Ankara Üniv Vet Fak Derg, **62**, 63-68, 2015

Effects of dietary organic or inorganic manganese, zinc, copper and chrome supplementation on the performance, egg quality and hatching characteristics of laying breeder hens*

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Summary: This study was conducted to compare the effects of organic or inorganic Mn, Zn, Cu and Cr mixture using two different levels (80, 60, 5 and 0.15 mg/kg and 40, 30, 2.5 and 0.07 mg/kg, respectively) on the performance, egg quality and hatching characteristics of laying breeder hens. The experiment began with a total of 360 hens from Barred Rock line at 50 weeks of age and continued for 16 weeks. Each of the four dietary treatments was randomly assigned to six replicates, which included 15 hens each. Different trace mineral applications did not result in a significant effect on performance parameters such as livability, body weight gain, egg production, egg weight and mass, feed intake, and feed conversion. No significant differences in egg quality parameters such as damaged egg rate, shape index, eggshell rate and thickness, albumen height and haugh unit were observed among the groups. However, the low level of trace minerals resulted in a significant reduction in eggshell breaking strength ($P < 0.05$). Different trace mineral applications did not show a significant effect on hatching and fertilised eggs ratio. The dietary supplementation of organic (chelated with methionine) Mn, Zn, Cu and Cr at high levels (80, 60, 5 and 0.15 mg/kg, respectively) increased the hatchability of the fertilised eggs and hatchability compared to that of the other groups ($P < 0.05$).

Key words: Breeder hen, egg quality, hatching characteristics, performance, trace mineral.

Damızlık yumurta tavuğu yemine organik veya inorganik mangan, çinko, bakır ve krom ilavesinin performans, yumurta kalitesi ve kuluçka özellikleri üzerine etkileri

Özet: Bu araştırma, iki farklı seviyede organik veya inorganik Mn, Zn, Cu ve Cr karışımının (sırasıyla, 80, 60, 5 ve 0.15 mg/kg ve 40, 30, 2.5 ve 0.07 mg/kg) damızlık yumurta tavuklarının performans, yumurta kalitesi ve kuluçka özelliklerine etkilerini karşılaştırmak amacıyla düzenlenmiştir. Araştırma, 50 haftalık yaşta, toplam 360 adet Barred Rock hattı ile başlatılmış ve 16 hafta sürdürülmüştür. 4 ana grup her biri 15 tavuk içeren 6 tekerrüre rastgele dağıtılmıştır. Farklı iz mineral uygulamaları yaşama gücü, canlı ağırlık kazancı, yumurta verimi, yumurta ağırlığı ve kütlesi, yem tüketimi ve yemden yararlanma gibi performans parametreleri üzerinde önemli bir etki göstermemiştir. Yumurta kalite parametrelerinden, hasarlı yumurta oranı, şekil indeksi, kabuk oranı ve kalınlığı, ak yüksekliği ve haugh birimi bakımından gruplar arasında önemli farklılıklar bulunmamıştır. Bununla birlikte, düşük iz mineral seviyesi kabuk kırılma mukavemetinde önemli bir azalmaya sebep olmuştur. ($P < 0.05$). Farklı iz mineral uygulamaları, kuluçkalık ve döllü yumurta oranları üzerine önemli bir etki göstermemiştir. Organik (metiyonin şelatı) Mn, Zn, Cu ve Cr' un yüksek (sırasıyla, 80, 60, 5 ve 0.15 mg/kg) düzeyleri çıkış gücü ve kuluçka randımanını diğer gruplara göre artırmıştır ($P < 0.05$).

Anahtar sözcükler: Damızlık tavuk, iz mineral, kuluçka özellikleri, performans, yumurta kalitesi.

Introduction

Trace minerals are essential in poultry diets because they participate in biochemical processes required for normal growth, development and eggshell formation. Zinc is a component of the carbonic anhydrase enzyme, which is crucial for supplying the carbonate ions during eggshell formation (26); manganese is the metal activator of enzymes that are involved in the synthesis of mucopolysaccharides and glycoproteins that contribute to the formation of the organic matrix of the shell (10), and copper plays the role of cofactor of the lysyl-oxydase enzyme that is important in the formation of collagen cross links present in the egg shell membrane (4). Chromium stimulates and regulates the action of insulin, which is involved in anabolic processes (22). In addition, chromium is required for activating certain enzymes and for stabilising proteins and nucleic acids (18). Trace minerals present in the egg and available to the developing embryo function either as catalytic or structural cofactors in enzymes and proteins. These enzymes and proteins are located in the cells of the embryo and its extraembryonic membranes. It is crucial

* This study was supported by General Directorate of Agricultural Research and Policy (TAGEM HAYSÜD/09/13/04/04)

to the survival of the embryo that the requisite amount of each essential trace mineral must be available at the appropriate time during its growth and development within the egg (29).

The trace mineral requirements of poultry are not well defined. Commercial diets typically supplement inorganic trace minerals at a rate much higher than those recommended by NRC (25). However, the usage of inorganic trace minerals has many disadvantages such as forming insoluble compounds of phytic phosphorus with trace elements, low digestibility, their polluting the environment by being thrown away with faeces, ration addition in an amount higher for meeting the demand and the cost resulting from this (14, 17, 36). Organic minerals are metal ions chemically linked to an organic molecule (amino acids), forming chemical structures with unique characteristics of stability and high mineral bioavailability (1). Several previous studies have shown that compared with inorganic form, the absorption rate of organic minerals in the gastrointestinal tract is higher (13, 23, 35). However, information on these organic minerals is still controversial**.**

This study was conducted to compare the effects of organic or inorganic Mn, Zn, Cu and Cr mixture at two different levels on performance, egg quality and hatching characteristics of laying breeder hens.

Materials and Methods

Experimental design and diets: This research was conducted using a 2 × 2 factorial design, and two different trace mineral forms (inorganic and organic) and two different levels (80, 60, 5 and 0.15 mg/kg and 40, 30, 2.5 and 0.07 mg/kg of Mn, Zn, Cu and Cr, respectively) were tested. A total of 360 brown, Barred Rock laying breeder hens were distributed to individual cages (25 × 47 × 55 cm) in the form of 4 groups and 6 replicates (15 hens in each replicate). The experiment began at 50 weeks of age of hens and continued for 16 weeks. Feed and water were supplied *ad libitum*. The house was environmentally full-controlled with 16 hours lighting. Organic trace minerals of the chelated type, depending on the methionine, were supplied from Redoks Chemical Mineral Prod. Co. (Istanbul, Turkey). Inorganic Mn, Zn and Cr were provided by their oxide sources and Cu was provided by its sulphate source. Inorganic mineral premixes were prepared using a micro-mixer (Çelik Makine, Ankara, Turkey) at 1800 rotations/min for 3 min. Basal diet was formulated according to NRC requirements (25) (Table 1). Crude nutrient, starch, and sugar analyses of feed ingredients were performed according to AOAC (2) procedures and their metabolisable energy were calculated using the equations of Janssen (12). The resulting values were used in the calculation of diet composition and chemical components. The amounts of Mn, Zn, Cu and Cr provided with feed ingredients were determined. 1 kg of feed mix without mineral premix was prepared according to experimental formulation at the micro-mixer. The Mn, Zn, Cu and Cr contents of the feed mix was analysed by the air-acetylene flame method (SM 3030 D,E,F,G,H,I; SM 3111B) using a Perkin-Elmer atomic absorption spectrometer (32).

Table 1. Composition of the basal diet.
Tablo 1. Bazal rasyonun bileşimi.

Basal diet[1]	Composition (%)[2]
Yellow corn	56.2
Soybean meal	20.9
Sunflower seed meal	9.6
Vegetable oil	1.0
Ground limestone	9.9
Di calcium phosphate	1.4
DL-Methionine	0.20
Salt	0.35
Salmonella inhibitor	0.20
Vitamin-mineral premix[3]	0.15
Mineral premix[4,5]	0.10
Calculated nutrient content	
ME (kcal/kg)	2751
Crude protein (%)	16.52
Ether extract (%)	3.22
Crude ash (%)	13.60
Calcium (%)	4.0
Available phosphorus (%)	0.37
Methionine (%)	0.44
Methionine+cystine (%)	0.72
Lysine (%)	0.82

[1] The contents of Mn, Zn, Cu and Cr in the feed mix without mineral premix were 12.3, 31.9, 5.3 and 0.88 mg/kg, respectively.
[2] In low trace mineral diets, inorganic or organic mineral premix are reduced by half.
[3] Vitamin-mineral premix provided per kilogram of diet; vitamin A, 15 000 IU; vitamin D_3, 5 000 IU; vitamin E, 50 mg; vitamin K_3, 10 mg; thiamine, 4 mg; riboflavin, 8 mg; pyridoxine, 5 mg; vitamin B_{12}, 0.025 mg; niacin, 50 mg; Ca-pantothenate, 20 mg; folic acid, 2 mg; biotin, 0.25 mg; ascorbic acid, 75 mg; choline, 175 mg; Mg, 35 mg; Fe, 40 mg; I, 2 mg; Co, 0.5 mg; Se, 0.2 mg.
[4] Inorganic mineral premix provided per kilogram of diet; Mn, 80 mg; Zn, 60 mg; Cu, 5 mg; Cr, 0.15 mg.
[5] Organic mineral premix provided per kilogram of diet; Mn, 80 mg; Zn, 60 mg; Cu, 5 mg; Cr, 0.15 mg.

Production, egg quality and hatchability parameters: The body weight of the hens was individually measured at the beginning and at the end of the experiment and body weight gain was calculated. Egg production, damaged eggs and hen deaths were recorded daily. Feed intake and egg weight were determined fortnightly. Egg mass was calculated from laying rate and egg weight (egg production (hen-day, %) x egg weight (g) / 100), and feed conversion was determined from feed intake and egg mass (feed intake (g/hen/day) / egg mass (g/hen/day)). Thirty-six eggs from each group were collected once fortnightly, and egg quality characteristics were determined 24 h after collection of the eggs. Egg shape index was determined by the equipment that measures the width: length ratio as a percentage. Eggshell rate was calculated from eggshell and egg weight (eggshell weight (g)/egg weight (g) x100). Eggshell thickness was measured after peeling off the membrane under the shell with Mitutoyo digital micrometer (digital 395 series with 0.001 mm sensitivity, Kawasaki, Japan) on three points at the equatorial region of the egg, and expressed as an average value. Eggshell breaking strength and haugh unit were measured by using Futura 3/A egg quality measuring system (Futura, Lohne, Germany). Hens were artificially inseminated at 60 weeks of age. Eggs were collected for 1 week from each subgroup 2 days after insemination. Sixty eggs from each subgroup were incubated. Hatching and fertilised egg rates, hatchability of fertilised eggs and hatchability were determined at the Poultry Research Station Hatchery.

Statistical analysis: The results of all experiments were analysed statistically using the analysis of variance procedures of the statistical program MİNİTAB Release 14 and the means were compared for significance by Duncan's multiple range test (7) at $P < 0.05$.

Results

The effects of Mn, Zn, Cu and Cr supplementation to diet in different forms and levels on the performance of laying breeder hens are shown in Table 2. No differences in livability, body weight gain, egg production, egg weight, egg mass, feed intake and feed conversion were observed among the experimental groups ($P > 0.05$).

The egg quality characteristics are presented in Table 3. No significant differences in damaged egg rate, shape index, eggshell rate, eggshell thickness, albumen height and haugh unit were observed among groups ($P > 0.05$). The low level of trace minerals (40, 30, 2.5 and 0.07 mg/kg Mn, Zn, Cu and Cr, respectively) significantly reduced eggshell breaking strength ($P < 0.05$). However, the form of trace minerals did not affect eggshell breaking strength.

The hatching characteristics are presented in Table 4. The supplementation of Mn, Zn, Cu and Cr to the diet in different forms and levels did not significantly affect the hatching and fertilised egg rates. However, the organic form of these trace minerals significantly increased the hatchability of fertilised egg and hatchability compared to their inorganic form; and the high level of trace minerals significantly increased the hatchability according to their low level ($P < 0.05$). Interaction between trace mineral form × level in terms of hatchability of fertilised egg and hatchability was statistically relevant. The supplementation of the organic Mn, Zn, Cu and Cr at high level increased the hatchability of fertilised eggs and hatchability according to the other groups ($P < 0.05$).

Discussion and Conclusion

The supplementation of inorganic or organic forms of trace minerals (Mn, Zn, Cu and Cr) to the diet at different levels did not affect the performance of laying hens such as livability, body weight gain, egg production, egg weight, feed intake and feed conversion, which is similar to the findings of previous studies (6, 8, 24, 31). Contrary to the present study, organic Mn, Zn and Cu resulted in an increase in egg weight when compared to their inorganic form at 72 to 80 weeks of age (20). In

Table 2. The effects of Mn, Zn, Cu and Cr supplementation to diet in different forms and levels on performance of laying breeder hens.
Tablo 2. Yeme farklı form ve seviyelerde Mn, Zn, Cu ve Cr ilavesinin yumurtacı damızlık tavukların performansı üzerine etkileri.

Treatments	Livability (%)	Body weight gain (g/hen)	Egg production (%/hen/day)	Egg weight (g/egg)	Egg mass (g/hen/day)	Feed intake (g/hen/day)	Feed conversion (g feed/g egg)
Inorganic+high	98.9±1.1	31.3±15.5	76.4±1.1	59.3±0.5	45.3±0.6	112.0±0.6	2.48±0.04
Inorganic+low	98.9±1.1	37.7±16.5	76.6±1.3	59.4±0.5	45.5±0.8	110.8±1.4	2.44±0.04
Organic+high	100±0.0	40.7±12.9	76.9±1.2	59.7±0.2	45.9±0.8	112.6±1.2	2.46±0.03
Organic+low	98.9±1.1	46.0±7.31	76.7±0.9	59.4±0.5	45.6±0.6	110.7±1.1	2.43±0.03
Trace mineral form							
Inorganic	98.9±0.8	34.5±10.8	76.5±0.8	59.4±0.4	45.4±0.5	111.4±0.7	2.46±0.03
Organic	99.4±0.6	43.3±7.12	76.7±0.7	59.6±0.3	45.7±0.5	111.6±0.8	2.44±0.02
P	0.570	0.521	0.82	0.64	0.65	0.837	0.719
Trace mineral level							
High	99.4±0.6	36.0±9.7	76.6±0.8	59.5±0.3	45.6±0.5	112.29±0.6	2.47±0.03
Low	98.9±0.8	41.8±8.7	76.6±0.8	59.4±0.3	45.5±0.5	110.72±0.8	2.44±0.03
P	0.570	0.671	0.97	0.83	0.89	0.160	0.421
Trace mineral form x level interaction							
P	0.570	0.971	0.872	0.661	0.725	0.766	0.857

Table 3. The effects of Mn, Zn, Cu and Cr supplementation to diet in different forms and levels on egg quality characteristics.
Tablo 3. Yeme farklı form ve seviyelerde Mn, Zn, Cu ve Cr ilavesinin yumurta kalite özellikleri üzerine etkileri.

Treatments	Damaged egg rate (%)	Shape index (%)	Eggshell rate (%/egg)	Eggshell thickness (10^{-2} mm)	Eggshell strength (Newton)	Albumen height (mm)	Haugh unit
Inorganic+high	5.5±0.6	77.1±0.1	8.94±0.08	30.8±0.2	34.4±0.5	6.6±0.08	81.0±0.6
Inorganic+low	5.0±0.4	77.1±0.2	9.00±0.06	30.9±0.2	33.9±0.4	6.5±0.09	80.0±0.7
Organic+high	4.5±0.4	77.1±0.1	8.96±0.06	30.7±0.2	35.0±0.4	6.6±0.08	80.9±0.5
Organic+low	5.0±0.2	77.3±0.2	8.93±0.06	30.5±0.2	33.4±0.4	6.5±0.07	80.5±0.4
Trace mineral form							
Inorganic	5.3±0.4	77.1±0.1	8.97±0.05	30.8±0.1	34.2±0.3	6.5±0.06	80.5±0.4
Organic	4.8±0.2	77.2±0.1	8.95±0.04	30.6±0.1	34.2±0.3	6.6±0.05	80.7±0.3
P	0.306	0.601	0.742	0.248	0.945	0.634	0.702
Trace mineral level							
High	5.0±0.4	77.1±0.1	8.95±0.05	30.8±0.1	34.7±0.3 [a]	6.6±0.06	80.9±0.4
Low	5.0±0.2	77.2±0.1	8.96±0.05	30.7±0.1	33.7±0.3 [b]	6.5±0.06	80.3±0.4
P	0.929	0.305	0.846	0.608	0.013	0.253	0.236
Trace mineral form x level interaction							
P	0.270	0.692	0.494	0.348	0.145	0.728	0.621

[a,b]: means within columns with no common superscripts are significantly different (P<0.05).

Table 4. The effects of Mn, Zn, Cu and Cr supplementation to diet in different forms and levels on hatching characteristics.
Tablo 4. Yeme farklı form ve seviyelerde Mn, Zn, Cu ve Cr ilavesinin kuluçka özellikleri üzerine etkileri.

Treatments	Hatching egg (%)	Fertilized egg (%)	Hatchability of fertilized egg (%)	Hatchability (%)
Inorganic+high	85.4±3.0	96.3±1.4	82.6±1.1 [b]	79.5±2.0 [b]
Inorganic+low	89.6±1.2	93.8±0.8	83.5±0.8 [b]	78.4±1.5 [b]
Organic+high	88.0±1.5	96.3±1.1	89.1±1.1 [a]	85.9±1.4 [a]
Organic+low	88.6±2.2	93.5±1.5	84.1±2.1 [b]	78.6±1.0 [b]
Trace mineral form				
Inorganic	87.5±1.7	95.0±0.8	83.0±0.6 [b]	78.9±1.2 [b]
Organic	88.3±1.3	94.9±1.0	86.6±1.4 [a]	82.2±1.4 [a]
P	0.761	0.991	0.012	0.036
Trace mineral level				
High	86.7±1.7	96.3±0.8	85.9±1.2	82.7±1.5 [a]
Low	89.1±1.2	93.7±0.8	83.8±1.1	78.5±0.9 [b]
P	0.304	0.08	0.137	0.008
Trace mineral form x level interaction				
P	0.557	0.738	0.044	0.045

[a,b]: means within columns with no common superscripts are significantly different (P<0.05).

another study, an increase in the egg laying rate was observed in hens of 96 to 103 weeks of age that received organic Cu or Cu+Mn+Zn, and an enhancement in egg weight was observed when the hens received organic Zn (27). Şahin et al. (34) reported that body weight, feed conversion and egg production were increased in laying hens at low temperatures by providing supplemental dietary organic Cr (0.10, 0.20 and 0.40 mg/kg) ($P < 0.01$). However, feed intake and egg weight were not influenced by the supplementation with dietary organic Cr ($P > 0.05$).

Egg quality characteristics, except for eggshell breaking strength, were not significantly affected by the different forms and levels of Mn, Zn, Cu and Cr ($P > 0.05$) in the present study. In accordance with the findings of the present study, the supplementation of organic Zn, Mn and Cu to the diet did not affect the eggshell rate and thickness (20). In addition, egg shape index and eggshell thickness (8, 34) or haugh unit (8, 24) were not influenced by supplemental dietary organic Cr levels (0.10 to 0.80 mg/kg). Gheisari et al. (11) showed that a corn-soybean diet supplemented with the organic forms of Zn, Mn and Cu at a dosage 50 % to 75 % lower than available levels is sufficient to maintain performance and egg quality characteristics in laying hens. On the other hand, Maciel et al. (20) recorded that when compared with inorganic form, organic trace minerals (Zn+Mn+Cu) decreased the damaged egg rate.

Dietary supplementation of 80 mg/kg Zn (21) or 100 mg/kg Zn (3) as Zn-methionine reduced shell defects in hens exposed to high temperatures. However, Kita et al. (16) did not find any positive effects of adding a Zn-methionine supplement in their study.

In this study, eggshell breaking strength was not significantly affected by the different forms of Mn, Zn, Cu and Cr ($P > 0.05$). However, the low level of these minerals adversely affected the breaking strength ($P < 0.05$). Similarly, Mabe et al. (19) reported that, 30, 30 and 5 mg/kg of Zn, Mn and Cu, respectively, from the organic source had lower eggshell breaking strength than 60, 60 and 10 mg/kg at 69-82 weeks of age.

The low level of Mn, Zn, Cu and Cr significantly decreased hatchability compared with their high level ($P < 0.05$) in the present study. Richards and Steele (28) reported that trace mineral deficiencies have been found to cause impaired growth, abnormal development of all of the major organ systems and, death of the embryo. Organic form of these trace minerals supplied higher hatchability of fertilised eggs and hatchability according to their inorganic form. The high level of organic Mn, Zn, Cu and Cr resulted in higher hatchability of fertilised eggs and hatchability than the high or low levels of inorganic form and the low level of the organic form ($P < 0.05$). These results are compatible with the findings of Rutz et al. (30), who reported that the supplementation of organic Mn and Zn (30 and 30 mg/kg) to broiler breeder hen diets increased the fertility and hatchability, and decreased embryo death according to the high level (100 mg/kg) of their inorganic forms. In a study of Favero et al. (9), the supplementation of organic or inorganic Zn, Mn and Cu at high levels (100, 100 and 10 mg/kg, respectively) to the control group decreased early embryo mortality in broiler breeder hens at 22-68 weeks of age ($P < 0.01$). On the other hand, Kidd et al. (15) reported that inorganic or organic Zn supplementation (80 mg/kg) to control diet statistically did not affect these parameters at 41-62 weeks of age.

According to the present hatchability results, organic trace minerals have greater bioavailability than their inorganic forms because, during embryogenesis, the developing embryo requires the trace minerals stored in the yolk to be transferred to developing tissues (29). The concentration of the trace minerals deposited in the hen's egg is dependent on the form and the amount of the mineral in the diet of the hen (33). The age of the hen and environmental conditions also influences the mineral content of the eggs (5).

In conclusion, the performance and egg quality characteristics of laying breeder hens were unaffected, except for the eggshell breaking strength, using various levels or forms of the dietary Mn, Zn, Cu and Cr minerals. The dietary supplementation of organic Mn, Zn, Cu and Cr at 80, 60, 5 and 0.15 mg/kg, respectively, had positive effects on hatching performance.

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Geliş tarihi: 13.12.2013 / Kabul tarihi: 05.06.2014

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