

## *Gyvulininkystė ir zootechnika • Animal Husbandry and Zootechnics • Животноводство и зоотехника*

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# Availability of inorganic and organic bound copper and zinc fed at physiological levels to fattening pigs

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To evaluate the availability of organic and inorganic copper and zinc, two experiments were conducted with 24 castrated crossbred pigs in each experiment, covering a body weight range from 71 kg to 81 kg. In both experiments, the animals were randomly allocated to 4 groups with 6 animals each. 10 mg copper and 30 mg zinc were added to the diets in experiment 1. In experiment 2, 20 mg copper and 70 mg zinc were supplemented. Copper and zinc were supplemented in both experiments as inorganic sulfates or as organic copper and zinc produced by chelation of copper and zinc with partially hydrolysed soybean protein in a 2 × 2 factorial arrangement. As the concentration of coeruloplasmin increased in experiment 1 and the concentration of copper in plasma was higher in experiment 2 when organic copper was supplemented, it can be concluded that organic copper has a higher availability as compared to inorganic copper. The addition of organic zinc to the diets increased copper digestibility in both experiments and plasma copper and coeruloplasmin in experiment 2. At 20 mg dietary copper and 70 mg dietary zinc, a higher digestibility of organic zinc could be observed as compared to inorganic zinc. It can therefore be concluded that the depressive effect of zinc on copper availability is lower for organic zinc as compared to inorganic zinc.

**Key words:** Cu / Zn availability, copper / zinc sulfate, organic copper, organic zinc, pig, digestibility, level in blood and tissues

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## INTRODUCTION

The negative effect of zinc on copper absorption and utilization is probably the most significant interaction among trace elements in monogastric animals. The antagonistic effect of excess dietary zinc on copper availability was first shown by Smith and Larson [34] in rats. The observation was confirmed in rats [18, 24, 28] and extended to chicks [35] sheep [31] and pigs [12, 30]. Zinc depresses copper absorption by enriching metallothionein in intestinal mucosa. This protein binds copper more strongly than zinc. Cu-metallothionein is not absorbed and is sloughed off with the mucosal cell [8, 10]. Providing copper in a readily available form not markedly influenced by copper antagonists could be an approach to overcome the depressive effect of zinc on the absorption of copper. The source of zinc might also have an influence on the interaction of copper and zinc. In different studies in pigs, copper and zinc amino acid complexes were compared to inorganic copper and zinc sulfate or copper and zinc oxide using either the rate of absorption and tissue concentration of

copper and zinc or animal performance as indicators of availability. Results of feeding trials reported previously are inconsistent. A comparison of organic to inorganic sources of copper was made, in most cases, at high levels of incorporation (>100 ppm) using weaned piglets. In several studies, an equal availability of copper–amino acid complexes was found as compared to copper sulfate [6, 37]. The same comparable results were reported [1] for finishing pigs. On the other hand [45], it was reported that pigs fed copper lysine had a higher daily gain than those fed copper sulfate, but liver copper was similar. When feeding 200 ppm copper [2], a one-fold higher concentration of copper in the liver of pigs fed copper lysine was found as compared to those fed copper sulfate. But for the levels of 100 and 150 ppm, liver copper did not differ between sources. In a new study [15], the efficacy of two chelated copper and zinc sources fed to weanling pigs was similar to inorganic sulfates in terms of growth performance. Furthermore, no differences in the availability of organic and inorganic zinc were reported by several research groups [5, 11, 29, 40] using physiological zinc concentrations in the feed. Some groups [41] observed a lower bone zinc when pigs were fed diets supplemented with zinc lysine compared to zinc sulfate. Feeding

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zinc at a pharmacological level [33] resulted in a higher zinc availability from zinc-sulfate as compared to zinc-lysine and zinc-methionine using liver and plasma zinc as indicators for the availability. No differences were found for bone zinc. Using high feed zinc levels [9] plasma zinc concentration in pigs fed zinc-methionine was higher than zinc oxide but equal to feeding zinc sulfate. Higher femoral and serum zinc were found, however [38], when feeding a zinc amino acid chelate compared to zinc sulfate. With rats [7], the utilization of copper from copper proteinate and copper lysine was higher than that of copper sulfate in the presence of high dietary zinc. These authors suggest that organic copper complexes might be absorbed via a mechanism which differs from inorganic copper and does not interfere with zinc. The aim of the present study was to assess the availability of copper and zinc in pigs fed diets with organic and inorganic copper and zinc supplements. For the evaluation of copper and zinc availability, the digestibility of copper and zinc was determined. In addition, the concentration of copper, zinc and coeruloplasmin as well as the activity of alkaline phosphatase in plasma were measured.

## MATERIALS AND METHODS

### Animals and diets

Two experiments were conducted with 24 castrated crossbred pigs (German Landrace × Pietrain) in each experiment. Animals were randomly allocated to 4 groups with 6 animals each in both experiments. In experiment 1 the diet was supplemented with 10 mg copper and 30 mg zinc. In experiment 2, 20 mg copper and 70 mg zinc were added to the diets. Animals in both experiments were housed individually in pens and fed diets based on corn, barley, broad beans, and soy bean meal (Table 1). The diets provided all nutrients as recommended by the National Research Council [24]. Copper and zinc were supplemented as copper and zinc sulfate or as organic copper and zinc produced by chelatisation of copper and zinc with partially hydrolyzed soy bean protein in a 2 × 2 factorial arrangement. The experimental design and chemical analysis of the diets is presented in Table 2. Daily feed allowance was restricted (90 g/kg<sup>0.75</sup>) to guarantee the complete consumption of feed. Water was offered ad libitum. The concentration of cop-

Table 1. Composition of the basal diet

Ingredient	g/kg
Corn	480
Barley	270
Soybean meal	140
Broad beans	50
Soybean oil	20
Premix <sup>a</sup>	40

<sup>a</sup> Mineral/vitamin premix supplied per kg: vit. A 12000 IU; vit. D 2000 IU; vit. E 75 mg; vit. K 3 mg; vit. B<sub>1</sub> 3 mg; vit. B<sub>2</sub> 8 mg; vit. B<sub>6</sub> 5 mg; vit. B<sub>12</sub> 40 µg; niacin 40 mg; Ca-D-panthothenate 20 mg; folic acid 1 mg; biotin 0.25 mg; choline chloride 400 mg; Se 0.4 mg; J 0.4 mg; butylhydroxytoluol 150 mg; L-lysine-HCl 2.3 g; methionine + cysteine 0.36 g; tryptophane 0.29 g; NaCl 1.37 g; CaCO<sub>3</sub> 5.01 g; Mn 10.2 mg; Fe 52 mg; TiO<sub>2</sub> 1g; copper sulfate or organic copper 10 and 20 mg; zinc sulfate or organic zinc 30 and 70 mg.

per and zinc in the drinking water did not reach detection limits. Pens were cleaned with drinking water each day. The air filter was checked and changed twice each week. 1 g TiO<sub>2</sub> per kg feed was used as an indigestible marker for the determination of the digestibility of copper and zinc in the body weight range of 71 to 81 kg. Faeces were collected 21 days after introduction of the experimental diets. They were spot-sampled for 10 days and stored at -20 °C until analysis. For the determination of copper, zinc, coeruloplasmin and the activity of the alkaline phosphatase in the plasma, blood from each pig was sampled from the anterior vena cava at the end of the experiment. Plasma was separated immediately by centrifugation for 10 minutes at 2000 g.

### Analytical methods

The crude nutrients of the diets were analysed according to a standard method [26]. For the determination of the minerals, trace elements and TiO<sub>2</sub> in the diets and in the faeces, the samples were ashed at 450 °C. For the measurement of copper and zinc in the plasma, 1 ml plasma was wetly digested at a temperature of 180 °C under pressure using nitric acid (suprapure) for oxidation. Mineral solutions and calibration standards from pure stock solutions were prepared in 1% nitric acid. Phosphorus was assayed by the vanadomolybdate procedure [26]. Sodium and potassium were determined by

Table 2. Chemical composition of the experimental diets (per kg dry matter)

Supplement per kg feed	10 mg Cu and 30 mg Zn				20 mg Cu and 70 mg Zn			
	Source of zinc <sup>a</sup>		Source of copper <sup>a</sup>		Source of zinc <sup>a</sup>		Source of copper <sup>a</sup>	
	I	O	I	O	I	O	I	O
Dry matter, %	90.2	89.9	90.0	90.1	90.1	90.2	90.2	90.1
Ash, g	50.5	46.8	49.4	50.8	51.2	53.4	48.5	47.0
Crude fat, g	52.8	51.1	54.3	55.9	55.5	53.5	51.2	51.7
Crude protein, g	174	175	174	170	175	173	173	175
Crude fiber, g	28.5	25.9	28.4	26.9	28.6	29.9	29.2	26.4
Na, g	2.88	2.65	2.95	2.98	2.74	2.86	2.57	2.93
K, g	8.12	8.20	8.38	8.27	8.30	8.51	8.44	8.56
Ca, g	6.90	6.85	6.88	6.91	6.93	6.95	6.89	6.82
Mg, g	1.70	1.69	1.72	1.66	1.68	1.65	1.69	1.61
P, g	5.38	5.60	5.37	5.56	5.51	5.49	5.55	5.39
Zn, mg	63.9	64.1	73.3	69.4	114.6	107.7	117.8	116.4
Cu, mg	14.9	14.8	19.4	17.9	25.4	24.5	28.2	27.9

<sup>a</sup> I = inorganic; O = organic.

Atomic Emission Spectrometry. The other elements, including TiO<sub>2</sub>, were measured by Atomic Absorption Spectrometry [26].

Immunoreactive coeruloplasmin was measured by rate nephelometry (Behring Nephelometer, BNA2) using reagents purchased from Behring (Marburg, Germany) according to the manufacturers' instructions.

Plasma alkaline phosphatase activity was determined using an enzymatic test kit of Boehringer Mannheim (MPR 3; 1442236). The results are expressed in units per litre (U/l). One unit is defined as the amount of enzyme required to produce 1 µmol of p-nitrophenol in 1 minute at 37 °C.

### Statistical analysis

Statistical analysis was carried out using the Mixed Procedure for analysis of variance of SAS (release 8.2). The effect of the source of copper and zinc and their interactions were evaluated for each experiment by a two-way analysis of variance. Least square means (LSmeans) and least significant differences (LSD) were computed for alpha = 0.05 for determination of statistical differences.

## RESULTS

Digestibility of copper and zinc, copper and zinc contents in the faeces, plasma zinc and copper, the activity of alkaline phosphatase and the concentration of coeruloplasmin in plasma are presented for the supplement of 10 mg copper and 30 mg zinc (experiment 1) in Table 3 and for the supplement of 20 mg copper and 70 mg zinc (experiment 2) in Table 4.

Organic copper decreased the digestibility of zinc and increased the concentration of coeruloplasmin in plasma as com-

pared to inorganic copper when 10 mg copper and 30 mg zinc were added to the diets. No effect on copper digestibility was found. Organic zinc increased the digestibility of copper as compared to inorganic zinc, but showed no effect on zinc digestibility or on the concentration of coeruloplasmin. No effects of the different sources of copper and zinc on plasma zinc and copper or on the activity of alkaline phosphatase were found.

After supplementing the diets with 20 mg copper and 70 mg zinc, organic copper increased the copper concentration in plasma as compared to inorganic copper. No effect of organic copper on copper and zinc digestibility or on the concentration of coeruloplasmin was found. When compared to inorganic zinc, organic zinc increased the digestibility of copper and zinc and decreased the content of copper and zinc in the faeces. Furthermore, plasma copper and coeruloplasmin were higher when organic zinc was added to the diets. No effects of the different sources of copper and zinc on plasma zinc or on the activity of alkaline phosphatase were found.

## DISCUSSION

### Parameters for the evaluation of the availability of copper and zinc

In this study, the digestibility of copper and zinc, the concentration of copper and zinc in plasma and the enzymes' alkaline phosphatase and coeruloplasmin were measured to evaluate the availability of copper and zinc from an organic and inorganic source. The digestibility of zinc and copper in pigs has been proved to be useful to assess copper and zinc availability [1, 27]. Coeruloplasmin and plasma copper, being closely correlated, are often used to assess copper status [17]. Coeruloplasmin, a

Table 3. LSmeans of apparent digestibility of copper and zinc, content of copper and zinc in the faeces, plasma zinc and copper and activity of alkaline phosphatase (AP) and concentration of coeruloplasmin (CP) in plasma of pigs fed 10 mg copper and 30 mg zinc in organic or inorganic form (n. s. = not significant; p > 0.1)

	Cu		Zn		LSD <sup>a</sup>	p		
	Organic	Inorganic	Organic	Inorganic		Cu	Zn	Cu / Zn
Digestibility of Cu, %	29.8	28.2	37.1	20.8	4.862	n. s.	<0.001	0.054
Cu in the faeces, mg/d	25.0	26.0	24.8	26.1	3.137	n. s.	n. s.	n. s.
Digestibility of Zn, %	19.6	24.2	23.1	20.6	3.886	0.025	n. s.	0.036
Zn in the faeces, mg/d	115.4	112.5	114.7	113.2	7.445	n. s.	n. s.	0.024
Plasma Zn, mg/l	0.58	0.61	0.61	0.58	0.103	n. s.	n. s.	n. s.
AP, U/l	112.0	122.5	119.5	115.0	27.92	n. s.	n. s.	n. s.
Plasma Cu, mg/l	1.86	1.79	1.90	1.74	0.185	n. s.	0.084	n. s.
CP, mg/dl	17.0	13.6	16.5	14.1	2.563	0.011	0.058	n. s.

<sup>a</sup> LSD = least significant difference. Variants follow the scheme of Table 2.

Table 4. LSmeans of apparent digestibility of copper and zinc, content of copper and zinc in the faeces, plasma zinc and copper and activity of alkaline phosphatase (AP) and concentration of coeruloplasmin (CP) in plasma of pigs fed 20 mg copper and 70 mg zinc in organic or inorganic form (n. s. = not significant; p > 0.1)

	Cu		Zn		LSD <sup>a</sup>	p		
	Organic	Inorganic	Organic	Inorganic		Cu	Zn	Cu / Zn
Digestibility of Cu, %	22.6	23.8	32.2	14.2	3.392	n. s.	<0.001	n. s.
Cu in the faeces, mg/d	45.4	45.3	43.8	46.8	2.609	n. s.	0.025	n. s.
Digestibility of Zn, %	19.9	21.7	25.7	15.9	4.714	n. s.	<0.001	n. s.
Zn in the faeces, mg/d	206.8	201.0	193.4	214.5	12.26	n. s.	0.002	n. s.
Plasma Zn, mg/l	0.84	0.78	0.83	0.79	0.094	n. s.	n. s.	n. s.
AP, U/l	143.8	148.4	156.8	135.3	22.24	n. s.	0.057	n. s.
Plasma Cu, mg/l	1.96	1.79	2.06	1.70	0.166	0.043	<0.001	n. s.
CP, mg/dl	15.1	14.4	15.7	13.9	1.454	n. s.	0.016	n. s.

<sup>a</sup> LSD = least significant difference. Variants follow the scheme of Table 2.

Cu-dependent enzyme with ferroxidase activity, oxidizes Fe(II), being released from intracellular ferritins with reduction and formation of chelates. So it binds to trans-ferrin, which exclusively binds Fe(III) [16]. A strict decline of zinc in plasma has been observed in zinc-deficiency including lambs [22], calves [19] and baby pigs [20]. Besides the concentration in bone, plasma zinc is commonly used to assess zinc status [13, 23, 41, 42]. Low zinc supply depresses alkaline phosphatase activity in the serum of baby pigs [20], in the blood, serum and bones of cows [14], serum of lambs [32] and ewes [3] and bones of turkey poults [39].

#### Availability of copper

In our study, we found an increase of the concentration of caeruloplasmin in experiment 1 and a higher copper plasma concentration in experiment 2 when organic copper was added to the diets. This indicates a higher availability of organic copper compared to inorganic. The lower digestibility of zinc in experiment 1, caused by the addition of organic copper, might provide evidence that organic copper has a higher antagonistic effect on zinc availability than inorganic copper, resulting in a higher availability of organic copper. In spite of the strong negative effect of zinc on copper availability, the converse effect of copper on zinc may also occur. A high copper / zinc ratio in rat intestinal segments decreased zinc absorption [4]. The decrease of zinc digestibility in the presence of organic copper was not found in experiment 2. Higher dietary zinc might overcome the antagonistic effect of organic copper on zinc availability. As the addition of organic zinc to the diets increased the digestibility of copper in both experiments and the concentration of caeruloplasmin and copper in plasma in experiment 2, the negative effect of organic zinc on copper availability could be lower as compared to inorganic zinc. It might be possible that organic zinc cannot induce high concentrations of metallothioneine in the intestinal mucosa. This protein binds copper stronger than zinc; Cu-methallothioneine is not absorbed and is sloughed off with the mucosal cells [8, 10].

#### Availability of zinc

At 10 mg copper and 30 mg zinc, no effect of the addition of organic zinc on zinc availability could be found, in terms of neither zinc digestibility nor of plasma zinc or alkaline phosphatase activity. On the other hand, 20 mg dietary copper and 70 mg dietary zinc resulted in a higher zinc digestibility and a lower zinc content in faeces when the diets were supplemented with organic zinc. Zinc is absorbed according to need. Animals adjust to the increasing dietary intake of zinc by reducing its absorption and enhancing excretion via faeces [43]. Increased absorption during zinc depletion and inhibition during zinc overload both occur rapidly within a week related to change in supply [21, 36]. In this study with 70 mg/kg, supply exceeded requirement and the absorption of zinc was probably reduced. 30 mg zinc might be insufficient, and optimal absorption results. It can be assumed that zinc absorption at 70 mg dietary zinc could only be reduced when inorganic zinc was supplemented, resulting in a higher digestibility of organic zinc. This might also be indicated by the same digestibility of organic zinc at both supplementation levels (24% on average). The dose-dependent absorption of inorganic

zinc could be a possible explanation for an inconsistency of results of feeding trials with inorganic and organic zinc at high and low dietary concentrations reported in previous studies and shown also in our study.

Zinc uptake was studied from perfused jejunal, ileal and colonic segments of rats [44]. The authors suggest that intestinal zinc absorption may be mediated by amino acids through the formation of complexes with zinc. Perfusion with tryptophan, histidine, cysteine and proline in the small intestine achieved a higher zinc uptake compared with the results after perfusion with their respective homologues. Both mediated and non-mediated transport mechanisms were involved in zinc uptake when the perfusate contained one of the amino acids. When the perfusate contained one of the amino acid homologues, only non-mediated transport mechanisms appeared to be activated. In our study, organic bound zinc may also be absorbed by another mechanism other than inorganic zinc. For this mechanism, increased absorption during zinc depletion and inhibition during zinc overload are probably not possible.

#### CONCLUSIONS

Supplying 10 mg copper and 30 mg zinc per kg to fattening pigs (70kg) both as protein chelates or inorganic salts resulted in higher caeruloplasmin levels after addition of organic sources.

With 20 mg copper and 70 mg zinc, plasma levels of copper were higher in variants with organic copper.

Adding organic zinc improves availability of copper in both dosage frames.

With the variant of 20 mg zinc and 70 mg zinc per kg feed, zinc availability from the organic source was better than from inorganic salts.

Investigations of the bioavailability of zinc and copper from organic sources are dependent on standardized conditions of Zn / Cu relations and the level of trace elements included in the diet.

#### ACKNOWLEDGEMENTS

The authors wish to thank the H. W. Schaumann Stiftung for financial support. The determination of caeruloplasmin by Gert Huesgen (Laboratory for Clinical Chemistry, Leinfelden-Echterdingen) and the help of Myquerem Tafai (Institute of Animal Nutrition, Hohenheim University) regarding the statistics are also acknowledged.

Received 12 January 2007

Accepted 21 February 2007

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## NEORGANINIO IR ORGANINIO VARIO IR CINKO PRIEDŲ ĮSISAVINIMAS PENIMŲ KIAULIŲ ORGANIZME

### *S a n t r a u k a*

Siekiant ištirti neorganinio ir organinio vario ir cinko įsisavinimą kiaulių organizme buvo atlikti du bandymai su 24 mišrūnais kastratais, kurių svoris kito nuo 71 iki 81 kg. Abiejų bandymų metu atsitiktinės atrankos metodu buvo sudarytos 4 grupės po 6 paršus kiekvienoje. Pirmojo bandymo metu į kiaulių pašarus buvo įmaišyta po 10 mg vario ir 30 mg cinko, antrojo bandymo metu – 20 mg vario ir 70 mg cinko. Pagal specialią bandymų atlikimo schemą –  $2 \times 2$  tiriamų faktorių išdėstymą (t. y. 2 skirtingi Cu ir Zn šaltiniai  $\times$  2 skirtingos koncentracijos Cu ir Zn palyginti su kontroliniais neorganinio Cu ir Zn priedais). Abiejuose bandymuose Cu ir Zn buvo pridedami kaip neorganiniai sulfatai ir kaip organinis Cu ir Zn, kuris buvo pagamintas sujungus chelatinį kompleksą su iš dalies hidrolizuotais sojų pupelių baltymais. Kadangi padidėjusi koeruloplazmino koncentracija buvo nustatyta pirmojo bandymo metu, o vario koncentracija plazmoje buvo didesnė antrojo bandymo metu, kai racionas buvo papildytas organiniu variu, galima teigti, jog organinis varis pasisavintas geriau, palyginti su neorganiniu variu. Dėl organinio cinko priedo racione padidėjo vario virškinamumas abiejų bandymų metu bei vario ir koeruloplazmino koncentracija plazmoje antrojo bandymo metu. Įmaišius į kiaulių racioną 20 mg vario ir 70 mg cinko padidėjo organinio cinko virškinamumas, palyginus su neorganiniu cinku. Todėl galima daryti išvadą, kad slopinantis cinko poveikis vario įsisavinimui yra mažesnis organinio cinko atveju, palyginus su neorganiniu cinku.

**Raktažodžiai:** Cu / Zn įsisavinimas, vario / cinko sulfatas, organinis varis, organinis cinkas, kiaulė, virškinamumas, koncentracija kraujyje ir audiniuose