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Effects of feed supplementation with various zinc sources on mineral concentration and selected antioxidant indices in tissues and plasma of broiler chickens

Oksana Ivanišinová, Ľubomíra Grešáková, Miroslav Ryzner, Vladimíra Oceľová, Klaudia Čobanová

Slovak Academy of Sciences, Institute of Animal Physiology, Košice, Slovak Republic

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Abstract

The aim of this study was to compare the effects of organic dietary zinc (Zn) sources and zinc sulphate on mineral deposition, activity of total superoxide dismutase (SOD) and copper/zinc SOD in tissues of broiler chickens. The performance indicators and lipid peroxidation by measuring the contents of thiobarbituric acid reactive substances (TBARS) in tissues and plasma were also evaluated. Broilers were assigned to 4 treatment groups, each replicated × 6, with 9 birds per replicate. The control group was fed conventional basal diet (BD); the three other groups received identical BD supplemented with 120 mg Zn/kg in the form of zinc sulphate, zinc chelate of glycine hydrate (Zn-Gly), and zinc proteinate (Zn-Pro), respectively. After 5 weeks of dietary treatment, feed supplementation with Zn sulphate resulted in significantly higher average daily weight gain and final body weight, as well as improved feed conversion ratio compared to the Zn-Gly group. Intake of Zn-Pro significantly increased SOD activity ($P < 0.05$) in erythrocytes and lipid peroxidation ($P < 0.01$) in plasma. Activities of total SOD and Cu/Zn SOD in liver and kidney were not affected by Zn supplementation. Addition of Zn supplements to broiler diets did not influence concentrations of zinc, manganese and copper in plasma, liver, kidney or breast muscle, with the exception of Zn deposition in the liver being significantly higher ($P < 0.05$) in the Zn-Pro supplemented group. Results of our study show that organic zinc sources have effects comparable to inorganic zinc sulphate in broilers fed diets containing a higher Zn content.

Zinc chelate, tissue deposition, superoxide dismutase, lipid oxidation, poultry

Appropriate mineral feed supplementation is required for many physiological functions and can improve growth performance and health of chickens for fattening. Zinc is known to be an essential microelement influencing immune functions, gene expression, cell proliferation, growth, and fertility. Being an essential part of more than 300 known enzymes, zinc directly participates in metabolic pathways and is one of major components of cell defence against oxidative stress as an integral part of cytosolic Cu/Zn superoxide dismutase (Cu/Zn SOD) (Zago and Oteiza 2001; McDowell 2003).

Recently, organic sources of Zn have been introduced into animal nutrition due to their potentially higher bioavailability compared to the traditionally used inorganic forms. However, study results regarding the bioavailability of organic chelates remain controversial. Several studies show that organic Zn sources are more available to animals (Yenice et al. 2015; Sahraei et al. 2013; Yu et al. 2010; Rupić et al. 1997), contribute to elevation of Cu/Zn SOD activity in chicken liver (Ma et al. 2010) and improve growth performance of broilers (Feng et al. 2010). In contrast, other results indicate that organic chelates are comparable to standard Zn sulphate (Cao et al. 2000). Use of organic sources of trace elements (i.e. chelated amino acids, proteinates) in animal nutrition may prevent minerals from creating indigestible complexes with some dietary compounds, and reciprocal mineral antagonisms in the intestine which could reduce their absorption rate (Swiatkiewicz et al. 2014).

The current study was conducted to compare the effects of organic Zn sources with

Address for correspondence:
RNDr. Klaudia Čobanová, PhD.
Institute of Animal Physiology
Slovak Academy of Sciences
Šoltésovej 4-6, 040 01 Košice, Slovak Republic

Phone: +421 557 922 965
E-mail: boldik@saske.sk
http://actavet.vfu.cz/

inorganic Zn sulphate supplemented to conventional broiler diet for 5 weeks on the deposition of Zn, Cu and Mn as well as the activities of total and Cu/Zn SOD in tissues. Moreover, the extent of lipid peroxidation in plasma, liver and kidney measured as TBARS level was determined in broilers fed with a higher content of dietary Zn, and performance indicators were monitored during the whole experiment as well.

Materials and Methods

The experiment was carried out on a total of 216 broiler chickens (ROSS 308) obtained from a commercial hatchery (Budmerice, Slovakia). One-day-old chicks of both sexes were weighed and assigned to 4 treatment groups with each containing 6 replicates. Based on the body weight (BW), nine chicks were allotted to each replicate cage resulting in a similar mean initial BW per replicate. Birds of all groups received identical basal diet (BD) formulated to meet the requirements for broiler chickens (NRC 1994). All chicks were fed the starter diet from 1 to 19 days of age followed by the grower diet from 20 to 35 days of age. The BD applied in our

Table 1. Ingredients and chemical composition of basal diet fed to broiler chickens.

Item	Starter diet (Days 1 to 19)	Grower diet (Days 20 to 35)
Ingredient (%)		
Maize, ground (9% CP)	41.96	42.13
Soybean meal, extracted (48% CP)	28.00	30.00
Wheat, ground (12% CP)	22.00	22.00
Maize gluten (67% CP)	3.00	-
Limestone	1.50	1.40
Monocalcium phosphate	1.30	1.30
Sunflower oil	1.00	2.00
Premix[a]	0.30	0.30
Feed salt	0.40	0.40
Lysine	0.35	0.25
DL-Methionine	0.17	0.20
Allzyme SSF-poultry[b]	0.02	0.02
Nutrient composition		
Dry matter (g/kg)	884.0	889.3
Crude protein (g/kg)	198.0	187.0
Crude fibre (g/kg)	31.8	30.3
Zinc (mg/kg)	84.4	64.6
Manganese (mg/kg)	135.4	134.7
Copper (mg/kg)	22.7	21.5
Calcium (g/kg)	9.8	9.5
Phosphorus (g/kg)	4.7	4.7
Lysine (g/kg)	13.7	13.1
Methionine (g/kg)	5.4	5.3
Methionine + cysteine (g/kg)	9.3	9.1
Metabolizable energy (MJ/kg)	12.6	12.7

Dry matter, crude protein, crude fibre, zinc, manganese and copper are analysed data.
Experimental diets were supplemented with 120 mg Zn/kg in the form of zinc sulphate, zinc chelate of glycin hydrate (Zn-Gly) or zinc proteinate (Zn-Pro).
[a]The vitamin/mineral premix provided per kg of complete diet: vitamin A 13 500 IU, vitamin D_3 5 000 IU, vitamin K 4.2 mg, vitamin E 60.0 mg, thiamine 5.4 mg, riboflavin 7.5 mg, pyridoxine 4.8 mg, cyanocobalamin 0.03 mg, vitamin C 75.0 mg, niacin 54.0 mg, pantothenic acid 16.5 mg, biotin 0.2 mg, folic acid 1.8 mg, choline 90.0 mg, betaine 195.0 mg, I 1.2 mg, Zn 55.0 mg, Mn 115.0 mg, Cu 16.5 mg, Se 0.3 mg, Co 0.5 mg, Fe 81.6 mg.
[b]Allzyme®SSF Enzyme Complex, Altech, Inc., Nicholasville, Kentucky USA

experiment was a conventional diet commonly used in the nutrition of broiler chickens, and its composition and nutrient values are shown in Table 1. No supplemental zinc was added to BD for the control group, whereas the three other groups were fed identical BD supplemented with equal amounts of 120 mg Zn/kg complete feed from either Zn sulphate ($ZnSO_4 \cdot H_2O$, reagent grade, Sigma-Aldrich, USA), or Zn chelate of glycine hydrate (Zn-Gly; Glycinoplex-Zn 26%, Phytobiotics Futterzusatzstoffe GmbH, Eltville, Germany) or Zn proteinate (Zn-Pro; Bioplex®-Zn 15%, Alltech Inc., Nicholasville, KY, USA), respectively. Feed and drinking water were offered *ad libitum*.

All birds were kept under similar conditions of management throughout the experiment in accordance with the guidelines for the care of fattening broiler chickens (Aviagen's Manual, 2014). The lighting schedule was maintained at 23 h of light and 1 h of darkness during the first 7 days, followed by 6 h of a continuous period of darkness. Relative humidity varied from 60 to 70% and the temperature regimen was adjusted to the particular age of the chickens according to breeding recommendations.

All procedures were in accordance with the European Community guidelines (Directive 2010/63/EU) on the protection of animals used for scientific purposes and the experimental protocol was approved by the Ethics Committee of the Institute of Animal Physiology SASci and by the State Veterinary and Food Office (Ro-4160/13-221).

Weekly measurements of body weight and feed intake for each replicate of the treatments were taken to calculate the average daily feed intake, average daily weight gain and feed conversion ratio (FCR). On day 35, all birds were weighed individually and the final average body weight was calculated for each treatment. Mortality within each replicate was monitored daily throughout the experiment.

At the end of the experiment, two randomly selected chickens from each replicate (a total of 12 birds from each treatment) were slaughtered by electrical stunning followed by decapitation. Blood was collected into heparinised tubes and centrifuged for plasma samples at 1,180 × *g* for 15 min. Tissue samples (5–8 grams) were collected from identical areas of the liver, kidney and breast muscle, immediately flushed with ice-cold saline and quickly frozen. All tissue and plasma samples were stored at –70 °C until analysis.

Dry matter (DM) of diets and tissues was determined using the standard method of drying samples at 105 °C for 48 h. Feed was also analyzed for crude protein and crude fibre using standard procedures (AOAC 2005; methods 976.05, 973.18).

Superoxide dismutase (SOD; EC 1.15.1.1) activity in erythrocytes and the content of haemoglobin (Hb) were analyzed in fresh blood using a commercial kit from Randox (Randox Laboratories, UK). Liver and kidney samples for analysis of total SOD and Cu/Zn SOD activities were homogenized in ice-cold buffer (pH 7.4) containing 10 mM Tris and 0.25 M sucrose. Subsequently, homogenates were centrifuged at 10 000 × *g* for 30 min at 4 °C and supernatants were used for analysis of total SOD activity using the spectrophotometric method based on the autoxidation of pyrogallol (Marklund and Marklund 1974). For each sample, a parallel determination was performed in the presence of 1 mM KCN and the activity of Cu/Zn SOD was calculated as the activity inhibited by KCN. Protein concentration in the sample supernatants was measured using the spectrophotometric method published by Bradford (1976). The TBARS levels in plasma, liver and kidney were measured using the modified fluorometric method in accordance with Jo and Ahn (1998).

The contents of Zn, Mn, and Cu in samples were determined using a double-beam atomic absorption spectrometer (AAS-7000 series, Shimadzu, Kyoto, Japan). All samples except plasma were dried (at 105 °C for 48 h) and then ground for subsequent wet digestion in concentrated nitric acid and hydrogen peroxide (3:1) in a microwave digestion system (MWS-4, Berghof, Germany). Mineral deposition in tissue samples, Zn and Cu contents in plasma and the mineral content in diets were determined using flame atomic absorption spectrophotometry (FAAS). The AAS equipped with a graphite furnace atomizer GFA-7000 and deuterium lamp for background correction was used to measure the concentration of Mn in plasma.

Statistical analysis was done by one-way analysis of variance (ANOVA) with *post hoc* Tukey's multiple comparison test using GraphPad Prism Software (Version 2.02, 2008, USA). Differences between groups were considered significant at $P < 0.05$. Values in tables are given as means ± standard errors of the mean (SEM).

Results

The effects on growth performance of broiler chickens assessed from 0 to 35 days after supplementing the basal diet with inorganic and organic zinc are summarized in Table 2. The average daily feed intake was not influenced by supplemental Zn. Intake of dietary Zn sulphate resulted in improvement of the feed conversion ratio ($P < 0.001$) and increased final body weight ($P < 0.01$) compared to birds fed the diet enriched with Zn-Gly. The average daily weight gain was significantly higher in the Zn sulphate group than in the unsupplemented control ($P < 0.05$) and Zn-Gly group ($P < 0.001$).

Antioxidant indicators of broilers supplemented with Zn from different sources are given in Table 3. Activity of SOD measured in erythrocytes was significantly elevated in the Zn-Pro group compared to control birds ($P < 0.05$) and the Zn sulphate group ($P < 0.05$).

Table 2. Effect of different zinc sources on growing performance of broilers during the feeding period of 5 weeks (from 1 to 35 days of age).

Indicator	BD	Zn-sulphate	Zn-Gly	Zn-Pro
Feed intake (g/bird/day)	79.60 ± 1.06	81.26 ± 0.97	80.03 ± 1.14	80.81 ± 0.93
Weight gain (g/bird/day)	48.63 ± 0.47^{a}	51.47 ± 0.87^{b}	47.12 ± 0.78^{a}	49.24 ± 0.32^{ab}
Feed conversion ratio (g/g)	1.64 ± 0.01^{ab}	1.58 ± 0.03^{a}	1.70 ± 0.01^{b}	1.64 ± 0.02^{ab}
Initial body weight (g/bird)	46.47 ± 0.32	46.47 ± 0.41	46.57 ± 0.50	47.58 ± 0.72
Final body weight (g/bird)	1733.7 ± 16.5^{ab}	1832.0 ± 30.3^{b}	1683.7 ± 27.5^{a}	1781.4 ± 36.1^{ab}
Mortality (%)	0.00	1.85	0.00	1.85

Results are presented as mean ± SEM, ^{a,b} - values in a row with different letters in superscripts are significantly different ($P < 0.05$)

Table 3. Effect of different zinc sources on activity of SOD in erythrocytes and tissues, activity of Cu/Zn SOD in tissues and TBARS concentration in plasma and tissues of 35-day-old broiler chickens.

Indicator	BD	Zn-sulphate	Zn-Gly	Zn-Pro
SOD				
Erythrocytes (µkat/g Hb)	9.17 ± 1.04^{a}	9.16 ± 1.54^{a}	12.75 ± 0.67^{ab}	14.00 ± 1.30^{b}
Liver (µkat/mg protein)	0.83 ± 0.06	0.91 ± 0.06	0.88 ± 0.13	0.87 ± 0.04
Kidney (µkat/mg protein)	0.48 ± 0.02	0.48 ± 0.01	0.49 ± 0.01	0.50 ± 0.01
Cu/Zn SOD				
Liver (µkat/mg protein)	0.67 ± 0.04	0.71 ± 0.06	0.71 ± 0.10	0.67 ± 0.03
Kidney (µkat/mg protein)	0.32 ± 0.02	0.32 ± 0.01	0.33 ± 0.01	0.33 ± 0.02
TBARS				
Plasma (nmol/ml)	0.27 ± 0.02^{a}	0.25 ± 0.01^{a}	0.28 ± 0.01^{a}	0.37 ± 0.02^{b}
Liver (nmol/g protein)	151.5 ± 16.8	131.0 ±11.7	135.2 ± 12.3	163.8 ± 7.8
Kidney (nmol/g protein)	74.65 ± 2.19	71.84 ± 6.55	76.06 ± 7.32	89.74 ± 7.03

SOD – superoxide dismutase; Hb – haemoglobin; TBARS – thiobarbituric acid reactive substances; Results are presented as mean ± SEM; ^{a,b} - values in a row with different letters in superscripts are significantly different ($P < 0.05$)

Neither the SOD activity nor the Cu/Zn SOD activity in liver and kidney were affected by dietary Zn supplementation. Feed supplementation with Zn from Zn-Pro significantly increased the TBARS levels in plasma ($P < 0.01$) compared to all other groups. There was a tendency for higher TBARS values in liver and kidney of chickens receiving Zn-Pro, however, no significant differences between treatments were observed for this indicator.
The effects of various sources of dietary Zn on mineral concentration in plasma and tissues are presented in Table 4. Addition of Zn-Pro to the diet significantly increased Zn deposition in liver ($P < 0.05$) compared to control birds fed only BD, but Zn concentration in plasma, kidney and breast muscle was not affected by Zn supplementation. No differences in concentration of Mn and Cu in plasma and tissues were found between dietary treatments.

Discussion

After 35 days of feeding experimental diets, increased average daily weight gain was observed in broilers from the Zn sulphate group, with an increase in the final body weight compared to

Table 4. Concentration of zinc, manganese and copper in plasma and tissues of broilers supplemented with zinc from various sources.

Indicators	BD	Zn-sulphate	Zn-Gly	Zn-Pro
Plasma				
Zn (mg/l)	1.88 ± 0.07	1.96 ± 0.12	1.85 ± 0.10	1.99 ± 0.12
Mn (µg/l)	4.03 ± 0.30	3.76 ± 0.32	4.12 ± 0.80	5.19 ± 0.87
Cu (mg/l)	0.18 ± 0.02	0.21 ± 0.02	0.19 ± 0.01	0.23 ± 0.01
Liver (mg/kg DM)				
Zn	90.20 ± 2.09^{a}	99.74 ± 3.37^{ab}	96.67 ± 2.03^{ab}	101.00 ± 2.89^{b}
Mn	10.57 ± 0.77	10.65 ± 0.67	9.93 ± 0.50	11.14 ± 0.46
Cu	12.28 ± 0.32	13.47 ± 0.63	12.98 ± 0.65	12.22 ± 0.72
Kidney (mg/kg DM)				
Zn	96.92 ± 0.71	96.21 ± 2.25	98.18 ± 1.07	96.40 ± 1.04
Mn	10.39 ± 0.22	10.30 ± 0.30	10.82 ± 0.25	10.25 ± 0.42
Cu	11.91 ± 0.13	12.49 ± 0.15	12.45 ± 0.25	12.48 ± 0.26
Breast muscle (mg/kg DM)				
Zn	19.58 ± 0.46	19.45 ± 0.31	18.96 ± 0.30	20.65 ± 0.73
Mn	0.88 ± 0.09	0.93 ± 0.10	1.06 ± 0.07	0.93 ± 0.10
Cu	1.75 ± 0.23	1.81 ± 0.29	1.80 ± 0.25	1.83 ± 0.20

DM – dry matter; results are presented as mean ± SEM; ^{a,b} - values in a row with different letters in superscripts are significantly different ($P < 0.05$)

the Zn-Gly group. However, there was no difference in feed intake between treatments, which explains the improvement of feed conversion ratio in birds receiving inorganic Zn. These findings show that feed supplementation with Zn sulphate helps to improve the performance of broiler chickens, although significant difference compared to unsupplemented control birds was observed only in the average daily weight gain. Sahraei et al. (2013) recorded higher weight gain in broilers fed similar dietary Zn concentrations from sulphate compared to control birds from day 22 to 28, which is partially consistent with our results. They also reported improvement of the feed conversion ratio in the birds supplemented with Zn sulphate and Zn-Pro compared to the control group, and no significant difference in the final body weight between treatments, which was not confirmed in our experiment. Sunder et al. (2008) did not find any significant effect of Zn sulphate supplemented to the BD at graded doses up to 320 mg Zn/kg for 3 weeks on broiler performance.

Several mechanisms by which Zn can exert its antioxidant action in a biological system have been described (Powell 2000), but one of the most important functions of Zn is related to its antioxidative effect mediated by Cu/Zn SOD. Elevated activity of SOD in erythrocytes was observed in broilers fed dietary Zn-Pro compared to control birds and the Zn sulphate group, and was associated with higher TBARS values in plasma (Table 3). This finding could be explained by the ability of SOD to protect cells against oxidative damage (Fridovich 1995) and may in this case be a reaction of antioxidant defence mechanisms to a higher rate of lipid peroxidation. Zago and Oteiza (2001) reported that Zn as an important component of the antioxidant defence network prevents membrane damage from oxidation and can also partially inhibit formation of free radicals and other potentially reactive substances.

No response to Zn supplementation in activities of total and Cu/Zn SOD in the liver and kidney was observed in our experiment, which is in agreement with the results of Liao et al. (2013). Nevertheless, other authors have reported an increase in Cu/Zn SOD

activity while the concentration of malondialdehyde decreased in the liver of broilers after supplementation with 120 mg Zn/kg from Zn-Gly (Ma et al. 2010). Intake of diets with Zn dosage above the maximum EU authorized total contents (EC 2003) of this trace element in complete feed (150 mg Zn/kg) did not affect TBARS values in the liver and kidney of broilers. There was only a tendency for higher values of TBARS concentration in tissues of birds receiving Zn-Pro (Table 3).

The minerals are usually administered in the form of inorganic salt which has been traditionally considered as the most cost-effective. On the other hand, organic mineral sources can be used at lower inclusion levels in the diet without a negative impact on animal health and performance, while also decreasing the excess mineral excretion to the environment (Swiatkiewicz et al. 2014). Introduction of organic or chelated sources of trace elements into animal nutrition and their potential higher bioavailability compared to inorganic sources could alter mineral deposition in tissues. In this trial, no significant differences in the plasma, liver, kidney and muscle zinc deposition were found between inorganic or organic Zn sources supplied to the broiler feed. Significantly higher Zn concentration in the liver observed in the Zn-Pro group may be connected to elevated Zn absorption in the intestine. Yu et al. (2010) found that Zn absorption from Zn-Pro complex is higher than that of Zn-Gly and Zn sulphate due to different chelation strength of the organic Zn feed additives; however, there was no significant difference in Zn tissue deposition between organic Zn sources used in our experiment. Plasma Zn concentration in the supplemented groups was equal to that in the control birds due to sufficient Zn content in BD. Some authors have reported that 80–84 mg Zn/kg of complete feed is sufficient for optimal performance of broiler chickens (Huang et al. 2007; Sunder et al. 2008). Our results indicate that broiler performance as well as activity of Cu/Zn SOD and Zn deposition in tissues of the control (unsupplemented) birds were similar to those in the supplemented groups. Based on these findings we can consider Zn content in our BD (from 65 to 85 mg Zn/kg) as sufficient for optimal health and performance of broiler chickens.

Several studies have demonstrated (Ao et al. 2009; Sunder et al. 2011) that the extent of mineral absorption is dependent on interaction between the minerals which could either be synergistic (Zn and Mn) or antagonistic (Zn and Cu). Ao et al. (2009) reported that the antagonism between Zn and Cu occurred when the inorganic forms, but not organic forms of minerals were included in a chick diet. Addition of inorganic minerals to the diet may result in reducing their absorption due to competing with each other for binding ligands and mineral uptake sites in the gut mucosa (Santon et al. 2002). It has been shown that higher Zn intake can inhibit intestinal absorption and consequently also hepatic uptake of Cu (Gonzalez et al. 2005). In this trial, the concentrations of Cu and Mn in the liver, kidney and plasma were not influenced by feed supplementation with either inorganic or organic Zn sources. Thus it appears that the greater Zn content used in the diets of our broilers did not affect the intestinal absorption of Cu and Mn.

The results of our study indicate that organic zinc sources had a similar effect on mineral deposition and activity of Cu/Zn SOD in tissues as zinc sulphate in broilers with higher dietary Zn intake. Based on this result we can conclude that organic sources of zinc are comparable to the traditionally used inorganic source in nutrition of broiler chickens.

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