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THE EFFECT OF DIFFERENT DIETARY ZINC SOURCES ON MINERAL DEPOSITION AND ANTIOXIDANT INDICES IN RABBIT TISSUES

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Abstract: The purpose of this study was to compare the effect of dietary zinc from inorganic and organic sources on the concentration of Zn, Cu, Mn and Fe in plasma, tissues and faeces of rabbits. Simultaneously, the activities of total superoxide dismutase (SOD), specific Cu/Zn SOD, glutathione peroxidase (GPx), lipid peroxidation and total antioxidant capacity (TAC) in liver and kidney were also determined. Ninety-six 49-day-old broiler rabbits were allocated to 4 dietary treatments, each replicated 6 times with 4 animals per replicate. For the subsequent 6 wk, the rabbits were fed an identical basal diet (BD) supplemented with an equivalent dose of Zn (100 mg/kg) from different sources. Group 1 (control) received the unsupplemented BD, while the BD for groups 2, 3 and 4 was supplemented with Zn from Zn sulphate, Zn chelate of glycine hydrate (Zn-Gly) and Zn chelate of protein hydrolysate (Zn-Pro), respectively. The intake of dietary Zn sulphate resulted in an increase in Zn plasma concentration (1.85 *vs.* 1.48 mg/L; $P<0.05$) compared to the control group. Feeding the diets enriched with Zn increased the deposition of Zn in the liver ($P<0.05$), irrespective of the Zn source. The addition of Zn-Pro resulted in significantly higher Cu uptake in liver ($P<0.05$) than in the control and Zn sulphate group (56.0 *vs.* 35.0 and 36.7 mg/kg dry matter (DM), respectively). Neither Mn nor Fe concentration in plasma and tissues were affected by dietary Zn supplementation, with the exception of Fe deposition in muscle, which was significantly decreased ($P<0.05$) in rabbits supplemented with inorganic Zn sulphate compared to control and Zn-Gly group (9.8 *vs.* 13.3 and 12.2 mg/kg DM, respectively). Intake of organic Zn-Gly significantly increased the activities of total SOD (43.9 *vs.* 35.9 U/mg protein; $P<0.05$) and Cu/Zn SOD (31.1 *vs.* 23.8 U/mg protein; $P<0.01$) as well as TAC (37.8 *vs.* 31.2 μmol/g protein; $P<0.05$) in the kidney when compared to that of the control group. The presented results did not indicate any differences between dietary Zn sources in Zn deposition and measured antioxidant indices in rabbit tissues. Higher dietary Zn intake did not cause any interactions with respect to Mn, Cu and Fe deposition in liver and kidney tissues, but did increase the faecal mineral concentrations. Dietary organic Zn-Gly improved the antioxidant status in rabbit kidney.

Key Words: zinc, rabbit, mineral concentration, antioxidant enzymes, lipid peroxidation.

INTRODUCTION

The importance of zinc for animals can be attributed to several factors, mainly its involvement in growth and bone development, enzyme structure and function and gene expression, as well as immune competence (Suttle, 2010). Zinc is an essential nutrient, and therefore is commonly added as a supplement to animal diets. Dietary supplementation with inorganic zinc salts traditionally added to commercial animal feed provides sufficient amounts of the mineral to support normal growth, health and reproduction (Bao *et al.,* 2007; Casado *et al.,* 2011). Currently, there is much interest in using dietary organic mineral sources as a means of protecting against the formation of indigestible

complexes with some anti-nutritive dietary compounds in the intestine and against reciprocal mineral antagonisms, resulting in higher mineral absorption and subsequent utilisation by the body (Swiatkiewicz *et al.,* 2014). Recent data have indicated that inclusion of organic Zn sources in rabbit diets could improve growth performance and duodenum morphology, as well as reducing the incidence of diarrhoea in growing rabbits (Yan *et al.,* 2017). However, some studies have shown no differences in the bioavailability of Zn between organic and inorganic sources (Ivanišinová *et al.,* 2016). Our previous work demonstrated that dietary supplementation with 100 mg Zn/kg from inorganic or organic sources resulted in a significant increase in digestibility coefficients of Zn, Fe and Mn compared to unsupplemented rabbits and did not influence selected biochemical parameters in caecal fermentation (Chrastinová *et al.,* 2016). Nessrin *et al.* (2012) reported that growing rabbits are tolerant to excessive dietary doses of Zn (up to 400 mg/kg diet) and also respond positively to feed supplementation with a 100 mg Zn/kg diet in terms of significant improvement in live body weight gain and feed conversion ratio by 12.3 and 10.6%, respectively.

Zinc performs important antioxidative functions in biological systems, mainly as a structural component of the antioxidant enzyme Cu/Zn superoxide dismutase (SOD); it upregulates the synthesis of proteins with oxidant-scavenging capacity (e.g. thionein) and prevents the binding of redox active metals to oxidisable target cell molecules (Oteiza, 2012). Additionally, zinc exerts a protective role in the plasma membrane by stabilising protein sulfhydryl groups and is able to retard oxidative processes (Powell, 2000). However, no recent trials have been conducted to study the response of antioxidant status to diet supplementation with organic Zn sources in rabbits.

Based on the discrepancy of data related to bioavailability of dietary Zn sources, the present study aimed to compare the effect of inorganic and organic Zn sources supplemented to a conventional rabbit diet for 6 wk on the distribution of Zn and other minerals, including Cu, Fe and Mn, in the plasma, muscle, liver and kidney tissues of growing rabbits. Simultaneously, the activity of zinc-containing Cu/Zn SOD and antioxidative status were evaluated in animals fed with a higher content of dietary Zn.

MATERIALS AND METHODS

All procedures were carried out in accordance with established guidelines for the care and use of animals for scientific purposes. The experimental protocol was approved by the Institutional Ethical Committee and by the State Veterinary and Food Office of the Slovak Republic (4047/16-221).

Animals and experimental design

For this study, a total of 96 rabbits (meat line M9) at 49 d of age and initial body weight of 1643±12 g were used in a feeding trial that lasted 6 wk. All rabbits were kept in the same building equipped with heating and a forced ventilation system and placed in standard cages (0.61×0.34×0.33 m) with 2 animals in each cage. Throughout the experiment, the lighting schedule was maintained at 16 h of light and 8 h of darkness; the environmental temperature was adjusted in the range of 22±4°C and the relative humidity was about 70±5%.

The experimental animals in both sexes were randomly assigned to one of 4 dietary treatments comprising 1 control group and 3 experimental groups, each containing 6 replicates with 4 animals (2 cages) per replicate. Rabbits of all groups received an identical basal diet (BD) formulated to meet the nutrient requirements recommended by the National Research Council (NRC, 1977). The BD applied in the experiment is typical of diets commonly used in the nutrition of growing rabbits, and its main ingredients and chemical composition are presented in Table 1. Experimental diets included an unsupplemented BD (114 mg Zn/kg as fed basis), or the identical BD supplemented with equal amounts of 100 mg Zn/kg complete feed from either Zn sulphate ($ZnSO_4 \cdot H_2O$, reagent grade, Sigma-Aldrich, USA), Zn chelate of glycine hydrate (Zn-Gly; Glycinoplex-Zn 26%, Phytobiotics Futterzusatzsstoffe GmbH, Eltville, Germany) or Zn proteinate (Zn-Pro; Bioplex®-Zn 15%, Alltech Inc., Nicholasville, KY, USA). The diets were mixed, pelleted (pellets of 3.5 mm in diameter) and offered to the rabbits *ad libitum*. The analysed Zn concentration in supplemented diets was 208 (Zn sulphate treatment), 200 (Zn-Gly treatment), and 210 (Zn-Pro treatment) mg/kg of dry matter (DM), respectively. Animals had free access to fresh water from a nipple drinker.

Table 1: Ingredients and chemical composition of the granulated basal diet.

Ingredients	g/kg	Nutrient composition	g/kg
Lucerne meal	360	Dry matter	899
Extracted sunflower meal	55	Crude protein	178
Extracted rapeseed meal	55	Crude fibre	147
Wheat bran	90	Fat	36
Oats	130	Ash	97
Malt sprouts	150	Starch	129
DDGS[a]	50	Organic matter	848
Sodium chloride	3	Acid detergent fibre	185
Vitamin and mineral premix[b]	17	Neutral detergent fibre	316
Barley grains	80	Calcium	9.7
Limestone	10	Phosphorous	6.9
		Zinc (mg/kg)	114
		Manganese (mg/kg)	118
		Copper (mg/kg)	20.3
		Iron (mg/kg)	126
		Metabolisable energy (MJ/kg)[c]	11.4

[a]DDGS: dried distiller grains with solubles.
[b]The vitamin/mineral premix provided per kg of complete diet: Vitamin A 6000 IU; Vitamin D3 1000 IU; Vitamin E, 50 mg; Vitamin B1, 1.7 mg; Vitamin B2, 8.0 mg; Vitamin B6, 3.0 mg; Vitamin B12, 0.01 mg; Vitamin K3, 0.5 mg; biotin, 0.2 mg; folic acid, 0.5 mg; nicotinic acid, 70 mg; choline chloride, 700 mg; Mn, 50 mg; Fe, 40 mg; Cu, 30 mg; Se, 0.2 mg.
[c]Metabolisable energy was calculated by the equation of Wiseman et al. (1992).

Sample collection

At the end of the experiment, a total of 24 rabbits (6 rabbits/treatment; 1 male rabbit from each replicate) with the final weight of 3018 ± 18 g were selected for blood collection and subsequent slaughter. Blood samples were collected from the peripheral vein of the ear into heparinised tubes and centrifuged for plasma samples at $1180\times g$ for 15 min. Thereafter, the experimental animals were slaughtered immediately after electrical stunning by cutting the vessels of throat and subsequent bleeding. Tissue samples were taken from identical areas of liver, kidney cortex and muscle (*musculus longissimus dorsi*), flushed with ice-cold saline and quickly frozen. All tissues and plasma samples were stored at $-70°C$ until analysis. Faecal samples were collected for 24 h from each replicate (4 rabbits) and homogeneously mixed. The representative mixed sample (10% of the total faecal output) of respective replicate was dried in a forced-air oven (at 70°C for 48 h), then ground in an electrical grinder and stored in plastic containers until mineral analysis.

Chemical analysis and measurements

Crude protein (CP), crude fibre (CF), crude fat (CF), ash and organic matter of the BD were determined using standard procedures of AOAC (2005). Furthermore, the BD was analysed for neutral detergent fibre (NDF) and acid detergent fibre (ADF) using the method of Van Soest *et al.* (1991), with heat stable amylase pre-treatment, and starch was determined by the enzymatic method according to Salomonsson *et al.* (1984). Metabolisable energy was calculated by the equation of Wiseman *et al.* (1992). Dry matter of feed, tissues and faeces were obtained by the standard method of drying sample to a constant weight at 105°C.

Mineral (Zn, Mn, Cu, Fe) concentrations in feed, plasma, tissues and faeces were analysed using a double-beam atomic absorption spectrophotometer (AA-7000 Series, Shimadzu Co., Kyoto, Japan) with a graphite furnace (GFA-7000, Shimadzu Co., Kyoto, Japan). All samples except the plasma were dried (105°C for 48 h) and ground for subsequent wet digestion with a concentrated nitric acid and hydrogen peroxide mixture (3:1) in a microwave digestion system (MWS 4, Berghof Co., Germany). The plasma samples were only diluted (1:10) with 0.05% Triton X-100 solution (Gresakova *et al.,* 2016). The concentration of Mn in plasma as well as the Mn and Cu content in

muscle was measured using a graphite furnace atomic absorption spectrophotometer, with deuterium background correction and pyrolytic-coated graphite tubes. The certificate reference materials of bovine liver BCR-185R, bovine muscle ERM-BB184, pig kidney ERM-BB186 (Institute for Reference Materials and Measurements, Geel, Belgium) and human plasma ClinCheck Control (Recipe, Munich, Germany) were routinely run in each analysis to ensure the accuracy of the estimates. In order to improve the accuracy of the analysis and to eliminate matrix effects, mineral concentrations in the digested faeces samples were determined by flame atomic absorption spectrometry using the standard addition method.

The total SOD activity was assayed by a modified spectrophotometric method, based on the ability of the enzyme to inhibit the autoxidation of pyrogallol (Marklund and Marklund, 1974). Tissue samples were homogenised in ice-cold 10 mM TRIS buffer containing 0.25 M sucrose (pH 7.4) to make a 10% (w/v) homogenate. After centrifugation at $10\,000{\times}g$ for 30 min at 4°C, the supernatants were used for enzyme activity determination in liver and kidney cortex at 420 nm. The specific Cu/Zn SOD inhibition by KCN allows the determination of enzyme activity under the same condition. One unit of enzyme activity is expressed as the amount of enzyme required to inhibit the pyrogallol autoxidation by 50%, and the specific activity is expressed as unit of enzyme per mg of tissue protein.

Supernatants for measurement of the GPx activity and total antioxidant capacity (TAC) were obtained by tissue-sample homogenisation in ice-cold phosphate buffer saline (pH 7.4) and subsequent centrifugation at $13\,680{\times}g$ for 20 min at 4°C. Activity of GPx was measured spectrophotometrically according to the Paglia and Valentine (1967) method by monitoring the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm. A unit of enzyme activity is defined as the amount of enzyme required for the oxidation of 1 μmol of NADPH per minute. The determination of TAC was performed using the ferric reducing ability of tissue supernatant, as described by Benzie and Strain (1996), with some modifications. This method is based on the reduction of the ferric tripyridyltriazine (Fe^{3+} - TPTZ) complex to the ferrous (Fe^{2+}) form, monitored by measuring absorbance at 593 nm. The calibration curve was prepared using a ferrous sulphate solution, and the results were expressed in μmol Fe^{2+} formed per g protein. The extent of lipid peroxidation in tissues was determined by a modified fluorometric method according to Jo and Ahn (1998), using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, USA) to get the standard curve for the estimation of malondialdehyde (MDA) concentration. Protein concentration in the tissues was determined using the spectrophotometric method, as described by Bradford (1976).

Statistical analysis

All measurements were made in duplicate and results in tables are reported as means±standard error of means (SEM). The data were analysed by one-way analysis of variance (ANOVA) with the *post hoc* Tukey's multiple comparison test (GraphPad Prism, Version 5.02, 2008, USA) at a significance level of $P{<}0.05$.

RESULTS AND DISCUSSION

A substantial portion of research papers have shown that the inclusion of organic Zn into the diet did not improve feed intake and animal performance; however, a majority demonstrated higher bioavailability resulting in higher retention and lower mineral excretion (Swiatkiewicz *et al.,* 2014). Our previous paper presented results related to performance indices in rabbits supplemented with inorganic and organic Zn sources (100 mg Zn/kg) for 6 wk. There was no significant effect of dietary zinc sources on growth performance of rabbits on the 91st day of age. Weight gain was in the range of 31.3-33.0 g/d and feed conversion ratios were within the range 4.08-4.26 g/g (Chrastinová *et al.,* 2016).

In the present study, the Zn concentration in plasma was significantly increased only in rabbits fed a Zn sulphate-containing diet ($P{<}0.05$) over those fed unsupplemented diets, but no differences appeared between dietary Zn sources (Table 2). Our results are inconsistent with the study of Nessrin *et al.* (2012), who reported that supplemental dietary Zn by levels of 50, 100, 200 or 400 mg/kg as inorganic ZnO had no significant effect on plasma Zn concentration in growing rabbits. Yan *et al.* (2017) reported a higher concentration of Zn in the serum of rabbits receiving organic Zn as the Zn methionine form compared to the ZnSO$_4$ diet; however, no changes in this parameter were found when the diet was supplemented with Zn at 80 mg/kg as ZnGly or Zn lactate. Plasma or serum Zn concentrations are typically used as a biomarker of Zn status, although only about 0.1% of total body Zn is present in the plasma and its constant level

Table 2: Effect of different dietary zinc sources on zinc, manganese, copper and iron concentration in plasma, liver, kidney, muscle and faeces of rabbits.

	Treatment				
	BD	ZnSO$_4$	Zn-Gly	Zn-Pro	*P*-value
Zinc (mg/kg DM)					
Plasma (mg/L)	1.48±0.05[a]	1.85±0.08[b]	1.58±0.06[ab]	1.53±0.12[ab]	0.017
Liver	114±2[a]	132±3[b]	131±3[b]	149±6[c]	0.0001
Kidney	123±1	124±1	124±1	132±11	0.660
Muscle	30.5±0.8	31.5±1.1	33.3±2.9	30.9±1.4	0.707
Faeces[1]	336±3[a]	600±15[b]	625±10[b]	633±8[b]	<0.0001
Manganese (mg/kg DM)					
Plasma (µg/L)	5.84±0.21	6.46±0.14	6.20±0.24	6.41±0.40	0.440
Liver	7.39±0.26	7.80±0.52	6.76±0.26	7.10±0.41	0.335
Kidney	8.37±0.55	9.22±0.55	9.58±0.64	8.97±0.19	0.419
Muscle	0.31±0.02	0.30±0.05	0.32±0.03	0.33±0.05	0.962
Faeces[1]	378±17[a]	468±8[b]	456±20[b]	441±8[b]	0.002
Copper (mg/kg DM)					
Plasma (mg/L)	0.99±0.11	1.08±0.13	0.90±0.07	0.97±0.11	0.703
Liver	35.0±2.5[a]	36.7±1.9[a]	42.1±5.7[ab]	56.0±7.3[b]	0.027
Kidney	17.1±1.1	18.7±0.7	17.9±0.4	16.8±0.3	0.198
Muscle	1.05±0.05	1.09±0.07	0.98±0.03	0.95±0.02	0.161
Faeces[1]	57.7±0.7[a]	64.4±0.8[b]	62.1±0.6[b]	62.8±0.5[b]	<0.0001
Iron (mg/kg DM)					
Plasma (mg/L)	1.78±0.09	1.79±0.07	1.73±0.14	1.84±0.07	0.889
Liver	285±36	271±30	245±35	238±40	0.764
Kidney	170±9	167±15	193±5	176±7	0.242
Muscle	13.3±0.4[a]	9.8±0.7[b]	12.2±0.2[a]	11.5±0.5[ab]	0.001
Faeces[1]	931±7[a]	1150±87[b]	1033±12[ab]	1023±16[ab]	0.017

[ab]Means in the same row having different letter superscripts indicate a significant difference (*P*<0.05). Data are presented as means±standard error of the mean (SEM).
[1]Data represent means±SEM of 6 replicates (4 rabbits/replicate).

is regulated by homeostatic mechanisms with respect to the varying needs of individual cells and tissues (King *et al.,* 2016). Therefore, the tissue deposition of Zn and enzyme-related activities are generally considered as sensitive criteria for evaluating Zn bioavailability from different sources. In our trial, dietary supplementation of Zn in either inorganic or organic forms increased Zn deposition in the liver (*P*<0.05), with the highest values observed in rabbits receiving diets enriched with Zn-Pro (*P*<0.001), while the Zn concentration in kidney and muscle was not affected by dietary treatments (Table 2). These findings suggest that there are no differences between intestinal absorption of Zn from inorganic or organic sources. Our results are partially similar with the finding of Yan *et al.* (2017), who reported a higher liver Zn concentration in rabbits supplemented with Zn (80 mg/kg) from organic sources (Zn lactate, Zn methionine, Zn glycine) in comparison with inorganic sulphate; however, no differences between sources were noticed in muscle Zn concentration.

The excess Zn competes with other metal ions for enzyme binding sites and transporter proteins. It has been shown that high dietary Zn intake inhibits intestinal absorption and hepatic accumulation of Cu (Ao *et al.,* 2009). In the present experiment, supplemental Zn-Pro increased Cu concentration in the liver compared to the control (*P*<0.05) and Zn-sulphate groups (*P*<0.05); however, no differences between treatments were observed in plasma, kidney and muscle in this parameter. Our results indicate that an antagonism between Zn and Cu did not occur in rabbits fed inorganic or organic Zn at a level higher than the maximum authorised Zn content in complete feed. This finding

Table 3: Effect of different dietary zinc sources on the total superoxide dismutase activity (SOD), Cu/Zn superoxide dismutase activity (Cu/Zn SOD), glutathione peroxidase activity (GPx), malondialdehyde concentration (MDA) and total antioxidant capacity (TAC) in liver and kidney tissues of rabbits.

| | Treatment | | | | |
	BD	ZnSO$_4$	Zn-Gly	Zn-Pro	*P*-value
SOD (U/mg protein)					
Liver	45.2±0.6	45.7±1.3	46.5±1.1	48.9±3.0	0.554
Kidney	35.9±2.9[a]	40.8±1.3[ab]	43.9±0.5[b]	41.3±1.4[ab]	0.027
Cu/Zn SOD (U/mg protein)					
Liver	34.6±0.6	35.8±1.5	35.7±1.3	38.9±2.3	0.324
Kidney	23.8±2.1[a]	26.8±0.9[ab]	31.1±0.4[b]	28.8±1.4[ab]	0.006
GPx (U/g protein)					
Liver	20.0±0.9	20.0±0.5	18.8±0.7	19.6±0.8	0.613
Kidney	21.5±0.8	23.4±1.3	23.5±1.0	23.5±1.6	0.554
MDA (nmol/g protein)					
Liver	117±9	109±5	104±7	116±8	0.559
Kidney	136±7	120±9	149±5	126±13	0.190
TAC (µmol/g protein)					
Liver	51.8±5.0	52.3±3.6	55.5±5.1	56.5±1.8	0.808
Kidney	31.2±1.4[a]	34.2±0.4[ab]	37.8±1.0[b]	35.5±2.1[ab]	0.019

[ab]Means in the same row having different letter superscripts indicate a significant difference (*P*<0.05). Data are presented as means±standard error of the mean.

is not in line with the results of an experiment carried out on chickens, which showed that the antagonism between Zn and Cu was observed when inorganic forms, but not organic forms of the minerals were included in the diet (Ao *et al.,* 2009). In addition, a co-accumulation between Zn and Cu concentration was observed in the liver of rabbits supplemented with organic Zn-Pro. It seems that the higher intake of organic Zn in the Zn-Pro form perhaps did not interfere with Cu absorption in the duodenal mucosa, resulting in higher Cu (*P*<0.05) accumulation in the liver tissue. A similar relationship between Zn and Cu accumulation in liver was also observed in broiler chicks supplemented with 100, 50 and 25 mg of Zn-Gly, resulting in enhanced Zn and Cu uptake by the liver (Kwiecien *et al.,* 2017). Addition of Zn supplements to rabbit diets did not influence the Mn and Fe concentrations in plasma and selected tissues, with the exception of Fe deposition in muscle being significantly lower in the Zn-sulphate supplemented group compared to the control (*P*<0.01) and Zn-Gly group (*P*<0.05). However, no differences in Fe concentration in plasma, liver and kidney were observed between treatments. This indicates that higher intake of Zn from inorganic or organic source did not influence the tissue deposition of other minerals (Mn, Cu and Fe) measured in the current study.

Feeding organic mineral sources of trace elements instead of inorganic ones could provide alternative pathways for absorption, thus leading to a reduction in the excretion of minerals (Bao *et al.,* 2007). When trace minerals are supplemented in excess of requirement, more is excreted due to decreased efficiency of utilisation of that mineral (Spears, 1996; Skřivan *et al.,* 2005). In the current study, dietary treatments significantly influenced mineral concentrations in faeces (Table 2). Regardless of source, the addition of 100 mg Zn/kg to the diet increased the faecal concentration of Zn almost twofold in all supplemented groups compared to control animals (*P*<0.001). We can assume that higher dietary Zn intake resulted in a saturation of the carrier-mediated pathway of zinc absorption, and subsequently a large amount was excreted. It is well known that faecal excretion is the major route of unabsorbed dietary zinc and endogenous zinc excretion. An increase in faecal excretion of trace minerals is considered to be one of the mechanisms for maintaining the homeostasis of these minerals in the body (King *et al.,* 2000). However, increased Zn concentration in this trial was associated with higher faecal concentration of Mn (*P*<0.01) and Cu (*P*<0.001) in the supplemented groups, and Fe concentration was also significantly increased in the faeces of rabbits receiving inorganic Zn sulphate (*P*<0.01). It has been shown that higher dietary Zn induces the synthesis of intestinal metallothionein to bind excess Zn and other divalent cations, making them less available for absorption (Sunder *et al.,*

2013). It seems that for this reason a large amount of minerals was excreted via faeces. The higher Fe excretion ($P<0.01$) was accompanied by a decrease ($P<0.01$) in its deposition in the muscle tissue, suggesting a negative effect of mineral interactions when a higher level of Zn was supplemented in inorganic form.

Based on the antioxidative properties of Zn, the present study aimed to estimate the effect of inorganic and organic Zn sources on antioxidant status of rabbits by evaluating TAC, SOD and GPx activity, and MDA level in the liver and kidney tissues (Table 3). The activities of SOD, GPx and MDA level are involved in the antioxidant defence system and lipid peroxidation, considered to be important oxidative stress markers (Farombi *et al.,* 2004). Dietary treatment overall had no significant effect on antioxidant indices in rabbit liver. In contrast, Ma *et al.* (2011) observed increased Cu/Zn SOD and GPx activity as well as the reduction of MDA content in the liver of chickens supplemented with 90 or 120 mg Zn/kg from Zn-Gly. In the current study, the addition of Zn-Gly at the level of 100 mg Zn/kg of diet caused a significant increase in the activity of Cu/Zn SOD ($P<0.01$) and TAC ($P<0.05$) in rabbit kidney, even though the Zn concentration in this tissue was not affected by the treatment. No significant effect of dietary Zn on GPx activity and MDA level in the kidney was observed. Higher TAC indicates stronger antioxidant defence and might decrease potential oxidative damage in kidney tissue. This result might be attributed to the higher activity of Cu/Zn SOD in kidney observed in the Zn-Gly group, as mentioned above. Zn is involved in the formation of Cu/Zn SOD, and its activity is elevated in the case of supplementation of this mineral into the diet (Kwiecien *et al.,* 2017; Ma *et al.,* 2011). Our finding may also be explained by increasing the other cellular antioxidants (which were not measured during this study), which could contribute to the elevated TAC in the kidney. Zinc is a strong inducer of the endogenous antioxidant metallothionein in renal proximal tubular cells and plays a major protective role in the renal tissues and may prevent oxidative stress (Alscher *et al.,* 2005). In addition, Zn is involved in the regulation of glutathione (GSH) metabolism, which is considered as the most important antioxidant containing thiol group (Oteiza, 2012). Bulbul *et al.* (2008) reported that dietary intake of Zn in organic form was more effective in the protection of cell membrane against lipid peroxidation than the inorganic form of zinc and increased the glutathione level in plasma. There are several mechanisms which contribute to the antioxidant properties of zinc. However, its role in the upregulation of antioxidant genes via Nrf2 may be a major mechanism underlying the antioxidant action of zinc (Cortese *et al.,* 2008).

CONCLUSION

We can conclude that intake of diet with Zn dosage above the maximum EU authorised total contents (150 mg Zn/kg complete feed) does not indicate an adverse impact on the tissue deposition of other minerals (Mn, Cu and Fe) and had no negative effects on the antioxidant defence system in animals. In contrast, it improved the antioxidant status in the kidney of rabbits fed a diet enriched with organic Zn-Gly. Dietary organic zinc sources had a similar effect on Zn deposition and Cu/Zn SOD activity in tissues as zinc sulphate, and can be an alternative to traditionally used inorganic mineral salts in providing an efficacious source of Zn to enrich feed for rabbits.

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