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EFFECTS OF DIETARY SUPPLEMENTATION OF INORGANIC, ORGANIC OR NANO ZINC FORMS ON PERFORMANCE, EGGSHELL QUALITY, AND BONE CHARACTERISTICS IN LAYING HENS

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Abstract

This study was conducted to evaluate the efficiency of dietary zinc forms and dosages on egg production performance, egg quality, and bone characteristics in laying hens. Forty-two-week-old, 144 Lohmann LSL-Lite laying hens were allocated to 12 experimental groups in a 4 (forms) × 3 (dosages) factorial arrangement. Four zinc forms including zinc-sulphate and zinc-oxide as inorganic forms, zinc-glycine as organic form and nano zinc-oxide powder as nano form at different dosages (50, 75 and 100 mg per kg diet) were tested. Compared to the inorganic (zinc-sulphate) form, the zinc-glycine supplementation significantly depressed the egg weight, egg mass and feed conversion ratio. The eggshell thickness was significantly decreased by supplementation with nano zinc-oxide. The shear force of tibia was significantly decreased by zinc-glycine or nano zinc-oxide supplemented in the diet when compared to inorganic forms of zinc. On the other hand, the dietary 50 mg/kg dosage of zinc was sufficient for optimum performance and the dietary 75 mg/kg dosage of zinc significantly improved shear force of tibia in laying hens. Tibia zinc content increased with the dietary 100 mg/kg dosage of zinc. The interactions between zinc forms and dosages had a significant effect on egg weight, feed intake, feed conversion ratio, eggshell thickness, shear force and shear stress of bone, and tibia calcium concentration. The highest egg weight and the lowest eggshell thickness were observed for the group fed with nano Zn-oxide at 100 mg/kg in the diet. These results showed that nano zinc form supplementation negatively affects the eggshell thickness and bone mechanical properties. The zinc in nano form may not be suggested for feeding laying hens, but other forms of zinc could be used safely in layer diets.

Key words: zinc, nano, eggshell, bone, laying hens

Zinc (Zn) is an essential trace element for growth, bone development, enzyme structure and function, and eggshell formation in poultry. The Zn content of the diet is very low compared to the Zn requirement (35 mg/kg) of poultry (NRC, 1994). Therefore, in egg producers, Zn is added routinely to diets through premix and the amount included in the diet is generally higher than the Zn requirement of laying hens. This practice has caused concerns regarding optimisation of the genetic potential of modern breeds and environmental issues (Ao and Pierce, 2013).

Cracked-broken eggs proportion may be about 8% and this can cause major economic losses in the egg industry (Bain, 1997). Many studies have been focused on macro-minerals such as calcium (Ca) and phosphorus (P) and trace minerals. Also, trace minerals such as Zn play an important role in eggshell formation. Zinc is a cofactor and/or structural component of carbonic anhydrase which is very important for supplying the carbonate ions needed during eggshell formation (Nys et al., 1999); thus, the addition of Zn in the diet has been effective to increase eggshell quality (Zamani et al., 2005; Amem and Al-Daraji, 2011; Bahakaim et al., 2014).

The Ca which is necessary for eggshell formation is absorbed from the intestine. Additionally, a part of the absorbed Ca from intestine is stored in medullary bone, where it is later released for the calcification of eggshells (Etches, 1987; Webster, 2004; Jonchere et al., 2012). Therefore, the maintenance of bone health is important for the protection of hens' health and optimising eggshell quality. The Zn supplementation increases bone strength by favourably modulating bone and by inhibiting osteoclast differentiation (Yamaguchi and Kishi, 1996; Ovesen et al., 2001; Peretz et al., 2001; Hadley et al., 2010; Nagata and Lönnerdal, 2011).

Some researchers showed that the biomechanical properties and mineralisation of bone in birds was positively affected by Zn supplementation (Sunder et al., 2008; Idowu et al., 2011; Stofanikova et al., 2011; Sahraei et al., 2012) in diets.

Zinc bioavailability is 6–11% in monogastric animals (Brody, 1997). The bioavailability and tissue accumulation of Zn depend upon various factors such as its chemical form, feed composition, age and physiological state of hens, and interactions with other minerals (Leeson, 2005; Mezes et al., 2012). The most commonly used Zn for poultry diet is inorganic Zn in the form of sulphate due to its cost and commercial availability. Kidd et al. (1996) indicated that dietary organic sources of Zn might be absorbed intact and function differently to inorganic sources of Zn after absorption. Wedekind et al. (1992) reported that Zn-methionine as an organic form of Zn was more bioavailable than Zn-sulphate or Zn-oxide as inorganic forms of Zn.

As a general rule, the smaller the particles, the higher and the more effective their absorption, especially if the particle size is below 100 nm (Hett, 2004). Thus, it was hypothesised that nanoparticles were easier to absorb compared to their inorganic or organic counterparts (Hett, 2004). Nano trace element may enter the animal's body through direct penetration; therefore, its utilisation rate will be much higher than that of the ordinary inorganic trace elements (Huang et al., 2015). Sahoo et al. (2014) and Mohammadi et al. (2015) reported that the tibia Zn concentration in nanoparticle Zn samples was higher relative to the Zn-sulphate form of Zn.

There is very limited information available concerning the comparative effects of the supplementation of dietary nano Zn in laying hens. Therefore, the aim of this study was to determine the effects of Zn forms and dosages and their interactions on the egg production performance, eggshell quality, and bone mechanical and mineralisation in laying hens.

Material and methods

The criteria specified by the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals were obeyed during the experiments carried out on animals. A total of 144, 42-week-old, Lohmann LSL-Lite laying hens were randomly allocated into 12 treatment groups with 4 replicates each including three hens. Hens were fed on a basal diet, containing 16.0% crude protein, 11.73 MJ/kg ME and 33.40 mg Zn per kg diet (Table 1). Per kg basal diet was supplemented with 3 increasing dosages of Zn (added to the basal diet 16.6, 41.6 and 66.6 mg Zn to get total Zn contents 50, 75 and 100 mg, respectively) with Zn-sulphate (35%; Newsky Chemical Co. Ltd, Changsha, China), Zn-oxide (72%; Metaltek Metallurgy, Ankara, Turkey), Zn-glycine (26%; Phytobiotics GmbH, Eltville, Germany) or nano Zn-oxide sources (99+%; 35–45 nm; US Research Nanomaterials, Inc, Houston, USA). The experiment lasted for 12 weeks. The birds were housed in an environmentally controlled room equipped with 48 metal battery cages. Feed and water were offered *ad libitum* throughout the experiment. The lighting programme was 16h L: 8h D throughout the experimental period.

Table 1. Composition of basal diet (%, as fed)

Item	Contents
Ingredients (%, as fed)	
corn	50.70
wheat	10.00
soybean meal	16.50
sunflower meal	8.00
soybean oil	2.79
limestone	9.70
dicalcium phosphate	1.59
salt	0.35
mineral Premix[1]	0.10
vitamin Premix[1]	0.15
DL methionine	0.12
	100.00
Chemical composition	
metabolisable energy (MJ/kg)	11.73
crude protein (%)	16.01
lysine (%)	0.77
methionine (%)	0.40
methionine + cysteine (%)	0.68
calcium[2] (%)	4.12
total phosphorus[2] (%)	0.52
non-phytate phosphorus (%)	0.40
zinc[2] (mg/kg)	33.40

[1]Supplied per kg diet: Manganese: 60 mg; Iron: 30 mg; Copper: 5 mg; Selenium: 0.1 mg, Vitamin A: 8,800 IU; Vitamin D_3: 2,200 IU; Vitamin E: 11 mg; Nicotine acid: 44 mg; Cal-D-Pan: 8.8 mg; Riboflavin: 4.4 mg; Thiamin: 2.5 mg; Vitamin B_{12}: 6.6 mg; Folic acid: 1 mg; D-Biotin: 0.11 mg; Choline: 220 mg.

[2] Analysed value as feed.

*Calculations (crude protein, lysine etc.) were made based on the recommendations by the company which buys in feedstuffs.

The body weight of hens was determined by weighing the hens individually at the beginning and end of the experiment. Egg production (EP) was recorded daily. Feed intake (FI) was calculated as the mean for the subgroup for the 12-week trial period (FI = given total feed – remaining feed in manger). Egg mass (EM) was calculated from the EP and egg weight (EW) data using the formula: EM = (EP × EW)/ Period (days). The feed conversion ratio (FCR) was calculated using the formula; FCR = FI/EM.

Damaged eggs were calculated using the formula: damaged eggs (%) = [(total egg production (number) / damaged eggs (number)) / 100]. The eggs were examined to determine the EW and eggshell quality characteristics (shell breaking strength, shell weight, and shell thickness) for all collected eggs produced during the last three days of the study. Eggshell breaking strength was measured using a cantilever system by applying increasing pressure to the broad pole of the shell using an Egg Force Reader (Orka Food Technology Ltd., Ramat Hasharon, Israel). The eggs were broken to separate and weigh the eggshell. Eggshells were weighed using a 0.01 g precision scale. Eggshell weight was calculated using the formula: eggshell weight (%) = [(eggshell weight (g)/EW (g))/100]. Eggshell thickness (including the membrane) was determined at three points on the eggs (one point on the air cell and two randomised points on the equator) using a micrometre (Mitutoyo Inc., Kawasaki, Japan).

Hens (one hen per replicate and four hens per treatment group) were killed humanely by cervical dislocation, and then the left and right tibias with some attached flesh were collected. Bones were excised from all flesh and proximal cartilages were removed. While the left tibias were used for the determination of mineral contents, the right tibias were used for measuring the bone mechanical properties. The sample tibias were placed in a plastic container and stored at −20ºC until analysis. The tibias were thawed at room temperature for 6 h in an air-conditioned room before the measurements began. The tibia mechanical properties were determined from the load-deformation curve generated from a three-point bending test (ASAE, 2001) using an Instron Universal Testing Instrument (Model 1122; Instron, Canton, MA) and the Test Works 4 software package (version 4.02; MTS System Corporation, Eden Prairie, MN). The crosshead speed was constant at 5 mm per min. The full-scale load of the load cell was 5,000 Newtons (N). Shear force tests were performed on the tibia using a double-shear block apparatus. The shear force was exerted over a 6.35-mm (0.25-inch) section located at the centre of the diaphysis. These tests enabled the ultimate shear force and shear stress to be evaluated for each bone. The diameter and wall thickness of the tibia was measured using digital callipers (precision of 0.001 mm) at two points on the central axis of the tibia that was used to determine the mechanical properties. The shear stress was calculated using the formula: Shear stress = Shear force/(π × (diameter of tibia/2)^2 – (cavity diameter of tibia/2)^2. These mechanical properties of the bone are described by Wilson and Ruszler (1996) and Armstrong et al. (2002).

Tibia mineral contents were determined by using MarsXpress Technology Inside and an Inductively Coupled Plasma Atomic Emission Spectrometer (Vista AX CCD Simultaneous ICP-AES, Varian, Mulgrave, Australia). Approximately 200 mg dried sample (bone with marrow removed) was introduced into a burning cup and 5 mL nitric acid, 3 mL perchloric acid and 2 mL hydrogen peroxide were added. The sample was incinerated in a MARS 5 Microwave Oven (CEM, Corp., Mathews, NC, USA) at 190°C and 1.207 kPa pressure, and subsequently diluted to 25 mL with distilled water. The mineral concentrations were determined using an ICP-AES (Skujins, 1998).

Data were subjected to ANOVA using General Linear Model procedure in Minitab (2000). Duncan's multiple range tests were applied to separate means. Differences were considered as significant when P–value was less than 0.01 or 0.05.

Table 2. Effect of dietary zinc dosages and inorganic, organic or nano forms of zinc on performance in laying hens

Form	Dosage (mg/kg)	Body weight change (g)	Egg production (%)	Egg weight (g)	Egg mass (g/d/hen)	Feed intake (g/d/hen)	Feed conversion ratio (g feed/g egg)
Interactions							
Zn-oxide	50	79.67	95.68	64.89 ab	62.10	123.4 ab	1.99 bcd
Zn-oxide	75	53.00	97.49	60.90 cd	59.38	121.8 abc	2.05 bc
Zn-oxide	100	90.75	96.08	62.25 bcd	59.83	123.3 ab	2.07 ab
Zn-sulphate	50	68.25	95.98	64.73 ab	62.12	120.4 abc	1.94 cd
Zn-sulphate	75	120.33	95.98	66.05 a	63.38	121.8 abc	1.92 d
Zn-sulphate	100	67.17	96.65	65.20 ab	62.98	122.9 ab	1.95 bcd
Zn-glycine	50	67.00	95.08	64.69 ab	61.54	120.1 bc	1.96 bcd
Zn-glycine	75	58.58	96.39	59.80 d	57.63	124.8 a	2.17 a
Zn-glycine	100	19.83	95.68	62.34 bcd	59.64	117.4 c	1.97 bcd
Nano Zn-oxide	50	74.00	96.69	63.58 abc	61.48	123.6 ab	2.02 bcd
Nano Zn-oxide	75	66.75	96.79	62.72 bcd	60.68	121.7 abc	2.01 bcd
Nano Zn-oxide	100	87.67	97.11	66.15 a	64.23	124.4 ab	1.94 cd
SEM[1]		23.83	0.92	0.97	1.03	1.28	0.04
Main effects							
Zn-oxide		74.47	96.42	62.68 B	60.44 AB	122.9	2.04 a
Zn-sulphate		85.25	96.12	65.33 A	62.83 A	121.7	1.94 b
Zn-glycine		48.47	95.72	62.28 B	59.60 B	120.8	2.03 a
Nano Zn-oxide		76.14	96.86	64.15 AB	62.13 AB	123.2	1.99 ab
SEM[1]		14.07	0.52	0.69	0.68	0.87	0.02
	50	72.23	95.86	64.47 a	61.81	121.9	1.98 b
	75	74.67	96.66	62.37 b	60.27	122.5	2.04 a
	100	66.35	96.38	63.99 a	61.67	122.0	1.98 b
SEM[1]		12.61	0.45	0.63	0.66	0.79	0.02
Probabilities (P≤)							
Form		0.330	0.534	0.003	0.004	0.130	0.011
Dosage		0.891	0.491	0.015	0.107	0.795	0.049
Form × Dosage		0.417	0.110	0.021	0.110	0.015	0.017

[1]Pooled standard error of mean.

A, B – within a column, values not sharing a common letter are statistically different; P<0.01.

a, b, c, d – within a column, values not sharing a common letter are statistically different; P<0.05.

Results

The performance parameters are reported in Table 2 according to the forms and dosages of Zn. There were no significant differences in body weight change and EP between the treatment groups (P>0.05). The Zn forms were significantly affected: EW (P<0.01), EM (P<0.01) and FCR (P<0.05). The EW and FCR were improved in laying hens supplemented with Zn-sulphate, compared to those given Zn-oxide and Zn-glycine. Also, the EM was increased with Zn-sulphate in comparison with Zn-glycine. The different dietary Zn dosages as main factors have significantly affected the EW and FCR (P<0.05). The EW was significantly reduced with 75 mg/kg dietary Zn when compared to the other groups, while FCR was dramatically deteriorated with 75 mg/kg dietary Zn. The interactions between Zn forms and dosages had a significant effect on EW, FI and FCR (P<0.05), but not on other parameters (P>0.05). The highest EW were found in laying hens fed with nano Zn-oxide*100, whereas the lowest values were observed in birds receiving Zn-glycine*75. Additionally, FI was notably reduced in subgroups fed with Zn-glycine*100. The best FCR was observed in the Zn-sulphate*75 interaction group.

The eggshell quality parameters are shown in Table 3. All eggshell parameters were not significantly affected by forms, dosages or interactions with Zn except for eggshell thickness. The eggshell thickness (P<0.05) was dramatically reduced when birds were supplemented with Zn in the nano form, in comparison with the Zn-sulphate and Zn-glycine forms. The eggshell thickness was markedly increased (P<0.01) when hens were fed with 100 mg/kg Zn compared to the 75 mg/kg Zn group. The lowest eggshell thickness (P<0.05) was obtained in nano Zn-oxide*100 eggs, and the highest eggshell thickness was obtained in Zn-glycine*100 eggs.

The biomechanical properties and mineralisation of the bone in laying hens with Zn forms and dosages are shown in Table 4. The different Zn forms did not significantly affect the bone share stress, however, the shear force was increased in laying hens supplemented with Zn-sulphate compared to those receiving Zn-glycine or nano Zn-oxide (P<0.05). Similarly, the different dietary Zn dosages had significant effects only on the shear force from the bone properties (P<0.01). Also, shear force was significantly increased by 75 mg/kg Zn when compared to 50 or 100 mg/kg Zn. The interaction between Zn forms and dosages was significant with respect to shear force (P<0.01) and shear stress (P<0.05). The shear force and shear stress were found to be lower in Zn-oxide*100 bones, in comparison with Zn-oxide*75, Zn-sulphate*75 or Zn-sulphate*100 bones.

The forms and dosages of Zn as main factors and their combinations had no effect on P content of the tibia (P>0.05). As shown in Table 4, tibia Zn content was significantly higher in birds treated with the highest Zn (100 mg/kg) dosage compared to the other doses (P<0.05). The interactions between forms and dosages of Zn significantly affected the Ca content of tibia (P<0.001). The highest Ca content in the tibia was obtained for the group fed with Zn-oxide*50. Also, the lowest Ca content in the tibia was obtained for the group fed with Zn-oxide*100.

Table 3. Effect of dietary zinc dosages and inorganic, organic or nano forms of zinc on eggshell quality in laying hens

Form	Dosage (mg/kg)	Damaged eggs (%)	Egg breaking strength (kg)	Eggshell weight (g/100 g egg)	Eggshell thickness (µm)
Interactions					
Zn-oxide	50	2.02	3.91	9.69	377.0 cd
Zn-oxide	75	1.96	3.92	9.50	376.3 cd
Zn-oxide	100	1.14	4.29	10.19	394.0 ab
Zn-sulphate	50	1.14	4.31	9.80	388.2 abc
Zn-sulphate	75	1.90	4.17	9.61	380.6 cd
Zn-sulphate	100	1.44	4.11	9.71	381.5 bcd
Zn-glycine	50	2.66	4.23	9.59	380.3 cd
Zn-glycine	75	1.16	4.32	9.89	382.9 bcd
Zn-glycine	100	1.06	4.11	9.89	397.0 a
Nano Zn-oxide	50	1.15	4.07	9.67	378.3 cd
Nano Zn-oxide	75	1.04	4.24	9.62	374.6 cd
Nano Zn-oxide	100	1.44	4.20	9.37	374.2 d
SEM[1]		0.57	0.12	0.15	4.0
Main effects					
Zn-oxide		1.71	4.04	9.80	382.4 ab
Zn-sulphate		1.49	4.19	9.71	383.4 a
Zn-glycine		1.63	4.22	9.79	386.7 a
Nano Zn-oxide		1.21	4.17	9.55	375.7 b
SEM[1]		0.34	0.07	0.10	2.7
	50	1.74	4.13	9.69	380.9 ab
	75	1.51	4.16	9.65	378.6 b
	100	1.27	4.17	9.79	386.6 a
SEM[1]		0.29	0.06	0.09	2.4
Probabilities (P≤)					
Form		0.753	0.292	0.229	0.020
Dosage		0.546	0.877	0.461	0.027
Form × Dosage		0.486	0.184	0.062	0.034

[1]Pooled standard error of mean.

a, b, c, d – within a column, values not sharing a common letter are statistically different; P<0.05.

Table 4. Effect of dietary zinc dosages and inorganic, organic or nano forms of zinc on tibia characteristics in laying hens

Form	Dosage (mg/kg)	Tibia biomechanical properties		Tibia mineralization		
		shear force (N)	shear stress (N/mm^2)	zinc (mg/kg)	calcium (g/kg)	phosphorus (g/kg)
Interactions						
Zn-oxide	50	734 ABCD	51.85 abcd	166.3	299.6 A	110.6
Zn-oxide	75	849 A	63.21 a	183.3	287.6 ABCD	107.4
Zn-oxide	100	589 D	43.33 d	187.7	272.7 D	103.8
Zn-sulphate	50	644 D	49.52 bcd	170.6	277.1 CD	109.1
Zn-sulphate	75	822AB	56.23 abc	182.5	287.9 ABCD	107.1
Zn-sulphate	100	810 ABC	59.89 ab	186.4	286.8 ABCD	107.2
Zn-glycine	50	708 ABCD	50.79 bcd	166.3	286.4 ABCD	106.8
Zn-glycine	75	674 BCD	46.04 cd	170.9	287.1 ABCD	106.0
Zn-glycine	100	674 BCD	51.73 abcd	169.1	293.8 AB	109.2
Nano Zn-oxide	50	667 CD	51.04 abcd	170.8	290.7 ABC	108.8
Nano Zn-oxide	75	683 BCD	53.43 abcd	171.8	289.7 ABC	109.3
Nano Zn-oxide	100	665 CD	50.52 bcd	174.2	280.0 BCD	105.9
SEM[1]		33	3.47	5.1	3.4	1.7
Main effects						
Zn-oxide		724 ab	52.80	179.1	286.6	107.2
Zn-sulphate		759 a	55.21	179.8	283.9	107.8
Zn-glycine		685 b	49.52	168.8	289.1	107.3
Nano Zn-oxide		672 b	51.66	172.3	286.8	108.0
SEM[1]		26	2.31	3.3	2.6	1.1
	50	688 B	50.80	168.5 b	288.4	108.8
	75	757 A	54.73	177.1 b	288.0	107.4
	100	684 B	51.37	179.4 a	283.3	106.5
SEM[1]		23	2.06	3.0	2.2	0.9
Probabilities (P≤)						
Form		0.019	0.306	0.065	0.381	0.948
Dosage		0.009	0.276	0.026	0.092	0.224
Form × Dosage		0.001	0.018	0.629	0.001	0.278

[1]Pooled standard error of mean.
A, B, C, D – within a column, values not sharing a common letter are statistically different; P<0.01.
a, b, c, d – within a column, values not sharing a common letter are statistically different; P<0.05.

Discussion

The present study showed that the dietary incorporation of Zn-sulphate as an inorganic form of Zn induced significant amelioration in EW and FCR when compared with Zn-oxide and Zn-glycine forms of Zn. Also, EM was increased with additional Zn-sulphate as an inorganic form of Zn when compared with Zn-glycine form of

Zn. Generally, as shown in Table 2, the performance parameters of hens were deteriorated by the addition of Zn-glycine as an organic form of Zn to the diet, and the supplementation of the nano form of Zn to the diet did not affect these parameters, compared to those fed with inorganic forms of Zn. The results disagree with the findings of Tabatabaie et al. (2007) and Idowu et al. (2011), who showed that the feed efficiency was significantly improved at a concentration of the organic form of Zn compared to inorganic (Zn-oxide or Zn-sulphate). Bahakaim et al. (2014) indicated that the EM increased with the addition of organic Zn (Zn-methionine) compared to Zn-sulphate as inorganic form of Zn in laying hens. Also, Mohammadi et al. (2015) stated that the dietary zinc-nano-sulphate supplementation decreased body weight when compared to other Zn sources, but FCR was not influenced by treatment groups in broilers. Nevertheless, studies in previous years indicated that the EW (Hudson et al., 2004; Idowu et al., 2011), EM (Tabatabaie et al., 2007) and FCR (Bahakaim et al., 2014) were not affected by organic or inorganic forms of Zn. In the present study, EW and FCR significantly deteriorated in hens being fed 75 mg/kg Zn. Zamani et al. (2005) and Amem and Al-Daraji (2011) stated that EW was increased following supplementation with 50 mg/kg and 75 mg/kg Zn in the diet, respectively. Bahakaim et al. (2014) noted that the best FCR was recorded for hens fed diets supplemented with 50 mg/kg Zn. Kucuk et al. (2003) demonstrated that adding Zn (30 mg/kg) to the basal diet improved feed efficiency of broilers. The highest EW was observed for the group fed with nano Zn-oxide*100 in the diet. Also, the lowest EW was observed for the group fed with Zn-glycine*75 in the diet. The FI was significantly higher in the interaction group fed a diet containing Zn-glycine*75 than in groups fed Zn-glycine*50 or Zn-glycine*100. The best FCR was observed for the group fed with Zn-sulphate*75 in the diet. Also, the worst FCR was observed for the group fed with Zn-glycine*75 in the diet. This result disagrees with the findings of Tabatabaie et al. (2007), who noted that interactions between the different Zn sources and levels had no effect on EW and FCR in laying hens, but FI was in the group receiving 79 mg/kg organic Zn (adding 50 mg/kg). However, Bahakaim et al. (2014) reported that the lowest values of FI and FCR were recorded with layers fed diet supplemented with 150 mg/kg Zn-sulphate and 100 mg/kg Zn-methionine, respectively.

Zn plays an important role in the isthmus, where eggshell membranes are produced. Besides, dietary zinc improved eggshell quality because it is a component of the carbonic anhydrase enzyme, which is necessary for the formation of eggshells (Innocenti et al., 2004). On the other hand, it was observed in the present study that eggshell thickness was significantly decreased when birds were supplemented with the nano form of Zn, but this effect was not observed between organic and inorganic sources of Zn. Similar to the current study results, Tabatabaie et al. (2007) and Idowu et al. (2011) stated that the addition of different sources of Zn had no effect on eggshell thickness. However, Bahakaim et al. (2014) found that the eggshell thickness improved as a result of using organic Zn in diets. In the current study, it was also observed that the dietary 100 mg/kg Zn in diets induced a marked increase of eggshell thickness in laying hens compared to 50 and 75 mg/kg Zn contents. Similarly, Zamani et al. (2005) and Amem and Al-Daraji (2011) showed that supplementation of Zn (from 50 to 150 mg/kg) increased eggshell thickness in laying hens and broiler breeders. However, the previous studies by Kita et al. (1997) and Bahakaim et al. (2014) demonstrated that supplementing diets with Zn (from 50 to 150 mg/kg) did not affect eggshell thickness in laying hens. The interactions between forms and dosages of Zn were significant on the eggshell thickness, and it was increased with the Zn dosages added to the diets of the Zn-oxide or Zn-glycine forms of Zn, whereas it was reduced by Zn dosage added to the diets of the Zn-sulphate or nano Zn-oxide forms of Zn. Nevertheless, Bahakaim et al. (2014) reported that the eggshell thickness was affected by interaction sources (organic or inorganic) and levels (50, 100 or 150 mg/kg) of Zn.

The Zn, which is used by Zn-dependent enzymes such as alkaline phosphatase, collagenase and aminoacyl-tRNA synthetase, is essential for bone tissue and bone strength (Beattie and Avenell, 1992; Wang et al., 2002; Rossi et al., 2007; Shelton and Southern, 2007; Yamaguchi, 2010). The insufficient zinc content of feed caused a reduction of bone density and a deterioration of compact bone formation due to the role of Zn in protein synthesis (Scrimgeour et al., 2007; Shelton and Southern, 2007). The shear force of the tibia was significantly higher in the group fed diets containing the Zn-sulphate form of Zn in comparison with the organic or nano forms of Zn. This result was similar to that of Sahraei et al. (2012), who reported that the wall thickness and tibiotarsal index of tibia were significantly higher in chicks fed Zn-oxide as an inorganic Zn source than in those fed with Bioplex Zn as an organic Zn source. The lowest shear force was observed for the group fed with nano Zn-oxide in the diet. The present study showed that the 75 mg/kg dosage of Zn in the diet significantly increased shear force of the tibia when compared to 50 or 100 mg/kg Zn, which is in agreement with previous reports by Stofanikova et al. (2011) and Sahraei et al. (2012). Furthermore, Wang et al. (2002) noted that the lower Zn content in diet led to a reduction in bone density and bone length. Nevertheless, Swiatkiewicz and Koreleski (2008) found that bone biomechanical properties were not affected by Zn supplementation (30 mg/kg) in laying hens. Interactions between forms and dosages of Zn were significant on the shear force and shear stress of the tibia; the highest shear force and shear stress were obtained in hens fed the Zn-oxide form associated with a Zn dosage of 75 mg/kg. Sahraei et al. (2012) stated that the addition of 150 mg/kg Zn as Zn-oxide resulted in higher mechanical properties of the tibia than dosages (100 or 200 mg/kg) or sources (Bioplex Zn) of Zn. Midilli et al. (2015) showed that the modulus of elasticity and breaking stress were not affected by 75 mg/kg Zn as organic or inorganic forms. The tibia Zn concentration was only significantly increased with the highest Zn dose (100 mg/kg) added to the diets. Idowu et al. (2011) reported that the tibia Zn concentration in supplemented Zn samples in laying hens was significantly higher relative to the control group. Ao et al. (2006) and Rossi et al. (2007) demonstrated that dietary supplementation (from 5 to 60 mg/kg) of Zn as organic or inorganic forms increased tibia Zn content in broilers. Similarly, Mohanna and Nys (1999) and Sunder et al. (2008) noted that the concentration of Zn in tibia of broilers increased linearly with dosage of Zn (from 10 to 320 mg/kg) in broiler feeds. The results of the present study showed that the interactions between forms and dosages of Zn had a significant effect on the Ca contents of tibia. The Ca content of tibia was decreased with the Zn dosages added to the diets of the Zn-oxide or nano Zn-oxide forms of Zn, whereas an increase depending on Zn dosage added to the diets of the Zn-sulphate or Zn-glycine forms of Zn was observed. Sahoo et al. (2014) reported that the Ca concentration of tibia was not affected by sources (organic, inorganic or nano) or levels of Zn in broilers. Idowu et al. (2011) found that the Ca concentration in tibia was significantly increased in hens supplemented with organic Zn compared to those supplemented with inorganic Zn.

Previous studies had shown that organic or nano forms of Zn were more bioavailable than inorganic forms of Zn, but this effect, which is expected from organic or nano forms of Zn, was not observed in the present study. The NRC (1994) and Leeson and Summers (2005) stated that 35 and 50 mg/kg Zn was adequate to meet the requirements of laying hens, respectively. However, in most previous studies, there were no Zn groups added as a negative group. Therefore, the forms and dosages of Zn had a significant effect on performance, egg quality or bone parameters of hens when compared to other groups, because the Zn requirement of hens did not meet with the basal diet. Additionally, the high EP (average 96.30%) of the groups may have caused a Zn deficiency in hens because high EP may increase zinc requirements. Spears (1989) indicated that when an inadequate level of Zn was provided in the diet, the absorption of different forms of Zn was similar. The nano form of Zn in the diet and/or in the digestive system chelates with different inorganic or organic substances, and its absorption in the intestine may be decreased or excretion increased; however, this is not clear, and there is a need for more detailed studies. The differences between the studies may include the use of different Zn sources (e.g., Zn-methionine, Zn-proteinate or nano Zn-sulphate), and the genetic differences, ages and physiological states of birds (Wedekind et al., 1992; Hudson et al., 2004; Amem and Al-Daraji, 2011; Sahoo et al., 2014; Mohammadi et al., 2015).

In conclusion, the Zn-sulphate form of Zn had a positive effect on FCR, and inorganic forms of Zn increased the shear force of tibia, especially the sulphate form. The laying hens fed with Zn-glycine as the organic form of Zn significantly decreased performance (EW, EM and FCR) and shear force of tibia. Also, the eggshell thickness and shear force of tibia decreased with the addition of the nano form of Zn. These results showed that Zn-sulphate coupled to 50 mg/kg dosage of Zn in diet was optimal for sustaining performance, but for a good eggshell quality Zn-glycine should be added as organic form of Zn.

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