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Research article

Effects of organic zinc and manganese supplementation on eggshell quality and bone characteristic of laying hens during late laying cycle

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Abstract

Importance of the work: Problems with eggshell quality are common among aged laying hens.

Objectives: To investigate whether supplementation of zinc and manganese (inorganic versus organic) in varying combinations could improve egg production, egg quality and bone characteristics.

Materials & Methods: In total, 384 Lohmann Brown laying hens aged 64 wk were allocated to 4 dietary treatment groups, with each group consisting of 8 replicates with 12 laying hens each. The dietary treatments were corn-soybean meal basal diet supplemented with varying sources of zinc (80 parts per million, ppm) and manganese (100 ppm). The dietary treatments were: 1) 100% inorganic Zn and Mn; 2) 50% inorganic Zn + 50% organic Zn and 100% inorganic Mn; 3) 100% inorganic Zn and 50% inorganic Mn + 50% organic Mn; and 4) 50% inorganic Zn + 50% organic Zn and 50% inorganic Mn + 50% organic Mn.

Results: There were no significant ($p > 0.05$) effects of trace mineral sources (inorganic versus organic) on laying hen performance and egg quality. The supplementation of organic zinc significantly ($p = 0.0053$) increased the yolk zinc content, while the supplementation of organic manganese significantly ($p = 0.0020$) reduced the manganese excretion in the second period compared to those of the inorganic groups. However, these effects were not consistent. Feeding different sources of zinc and manganese had no significant ($p > 0.05$) effects on the tibia breaking strength and tibia mineralization.

Main finding: Based on the study conditions, supplementation of zinc and manganese in the laying hen diet during the late laying cycle, regardless of mineral source, had no effect on egg production and egg quality. However, the Mn concentration in the excreta considerably diminished in the 2nd period.

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Introduction

Eggshell quality is one of the most important characteristics in poultry egg production because it influences both the hatchability and economic profitability (Swiatkiewic et al., 2010). A major factor contributing to decreasing shell strength is increased egg size with hen age, while the eggshell weight remains the same (Washburn, 1982). In addition, problems with eggshell quality are common among aged laying hens (Nys, 2001) because they absorb Ca less efficiently than younger hens (Al-Batshan et al., 1994). In egg production, approximately 8% of all losses are caused by low eggshell quality (Klecker et al., 2002). Grobas et al. (1999) found that from the age of 22 wk to 57 wk, the percentage of broken eggs from brown egg-laying hens increased from 0.43% to 1.81%, while the shell thickness declined from 0.403 mm to 0.373 mm. Additionally, these eggs are often considered unsuitable for human consumption because of their low eggshell breaking strength and shell defects, which allow pathogenic bacteria to penetrate into the eggs more easily (Mabe et al., 2003). Normally, eggshell quality depends on dietary nutrients, such as macro minerals (Ca and P) and vitamin D3 (Swiatkiewicz and Koreleski, 2007). Zinc (Zn), manganese (Mn) and copper (Cu) are some of the trace minerals required in poultry diets, being available in both inorganic and organic forms.

Egg quality is improved in laying hens by supplementing the diet with organic trace mineral sources of Zn, Mn, or Cu (Bao et al., 2007) because trace minerals, such as Zn and Mn, play a key role in developing and maintaining the skeleton and eggshell. They are also essential components of many enzymes, vitamins and hormones (National Research Council, 1994). Furthermore, the trace minerals are cofactors of metalloenzymes responsible for carbonate and mucopolysaccharides synthesis that are important for eggshell formation (Swiatkiewicz and Koreleski, 2008). In laying hens, Zn is essential because it is a component of carbonic anhydrase and metalloenzymes (Mabe et al., 2003). Carbonic anhydrase contains 0.3% Zn (McDowell, 1992) and acts on carbonate to accumulate in the eggshell. A Zn deficiency affects metalloenzyme synthesis and leads to a reduction in carbonate deposition in the eggshell, thereby, decreasing the shell weight (Nys et al., 1999).

Most of the minerals used to supplement the diet of laying hens are generally derived from inorganic compounds, such as oxides, sulfates, carbonates and phosphates. These inorganic compounds have lower bioavailability than organic forms and the organic minerals are be more easily absorbed and retained by poultry because they have metal ions that are linked to organic molecules such as amino acids (Zamani et al., 2005). Metal amino acid chelates lead to improved bond strength and increased pH-stability and this binding creates a unique chemical structure characterized by high stability and increased absorption (Association of American Feed Control Officials, 2005). Higher bioavailability of organic minerals is enhanced by the absorption mechanism though the uptake of peptide or amino acid in the intestine and by being protected from binding and forming indigestible complexes with dietary constituents, such as phytate (Swiatkiewicz et al., 2001). Stable chelation enhances the solubility and availability of absorbed trace minerals, thus reducing the excretion of trace minerals and preventing them from potentially polluting the environment (Zamani et al., 2005). This could benefit laying hens during the late laying cycle (age > 60 wk) when nutrient absorption and utilization efficiency declines (Xiao et al., 2015).

Klecker et al. (2002) indicated that supplementation with chelated Zn and Mn resulted in a significant ($p < 0.05$) increase in laying performance and a highly significant ($p < 0.01$) increase in the strength, weight and thickness of the eggshell. Similarly, Mabe et al. (2003) and Stefanello et al. (2014) indicated that supplementation of inorganic and organic forms of Zn and Mn improved egg production and eggshell quality. Manangi et al. (2015) found that feeding laying hens with chelated trace minerals improved shell breaking strength ($p < 0.05$), shell thickness ($p = 0.08$ at wk 68; $p = 0.03$ at wk 74), and tibia breaking strength ($p = 0.07$) compared to inorganic trace mineral. However, Maciel et al. (2010) found no effects of organic trace minerals on egg production, feed intake and the feed conversion ratio ($p > 0.05$). The supplementation of Zn and Mn in organic trace minerals (OTM) and inorganic trace minerals (ITM) forms has been widely studied in laying hen both in the high egg production and late laying periods. However, there is limited study on supplementation using an OTM and ITM mixture of Zn and Mn in laying hen during their late laying cycle.

The aim of this study was to determine whether the supplementation of inorganic and organic forms of Zn and Mn in varying combinations could improve egg production, egg quality and bone characteristics of laying hens during their late laying cycle.

Materials and Methods

The procedures in this study were reviewed and approved by the Ethics Committee of Animal Care and Use for Scientific Research at Kasetsart University (Approval No. ACKU 60-AGK-014).

Animals and husbandry

A feeding trial was conducted to evaluate the effects of feeding an organic trace mineral (OTM) of manganese (Mn) and zinc (Zn) compared to a commercially used inorganic trace mineral (ITM) on eggshell quality and bone characteristics of laying hens during their late laying cycle. In total, 384 Lohmann Brown laying hens aged 64 wk were allocated to battery cages (three birds per battery cage) and housed in a curtain-sided, evaporative cooling system house for the four 28-day periods of the experiment. A lighting program was provided according to the breeder's recommendation (16 h light and 8 h darkness).

Experimental diets

The study was conducted using a completely randomized design (CRD). All laying hens were randomly allocated into 4 dietary treatments, each consisting of 8 replicates with 12 birds per replicate (4 battery cages). The inorganic minerals were ZnO and MnO, with the organic sources being Zn and Mn amino acid complexes (ZnAA and MnAA). Organic trace minerals are unique because they are bound to multiple amino acids (ligands) and oligo-peptides rather than the single amino-acid linked compounds (Vieira, 2008). The dietary treatments were a corn-soybean meal basal diet supplemented with two sources of Zn (80 parts per million, ppm) and Mn (100 ppm) and their combination (Table 1).

Diet formulation and feed processing

A practical corn-soybean meal diet was fed to the laying hens from age 64 wk until age 80 wk. All experimental diets were formulated according to the nutrient specifications recommended by the breeder guide (Lohmann Tierezucht, n.d.). The ingredient compositions of the experimental diet and calculated nutrient analyses are shown in Table 2.

Table 1 Dietary treatment with different sources and levels of zinc (Zn) and manganese (Mn)

Treatment	Inorganic form		Amino acid chelated (organic form)	
	Zn (%)	Mn (%)	Zn (%)	Mn (%)
1 (Control)	100	100	-	-
2	50	100	50	-
3	100	50	-	50
4	50	50	50	50

Table 2 Ingredient and nutrient composition of experimental diets

Ingredient (%)	Treatment			
	1 (Control)	2	3	4
Corn	55.599	55.599	55.599	55.599
Rice bran	5.00	5.00	5.00	5.00
Soybean oil	0.50	0.50	0.50	0.50
SBM 44 % crude protein	21.00	21.00	21.00	21.00
Canola	5.00	5.00	5.00	5.00
Meat and bone (50%)	3.00	3.00	3.00	3.00
DL-methionine	0.20	0.20	0.20	0.20
Choline chloride 50%	0.001	0.001	0.001	0.001
$CaCO_3$	8.25	8.25	8.25	8.25
MCP	0.90	0.90	0.90	0.90
Salt	0.30	0.30	0.30	0.30
Premix	0.25	0.25	0.25	0.25
Chemical composition of diets (%)				
Moisture	10.48	10.57	10.43	10.35
Crude Protein	17.65	17.58	17.86	17.62
Ether Extract	4.12	4.29	4.00	4.16
Crude fiber	4.00	4.10	4.30	4.20
Ash	13.59	13.48	13.45	13.57
Calcium	4.39	4.31	4.12	4.05
Phosphorus	0.7	0.8	0.78	0.81
Gross energy (Kcal/kg)	4037.11	4050.52	4047.32	4098.16
Zn (ppm)	156.49	143.27	152.27	142.91
Mn (ppm)	156.43	145.60	152.68	142.37

ppm = parts per million

Premix contained (per kilogram Premix): 5,000,000 IU vitamin A, 1,200,000 IU vitamin D_3, 4,000 IU vitamin E, 0.6 g vitamin K_3, 0.8 g vitamin B_1, 2.0 g vitamin B_2, 1.2 g vitamin B6, 0.0025 g vitamin B_{12}, 5 g niacin, 3.76 g pantothenic acid, 0.20 g folic acid, 0.036 g biotin, 16 g Fe, 4 g Cu, 0.8 g I, 0.08 g Co, 0.04 g Se.

Feed and water were provided *ad libitum*. Diets in mash form were fed twice daily at 0700 hours and 1500 hours. Representative samples of experimental diets were collected from each batch and then pooled at the end of the experiment for nutrient composition analysis.

Laying hen performance

The weight of all hens was recorded at the beginning and the end of the experiment and used to determine the body weight gain. The number of eggs laid by hens in each replicate was recorded daily and calculated as a percentage of hen-day egg production. In each period of the experiment, egg production (percentage), feed intake (grams per hen per day), and the number of dead birds were recorded. Egg mass (grams of egg per hen per day), feed conversion ratio (FCR; kilograms feed per kilogram eggs) and the total number of eggs were calculated for the entire 16 wk, according to North and Bell (1990) methods.

Egg quality

The numbers were recorded daily of dirty eggs, cracked eggs, shell-less eggs and soft-shelled eggs in each replication. Egg quality was evaluated by the breaking method to measure egg weight, albumen weight, yolk weight and shell weight on the last three consecutive days of each period of the experiment. The eggs were broken and the interior contents were assessed for albumen height, Haugh unit and yolk color using a digital egg tester (DET6000, Nabel; Kyoto, Japan). The eggshells without membranes were washed with tap water and dried at room temperature. Once completely dried, the shell thickness was measured using a Digimatic micrometer (Mitutoyo Corporation; Kanagawa, Japan). The zinc content of the egg yolk was measured at the end of periods 2 and 4 using the Association of Official Analytical Chemists (2012) methods.

Bone-breaking strength and ash content

At the end of the experiment, one hen from each replicate (each 8 hens per treatment) was sacrificed, the left and right tibia were dissected and soft tissues were cleaned off. The tibia samples were wrapped individually in cling wrap, sealed in plastic bags to prevent exposure to air, labeled and stored at -20 °C for subsequent bone breaking strength (American Society of Association Executives, 2005) and bone ash (Association of Official Analytical Chemists, 1990) measurements. The center of each bone was aligned with the breaking probe, which approached at 10 mm/min and the supports for each bone were 40 mm apart (Sun et al., 2013). The ailure point (peak) of each loading curve was used to determine the breaking strength. After the breaking strength had been measured and recorded, the bones were dried in an oven at 105 °C for 24 h. The bones were subjected to fat extraction with ether for 24 h and for further ash, Ca and P content analysis, according to the Association of Official Analytical Chemists (1990) methods. The trace mineral (Zn and Mn) contents of representative bones were analyzed based on Inductively Coupled Plasma Optical Emission spectroscopy according to Association of Official Analytical Chemists (2012) methods. The trace mineral contents were expressed as the percentage ash of dry fat-free weight, as described by Elaroussi et al. (1994).

Mineral contents in excreta

At the end of the 2nd and 4th periods, the fresh excreta from each replicate (four battery cages per replicate) were collected, pooled and 200 g of representative excreta was sampled, sealed in plastic bags to prevent exposure to the air and labeled and stored at -20 °C for moisture and mineral content analyses, based on Association of Official Analytical Chemists (2012) methods.

Statistical analysis

Treatments were assigned following the completely randomized design (CRD). The PROC GLM procedure of the SAS Studio 3.8 Basic Edition software (SAS University Edition, 2018) was used to analyze the effects of the experimental diet on layer performance, egg and eggshell quality and bone characteristics. Whenever the treatment effects were statistically significant, the means were compared using the Duncan's new multiple range test. The tests are considered significant at $p < 0.05$.

Results and Discussion

Laying hen performance

Throughout the experiment, there were no significant ($p > 0.05$) differences in body weight gain, feed intake, feed conversion ratio, hen day egg production, hen-housed egg production, egg weight, egg mass and percentage of livability when metal amino acid with Zn and Mn was partially and completely used to substitute for inorganic Zn and Mn (Table 3).

Table 3 Effects of organic zinc (Zn) and manganese (Mn) on layer performance and egg production

Parameter	Treatment				*p*-value	Pool SE
	1 (Control)	2	3	4		
Body weight (BW)						
Initial BW (g)	1882.85±39.71	1885.71±35.67	1886.25±46.30	1887.62±51.12	0.9969	7.71
Final BW (g)	1902.28±47.79	1899.48±70.63	1900.42±53.47	1906.47±33.22	0.9938	9.37
Body weight change (g)	19.44±47.00	13.77±92.44	14.17±31.75	18.85±42.59	0.9957	10.3
Productive performance						
Feed intake (g/d/hen)	111.34±1.50	110.11±1.84	110.51±4.14	109.61±3.50	0.6932	0.52
Hen-day production (%)	84.11±4.66	82.52±4.29	82.44±5.28	80.00±4.33	0.3816	0.82
Hen-housed production (%)	84.11±4.65	82.51±4.29	82.41±5.28	80.00±4.33	0.3831	0.82
Egg weight (g)	63.94±0.93	63.21±1.89	63.59±1.22	64.69±1.30	0.1977	0.24
Egg mass (g)	53.74±2.78	52.08±2.26	52.35±2.83	51.68±3.33	0.5054	0.5
FCR egg weight	2.082±0.116	2.117±0.100	2.101±0.093	2.135±0.132	0.8004	0.02
FCR egg mass	2.472±0.291	2.554±0.254	2.541±0.285	2.691±0.38	0.549	0.05
Livability (%)	99.99±0.026	99.99±0.029	99.97±0.039	100.00±0.000	0.2496	0.00

FCR = feed conversion ratio

Similarly, Swiatkiewicz and Koreleski (2008) observed that complete or partial substitution of inorganic Zn oxide and Mn oxides with their amino acid complex forms had no significant ($p > 0.05$) effects on laying performance parameters. Fernandes et al. (2008) found that the feed intake and feed conversion ratio were not influenced by any supplementation level of organic trace minerals (250 ppm or 500 ppm) compared to birds fed exclusively inorganic sources. Likewise, supplementation of Zn-Met or its combination with other chelated minerals had no beneficial effects on laying performance (Lim and Paik, 2003). In addition, Manangi et al. (2015) found that feed intake, egg production and FCR did not differ between hens fed with either chelated or inorganic trace minerals. However, Klecker et al. (2002) reported that supplementation of chelated Mn and Zn at 20% significantly ($p < 0.05$) improved laying performance (92.65 %), while supplementation at 40% significantly ($p < 0.05$) improved the production of eggs (92.15%) compared to the control group (90.91%).

The supplementation of organic minerals (Zn and Mn) did not affect the performance of the laying hens due to the fact that the levels of Zn and Mn in overall treatment were sufficient for laying performance, as recommended in the breeder's guidelines (Lohmann Tierezucht, n.d.). Specifically, Zn at 60 ppm and Mn at 100 ppm and different sources of minerals did not change the overall nutritional composition of the diets. Due to the similar nutritional values, the laying hens of all experimental groups received nutrients for their survival and performance of similar quantities throughout the experiment.

Egg quality and egg compositions

The supplementation of organic and inorganic forms of Zn and Mn and their combinations did not result in significant ($p > 0.05$) changes in the egg quality and egg composition of laying hens during their late laying cycle (Table 4).

Table 4 Effects of organic zinc (Zn) and manganese (Mn) supplement on egg quality and egg composition

Parameter	Treatment				*p*-value	Pooled SE
	1 (Control)	2	3	4		
Egg quality						
Cracked (%)	0.76±0.40	1.15±1.42	0.96±0.87	1.36±1.02	0.6711	0.18
Dirty (%)	0.33±0.20	0.76±1.23	0.88±1.35	0.38±0.20	0.5623	0.16
Shell-less egg (%)	0.17±0.24	0.1±0.17	0.18±0.26	0.19±0.31	0.8759	0.04
Soft-shelled egg (%)	0.37±0.37	0.23±0.24	0.27±0.36	0.56±0.61	0.4006	0.07
Eggshell breaking strength (N)	37.81±2.41	38.4±1.72	38.08±2.35	37.49±1.73	0.838	0.37
Albumen height (mm)	7.37±0.53	7.19±0.27	7.46±0.23	7.20±0.32	0.3746	0.06
Haugh unit	84.19±3.06	83.36±1.63	84.85±1.32	82.98±1.69	0.2795	0.36
Yolk color (Roche scores)	7.32±0.15	7.26±0.10	7.42±0.10	7.36±0.15	0.1007	0.02
Shell thickness (mm)	0.336±0.009	0.338±0.012	0.336±0.005	0.332±0.007	0.5593	0.00
Egg composition						
Yolk weight (%)	24.70±0.75	25.11±0.64	24.65±0.32	24.74±0.64	0.4181	0.11
Albumen weight (%)	62.78±0.88	62.46±0.73	63.06±0.47	62.89±0.61	0.3757	0.12
Eggshell weight (%)	12.56±0.22	12.41±0.20	12.30±0.25	12.36±0.18	0.1243	0.04

Similar results were observed by Mabe et al. (2003), who reported that feeding diets supplemented with organic and inorganic Zn, Cu, and Mn had no significant effect on the egg and eggshell quality of aged laying hens. Swiatkiewicz and Koreleski (2008) showed that substituting Zn and Mn oxides with metal amino acids improved eggshell breaking strength ($p < 0.05$) in the late phase of laying cycle (during age 62–70 weeks). However, Gheisari et al. (2011) reported that supplementation of the sulfate forms of Zn and Mn at 40 mg/kg significantly ($p < 0.05$) decreased the eggshell thickness and increased the broken eggs percentage compared with those supplemented with the same level of metal-amino acid complexes. The difference in research results was probably due to the great variety of chelated molecules and their bioavailability and stability, as well as their metabolism in the animal's body (Maciel et al., 2010).

Zinc deposition in yolk

At the end of the 2nd period, birds fed the diet containing 50% organic Zn (treatments 2 and 4) had a higher Zn content within the yolk than those in the inorganic Zn groups ($p < 0.01$; Table 5). However, no significant ($p > 0.05$) difference was observed among dietary treatments at the end of the 4th period. Similar results were reported by Venglovská et al. (2014), who stated that the supplementation of laying hens with diets containing inorganic and organic Mn sources had no significant effect on the Zn content of egg yolk. Mabe et al. (2003) supplemented inorganic and organic Zn and Mn at 30 mg/kg and 60 mg/kg, respectively, and found no significant difference in Zn and Mn deposition in the yolk of aged laying hens. There is a physiological limitation to Zn accumulation in the egg yolk because Zn interferes with the iron metabolism in chicks. An excess amount of Zn could lead to iron-deficient chicks; these chicks would be more susceptible to the effects of zinc toxicity than iron-adequate chicks (Sogunle et al., 2018). The accumulation of various nutrients in the yolk may have a direct effect on the embryo. Increasing the amount of Zn in the egg bubble may be a mechanism to control the accumulation of Zn in foods with higher amounts. However, the Zn in the yolk must be at an appropriate level so that the intake of Zn into the egg is not too large (Grau et al., 1979).

Bone characteristics

Throughout the experimental period, the supplementation of inorganic and organic forms of Zn and Mn did not significantly ($p > 0.05$) affect the tibia breaking strength and tibia mineralization (Table 6). Oliveira et al. (2015) injected organic forms of Zn, Mn and Cu *in ovo* and found no significant effects on the broiler hatchability and bone parameters, tibia characteristics, bone breaking strength and tibia mineral deposition. Similar results were observed from feeding inorganic and organic Mn by Xiao et al. (2015) on the tibia Mn content of laying hens ($p > 0.05$). However, Manangi et al. (2015) reported an improvement in tibia

Table 5 Effects of organic zinc (Zn) and manganese (Mn) supplement on Zn deposition

Parameter	Treatment				*p*-value	Pooled SE
	1 (Control)	2	3	4		
Yolk-P2 (DM, mg/kg)	68.23±2.20[b]	71.59±2.31[a]	67.93±1.90[b]	73.15±2.01[a]	0.0053	0.47
Yolk-P4 (DM, mg/kg)	92.54±4.01	91.96±2.00	90.88±2.52	91.28±1.26	0.7122	0.54

DM = dry matter

Mean ±SD within a row superscripted with different lowercase letters are significantly ($p < 0.01$) different.

Table 6 Effects of organic zinc (Zn) and manganese (Mn) supplement on tibia breaking strength and tibia mineralization

Parameter	Treatment				*p*-value	Pooled SE
	1 (Control)	2	3	4		
Tibia breaking strength (N)						
Tibia left	163.40±28.72	164.40±22.60	184.29±42.48	173.65±37.26	0.5781	5.95
Tibia right	163.41±23.24	169.35±25.18	180.17±37.93	178.09±45.14	0.7413	6.03
Tibia mineralization						
Ash (%DM)	64.27±1.37	63.92±1.24	65.17±2.02	64.65±2.07	0.5119	0.30
Ca (%DM)	24.90±0.69	25.12±1.30	25.30±0.85	25.87±0.76	0.2637	0.17
P (%DM)	10.30±0.54	10.24±0.31	10.46±0.40	10.28±0.44	0.7621	0.08
Zn (%DM)	190.74±25.71	201.83±17.41	202.95±21.31	204.08±23.03	0.6044	3.90
Mn (%DM)	8.42±1.43	8.57±0.68	8.49±1.24	8.57±1.31	0.9929	0.21

DM = dry matter

breaking strength of laying hens fed with chelated trace minerals (Zn-, Cu- and Mn-[2-hydroxy-4-(methylthio) butanoic acid]) compared to inorganic trace minerals (p = 0.07). These results indicated that in older hens, the bone properties are less sensitive to zinc and manganese availability in their diet. Regarding the nutritional factors, Swiatkiewicz and Koreleski (2008) showed the beneficial effects of active forms of vitamin D3 and particulate limestone on the bone quality of laying hens.

Zinc and manganese content in excreta

There were no significant (p > 0.05) effects from feeding inorganic and organic forms of Zn and Mn and their combinations on the excreted Zn content (Table 7). However, at the end of the 2nd period, the Mn content in the excreta of laying hens fed with organic Mn both at 50% and 100% in their diets was lower than that of those fed with inorganic Mn (p < 0.01). The present result was in accordance with the report by Carvalho et al. (2015) which indicated that the supplementation of organic trace minerals reduced trace mineral excretion compared to that of the inorganic mineral group. Organic minerals will not disintegrate under gastric acidic conditions and gastrointestinal defense, with the absorption of other minerals and increased absorption of inorganic mineral resources (Swiatkiewicz et al., 2014). However, at the end of the 4th period, no significant (p > 0.05) effect on Mn excretion in feces was observed.

Feeding organic Zn and Mn in the diets, using both partial and complete substitution of inorganic Zn and Mn, during the late laying cycle did not show any effects on the production performance, egg and eggshell quality as well as bone characteristics. Feeding the organic form of Zn increased the yolk Zn content, while the organic form of Mn reduced Mn excretion but these effects were not consistent throughout the experimental period.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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Table 7 Effects of organic zinc (Zn) and manganese (Mn) supplement on Zn and Mn in feces

Parameter	Treatment				p-value	Pooled SE
	1 (Control)	2	3	4		
Zn (DM, mg/kg) period 2	288.82±18.14	278.56±21.80	280.73±26.19	271.09±13.29	0.5559	3.9
Zn (DM, mg/kg) period 4	285.74±24.44	253.49±27.82	288.5±40.25	274.08±47.59	0.2254	6.41
Mn (DM, mg/kg) period 2	349.24±17.16^{a}	331.04±16.49^{ab}	317.08±16.77^{bc}	309.10±12.55^{c}	0.002	3.09
Mn (DM, mg/kg) period 4	329.36±18.94	307.95±9.76	319.44±37.13	321.22±30.10	0.4491	4.63

DM = dry matter

Mean ±SD within a row superscripted with different lowercase letters are significantly (p < 0.01) different.

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