Iranian Society of Animal Science

Journal of Livestock Science and Technologies

Shahid Bahonar University of Kerman

ISSN: 2322-3553 (Print) ISSN: 2322-374X (Online)

Paper type: Original Research

Various zinc sources and levels supplementation on performance, egg quality and blood parameters in laying hens

Azam Mehrabani Mamdooh, Sara Mirzaie goudarzi*, Ali Asghar Saki and Hassan Aliarabi

Department of Animal Science, Faculty of Agriculture, Bu-Ali University, Hamedan, Iran

*Corresponding author,
Tel.: +98 9188520885
E-mail address:
smirzaie@basu.ac.ir

Received: 18 May 2021,
Accepted: 14 Sep 2021,
Published online: 22 Nov 2021,

ORCID
Azam Mehrabani Mamdooh
0000-0002-1689-4534
Sara Mirzaie goudarzi
0000-0001-8118-9922
Ali Asghar Saki
0000-0002-6789-1288
Hassan Aliarabi
0000-0001-8327-0628

Abstract The objective of this study was to investigate the effects of dietary zinc (Zn) source and level of supplementation on performance, egg quality, blood parameters, and egg yolk Zn content in laying hens from 28 to 36 weeks of age. Two hundred laying hens (Hy-Line W-36) were weighed individually and placed randomly in cages with five treatments, five replicates of 8 birds in each using a 2×2+1 factorial arrangement. Treatments consisted of a control diet (corn-soybean meal as a basal diet without Zn supplementation) and the diet supplemented with 80 and 120 mg of Zn/kg, added as either Zinc-sulfate (ZnSulf) or Zn-methionine (ZnMet), respectively. Treatments did not impact on the laying hen performance. Eggshell thickness was improved (P<0.001) by ZnMet in comparison to ZnSulf. Eggshell thickness improved by increasing Zn from 80 to 120 mg/kg of the diet (P<0.05). Plasma HDL (P<0.001), alkaline phosphatase activity (P<0.05), total protein, and albumin concentrations (P<0.001) increased by ZnMet in comparison to ZnSulf supplementation. Plasma Fe, Cu, Zn, and P, and Zn contents of the egg yolk were not affected by Zn sources or levels. Plasma Ca was higher in hens receiving 120 mg/kg ZnMet/kg than other treatments (P<0.01). In conclusion, more positive effects on eggshell quality and some blood parameters were found by dietary ZnMet supplementation at 80 or 120 mg/kg diet than ZnSulf in laying hens under the conditions of this study.

Keywords: alkaline phosphatase, eggshell thickness, layer, zinc methionine, zinc sulfate effects

Introduction

Knowledge of the essential nutrient requirements of laying hens at peak production is of particular interest in assuring optimal egg production and interior quality to prevent significant losses to the commercial egg industry production. Identification of factors affecting the attaining and maintenance of peak production, overall performance, and egg quality in layaers is of prime concern (Roland, 1998; Coutts et al., 2007). Zinc is a crucial trace mineral playing a vital role in numerous additional biological processes, such as enzyme action, cell membrane stabilization, gene expression, and cell signaling (Prasad, 2009). It acts as a cofactor of more than 300 enzymes (Keith et al., 2000). Hence, almost all metabolic pathways are in some way reliant on at least one zinc requiring protein (McCall et al., 2000). Zinc is a cofactor of carbonic anhydrase, which is needed for the formation of eggshells (Scheideler, 2008; Yilmaz Dikmen et

al., 2015). Moreover, zinc is involved in the deposition of albumen and the production of eggshell membranes in the magnum and isthmus, respectively (Tabatabaie et al., 2007). Zinc also plays an essential role in the uterus (Zinpro, 2002). Natural Zn concentrations in feedstuffs are generally lower than the daily Zn requirement for poultry, leading to the necessity of dietary Zn supplementation. Most mineral sources used in laying hen diets are inorganic compounds such as oxides, sulfates, and carbonates. During recent years, there has been a trend towards increasing use of organic trace minerals as supplements by livestock producers and feed manufactures due to improved performance and health. This is based on the higher bioavailability of organic Zn compared to inorganic salts (Spears, 2003; Mohanta and Garg, 2014). Zinc methionine, an organic source of Zn, has become increasingly popular in poultry production. Dietary supplementation with optimal levels of ZnMet was reported to promote the laying performance and egg quality, increase the hatchability, and enhanced resistance to diseases and stress in poultry (Wang et al., 2015; Jahanian and Rasouli, 2014; Abd El-Hack et al., 2018; Li et al., 2019; Chen et al., 2018). Marie (2016) found that supplementing the hen's diets with a combination of organic (zinc-amino acid, (Zn-AA)) and inorganic (ZnSulf) sources of Zn reduced egg breakage and improved both hen-day egg production (EP) and shell quality.

However, available data and literature concerning the effects of ZnMet in laying hens are limited, and the practical results of organic trace mineral supplementation in poultry diets remain controversial, due to the differences between the Zn sources and supplementation rates (Stefanello et al., 2014). High bioavailability of organic Zn sources in laying hens may lead to improved laying performance than inorganic forms. To test the hypothesis, this study aimed to compare the effects of diets containing different levels of organic or inorganic Zn on the performance, egg quality, blood parameters, and egg yolk Zn content in laying hens.

Materials and methods

Experimental procedures were conducted according to the Bu-Ali Sina University Animal Ethics Committee Guidelines.

Birds, experimental diets and management

Two hundred Hy-Line W-36 laying hens were allocated into 50 cages equipped with two nipple drinkers and an open trough feeder. At 28 wk of age, two adjacent cages were assigned at random to 5 experimental treatments (5 replicates of 8 layers in each), and the hens were fed their corresponding diets.

A completely randomized design, arranged as a 2×2+1 factorial, included a control diet (basal diet based on a corn-soybean meal without supplementation of Zn), and the basal diet was supplemented with either 80 or 120 mg Zn/kg, added as ZnSulf or ZnMet from 28 to 36 weeks of age. The basal diet was formulated based on the recommendations by Hy-Line W-36 International (Hy-Line, 2015) except for Zn. Composition of the basal diet is shown in Table 1.

Table 1. Composition of the experimental basal diet (% as-fed basis, unless otherwise indicated)

Ingredient	(%)[1]
Corn	57.68
Soybean meal (45.33% CP)	27.41
Soy oil	1.87
Dicalcium phosphate	2.07
Oyster shell	9.89
Sodium chloride	0.40
Mineral premix[2]	0.25
Vitamin premix[3]	0.25
DL-Methionine	0.18
Calculated composition	
AME_n (kcal/kg)	2750
Crude protein	17.02
Calcium	4.26
Available phosphorus	0.50
Sodium	0.18
Lysine	0.81
Methionine	0.41
Methionine +cysteine	0.66
Threonine	0.55
Tryptophan	0.17
Valine	0.72
Isoleucine	0.65
Analyzed composition	
Dry matter	91.66
Total ash	11.70
Crude protein	16.64
Crude fiber	2.56
Zinc (mg/kg)	45.19
Copper (mg/kg)	11.63
Iron (mg/kg)	166.45

[1]Basal diet (control) formulated without Zn supplementation and the inorganic zinc in control provided per Kg of diet: Zinc Sulfate 80 and 120 mg. The organic Zinc in other 2 treatments provided per Kg of diet: Zn-methionine; 80 and 120 mg.

[2]Supplied per kilogram of diet: 150 mg Mn ($MnSO_4 \cdot H_2O$), 72.7 mg Fe ($FeSO_4 \cdot H_2O$), 13.99 mg Cu ($CuSO_4 \cdot 5H_2O$), 0.815 mg; I (KI), 8.8 mg; Se (Na_2SeO_3).

[3]Supplied per kilogram of diet: vitamin A (trans-retinyl acetate), 8,800 IU; vitamin D_3 (cholecalciferol), 2,500 IU; vitamin E (allrac-α-tocopherol acetate), 6.6 mg; thiamine (thiamine mononitrate), 1.5 mg; riboflavin, 4.4 mg; pyridoxine (pyridoxine HCl), 2.5 mg; vitamin B12 (cyanocobalamin), 0.08 mg; vitamin K (bisulfate menadione complex), 2.5 mg; nicotinic acid, 20 mg; pantothenic acid (d-calcium pantothenate), 8 mg; folic acid, 1.1 mg; biotin, 0.15 mg; choline (chloride chloride).

An analytical grade $ZnSO_4 \cdot 7H_2O$ (Merck, Germany) was used as the inorganic source of Zn. The organic source of Zn was Zn-methionine (18% Zn: by a modified method of United States Patent with Publication Number: US7087775 B2) (Alimohamady et al., 2018). Briefly, for the synthesis of organic zinc supplement in the form of zinc-methionine, high purity zinc sulfate salt and methionine were used. Then chelate formation was eval-

uated using the Fourier Transform Infrared Spectroscopy (FT-IR) as described by De Souza et al. (2007). The average temperature and relative humidity were maintained at 20-22 ºC and 50-60%, respectively. The lighting program consisted of 16 hours of light: 8 hours of dark. Water and feed were offered *ad libitum*.

Laboratory analysis

A basal diet sample was ground by a 1-mm screen laboratory mill (Cyclotech Mill, Tecator, Sweden) followed by chemical analysis using the AOAC standard methods (1990); for dry matter (method 967.03), total ash (method 942.05), and crude protein (method 976.06). Crude fiber (CF) was measured sequentially using a filter bag system (Ankom Technology, Macedon, NY) (Van Soest et al., 1991). The Zn, Cu, and Fe contents of the basal diet, and plasma samples were estimated utilizing an air-acetylene flame on an atomic absorption spectrophotometer (Varian spectra AA220, Australia) as described by Salama et al., (2003) and Rimbach et al., (1998), respectively. Yolk contents from each egg were frozen (−18°C) for storage, then thawed, mixed, dehydrated in a forced-draft oven at 55°C for 96 h, ground through a 0.5-mm sieve, and sampled. A representative sample of each egg yolk (250 mg) was weighed and digested with a mixture of 2 mL of 30% H_2O_2 and 4 mL of 70% HNO_3 for 6h in a 100°C bath according to AOAC International (AOAC, 1999). The digested samples were analyzed for Zn content using an inductively coupled plasma atomic emission spectroscopy instrument (Variant Spectr AA220, Australia).

Performance and egg quality

Egg production and egg weight (EW) were recorded daily, and feed intake (FI) was determined weekly. This information was used to calculate the average daily feed intake (ADFI), egg mass (EM), and feed conversion ratio (FCR).

Egg quality was measured by collecting six eggs from each replicate in the last two days of the 28, 30, 32, 34, and 36 weeks of age. Then, the average value in each period was adjusted for further analysis. The eggs were individually weighed, and the egg external (shape index, shell thickness and shell weight), and internal quality (Haugh unit and yolk color) were studied. The shell was separated from the yolk and albumen, dried overnight at 60°C, and weighed (Grobas et al., 2001). Eggshell thickness was measured using a digital micrometer (Echometer 1061, Robotmation Company, Tokyo, Japan). Eggshell ratio was expressed as shell weight to egg weight. The Haugh unit was calculated by using the egg weight (g) and albumen height (AH/mm) (Haugh, 1937). Yolk color evaluated visually by the COLOR FAN method, was determined against a white, nonreflective surface, in order to eliminate the influence of adjacent colors, using indirect daylight and no artificial light. The use of indirect light prevents distracting reflections from the glossy surface of the yolk. The blades of the DSM Yolk FanTM were held immediately above the egg yolk and viewed vertically from above, with the blade numbers facing down and the yolk positioned between the tips of the blades (DSM, 2016).

Blood parameters

At 36 weeks of age, 3 mL of blood samples were drawn from the brachial vein of 15 birds per treatment (n=5) into heparinized tubes. Blood samples were centrifuged for 10 min (5000×g), and plasma was collected and frozen at –20ºC until biochemical analysis. Plasma LDL, HDL, triglyceride, glucose, cholesterol, total protein, albumin, calcium, and phosphorus were measured using commercial kits (Pars Azmon, Iran) and an atomic absorption spectrophotometer (Varian Spectr AA220, Australia). Alkaline phosphatase activity was estimated according to the recommendations of the German Society of Clinical Chemistry and the International Federation of Clinical Chemistry (IFCC), using a commercial kit (Pars Azmon, Iran) by an atomic absorption spectrophotometer (Varian Spectr AA220, Australia).

Statistical analysis

This experiment was conducted as a completely randomized design with five treatments in a 2×2+1 factorial arrangement. The main effects were analyzed by ANOVA using the GLM procedure of the SAS statistical package (SAS, 2012). The treatments included the control (basal diet without supplementary Zn), and the basal diet supplemented with 80 and 120 mg Zn/kg, added as either ZnSulf or ZnMet as the main effects. The Duncan's multiple range test was used to compare the means ($P<0.05$). The data were analyzed for the main effects and interactions between Zn source and Zn level. Data on performance and egg quality were analyzed as repeated measures. The statistical model used was:

$$Y_{ijkl} = \mu + S_i + L_j + S_i \times L_j + W_k + S_i \times W_k + L_j \times W_k + W_k \times S_i \times L_j + Ea_{ijk}$$

where, Y_{ijkl}: represents the dependent variable analyzed; μ: is the overall mean, S_i: is the effects of i^{th} source of Zn; L_j: is the effects of the j^{th} level of Zn; $S_i \times L_j$: the interaction between source and level of Zn; W_k: effect of time (week); $S_i \times W_k$: the interaction between source of Zn and time (week); $L_j \times W_k$: the interaction between level of Zn and time (week); $W_k \times S_i \times L_j$: the interaction between time (week) and source and level of Zn; Ea_{ijk}: is the random residual error.

Results and discussion

Performance and egg quality

No interactions effects were detected between Zn source

and level on the performance traits. The ADFI, EP, EW, EM, and FCR were not affected by the source or level of Zn in the diet (Table 2).

Table 2. Effects of dietary Zn source and level on productive performance of laying hens from 28 to 36 weeks of age

	ADFI[1] (g/hen/day)	Egg production (%)	Egg weight (g)	Egg mass (g/day)	FCR[2]
Zn source					
ZnSulf	97.71	92.69	60.91	56.49	1.731
ZnMet	98.70	93.10	60.67	56.47	1.753
Zn level (mg/kg)					
80	98.51	92.72	61.03	56.61	1.747
120	97.90	93.06	60.55	56.35	1.738
SEM[3]	0.485	0.864	0.412	0.651	0.0162
Treatments[4]					
T1	97.16	93.74	61.02	57.18	1.702
T2	97.87	92.93	61.49	57.16	1.719
T3	97.55	92.45	60.34	55.81	1.743
T4	99.15	92.52	60.58	56.05	1.775
T5	98.26	93.67	60.77	56.89	1.732
SEM	0.688	1.188	0.586	0.868	0.023
P value					
Zn source	0.1675	0.7427	0.6835	0.9878	0.3531
Zn level	0.3892	0.7843	0.4246	0.7862	0.6919
Zn source × Zn level	0.6879	0.5139	0.2682	0.2523	0.1647
Treatment	0.3348	0.8936	0.6908	0.7009	0.2717

[1]ADFI: Average daily feed intake; [2]Feed conversion ratio (ADFI/egg mass); [3]SEM: standard error of the mean. [4]Treatments: T_1 basal diet (control), T_2 basal diet+80 mg ZnSulf/kg diet, T_3 basal diet+ 120 mg ZnSulf/kg diet, T_4 basal diet+ 80 mg ZnMet/kg diet, T_5 basal diet+ 120 mg ZnMet/kg diet.

In agreement with this data, Tsai et al. (2016) reported that FI, EP, EW, and FI/EM were not influenced by different zinc sources (ZnO, ZnMet, or nanosize ZnO). Stefanello et al. (2014) reported no effect of supplementation of Mn, Zn, and Cu in the diet of laying hens on feed intake and FCR, which is consistent with our results. Tabatabaie et al. (2007) observed that source (zinc sulfate or Albino-Zn) or level (25 and 50 mg/kg) of zinc supplements did not affect EP, EW, or FCR in laying hens. Li et al. (2019) showed that ZnMet did not affect the egg weight compared to the control. In contrast, Abedini et al. (2018) stated that laying hens fed diets supplemented with ZnMet and zinc oxide nanoparticles had greater EP and EM than the control group, while no significant effect was reported for FI, FCR, and EW. This may be attributed to the high bioavailability of organic Zn. Another theory postulates that dietary Zn possibly improves EP by interacting with the endocrine system. Hence, it is essential for progesterone synthesis, and its deficiency can induce an excessive secretion of prolactin that initiates broodiness and stops egg production (Park et al., 2004). Part of these contradictory results may arise from differences in hen age, dietary composition, the concentration of examined microelements in the basal diets, and source of organic mineral (amino acid complex, proteinate etc. Gheisari et al., 2011). As shown in Table 3, the egg traits were not affected by Zn source except for shell thickness ($P<0.001$) which was improved to a greater degree by ZnMet than by ZnSulf. Increasing the dietary zinc levels from 80 to 120 mg/kg increased the shell thickness ($P<0.05$). The most significant improvement in shell thickness was achieved ($P<0.001$) by birds fed the diets supplemented with ZnMet or ZnSulf in comparison with the control.

In agreement with the data reported here, Stefanello et al. (2014) observed an increase in eggshell thickness by increasing some trace minerals in the diet (Zn, Cu, and Mn). The greater shell thickness was found by organic mineral forms such as proteinates which may be due to the higher thickness of the palisade layer and a lower mammillary density (Gheisari et al., 2011). This study confirmed the beneficial effect of supplementing Zn in laying hen diets. Zn plays a crucial role in eggshell for-

mation in the magnum and uterus during the deposition of albumen in the isthmus and eggshell membranes in the uterus. In this respect, Zinpro (2002) indicated that Zn deficiency affected the quality of the epithelium due to the role of zinc in protein synthesis. Zinc also indirectly affects the epithelial secretions by affecting the structure of the epithelium or directly during the synthesis of eggshell membranes. Mabe et al. (2003) showed an improvement in egg quality by using 60 mg zinc /kg in laying hen diets. Trace elements may affect eggshell quality by their catalytic properties as critical enzymes involved in the process of membrane and eggshells formation or by interacting directly with the calcite crystals in the formation of eggshells. Zinc is a component of the carbonic anhydrase, which is crucial - for supplying carbonate ions during eggshell formation (Mabe et al., 2003; Nys et al., 2004). There were no significant differences in egg weight components and shell thickness, but albumen height and Haugh units were higher in birds receiving organic zinc at 25 or 50 mg/kg than the control group (Tabatabaie et al., 2007).

No significant differences were observed in egg quality parameters due to zinc sources except the Haugh unit values when laying hens were fed diets that included zinc oxide, zinc sulfate, zinc carbonate, and zinc proteinate at 140 mg/kg diet (Idowu et al., 2011). Zhao et al. (2016) reported decreases in the albumen height and Haugh unit by ZnSulf and ZnO compared to the control group.

Table 3. Effects of dietary Zn source and level on egg quality from 28 to 36 weeks of age

	Haugh unit	Yolk color	Shape index (%)	Shell thickness (mm)	Shell ratio (%)	Shell weight (g)
Zn source						
Zn Sulf	86.04	4.82	75.92	0.542[b]	9.32	5.76
ZnMet	84.95	4.91	76.00	0.579[a]	9.29	5.67
Zn level (mg/kg)						
80	85.56	4.81	75.73	0.556[b]	9.32	5.77
120	85.43	4.92	76.19	0.566[a]	9.28	5.67
SEM[1]	0.399	0.064	0.254	0.0026	0.063	0.045
Treatments[2]						
T1	88.50	4.70	76.35	0.509[d]	9.10	5.65
T2	86.24	4.70	75.86	0.540[c]	9.35	5.83
T3	85.84	4.95	75.99	0.544[c]	9.28	5.69
T4	88.84	4.92	75.60	0.571[b]	9.30	5.70
T5	85.02	4.90	76.40	0.587[a]	9.11	5.65
SEM	0.563	0.090	0.339	0.0043	0.089	0.065
P value						
Zn source	0.0710	0.3053	0.8272	<0.0001	0.3475	0.4127
Zn level	0.8224	0.1881	0.2153	0.0148	0.3550	0.4237
Zn source × Zn level	0.6340	0.1082	0.3627	0.0490	0.3243	0.3087
Treatment	0.2197	0.1333	0.4352	<0.0001	0.4222	0.5078

[1] SEM: standard error of the mean.
[2]Treatments: T_1 basal diet (control), T_2 basal diet+ 80 mg ZnSulf/kg diet, T_3 basal diet+ 120 mg ZnSulf/kg diet, T_4 basal diet+ 80 mg ZnMet/kg diet, T_5 basal diet+ 120 mg ZnMet/kg diet.
a,b,c,d: Within columns, mean values with common superscript (s) are not different (P>0.05).

Blood parameters

No changes were found in blood parameters by Zn sources or levels except for HDL (P<0.001), ALP (P<0.05), total protein, and albumin (P<0.001) contents, which were significantly increased by ZnMet. However, HDL (P<0.01), total protein (P<0.001), and albumin (P<0.01), were higher in the 80 and 120 mg/kg ZnMet treatments. Plasma glucose (P<0.05) was higher in 120 mg/kg ZnMet than other treatments (Table 4).

Bahakaim et al. (2014) reported that plasma total protein and albumin increased by higher Zn levels. These results may be due to the important function of Zn in protein synthesis (Ibs and Rink, 2003), which is in agreement with our results. Hazim and Mahmood (2011) pointed out the addition of zinc to the diet of broiler bre - eder hens increased blood plasma cholesterol, protein, calcium and phosphorus concentration and alkaline phosphatase activity in comparison with the control group. Idowu et al. (2011) observed no significant differences between Zn sources in blood glucose levels. Yilmaz Dikmen et al. (2015) found that dietary supplementation of ZnAA-MnAA chelate did not significantly affect plasma total protein, glucose, total cholesterol, Ca, P, and Zn levels. Feng et al. (2010) reported increases in serum total protein and calcium concentration by zinc glycine with no effect on albumin or phosphorus. However, Li et al. (2019) found decreased ionic calcium concentration, and improved ALP activity of ALP due to 80 mg/kg zinc sulfate supplementation in the layer hen diet. Because ALP is a zinc-containing enzyme and zinc is essential for ALP activity (Yu et al., 2005), this element plays an essential role in calcium storage in bones. Shell formation requires

several times as much Ca as exists in the extra-cellular pool (Bar, 2008). This was due to the Zn binding capacity in the serum, which has been used as a good indicator of Zn status.

Table 4. Effects of dietary Zn source and level on blood parameters of laying hens at 36 weeks of age

	LDL[1] (mg/dL)	HDL[2] (mg/dL)	TG[3] (mg/dL)	ALP[4] (U/L)	Glucose (mg/dL)	Cholesterol (mg/dL)	Total protein (g/dL)	Albumin (g/dL)
Zn source								
ZnSulf	46.25	19.59[b]	129.7	282.5[b]	230.0	85.78	2.93[b]	2.54[b]
ZnMet	43.81	21.55[a]	142.2	307.4[a]	246.5	90.57	4.23[a]	3.34[a]
Zn level (mg/kg)								
80	50.38	20.50	137.8	291.3	234.2	82.32	3.55	2.91
120	51.56	20.65	134.1	298.7	242.3	94.03	3.64	2.97
SEM[5]	5.365	0.303	5.67	6.55	8.18	7.849	0.201	0.082
Treatments[6]								
T1	45.00	19.51[b]	112.6	281.1	200.2[b]	83.02	2.98[b]	2.57[b]
T2	35.91	19.52[b]	134.8	280.5	234.4[ab]	78.39	2.97[b]	2.58[b]
T3	53.59	19.66[b]	124.5	284.5	225.7[ab]	93.16	2.93[b]	2.51[b]
T4	41.06	21.48[a]	140.8	302.0	234.0[ab]	86.24	4.12[a]	3.24[a]
T5	46.53	21.63[a]	143.7	312.8	258. 9[a]	94.89	4.34[a]	3.44[a]
SEM	8.044	0.481	7.95	9.27	11.29	11.282	0.255	0.115
P value								
Zn source	0.7523	0.0003	0.1342	0.0102	0.1752	0.6720	0.0004	<0.0001
Zn level	0.1046	0.7422	0.6492	0.4237	0.4932	0.3071	0.7580	0.5731
Zn source × Zn level	0.3301	0.9925	0.4225	0.7119	0.1671	0.7866	0.6544	0.2524
Treatment	0.4792	0.0051	0.0669	0.0646	0.0260	0.8240	0.0007	0.0016

[1]LDL: Low-density lipoprotein, [2] HDL: High-density lipoprotein, [3] TG: Triglycerides, [4]ALP: Alkaline phosphatase; [5]SEM: standard error of the mean. [6]Treatments: T_1 basal diet (control), T_2 basal diet+ 80 mg ZnSulf/kg diet, T_3 basal diet+ 120 mg ZnSulf/kg diet, T_4 basal diet+ 80 mg ZnMet/kg diet, T_5 basal diet+ 120 mg ZnMet/kg diet.

a,b: Within columns, mean values with common superscript (s) are not different (P>0.05).

In agreement with the results of the current study, Abedini et al. (2018) reported that Zn supplementation significantly affected the total serum protein and albumin levels. Prasad (2014) stated that Zn deficiency may cause abnormalities in nucleic acid synthesis and the activity of many enzymes. Therefore, the higher serum proteins in the groups receiving Zn could be due to the role of Zn in protein synthesis.

Blood mineral contents

Plasma Fe, Cu, Zn, and P concentrations were not affected by zinc source and level. Interaction effects between the zinc source and level wre significant for plasma concentration of Ca. The greatest plasma Ca was recorded for hens fed with 120 mg ZnMet (P<0.01; Table 5). Bartlett and Smith (2003) also observed that levels of dietary Zn did not affect the blood content of zinc in birds exposed to high ambient temperature. On the contrary, Bahakaim et al. (2014) showed that ZnMet significantly increased the plasma zinc concentration. This may be due to the higher availability of ZnMet compared to ZnSulf. Li et al. (2019) reported that concentrations of serum P, and Zn were not affected by Zn-Met. Abedini et al. (2018) observed that dietary Zn supplementation did not affect the serum concentrations of several minerals (Ca, P, Mn, Fe, and Cu). In agreement with the data reported herein, supplemental Zn did not affect the serum Zn levels in broilers when the basal diet was supplemented with Zn (Tronina et al., 2007). The discrepancies between various studies might be attributed to the dietary Zn concentration, source or -duration of Zn feeding, and previous body stores.

Zn content of the egg yolk

No effect of Zn source or supplementation level was found on the Zn content of the egg yolk. However, the Zn content of the egg yolk was numerically increased by 120 mg/kg ZnMet in comparison with other treatments (Table 5).

It was demonstrated that increasing the supplemental Zn level from 0 to 120 mg/kg of ZnMet increased the egg Zn content by 23.2 % compared to the control group (unsupplemented Zn diet). The Zn content of egg increased by dietary supplementation of ZnO-NPs and ZnMet (Abedini et al., 2018).

Bahakaim et al. (2014) indicated that increasing the Zn level from 0 to 150 mg/kg of organic source (ZnMet) increased the Zn egg content significantly without any adverse effects on egg production. Kim and Patterson (2005) and Plaimast et al. (2008) showed that zinc storage in egg increased linearly with increasing the zinc levels in the diet. Mabe et al. (2003) observed a significant increase in Zn egg yolk concentration with 60 mg/kg zinc in the diet. In contrast, Skrivan et al. (2005) reported no significant effect on the yolk Zn content by adding 80 mg/kg Zn to the diet of laying hens; in agreement with the results reported herein, Plaimast et al. (2008) found the greatest concentration of egg Zn when layers received a diet supplemented with organic Zn, being due to the higher bioavailability of organic Zn.

Table 5. Effects of dietary Zn source and level on mineral content of the blood and Zn content of the egg yolk

	Plasma					Yolk
	Zn (mg/dL)	Cu (µg/dL)	Fe (mg/dL)	Calcium (mg/dL)	Phosphorous (mg/dL)	Zn (mg/kg)
Zn source						
ZnSulf	7.25	1.07	152.87	13.51[b]	6.57	69.48
ZnMet	7.51	1.06	156.65	15.23[a]	6.09	75.12
Zn level (mg/kg)						
80	6.81	1.08	156.87	13.50[b]	6.25	69.17
120	7.95	1.06	152.65	15.24[a]	6.45	75.88
SEM[1]	0.854	0.061	9.762	0.513	0.255	3.432
Treatments[2]						
T1	6.47	1.16	154.28	13.21[b]	5.41	64.70
T2	6.47	1.09	150.32	13.24[b]	6.56	67.62
T3	8.03	1.06	155.42	13.79[b]	6.58	72.06
T4	7.14	1.07	163.43	13.76[b]	5.85	70.71
T5	7.88	1.07	149.88	15.69[a]	6.32	79.71
SEM	1.105	0.080	15.390	0.666	0.363	4.351
P value						
Zn source	0.8361	0.8972	0.7887	0.0312	0.2000	0.2846
Zn level	0.3545	0.8251	0.7654	0.0287	0.5077	0.1855
Zn source × Zn level	0.7315	0.9634	0.5093	0.1206	0.5509	0.6448
Treatment	0.7627	0.8717	0.9741	0.0073	0.1441	0.1914

[1]SEM: standard error of the mean. [2]Treatments: T_1 basal diet (control), T_2 basal diet+ 80 mg ZnSulf/kg diet, T_3 basal diet+ 120 mg ZnSulf/kg diet, T_4 basal diet+ 80 mg ZnMet/kg diet, T_5 basal diet+ 120 mg ZnMet/kg diet.
a,b: Within columns, mean values with common superscript (s) are not different (P>0.05).

Conclusions

Dietary zinc as ZnMet and supplemented at 120 mg/kg improved the eggshell quality and some blood parameters in laying hens.

Acknowledgments

Our special thanks to Bu-Ali Sina University for providing facilities and financial support for this study.

References

Abd El-Hack, M.E., Alagawany, M., Salah, A.S., Abdel-Latif, M.A. Farghly, M.FA., 2018. Effects of dietary supplementation of zinc oxide and zinc methionine on layer performance, egg quality, and blood serum indices. *Biological Trace Element Research* 184, 456-462.

Abedini, M., Shariatmadari, F., Karimi Torshizi, M.A. Ahmadi, H., 2018. Effects of zinc oxide nanoparticles on the egg quality, immune response, zinc retention, and blood parameters of laying hens in the late phase of production. *Journal of Animal Physiology and Animal Nutrition* 102, 736-745.

Alimohamady, R., Aliarabi, H., Bruckmaier, R.M. Christensen, R.G., 2018. Effect of different sources of supplemental zinc on performance, nutrient digestibility, and antioxidant enzyme activities in lambs. *Biological Trace Element Research* 189, 75-84.

AOAC. 1990. Official Methods of Analysis. 15th edition. Association of Official Analytical Chemists, Arlington, VA, USA.

AOAC. 1999. Macro and micro elements in plant tissues by ICPAES. In: Analytical Techniques for Inorganic Contaminants. AOAC Int., Arlington, VA. Pages 110–116.

Bahakaim, A.S.A., Abdel Magied, A., Sahar, M., Osman, H., Amal, S., Omar, N., Abdel Malak, Y. Ramadan, A., 2014. Effect of using different levels and sources of zinc in layer's diets on egg zinc enrichment. *Egyptian Poultry Science* 34, 39-56.

Bar, A., 2008. Calcium homeostasis and vitamin D metabolism and expression in strongly calcifying laying birds. *Comparative Biochemistry and Physiology* Part A 151, 477-490.

Bartlett, J.R. Smith, M.O., 2003. Effects of different levels of zinc on the performance and immunocompetence of broilers under heat stress. *Poultry Science* 82, 1580-1588.

Chen, N.N., Liu, B., Xiong, P.W., Guo, Y., He, J.N., Hou, C.C., Ma, L.X. Yu, D.Y., 2018. Safet evaluation of zinc methionine in laying hens: Effects on laying performance, clinical blood parameters, organ development, and histopathology. *Poultry Science* 97,

1120-1126.

Coutts, J.A., Wilson, G.C., Fernandez, S., 2007. Optimum Egg Quality. M. Publishing, Sheffield, UK.

De Souza, A.R., Martins, L.P., de Faria, L.C., Martins, M.E.P., Fereira, R.N., Da Silva, A.M.L., Gil, E.S. Da Conceição, E.C., 2007. Studies on the bioavailability of zinc in rats supplementated with two different zinc-methionine compounds. *Latin American Journal of Pharmacy* 26, 825-830.

DSM, 2016. Egg yolk pigmentation guideline. Available at https://www.dsm.com/markets/anh/en_US/infocenter - news.markets.dsm.categories.dsm.html [Verified 4 December 2017].

Feng, J., Ma, W.Q., Niu, H.H., Wu, X.M., Wang, Y. Feng, J., 2010. Effects of zinc glycine chelate on growth, hematological, and immunological characteristics in broilers. *Biological Trace Element Research* 133, 203-211.

Gheisari, A.A., Sanei, A., Samie, A.H., Gheisari, M.M. Toghyani, M., 2011. Effect of diets supplemented with different levels of manganese, zinc, and copper from their organic or inorganic sources on egg production and quality characteristics in laying hens. *Biological Trace Element Research* 142, 557-571.

Grobas, S., Mendez, J., De Blas, C. Mateos, G.G., 2001. Influence of source and percentage of fat added to diet on performance and fatty acid composition of egg yolks of two strains of laying hens. *Poultry Science* 80, 1171-1179.

Haugh, R.R., 1937. The Haugh unit for measuring egg quality US. *Egg Poultry Magazine* 43, 522-555.

Hazim, J. Mahmood, H.M., 2011. Effect of dietary zinc on certain blood traits of broiler breeder chickens. *International Journal of Poultry Science* 10, 807-813.

Hy-Line International., 2015. Commercial Management Guide for Hy-Line W-36 Hens. Hy-Line International, West Des Moines, IA.

Ibs, K.H. Rink, L., 2003. Zinc alters immune function. *The Journal of Nutrition* 133(5 Suppl 1), 1452S-1456S.

Idowu, O., Ajuwon, R., Oso, A. Akinloye, O., 2011. Effects of zinc supplementation on laying performance, serum chemistry and Zn residue in tibia bone, liver, excreta and egg shell of laying hens. *International Journal of Poultry Science* 10, 225-230.

Jahanian, R. Rasouli, E., 2014. Effects of dietary substitution of zinc-methionine for inorganic zinc sources on growth performance, tissue zinc accumulation and some blood parameters in broiler chicks. *Journal of Animal Physiology and Animal Nutrition* 99, 50-58.

Keith, A.M., Huang, C.C. Fierke, C.A., 2000. Function and mechanism of zinc metalloenzymes. *Journal of* ---

Nutrition 130, 14375-14465.

Kim, K.W. Patterson, P.H., 2005. Effects of dietary zinc supplementation on hen performance, ammonia volatilization, and nitrogen retention in manure. *Journal of Environmental Science and Health* 40, 675-686.

Li, L.L., Gong, Y.J., Zhan, H.Q., Zheng, Y.X. Zou, X.T., 2019. Effects of dietary Zn-methionine supplementation on the laying performance, egg quality, antioxidant capacity, and serum parameters of laying hens. *Poultry Science* 98, 923-931.

Mabe, I., Rapp, C., Bain, M.M. Nys, Y., 2003. Supplementation of a corn-soybean meal diet with manganese, copper and zinc from organic or inorganic sources improves egg shell quality in aged laying hens. *Poultry Science* 82, 1903-1913.

Marie, M.K., 2016. The Effects of zinc supplementation from two sources on egg quality and bone health in laying hens. Theses and Dissertations in Animal Science. 123. http://digitalcommons.unl.edu/animalscidiss.

McCall, K,A., Huang, C. Fierke, C. A., 2000. Function and mechanism of zinc metalloenzymes. *The Journal of Nutrition* 130, 1437S–1446S.

Mohanta, R.K. Garg, A.K., 2014. Organic trace minerals: Immunity, health, production and reproduction in farm animals. *Indian Journal of Animal Nutrition* 31, 203-212.

Nys, Y., Gautron, J., Garcia-Ruiz, J.M. Hincke, M.T., 2004. Avian eggshell mineralization: Biochemical and functional characterization of matrix proteins. *Comptes Rendus Palevol* 3, 549-562.

Park, S., Birkhold, S., Kubena, L., Nisbet, D. Ricke, S., 2004. Effects of high zinc diets using zinc propionate on molt induction, organs, and post molt egg production and quality in laying hens. *Poultry Science* 83, 24-33.

Plaimast, H., Sirchakwal, P., Puwastien, P. Kijparkorn, S., 2008. Effect of supplementary zinc form organic sources on zinc deposition in eggs and laying performance. *Thai Journal of Veterinary Medicine* 38, 47-53.

Prasad, A.S., 2009. Zinc: role in immunity, oxidative stress and chronic inflammation. *Current Opinion in Clinical Nutrition and Metabolic Care* 12, 646-652.

Prasad, A.S., 2014. Zinc: an antioxidant and anti-inflammatory agent: Role of zinc in degenerative disorders of aging. *Journal of Trace Elements in Medicine and Biology* 28, 364-371.

Rimbach, G., Walter, A., Most, E. Pallauf, J., 1998. Effect of microbial phytase on zinc bioavailability and cadmium and lead accumulation in growing rats. *Food and Chemical Toxicology* 36, 7-12.

Roland, D.A., 1988. Eggshell breakage: Incidence and economic impact. *Poultry Science* 67, 1801-1803.

Salama, A.A.K., Caja, G., Albanell, E., Such, X., Casals, R. Plaixats, J., 2003. Effects of dietary supplements of zinc methionine on milk production, udder health and zinc metabolism in dairy goats. *Journal of Dairy Research* 70, 9-17.

SAS, 2012. SAS User's Guide: Statistics. Version 9.4. SAS Institute Inc., Cary, North Carolina. USA.

Scheideler, S.E., 2008. Trace mineral balance in poultry. Midwest Poultry Federation Convention, St. Paul, Minnesota, 18-20 March.

Skrivan, M., Skrivanove, V. Marounek, M., 2005. Effect of dietary iron, zinc and copper in layer feed on distribution of these elements in eggs, liver, excreta, soil, and herbage. *Poultry Science* 84, 1570-1575.

Spears, J.W., 2003. Trace mineral bioavailability in ruminants. *Journal of Nutrition* 133, 1506S-1509S.

Stefanello, C., Santos, T.C., Murakami, A.E., Martins, E.N. Carneiro, T.C., 2014. Productive performance, eggshell quality, and eggshell ultrastructure of laying hens fed diets supplemented with organic trace minerals. *Poultry Science* 93, 104-113.

Tabatabaie, M.M., Aliarabi, H., Saki, A.A., Ahmadi, A. Hosseini, S., 2007. Effect of different levels and sources of zinc on egg quality and layer performance. *Pakistan Journal of Biological Sciences* 10, 3476-3478.

Tronina, W., Kinal, S. Lubojemska, B., 2007. Effect of various forms of zinc applied in concentrate mixtures for broiler chickens on its bioavailability as well as meat composition and quality. *Polish Journal of Food and Nutrition Science*s 57, 577-581.

Tsai, Y.H., Mao, S.Y., Li, M.Z., Huang, J.T. Lien, T.F., 2016. Effects of nanosize zinc oxide on zinc retention, eggshell quality, immune response and serum parameters of aged laying hens. *Animal Feed Science and Technology* 213, 99-107.

Van Soest, P.J., Robertson, J.B. Lewis, A., 1991. Methods for dietary fiber, neutral detergent fiber, and non-starch polysaccharides in relation to animal nutrition. *Journal of Dairy Science* 74, 3583-3597.

Wang, X.Z., Liu, D.Y., Zhanga, W., Wang, C.J., Cakmakc, I. Zou, C.Q., 2015. An effective strategy to improve grain zinc concentration of winter wheat, Aphids prevention and farmers' income. *Field Crops Research* 184, 74-79.

Yilmaz Dikmen, B., Sozcu, A., Ipek, A. Ahan, U.S., 2015. Effects of supplementary mineral amino acid chelate (ZnAA-MnAA) on the laying performance, egg quality and some blood parameters of late laying period layer hens. *Kafkas Universitesi Veteriner Fakultesi Dergisi* 21, 155-62.

Yu, Z.P., Le, G.W. Shi, Y.H., 2005. Effect of zinc sulphate and zinc methionine on growth, plasma growth hormone concentration, growth hormone receptor and insulin-like growth factorigene expression in mice. *Clinical and Experimental Pharmacology and Physiology* 32, 273-278.

Zhao, Y., Li, L., Zhang, P.F., Liu, X.Q., Zhang, W.D., Ding, Z.P., Wang, S.W., Min, L.J. Hao, Z. H., 2016. Regulation of egg quality and lipids metabolism by zinc oxide nanoparticle. *Poultry Science* 95, 920-933.

Zinpro., 2002. Trace minerals for laying hens. Available at http://www.availzmc.com/technical/layer/page 2 html.