities and changes in hair texture. The subsequent reproductive performance of the heifers was also adversely affected by Mo but not by Fe, since conception rates in groups Mo and FeMo were greatly reduced when the animals were inseminated at the end of the 32-week experimental period (Phillippo *et al.* 1982).

The absence of these lesions in the Fe-treated animals highlights the inadequacy of current procedures to diagnose clinical Cu deficiency. Even the assay of erythrocyte SOD content to detect the onset of growth inhibition, as proposed by McMurray (1980), has limitations, since the decreases in the levels of this enzyme were the same in the calves given Fe or Mo. Assay of this enzyme does not appear to offer any advantage over the measurement of erythrocyte Cu concentrations. The absence of any increase in liver Fe content in the Mo-treated calves also indicates that there is no relationship between growth failure in Cu-deficient animals and excessive accumulation of Fe in the liver (Mills, 1980).

The reason why Mo and Fe exert such different clinical effects on calves which are ostensibly of the same Cu status is not known. It may be that Mo induces a more severe Cu deficiency, which cannot be recognized by conventional measurements of blood or liver Cu concentrations. Alternatively, Mo may disturb Cu metabolism at specific sites in the body, where Fe is without effect. It is well-established that systemic Cu metabolism is affected in ruminants given relatively large amounts of Mo in their diet (Smith & Wright, 1974; Bremner & Young, 1978) and in rats given thiomolybdates and oxythiomolybdates (Mills, El-Gallad & Bremner, 1981; Mills, El-Gallad, Bremner *et al.* 1981; Bremner, Mills *et al.* 1982). Another possibility is that Mo can exert direct effects on certain metabolic processes, independently of any disturbance in Cu metabolism. The amelioration of this form of molybdenosis by Cu supplementation could be atrributed to inhibition of Mo absorption or to restriction in the access of active forms of Mo to sensitive sites (Mason *et al.* 1978; Mills, El-Gallad & Bremner, 1981).

Unfortunately, little is known of the mechanisms whereby Fe disturbs Cu metabolism and it is not clear whether the effect of Fe is on the absorption or the hepatic retention of Cu. It does not depend on any reduction in food intake and there is some evidence of differences between species in response to variations in Fe supply. Thus, liver Cu concentrations in guinea pigs were reduced by 65% when their daily Fe intake was increased ten-fold to over 8 mg/d (Smith & Bidlack, 1980) whereas liver Cu accumulation by pigs was unaffected by Fe unless they were given diets with 250 mg Cu/kg (Hedges & Kornegay, 1973). At lower Cu intakes, the response of the pigs was similar to that found in rats, where ^{64}Cu absorption and tissue Cu concentrations were unchanged or decreased only slightly by a dietary supplement of 1000 mg Fe/kg diet (Bremner & Young, 1981). However, supplementary Fe did greatly reduce ^{64}Cu absorption and tissue Cu levels in rats when their diet was also supplemented with sulphide, the magnitude of the effect then being much closer to that observed in the calves in this experiment (Bremner, Young *et al.* 1982).

Since dietary supplementation of rats with both Mo and S^{2-} also mimics certain of the effects of Mo in ruminants (Mills, El-Gallad & Bremner, 1981) it is tempting to suggest that the inhibitory effect of Fe on Cu utilization in calves is, like Mo, dependent on interaction with S^{2-} in the rumen, with resultant decreases in Cu absorption. Some support for this view is provided by the observations that liver Cu concentrations in preruminant calves are not affected by the inclusion of Fe (500 mg/kg DM) in their milk-substitute ration and that the biliary excretion of Cu by weaned calves is unaffected by dietary Fe supplementation (I. Bremner, M. Phillippo, W. R. Humphries & D. S. Graca, unpublished

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press

results). Experiments are in progress to determine whether the effect of Fe on Cu metabolism in calves is indeed influenced by variations in dietary S supply.

The existence of a common step in the Mo–Cu and Fe–Cu interactions in calves could perhaps explain why there was a highly significant interaction between Fe and Mo in the present experiment and why there were no major or consistent additive effects of the two antagonists on Cu metabolism in the calves in group FeMo. However, it cannot be excluded that each antagonist alone was having the maximum possible effect on the absorption of Cu and on the liberation of hepatic reserves of Cu, so that there was no scope for further change. It will be necessary to determine the antagonistic effect of lower levels of both antagonists before this can be resolved. The findings will be of considerable importance to those concerned with the estimation of the requirements of ruminants for Cu and with the quantitation of the effects of specific dietary components on the utilization of Cu.

The authors acknowledge the interest, which Dr C. F. Mills has shown in this research and thank Messrs I. McDonald, W. Lawson and J. Mathieson for assistance with statistical, haematological and Mo analyses. Dr Bain, Macaulay Institute for Soil Research, kindly carried out the S analyses on the diets.

REFERENCES

Agricultural Research Council (1976). *The Nutrient Requirements of Livestock, no. 4, Composition of British Feedingstuffs*. London: Agricultural Research Council.
Beauchamp, C. & Fridovich, I. (1971). *Analyt. Biochem.* **44**, 276.
Bingley, J. B. (1959). *J. agric. Fd Chem.* **7**, 269.
Bremner, I. & Davies, N. T. (1980). In *Digestive Physiology and Metabolism in Ruminants*, p. 409 [Y. Ruckebusch & P. Thivend, editors]. Lancaster: MTP Press Ltd.
Bremner, I., Mills, C. F. & Young, B. W. (1982). *J. inorg. Biochem.* **16**, 109.
Bremner, I. & Young, B. W. (1978). *Br. J. Nutr.* **39**, 325.
Bremner, I. & Young, B. W. (1981). *Proc. Nutr. Soc.* **40**, 69A.
Bremner, I., Young, B. W. & Mills, C. F. (1982). *Proc. Nutr. Soc.* **41**, 82A.
Campbell, A. G., Coup, M. R., Bishop, W. H. & Wright, D. E. (1974). *N.Z. Jl agric. Res.* **17**, 393.
Coup, M. R. & Campbell, A. G. (1964). *N.Z. Jl agric. Res.* **7**, 624.
Grun, M., Anke, M., Hennig, A., Seffner, W., Partschefeld, M., Flachowsky, G. & Groppel, B. (1978). *Archiv für Tierernährung* **28**, 341.
Healy, W. B. (1972). *N.Z. Jl agric. Res.* **15**, 289.
Hedges, J. D. & Kornegay, E. T. (1973). *J. Anim. Sci.* **37**, 1147.
Humphries, W. R., Young, B. W., Phillippo, M. & Bremner, I. (1981). *Proc. Nutr. Soc.* **40**, 68A.
Kennedy, R. P. (1927). J. biol. Chem. **74**, 385.
McMurray, C. H. (1980). In *Biological Roles of Copper*, Ciba Fdn Symp. no. 79, p. 183 [D. Evered and G. Lawrenson, editors]. Amsterdam: Excerpta Medica.
Mason, J., Lamand, M., Tressol, J. C. & Lab, C. (1978). *Ann. Rech. Vet.* **9**, 577.
Mills, C. F. (1980). In *Biological Roles of Copper*, Ciba Fdn Symp. no. 79, p. 49 [D. Evered and G. Lawrenson, editors]. Amsterdam: Excerpta Medica.
Mills, C. F., Dalgarno, A. C., Bremner, I. & El-Gallad, T. T. (1977). *Proc. Nutr. Soc.* **36**, 105A.
Mills, C. F., Dalgarno, A. C. & Wenham, G. (1976). *Br. J. Nutr.* **35**, 309.
Mills, C. F., El-Gallad, T. T. & Bremner, I. (1981). *J. inorg. Biochem.* **14**, 189.
Mills, C. F., El-Gallad, T. T., Bremner, I. & Wenham, G. (1981). *J. inorg. Biochem.* **14**, 163.
Phillippo, M., Humphries, W. R., Bremner, I. & Young, B. W. (1982). *Proc. Nutr. Soc.* **41**, 80A.
Quin, B. F. & Woods, P. H. (1979). *Analyst, Lond.* **104**, 552.
Roberts, H. E. (1976). *Vet. Rec.* **99**, 496.
Ruutu, R. (1975). *Clinica chim. Acta* **61**, 229.
Smith, B. P., Fisher, G. L., Poulos, P. W. & Irwin, M. R. (1975). *J. Am. vet. med. Ass.* **166**, 682.
Smith, B. S. W. & Wright, H. (1974). *Clin. Chim. Acta*, **50**, 359.
Smith, C. H. & Bidlack, W. R. (1980). *J. Nutr.* **110**, 1398.
Standish, J. F., Ammerman, C. B., Palmer, A. Z. & Simpson, C. F. (1971). *J. Anim. Sci.* **33**, 171.
Standish, J. F., Ammerman, C. B., Simpson, C. F., Neal, F. C. & Palmer, A. Z. (1969). *J. Anim. Sci.* **29**, 496.
Suttle, N. F. (1975). *Br. J. Nutr.* **34**, 411.
Suttle, N. F., Alloways, B. J. & Thornton, I. (1975). *J. agr. Sci., Camb.* **84**, 249.

Printed in Great Britain

https://doi.org/10.1079/BJN19830013 Published online by Cambridge University Press