

# Zinc supplementation improves antioxidant status, and organic zinc is more efficient than inorganic zinc in improving the bone strength of aged laying hens

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## Abstract

**Background:** There have been some reports indicating that supplementation of zinc could alleviate the negative effects of age on egg quality in laying hens. However, information regarding these positive effects on health and zinc deposition in the body is limited.

**Objectives:** The aim of the present study was to investigate the effect of organic and inorganic sources of zinc on the antioxidant activity, bone strength, and zinc deposition in the tissues of older laying hens.

**Methods:** In a completely randomized design, 175 Leghorn laying hens (w36) aged 80 weeks were allocated into seven treatment groups and five replications: control (without zinc supplementation), zinc sulphate treatments (15, 30, and 45 mg/kg), and organic zinc treatments (15, 30, and 45 mg/kg).

**Results:** There was a significant increase in feed intake in the zinc sulphate and organic zinc treatments compared to the control treatment ( $p < 0.05$ ). The egg mass in organic and sulphate zinc showed a significant increase. The feed conversion ratio was decreased significantly in the organic zinc treatments ( $p < 0.05$ ). Both organic and sulphate zinc supplements enhanced serum superoxide dismutase activity as an antioxidant index ( $p < 0.05$ ). The cortical thickness of the tibia was improved in laying hens receiving 30 mg/kg organic zinc. Supplementation of zinc could lead to an increase in zinc deposition in tissues, and organic zinc boosts bone strength.

**Conclusion:** Zinc supplementation can improve antioxidant activity, feed intake, and feed conversion ratio and enhance egg mass and optimal absorption of zinc in tissues. The use of 30 mg/kg organic zinc is recommended for improving the cortical thickness of the tibia in aged laying hens.

## KEYWORDS

bone strength, laying hen, superoxide dismutase, zinc reserves, zinc supplementation

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## 1 | INTRODUCTION

Zinc is vital for growth and development, egg quality, bone health, immune function in laying hens (Yu et al., 2020), and the antioxidant protection system (Vakili & Rashidi, 2011). Cell growth, differentiation, reproduction, carbohydrate, and protein metabolism involve zinc, which has an important role in the isthmus during the formation of eggshell membranes and in the magnum during albumen deposition. zinc is considered a cofactor for carbonic anhydrase supplying carbonic ions during shell formation. Carbonic anhydrase inhibitors enhance the production of shell-less eggs and reduce bicarbonate ion secretion. zinc deficiency results in economic damage in the poultry industry, reduction or cessation of egg production and poor hatchability (Ogbuewu & Mbajiorgu, 2022).

The amount of a nutrient that reaches the systemic circulation and is involved in metabolism after being ingested and absorbed is called bioavailability (Ammerman et al., 1995). Many authors have discussed Zn bioavailability and accumulation, and there is a difference in the absorption of organic and inorganic supplements (Suttle, 2010). Mineral zinc can be generated as sulphates, oxides, carbonates, or chlorides, while organic zinc is available as the metal ion complexes attached to organic molecules (Attia et al., 2013). Zinc methionine, zinc lysine, and zinc threoninate are zinc amino acid chelates that improve absorption and availability by reducing antagonism compared to the inorganic form, which may react with no absorbable compounds in the gastrointestinal tract (Neto et al., 2020). Generally, chelated organic elements are regarded as rare elements that, compared to mineral reserves, have a higher tissue availability (Mondal et al., 2009). Mabe et al. (2003) found basic differences between the mineral and organic zinc reserves; however, regardless of the origin, zinc, manganese, and copper supplements increased the eggshell breaking strength in aged laying hens. In addition, it has been suggested that the organically generated zinc has been prioritized in cases in which the dietary levels of trace elements are pushed to the margins. Additionally, organic zinc (as a complex of metal amino acids) has been very effective in improving the feed conversion ratio, broken egg percentage, shell thickness, and Haugh units in young laying hens.

The findings of the meta-analysis of 11 research pieces indicate that dietary zinc supplementation improved hen day egg production, feed conversion ratio, egg mass, egg weight, eggshell thickness, Haugh unit score, and blood zinc concentrations in laying hens, while no significant effect on feed intake and eggshell weight was observed. In addition, subgroup analysis revealed significantly higher hen daily egg production in laying hens fed a diet supplemented with zinc at 100 mg/kg feed. The subgroup findings indicate that specific parameters (zinc form, hen age at the start of the trial, supplementation duration, and inclusion level) were associated with the discrepancies observed between the experiments. Compared with the controls, the observed significant improvement in egg production and quality traits in laying hens fed zinc-supplemented diets will assist in the sustainable use of zinc and policy advancements in the egg production industry. It is recommended to use the regression function in determining zinc inclusion

levels for optimal quality and egg production in laying hens) Ogbuewu & Mbajiorgu, 2022).

Previous researchers observed no differences in bone quality and eggshell thickness when replacing inorganic zinc with zinc amino acid complexes (Gheisari et al., 2011; Swiatkiewicz & Koreleski, 2008). Various investigations have examined and compared the effects of different forms of mineral and organic zinc; however, the results are controversial in the scientific community regarding the origin of zinc supplementation when feeding laying hens. The disparity in the results of the investigations can be attributed to the differences in the employed research models in terms of the strain of chicken used, hen age, experimental and basal diet composition, replacement amount, and the origin of the organic mineral used (Gheisari et al., 2011; Ogbuewu & Mbajiorgu, 2022). The natural presence of zinc in raw materials alone is not enough to satisfy the zinc requirements (Attia et al., 2013). On the other hand, dietary supplementation of organic zinc could alleviate the negative effects of age on egg quality characteristics in laying hens (Behjatian Esfahani et al., 2021). Previous studies have shown that organic zinc has more advantages than inorganic zinc in improving eggshell quality. However, the optimum supplementation amount has not been fully studied. The present study was designed to evaluate the effect of zinc on the antioxidant activity, bone strength, and zinc deposits in late-phase laying hens.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design

In the current study, 175 W36 Leghorn laying hens aged 80 weeks were chosen for the experiment. This experiment was performed in a commercial poultry house. The hens were kept in wire cages 52 cm long, 34 cm wide, and 30 cm high on three floors with a maximum temperature of 25°C, a humidity of 56%, and a lighting period of 16 h. There were two nipple drinkers in each cage, and the feed was freely available to the hens. To conduct this research, a mineral supplement of zinc sulphate (5H<sub>2</sub>O) with 34.12% pure zinc as the mineral base in the diet and Zinpro Company supplement with 12% pure zinc as an organic source in the diet were used.

The diets were adjusted based on the requirements recommended for the strain and after reviewing the research conducted in this field according to Table 1. Zinc-free vitamins and mineral supplements were purchased.

The birds were fed the experimental diets in seven treatments with five replications. Each replication consisted of cages (five hens per cage), with 25 hens in each treatment. The hens under the experiment were first weighed, and those with the same weight were used for treatment to standardize the experimental conditions. In addition, for adaptation, the control diet was fed to the hens for 2 weeks. These diets, which contained different levels of mineral reserves and organic chelating agents, were prepared as shown in Table 2.

The amounts (percentage) of the zinc element provided in the sulphate zinc and organic zinc sources used for the experiment are shown

**TABLE 1** Diet ingredients and chemical analysis

Ingredients	%
Corn	53.0
Soybean	25.0
Calcium carbonate	10.0
Vegetable oil	1.40
Wheat bran	6.08
Dicalcium phosphate	2.20
Salt	0.25
Sodium Bicarbonat	0.15
Vitamin supplement	0.25
Mineral supplement	0.25
DL-methionine	0.14
Lysine	0.06
Chemical analysis	
Metabolizable energy (kcal/kg)	2810
Crude protein	15.00
Calcium	4.65
Available phosphorus	0.4
Sodium	0.18
Methionine	0.32
Methionine-cysteine	0.65
Lysine	0.66
Arginine	0.90
Threonine	0.59

Note: Vitamin supplement composition for each kilogram of diet includes 3,200,000 U/kg of vitamin A, 1,320,000 U/kg of vitamin D3, 8000 U/kg of vitamin E, 1000 mg/kg of vitamin K3, 1000 mg/kg of vitamin B1, 2200 mg/kg of vitamin B2, 3200 mg/kg of vitamin Kalban, 12,000 mg/kg of niacin, 1600 mg/kg of B6, 360 mg/kg of B9, 9 mg/kg of B12, 30 mg g/kg biotin, 44,000 mg/kg choline, antioxidant 3000 mg/kg. The mineral supplement without Zn for each kilogram of basic diet included 79.01 mg of Mn, 3200 mg of copper, 480 mg of iodine, 88 mg of selenium, and 16000 mg of iron. The amount of Zn in the basic diet measured in the laboratory was 63.58 mg/kg.

**TABLE 2** Experimental treatments

Treatment	Zinc sulphate	AA-zinc (%)	Total zinc (mg/kg)
T1	0	0	63.58
T2	15	0	78.58
T3	30	0	93.58
T4	45	0	108.58
T5	0	15	78.58
T6	0	30	93.58
T7	0	45	108.58

Note: Treatment 1: control (basal diet), treatment 2: basal diet with 15 mg/kg zinc sulphate, treatment 3: basal diet with 30 mg/kg zinc sulphate, treatment 4: basal diet with 45 mg/kg zinc sulphate, treatment 5: basal diet with 15 mg/kg organic zinc, treatment 6: basal diet with 30 mg/kg organic zinc, treatment 7: basal diet with 45 mg/kg organic zinc.

in Table 3. Organic zinc supplement, as an amino acid-zinc chelate (AA-Zn) with 12.5% zinc, was obtained from Zinpro Co.

## 2.2 | Production performance

Body weight was measured by weighing all hens individually at the onset and end of the experiment. Mortality was calculated during the experiment. Egg production was recorded daily from 80 to 92 weeks of age. All eggs were weighed by an electronic balance (Sartorius, accuracy 0.001). Feed intake was recorded weekly. The feed conversion ratio was calculated as the ratio of the mass of feed consumed per egg mass produced. According to the following formula, egg mass production was determined based on the percent of hen-day production. Egg production was calculated based on hen houses as shown in the formula below:

$$\text{Average egg mass} = \% \text{hen-day production} \times \text{Average egg weight in gram}, \quad (1)$$

$$\text{Hen house egg production} = \frac{\text{Total number of eggs laid during the period}}{\text{Total number of hens housed at the beginning of laying period}} \quad (2)$$

## 2.3 | Antioxidant activities

To evaluate the antioxidant status, the activity of serum superoxide dismutase (SOD) enzyme was assessed using a Randsel SOD diagnostic kit (Randox, Crumlin, UK) in Mad laboratory. At the end of the experimental period, two hens were selected from each repetition to collect their blood samples (10 ml) during slaughtering.

## 2.4 | Bone strength

The bone strength samples were collected at the end of the experiment. In brief, 10 hens/treatments were selected and sacrificed by rapid cervical dislocation, and the right tibia was removed to analyze tibia breaking strength using an Instron device (Model H5KS; Tinius Olsen Company).

## 2.5 | Zinc deposition in body tissues

Two hens were selected from each replication to evaluate the level of zinc deposition in body tissues at the end of the experiment, and 10 ml of blood was collected from each hen during slaughter. Before slaughter, all experimental hens were fasted for 10 h to improve the accuracy of blood factor measurements. Serum samples were centrifuged at 3000 rpm for 10 min to separate the serum and were stored at  $-20^{\circ}\text{C}$  until analysis. Moreover, to evaluate the amount of zinc in the bones,

**TABLE 3** Amounts of provided zinc element in the mineral and organic reserves used for the experiment (%)

Treatment	Zinc sulphate supplement	Organic zinc supplement
Control (basal diet)*	0	0
A basal diet with 15 mg mineral zinc	4.53	0
A basal diet with 30 mg mineral zinc	9.06	0
A basal diet with 45 mg mineral zinc	13.6	0
A basal diet with 15 mg organic zinc	0	12.5
A basal diet with 30 mg organic zinc	0	25
A basal diet with 45 mg organic zinc	0	37.5

\*The amount of basal diet analyzed in the laboratory was 63.58 mg/kg. The Hy-Line strain need is 80 mg, according to the strain breeding guide (2016).

the tibia was dried in an oven for 24 h and then placed in an oven at 550°C to prepare the ash. The blood sample, tibia, yolk, and liver of two replication samples were selected and sent to the laboratory to determine the zinc level. The zinc levels of the serum and other tissues were analyzed by inductively coupled plasma–optical emission spectrometry (Optima 8300, PerkinElmer, Waltham, MA, USA) according to the method suggested by Qiu et al. (2020).

## 2.6 | Statistical analysis

This experiment was conducted using a simple complete random design (Performance traits) for 12 weeks by using the following model:

$$Y_{ij} = \mu + T_i + e_{ijk} \quad (3)$$

These SOD, bone strength, and zinc deposition experiments were conducted in the form of a simple complete random design with several observations in each repetition. The SOD, bone strength, and zinc deposition samples were collected at the end of the experiment by using the following model:

$$Y_{ijk} = \mu + T_i + e_{ijk} + \varepsilon_{ijk}, \quad (4)$$

where  $\mu$  demonstrates the mean trait,  $T_i$  indicates the treatment effect, and  $\varepsilon_{ijk}$  and  $e_{ijk}$  illustrate the effect of sampling and experimental error. The data were analyzed by using GLM in SAS statistical software. Tukey's test was utilized to compare the means of various treatments when the mean difference was significant at the level of  $p < 0.05$ .

## 3 | RESULTS AND DISCUSSION

### 3.1 | Production performance

The result of the performance of laying hens showed (Table 4) a significant difference in aged laying hens' production traits. Based on the obtained results, the feed intake, egg production (%), egg weight, egg mass, and conversion ratio were affected by the experimental treatments ( $p < 0.05$ ). The results regarding the performance of laying hens are contrary to the findings of Stefanello et al. (2014), who reported

that mineral trace elements in laying hens' diet had no effects on feed intake, feed conversion ratio, and egg production. Manangi et al. (2015) also reported no effects for rare organic minerals. In a study on 96 Hy-Line W-36 Leghorn hens, the effects of adding zinc to the diet of laying hens on their performance and behaviour were evaluated for 10 weeks using four treatments (40, 70, 100, and 130 mg/kg zinc per diet). The results indicated that egg production in the hens receiving 130 mg/kg zinc was significantly higher than that in the hens receiving 40 and 70 mg/kg zinc ( $p < 0.05$ ). Moreover, the hens receiving 130 mg/kg zinc had a lower feed intake than the other two treatments ( $p < 0.05$ ).

Treatments 2, 4, 5, 6, and 7 demonstrated the highest feed intake. It has been declared that zinc influences appetite because anorexia is one of the symptoms of zinc deficiency (Behjatian Esfahani et al., 2021). The results of this study agree with the findings of Stefanello et al. (2014) and Manangi et al. (2015) that they found no significant effects of supplemental inorganic and organic trace minerals (Mn, Zn, and Cu) respectively in laying hens on feed intake. Nevertheless, Gheisari et al. (2011) reported that the age of hens had a vital impact on the average daily feed intake.

The findings demonstrated a significant difference between treatment 4 (45 mg/kg zinc sulphate) and treatment 1 (basal diet) regarding egg production ( $p < 0.05$ ; Table 4). The cause of the enhancement in egg production can be ascribed to the impact of zinc on egg production as a result of the impact of zinc on albumin deposition in the formation of eggshell layers in the isthmus, magnum, and eggshell formation in the uterus and the impact of zinc on the enhancement in FSH and LH hormones, progesterone, and oestrogen (Park et al., 2004). Improvement in egg production is also probably due to the impact of zinc on the secretion of reproductive hormones. A considerable enhancement in the concentration was observed in the experiment on broiler oestrogen and progesterone breeders by adding different zinc levels to the diet. Ovarian oestrogen releases oviduct growth and enhances proteins, vitamins, blood calcium, fats, and nutrients required for egg formation.

In addition, progesterone induces mature follicle ovulation (Behjatian Esfahani et al., 2021). EL-Katcha et al. (2018) reported that adding 30 ppm zinc nanoparticles to the diet increases egg production. Abedini et al. (2018) conducted an experiment on 288 Leghorn laying hens aged 64 weeks with four treatments. The experimental diets included soybean meal (without zinc supplementation) and a basal diet with 80 mg/kg ZnO, ZnO nanoparticles, and Zn-methionine. The results

**TABLE 4** Comparison between the increasing levels of the chelated organic and mineral zinc in terms of performance traits of aged laying hens

Treatment	Feed intake (g)	Hen day egg production (%)	Egg weight (g)	Egg mass (g)	Conversion ratio
1	107.6 <sup>b</sup>	62.7 <sup>b</sup>	65.4 <sup>b</sup>	40.9 <sup>b</sup>	2.62 <sup>a</sup>
2	108.8 <sup>a</sup>	65.5 <sup>ab</sup>	66.4 <sup>ab</sup>	43.4 <sup>ab</sup>	2.51 <sup>ab</sup>
3	108.7 <sup>ab</sup>	64.6 <sup>ab</sup>	66.6 <sup>ab</sup>	43.1 <sup>ab</sup>	2.52 <sup>ab</sup>
4	109.3 <sup>a</sup>	72.2 <sup>a</sup>	67.5 <sup>a</sup>	48.6 <sup>a</sup>	2.26 <sup>b</sup>
5	109.4 <sup>a</sup>	70.1 <sup>ab</sup>	67.9 <sup>a</sup>	47.6 <sup>a</sup>	2.32 <sup>b</sup>
6	109.1 <sup>a</sup>	70.1 <sup>ab</sup>	68.4 <sup>a</sup>	47.9 <sup>a</sup>	2.30 <sup>b</sup>
7	108.9 <sup>a</sup>	68.7 <sup>ab</sup>	68.4 <sup>a</sup>	46.9 <sup>ab</sup>	2.32 <sup>b</sup>
SEM	0.274	0.484	0.388	1.42	0.065
p-Value	<0.0009	<0.0151	<0.0001	<0.0022	<0.0012

Note: The means with dissimilar superscript letters in each column are significantly different ( $p < 0.05$ ). Treatment 1: control (basal diet), treatment 2: basal diet with 15 mg/kg of zinc sulphate, treatment 3: basal diet with 30 mg/kg of zinc sulphate, treatment 4: basal diet with 45 mg/kg of zinc sulphate, treatment 5: basal diet with 15 mg/kg of organic zinc, treatment 6: basal diet with 30 mg/kg of organic zinc, treatment 7: basal diet with 45 mg/kg of organic zinc. Abbreviation: SEM, standard error mean.

indicated that the egg production and mass in the treatments containing Zn-methionine and ZnO nanoparticles were significantly higher ( $p < 0.05$ ). Therefore, it is obvious that dietary supplementation with zinc sources could significantly improve egg production.

The mean egg weight at the end of the experimental period was significantly higher in treatment 4 (45 mg/kg of zinc sulphate), treatment 5 (15 mg/kg chelated organic zinc), treatment 6 (30 mg/kg chelated organic zinc), and treatment 7 (45 mg/kg chelated organic zinc) ( $p < 0.05$ ) than in the control treatment and other treatments ( $p > 0.05$ ). L. Li, Gong, et al. (2019) stated that the treatment containing 80 mg/kg zinc in the diet led to higher egg weight.

It should be noted (Table 4) that at all ages, treatments with 45 mg/kg sulphate zinc had a higher egg mass than inorganic zinc and the control group ( $p < 0.05$  and  $p < 0.05$ , respectively). This egg mass enhancement can be due to the effect of zinc on several physiological and biochemical processes. Zinc is needed to function normally in enzymes, protein structures, hormones, and development and growth. This improvement can result from zinc participation in the body's metabolism of energy and protein (Behjatian Esfahani et al., 2021). Because egg mass is related to egg production, the egg mass was improved by enhancing egg production (Table 4) in hens fed a diet enriched with zinc sulphate and organic zinc compared with the control. The results of this study agreed with those of Behjatian Esfahani et al. (2021), who declared that egg mass increased considerably due to the use of organic zinc sources in the diet.

The results (Table 4) demonstrated that the lowest conversion ratio was observed in treatments 4, 5, 6, and 7 ( $p < 0.05$ ). Abd El-Hack et al. (2018) reported that Zn-Met improved the feed conversion ratio in laying hens. The addition of organic zinc to the diet also led to the improvement of the feed conversion ratio ( $p < 0.05$ ) (Sorosh et al., 2018). In another study, Jialing et al. (2020) stated that the use of a diet with one third of the commercial level of rare minerals (iron, copper, manganese, and zinc) reduced the number of daily eggs and egg mass and increased the ratio of feed intake to egg production compared to the other treatments ( $p < 0.05$ ). Therefore, dietary organic zinc plays a

vital role in increasing the digestion and absorption of nutrients in the gastrointestinal tract (Behjatian Esfahani et al., 2021), which improves the performance of laying hens in the present experiment.

### 3.2 | Antioxidant status

The antioxidant effects of different zinc sources and doses on laying hens are reported in Table 5. According to the results of this experiment, SOD was significantly affected by the experimental treatments ( $p < 0.05$ ). SOD, which is the primary antioxidant enzyme that protects cells from oxidative stress, increases the antioxidant ability to eliminate overactive reactive oxygen species and reduce lipid peroxidation in poultry (Zhu et al., 2017). In a previous study, hens supplemented with zinc at a concentration of 70 mg/kg dry matter (DM) had higher Zn21 and Cu-ZnSOD ( $p < 0.05$ ) contents and tended to have higher serum concentrations of SOD than hens supplemented with zinc at a concentration of 35 mg/kg DM; however, the source of zinc did not have an effect (YU et al., 2020). One of the most important actions of zinc is its participation in the antioxidant defence system. Oxidative damage to the cell membrane occurs through free radicals during zinc deficiency (Prasad & Kucuk, 2002). On the other hand, zinc is a cofactor of the main antioxidative enzyme Cu Zn-superoxide dismutase; it may play a key role in suppressing free radicals and in inhibiting NADPH-dependent lipid peroxidation (Prasad, 1997) as well as in preventing lipid peroxidation via the inhibition of glutathione depletion (Gibbs et al., 1985). One of the proposed mechanisms of zinc's function is its capacity to replace transition metals (Fe, Cu) from binding sites. Zinc can compete with iron and copper to bind to the cell membrane and decrease the production of free radicals, thus exerting a direct antioxidant action (Tate et al., 1999). Zinc induces the production of metallothionein, an effective scavenger of hydroxyl radicals, and it has been suggested that Zn-metallothionein complexes in islet cells provide protection against immune-mediated free-radical attack (Shaheen & Abd El-Fattah, 1995). Zinc is an essential component of SOD, which

**TABLE 5** Effects of different sources of organic and inorganic zinc on antioxidant activity in old laying hens

Parameters	Experimental diets							SEM	p-Value
	T1	T2	T3	T4	T5	T6	T7		
SOD (U/ml)	221.2 <sup>b</sup>	285.7 <sup>ab</sup>	332.0 <sup>a</sup>	341.4 <sup>a</sup>	314.4 <sup>a</sup>	279.2 <sup>ab</sup>	306.6 <sup>a</sup>	0.694	0.0001

Note: Values in a row with no common superscript letter are significantly different ( $p < 0.05$ ). Treatment 1: control (basal diet), treatment 2: basal diet with 15 mg/kg zinc sulphate, treatment 3: basal diet with 30 mg/kg zinc sulphate, treatment 4: basal diet with 45 mg/kg zinc sulphate, treatment 5: basal diet with 15 mg/kg organic zinc, treatment 6: basal diet with 30 mg/kg organic zinc, treatment 7: basal diet with 45 mg/kg organic zinc.

Abbreviation: SEM: standard error mean.

**TABLE 6** Effect of feeding supplemental inorganic and organic zinc on bone strength in old laying hens

Treatment	Force of break (N)	Work to break point (J)	Bend to break point (mm)	Cortical thickness (mm)
T1	212.3	223.3	0.146	2.00 <sup>b</sup>
T2	143.2	189.8	0.147	2.61 <sup>b</sup>
T3	193.8	200.8	0.124	3.10 <sup>b</sup>
T4	148.6	179.3	0.148	3.85 <sup>b</sup>
T5	229.1	177.5	0.109	4.29 <sup>b</sup>
T6	131.6	197.9	0.192	5.94 <sup>a</sup>
T7	212.3	224.8	0.138	4.82 <sup>b</sup>
SEM	28.8	33.6	0.02	0.21
p-Value	0.0880	0.9040	0.3480	0.0001

Note: Treatment 1: control (basal diet), treatment 2: basal diet with 15 mg/kg zinc sulphate, treatment 3: basal diet with 30 mg/kg zinc sulphate, treatment 4: basal diet with 45 mg/kg zinc sulphate, treatment 5: basal diet with 15 mg/kg organic zinc, treatment 6: basal diet with 30 mg/kg organic zinc, treatment 7: basal diet with 45 mg/kg organic zinc.

Abbreviation: SEM, standard error mean.

has an important function in the detoxification of superoxide free radicals and the protection of cells against oxidative stress by catalyzing the conversion of superoxide anion ( $O_2^-$ ) to  $H_2O_2$  (YU et al., 2020). Zinc has antioxidant properties and is essential for the immune system (Ogbuewu & Mbajior, 2022).

### 3.3 | Bone strength

The results regarding the effects of inorganic and organic zinc supplementation on tibia bone strength are presented in Table 6. According to these results, no significant differences were observed among the experimental treatments ( $p > 0.05$ ). However, the cortical thickness of the bone with 30 g of chelated organic zinc (T6) was significantly increased compared to that in the control and the other experimental treatments.

Zinc deficiency (10 mg/kg) in young hens has a limiting effect on bone formation. In broilers, an increase in the zinc level up to 100 mg/kg led to a significant improvement in bone strength and lowered injury risks. Manangi et al. (2015) reported no significant association between chelated trace minerals (CTM) diet and the mineral source on this parameter. However, they showed that the use of CTM resulted in the strongest bone strength values. Substitution of inorganic Zn and Mn with amino acid complexes in birds aged up to 70 weeks (Stefanello et al., 2014) had no significant effect on the tibia bone, and the authors

speculated that bone properties in older birds might be less sensitive to the availability of Zn and Mn in the diet than eggshells.

The decrease in eggshell quality of aged laying hens is one of the most critical issues in the poultry industry. Although the majority of nutrition research on shell quality has focused on macronutrients such as calcium, phosphorus, and vitamin D<sub>3</sub>, several enzymes associated with trace elements are calcified and involved in eggshell formation. These trace elements include zinc and manganese, which are responsible for producing carbonate and mucopolysaccharides as cofactors of metalloenzymes and play an important role in forming eggshells (Park et al., 2004; Swiatkiewicz & Koreleski, 2008).

In terms of tibia strength, the treatment group receiving 30 mg/kg organic zinc showed a significant increase in the level of flexion and the thickness of the bone tissue ( $p < 0.05$ ); therefore, it can be concluded that the hens in this treatment group had higher bone strength than the other treatments. Moreover, the results related to shell thickness revealed that the eggshell thickness in the treatment group receiving 30 mg/kg organic zinc was increased. However, this difference was not significant, which could be due to the different bioavailabilities of zinc in the shell and bone. The antagonistic relationships between zinc and calcium and how the carbonic enzyme anhydrase works in response to these two elements must be investigated.

Cufadar et al. (2020) stated that different levels of zinc and their interactions had no effects on the tibia weight, tibia pressure, and tibia breaking strength as mechanical parameters of the tibia ( $p > 0.05$ ).

**TABLE 7** Zinc deposition in the body tissues of aged laying hens

Treatments	Bone (tibia)	Yolk	Egg shell	Serum*	Liver
T1	173.4 <sup>b</sup>	17.4 <sup>b</sup>	0.56	6.47 <sup>b</sup>	83.5 <sup>b</sup>
T2	213.9 <sup>a</sup>	29.5 <sup>ab</sup>	0.57	6.94 <sup>ab</sup>	111.5 <sup>a</sup>
T3	216.5 <sup>a</sup>	40.6 <sup>a</sup>	0.63	7.34 <sup>a</sup>	81.6 <sup>ab</sup>
T4	205.1 <sup>a</sup>	54.2 <sup>a</sup>	0.56	5.38 <sup>b</sup>	137.9 <sup>a</sup>
T5	236.2 <sup>a</sup>	46.2 <sup>a</sup>	0.61	6.93 <sup>ab</sup>	101.3 <sup>ab</sup>
T6	193.1 <sup>a</sup>	37.6 <sup>a</sup>	1.01	6.16 <sup>ab</sup>	74.4 <sup>ab</sup>
T7	210.1 <sup>a</sup>	65.3 <sup>a</sup>	1.18	7.36 <sup>a</sup>	64.2 <sup>ab</sup>
SEM	3.56	3.08	0.20	0.20	5.11
p-Value	0.088	0.040	0.348	0.0001	0.030

Note: The means with dissimilar superscript letters in each column are significantly different ( $p < 0.05$ ), SEM: Standard Error of Mean. Treatment 1: control (basal diet), treatment 2: basal diet with 15 mg/kg zinc sulphate, treatment 3: basal diet with 30 mg/kg zinc sulphate, treatment 4: basal diet with 45 mg/kg zinc sulphate, treatment 5: basal diet with 15 mg/kg organic zinc, treatment 6: basal diet with 30 mg/kg organic zinc, treatment 7: basal diet with 45 mg/kg organic zinc.

\*per ppm/ml.

Replacement of zinc and manganese oxides with micronutrient amino acid complexes also did not affect the physical and geometric parameters of the tibia and ash content in the tibia and toes (Swiatkiewicz & Koreleski, 2008). Manangi et al. (2015) showed that mineral elements such as calcium, zinc, copper, and manganese led to the highest bone strength, although the difference was not statistically significant ( $p = 0.01$ ). These results indicate other advantages of using organic sources of zinc over inorganic sources.

Nevertheless, adding zinc has been declared to enhance the utilization of Ca in hens and improve the qualitative parameters of the eggshell (Klecker et al., 2002). It has also been reported that zinc supplementation increases eggshell quality since it is an element of the carbonic anhydrase enzyme, supplying carbonate ions during eggshell formation (Ogbuewu & Mbajiorgu, 2022). Moreover, it has been indicated that the secretion of alkaline phosphatase is decreased under stress conditions, and this enzyme interacts with zinc in bone calcium storage (Rajabi & Torki, 2021).

### 3.4 | Zinc deposition in body tissues

Table 7 presents the zinc deposition in the body tissues of aged laying hens. The zinc deposition was remarkably different between the yolk, tibia, liver, and serum treatments ( $p < 0.05$ ).

Abedini et al. (2018) investigated three sources of zinc, namely, zinc oxide, zinc oxide nanoparticles, and methionine-zinc, and reported that zinc deposition in the tibia, liver, pancreas, eggs, and faeces was significantly different among the treatments ( $p < 0.01$ ). Zinc has a protective effect against oxidative damage in pancreatic tissue, and it may help the pancreas function properly, including secretion of digestive enzymes, thus improving the digestibility of nutrients (Onderci et al., 2003).

### 3.5 | Bone (tibia) deposition

The measurement of zinc bioavailability in the right tibia showed that this parameter was significantly higher in treatments containing sulphate bone (tibia) deposition and organic zinc than in the control treatment ( $p < 0.05$ ). Treatment 5 (containing 15 mg/kg organic zinc) had the highest zinc concentration of 236.23 ppm. Cufadar et al. (2020) examined three sources of zinc (zinc oxide, zinc proteins, and zinc oxide nanoparticles) at 20, 40, 60, 80, and 100 mg/kg levels in laying hen diets. They indicated that different sources of zinc had a significant effect on the zinc levels, although they did not significantly affect the calcium and phosphorus content of the tibia ( $p > 0.05$ ). L. Li, Abouelezz, et al. (2019), in a study on 576 Lingnan parent broiler hens aged 58 weeks, reported that the hens receiving 24–48 mg/kg zinc had a higher tibia breaking strength than those in the other experimental treatments. In addition, they reported that the increase in Zn-Met-induced calcium deposition might be due to the increase in zinc content in the serum and tissues, which is attributed to the rise in serum calcium and albumin concentrations and the concentration of calcium, albumin, and CaBP-D28k mRNA in the eggshell gland (ESG) (L. Li, Liping, et al., 2019).

### 3.6 | Yolk deposition

In addition, the zinc bioavailability level in the yolk in the sulphate and organic zinc supplementation treatments had a significant increase compared to that in the control treatment, except for treatment 2 ( $p < 0.05$ ). L. Li, Liping, et al. (2019) compared three levels of 0, 40, and 80 mg of zinc sulphate and methionine-zinc. They stated that the treatment containing 80 mg/kg methionine-zinc led to a higher calcium deposition on the transverse section of the eggshell and had a higher zinc content in the yolk and serum ( $p < 0.05$ ).

Saleh et al. (2020) evaluated laying hens receiving a mixture of 0.5 and 1 g of sulphate and organic zinc, manganese, and copper compared to the control treatment and reported that the yolk's zinc and copper levels were significantly increased compared to the control treatment ( $p < 0.05$ ), while manganese was only numerically increased ( $p > 0.05$ ).

### 3.7 | Shell deposition

Based on the obtained results, there were no significant differences between the control treatment and the treatments containing sulphate and organic zinc supplements in terms of the shell's zinc levels ( $p > 0.05$ ). However, hens receiving 30 and 45 mg/kg organic zinc treatments had the highest numerical values of zinc in their shells.

### 3.8 | Serum deposition

The treatment containing 45 mg/kg sulphate zinc (treatment 4) led to a significant decrease in serum zinc compared to the control treatment

(5.38 ppm). The treatment containing 45 mg/kg organic zinc (treatments 3 and 7) led to a significant increase compared to the control treatment (7.36 ppm). Abd El-Hack et al. (2018) reported that ZnO or Zn-Met supplementation increased serum zinc compared to the control group, and there were no differences between the supplemental doses of zinc. In the current study, serum zinc was enhanced in the treatment group receiving 30 mg/kg zinc sulphate and 45 mg/kg organic zinc, while there was no increase in the other treatments.

### 3.9 | Liver deposition

The zinc bioavailability in the liver showed a significant difference, and treatments 2 (15 mg/kg zinc sulphate) and 4 (45 mg/kg zinc sulphate) had the highest zinc levels of 111.51 and 137.99 ppm, respectively ( $p < 0.05$ ). Diet zinc sources significantly affected the liver zinc levels ( $p < 0.05$ ). Zinc deposition in body tissues is a part of the digested and absorbed elements used in animal metabolism. Therefore, selecting a precise criterion is of great importance in estimating bioavailability. The bioavailability of different mineral combinations changes when added to organic or inorganic carriers (Ammerman et al., 1995). The bioavailability changes with the alterations of the absorption mechanism, for example, transmission through the cell wall by diffusion, are much less efficient than the translocation of amino acid-bonded minerals.

Furthermore, it should be noted that there are some special interactions between high- and low-consumption mineral elements that can lead to antagonism or cooperation between them. Organic Zn sources are more biologically available in the digestive system than inorganic sources, resulting in less Zn excretion (Ogbuewu & Mbajorgu, 2022). However, the similar results regarding the zinc reserves in body tissues both in supplemental inorganic and organic of zinc. Qifang et al. (2020) studied the effects of two organic sources of amino acid-zinc and zinc sulphate at two concentrations of 35 and 70 mg/kg. They reported that the zinc content of the eggs increased linearly for both sources.

Bioavailability can be specified as the degree to which an ingested nutrient in a specific source is absorbed in a form that the animal can utilize in metabolism (Ammerman et al., 1995). The relative bioavailability value (RBV), or the bioavailability of a nutrient within one source, is evaluated concerning the bioavailability of the same nutrient in a standard source. Evaluating mineral storage or deposition into chosen tissues is the utmost common variable evaluated in trace mineral RBV experiments (Richards et al., 2015). Zinc in chelate creates a soluble complex with zinc, and as a result, it is readily available to the animal. An alternative possibility is that organic Zn sources are absorbed through amino acid or peptide transport systems, leading to higher bioavailability and digestibility (Behjatian Esfahani et al., 2021). Consequently, higher bioavailability zinc probably has a significant role in enhancing the absorption of nutrients and digestion and improving the egg quality and performance of laying hens in the present experiment.

In previous studies, the addition of 30 ppm zinc oxide also increased the level of zinc in the excreta (Attia et al., 2013). Tsai et al. (2016) con-

cluded that 60 mg/kg nanosized zinc oxide can enhance zinc absorption and showed its feeding effectiveness in aged layers. Moreover, the recommended level of MHA-Zn in the diet of aged laying hens is 40 mg/kg (Min et al., 2018). The highest level of supplementation (120 ppm) was correlated with high zinc excretion for both zinc sulphate and organic zinc. This indicates that the hens are not retaining a large portion of the zinc that is fed. Overall, the zinc content of the manure decreased for all treatments during the trial period. This could indicate that as the hen's body adapts to the diet, it can better utilize the nutrients in the feed and therefore show decreased manure zinc excretion.

The linear regression analysis showed a strong linear relationship between zinc level in the diet and feed intake and egg weight and a poly relationship between zinc level and egg production and egg mass. The prediction equations will be valuable to producers who may be interested in feeding higher levels of zinc for the proposed benefits but also want to monitor zinc levels in the manure. This will provide them with an accurate indicator of zinc excretion in manure based on the zinc source and content of the proposed diet fed. In this experiment, the linear regression analysis of zinc source showed a strong linear relationship between zinc source and performance traits. The levels of 30 mg/kg organic zinc and 45 mg/kg zinc sulphate supplementation were highly correlated with performance traits. According to our findings, organic zinc was more efficient than inorganic zinc in improving the bone strength of laying hens.

## 4 | CONCLUSION

The results of this study denote the importance of supplementary dietary zinc from different sources that improve feed intake and antioxidant activity in older laying hens. Zinc supplements increased egg weight, and organic zinc supplements boosted egg mass and decreased the feed conversion ratio. Zinc deposition was increased in the tibia, egg yolk, and liver by Zinc supplementation. It can be concluded that dietary supplementation with zinc could alleviate the negative effects of age on antioxidant status and bone characteristics in laying hens. The optimum supplementation amount was 30 mg/kg organic zinc in the late phase of laying, which improved the cortical thickness of the bone in older laying hens.

### AUTHOR CONTRIBUTIONS

Reza Vakili contributed to the conception of the study; Aidin Dokht Niknia performed the experiments; Reza Vakili and Abdol Mansour Tahmasbi contributed significantly to the analysis and manuscript preparation.

### CONFLICT OF INTEREST

All authors have no conflicts of interest.

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### DATA AVAILABILITY STATEMENT

The related data sheet will be available on legal request.



## ETHICS STATEMENT

All applicable international, national and institutional guidelines for the care and use of animals were followed. The protocol for animal care and use was approved by the Animal Ethics Committee of the Agricultural Research Institute of Iran.

## PEER REVIEW

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