Influence of dietary zinc and manganese chelate on productive and reproductive performance of layer chicken

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Abstract

Zinc (Zn) and Manganese (Mn) are two essential trace minerals for layer chickens. Present study was conducted to assess the effect of different sources of Zn and Mn on general performance, egg quality and reproductive performance of layers. One hundred and twenty RIR pullets (108 hens and 12 cocks) were randomly distributed in four experimental groups. Each group consisted of 30 birds in three replicates, each having 10 birds (9 hens and 1 cock). The first group was supplied with traditional mineral mixture as per requirement of BIS (1992) and considered as control (CTR). Other three treatment groups were supplied with same mineral mixture with high level (50%) of Mn and Zn (TMZ), amino acid chelate (Zn and Mn) as per requirement (TIC) and organic chelate of Zn and Mn (TOC) half of the requirement respectively. First laying age was significantly ($P<0.01$) lower in case of both the chelate supplemented groups. Feed efficiency ($P<0.01$), weight of eggs ($P<0.01$), shape index ($P<0.01$), albumen index ($P<0.01$), yolk index ($P<0.01$), shell thickness ($P<0.05$) and Haugh unit ($P<0.01$) were significantly higher in both the chelated Zn and Mn supplemented groups. However, membrane thickness, shell, yolk and albumen percentage values were not significantly varied among groups. No significant difference was found in fertility percentage of eggs among the groups but hatchability was improved significantly ($P<0.05$) in organic source chelate supplementation. It is concluded that supplementation of chelated Zn and Mn may improve overall performance, egg quality and hatchability in RIR layer chickens.

Key words: Chelates, Egg production, Layer chicken, Manganese, Zinc

INTRODUCTION

Mineral is one of the most essential nutrients needed for various metabolic and body functions to maintain optimum growth and egg production in layer chicken. In addition to macro minerals like Ca and P along with vitamin D₃, trace elements like Zn and Mn also have a major role in affecting the eggshell quality (Swiatkiewicz and Koreleski, 2008). Zn and Mn also play a vital role in various metabolic, biochemical and enzymatic pathways leading to better growth, egg production and feed efficiency. They have a very vital role to play in the mechanism of nutrient circulation in the animal. In case of laying hen enzyme carbonic anhydrase from the shell gland of the oviduct is essential for

eggshell formation and calcification of bone from carbonate ions (Leeson and Summers, 2009). Zinc is a major component of carbonic anhydrase. The significant role Zn plays is in reproduction, development of blood cells, immune system function and bone development (Hafeez, 2015). Deficiency of Zn may cause a drop in egg production and poor eggshell quality (Favero *et al.*, 2013). Mn is also a vital trace element having an essential role in growth, normal brain function, reproduction, amino acid metabolism, biosynthetic processes, physiological function and various enzymatic systems (Yildiz *et al.*, 2011). In poultry, Mn is also required for bone plate growth and bone cartilage formation (Zamani *et al.*, 2005). Reduced egg production

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and eggshell deformities are observed in Mn deficiency in laying hen (Mabe *et al.*, 2003). The requirements for Zn and Mn may not be fulfilled by the concentrations available in the regular feed and hence require supplementation to ensure adequate intake of minerals. Again bioavailability of minerals is related to the chemical form of the supplement along with other factors (Dobrzanski *et al.*, 2003). The use of various sources of trace minerals, different processing forms, and interaction among the minerals can influence its bioavailability. The researchers are trying to bind the minerals with amino acids, organic acids and other binders or chelators to increase the absorption of minerals and thereby making them more bioavailable (Gheisari *et al.*, 2011). A balance between dietary mineral supplementation and their different forms is important for better bioavailability. Few studies reported that substituting inorganic Zn and Mn with organic proteinates (Bioplex Zn and Bioplex Mn) improved laying performance, egg weight and egg mass in poultry (Siske *et al.*, 2000). A significant improvement in uniformity of the laying curve was also observed. In view of the above facts, to compare the efficacy of various forms and levels of Zn and Mn supplement in the diet of layer chicken, the present study was undertaken to evaluate the effect on overall performance, egg quality, fertility and hatchability of eggs in layer chickens.

MATERIALS AND METHODS

One hundred and twenty, 16 week old pullets (108 hens and 12 cocks) belonging to the Rhode Island Red (RIR) strain were brought from Government Poultry Farm and were randomly assigned to four experimental groups - one control group and three treatment groups with three replicates, with ten birds (9 hens and 1 cock) in each replicate. Each group was housed separately allowing 5ft x 6ft deep litter floor partitioned by iron net within a layer house. The first group was supplied with the traditional mineral mixture as per the requirement of BIS (1992) and considered as control (CTR). The other three treatment groups

were provided with the same mineral mixture with high level (50%) of Mn and Zn (TMZ), amino acid chelate (Zn and Mn) as per requirement (TIC) and organic chelate of Zn and Mn (TOC) half of the requirement respectively. Birds were fed the grower (up to 20th week of age) and layer (21st to 42nd week of age) ration (Table 1). Diets were prepared to meet the nutrient requirements as per BIS (1992) except Zn and Mn in particular treatment concerned. Birds were exposed to 18 hours of light and 6 hours of darkness throughout the experiment. All the birds were fed accurately weighed feed material twice a day, and the leftover feed, if any, was collected in the next day morning to determine the residue and actual consumption of feed per day per bird. All the birds were offered water *ad libitum* in clean water troughs every day throughout the experimental period.

Diets for both the grower and layer birds were prepared by adding trace mineral mixture to the respective group. The trace minerals were supplemented as per the requirement of BIS (1992) without considering the availability of trace minerals from the feed. The composition of trace mineral mixture of control diet for both grower and layer birds are given in Table 2. The diets of the TMZ group contained 50% more Zn and Mn than the requirement as per the BIS standard (1992). In the rest two groups, Zn and Mn were supplemented from commercially procured amino chelate and organic chelate (Alltech[®]) respectively. The procedure adopted for extracting trace minerals in feed was done by wet oxidation as per the method suggested by AOAC (1970) for estimation of Zn and Mn with the help of Atomic Absorption Spectrophotometer (Perkin Elmer Analyst 100). Productive and reproductive performances of various experimental groups were calculated for the period of 22 wks (21st to 42nd wk of age). Twelve eggs were randomly collected per treatment group for egg quality measurement. Eggs were collected mainly in the morning time daily and marked. They were kept in a dried, clean container with the dust of rice bran at room temperature. To evaluate the

Table 1. Ingredient and nutrient composition of basal diet for grower and layer birds

Particulars	kg/100 kg feed	
Ingredient composition	Grower	Layer
MAIZE	39.000	48.680
DORB	39.780	16.920
SOYABEAN MEAL	10.320	17.510
TIL CAKE	2.870	5.420
FISH MEAL	3.740	2.670
LIMESTONE POWDER	1.000	1.000
DICALCIUM PHOSPHATE	1.000	1.000
OYSTER SHELL	2.000	6.000
NICOMIX*	0.025	0.025
DL-METHIONINE	0.025	0.050
CHOLINE CHLORIDE	0.164	0.150
SOYABEAN OIL	-	0.500
NUTRIENT COMPOSITION	GROWER	LAYER
CP (%) ¹	16.20	18.23
CF (%) ¹	7.52	4.99
EE (%) ¹	1.51	2.50
NFE (%) ²	65.95	62.82
TOTAL ASH (%) ¹	8.82	11.46
ME (KCAL/KG) ²	2552.36	2622.23
CA (%) ¹	1.89	3.371
AVAILABLE P (%) ²	0.68	0.57
ZINC (MG/KG)	40.65	35.74
MANGANESE (MG/KG)	47.98	29.03

*Each gram of Nicomix contained Vitamin A- 40,000IU, Vitamin D3- 6,000 IU, Vitamin B1- 3.2mg, Vitamin B2- 20mg, Vitamin B6- 6.4mg, Vitamin B12- 82 mcg, Niacin- 48mg, Calcium pantothenate- 32mg, Vitamin K- 4mg, Vitamin E- 32mg and Folic acid- 3.2mg; ¹Estimated values ; ²Calculated values

Table 2. Composition of trace mineral mixtures used in different groups

Particulars	Quantity (g/100kg feed)							
	CTR		TMZ		TIC		TOC	
	Grower	Layer	Grower	Layer	Grower	Layer	Grower	Layer
Ferrous sulphate (FeSO ₄ , 7H ₂ O)	45.00	37.50	45.00	37.50	45.00	37.50	45.00	37.50
Copper sulphate (CuSO ₄ , 5H ₂ O)	3.60	3.60	3.60	3.60	3.60	3.60	3.60	3.60
Potassium iodide (KI)	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
Sodium selenite (Na ₂ SeO ₃)	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Zinc sulphate (ZnSO ₄ , 7H ₂ O)	12.50	18.75	18.75	28.13	-	-	-	-
Manganese sulphate (MnSO ₄ , H ₂ O)	13.90	15.30	20.85	22.94	-	-	-	-
Amino chelate of zinc	-	-	-	-	31.15	46.73	-	-
Amino chelate of manganese	-	-	-	-	35.08	38.06	-	-
Bioplex Zinc	-	-	-	-	-	-	10.62	15.94
Bioplex Manganese	-	-	-	-	-	-	9.01	9.91

(Chelates procured commercially from Alltech[®])

external egg quality, egg weight and shape index was measured. The length and width of the eggs at the broadest points were measured with the help of a digital calliper (Mitutuyo, Japan). The shape index (SI) was calculated following the formula of Shultz (1953). To assess the internal quality of the egg, albumen index, yolk index, shell thickness, membrane thickness, shell, yolk and albumen percentage, Haugh unit, air cell and pH were measured following standard procedures. The method suggested by Heiman and Carver (1936) with slight modifications was adopted to determine albumen index (AI). The method of Sharp and Powell (1930) was used to determine the yolk index. Haugh unit (HU) was calculated by the formula of Haugh (1937).

The relative yolk weight, shell weight and albumen weight of the egg were measured using standard procedures. The shell thickness and membrane thickness of the egg were measured by digital gauge following standard procedure. The assessing of air cell was done as per the method outlined by Central Poultry Training Institute, Hessarghatta, Bangalore, India with some modification. After proper mixing of albumen and yolk, the pH of whole egg was estimated with the help of a digital pH meter (Systronic, Model No: 335).

The fertility of eggs was measured by candling method. The number of infertile eggs was recorded to estimate fertility percentage. For hatching, 20 eggs from each replicate were selected randomly from each replicate group.

The data were analysed by one-way analysis of variance (ANOVA) using SPSS (1997) to compare different parameters due to different treatments where the only source of variation is the type of mineral supplements. Duncan's test at 5% of significance was further followed to have group comparison between means of varying mineral types through homogenous subsets of such means.

RESULTS

The effects of different levels and forms of Zn and Mn on the general performance of layer

chickens (21wk to 42wk) are presented in Table 3. The average gain in body weight (g) was 496.88 ± 3.39 , 552.12 ± 1.39 , 603.21 ± 1.58 and 650.31 ± 2.60 respectively. The percentage of egg production was significantly higher ($P < 0.05$) in the TOC group over the CTR group. Total number of eggs produced was also higher ($P < 0.05$) in the TOC group (1211.33 ± 31.18) in comparison to the CTR group (1008.00 ± 35.81). Dry matter consumed (g) per kg of egg was 2897.18 ± 94.42 , 2762.00 ± 82.10 , 2560.53 ± 73.30 and 2308.58 ± 75.96 respectively, and it was significantly lower ($P < 0.05$) in both chelate supplemented groups. Calculated FCR for egg production was 2.89, 2.76, 2.56 and 2.30 respectively. Effects on egg quality and reproduction performance of layer chickens are presented in Table 4. The average weight (g) of eggs was 55.70 ± 0.17 , 55.92 ± 0.13 , 57.94 ± 0.09 and 58.91 ± 0.09 in CTR, TMZ, TIC and TOC groups respectively. Both TIC and TOC groups significantly ($P < 0.01$) varied from both CTR and TMZ groups, and there was a significant ($P < 0.01$) difference between TIC and TOC groups but no significant difference was found between CTR and TMZ groups. Statistical analysis revealed that shape index in TIC (74.59 ± 0.26) and TOC (75.96 ± 0.18) groups were significantly higher ($P < 0.01$) than CTR (70.71 ± 0.44) and TMZ (71.23 ± 0.31) groups. However, TOC groups differed significantly from the TIC group, and there was no significant difference between CTR and TMZ groups. Data on average albumen index was significantly ($P < 0.01$) varied among the groups, which showed a similar trend of increasing shape index in CTR, TMZ, TIC and TOC groups. The average yolk index was highest in the TOC group (0.51 ± 0.01). A significant difference ($P < 0.01$) was observed among the experimental groups showing a gradual increase of yolk index in CTR, TMZ, TIC and TOC groups in a similar manner of shape index values. The average shell thickness (mm) value in TOC (0.45 ± 0.02) group was significantly ($P < 0.05$) higher than CTR (0.38 ± 0.02) and

Table 3. Effect of various forms and levels of zinc and manganese on general performance of RIR layer chickens (21 to 42 wks of age)

Particulars	CTR	TMZ	TIC	TOC	Level of significance
Initial body weight (g)	1504.18 ±4.29	1498.18 ±3.55	1497.83 ±4.35	1499.78 ±2.27	NS
Final body weight (g)	2001.05 ^d ±1.44	2050.30 ^c ±2.60	2101.04 ^b ±2.78	2150.09 ^a ±1.93	**
Average gain in body weight (g)	496.88 ^d ±3.39	552.12 ^c ±1.39	603.21 ^b ±1.58	650.31 ^a ±2.60	**
Dry matter consumed per bird (g)	18022.53 ±114.49	18126.15 ±114.47	18239.44 ±148.89	18331.69 ±199.19	NS
Dry matter intake/bird/day (g)	104.03 ^c ±0.63	105.84 ^{bc} ±0.77	108.22 ^b ±0.82	113.18 ^a ±1.03	**
Percentage of egg production (%)	72.73 ^b ±2.58	76.36 ^b ±2.65	80.04 ^{ab} ±2.54	87.40 ^a ±2.25	*
Total number of eggs (nos.)	1008.00 ^b ±35.81	1058.33 ^b ±36.71	1109.33 ^{ab} ±35.22	1211.33 ^a ±31.18	*
Dry matter consumed per kg of eggs (g)	2897.18 ^a ±94.42	2762.00 ^a ±82.10	2560.53 ^{bc} ±73.30	2308.58 ^c ±75.96	*
First laying age (days)	134.00 ^a ±0.58	134.00 ^a ±0.58	132.00 ^b ±0.58	130.00 ^c ±0.58	**

Means bearing different superscripts in the same row differ significantly (*P<0.05; **P<0.01; NS- Non significant)

Table 4. Effect of various forms and levels of zinc and manganese on the egg quality and reproductive parameters in RIR layer chickens (21 to 42 wks of age)

Particulars	CTR	TMZ	TIC	TOC	Level of significance
Weight of egg (g)	55.70 ^c ±0.17	55.92 ^c ±0.13	57.94 ^b ±0.09	58.91 ^a ±0.09	**
Shape Index	70.71 ^c ±0.44	71.23 ^c ±0.31	74.59 ^b ±0.26	75.96 ^a ±0.18	**
Albumen Index	0.10 ^c ±0.00	0.11 ^c ±0.00	0.14 ^b ±0.00	0.15 ^a ±0.00	**
Yolk Index	0.44 ^c ±0.01	0.45 ^c ±0.00	0.48 ^b ±0.00	0.51 ^a ±0.01	**
Shell thickness (mm)	0.38 ^c ±0.02	0.39 ^{bc} ±0.02	0.42 ^{ab} ±0.02	0.45 ^a ±0.02	*
Membrane thickness (mm)	0.07±0.00	0.08±0.00	0.08±0.00	0.09±0.00	NS
Shell %	10.34±0.13	10.50±0.15	10.56±0.14	10.66±0.16	NS
Yolk %	27.17±0.12	27.21±0.26	27.19±0.40	27.26±0.14	NS
Albumen %	62.49±0.25	62.29±0.41	62.25±0.26	62.08±0.31	NS
Haugh Unit	87.57 ^c ±0.43	87.84 ^c ±0.79	89.92 ^b ±0.45	91.95 ^a ±0.65	**
Air cell (mm)	1.00±0.00	1.00±0.00	1.00±0.00	1.00±0.00	NS
pH	7.02±0.03	6.99±0.03	7.01±0.02	7.01±0.04	NS
Fertility %	81.67±1.67	86.67±3.33	91.67±4.41	96.67±3.33	NS
Hatchability %	80.00 ^b ±2.89	83.33 ^b ±1.67	88.33 ^{ab} ±4.41	95.00 ^a ±2.89	*

Means bearing different superscripts in the same row differ significantly. (*P<0.05; **P<0.01; NS- Non significant)

TMZ (0.39 ± 0.02) groups, but there was no significant difference between TIC (0.42 ± 0.02) and TOC groups, though TIC group differ significantly only from CTR group. No significant difference was observed in membrane thickness, shell, yolk and albumen percentage, air cell and pH of the egg content. The average Hough unit was found to be 87.57 ± 0.43 , 87.84 ± 0.79 , 89.92 ± 0.45 and 91.95 ± 0.66 in CTR, TMZ, TIC and TOC groups respectively. Results revealed a significantly ($P < 0.01$) higher value in TOC and TIC groups.

There was no significant difference observed in fertility percentage. The average percentage of hatchability of eggs was 80.00 ± 2.89 , 83.33 ± 1.67 , 83.33 ± 4.41 and 95.00 ± 2.89 in CTR, TMZ, TIC and TOC groups respectively. Analysis showed a significant ($P < 0.05$) difference between TOC, and CTR and TMZ group, though no significant difference was observed between TIC and TOC groups.

DISCUSSION

Considering the role of Zn and Mn supplementation on production and reproduction performance of laying hen, particularly on egg quality, fertility and hatchability, present study was carried out to evaluate the efficacy of different forms and combination of Zn and Mn and the findings of this study do agree with others workers (Darvishi *et al.*, 2020; Min *et al.*, 2018). Increased body weight gain might be due to more feed consumption, more nitrogen retention and organic matter retention. Growth retardation is universally observed in Zn and Mn deficiency. Increased body weight gain is in agreement with the works of other researchers (Rutz *et al.*, 2003; Min *et al.*, 2018; Darvishi *et al.*, 2020). Improved feed efficiency in terms of dry matter consumed per kg of eggs in chelate supplemented groups was also reported by Darvishi *et al.* (2020). FCR for egg production (kg feed/kg egg mass) in this study were within range (2.1 to 3.4) as reported by other workers in RIR (Khatun *et al.*, 2005;

Akhtar *et al.*, 2007). Significantly higher egg production percentage was observed in case of the TOC group. In a study in RIR conducted from 28 to 45 wks of age, egg production percentage was reported as 79% in the control group (Khatun *et al.*, 2005). However, in the present study, the egg production percentage values were comparatively higher in all the experimental groups as the experimental period was considered only 22 weeks (i.e. 21wk to 42wk). Significantly higher egg weight was recorded in TIC and TOC groups, and ascending order of values of average egg weight in CTR, TMZ, TIC and TOC groups was noticed. Siske *et al.* (2000) reported that 20%, 40%, and 50% substitution of inorganic minerals with organic forms including Bioplex™ Mn improved egg production. The present study showed that the average Hough unit was significantly higher in chelate supplemented groups than the conventional inorganic source of Zn and Mn supplemented groups. The shape index was also significantly higher in the TOC group in present study. The present finding related to shell thickness was in closer agreement with the findings of Sazzad *et al.* (1994), Rapp *et al.* (2002) and Swiatkiewicz and Koreleski (2008). The increase in shell thickness might be due to an increase in carbonic anhydrase and alkaline phosphatase activity. Zn being a co-factor of this enzyme makes both activity and proper function of this enzyme potentially sensitive to trace elements, their interaction and availability. The presence of Mn has an activating effect on alkaline phosphatase, explaining the importance of these elements in the proper formation of eggshell. Ochrimenko *et al.* (1992) noted a positive effect of Mn supplements on calcification and eggshell strength. Though statistical analysis did not find any significant difference among the groups for average shell percentage, an ascending value was obtained, reflecting ascending increment in shell weight along with an increase in egg weight. The present finding related to shell, yolk and albumen percentage was in close agreement with the findings of Rapp *et al.* (2002) and Rutz

et al. (2003). The Haugh unit value is used to assess the egg protein quality based on the height of its egg white (albumen). The average HU in the TOC group was significantly higher than the other groups, and HU in the TIC group was significantly higher than the HU values of both CTR and TMZ groups but significantly lower than the TOC group, although no significant difference was observed between CTR and TMZ groups. Overall an ascending value of HU was observed in CTR, TMZ, TIC and TOC groups in the present study reflecting good quality of eggs in case of supplementation of Zn and Mn in chelated form. Though some studies found no significant variation in the Hough unit (Yenice *et al.*, 2015; Zapata, 2016; Darvishi *et al.*, 2020), some other studies reported a similar trend of present findings (Idowu *et al.*, 2011; Abd *et al.*, 2018). Non-significant improvement in fertility and significant improvement in hatchability in chelate form of Zn and Mn supplemented groups is in agreement with other published

work (Yenice *et al.*, 2015). Siske *et al.* (2000) observed increase fertilization of hatching eggs in cockerels substituting 50% of the inorganic Zn, Mn and Se with organic chelates. The present finding was supported by Rutz *et al.* (2003), who observed 3.4% increases in hatchability in the organic mineral supplemented group than the control group.

The present study revealed that supplementation of chelated Zn and Mn improved egg weight, feed efficiency, first laying age, egg quality (egg weight, shape index, albumen index, yolk index, shell membrane thickness, Haugh unit) and hatchability as compared to inorganic sources in RIR layer chickens. Therefore, it can be concluded that chelated Zn and Mn may improve overall productive performance, egg quality and hatchability in layer chickens.

Conflict of interest: Authors have no conflict of interest in this study.

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