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COPPER, ZINC AND MOLYBDENUM IN LIVERS OF NORWEGIAN CATTLE AT SLAUGHTER

By

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FRØSLIE, ARNE, GUNNAR NORHEIM and ERLING WAASJØ: *Copper, zinc and molybdenum in livers of Norwegian cattle at slaughter.* Acta vet. scand. 1980, 21, 62—70. — The concentrations of copper, zinc and molybdenum were measured in samples of cattle liver from 10 slaughter-houses in Norway. A total of 335 samples were analysed. A clear accumulation of copper with age was found, the average copper level in the younger animals (≤ 3 years, $n = 194$) being $30 \mu\text{g Cu/g}$ liver wet weight, and in the older ones (> 3 years, $n = 141$) $59 \mu\text{g Cu/g}$. The range in the copper values found was considerable, though significant differences between some of the districts were recorded. Copper concentrations were classified as low ($\leq 10 \mu\text{g Cu/g}$) in 9.6 % of the samples. Zinc showed no accumulation with age, nor were there any differences in zinc levels found in animals from different districts, the average level being $32 \mu\text{g Zn/g}$ liver wet weight. The picture was the same for molybdenum, no differences between age groups or districts being found. The average level was $1.0 \mu\text{g Mo/g}$ liver. There was no significant correlation between levels of copper, zinc or molybdenum.

The supply of copper and zinc to cattle in Norway seems close to sufficient, but copper- and zinc-fortified mineral supplementation of cattle feed is still to be recommended. There seems to be no need for molybdenum supplementation in cattle.

copper; zinc; molybdenum; cattle; liver.

Copper-related deficiency diseases in cattle in Norway were first described by *Ender* in 1942. Since then he and his coworkers have reported copper deficiency in cattle in several districts in the country (*Havre et al.* 1960, *Havre & Dynna* 1961, *Dynna & Havre* 1963, 1966, *Havre* 1970). These reports were mainly based on clinical examinations of the animals and chemical analysis of grass. Only a very few analyses of liver or blood samples have been carried out.

In the present paper, the results of analyses of bovine liver for copper and for the interfering elements zinc and molybdenum are reported.

MATERIALS AND METHODS

Samples of bovine liver were collected at 10 slaughter-houses, the locations of which are given in Table 1. The samples originated from slaughtered animals varying in age from 3/4 to 7 years according to estimations by the meat inspectors. Copper, zinc and molybdenum levels in the liver samples were determined as described by *Frøslie & Norheim (1976)* and *Norheim & Waasjø*

Table 1. The concentrations of copper, zinc and molybdenum ($\mu\text{g/g}$ wet weight) in bovine liver from 10 districts in Norway. The copper levels are grouped according to age. The number of samples with copper concentrations below 10 $\mu\text{g/g}$ are given.

Location of slaughter-houses	Copper					Zinc			Molybdenum		
	age (years)	number	mean $\mu\text{g/g}$	range	number $\leq 10 \mu\text{g/g}$	number	mean $\mu\text{g/g}$	range	number	mean $\mu\text{g/g}$	range
Sarpsborg	≤ 3	18	31	8.6—81	2	30	34	25—97	10	1.1	0.8—1.3
	> 3	12	61	16—97	0						
Oslo	≤ 3	18	39	6.4—97	1	30	32	14—44	10	1.0	0.6—1.1
	> 3	12	85	37—105 ¹	0						
Lillehammer	≤ 3	23	35	10—69	2	30	31	25—51	10	1.1	0.9—1.2
	> 3	7	82	43—120	0						
Skien	≤ 3	21	26	1.7—43	3	42	32	25—102			
	> 3	21	58	4.0—120	1						
Stavanger	≤ 3	17	18	3.4—45	5	32	30	24—44	10	1.0	0.7—1.2
	> 3	15	51	6.8—98	1						
Bergen	≤ 3	17	34	7.8—82	2	30	30	14—54	10	1.0	0.7—1.1
	> 3	13	53	6.4—102	2						
Ålesund	≤ 3	20	37	14—80	0	41	33	25—90			
	> 3	21	74	13—107	0						
Steinkjer	≤ 3	21	30	3.0—71	2	30	32	23—49	10	1.0	0.8—1.1
	> 3	9	66	24—95	0						
Bodø	≤ 3	19	16	3.0—63	9	30	32	24—54	9	1.0	0.7—1.2
	> 3	11	37	12—68	0						
Tromsø	≤ 3	20	28	10—49	1	40	31	23—54			
	> 3	20	45	4.8—73	1						
Sum	≤ 3	194	30	1.7—97	27	335	32	14—102	69	1.0	0.6—1.3
	> 3	141	59	4.0—120 ¹	5						

¹ One single value of 240 $\mu\text{g Cu/g}$ is excluded from the calculations.

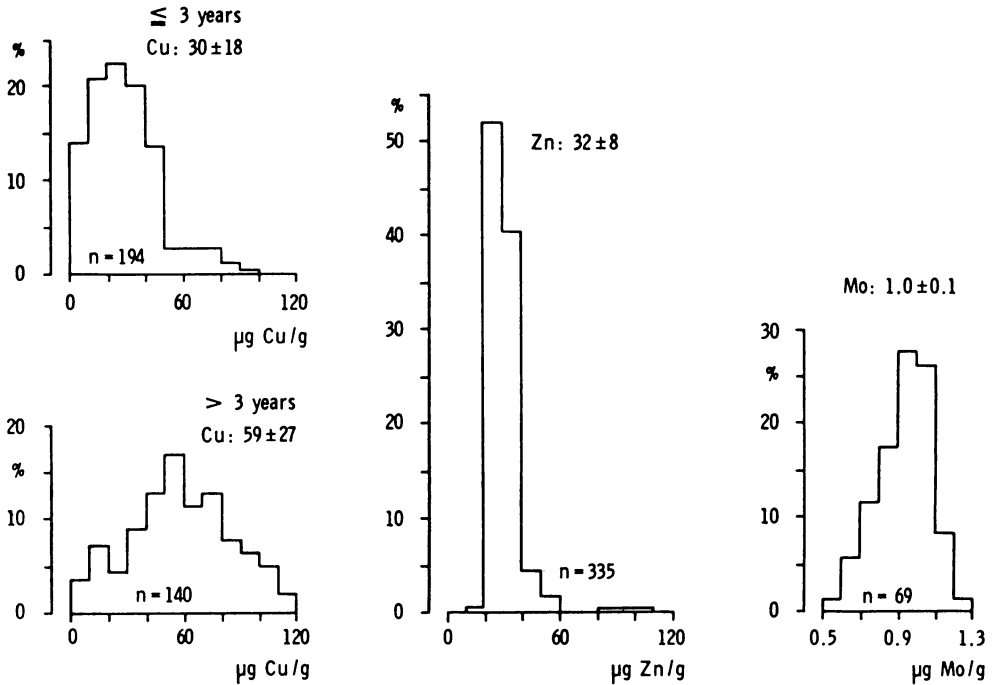


Figure 1. Histograms showing the distribution of samples as a function of the hepatic concentrations of copper (left), zinc (middle) and molybdenum (right) in Norwegian cattle at slaughter. The copper levels have been split up according to the age of the animals, those ≤ 3 years (upper curve) and those > 3 years of age (lower curve). The mean values, the standard deviations and the number of samples are given. One single copper concentration of 240 $\mu\text{g/g}$ in a sample from the group of older animals has been excluded from these calculations.

(1977). All concentrations are expressed on a wet weight basis in $\mu\text{g/g}$.

RESULTS

Analytical results are shown according to district in Table 1, while histograms of the samples are given in Fig. 1.

As the variation in liver copper concentration varied with age, the material was split in two age groups, animals younger or older than three years. Liver copper levels in the older animals were about twice as high as those in the younger animals, 59 ± 27 $\mu\text{g/g}$ and 30 ± 18 $\mu\text{g Cu/g}$, respectively. There were great variations within the groups from the different districts and a con-

siderable overlapping in the range of levels recorded. The differences between the two age groups were significant for all districts ($P < 0.05$ — $P < 0.001$).

There was also certain differences in the copper concentration in animals from different districts. The values recorded in young animals at Stavanger and Bodø were only about half those from other districts ($P < 0.05$ — $P < 0.001$). The differences recorded between the other eight districts were not significant. The differences in copper levels are also illustrated by the frequencies of samples below 10 $\mu\text{g/g}$. As seen from the table, the number of such samples was highest in Stavanger and Bodø. Copper levels below 10 $\mu\text{g/g}$ were found in 27 samples (14 %) from young animals, while in older animals only five samples (about 3.6 %) were below this level.

The zinc values varied less than the copper values. The mean value for all samples was $32 \pm 8 \mu\text{g Zn/g}$. No significant differences were found between age groups or between different districts.

As regards molybdenum, there were no significant differences between age groups or geographical areas. Mean and standard deviation of all the samples ($n = 69$) was $1.0 \pm 0.1 \mu\text{g Mo/g}$. No significant correlation between molybdenum and zinc, molybdenum and copper, or copper and zinc was found in the present material ($r < 0.15$).

DISCUSSION

According to this investigation, the copper status in most Norwegian cattle is satisfactory, although copper levels in 9.6 % of the tested livers are considered to have been low ($\leq 10 \mu\text{g Cu/g}$).

The optimal level of copper in bovine liver seems to vary between 100 and 200 $\mu\text{g/g}$ dry weight (d.w.) (Rosenberger 1970, Blood *et al.* 1979). Applying a figure of about 30 % dry matter in bovine liver, this is equivalent to 30—60 $\mu\text{g/g}$ wet weight (w.w.). The authors mentioned above state the marginal level for deficiency to be 12 $\mu\text{g/g}$ d.w. ($\approx 3.5 \mu\text{g/g}$ w.w.). The *Committee on Mineral Nutrition* (1973) in the Netherlands states the normal value in bovine liver to be 150 $\mu\text{g Cu/g}$ d.w. ($\approx 45 \mu\text{g/g}$ w.w.), the marginal level 25 $\mu\text{g Cu/g}$ d.w. ($\approx 8 \mu\text{g/g}$ w.w.) and the deficiency level 3 $\mu\text{g Cu/g}$ d.w. ($\approx 1 \mu\text{g/g}$ w.w.). According to *Blood et al.* levels below 30 $\mu\text{g Cu/g}$ d.w. ($\approx 10 \mu\text{g Cu/g}$ w.w.) should

be classified as low. The last-mentioned level is used in the present investigation as a criterion for suboptimal copper supply.

As regards the copper status of domestic ruminants in Norway, there is a fundamental difference between sheep, on the one hand, and cattle and goats on the other (Frøslie & Norheim 1976, Frøslie 1977, Sjøli & Frøslie 1979). Average copper values in sheep are considerably higher than in goats and cattle. The range in values is wider, extending from submarginal levels to levels exceeding those at which toxicosis occurs. These differences probably reflect species differences in the ability to accumulate copper (Hill 1977 a, b), though nutritional considerations are probably also of some importance. These matters are not yet fully understood.

A difference in copper levels has been demonstrated between sheep from typical inland areas and those from coastal areas (Frøslie). Copper toxicosis in sheep is mainly a problem of typical inland areas. In the Rogaland county in southwestern Norway, copper levels in both sheep and cattle are relatively low. In other districts the copper status of sheep does not relate very well to that of cattle. For example, copper levels in sheep from the Bodø region are rather high, and yet values found in cattle from this region were lower than in any of the other groups of cattle examined. This may reflect pronounced regional differences concerning natural and cultivated pastures, sheep grazing to a large extent on natural pastures. The fact that regional differences were less pronounced in cattle may also be due to more extensive use of concentrates in cattle than in sheep. Only small amounts of concentrates are used in sheep, while about half the total amount of concentrates distributed in Norway is included in standard feed mixtures for cattle (Statens Kornforretning 1978). These mixtures contain copper and other minerals. Standard mineral mixtures for ruminants and horses contain 1,000 mg Cu/kg. Mineral supplements which have not been standardized usually also contain copper. Had these sources of copper been in general use in cattle nutrition in Norway, regional differences in bovine copper status would have been small, and problems related to deficient copper supply would probably not have arisen. However, the present investigation has shown that copper levels are still somewhat low in parts of the Norwegian cattle population, especially in the younger animals. The age-related copper accumulation in cattle corresponds to the situa-

tion in sheep (*Frøslie & Norheim 1976*) and swine (*Frøslie & Norheim 1977*). Cases of chronic sickness responsive to copper supplementation have been reported in certain districts of the country (*Øverby* personal communication 1979, *Larssen* personal communication 1979). Such reports supply evidence of occurrence of copper deficiency disorders.

Rosenberger reported a zinc level in livers of healthy calves of 125–170 $\mu\text{g Zn/g d.w.}$ (∞ 38–51 $\mu\text{g/g w.w.}$), while the concentration in livers of calves suffering from parakeratosis was 100–125 $\mu\text{g/g d.w.}$ (∞ 30–38 $\mu\text{g/g w.w.}$). If these deficiency limits are relevant for cattle at slaughter, a large part of the cattle population in Norway is deficient in zinc. More than 50 % of the liver samples were below 30 $\mu\text{g Zn/g w.w.}$ The local occurrence in Norway of a complex zinc-copper deficiency condition has been described (*Dynna & Havre 1963*), but apart from this, zinc deficiency in cattle is not considered to be a problem in Norway (*Krogh* personal communication 1979). Standard mineral mixtures contain 2,400 mg Zn/kg. Liver levels of zinc in cattle are, however, significantly lower than those in sheep and swine (*Frøslie & Norheim 1976, 1977*), but about the same as those found in goat (*Søli & Frøslie*). The distribution of copper- and zinc-binding proteins in cattle liver with zinc concentrations below 40 $\mu\text{g/g}$ (*Mjør-Grimrud & Norheim 1980*) was quite similar to that found in zinc deficient sheep by *Bremner & Marshall (1974)*.

Hellesnes et al. (1975) found regional differences in zinc concentrations in bovine liver. Corresponding variations were not found in the present investigation. The lowest levels found by *Hellesnes et al.* were in liver samples from the Lillehammer region, $133 \pm 24 \mu\text{g Zn/g d.w.}$ (∞ 40 $\mu\text{g/g w.w.}$), and the highest in samples from the Tromsø region, $250 \pm 38 \mu\text{g Zn/g d.w.}$ (∞ 75 $\mu\text{g/g w.w.}$). These figures are considerably higher than in the present material.

According to *Underwood (1977)* molybdenum levels in the livers of a majority of animal species are in the range 2–4 $\mu\text{g/g d.w.}$ (∞ 0.6–1.2 $\mu\text{g/g w.w.}$). The levels found in the present investigation, and those found in other farm animals (*Frøslie & Norheim 1976, 1977, Søli & Frøslie*), are within these limits. *Rosenberger* states that the molybdenum levels in cases of clinical molybdenosis lie between 2.5 and 5.0 $\mu\text{g Mo/g liver tissue w.w.}$ Such high levels have, to the authors' knowledge, never

been measured in bovine liver in Norway. *Brockman* (1977), however, reported that 10 of 40 livers of slaughtered cattle from Saskatchewan, Canada, had molybdenum levels greater than 2.0 µg/g w.w. In the same material, comprising 152 samples, he found that 67 % of the samples had copper concentrations below 10 µg/g liver w.w. In such districts molybdenum loading is probably a major factor in the development of copper deficiency. This is in contrast to the situation in Norway.

It can be concluded from the present investigation that the supply of copper and zinc to the majority of cattle in Norway is close to sufficient, but copper- and zinc-fortified mineral supplementation is still to be recommended. There seems to be no need for molybdenum supplementation in cattle.

REFERENCES

- Blood, D. C., J. A. Henderson & O. M. Radostits*: Veterinary Medicine. A textbook of the diseases of cattle, sheep, pigs and horses. 5th Ed., Baillière Tindall, London 1979, 1135 pp.
- Bremner, I. & R. B. Marshall*: Hepatic copper- and zinc-binding proteins in ruminants. 1. Distribution of Cu and Zn among soluble proteins of livers of varying Cu and Zn content. *Brit. J. Nutr.* 1974, 32, 283—291.
- Brockman, R. P.*: Concentration of copper in livers of Saskatchewan cattle at slaughter. *Canad. vet. J.* 1977, 18, 168—170.
- Committee on Mineral Nutrition*: Tracing and treating mineral disorders in dairy cattle. Centre for Agricultural Publishing and Documentation. Wageningen 1973, 27—31.
- Dynna, O. & G. N. Havre*: Interrelationship of zinc and copper in the nutrition of cattle. A complex zinc-copper deficiency. *Acta vet. scand.* 1963, 4, 197—208.
- Dynna, O. & G. N. Havre*: Some observations of a complex zinc-copper deficiency in cattle. 9th Int. Grassld Congr. Sao Paulo 1964. *Proc.* 1966, 8; *i.p.*; 498, 717—721.
- Ender, F.*: Undersøkelser over slikkesykens etiologi i Norge. (Investigations on the etiology of pica in Norway). *Norsk Vet.-T.* 1942, 54, 3—27, 78—127, 137—158.
- Frøslie, A.*: Kobberstatus hos sau i Norge. (Copper status of sheep in Norway). *Norsk Vet.-T.* 1977, 89, 71—79.
- Frøslie, A. & G. Norheim*: The concentrations of molybdenum and zinc in liver in relation to copper accumulation in normal and copper poisoned sheep. *Acta vet. scand.* 1976, 17, 307—315.
- Frøslie, A. & G. Norheim*: The concentrations of copper, zinc and molybdenum in swine liver and the relationship to the distribution of soluble copper- and zinc-binding proteins. *Acta vet. scand.* 1977, 18, 471—479.

- Havre, G. N.*: A combined copper-zinc deficiency in cattle: Clinical symptoms. In Trace Element Metabolism in Animals. — 1st Ed., C. F. Mills, E. & S. Livingstone, Edinburgh and London 1970, 117—119.
- Havre, G. N. & O. Dynna*: The occurrence of conditioned and simple copper deficiency in cattle and sheep in Setesdalen, a valley in the southern part of Norway. II. Acta vet. scand. 1961, 2, 375—398.
- Havre, G. N., O. Dynna & F. Ender*: The occurrence of conditioned and simple copper deficiency in cattle and sheep in Setesdalen, a valley in the southern part of Norway. Acta vet. scand. 1960, 1, 250—276.
- Hellesnes, I., B. Underdal, G. Lunde & G. N. Havre*: Selenium and zinc concentrations in kidney, liver and muscle of cattle from different parts of Norway. Acta vet. scand. 1975, 16, 481—491.
- Hill, R.*: Copper toxicity. I. Brit. vet. J. 1977 a, 133, 219—224.
- Hill, R.*: Copper toxicity. II. Brit. vet. J. 1977 b, 133, 365—373.
- Mjør-Grimsrud, M. & G. Norheim*: Fractionation of soluble copper- and zinc-binding proteins from cattle liver. Acta vet. scand. 1980, 21, 71—78.
- Norheim, G. & E. Waasjøl*: On the determination of molybdenum with dithiol in biological materials with high levels of copper and iron. Fresenius Z. anal. Chem. 1977, 286, 229—231.
- Rosenberger, G.*: Krankheiten des Rindes. (Diseases of cattle). Paul Parey, Berlin 1970, 1390 pp.
- Statens kornforretning*: Korn, mel, kraftfôr. (Grain, flour, concentrates). Utdrag av årsmelding 1977. Oslo 1978, 28 pp.
- Sjøli, N. E. & A. Frøslie*: Copper, zinc and molybdenum in goat liver. Acta vet. scand. 1979, 20, 45—50.
- Underwood, E. J.*: Trace elements in human and animal nutrition. 4th Ed., Acad. Press, New York 1977, 545 pp.

SAMMENDRAG

Kobber, sink og molybden i lever fra norsk storfeslakt.

Det ble samlet i alt 335 leverprøver fra storfe ved 10 forskjellige slakterier i Norge. Dyrenes alder ble anslått ved kjøttkontrollen. Alle prøvene ble analysert med hensyn på kobber og sink. Molybdeninnholdet ble bestemt i 69 prøver. For alle distriktene ble det påvist en klar kobberakkumulering med alderen. Gjennomsnittsnivået for unge dyr (≤ 3 år, $n = 194$) var $30 \mu\text{g Cu/g}$ lever våtvekt, mens det for eldre dyr (> 3 år, $n = 141$) var $59 \mu\text{g Cu/g}$ lever. Det var en betydelig spredning i kobbernivåene i leverprøvene fra de forskjellige slakteriene. For unge dyr slaktet i Bodø og Stavanger var alikevel kobbernivåene signifikant lavere enn i unge dyr slaktet ved andre slakterier. I 9,6 % av prøvene må kobbernivåene klassifiseres som lave ($\leq 10 \mu\text{g Cu/g}$). For sink ble det ikke funnet noen akkumulering med alderen. Det ble

heller ikke funnet noen forskjeller i sinknivået i prøvene fra de forskjellige distriktene. Det gjennomsnittlige sinknivået var $32 \mu\text{g Zn/g}$ lever våtvekt. Heller ikke for molybden ble det funnet noen aldersakkumulering eller noen forskjeller mellom distriktene. Det gjennomsnittlige molybdennivået var $1,0 \mu\text{g Mo/g}$ lever. Det ble ikke funnet noen korrelasjon mellom konsentrasjonene av kobber, sink eller molybden i prøvene.

Tilskuddet av kobber og sink til storfe i Norge er nær opp til behovet, men det må fortsatt anbefales å bruke mineralblandinger tilsett kobber og sink. Det synes ikke å være noe behov for tilskudd av molybden til storfe.

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