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INTERRELATIONSHIP OF ZINC AND COPPER IN THE NUTRION OF CATTLE

A COMPLEX ZINC-COPPER DEFICIENCY

By

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The authors have previously described a simple and a conditioned form of copper deficiency appearing in Setesdalen, a valley in the southern part of Norway. (1960 & 1961.)

Since then the investigations have proceeded in Sigdal, another valley in South Norway. In this district an unthriftiness in young cattle is observed, clinically similar to the simple form of copper deficiency. From farms where this unthriftiness syndrome occurs, rather low copper values are found in samples of grass and hay. However, the unthriftiness on these farms is not always so pronounced as used to be the rule in Setesdal, with correspondingly low copper values in the hay, in spite of the fact that the main feeding of young cattle in Sigdal, as in Setesdal, is hay.

The depigmentation of the hairlayer in cattle observed in Setesdal occurred like islands, about the size of a hand, around the eyes and down the cheeks. In Sigdal, however, the fading is localized to a ring around the eyes, looking like spectacles, "copper spectacles". Sometimes a greyish thickening of the skin is also observed, with loss of hair, localized to the bridge of the nose from the borderline between nose and muzzle. Moreover, the simple copper deficiency in Setesdal occurred in the spring season only, while in Sigdal the disorder is occurring throughout the year.

In the syndrome observed in Sigdal, copper treatment seems to have a beneficial effect, but the response is not nearly as dra-

matic as observed in Setesdal. Sometimes the treatment fails completely. Administration of vitamin A is found to give effect through the period of administration, but residivation is the result as soon as the administration has ceased.

By analyzing hay and grass samples from Sigdal, these were found to be low in copper. In addition, the content of zinc was also found to be low, in fact significantly lower than in grass samples from Setesdal. The low zinc content in the diet and the observed skin symptoms on nose and muzzle, indicating a parakeratosis, led to a study of the eventual influence of zinc in the present deficiency syndrome.

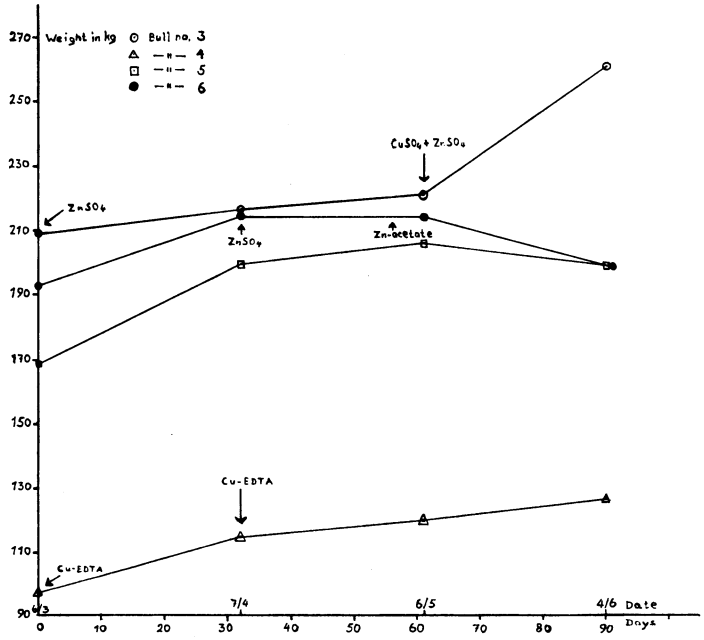
As far as the authors know, observations of zinc deficiency in cattle are very scarce. *Legg and Sears* (1960) described parakeratotic symptoms in cattle due to deficiency of zinc in the diet. In 1950—53, *Ender* studied an unthriftiness syndrome in cattle, observed on an island on the western coast of Norway. Results obtained in his study could indicate a lack of zinc as being at least one of the factors involved in that deficiency syndrome. In 1960, *Larssen* observed a special unthriftiness in Lofoten, on the north coast of Norway, a syndrome where one of the symptoms was the described alterations of the skin of the nose. Different observations indicating a lack of zinc as being one of the causes, led one of us (*Havre*) to collect a great number of soil and grass samples from this and surrounding districts. The results will be published later. *Haaranen* (1962) reports curing of an itching tail root eczema in cattle with zinc, the calcium excretion in the urine being exceptionally high. The influence of excess of zink in the diet of rats has been studied by *Smith and Larson* (1946), and by *Grey and Ellis* (1950). The latter proved very interesting relationships between zinc, copper, molybdenum and lead in the metabolism of rats. The effect of excess of dietary zinc upon copper metabolism in rats has also been studied by *Cox and Harris* (1960). In pigs zinc deficiency, in combination with a high calcium content of the diet, resulting in parakeratosis, is well known.

OWN INVESTIGATIONS

On a small farm in Sigdal the described symptoms were observed in a pronounced degree, especially in two young bulls which were isolated on the farm in order to carry out feeding experiments with controlled supplements of copper and zinc. One of the bulls, no. 3, was 8—9 months old, the other, no. 4,

was about 6 months old. In no. 3, in particular, the "copper spectacles" and the typical skin symptoms on the bridge of the nose were fully developed. Two healthy bulls, about 6 months old, were added to the group as no. 5 and 6. The basal diet for all the animals was hay ad libitum and 1 kg. barley or oatmeal daily.

The mineral content of the hay was determined and gave the following results: Zn: 28 p.p.m. — Cu: 3 p.p.m. — Mo: 0.69 p.p.m. — Ca: 0.382 %.



F i g. 1. Weight gain of the four experimental bulls.

Later in the experimental period the hay store had to be supplemented with hay from another farm, having the following mineral content: Zn: 42 p.p.m. — Cu: 2.7 p.p.m. — Mo: 0.22 p.p.m. — Ca: 0.628 %. This diet was thus exceptionally low in copper, low in zinc, and normal in molybdenum and calcium content. Barley and oatmeal are, according to earlier experience, not very high in copper and zinc content.

Bull no. 3 was administered zinc orally as 40 g. zinc sulphate monthly from the 20th of February to the 5th of May. From that time copper was administered in addition at a rate of 5 g. copper

Table 1.

Mineral content of hay and grass from Sigdal. Values calculated on dry matter basis.

Journ. no.	Material	Pb p.p.m.	Sn p.p.m.	Mo p.p.m.	Cu p.p.m.	Ni p.p m.	Mn p.p.m.	Cr p.p.m.
1D60	Hay	9.5	< 3.8	1.3	8.2	1.3	130	0.13
2D60	„	7.4	< 3.5	2.8	5.7	0.57	114	0.23
3D60	Grass Fescue	17	< 2.5	1.3	5.1	1.7	169	0.085
4D60	Grass Timothy	7.7	< 3.5	1.2	12	0.95	71	< 0.12
4D60	Grass Clover	1.3	< 3.8	3.1	11	0.75	380	0.19
5D60 a	Hay	0.50	< 1.6	0.34	3.4	0.16	50	0.16
5D60 b	„	< 0.50	< 1.5	< 0.15	2.8	< 0.15	43	0.051
5D60 c	„	0.55	< 1.7	1.1	3.3	0.17	55	0.11
6D60	Hay Timothy	2.7	< 2.0	0.20	5.6	0.82	200	0.068
7D60	Hay Tim & Clov	0.65	< 2.0	0.20	2.3	0.52	200	0.13
8D60	Hay	1.9	< 1.9	0.44	3.4	0.40	62	0.30
9D60	Hay Timothy	1.0	< 1.8	0.30	6.1	0.92	120	0.12
10D60	Hay Agrostis	21	2.1	0.50	6.4	1.4	710	1.4
163/61-S	Hay	1.2		0.23	2.9			
164/61-S	Hay Timothy	0.61		0.20	1.9			
165/61-S	„	1.7		0.86	2.3			
166/61-S	Hay	2.2		0.52	3.7			
74/62-S	Hay	27		0.22	2.7			
75a/62-S	Hay Timothy	< 0.7		0.37	1.5			
75b/62-S	Hay	2.5		0.87	2.3			

sulphate monthly. The experiment was running up to the 4th of June. The animal was dosed every second day with 2.7 g. zinc sulphate and 0.33 g. copper sulphate respectively, as water solutions, which corresponds to a daily supplement of 0.55 g. zinc and 0.065 g. copper.

On the 6th of March and the 7th of April, bull no. 4 was injected 45 mg. copper sub-cutaneously in the form of the copper complex of EDTA dispersed in oil (Glaxo product). According to own experience as well as that of other authors (*Cunningham* 1957, *Allcroft* 1957 and 1959), the amounts of copper injected in

Table 1.

Mineral content of hay and grass from Sigdal. Values calculated on dry matter basis.

V p.p.m.	Sr p.p.m.	Ba p.p.m.	Li p.p.m.	Ti p.p.m.	Zn p.p.m.	Ca %	Ash % of dry matter	Dry matter %	pH in soil
< 0.6	45	32	0.63	3.8	49		12.63	95.84	
< 0.6	29	23	0.11	3.4	45		11.42	96.44	
< 0.4	21	23	0.42	8.5	29		8.45	95.93	5.4
< 0.6	36	12	0.12	6.6	43		11.84	96.05	5.7
< 0.6	38	32	< 0.13	5.0	83		12.51	96.03	
< 0.3	34	11	0.16	3.4	23		5.24	96.61	
< 0.3	10	6.7	0.15	1.8	19		5.11	97.04	
0.28	55	8.0	0.17	36	22		5.51	96.67	
< 0.3	61	27	0.068	6.1	32		6.78	96.62	5.3
0.26	65	39	0.065	13	33		6.52	96.52	
1.0	25	12	0.37	25	33		6.21	97.18	
< 0.3	22	40	0.40	10	35		6.13	96.93	
1.1	36	71	0.71	21	50		7.11	95.86	
					46		5.86	95.14	
					46		6.15	94.98	
					24	0.331	5.73	95.05	
					34	0.433	7.46	94.82	
					37	0.628	5.47	93.61	
					28		7.30	94.25	
					34		5.86	93.20	

this form should be sufficient to compensate for a "copper pine".

Bull no. 5 was kept on the basal diet for control.

Bull no. 6 was kept on the basal diet until the 7th of April, when zinc administration was started. The dosages were as in bull no. 3, with the exception that, from the 1st of May, zinc sulphate was changed to equivalent amounts of zinc acetate, in order to exclude an eventual influence of the sulphate group.

The weight gain of bull no. 3 during the 2½ months of zinc administration alone has been constant but far too small. From the 20th of February to the 6th of May the weight gain amounted

to 12 kg. During the next month, i. e. after administration of copper, the weight increased by 41 kg.

The skin symptoms on the bridge of the nose disappeared already during the zinc administration, but the "copper spectacles" persisted throughout the entire experimental period. According to general experience, however, the latter symptom is very persistent. Immediately after the administration of copper, the appetite of the bull increased remarkably.

Bull no. 4, receiving copper injections, had a constant weight gain throughout the experimental period. From the 6th of March to the 6th of May the increase of weight was 23 kg. and during the next month 7 kg. This bull was, however, of a very tender constitution. During the two months from the 6th of March to the 6th of May, bull no. 5, the control animal, kept on basal diet, increased his weight by 38 kg., and then stagnated.

Bull no. 6, receiving zinc supplements, had during the same two months period increased his weight by 22 kg. only, and then stagnated. The results are presented in Fig. 1.

In order to study the mineral status in grass and hay, representing the main feeding of young cattle in Sigdal, a number of samples were subjected to a semi-quantitative spectrographic analysis of 14 different elements. The results are set out in *Table 1*. The table shows that the copper content in several samples is below the "critical level", which in the authors' previous papers, in accordance with Cunningham, is put at 5 p.p.m. In none of the samples, however, the molybdenum content is high enough to cause a conditioned copper deficiency. The manganese content is also low in some samples, though not exceptionally low, and finally the zinc content seems to be low in most of the samples compared with what is normally found in Norwegian herbage material. (Values above 40—50 p.p.m. is regarded as normal.) These facts, together with the clinical observations, in particular the skin alterations, directed the interest to the elements copper, zinc and manganese. In addition, the high figures of lead are also a striking feature of the mineral status in these samples, and the authors are aware of a possible role played by lead in the deficiency syndrome in Sigdal. The other elements presented in Table 1 seem to be of no interest at present.

After the end of the feeding experiments twenty samples of the grass material collected in Setesdal during 1958 were selected at random, representing the areas of simple copper deficiency

Table 2.
Mean values of copper and zinc in herbage from Setesdal and Sigdal. The values are given as p. p. m. on dry matter basis.

District	Element	Number of samples	Mean	Standard error of mean	Range
Setesdal	Cu	20	5.8	0.51	2.5— 9.8
	Zn	20	49	2.7	33 —80
Sigdal	Cu	20	4.6	0.66	1.5—12
	Zn	20	37	3.2	19 —83

as well as conditioned copper deficiency. These samples, having previously been analyzed in copper, were now subjected to analysis of zinc in order to see whether the zinc content in Setesdal grass was significantly different from the content of zinc in Sigdal grass, represented by the twenty samples presented in Table 1. The results are set out in *Table 2* and reveal highly significant difference, $1\ \% > P > 0.5\ \%$, the zinc content of Sigdal grass being the lowest. The copper content is fairly the same in the two materials.

DISCUSSION

Already in 1960 it was clear that a form of copper deficiency was occurring in Sigdal, but also that this form scarcely was a simple one since administration of copper was not always a success. Molybdenum as a conditioning factor is well known, first described by *Ferguson et al.* (1938, 1943), and since then studied by several other research workers. The present authors described this deficiency in Setesdal (*Havre et al.* 1960 and 1961).

In the syndrome of copper deficiency occurring in Sigdal it was not likely that molybdenum was the conditioning factor. The skin alterations on the bridge of the nose might indicate a parakeratosis, and two samples of skin were subjected to a histological examination. The examination was carried out by the Department of Pathology, the Veterinary College of Norway. The results gave, however, only little support to the hypothesis of parakeratosis. It was concluded that a moderate hyperkeratosis might be proved, but it could not be stated with certainty. Owing to the low values of zinc and copper in the grass from Sigdal, it was suggested, however, that a combined copper and zinc deficiency might occur, and it was decided to study the influence of

zinc and copper administration upon animals kept on a basal diet of the copper and zinc deficient hay produced in Sigdal. (The experiment described above.)

Bull no. 3 in this experiment was administered zinc with the intention, in that way, to produce a simple copper deficiency. Bull no. 3 and bull no. 4 had, apart from the difference in age, the same basis when starting the experiment. Fig. 1 shows that bull no. 3 has increased his weight to a lesser extent than has no. 4, despite the fact that no. 3 should be in a better age of growing. A real zinc toxicity is not to be expected, as the amounts of zinc received by the animal from basal diets and supplement do not exceed the amounts normally occurring in good quality hay, rich in zinc. Moreover, the same supplements of zinc were also given during the last period of the experiment when bull no. 3 had his striking increase in weight.

In comparing bulls nos. 5 and 6, it will be seen that the weight gain is fairly equal in the two animals during the first month, and about what is normally expected. The fact that copper deficiency is not developing during the first month is quite natural, as these healthy animals must be expected to have a copper reserve in the liver. From the time of administering zinc to bull no. 6, a difference is observed, as this bull is now distinctly retarded in the growth.

These comparisons may suggest that zinc supplements to a diet, increasing the zinc intake to a normal level, will aggravate a persistent copper deficiency. It could hardly be observed, however, that the bulls nos. 3 and 6, receiving zinc supplements, developed a simple copper deficiency similar to that observed in Setesdalen, and consequently it seems that the effect of zinc and copper is not cumulative, but rather of a complex nature. A possible influence of other conditioning factors must, however, be kept in mind.

It may be objected that the heavy weight gain of bull no. 3, after administration of copper, may be due to copper alone, but this seems unlikely when referring to bull no. 4, receiving adequate copper amounts by sub-cutaneous injections during the experimental period. It seems more pertinent to suppose that the effective factor is the combination of copper and zinc. Practical experience, in about 30 cases, has also revealed that a combination of these two elements is necessary to cure the unthriftiness occurring in Sigdal.

For instance: During the winter 1962, a herd of four young cattle was treated with copper injections, without remarkable results. When turning to pasture, one of the calves, being quite miserable, was treated with zinc and copper. One month later, the three other calves were in the same bad condition, one of them recumbent and with scouring. For this animal a blood transfusion was necessary, and then all these calves were treated with copper and zinc. Since then all four animals have been in an excellent condition.

The gain of weight observed after copper and zinc supplement to bull no. 3 in the experiment has later been verified in practice, in that a young bull, about 1 year old, after copper-zinc administration, increased his weight by 44 kg. during one month.

The remedy used in practice is a mixture of copper sulphate and zinc sulphate, consisting of 20 g. copper sulphate and 160 g. zinc sulphate dissolved in 1 l. water. 15 ml. of this mixture is dosed orally every second day. The disorder cured in this way is mostly restricted to young animals in the age of growth. These doses are experimental, used until now, but it is hoped to find a form of administration which makes it possible to dose the animals weekly only, or perhaps monthly.

Table 2 shows that the zinc level in randomly selected samples from Setesdal is significantly higher ($1\ \% > P > 0.5\ \%$) than in hay from Sigdal and, moreover, that the copper levels are rather equal in the two materials. This may explain the already mentioned fact that the symptoms of copper deficiency in Sigdal are not as pronounced as in Setesdal. When the symptoms do appear, however, they are often more acute. It thus seems that a low zinc content in the diet has a quenching effect upon the symptoms, until a certain limit. Beyond this limit, however, the symptoms will appear more acute.

Haemoglobin and haematocrit determinations were carried out during the experimental period. No effect upon the haemoglobin formation, nor upon the haematocrit values, could be observed. The values were all normal.

The conclusions which appear reasonable to draw from the investigations are that in feeding to young cattle the copper and zinc dificient hay growing in Sigdal, an aggravation of the deficiency is the result when adequate amounts of zinc are added, while a slight improvement may be observed when only copper

is supplemented. By adding both of these elements, a full recovery is obtained.

The interrelationship of zinc and copper is obviously a complex one. Other factors may also play a role, in particular attention must be paid to the elements molybdenum, manganese, lead, calcium and phosphorus. The investigation series reported here is thus of a preliminary character. Further research work is necessary to gain information about the complex trace element metabolism, and work is proceeding.

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SUMMARY

The paper describes a complex zinc-copper deficiency in young cattle. The symptoms are general unthriftiness, reduced growth and a fading of the skin around the eyes. Sometimes an alteration of the skin on the bridge of the nose from the borderline between nose and muzzle is also observed. The last symptom resembles parakeratotic alterations.

In the district where this deficiency is observed the copper and zinc levels in the hay and grass are found to be rather low, on an average below 5 p.p.m. and 40 p.p.m., respectively.

Copper administration is sometimes observed to cause a slight improvement, but often the treatment fails. However, when a mixture of copper and zinc, as sulphate or acetate, is supplemented to the diet, the effect is remarkable. About 30 cases have been treated, all with successful results.

In an experiment with 4 young bulls it was demonstrated that a copper supplemented bull had a weight gain similar to, or slightly better, than the control animal, kept on the basal diet of hay from the deficient areas, low in copper and zinc. Two animals, supplemented with zinc as sulphate and acetate, stagnated in growth shortly after the zinc administration was started. When one of these animals, during the last part of the experiment, was given copper sulphate in addition to zinc, the weight suddenly increased strikingly.

A number of analytical data concerning the mineral status of hay grown in these areas are presented, and it is discussed whether other elements may be of importance in the deficiency syndrome.

ZUSAMMENFASSUNG

Der wechselseitige Einfluss von Zink und Kupfer in der Ernährung des Rindes.

Ein Zink-Kupfermangel-Komplex.

In dem vorliegenden Artikel wird ein Zink-Kupfermangel beim Jungvieh beschrieben. Die Symptome sind allgemeines Unbehagen, Wachstumshemmung und Blässe der Haarschicht ringsum die Augen. Zuweilen nimmt man auch Hautveränderungen am Näsenrücken von der Grenzlinie zwischen der Nase und dem Maul wahr. Das letztgenannte Symptom hat eine gewisse Ähnlichkeit mit parakeratotischen Hautveränderungen.

In dem Distrikt, in welchem diese Krankheit beobachtet wird, wurde der Kupfer- und Zinkgehalt im Heu und Gras als niedrig befunden, das Kupferniveau lag nämlich durchschnittlich unter 5 p.p.m. und dasjenige von Zink unter 40 p.p.m.

Eine Kupferbehandlung scheint mitunter eine schwache Besse-

rung des Zustandes zu geben, oft ist eine solche Therapie jedoch ohne Effekt. Anderseits ergibt die orale Verabreichung einer Mischung von Kupfer und Zink, als Sulfat oder Acetat, eine bemerkenswerte Wirkung. Zirka 30 Fälle wurden auf diese Weise behandelt, und zwar alle mit demselben günstigen Resultat.

In einem Fütterungsversuch mit 4 Ochskälbern zeigte das eine Tier, das Kupferzuschuss erhielt, eine Gewichtszunahme, die derjenigen des auf der Grunddiät gehaltenen Kontrolltieres gleichkam oder etwas höher lag. Diese Grunddiät bestand aus kupfer- und zinkarmem Heu aus dem Mangelgebiet. Die beiden übrigen Tiere, die Zinkzuschuss als Sulfat und Acetat erhielten, zeigten gleich nach Einsetzen des Zinkzuschusses eine Wachstumsstagnation. Nachdem dem einen von diesen Tieren im letzten Teil des Versuches Kupfersulfat als Zuschuss zum Zinksulfat verabreicht worden war, stieg das Gewicht plötzlich und markiert an.

In diesem Artikel wird eine Anzahl von analytischen Daten vorgelegt, die ein Bild des Mineralgehalts im Heu und Gras aus diesem Mangelgebiet geben, und es wird diskutiert, inwieweit andere Elemente in dem vorliegenden Mangelsyndrom von Bedeutung sein können.

SAMMENDRAG

Innbyrdes virkning av sink og kopper i ernæringen hos storfe. En kompleks sink-koppermangel.

Artikkelen beskriver en sink-koppermangel hos ungfe. Symptomene er generell utrivelighet, redusert vekst og en avbleking av hårlaget rundt øynene. Av og til iakttas også hudforandringer på neseryggen fra grenselinjen mellom nese og mule. Det siste symptom har en viss likhet med parakeratotiske hudforandringer.

I det distrikt hvor denne sykdommen er observert, er kopper- og sinknivået i høy og gras funnet å være heller lavt, i middel under 5 p.p.m. kopper og 40 p.p.m. sink.

Kopperbehandling synes av og til å gi en svak bedring av tilstanden, men ofte er en slik behandling uten effekt. På den annen side, når der oralt gis en blanding av kopper og sink, som sulfat eller acetat, er virkningen bemerkelsesverdig. Ca. 30 kasus er behandlet på denne måte, alle med det samme gunstige resultat.

Ved et fôringsforsøk på 4 oksekalver ble det vist at det ene dyr, som fikk koppertilførsel, hadde en vektøkning lik, eller noe høyere enn kontrolldyret, som ble holdt på basaldietten. Denne besto av kopper- og sinkfattig høy fra mangelområdet. De to øvrige dyr, som ble gitt sinktilskudd som sulfat og acetat, stagnerte i vekst straks etter at sinktilførselen startet. Da det ene av disse dyr, i siste del av forsøket, ble gitt koppersulfat i tillegg til sinksulfat, steg vekten plutselig og markert.

I artikkelen fremlegges et antall analytiske data som gir et bilde av mineral status i høy og gras fra dette mangelområdet, og det diskuteres hvorvidt andre elementer kan være av betydning i det foreliggende mangelsyndrom.

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