Original Article

Effect of organic or inorganic Zinc and Manganese sources on performance and egg quality traits of laying hens

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ABSTRACT

The objective of this research was to evaluate the effect of levels and sources of dietary Zn and Mn forms organic or inorganic on performance and egg quality traits in laying hens. Experiment was carried out with 192 hens and 8 treatments and 6 replicated of 4 hens in a 14 weeks trial. Experimental treatments basal diet was supplemented with inorganic forms of Zn (Zn-O) and Mn (Mn-O) were gradually replaced with their organic forms in 0, 50 or 100% (Amino acid complexes Zn-AA and Mn- AA). Treatments were used: (T1) BD supplemented with 100% Zn and Mn in inorganic form (control group), (T2) BD without Zn and Mn in inorganic form, (T3) BD supplemented with 50% Zn and Mn in inorganic form, (T4) BD supplemented with 100% Zn and Mn in organic form, (T5) BD supplemented with 50% Zn and Mn in organic form, (T6) BD supplemented with 50% Mn in inorganic form and 50% Mn in organic form, (T7) BD supplemented with 50% Zn in inorganic form and 50% Zn in organic form, (T8) BD supplemented with 50% Mn in inorganic + 50% Mn in organic form and 50% Zn in inorganic + 50% Zn in organic form. The experimental results showed that feed intake, egg weight and egg shape index were significantly increased with organic Zn or Mn sources and organic sources in combination with inorganic trace minerals (Zn and Mn) ($P<0.05$).

Keywords: Egg quality traits, Inorganic or organic sources, Laying performance, Manganese, Zinc

Introduction

It has been documented that eggshell quality is related to macrominerals (Ca , P) and vitamin D_3 , but nowadays it is well_known that trace elements are also very important in the mineralization process , zinc, copper and manganese organic or inorganic sources could affect the mechanical properties of eggshell [1]. High breaking strength of eggshell and absence of shell defects are essential for protection against the penetration of pathogenic bacteria such as salmonella sp into eggs copper, zinc and manganese are biologically important in poultry formation of enzymes, immune response, bone development, integrity, eggshell formation and protection against oxidative stress are all mineral dependent processes .The bioavailability of minerals is strongly related to the chemical form, feed composition, age and physiological state of the bird and mineral interactions [2]. Inorganic trace minerals are the most common source of trace minerals supplemented in poultry diets.The rate of dietary trace mineral supplementation is approximately 0.25% of the total diet [3, 4]. These elements are added to feed in the form of inorganic salts, such as sulfates, oxides, carbonates and chlorides [5]. Also, they stated that intense usage of inorganic salts may reduce their relative biological availabilities and their excessive use may cause environmental pollution due to increased mineral excretion. Over the years, organic trace mineral research has focused on biological value and bioavailability in several species of livestock and poultry. Chelates are molecules resulting from a covalent band between

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metal ions and a ligand such as proteins or carbohydrates [6]. Organic trace minerals are metal complexes, metal amino acid complexes, metal amino acid chelates, metal proteinates, metal poly saccharide complexes, metal propionates and yeast derivate complexes. Commercially, metal complexes are the form available in the market. The use of organic minerals results in improved intestinal absorption. This is related to the small organic molecules and the reduction of the combination of insoluble complexes with ionic trace elements [7, 8]. Manganase is a vital trace mineral and it plays a significant role in body, bone growth, normal brain function, reproduction, digestion, physiological function, biosynthetic processes within the body, amino acid metabolism and various enzymatic systems [9]. Additionally, manganese is required in the formation of mucopolysaccharides that are activated for the glycosyl transferases, which are constituents of proteoglycans. These proteins are necessary for bone plate growth and bone cartilage formation of poultry [10]. Deficiency of manganese causes reduced egg production, eggs produced with thinner shells and translucent areas and abnormalities in eggshell ultrastructure [11]. Also, absorption in the intestinal tract is low. Manganase is absorbed by binding divalent metal ion transporters [5]. Zinc is a component of carbonic anhydrase, an essential enzyme for eggshell formation and calcification of bone from carbonate ions. Carbonic anhydrase is located in the shell gland of the oviduct in laying hens [12]. Additionally, other vital metalloenzymes, which are carboxypeptidases and DNA polymerases [13, 14]. Deficiency of this enzyme causes a lowered bicarbonate ion secretion that decreases eggshell weight. Zinc plays a major role in erythropoiesis, as it activates the synthesis of alfa-aminolevunilic acid dehyratese [5]. Zinc is absorbed in the small intenstine of monogastric animals and a small amount of absorption occurs in the proventriculus of chicks [15]. Moreover, zinc is distributed throughout the body and plays a role in the following functions; reproduction, development of blood cells, immune system function and bone development [16]. A zinc deficiency can cause adverse effecs on erythropoiesis in the marrow. Reduction of egg production and eggshell quality [14]. The chemical form and concentration of zinc are essential for preventing mineral antagonisms. For example, in the case of high calcium intake, intestinal zinc absorption is reduced and excess zinc intake can reduce cu absorption [2]. Current reports indicate that feeding laying hens with organic sources of trace minerals can improve egg quality, especially as related to shell quality also egg production and growth [17]. Moreover, several reports show that organic sources have greater bioavailability in comparision to inorganic sources trace minerals. Some minerals are necessary in small quantities to improve eggshell. The trace elements Zn, Mn and Cu are necessary for the organic matrix of the eggshell and subsequently, the mechanical properties. Also, they are involved in the interaction with the crystals of calcium during shell formation [9, 18].

Materials and Methods

Animals and husbandry

In the experiment have been selected 192 strains of L.S. L LITE laying hens in age of 68 weeks. On the basis of their body weight the birds were assigned evenly into 8 dietary treatments, each replicated 6times with 4 hens per replicate.All birds were fed resctricted amount of feed (100 g/day) during whole experiment while water was offered ad- libitum. A constant lighting regimen of 15 houre light (L): 9 houre darkness (D) was maintained throughout the adaptation and experimental periods. Environmental temperature was kept at 19–24°C and relative air moisture at 60–70%.

Experimental design and diets

The test carried out in a completely randomized design. All hens were fed the same basal mash diet (BD). The basal diet was formulated to meet requirements of hens, except for Zn and Mn (vitamin-mineral premix without these microelemnts was used) (Table 1). A period of 2 weeks was used for the adaptation of birds to feeding only the basal diet (BD). Basal diet contained in 1 kg 60 mg Zn and 100 mg Mn. All experimental diets were supplemented with inorganic forms Zn and Mn (Zn-O and Mn-O), were substituted by their organic sources in 0, 50 or 100% (Amino acid complexes Zn-AA and Mn-AA). For two weeks of adaption period, all experimental groups were fed with basal diet based on corn-soybean meal, formulated by WUFFDA software. The experiment lasted 14 weeks was adaption period. Treatments were used; (T1) BD supplemented with 100% Zn and Mn in inorganic form (control group), (T2) BD without Zn and Mn in inorganic form, (T3) BD supplemented with 50% Zn and Mn in inorganic form, (T4) BD supplemented with 100% Zn and Mn in organic form, (T5) BD supplemented with 50% Zn and Mn in organic form, (T6) BD supplemented with 50% Mn in inorganic form and 50% Mn in organic form, (T7) BD supplemented with 50% Zn in inorganic form and 50% Zn in organic form, (T8) BD supplemented with 50% Mn in inorganic + 50% Mn in organic form and 50% Zn in inorganic + 50% Zn in organic form.

Data collection and measurements

The laying hens were weighed individually at the beginning and at the end of the experiment. During the experiment, weekly (12 weeks) egg production percentage, egg weight, egg mass weight, feed intake and feed conversion ratio were calculated at the end of experiment. Two eggs from each replicate (12 eggs per treatment) were collected to determine the egg quality parameters, which were made on all collected eggs produced during two consecutive days of per at the end of 28 days period during the experiment. In order to examine egg quality traits, egg weight was obtained by weighting with a digital scale with an accuracy0.01 (g) and egg shape index was determined by equipment (Digital caliper) that calculated the width: length ratio as a percentage. Also, egg specific gravity was determined through floating the eggs in salty water. Haugh unit was calculated by the following formula: Haugh unit = 100 log ($H+5/57-1/37W^{0/37}$). Where (H) is albumen height (mm) and (W) is egg weight (g). Albumen height was measured with a tripod micrometer. Yolk color was measured by color fan roche.

Statistical analysis

The data were subjected to ANOVA of variance procedures appropriate for a completely randomized design using the general liner model (G LM), procedures of SAS institute. Means were compared using the Duncan multiple range test. [19, 20] Statements of statistical significant are based on ($P<0.05$).

Results

The effects of levels and sources of dietary inorganic and organic Zn and Mn supplementation on performance of laying hens in 12 weeks (table 2). The results showed that none of treatments had no significant effect on egg production, egg mass weight and feed conversion ratio ($p>0.05$). It should be noted that only treatment 7, in which base diet contains a mixture of organic and inorganic forms Zn and Mn had the highest egg production (65.79%) and egg mass weight (40.57g) and had the lowest feed conversion ratio (2.37), but these effects were not significant. Also, the results of laying performance indicated that treatments 4, 7 and 8 versus treatment 2 had a significantly effect on egg weight and treatment 4 versus 1had a significantly effect on feed intake. On the other hand, egg weight and feed intake were significant increased when a mixture of organic and inorganic forms Zn and Mn in their base diet were used ($p<0.05$). other treatments had no significant effect on these functional traits.Effect of dietary supplementation of inorganic and organic Zn and Mn on egg quality traits of laying hens are presented in tables (3, 4 and 5). During the first and second 28 day collection period, the results should that treatments had no significant effects on egg quality parameters ($p>0.05$). During the third 28 day collection period, egg weight was higher ($p<0.05$) for hens fed the treatment 6 than from hens fed the other treatments. In the same period, Egg shape index was higher ($p<0.05$) for hens fed the treatment 4 and 5 than the other treatments. But were not affected on egg specific gravity, haugh unit and yolk color by different levels and source Zn and Mn supplementation from organic and inorganic ($p>0.05$).

Discussion

The objective of this research was to evaluate the effect of decreasing levels of dietary Zn and Mn form organic and inorganic sources on performance and egg quality traits in laying hens at the end of cycle production. These results do agree with those of other researchers like[21] that determined the effects of methionine hydroxyl analogue chelated Zn (MHA-Zinc) on laying performance, egg quality, mineral deposits and the activities of containing enzyms on aged laying hens at 57 week-old laying hens, were fed a basal diet (Zn, 35.06 mg/kg). Their results showed that dietary Zn, did not affect egg weight, feed intake and feed conversion ratio. [22] evaluated effects of organic and inorganic zinc supplementation in layer breeder and examined performance and egg quality. Treatments received 60 mg/kg of one of the following Zn supplements: Availa-Zn, Zn-so_4, Zn-Redox min, Zn-o, Zn-o and Zn-cl_2.Their findings showed that dietary supplementation could raise the egg weight and feed conversion ratio. It is concluded that Availa-Zn added to diets had negative effects on egg shape index, but the Zn-so_4 supplemented had positive effects on egg shape index. The Zn-Redox min supplement had positive effects on egg shape index and haugh unit. [23] investigated the effects of dietary Mn-Met supplementation on egg quality of laying hens at 53 weeks of age, were fed a diet supplemented with 60 mg/kg Mn in the form of Mn-So4 and in other experimental groups were fed a diet supplemented with (20, 40, 60 and 80 mg/kg) Mn-Met. Dietary Mn-Met treatments significantly affected the hugh unit and yolk color. [24] the effect of reduced levels of Cu, Zn and Mn in combination from organic mineral source on performance, eggshell quality and mineral retention in laying hens 39 to 50 weeks-old. In all experimental periods supplementation of these elements in combination did not significantly influence the egg production, egg weight and feed conversion ratio of hens. [25] evaluated Zinc methionine supplementation on the performance and egg quality in laying hens from 22 to 34 weeks-old of age with (25, 50, 75 or 100 mg/kg) Zinc-Met. The results showed that no significant differences were observed on feed conversion ratio. However, highly significant impact was observed on feed intake, egg production, egg weight and egg mass weight were increased in the group fed dietary supplemented with the highest level of Zinc-Met (100 mg/kg) as compared to other groups and all egg quality traits (haugh unit, egg weight, egg specific gravity and yolk color) were statistically affected as a response to dietary Zn-Met supplementation except egg shape index. [26] investigated the effect of diets supplemented with different Zn sources on performance and egg quality in laying hens 52 weeks of age. Basal diet supplemented with 80 mg/kg (Zn-O nanoparticles, Zn-O and Zn-Met). The results indicated that dietary Zinc supplementation had no effect on feed intake, feed conversion ratio. However, the greatest egg production, egg weight, egg mass weight and haugh unit observed in Zinc-Met treatment. [27] to investigate the impact of long-term inclusion of the trace elements Cu, Zn and Mn chelated with the hydroxyl analogue of methionine comparison with Mn-sulfate on layer performance, eggshell quality at 20 to 60 weeks-old. Data for feed intake and egg production showed no effect of treatment calculated feed conversion ratio. [28] evaluated the effect the indusion of organic Cu, Mn and Zn in the diet of layin at 100 weeks-old on mineral excretion, egg production and egg quality. There was no effect of organic trace mineral supplementation on egg mass weight. Also, there was no effect of organic trace mineral supplementation on egg specific gravity. similarly, [29] reported that Mn levels (0, 25, 50, 100 or 200 mmg/kg) or sources (Mn-sulfate or Mn-AA) had no effects on egg production, egg weight, egg mass weight and feed conversion ratio in laying hens. [30] stated that different amounts of trace mineral supplements organic or inorganic Zn, Mn, Cu and Cr at 50 weeks of age in laying hens did not lead to any significant differences between their experimental treatments relative to feed intake and feed conversion ratio and no significantly differences in egg weight, haugh unit and egg shape index. [6] found that supplemental (120 mg/kg) Mn feed did not appear any improvements in egg production, egg weight, egg mass weight and feed conversion ratio in laying hens at 20 weeks-old with (Mn-Sulfate or Mn-Bioplex) , but egg weight were significantly increased with a mixture of minerals. [31] carried out effects of supplementary mineral amino acid chelate Zn and Mn (Zn-AA and Mn-AA) on the laying period layer hens at 64 weeks of age at the end of experiment, there were no significantly difference among the groups in feed intake, feed conversion ratio, but egg production, egg weight and egg mass weight were statistically significant. [18] reported the effect of supplementing hen diets with trace minerals from inorganic and organic sources Zn, Mn and Cu between 47 to 52 weeks of age on performance and eggshell quality of laying hens. The recommended levels were (30, 30, 5 mg/kg) of Zn, Mn and Cu, respectively. That increasing dietary these minerals to (60, 60, 10 mg/kg) did not affect on egg production, egg mass weight, feed conversion ratio and feed intake and there was no effect on egg specific gravity. [32] reported that supplemental Zn, Mn and Cu levels from inorganic and organic sources on the performance and egg quality at 48 weeks of age commercial hens. The results showed that supplemental did not affect on egg weight, egg mass weight and feed intake. Also, did not affect haugh unit. [33] Also, reported supplementation of different sources of Zn, Mn and Cu (organic or inorganic) on laying hens at 37 weeks of age, did not affect egg production, feed conversion ratio and feed intake. [9] observed the effects of dietary organic (Mn-Sulfate) sources at five increasing doses with organic (Mn-Bioplex) (15, 30, 45, 60 and 75 mg/kg) at 45 weeks of age on performance, egg quality and bone mineralization. They reported increasing dietary this element did not affect on egg production, egg mass weight and feed conversion ratio, whereas the feed intake and egg weight were significantly increased. [34] that fed diets supplemented with different levels of Mn, Zn and Cu froms (organic or inorganic) sources on laying performance and quality characteristics at 38 weeks of age were used. Their results stated that diet supplemented with the organic form of these elements, with concentrations 50% to 75% lower than the (NRC 1994) recommendation was sufficient to maintain laying performance and resulted in improved eggshell. [35] examined the effect of using microminerals Zn, Mn and Cu in organic or inorganic forms on the performance and egg quality laying hens 72 to 80 weeks of age. They reported that no effect of diets on egg production, feed intake and feed conversion ratio. [1] evaluated the effects of organic (Amino acid complexes) and inorganic (Mno and Zno) sources added to the feed of laying hens in 25-70 weeks of age with concentrations zero, 50 and 100% the (NRC 1994) recommendation. The results showed that this supplementation did not affect on egg production, egg mass weight, egg weight and feed conversion ratio. [36-38] reported that feed intake was not affected by supplementation level of organic trace mineral blend per kg of product (Se, Zn, Mn) compared to inorganic sources, but egg weight increased. The results indicated that increasing dietary trace minerals supplementation did not affect egg production, egg specific gravity, egg shape index. Therefore, our results different from the results of these studies, perhaps owing to differences among bird species, ages of birds used in the studies and or amounts of Zn and Mn added to the feed. Improvement in egg production and egg quality traits observed in the present study maybe due to supplementation of organic sources of Zn and Mn which could be due to higher bioavailability from organic source than from inorganic sources.

Conclusion

Overall, In the present study, organic sources zinc and manganese showed higher bioavailability compared with their inorganic sources when fed to laying hens.The use of 50% inorganic and organic minerals affects positively on laying performance and egg quality traits.

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Table 1: Nutritional compostition of the experimental diets (Basal diet)

Ingredient (%)	Value
Corn grain (7.5%)	62.16
Soybean meal (44%)	**24.40**
Dicalcium phosphate	**1.49**
Lime stone	**8.80**
Common Salt	**0.19**
$NaHco_3$	**0.16**
Soybean oil	**2.12**
Vitamin - Mineral premix	**0.50**
DL- Met	**0.15**
L-Lysine	**0.03**
Total	**1000**
Nutrients	**Calculated composition (% or as shown)**
Metabolizable energy (kcal/kg)	**2880**

Crude protein (%)	**15.70**
Digestible Methionine+Cystine (%)	**0.70**
Digestible Methionine (%)	**0.38**
Digestible Lysine (%)	**0.74**
Calcium (%)	**3.95**
Available phosphorus (%)	**0.36**

Vitamin supplement (per kg): Vitamin A 8.000 UI, Vitamin E 15.000 mg, Vitamin D3 2.300 UI, Vitamin K3 1.000 mg, Vitamin B1 200 mg, Vitamin B2 3.000 mg, Vitamin B6 1.700 mg, Vitamin B12 10.000 mcg, Niacin 20.000 mg, Folic acid 500 mg, Biotin 15,00 mg.
Inorganic mineral supplement (per kg): Manganese 120.000 mg, Zinc 120.000 mg, Iron 60.000 mg, Copper 18.000 mg, Iodine 2.000 mg, Calcium 9.600 mg.

Table 2: Effect of organic or inorganic Zn and Mn sources on performance in laying hens (82 Weeks of age)

Treatments	Egg Production (%)	Egg Weight (g)	Egg mass Weight (g)	Feed Intake (g)	FeedConversion Ratio (g/g)
T1 (control)	62.16	60.43^{ab}	37.81	99.13^{c}	**2.42**
T2	52.16	59.06^{b}	32.18	101.61^{abc}	**2.95**
T3	58.38	60.42^{ab}	38.61	101.31^{bc}	**2.42**
T4	57.32	61.68^{a}	33.76	105.76^{a}	**2.93**
T5	63.15	59.34^{ab}	37.46	102.83^{abc}	**2.54**
T6	59.61	61.31^{ab}	36.69	103.34^{abc}	**2.61**
T7	65.79	61.74^{a}	40.57	104.47^{ab}	**2.37**
T8	62.27	61.91^{a}	38.61	104.56^{ab}	**2.50**
P-value	0.45	0.05	0.37	0.03	**0.23**
SEM	4.27	0.78	2.61	1.35	**0.19**

P= P-value SEM= Standard Error Mean Means in the same row with different superscripts differ significantly (P<0.05)

Table 3: Effect of organic or inorganic Zn and Mn sources on parameters of egg quality traits in laying hens (from 71 to 74 Weeks of age)

Treatments	Egg Weight (g)	Egg Shape Index (%)	EggSpecific Gravity (g/cm^3)	Haugh Units (%)	Yolk Color
T1 (control)	56.78	72.68	1.076	65.27	**4**
T2	58.76	69.28	1.076	64.97	**4**
T3	58.68	74.94	1.076	65.21	**4**
T4	58.43	75.54	1.078	65.11	**5**
T5	59.80	76.22	1.078	66.25	**4**
T6	61.54	75.57	1.088	65.77	**4**
T7	60.48	73.48	1.088	66.19	**4**
T8	59.96	73.17	1.088	66.37	**5**
P-value	0.38	0.87	0.89	0.89	**0.88**
SEM	1.42	0.82	0.002	0.53	**0.15**

P= P-value SEM= Standard Error Mean Means in the same row with different superscripts differ significantly (P<0.05)

Table 4: Effect of organic or inorganic Zn and Mn sources on parameters of egg quality traits in laying hens (from 75 to 78 Weeks of age)

Treatments	Egg Weight (g)	EggShape Index(%)	Egg Specific Gravity (g/cm^3)	HaughUnit (%)	Yolk Color
T1 (control)	58.43	74.37	1.075	66.08	**4**
T2	61.54	74.48	1.077	65.25	**4**
T3	60	75.44	1.076	65.41	**4**
T4	59	75.03	1.076	65.66	**4**
T5	61.87	74.48	1.076	65.25	**5**
T6	61.10	74.80	1.077	65.41	**4**
T7	60.50	74.20	1.076	65.25	**4**
T8	62.74	75.07	1.078	65.41	**4**

P-value	0.47	0.96	0.93	0.94	**0.93**
SEM	1.51	0.81	0.001	0.51	**0.14**

P= P-value SEM= Standard Error Mean Means in the same row with different superscripts differ significantly (P<0.05)

Table 5: Effect of organic or inorganic Zn and Mn sources on parameters of egg quality traits in laying hens (from 79 t o 82 Weeks of age)

Treatments	Egg Weight (g)	Egg Shape Index(%)	Egg Specific Gravity (g/cm^3)	Haugh Unit (%)	Yolk Color
T1 (control)	56.98^c	73.63^b	1.078	66.83	**5**
T2	60.01^{bc}	76.84^{ab}	1.078	65.91	**4**
T3	57.76^c	76.17^{ab}	1.076	65.67	**4**
T4	57.64^c	77.76^a	1.088	65.83	**5**
T5	57.80^c	78.25^a	1.078	66.83	**4**
T6	64.06^a	76.76^{ab}	1.078	65.83	**4**
T7	62.07^{ab}	74.81^{ab}	1.078	65.67	**4**
T8	58.93^{bc}	73.67^b	1.088	65.67	**4**
P-value	0.002	0.04	0.90	0.50	**0.49**
SEM	1.34	1.25	0.001	0.52	**0.14**

P= P-value SEM= Standard Error Mean Means in the same row with different superscripts differ significantly (P<0.05).

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