Studies on copper and cobalt in dairy calves

BY J. K. MARO* AND J. A. KATEGILE
Animal Science Department, University of Dar es Salaam, P.O. Box 643, Morogoro, Tanzania
AND H. HVIDSTEN
Agricultural University of Norway, Boks 25 1432 Aas-NLH, Norway

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1. Studies on the effect of supplementing copper and cobalt were conducted in 4- to 10-month-old dairy calves during successive indoor and outdoor feeding periods on conserved forages and fresh pastures respectively.

2. No significant differences in live-weight gains or serum Cu were recorded during the indoor period of 40 d.

3. Both live-weight gains and serum Cu were significantly elevated ($P < 0.05$) by Cu supplementation during the following grazing period of 70 d.

4. Supplementation with Co alone had no effect on any of the measurements taken, but it had an additive effect on weight gains to Cu supplementation during the grazing period.

5. It is concluded that Cu deficiency at marginal level is prevalent in the Mbeya area of Tanzania and that this is due to low Cu and high molybdenum in herbage.

Copper and cobalt deficiencies have been recognized for a long time in ruminants (Underwood, 1977) and various incidences of conditions such as 'Kipsiepsiep' which is similar to swayback and 'Nakuruitis' or more commonly enzootic marasmus due to Co deficiency have been reported in East Africa (French, 1952; Hedger *et al.* 1964).

Clinically, the manifestations range from depressed appetite, lack of thrift, poor growth, diarrhoea, bone abnormalities and depigmentation to anaemia (Becker *et al.* 1953; Harve *et al.* 1960); however, it is thought that subclinical cases might be more prevalent than clinical cases (McDowell, 1976) and result in hidden losses which are of great economic significance. In cattle, Morgan *et al.* (1962) and Thornton *et al.* (1972*a*) reported that Cu supplementation resulted in a substantial improvement in live-weight gains and blood Cu levels in animals which lacked obvious clinical symptoms. Similarly, Fuller & McAlpine (1961) cited a tremendous improvement in weight gains in apparently normal sheep which were supplemented with Co.

Naik (1965) reviewed previous work done on the positive effects of Cu and Co supplementation to cattle as depicted by weight gains, milk production and conception rates in the Iringa and Tanga regions of Tanzania. Recently A. G. Barwell & K. T. Sanderson (unpublished results) reported a 24% improvement in carcass yield with Co supplementation in sheep in Southern Tanzania. In Kenya, Hedger *et al.* (1964) observed an elevation in blood Cu from 0·26 to 0·91 mg/l in sheep and goats treated with 50 mg Cu as the EDTA salt over a 5-month period.

Pastures containing less than 5 mg Cu/kg dry matter (DM) are considered to be deficient (Cunningham, 1955) while 0·07 mg Co/kg is regarded as the lower limit of adequacy for both cattle and sheep (Ssekaalo, 1972). Most Cu deficiencies are, however, conditioned mainly by molybdenum or sulphur or both (Suttle, 1975), and results reported in Kenya (Hedger *et al.* 1964) show that although pasture Cu content in some two areas was high

* Present address: Uyole Agricultural Centre, P.O. Box 400, Mbeya, Tanzania.

at 9·4 and 12·8 mg/kg, the high Mo content of 3·9 and 5·6 mg/kg and sulphate levels of 8·2 and 6·2 g/kg precipitated swayback in sheep.

Results from a survey conducted in Southern Tanzania (E. Jensson & C. W. Rombulow-Pearse, unpublished results) indicate that hydrochloric acid-extractable Cu of approximately 72% of 123 samples analysed contained less than 5 mg/kg soil with a range of 0·7–2·7 mg/kg. This experiment was intended, therefore, to study the effect of supplementing Cu and Co on the growth rate and serum Cu levels in weaned dairy calves fed on forages grown at Uyole Agricultural Centre, which is in Southern Tanzania.

EXPERIMENTAL

Site

The experiment was conducted at Uyole Agricultural Centre, Mbeya, which lies approximately latitude 8° 55′ South and longitude 33° 22′ East at an altitude of 1700–1900 m above sea level. The annual rainfall is 970–1260 mm and the soils are of volcanic origin, with a slightly acidic pH of 6·5.

Feeding and management of the animals

Thirty-two female Friesian calves aged between 4 and 10 months and weighing an average of 99 kg were allocated to four treatments: i.e. control, Cu, Co, Cu+Co groups with respect to their body-weights in a randomized block experiment. Two experimental periods of indoor feeding and grazing were involved, with the former being preceded by a 36 d preliminary period.

During the 45 d indoor feeding experiment, each group was housed separately in a 12 × 6 m pen and group fed on Rhodes (*Chloris gayana*) hay and Elephant grass (*Pennisetum purpureum*) silage *ad lib.*, while concentrate feeding was restricted to 12 kg/group, which was fed at 09·00–10.00 hours daily. The concentrate consisted of (g/kg): 450 maize, 160 rice polishings, 280 lupins (*Lupinus albus*), 70 sunflower cake, 25 bone meal, 15 common salt (sodium chloride).

Cu and Co supplements were incorporated in the concentrate mixture to supply 10 mg Cu and 0·3 mg Co/kg in fresh diet in the form of hydrated copper sulphate ($CuSO_4.5H_2O$) and cobalt nitrate ($Co(NO_3)_2.6H_2O$) respectively.

During the grazing period, the animals were run together on Rhodes grass pastures lightly oversown with *Desmodium intortum* and were each morning sorted out into respective groups and fed concentrate mixtures at the rate of 4 kg/group.

Weighing was done at approximately 1 month intervals.

Sample collection, preparation and analysis

Random samples of hay, silage and concentrate fed to controls were taken from feeding troughs, bulked, dried in a forced-air oven at 65°, then ground in a hammer mill to pass through a stainless-steel 1 mm sieve. For pastures, samples were taken diagonally across the paddock and treated as described previously.

The animals were bled from the jugular vein using sterile stainless-steel needles at the start of the mineral supplementation and thereafter at intervals of 28–30 d. A whole-blood sample was taken in a Bijou bottle containing EDTA for haemoglobin determination, while another portion was collected by decanting and centrifuging. The serum samples were stored in a deep-freezer at −20° before analysis.

Haemoglobin was determined by the cyanmethaemoglobin technique described by Cartwright (1968). Cu and Co in the feeding stuff were determined by the atomic absorption

Table 1. *Analytical composition (g/kg dry matter* (DM))

Samples and date of collection	Crude protein*	Total S	mg/kg DM Copper	Molybdenum	Cobalt
Hay	50	1·3	4·6	2·3	1·0
Silage	51	0·7	2·6	3·8	1·0
Pastures	140	1·4	3·8	2·8	1·1
Concentrates	160	1·1	5·6	2·8	1·5
Pasture					
15 January 1979	161	1·7	4·6	4·4	1·2
16 February 1979	146	1·2	4·7	3·8	1·0
9 March 1979	131	1·2	3·4	1·6	1·2
26 March 1979	—	1·2	3·3	1·6	0·8
Pasture regrowth					
12 April 1979	116	1·5	2·8	1·6	1·2
30 April 1979	146	1·4	—	3·8	—

* Nitrogen × 6·25.

spectrophotometer (Pye Unicam SP 191) after ashing in a muffle furnace at 450° followed by digestion with 6 M-HCl. Serum samples were analysed for Cu according to the instruction manual of Pye Unicam (1975). Mo was determined colorimetrically without extraction after the procedure described by Sandell (1950). Total S was determined turbidimetrically after Tabatabai & Bremner (1970), crude protein (nitrogen × 6·25) by the macro-Kjeldahl procedure (Pearson, 1970) and DM was determined by drying in an oven at 105° for at least 24 h.

The results were analysed statistically for variance in accordance with Snedecor & Cochran (1967) and the means tested using Studentized Q test.

RESULTS

Mineral and crude protein content of feeding stuffs

The crude protein in hay and silage was particularly low at 50 g/kg (Table 1). Cu content varied from 2·6 to 5·6 mg/kg with a mean of 3·9 mg, while Mo ranged from 1·6 to 4·4 mg/kg with an average of 2·9 mg. Co content was generally high, ranging from 0·8 to 1·5 mg/kg, and the highest concentration was recorded in the concentrates.

There was a significant decrease in the crude protein content of pastures with advancing maturity, while Cu and Mo contents showed more rapid decreases.

Live-weight gains

The mean daily weight gains during the experimental periods are presented in Table 2, and Fig. 1 illustrates the total body-weight gains during the whole period.

Animals supplemented with both Cu and Co had the highest weight gains, followed by animals supplemented with Cu alone. At the end of the indoor trial, the live-weight gains did not differ significantly ($P = 0·1$) between treated and non-treated animals, though animals treated with either Cu or Cu + Co had higher weight gains than the rest.

Cu supplementation alone had a positive significant effect ($P < 0·05$) on weight gains during the grazing period, whereas Co alone did not significantly improve weight gains. However, there was an additive response to Co in combination with Cu supplementation (Fig. 1).

Table 2. *The effect of copper and cobalt supplementation on daily weight gains of calves (kg/d)*

Period	Duration of feeding (d)	Treatment† Control	Cu	Co	Cu+Co	Statistical significance of treatment	SED
Indoor	30	0·40	0·48	0·39	0·51	**	0·043
	45	0·41	0·44	0·39	0·49	NS	0·045
Grazing	28	0·50	0·56	0·54	0·62	NS	0·048
	38	0·51	0·58	0·54	0·64	**	0·038
	70	0·49	0·56	0·51	0·59	*	0·035

NS, not significant.

** $P < 0{\cdot}10$, * $P < 0{\cdot}05$.

† For details, see p. 26.

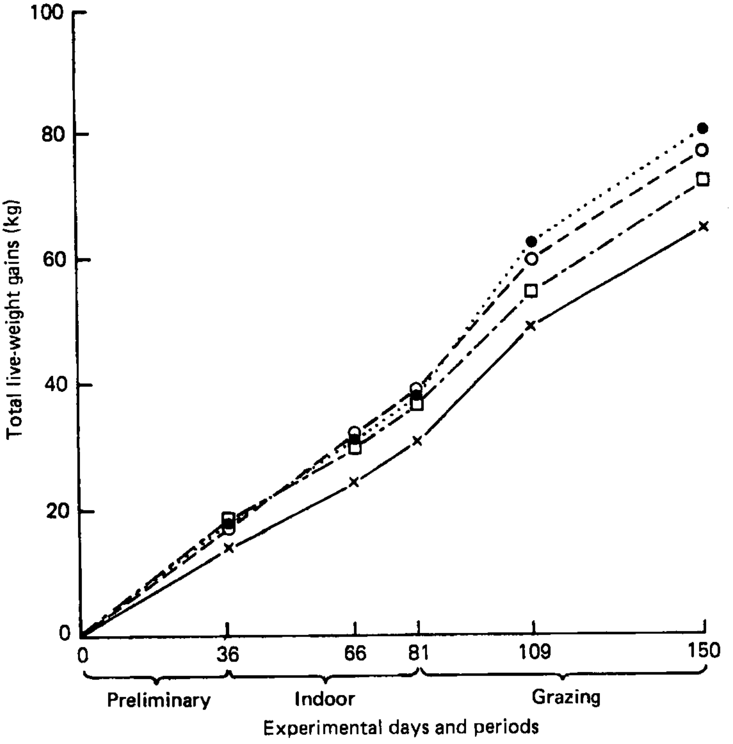

Fig. 1. Total weight gains of calves: (×—×) control, (○----○) copper supplemented, (□–·–·–□) cobalt supplemented, (●····●) copper and cobalt supplemented.

Serum Cu

The initial (pre-experimental) blood serum Cu differed significantly between groups ($P < 0{\cdot}01$) (Table 3). With the onset of indoor feeding serum Cu increased in all groups, and this was evident after 27 d. The rate of increase was most dramatic in animals supplemented with Cu alone (Fig. 2). Obvious treatment trends showed after 9 d of grazing with a high serum Cu in the two groups supplemented with Cu, and this pattern persisted for

Table 3. *Mean serum copper levels (mg/l) in calves supplemented with Cu and Co*

Period	Duration of feeding (d)	Treatment† Control	Cu	Co	Cu+Co	Statistical significance of treatment	SED
Initial	0	6·0	4·6	7·3	7·0	**	0·66
Indoor	27	8·5	7·6	8·9	9·4	NS	0·64
	45	6·1	9·8	7·9	9·6	**	0·75
Grazing	34	5·8	8·8	7·3	9·0	*	1·02
	63	6·3	9·3	6·4	9·0	*	1·08

NS, Not significant.

Tested at $*P < 0.05$, $**P < 0.01$.

† For details, see p. 26.

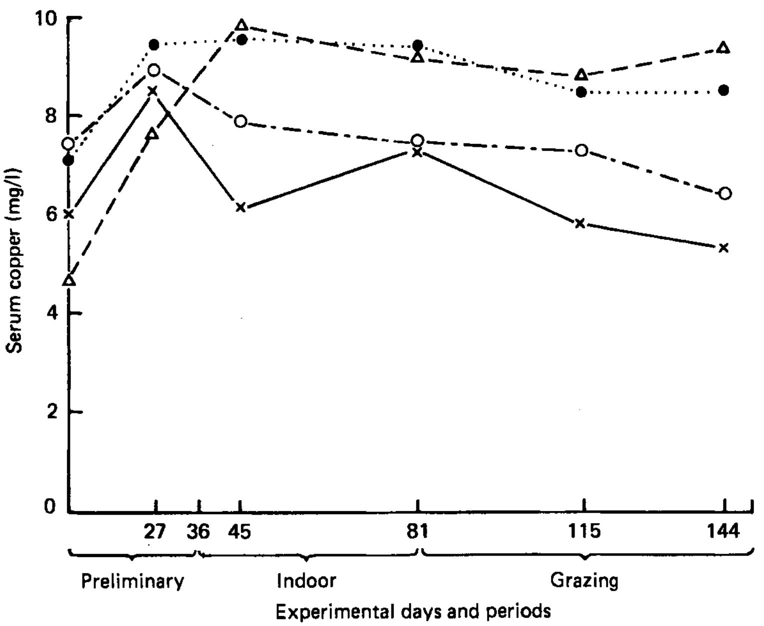

Fig. 2. Serum copper concentration in calves: (×—×) control, (△---△) copper supplemented, (○–·–·–○) cobalt supplemented, (●····●) copper and cobalt supplemented.

the rest of the experimental period, indicating the enhancing effect of dietary mineral supplementation on serum Cu.

Haemoglobin concentration

Haemoglobin values were very variable in all animals (Table 4) but the controls maintained some slightly lower mean concentration. A slight decline occurred during the first 27 d of the experiment and this was followed by a more rapid decline, so that the blood samples taken approximately 9 d after the start of the experiment had an over-all mean of 90 g/l compared with the initial mean of 118 g/l. However, no gross features of anaemia were observed in any of the control calves.

Table 4. *Mean haemoglobin concentration (g/l) in calves*

Period	Duration of feeding (d)	Treatment† Control	Cu	Co	Cu+Co	Statistical significance of treatment	SED
Initial	0	109	116	119	124	*	6·26
Indoor	27	101	113	110	114	*	5·51
	45	87	90	93	90	NS	3·90
Grazing	34	95	96	99	107	NS	5·91
	63	91	95	88	103	**	4·65

NS, Not significant.

Tested at $*P < 0·10$, $**P < 0·05$.

† For details, see p. 26.

DISCUSSION

The Cu content in hay, silage, pastures and concentrates at Uyole is on the deficiency level according to Havre *et al.* (1960), as similar levels precipitated Cu deficiency symptoms in cattle at Setesdalen in Norway. The values for Mo and total S content of the feeding stuffs (Table 1) suggest that Mo levels are high enough to be a significant complexing factor (Matrone, 1970). It is therefore most probable that Cu deficiency at the Uyole farm must be due to Cu insufficiency and Mo toxicity. The response in terms of increased growth rate and serum Cu levels upon supplementation further confirms the existence of Cu deficiency. However, lack of acute deficiency symptoms in grazing animals at the Uyole Centre and the surrounding farm shows that this deficiency is marginal (Becker *et al.* 1953; Havre *et al.* 1960; McDowell, 1976).

The Co content in the feeding stuff was, however, much higher than has been reported in most instances. Ssekaalo (1972) reported that Rhodes grass in Uganda contained 0·13 mg Co/kg, while Chamberlain (1955) found a range of 0·04–0·63 mg/kg for different East African feeding stuffs.

The weight gains recorded during the indoor period do not seem to indicate any added advantage to mineral supplementation, and some reports (Hartmans & Bosman, 1970; Roberts, 1976) indicate that Cu in conserved forages might be more available than from fresh pastures. It is also possible that the preliminary period was inadequate for depleting the animals of Cu reserves, as 2–3 months might elapse before animals show an obvious loss of condition due to Cu deficiency (Field, 1957).

Since the feeding stuffs were adequate in Co and consistently low in Cu, the response to the latter was expected but not the added advantage due to excessive Co used together with Cu, and this synergistic effect requires further investigation. It is possible that animals supplemented with both elements had an improved retention of Cu compared with those supplemented with Co alone, as was suggested by Becker *et al.* (1953) and Ammerman (1970).

Cu supplementation was effective in maintaining significantly higher serum Cu during grazing (0·88–0·90 mg/l) while the non-supplemented animals maintained lower levels (0·50–0·74 mg/l). It can be discerned from the results that subclinical Cu deficiency occurs in cattle at Mbeya and that such instances can be detected through Cu analysis, whereas levels below 0·8 mg/l would be suspect. Similar observations have been reported in the literature (Thornton *et al.* 1972*b*). However, much lower serum Cu levels were reported in Cu-deficient goats (Hedger *et al.* 1964) and adult cattle (Field, 1957). Statistical analysis showed a significant correlation between serum Cu and live-weight gains ($r + 0{\cdot}56$, $P < 0{\cdot}05$) and this agrees with an earlier report of Thornton *et al.* (1972*b*).

Both Cu and Co deficiencies often lead to anaemia (Ammerman, 1970), the former element having an enhancing effect on haemoglobin formation (Underwood, 1977). The normal haemoglobin levels reported in the literature are, however, highly variable and range from 95–133 g/l (Field, 1957; Wood & Kramer, 1968) and in the latter study levels as low as 75 g/l haemoglobin were reported in normal animals. Despite some rather low haemoglobin values no anaemia was detected in this present experiment.

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REFERENCES

Ammerman, C. B. (1970). *J. Dairy Sci.* **53**, 1097.
Becker, R. B., Arnold, P. T. D., Kirk, W. G., Davis, G. K. & Kidder, R. W. (1953). *Flo. Agric. Exp. Sta. Bull.* no. 513.
Cartwright, G. E. (1968). *Diagnostic Laboratory Hematology*, 4th ed., p. 42. New York: Grue and Stratton.
Chamberlain, G. T. (1955). *E. Afr. agric. J.* **21**, 103.
Cunningham, I. J. (1955). In *Advances in Veterinary Science*, vol. 2, p. 139 [C. A. Brandly and E. L. Jugher, editors]. New York: Academic Press.
Field, H. I. (1957). *Vet. Rec.* **69**, 788.
French, M. H. (1952). *E. Afr. agric. J.* **18**, 4.
Fuller, R. G. & McAlpine, V. W. (1961). *Cobalt in Animal Feeding*. Brussels: Centre d'Information du Cobalt.
Hartmans, J. & Bosman, M. S. M. (1970). In *Trace Element Metabolism in Animals*, p. 362 [C. F. Mills, editor]. Edinburgh: E. & S. Livingstone.
Havre, G. N., Dynna, O. & Ender, F. (1960). *Acta vet. scand.* **1**, 250.
Hedger, R. S., Howard, D. A. & Burdin, M. L. (1964). *Vet. Rec.* **76**, 493.
Matrone, G. (1970). In *Trace Element Metabolism in Animals*, p. 354 [C. F. Mills, editor]. Edinburgh: E. & S. Livingstone.
McDowell, L. R. (1976). In *Beef Cattle Production in Developing Countries*, p. 216 [A. J. Smith, editor]. Edinburgh: Centre for Tropical Veterinary Medicine.
Morgan, D. E., Clegg, A., Brooksbank, N. H. & McLea, C. T. (1962). *Anim. Prod.* **4**, 303.
Naik, A. K. (1965). *E. Afr. agric. for. J.* **31**, 175.
Pearson, D. (1970). *The Chemical Analysis of Foods*, 6th ed., p. 9. London: J. & A. Churchill.
Pye Unicam (1975). *Atomic Absorption Spectrophotometry with the Pye Unicam SP 190 and SP 1900*, p. 27. New York: Pye Unicam.
Roberts, H. F. (1976). *Vet. Rec.* **99**, 496.
Sandell, E. B. (1950). *Colorimetric Determination of Traces of Metals*, 2nd ed., p. 458. New York: Interscience Publishers.
Snedecor, G. W. & Cochran, W. G. (1967). *Statistical Methods*, 6th ed. Ames: The Iowa State University Press.
Ssekaalo, H. (1972). *Trop. Agric. Trin.* **49**, 221.
Suttle, N. F. & Field, A. C. (1975). *Br. J. Nutr.* **34**, 441.
Tabatabai, M. A. & Bremner, J. M. (1970). *Agron. J.* **62**, 805.
Thornton, I., Kershaw, G. F. & Davies, M. K. (1972*a*). *J. agric. Sci., Camb.* **78**, 157.
Thornton, I., Kershaw, G. F. & Davies, M. K. (1972*b*). *J. agric. Sci., Camb.* **78**, 165.
Underwood, E. J. (1977). *Trace Elements in Human and Animal Nutrition*, 4th ed. London: Academic Press.
Wood, A. S. & Kramer, J. W. (1968). *Trop. agric. Trin.* **45**, 187.

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