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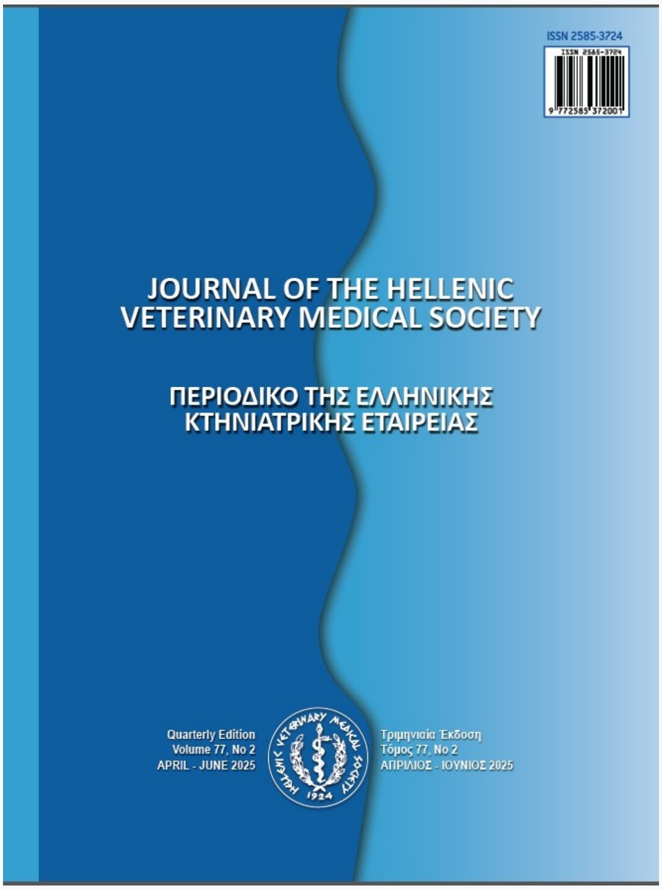

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Investigating the effects of organic and inorganic forms of zinc, copper, and manganese on the productive trait and reproductive performance of broiler breeder hens

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ABSTRACT: Trace minerals are essential for the birds growth, and are effective in its performance. The current study investigates the effects of organic and inorganic forms of copper, zinc, and manganese on the production traits and reproductive performance of Ross 308 broiler breeders aged 33 to 40 weeks. In this study, 240 hens and 30 roosters were used. This research was carried out as a factorial experiment (2×3) with three types of minerals (copper, zinc, and manganese) and two forms (organic and inorganic) in a completely randomized design with six treatments, five replications, and 8 hens and one rooster in each replication. The research was conducted as a factorial experiment in the form of a completely randomized design. The factors included the A factor (2 levels of organic and inorganic forms) and the B factor (3 levels of zinc, copper, and manganese). The number of treatments, repeats, and pens were 6, 5, and 30, respectively. The results of the study showed that the use of diets containing organic forms of elements had a significant effect on egg production traits, egg mass, egg weight, hatchable eggs, fertility rate, hatchability, and hatched chick weight ($p<0.05$). In general, the effect of the organic form of elements on the studied traits was greater than the inorganic form of the studied elements, and the highest and lowest impact of the treatments on the studied traits were related to organic zinc and inorganic manganese, respectively. Based on the present results, the use of organic forms of inorganic elements (especially organic zinc) is recommended to improve the production and reproductive traits of broiler breeders.

Keyword: Broiler breeder; Inorganic; Organic; Trace elements; Zinc.

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INTRODUCTION

Limited studies have examined the effects of organic versus inorganic forms of copper, zinc, and manganese on broiler breeder's production and reproductive performance. Minerals (Zn, Mn, and Cu) are essential for growing of chicken. Due to the low cost and amount needed in the feed, the poultry industry has established safety trace mineral margins to formulate feeds, consequently increasing mineral losses to the environment (Rezapour et al., 2024). Recent studies found Fe, Cu, Zn, and Mn requirements of broiler breeder hens using inorganic sources (Noetzold et al., 2020). Organic minerals can positively impact egg production, and fertility rates in poultry (Londero et al., 2020). In a separate investigation, the effects of chelated organic elements on broiler breeders and progeny were examined. Findings indicated that organic elements enhanced fertility, elevated hatchability, and diminished offspring mortality, proving more efficacious than mineral sources in avian diets (Faghih Mohammadi et al., 2022). Another study investigated the effects of dietary zinc sources in the form of zinc sulfate ($ZnSO_4$) compared to organic zinc amino acid complex on the reproductive performance of broiler breeders. This study showed that egg specific weight, egg production, and chick production (hatchability) were more optimal when long-term dietary zinc was consumed (Zhang et al., 2017). The provision of essential nutrients in the diet of broiler breeders is crucial for achieving high production levels with optimal efficiency (Avila., 2023). Inadequate levels of minerals significantly impact bone tissue formation and health, growth, and development (Ghasemi et al., 2020). Zn, Mn, Cu, and iron on broiler breeder play important roles in digestive, biosynthetic, and physiological processes within the body (Wang et al., 2019). Zn deficiency leads to decreased feather growth, leg joint swelling, shortened leg bones, and reduced growth, production, and hatchability (Sharma et al., 2023). Inadequate Zn levels decrease eggshell quality, leading to reduced egg production and hatching rates. Research indicates that an appropriate Zn in eggs is necessary for normal embryo production and chicken development. Feeding chickens with low zinc concentrations (<11 mg/kg) significantly increases embryo mortality and results in poor chick quality (Zhang et al., 2017). Eggs from copper-deficient chicken's exhibit abnormal size and shape, lack proper shells, or display wrinkling. Rough shells are attributed to abnormalities in the shell membrane (Wang et al., 2016). A study investigated the effects of mineral or organic nutrition, specifically Zn and Cu, on chicken performance and mineral content in chicken tissue. Chickens fed with both mineral sources of copper and Zn showed a lower feed conversion ratio than those fed with only the mineral source of Zn (Ao et al., 2009). Mn is vital for growth and reproduction in laboratory animals, playing a crucial role in collagen formation, bone development, urea formation, eggshell production, and immune system function (Zhang et al., 2021). Mn deficiency manifests as abnormal bone formation, disturbances in carbohydrate and fat metabolism, growth retardation, dermatitis, and fertility failure (Kimura., 2016). Maternal chickens deprived of Mn produce chicken embryos with incomplete skeletons (Miao et al., 2021).

The present study aims to the effects of organic and inorganic forms of Zn, Mn, and Cu on the production traits and reproductive performance of Ross 308 broiler breeders aged 33 to 40 weeks.

MATERIALS AND METHODS

Birds and experimental conditions: In the study, 270 birds were used. They were consisting of 240 hens and 30 roosters of the Ross 308 broiler breeders. They were randomly assigned to 6 experimental treatments with 5 repeats, resulting in 30 pens (each housing 8 hens and one rooster). The experimental area was designed with forty 1.5×2.5 m pens, separated by a fences. This research was conducted at Islamic Azad University-Qaemshahr branch, August to September 2023. The study was conducted on Ross 308 broiler breeders from 33-40 weeks of age (peak production).Weeks 29 to 33 were considered as adaptation period.

Birds were exposed to 14 h of light and 10 h of darkness. The roosters were collected from the experimental units before darkness, and the next day, after lighting and feeding, one rooster was randomly added to each experimental unit. Other environmental parameters were the same for all treatments including temperature (22°C), drinkers (1 nipple drinkers/9 birds), feeder space (15 cm/bird), bird density (3.5-5.5 bird/m^2), humidity (60–65%), light (60 lux) and ventilation. The amount of water and feed was the same in all pens.

Ethics declarations: The present study was conducted in accordance with the procedures for sample collection and management, as well as the ethical, scientific, and administrative standards for animal research established by the National Committee for Ethics in Biomedical Research of Iran (2018).

Experimental treatments: All diets were formulated using the Ross 308 broiler breeders' handbook (2021) and the NRC diet software (1994). The formulated diet (Table 1) contained the specific composition of the supplements. According to the Ross 308 broiler breeders' handbook, the requirements of Zn, Cu, and Mn of hens during the study were 16 mg/kg, 90 mg/kg, and 130 mg/kg, respectively. In the experimental treatments, the under-study trace elements were supplied by their organic and inorganic forms as follows. The inorganic mineral forms were Cu sulfate (CuSO4, 5H2O), Zn sulfate (ZnSO4, H2O), and Mn sulfate (MnSO4, H2O). GivanChemi® (GivanChemi®, Tehran, Iran) formulated them. The organic forms of mineral elements were in the form of Cu-amino acid (Zinpro® Availa® Cu-10.0%), Zn-amino acid (Zinpro® Availa® Zn-12.0%), and Mn-amino acid (Zinpro® Availa® Mn-8.0%). ZINPRO (Zinpro®, USA) formulated them. The required amounts of inorganic mineral in sulfate form for Ross broiler breeder hens were determined according to the Ross 308 (2021) catalog. The recommended values were 16 mg/kg for Cu, 90 mg/kg for Zn, and 130 mg/kg for Mn. Given the high bioavailability of inorganic minerals when combined with organic compounds, the amounts needed to satisfy the birds' nutritional requirements for inorganic minerals were lower. According to the manufacturer's catalog, the bioavailability percentages for organic Cu, organic Zn, and organic Mn were 120%, 185%, and 176%, respectively. Consequently, the recommended amounts of elements in organic form, as specified by the manufacturer in this study, were 13.33 mg/kg for Cu, 48.65 mg/kg for Zn, and 73.86 mg/kg for Mn. The experimental treatments were as follows: 1) inorganic Zn (Znso4), 2) organic Zn (Zn-methionine), 3) inorganic Cu (Cuso4), 4) organic Cu (Cu-methionine), 5) inorganic Mn (Mnso4), and 6) organic Mn (Mn-methionine).

Egg production percentage: It should be collected the eggs at least 2 times during the day, according to the Ross 308 guide. The daily production percentage in each experimental pen was calculated after egg collection, which occurred at 11:00 AM and 7:00 PM to prevent spoilage and experimental errors. At the end of the week, the average weekly production percentage of each pen was calculated using the formula (Kim et al., 2020):

Percentage of egg production= (number of chickens in each pen/ number of eggs in each pen) ×100

Egg weight: Every week, 2 eggs per pen were

Table 1 Composition of basal diet supplied to broiler breeder hens from 33 to 40 week of age

Ingredient composition	**(%)**	**Nutrient composition**	**33-40 week**
Corn	60.00	Metabolizable energy (*kcal/kg*)	2795.00
Soybean meal (44%)	17.50	Crude protein (%)	14.45
Wheat meal	9.5	Lysine (%)	0.68
Soybean oil	3.00	Methionine (%)	0.41
Dicalcium phosphate	1.25	Methionine + cysteine (%)	0.66
Oyster shell powder	4.52	Threonine (%)	0.56
Calcium carbonate	3.00	Calcium (%)	3.20
Sodium bicarbonate	0.25	Available phosphorus (%)	0.36
Salt	0.22	Sodium (%)	0.18
Methionine	0.17	Chlorine (%)	0.18
Threonine	0.04	Potassium (%)	0.70
Choline chloride	0.05	DEB[3] (mEq/kg)	247
Mineral premix[1]	0.25		
Vitamin premix[2]	0.25		

1- A mineral supplement without copper, zinc and manganese has been used in these diets. 2- The composition of vitamin supplements and mineral supplements for each kilogram of diet includes: Vitamin A, 10000 units., D3, 3200 units., E, 130 units., K, 9 mg., B12, 0.07 mg., Riboflavin, 20 mg., Calcium pantothenate, 15 mg., Niacin, 70 mg., choline chloride, 1000 mg., biotin, 0.6 mg., thiamine, 6 mg., pyridoxine, 8 mg. folic acid, 5 mg., iodine, 2 mg., iron, 50 mg., selenium, 200 micrograms., cobalt, 500 micrograms. 3- DEB: Dietary Electrolyte Balance.

taken as a sample and their quantitative characteristics were calculated in the laboratory. Egg weight was measured using a precise digital scale with an accuracy of 0.001 grams. The weight of the eggs of each experimental pen was calculated daily and at the end of each day using the formula (Oso et al., 2020):

Average daily egg weight= (number of eggs per pen/ total egg weight produced per pen)

Feed intake (FI): To estimate the weekly FI by the birds, the feed given in each meal was carefully weighed, and the remaining feed was also weighed. The weekly FI was determined from the difference between the feed given during the week and the remaining feed at the end of the week. The average FI in each pen was calculated by dividing the amount of feed allocated to each pen by the number of birds in that pen (Rezapour et al., 2024).

FI= [weekly feed in (g) - weekly feed remaining (g)]/ number of birds per pen

Egg mass: Calculation of egg mass based on the percentage of egg production and egg weight of each pen is calculated daily as follows (Kim et al., 2020):

Egg mass per experimental pen= (percentage of egg production per pen× Weight of eggs produced per pen) ÷100

Feed conversion ratio (FCR): The FCR was calculated based on the daily feed intake and egg mass production per pen using the formula below. Weekly averages were recorded. So, the weekly FCR was calculated (Macellineet al., 2022).

FCR of each pen= feed intake of each pen/ egg mass of each pen

Hatchable eggs: To calculate the number of hatchable eggs (weekly at 15-18°C and 75-80% humidity), small eggs, two-yolk, dirty, and bulbous eggs were separated, after weighing. So, hatchable eggs were selected and recorded (Abou-Kassem et al., 2024).

Fertility rate, and hatchability: The percentage of fertility rate and hatched chicks were calculated every 7 days. The fertility rate percentage was evaluated using candling on day 7 after laying the eggs in the incubator (Nowaczewski et al., 2022).

Fertility rate= number of fertilized eggs/ number of hatchable eggs x100

Hatchability= number of chicks hatched/ number of hatchable eggs x100

Chicken's weight: The chickens of each pen were randomly weighed and recorded every week immediately after hatching (Souza da Silva et al., 2021).

Statistical model and analysis: The data obtained from the research were analyzed using a factorial experiment (2×3) in the form of a completely randomized design and SAS(V9.2) software program. The LSMeans procedure was used to compare the treatments' means. The statistical model used is as follows:

$y_{ijk}=\mu+A_i+B_j+(AB)_{ij}+e_{ijk}$

Where, y_{ijk} is value of each observation, μ is the population mean, A_i is the effect of the A factor (two levels of organic and inorganic forms), B_j is the effect of the B factor (three levels of the effect of zinc, copper, and manganese elements), $(AB)_{ij}$= Interaction effect of two factors, and e_{ijk} is the residual effect.

RESULTS

Egg production: The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on egg production percentage during weeks 33-36, 37-40, and the whole period (33-40 weeks) were significant ($p<0.05$) (Tables 2-4). During weeks 33-36, Zn (91.26%) and Cu (90.87%) had a more significant effect on egg production percentage compared to Mn (88.91%) ($p<0.05$). Also, during weeks 37-40, the effect of Zn (91.86%) and Cu (90.97%) on the percentage of egg production was greater than that of Mn (89.12%) ($p<0.05$). In the whole period, Zn (91.47%) and copper (90.90%) had a greater effect on egg production percentage compared to Mn (89.05%) ($p<0.05$). During weeks 33-36 and 37-40 and throughout the whole period (33-40 weeks), the effect of organic forms on egg production percentage was greater than that of inorganic forms ($p<0.05$). The effect of organic Zn on egg production percentage during weeks 33-36 (91.46%) and 37-40 (91.92%) and throughout the whole period (91.65%) was greater than other organic and inorganic trace elements ($p<0.05$). The effect of organic Cu on egg production percentage in weeks 33-36 and 37-40 (90.48% and 90.61%) was higher than that of inorganic Cu (90.17% and 90.28%) ($p<0.05$). On the other hand, the effect of organic Mn on egg production percentage in weeks 33-36 and 37-40 (88.96% and 89.04%) was higher than that of inorganic Mn (88.40% and 88.53%) ($p<0.05$).

Egg weight: The effect of trace elements (Zn,

Table 2 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen production traits at 33-36 weeks

Treatment	Egg production (%)	Egg mass (g)	Egg weight (%)	FI[1](g)	FCR[2]
Trace elements					
Zinc	91.26[a]	49.62[a]	54.38[a]	163.24	3.28
Copper	90.87[a]	47.23[b]	51.98[b]	162.51	3.44
Manganese	88.91[b]	45.35[b]	51.01[b]	162.48	3.58
SEM	0.62	0.53	0.35	2.48	0.21
P. value	0.04	0.03	0.02	0.18	0.23
Mineral forms					
Inorganic Minerals	88.71[b]	45.49[b]	51.28[b]	162.17	3.56
Organic Minerals	91.20[a]	50.11[a]	54.95[a]	163.31	3.25
SEM	0.73	0.57	0.49	3.17	0.24
P.value	0.04	0.03	0.02	0.21	0.21
Trace elements × Mineral forms					
Inorganic zinc	90.03[b]	47.73[b]	53.02[b]	163.17	3.41
Organic zinc	91.46[a]	50.27[a]	54.97[a]	163.45	3.25
Inorganic copper	90.17[b]	46.55[b]	51.63[c]	162.75	3.57
Organic copper	90.48[b]	47.19[b]	52.16[b]	162.93	3.45
Inorganic manganese	88.40[c]	45.31[b]	51.26[c]	162.54	3.58
Organic manganese	88.96[bc]	46.57[b]	52.35[bc]	162.67	3.49
SEM	0.45	0.46	0.27	2.51	0.15
P.value	0.04	0.03	0.02	0.19	0.22

1. Feed intake, 2. Feed conversion ratio, Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value, FCR: Feed conversion ratio.

Cu, and Mn), mineral forms (organic and inorganic), and their interaction on egg weight percentage during weeks 33-36, 37-40, and the whole period (33-40 weeks) were significant ($p<0.05$) (Tables 2-4). During weeks 33-36, the effect of Zn (54.38%) on the egg weight percentage was greater than that of Cu (51.98%) and Mn (51.01%) ($p<0.05$).In weeks 37-40, the effect of Zn (58.72%) on egg weight percentage was greater than that of Cu (55.49%) and Mn (55.20%) ($p<0.05$). In the whole period, Zn (56.22%) had a greater effect on egg weight percentage compared to Cu (53.51%) and Mn (53.17%) ($p<0.05$). During weeks 33-36 and 37-40 and throughout the whole period, the effect of organic forms on egg weight percentage was greater than that of inorganic forms ($p<0.05$).The effect of organic zinc on egg weight percentage in weeks 33-36 (54.97%) and 37-40 (58.91%) and throughout the whole period (56.61%) was greater than other organic and inorganic trace elements ($p<0.05$). The effect of organic Cu on egg weight percentage in weeks 33-36 and 37-40 (52.16% and 55.83%) was higher than that of inorganic Cu (51.63% and 56.07%) ($p<0.05$). On the other hand, the effect of organic Mn on egg weight percentage in weeks 33-36 and 37-40 (52.35% and 56.42%) was higher than that of inorganic Mn (51.26% and 55.48%) ($p<0.05$).

Egg mass: In Tables 2-4, the effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on egg mass during weeks 33-36, 37-40, and the whole period (33-40 weeks) were significant ($p<0.05$). In weeks 33-36, the effect of Zn (49.62g) on egg mass was greater than that of Cu (47.23g) and Mn (45.35g) ($p<0.05$). Also, during weeks 37-40, the effect of Zn (53.94g) on egg mass was greater than that of Cu (50.47g) and manganese (49.19g) ($p<0.05$). In the whole period, Zn (51.42g) had a greater effect on egg mass compared to Cu (48.64g) and Mn (47.34g) ($p<0.05$). During weeks 33-36 and 37-40 and throughout the whole period (33-40 weeks), the effect of organic forms on egg mass was greater than that of inorganic forms ($p<0.05$). The effect of organic Zn on egg

Table 3 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen production traits at 37-40 weeks

Treatment	Egg production (%)	Egg mass (g)	Egg weight (%)	FI[1](g)	FCR[2]
Trace elements					
Zinc	91.86[a]	53.94[a]	58.72[a]	167.61	3.10
Copper	90.97[a]	50.47[b]	55.49[b]	167.08	3.31
Manganese	89.12[b]	49.19[b]	55.20[b]	167.12	3.39
SEM	0.59	0.49	0.48	2.48	0.19
P.value	0.04	0.02	0.02	0.24	0.26
Mineral Forms					
Inorganic Minerals	88.95[b]	49.48[b]	55.61[b]	167.17	3.37
Organic Minerals	91.85[a]	53.98[a]	58.78[a]	167.92	3.11
SEM	0.68	0.55	0.60	2.89	0.22
P.value	0.04	0.02	0.02	0.19	0.24
Trace elements × Mineral forms					
Inorganic zinc	90.12[b]	51.38[b]	57.02[b]	167.87	3.26
Organic zinc	91.92[a]	54.15[a]	58.91[a]	168.12	3.10
Inorganic copper	90.28[b]	50.40[b]	55.83[c]	167.28	3.31
Organic copper	90.61[b]	50.80[b]	56.07[b]	167.53	3.29
Inorganic manganese	88.53[c]	49.11[b]	55.48[c]	167.23	3.40
Organic manganese	89.04[bc]	50.23[b]	56.42[c]	167.48	3.33
SEM	0.38	0.41	0.37	2.13	0.16
P.value	0.04	0.02	0.02	0.21	0.21

1. Feed intake, 2. Feed conversion ratio, Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value. FCR: Feed conversion ratio.

mass in weeks 33-36 (50.27g) and 37-40 (54.15g) and throughout the whole period (52.98g) was greater than other organic and inorganic trace elements ($p<0.05$). Also, the effect of organic Cu on egg mass in weeks 33-36 and 37-40 (47.19% and 50.80%) was higher than that of inorganic Cu (46.55% and 50.40%) ($p<0.05$). On the other hand, the effect of organic Mn on egg mass in weeks 33-36 and 37-40 (46.57% and 50.23%) was higher than that of inorganic Mn (45.31% and 49.11%) ($p<0.05$).

Feed intake and feed conversion ratio: In Tables 2-4, the effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on FI and FCR during weeks 33-36, 37-40, and the whole period (33-40 weeks) were not significant ($p>0.05$).

In weeks 33-36, the highest and lowest FI were observed in treatments containing organic Zn (163.45g) and inorganic Mn (162.54g), respectively. Also, in weeks 37-40, the highest and lowest FI were observed in treatments containing organic Zn (168.12g) and inorganic Mn (167.23g), respectively. During the whole period, the highest and lowest FI were observed in treatments containing organic Zn (165.75g) and inorganic Mn (165.11g), respectively.

In weeks 33-36, the highest and lowest FCR were observed in the treatments containing organic Zn (3.25) and inorganic Mn (3.58), respectively. Also, in weeks 37-40, the highest and lowest FCR were observed in the treatments containing organic Zn (3.10) and inorganic Mn (3.40), respectively. During the whole period, the highest and lowest FCR were observed in the treatments containing organic Zn (3.19) and inorganic Mn (3.51), respectively.

Hatchable eggs: In Tables 5-7, the effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on hatchable egg percentage during weeks 33-36, 37-40, and 33-40 were significant ($p<0.05$). During week 33-36, the effect of Zn (95.23%) on the hatchable egg percentage was greater than that of Cu (92.30%) and Mn (91.99%) ($p<0.05$). In week 37-40, the effect

Table 4 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen production traits at 33-40 weeks (whole period)

Treatment	Egg production (%)	Egg mass (g)	Egg weight (%)	FI[1](g)	FCR[2]
Trace elements					
Zinc	91.47^{a}	51.42^{a}	56.22^{a}	165.80	3.22
Copper	90.90^{a}	48.64^{b}	53.51^{b}	165.12	3.39
Manganese	89.05^{b}	47.34^{b}	53.17^{b}	165.08	3.48
SEM	0.65	0.51	0.41	2.58	0.19
P.value	0.04	0.02	0.03	0.19	0.19
Mineral forms					
Inorganic Minerals	88.82^{b}	47.50^{b}	53.48^{b}	165.12	3.47
Organic Minerals	91.65^{a}	52.17^{a}	56.93^{a}	165.48	3.17
SEM	0.70	0.56	0.60	3.38	0.22
P.value	0.04	0.02	0.03	0.23	0.17
Trace elements × Mineral forms					
Inorganic zinc	90.09^{b}	50.16^{b}	55.90^{b}	165.14	3.27
Organic zinc	91.65^{a}	52.98^{a}	56.61^{a}	165.75	3.19
Inorganic copper	90.22^{b}	48.24^{bc}	53.48^{c}	165.08	3.42
Organic copper	90.52^{b}	49.25^{bc}	54.41^{b}	165.31	3.35
Inorganic manganese	88.49^{c}	47.03^{c}	53.15^{c}	165.11	3.51
Organic manganese	88.58^{bc}	48.70^{bc}	54.98^{bc}	165.39	3.39
SEM	0.47	0.44	0.34	2.17	0.18
P.value	0.04	0.02	0.03	0.21	0.21

1. Feed intake, 2. Feed conversion ratio, Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value, FCR: Feed conversion ratio.

of Zn (95.49%) on hatchable egg percentage was greater than that of Cu (92.41%) and Mn (92.16%) ($p<0.05$). In week 33-40, Zn (95.92%) had a greater effect on hatchable egg percentage compared to Cu (92.63%) and Mn (92.40%) ($p<0.05$). During weeks 33-36, 37-40, and 33-40, the effect of organic forms on hatchable egg percentage was greater than that of inorganic forms ($p<0.05$). The effect of organic Zn on egg weight percentage in weeks 33-36 (95.34%) and 37-40 (95.56%), and 33-40 (95.82%) was greater than other organic and inorganic trace elements ($p<0.05$). The effect of the organic form of Zn, Cu, and Mn on egg weight percentage was greater than their inorganic form. Also, the effect of organic Cu on hatchable egg percentage in weeks 33-36 and 37-40 (93.09% and 93.52%) was higher than that of inorganic Cu (92.41% and 92.80%) ($p<0.05$). On the other hand, the effect of organic Mn on hatchable egg percentage in weeks 33-36 and 37-40 (92.90% and 93.26%) was higher than that of inorganic Mn (90.30% and 90.71%) ($p<0.05$).

Fertility rate: The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on fertility rate percentage during weeks 33-36, 37-40, and 33-40 were significant ($p<0.05$) (Tables 5-7). During week 33-36, the effect of Zn (92.54%) on fertility rate percentage was greater than that of Cu (90.12%) and Mn (89.95%) ($p<0.05$). In week 37-40, the effect of zinc (92.77%) on fertility rate percentage was greater than that of Cu (90.28%) and Mn (90.10%) ($p<0.05$). In week 33-40, Zn (92.92%) had a greater effect on fertility rate percentage compared to Cu (90.44%) and Mn (90.28%) ($p<0.05$). During weeks 33-36, 37-40, and 33-40, the effect of organic forms on fertility rate percentage was greater than that of inorganic forms ($p<0.05$). The effect of organic Zn on fertility rate percentage in weeks 33-36 (92.46%), 37-40 (92.63%), and 33-40 (92.86%) was greater than other organic and inorganic trace elements ($p<0.05$). Also, the effect of organic Cu on fertility rate percentage in weeks 33-36 and 37-40 (90.06% and 90.37%) was higher than that of inorganic Cu (89.66% and

Table 5 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen reproductive performance at 33-36 weeks

Treatment	Hatchable eggs (%)	Fertility rate (%)	Hatchability (%)	Chick weight (g)
Trace elements				
Zinc	95.23[a]	92.54[a]	91.57[a]	44.19[a]
Copper	92.30[b]	90.12[b]	88.50[b]	42.11[b]
manganese	91.99[b]	89.95[b]	88.36[b]	41.91[b]
SEM	0.23	0.23	0.19	0.18
P.value	0.01	0.01	0.02	0.02
Mineral forms				
Inorganic Minerals	90.40[b]	89.99[b]	88.45[b]	42.20[b]
Organic Minerals	94.70[a]	92.35[a]	91.19[a]	44.11[a]
SEM	0.34	0.25	0.22	0.23
P.value	0.01	0.02	0.02	0.03
Trace elements × Mineral forms				
Inorganic zinc	92.81[b]	91.98[a]	90.89[a]	42.82[b]
Organic zinc	95.34[a]	92.46[a]	91.53[a]	44.30[a]
Inorganic copper	92.41[b]	89.66[b]	88.57[b]	42.19[b]
Organic copper	93.09[b]	90.06[b]	88.96[b]	42.87[b]
Inorganic manganese	90.30[c]	89.60[b]	88.45[b]	41.69[b]
Organic manganese	92.90[b]	89.99[b]	88.83[b]	41.97[b]
SEM	0.20	0.18	0.14	0.13
P.value	0.01	0.02	0.03	0.03

Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value.

90.03%) ($p<0.05$). On the other hand, the effect of organic Mn on fertility rate percentage in weeks 33-36 and 37-40 (89.60% and 89.78%) was higher than that of inorganic Mn (89.99% and 90.17%) ($p<0.05$).

Hatchability: In Tables 5-7, the effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on hatchability percentage during weeks 33-36, 37-40, and 33-40 were significant ($p<0.05$). During week 33-36, the effect of Zn (91.57%) on hatchability percentage was greater than that of Cu (88.50%) and Mn (88.36%) ($p<0.05$). In week 37-40, the effect of Zn (91.81%) on hatchability percentage was greater than that of Cu (88.69%) and Mn (88.50%) ($p<0.05$). In week 33-40, Zn (92.13%) had a greater effect on hatchability percentage compared to Cu (88.89%) and Mn (88.72%) ($p<0.05$). During weeks 33-36, 37-40, and 33-40, the effect of organic forms on hatchability percentage was greater than that of inorganic forms ($p<0.05$). The effect of organic and inorganic Zn on hatchability percentage in weeks 33-36 (91.53% and 90.89%), 37-40 (91.70% and 91.05%), and 33-40 (91.91% and 91.31%) was greater than other organic and inorganic trace elements ($p<0.05$). Also, the effect of organic Cu on hatchability percentage in weeks 33-36 and 37-40 (88.96% and 89.34%) was higher than that of inorganic Cu (88.57% and 88.98%) ($p<0.05$). On the other hand, the effect of organic Mn on hatchability percentage in weeks 33-36 and 37-40 (88.83% and 89.14%) was higher than that of inorganic Mn (88.45% and 88.87%) ($p<0.05$).

Chicks Weight: The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on chick weight during weeks 33-36, 37-40, and 33-40 were significant ($p<0.05$) (Tables 5-7). In week 33-36, the effect of Zn (44.19g) on chick weight was greater than that of Cu (42.11g) and Mn (41.91g) ($p<0.05$). In week 37-40, the effect of Zn (44.94g) on chick weight was greater than that of Cu (42.30g) and Mn (42.15g) ($p<0.05$). In week 33-40, Zn (46.19g) had a greater effect on chick weight compared to Cu (42.54g) and Mn (42.40g)

Table 6 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen reproductive performance at 37-40 weeks

Treatment	Hatchable eggs (%)	Fertility rate (%)	Hatchability (%)	Chick weight (g)
Trace elements				
Zinc	95.92[a]	92.92[a]	92.13[a]	46.19[a]
Copper	92.63[b]	90.44[b]	88.89[b]	42.54[b]
Manganese	92.40[b]	90.28[b]	88.72[b]	42.40[b]
SEM	0.24	0.27	0.19	0.20
P.value	0.01	0.01	0.02	0.02
Mineral forms				
Inorganic Minerals	90.80[b]	90.31[b]	88.91[b]	42.59[b]
Organic Minerals	94.99[a]	93.67[a]	91.59[a]	44.66[a]
SEM	0.27	0.29	0.23	0.23
P.value	0.01	0.02	0.02	0.03
Trace elements × Mineral forms				
Inorganic zinc	93.12[b]	92.40[a]	91.31[a]	43.26[b]
Organic zinc	95.82[a]	92.86[a]	91.91[a]	44.79[a]
Inorganic copper	92.80[b]	90.03[b]	88.98[b]	42.65[b]
Organic copper	93.52[b]	90.37[b]	89.34[b]	43.42[b]
Inorganic manganese	90.71[c]	90.02[b]	88.87[b]	42.20[b]
Organic manganese	93.26[b]	90.33[b]	89.14[b]	42.49[b]
SEM	0.18	0.23	0.16	0.13
P.value	0.01	0.02	0.03	0.03

Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value.

($p<0.05$). During weeks 33-36, 37-40, and 33-40, the effect of organic forms on chick weight was greater than that of inorganic forms ($p<0.05$). The effect of organic Zn on chick weight in weeks 33-36 (44.30g), 37-40 (44.55g), and 33-40 (44.79g) was greater than other organic and inorganic trace elements ($p<0.05$). Also, the effect of organic Cu on chick weight in weeks 33-36 and 37-40 (42.87% and 43.42%) was higher than that of inorganic Cu (42.19% and 42.65%) ($p<0.05$). On the other hand, the effect of organic Mn on chick weight in weeks 33-36 and 37-40 (41.97% and 42.49%) was higher than that of inorganic Mn (41.69% and 42.20%) ($p<0.05$).

DISCUSSION

The current study finds that organic trace elements, particularly Zn, were more effective than inorganic trace elements in increasing egg production percentage. The inclusion of these compounds in the diets is justification for the mentioned trait improvement. Organic Zn in the diet affects egg production percentage through its interaction with the endocrine system (Dikmen et al., 2016). Also, it is reported that the addition of organic Zn to the ration has a favorable influence on egg production in laying hens (Khajaren et al., 2006). Another research showed that the use of organic trace elements, rather than inorganic trace elements, improved the egg production of broiler breeders (Youssefi et al., 2014). It is reported that there is a positive effect on egg production in laying hens when the ration is supplemented with organic Zn. In the research conducted on brown laying hens aged 1 to 62 weeks, increasing the amount of organic Zn in the diet from 120 to 210 mg/kg increased egg production from 1.21 to 1.76% (Prasad and Kucuk, 2002). The results of the mentioned studies are consistent with the findings of the present research and confirm the positive effect of organic Zn on egg production. Zn plays a crucial role in egg formation. A Zn deficiency can impact the epithelium's quality as Zn is essential for protein synthesis. Zn also has an indirect influence on epithelial secretions, either by altering the epithelium's

Table 7 The effect of trace elements (Zn, Cu, and Mn), mineral forms (organic and inorganic), and their interaction on Ross 308 broiler breeder' hen reproductive performance at 33-40 weeks (whole period)

Treatment	Hatchable eggs (%)	Fertility rate (%)	Hatchability (%)	Chick weight (g)
Trace elements				
Zinc	95.49[a]	92.77[a]	91.81[a]	44.94[a]
Copper	92.41[b]	90.28[b]	88.69[b]	42.30[b]
Manganese	92.16[b]	90.10[b]	88.50[b]	42.15[b]
SEM	0.21	0.24	0.17	0.23
P.value	0.01	0.01	0.02	0.02
Mineral forms				
Inorganic Minerals	90.58[b]	90.16[b]	88.69[b]	42.38[b]
Organic Minerals	94.80[a]	93.51[a]	91.35[a]	44.42[a]
SEM	0.26	0.27	0.22	0.27
P.value	0.01	0.02	0.02	0.03
Trace elements × Mineral forms				
Inorganic zinc	92.93[b]	92.19[a]	91.05[a]	42.95[b]
Organic zinc	95.56[a]	92.63[a]	91.70[a]	44.55[a]
Inorganic copper	92.58[b]	89.84[b]	88.74[b]	42.42[b]
Organic copper	93.24[b]	90.10[b]	89.10[b]	43.20[b]
Inorganic manganese	90.48[c]	89.78[b]	88.65[b]	41.99[b]
Organic manganese	93.03[b]	90.17[b]	88.94[b]	42.25[b]
SEM	0.18	0.21	0.14	0.18
P.value	0.01	0.02	0.03	0.03

Non common superscripts in each column are indicate the significant statistical difference ($p<0.05$), SEM: Standard error of the mean, P.value: Probability value.

structure or directly during eggshell membrane synthesis (Liao et al., 2018). Organic Zn is more easily absorbed in the intestine, making it more effective (Durmus et al., 2004).

In the recent researches, Zn particularly organic Zn, led to a greater increase in egg weight compared to other trace elements. It is reported that the utilization of zinc-methionine and manganese-methionine in the diet of laying hens led to a substantial increase in egg weight (Khoshbin et al., 2023). A study on broiler breeders reported that different levels of organic zinc produced a significant difference in egg weight (Mahmood & Hazim, 2011). In contrast to the current study, it found that adding 60, 10, and 60 mg/kg of zinc, manganese, and copper to the basic diet did not result in a significant effect on egg weight in laying hens (Mabe et al., 2003). In a study, an increase in egg weight was observed in copper-deficient birds due to an increase in egg white content. Lacking vitamins and minerals supplement with only 24 ppm Zn, 15 ppm Mn, and 6 ppm Cu have been shown to decrease egg weight in old broiler breeders (62-74 weeks). This Low weight was not seen in young broiler breeders (30-40 weeks) (Inal et al., 2001).

Based on the results of the present research, the effect of zinc especially organic Zn was more effective than other trace elements on egg mass. The egg mass is affected by two factors egg weight and production percentage, and due to the influence of these two parameters, egg mass has also increased. Considering the role of Zn in the deposition of albumin in the magnum and the production of egg white and that the egg weight comes from its contents, it can be said that Zn and Mn can increase the egg mass by increasing the white (Klecker et al., 2002).

In the current study, organic and inorganic trace elements showed no significant effect on FI and FCR ($p>0.05$). The reduction in FI can occur due to Cu and Zn deficiency (Jahanian et al., 2008). The antagonistic relationship between Zn and Cu was reported (Hall et al., 1979).It has been stated that Mn has no affect on FI (Li et al., 2008). A study indicated that the organic minerals (Mn, Zn, and Cu) did not affect FI (Xiao et al., 2015). Research has shown that the consumption of organic Mn (Wang et al., 2012) and Zn (Pimentel et al., 1991). Supplements had no significant impact on FI in broilers. Another study investigating the effects of organic minerals, including Mn, selenium, and chromium, on FI in broiler chickens did not observe significant differences (Mohammadi et al., 2015). Moreover, research on laying hens (Sohrabi et al., 2018) found that organic chromium, Mn, and Zn did not affect FI. Found that the use of organic chelates of Zn and Mn, compared to their mineral sources, did not significantly impact the average FI of broiler chickens (Rahimi Fatehkohi et al., 2018). Franklin (2021) found that reducing inorganic levels of these minerals had no negative effect on broiler performance, while Gheisari (2010) reported that lower levels of inorganic sources could support broiler performance. Another study found that lower levels of organic Mn were required for optimal performance compared to inorganic Mn. These studies suggest that both organic and inorganic forms of these minerals can support broiler performance, with potential cost savings and environmental benefits from reducing inorganic levels (Carvalho., 2021).In another study, no significant effect was found by 50% replacement of inorganic trace minerals (Zn, Mn, and Cu) with organic sources (Maciel et al., 2010) on FCR in laying hens. Improvement in FCR when replacing more than one inorganic mineral simultaneously with organic sources has been reported in previous research. It was found that partial replacement (50%) of Zn, Mn, and Cu with organic sources (Zn-proteinate, Mn-methionine hydroxyl analogue chelate, and Cu-methionine hydroxyl analogue chelate) reduced FI, increased BWG, and improved FCR in Arbor Acres female broilers (El-Husseiny et al., 2012).

According on the present research results, the effect of Zn was more effective than other trace elements on Hatchable eggs. Trace element supplements are frequently fed to laying hens to reduce eggshell breakage (Nys et al., 2004) and eggshell quality incomplete sentence in other studies with the use of AACM (amino acid complexed minerals) in broiler breeders (Araújo et al., 2019) and laying hens (Qiu et al., 2020) compared to inorganic mineral sources. Lastly, found that dietary Zn supplementation significantly improved fertility traits (Mojaverian et al., 2025) and sperm egg penetration in broiler breeders (Amen and Al-Daraji., 2011). Similarly, found that organic sources of these minerals improved eggshell quality and decreased early embryo mortality (Favero et al., 2013). Xie (2014) found that Mn supplementation, particularly in the inorganic form, improved eggshell quality. However, Aksu (2011) found that lower levels of organically complexed minerals did not negatively impact performance, suggesting that the type and level of supplementation may play a role in eggshell quality. Eggshell quality has also been considered as a factor that affects hatchability (Roque and Soares, 1994), where thinner eggshell, low specific gravity, and poor breaking strength are correlated with lower hatchable (Liao et al., 2013).

According on the present research results, the effect of Zn was more effective than other trace elements on fertility and hatchability. The hatchability percentage, which can be considered an indicator of reproductive success and efficiency, signifies the positive strides and advancements in the broiler breeders'. A study on brown laying hens aged 1 to 62 weeks showed that rations with 60 (control), 90, 120, 150, 180, and 210 mg/kg of dry matter had a significant effect on hatchability percentage ($p<0.05$) (Durmus et al., 2004). Another study involving 45-week-old broiler breeders and different zinc levels in their diet (0, 50, 75, and 100 mg/kg) showed a significant effect on the overall performance (total chicks hatched compared to total eggs incubated) and the hens' ability to hatch fertile eggs (total chicks compared to eggs from fertile hens). The group receiving 100 mg/kg of Zn exhibited the highest chicken performance (75.67%) and fertile eggs (93.28%) (Mahmood & Hazim, 2011). Furthermore, Zhu et al. (2015) supplementing 120-ppm Mn on a deficient diet (14.3-ppm Mn in a corn-soybean meal diet) have observed improvements in hatchability of eggs from broiler breeder hens (88.8–95.1%). But A separate study on broiler breeders at 41 weeks of age, conducted over 22 weeks, found that supplementing a basic diet containing 72 mg/kg of zinc with an additional 152 mg/kg of inorganic and organic Zn (Zn-oxide and Zn-methionine) did not significantly affect hatchability. The limited number of records may have contributed to the ineffectiveness of Zn supplementation on hatchability (Kidd et al., 1992). Based on the present research results, the effect of Zn especially organic Zn was more effective than other trace elements on chick's weight. The egg's weight is one of the factors that affect the chick's weight that hatches from it. Usually, heavier egg production leads to the production of larger and higher-quality chickens. A study on broiler breeders at 41 weeks old for 22 weeks showed that supplementing the basic ration (72 mg/kg) with 152 mg/kg of Zn (Zn-oxide and Zn-methionine) did not significantly affect egg and freshly chicks weight (Kidd et al., 1992).

CONCLUSIONS

The utilization of Zn, Cu, and Mn organic elements resulted in increased egg production, egg weight, and egg mass, along with improved chick weight and fertility rate. Paying attention to the bioavailability of copper, zinc, and manganese in the diet is important for maximizing the reproductive and production performance of breeders. Copper, zinc, and manganese in organic form are more bioavailable than their inorganic counterparts. Therefore, poultry can absorb and utilize them more effectively. The reproductive performance of broiler breeders significantly affects the quality of the chicks produced. Based on the results of the present study, organic zinc increased the percentage of production, weight, and mass of eggs. It also increased the percentage of hatchable eggs, the percentage of fertility rate, and the weight of chicks produced. Therefore, the use of organic forms of copper, manganese, and especially zinc in the diet of broiler breeders is recommended. Additionally, the organic form of these elements significantly contributed to the increase in egg production, egg weight, and egg mass. Moreover, the inorganic form of zinc increased hatchability. Organic Zn improved the studied traits and, in comparison to other factors, had the most significant effect on them.

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CONFLICT OF INTEREST

The authors do not have any potential conflicts of interest to declare.

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