Acta vet. scand. 1977, *18*, 471—479.

From the National Veterinary Institute, Oslo, Norway.

THE CONCENTRATIONS OF COPPER, ZINC AND MOLYBDENUM IN SWINE LIVER AND THE RELATIONSHIP TO THE DISTRIBUTION OF SOLUBLE COPPER- AND ZINC-BINDING PROTEINS

By

Arne Frøslie and *Gunnar Norheim*

FRØSLIE, ARNE and GUNNAR 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. — The concentrations of copper, zinc and molybdenum were measured in liver samples from 21 normal slaughter pigs (average age about 6 months) and in 36 sows (average age about 2 years). The following mean values were found: Slaughter pigs: 15 ± 8 μg Cu/g, 45 ± 7 μg Zn/g and 1.0 ± 0.2 μg Mo/g wet weight; sows: 46 ± 70 μg Cu/g, 70 ± 26 μg Zn/g and 1.3 ± 0.3 μg Mo/g wet weight. The concentrations of all 3 elements were significantly higher in the sows than in the young pigs. There was no correlation between the concentrations of copper, zinc or molybdenum. The recorded copper levels in the slaughter pigs were in accordance with the levels of non-supplemented pigs given in the literature. The soluble hepatic copper- and zinc-binding proteins were separated into 3 different fractions by gel filtration. With increasing copper and zinc levels in the liver, a higher relative amount of these elements were found in the low molecular weight fraction.

c o p p e r; z i n c; m o l y b d e n u m; s w i n e; l i v e r; p r o t e i n b i n d i n g.

It is well known that tolerance to feed copper supplementation varies among animal species. Sheep, especially, are very susceptible to copper, and chronic cumulative poisoning may arise at levels higher than 20—30 mg Cu/kg in the feed (*Clarke & Clarke* 1975). Swine, on the other hand, have a higher tolerance to copper, and supplementation as high as 250 mg Cu/kg fodder is used as a feed additive to growing swine, to improve daily weight gain and feed conversion efficiency without significant risk of toxic accumulation of copper (*Meyer & Kröger* 1973, *Braude* 1975, *Castell et al.* 1975, *Hansen & Bresson* 1975, *Prothmann* 1975).

The accumulation of copper in the liver is an essential step in copper toxicosis (*Clarke & Clarke*), and hepatic copper levels, as well as the levels of zinc and molybdenum in sheep in Norway, have been investigated previously (*Frøslie & Norheim* 1976, *Frøslie* 1977). The distribution of the soluble hepatic proteins binding these elements has also been studied (*Norheim & Søli* 1977, *Søli et al.* 1977).

In this article, the results of similar investigations on swine liver are presented.

MATERIALS AND METHODS

Liver samples from 21 normal slaughter pigs (about 6 months old) and 36 sows (average age about 2 years) were collected at a slaughter-house in Oslo. The total concentrations of copper, zinc and molybdenum were determined as described by *Frøslie & Norheim* (1976) and *Norheim & Waasjø* (1977). Selected samples from 5 slaughter pigs and 11 sows were subjected to gel filtration on Sephadex G-75 Superfine (Pharmacia). The analytical procedures have been described previously (*Norheim & Steinnes* 1975, 1976, *Norheim & Søli* 1977). Copper and zinc were determined by atomic absorption spectroscopy by direct aspiration of the eluate samples.

RESULTS

The mean values and the concentration ranges of copper, zinc and molybdenum in the livers of the slaughter pigs and the sows are presented in Table 1. As seen from the table, all the elements are present in higher concentrations in the sows than in the slaughter pigs, the differences being highly significant for zinc and molybdenum and also significant for copper. Only small variations were seen in the concentrations of the 3 elements in the group of slaughter pigs, while the concentrations in sows' livers varied within rather wide limits. There was no correlation between the copper and zinc on one hand, and copper and molybdenum concentrations on the other. The correlation coefficients were 0.26 and 0.18, respectively.

The soluble hepatic copper-binding proteins were separated

Table 1. The mean values and concentration ranges (μg/g wet weight) of copper, zinc and molybdenum in the liver of slaughter pigs (average age about 6 months) and sows (average age about 2 years). The differences between the 2 groups of swine were tested statistically (*t*-test) and the levels of significance (P) are indicated in the table.

		Copper		Zinc		Molybdenum	
	Number	mean ± s	range	mean ± s	range	mean ± s	range
Slaughter pigs	21	15 ± 8	6—35	45 ± 7	33—62	1.0 ± 0.2	0.6—1.3
Sows	36	46 ± 70	4—310	70 ± 26	30—148	1.3 ± 0.3	0.8—1.9
P (*t*-test)		< 0.05		< 0.001		< 0.001	

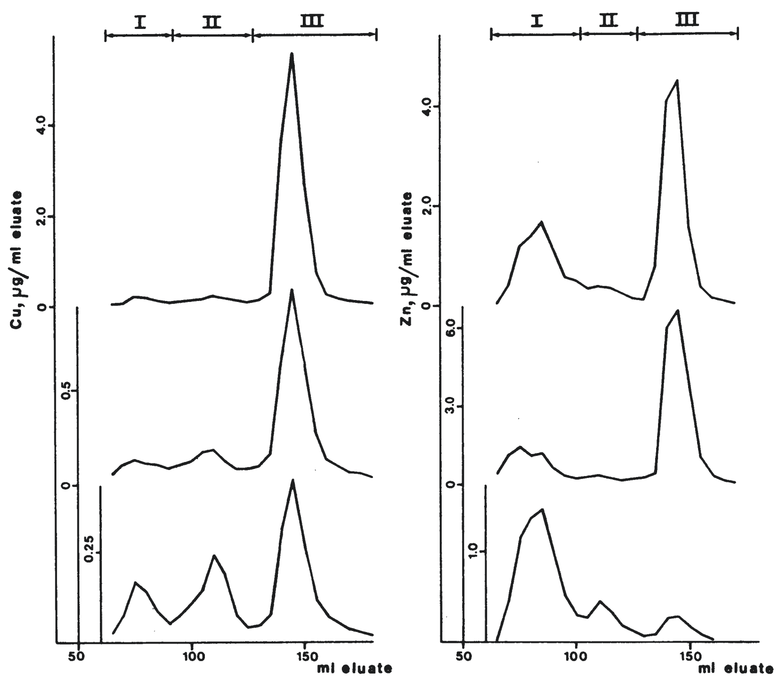

Figure 1. The distribution of copper- (left) and zinc-binding proteins (right) from swine livers after gel filtration on Sephadex G-75. Lower curves: 12 μg Cu/g and 30 μg Zn/g. Middle curves: 41 μg Cu/g and 148 μg Zn/g. Upper curves: 310 μg Cu/g and 93 μg Zn/g. The borders between Fractions I, II and III are indicated on top of the figure. The ordinate axes are individually dimensioned.

into 3 different fractions. The approximate molecular weights were > 65,000 (Fraction I), 35,000 (Fraction II), and 10,000 (Fraction III). Generally, the amount of copper in each fraction increased with increasing liver copper levels, but relatively, only Fraction III copper concentration increased. This latter fraction contained more than half of the copper present in the soluble protein fraction.

The soluble hepatic zinc-binding proteins were also separated into 3 main fractions with the same approximate molecular weights as the copper-binding proteins. The position of the maximum zinc concentration in the first high molecular weight fraction varied slightly with the zinc level. The amount of zinc found in the first 2 fractions (Fractions I and II) showed rather small variations, while in Fraction III the concentration increased considerably with increasing zinc levels. In relative terms, however, a marked decrease was seen in Fraction I, while a marked increase was seen in Fraction III.

Table 2. The relative amounts of copper in Fractions I, II and III after gel filtration on Sephadex G-75 at different levels of total copper in 7 representative liver samples.

Cu (μg/g wet weight)	9	12	32	57	95	225	310
Fraction I (%)	21	16	11	9	6	4	5
Fraction II „	25	29	16	22	12	5	6
Fraction III „	54	55	73	69	82	91	89

Table 2 gives the relative amounts of copper in Fractions I, II and III at different hepatic copper levels. The samples are representative for the whole group investigated, and no systemic differences were seen between slaughter pigs and sows. The volumes eluted in each fraction are indicated in Fig. 1. Table 3 gives the relative amounts of zinc in the 3 fractions at different zinc levels. The selected samples reflect the variations in copper and zinc levels shown in Table 1.

In the liver specimens investigated by gel filtration, there was a good correlation between the amount of copper or zinc found in Fraction III and the total hepatic concentration of copper or zinc, the correlation coefficients being 0.93 and 0.97, respectively.

Table 3. The relative amounts of zinc in Fractions I, II and III after gel filtration on Sephadex G-75 at different levels of total zinc in 7 representative liver samples.

Zn (μg/g wet weight)	30	41	48	55	88	105	148
Fraction I (%)	83	71	55	54	40	35	27
Fraction II „	13	11	11	6	3	6	7
Fraction III „	4	18	34	40	57	59	66

The amount of copper recovered in the protein extract decreased with increasing liver copper levels, while the extractable amount of zinc showed small variations. All copper and zinc present in the protein extract was recovered at an elution volume less than 200 ml on gel filtration.

A very careful examination of Fraction III in the copper- and zinc-protein spectra revealed that there seemed to be a slight shift in the maximum concentrations of the 2 elements. The maximum concentration was, in most cases, found in the same 5 ml fraction, but the shape of the peak was slightly different. This may indicate that more than 1 metal-binding protein may be present in this low molecular weight fraction.

DISCUSSION

The hepatic concentrations of zinc and molybdenum, and especially copper, seem to increase with increasing age of the pigs. However, high level copper supplementation is not in use in Norway, and copper, as sulphate, is added to the swine diets at concentrations of only 7.5—12.5 mg Cu/kg. There is probably no significant difference in the copper supply of growing swine and sows.

The low, and rather constant, level of copper in the livers of slaughter pigs, a total range of 6—35 μg/g wet weight, is in good agreement with the copper status of the diet, and corresponds very well with the levels of non-supplemented growing swine reported in the literature (*Meyer & Kröger* 1973, *Prothmann* 1975). According to *Prothmann,* who refers to more than 30 authors, the liver copper concentration in slaughter pigs on non-supplemented diets is 36 (9—170) μg Cu/g dry weight, (about 11 (3—51) μg Cu/g wet weight*), while a supplemen-

* The dry weight in liver is estimated to be about 30 %.

tation of 250 mg Cu/kg fodder, as sulphate, results in liver concentrations of 708 (32—4657) μg Cu/g dry weight (about 210 (10—1400) μg Cu/g wet weight). It is obvious from these data that there are very great differences in the accumulation of copper from high-level supplementation diets from one experiment to another. Likewise, there is a great difference between different copper compounds in their tendency to accumulate in the body (*Prothmann, Meyer et al.* 1976). *Hansen & Bresson* (1975) recorded only a small elevation of the liver copper concentration, from 4.1 to 6.1 μg Cu/g wet weight, in bacon pigs supplemented with 32 mg Cu/kg fodder, as sulphate. *Castell et al.* (1975) analyzed 500 liver samples from growing-finishing pigs from 100 swine units in Canada, after supplementation with 0.05 % or 0.08 % copper sulphate (125 or 200 mg Cu/kg) in the feed. Six % of the samples contained levels in excess of the Canadian tolerance limit (150 mg Cu/kg fresh weight), and most of these were from pigs fed the 0.08 % copper supplement.

The concentrations of zinc and molybdenum in the livers of slaughter pigs are also in agreement with the values given in the literature (*Underwood* 1971). According to this author, the normal value for zinc in swine liver is 40 μg/g wet weight. *Prothmann* gave values from about 80—250 μg Zn/g dry weight (about 24—75 μg Zn/g wet weight), and in her own experiments, she found 180 μg/g dry weight on average (about 54 μg/g wet weight).

The high liver copper concentration in some of the sows in the present investigation is somewhat surprising taking into account the low copper supplementation. It seems that when the swine have passed the growth period they are able to accumulate copper, as well as zinc and molybdenum, to a higher degree than during the growth phase.

It has been shown that copper supplementation also increases the liver zinc concentration (*Prothmann, Hansen & Bresson*). However, no correlation was found between copper and zinc, or copper and molybdenum in the present investigation. Neither did any correlation exist between copper and zinc, nor copper and molybdenum in adult sheep (*Frøslie & Norheim* 1976).

The distribution of the copper- and zinc-binding proteins, into 3 distinct fractions, is in good agreement with the results presented by *Bremner* (1976). In pigs fed a low zinc diet, however, *Bremner* showed that very little copper and zinc was found in the low molecular weight fraction (Fraction III). In the present investigation, it was shown that, in normal slaughter pigs and sows, the main part of the copper present in the soluble protein fraction is bound to low molecular weight proteins. With increasing copper and zinc levels, an increasing percentage of the copper and zinc was found in the low molecular weight fraction. It is assumed that metallothionein-like proteins are responsible for this binding.

Although the variation in the hepatic zinc level is less than the corresponding variation in the copper level, a very marked change was seen in the zinc-protein spectra. The greatest variation is seen in the low molecular weight zinc-binding fraction.

Taking previous investigations on slaughter sheep into consideration (*Frøslie & Norheim, Norheim & Søli* 1977), it can be concluded that the hepatic copper concentration range is much greater in sheep than in pigs. However, a greater range in the zinc and molybdenum levels is seen in pigs. Similarities are also seen in the copper-protein spectra, but in sheep, the zinc-protein spectra were dependent on the copper status, and zinc disappeared from the low molecular weight fraction when the hepatic copper level increased.

It may be concluded that the low molecular weight copper- and zinc-binding protein fraction is important in the detoxication, metabolism and storage of excess intake of the 2 elements. It has also been demonstrated that the amounts of copper and zinc in the 3 metal-binding fractions are, contrary to the results for sheep, independent of each other. Finally it is assumed that several low molecular weight proteins are responsible for the metal-binding capacity in Fraction III.

ACKNOWLEDGEMENT

The authors are indepted to Milica Mjør-Grimsrud, civ.ing., for carrying out the gel filtration analyses.

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SAMMENDRAG

Kobber, sink og molybden i svinelever og relasjonen til fordelingen av kobber og sink i løselige proteinfraksjoner.

Kobber, sink og molybden er analysert i lever fra 21 slaktegriser og 36 purker slaktet i Oslo. For slaktegris, som vanligvis slaktes ved 6 måneders alder, fikk en følgende resultater: 15 ±8 µg Cu/g, 45 ± 7 µg Zn/g og 1.0 ± 0.2 µg Mo/g beregnet på våtvekt. Purker som føres til slakt i Norge har en gjennomsnittsalder på ca. 2 år og for disse fikk en følgende resultater: 46 ± 70 µg Cu/g, 70 ± 26 µg Zn/g og 1.3 ± 0.3 µg Mo/g beregnet på våtvekt. Det var signifikant forskjell på de 2 gruppene for alle 3 elementene. Kobberinnholdet i lever fra slaktegris stemte bra med angivelser for gris uten spesielt kobbertilskudd som finnes i litteraturen. Innholdet av sink og molybden stemte også bra med litteraturangivelsene. Kobbertilsetning, opptil 250 mg Cu/kg fór, nyttes som vekststimulerende fórtilsetning i mange land, men slik „high level" kobbertilsetning til fór er ikke tillatt i Norge. Ut fra dette er det overraskende med det høye kobberinnholdet i lever fra en del purker.

De løselige kobber- og sinkholdige leverproteiner ble separert i 3 distinkte fraksjoner ved gelfiltrering. Fraksjonenes omtrentlige molekylvekter var: fraksjon I > 65.000, fraksjon II 35.000 og fraksjon III 10.000. Ved økende innhold av kobber og sink i leveren var det en relativ økning av de respektive elementer i den lavmolekylære fraksjonen. Økende kobberinnhold i leveren hadde derimot tilsynelatende ingen innflytelse på fordelingen av sink, slik som det tidligere er påvist hos sau.

(Received June 6, 1977).

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