

Effects of Supplementary Mineral Amino Acid Chelate (ZnAA - MnAA) on the Laying Performance, Egg Quality and Some Blood Parameters of Late Laying Period Layer Hens

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Abstract

This study was carried out to determine the effect of supplementary mineral amino acid chelate (ZnAA - MnAA) on the performance, egg quality and some blood parameters of late laying period layer hens. A total of 320, 64 wk of age Lohmann LSL laying hens, were divided into 4 groups with four replicates with 20 hen within each group. The control group was fed with basal diet and treatment groups were fed basal diet containing 1, 2 and 4 g/kg mineral amino acid chelate (ZnAA - MnAA) for 12 weeks. At the end of the experiment, there were no statistically difference among the groups in live weight, feed intake, feed conversion ratio, damaged egg ratio, mortality ($P>0.05$). Egg weight ($P<0.01$), albumen weight ($P<0.01$), shell weight ($P<0.05$), yolk index ($P<0.01$), yolk ratio ($P<0.01$), albumen ratio ($P<0.01$), and shell ratio ($P<0.05$) were found significant. The dietary supplementation of amino acid chelate (ZnAA - MnAA) were not significantly affected serum total protein, glucose, total cholesterol, Calcium (Ca), Phosphorus (P), Zinc (Zn) ($P>0.05$). The only statistical difference was found on the plumage score of the back region of hens ($P<0.05$). Also plumage coloring difference was statistically significant among groups ($P<0.01$). In conclusion, the supplementation of 1 g/kg mineral amino acid chelate (ZnAA-Mn AA) increased egg weight and had favourable effects on interior and exterior egg quality of laying hens in the late laying period.

Keywords: Chelate, Egg production, Egg quality, Mn-Aminoacid, Zn-Aminoacid

Geç Yumurtlama Dönemindeki Yumurtacı Tavuk Yemlerine Mineral Aminoasit Şelat (ZnAA - MnAA) Katkısının Performans, Yumurta Kalitesi ve Bazı Kan Parametreleri Üzerine Etkileri

Özet

Bu çalışma geç yumurtlama dönemindeki yumurtacı tavukların yemlerine mineral aminoasit şelat (ZnAA - MnAA) katkısının performans, yumurta kalitesi ve bazı kan parametreleri üzerine etkisini belirlemek amacıyla yapılmıştır. Çalışmada 320 adet 64 haftalık yaşta lohmann LSL beyaz yumurtacı tavuk 1 kontrol ve 3 uygulama grubu olacak şekilde 4 gruba ve her grup 20 tavuk içeren 4 tekrerrür içermektedir. Kontrol grubu bazal rasyon ile, uygulama grupları ise 1, 2 ve 4 g/kg mineral amino asit şelat (ZnAA - MnAA) içeren bazal rasyonla 12 hafta beslenmiştir. Araştırma sonunda, canlı ağırlık, yem tüketimi, yemden yararlanma oranı, hasarlı yumurta oranı, ölüm oranı bakımından gruplar arasında istatistik açıdan bir farklılık görülmemiştir ($P>0.05$). Yumurta ağırlığı ($P<0.01$), ak ağırlığı ($P<0.01$), kabuk ağırlığı ($P<0.05$), sarı indeksi ($P<0.01$), sarı oranı ($P<0.01$), ak oranı ($P<0.01$), kabuk oranı ($P<0.05$) değerleri istatistik açıdan önemli bulunmuştur. Rasyona mineral aminoasit şelat (ZnAA - MnAA) ilavesi grupların kan serumu toplam protein, glukoz, toplam kolesterol, Kalsiyum (Ca), Fosfor (P), Çinko (Zn) değerlerini istatistik açıdan etkilememiştir ($P>0.05$). Tüy skorlaması bakımından gruplar arasında farklılık sadece sırt bölgesi tüylerinde istatistik açıdan önemli bulunmuştur ($P<0.05$). Ayrıca gruplar arasında tüy renklenmesi de istatistik açıdan önemli bulunmuştur ($P<0.01$). Sonuç olarak, geç yumurtlama dönemindeki tavuk rasyonuna 1 g/kg mineral amino asit şelat katkısı (ZnAA-Mn AA) yumurta ağırlığını artırmış ve yumurta iç ve dış kalite özellikleri üzerine olumlu etkileri bulunmuştur.

Anahtar sözcükler: Mn-Aminoasit, Şelat, Yumurta kalitesi, Yumurta verimi, Zn-Aminoasit

INTRODUCTION

The drastic changes in egg quality is observed with the increasing flock age. As the hen ages, the thickness of the

shell usually declines. Older flocks lay larger eggs, which break easily ^[1]. Maintaining eggshell quality is a complex activity, it is impossible, even with current knowledge, to correct all eggshell quality problems. Most of the scientific



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papers main topic is the improving the production and quality of eggs specially late laying period [2,3]. It has been suggested that part of losses in egg production are directly connected with low eggshell quality [4] and also the internal quality of eggs is very important for the consumers [5].

Traditionally trace minerals have been supplemented to animal diets as inorganic salts. However, because of their preintestinal absorption and after absorption in cellular metabolism due to higher bioavailability when compared with the same minerals in inorganic form, in recent years organic minerals have began to gain importance [6,7]. The term "organic mineral" is used to denote minerals forms chelated to an organic molecule, with the intention of increasing mineral bioavailability in animal diets [8].

Zn and Manganese are the main trace minerals involved in the metabolic process of eggshell formation. Zn plays an important role in poultry, particularly for layers, as a component of a number of metalloenzymes such as carbonic anhydrase which is essential for eggshell formation in the hens shell gland [9]. The Zn deficiency symptoms are suppressed immune system, poor feathering and dermatitis, infertility and poor shell quality in poultry. Deficiency of manganese could perosis, bone shortening [9] and increases incidence of thin-shelled and shell-less eggs in laying hens [10]. Therefore, supplementing the diet with highly bioavailable minerals like trace elements mineral-amino acid chelated or complexes were expected to increase the eggshell quality [2]. Researchers are reported that amino acids in the organic-mineral chelate are also used as a source of amino acid [11,12].

The trace minerals have been supplemented to animal diets as inorganic salts. In recent years, there has been studies on use of chelated or organic form of trace minerals in animal diets. This is because use of organic trace minerals in animal diets is improved growth, reproduction and health in ruminants, broilers and laying hens [13]. Lima *et al.* [14] reported that supplementation of Zn-Met chelate in single or in combination with Mn-Met chelate increased specific gravity of egg, eggshell strength and eggshell thickness. Park *et al.* [15] and Hudson *et al.* [16], in excess, Zn has detrimental effect on egg production, affecting the absorption of nutrients, and causing lesions in the pancreas and gizzard of laying hens. Abnormal accumulation of Zn in the liver, result with decrease in plasma Zn concentration [17].

Feather cover is important for its insulation value and protection from scratches and injury to the hen's skin. Severe feather pecking has been demonstrated in hens that were fed with low mineral, protein or amino acid level in the diet [18]. Increasing the dietary protein and amino acid contents resulted in improved plumage score [19].

Although many studies have demonstrated benefits of metal amino chelate on animal metabolism, but the

research on their effects on late laying hen performance is limited. This study was conducted to determine the effect of supplementary mineral amino acid chelate (ZnAA - MnAA) on the performance, egg quality and some blood parameters of laying hens in the late laying period.

MATERIAL and METHODS

The experiment was carried out on 320 hens of Lohmann LSL white laying strain at the age of 64 weeks and experiment was conducted during 12 week. Before the experiment hens were placed cages for adaptation and controlled their egg production values during two week.

The hens were weighed on a digital scale and randomly assigned into 4 dietary treatment groups which are all reared at first floor of the battery cages. One control (0 g/kg) and three levels of L1 (1 g/kg), L2 (2 g/kg) and L3 (4 g/kg) mineral amino acid chelate containing diet were used to feed the hens in each group (n=80). The size of each cage was 50x45x45 cm. Each treatment group consisted of 4 replicates in 4 different cages (5 birds per cage).

All experimental procedures were in accordance with established standards for the care and use of animals for research purposes. The experiment was conducted under the protocol which was approved by Uludağ University Animal Use Local Ethical Committee (No: 2014-07-01).

Management

In the study a standard commercial layer diet was used for a basal diet (16% CP and 2.700 ME kcal/kg). The diet was formulated to meet or exceed National Research Council [20] specifications. The chemical composition of the basal diet fed to the hens were given in *Table 1*. The control group (Control) was feed with a basal diet and other groups were fed with basal diet supplemented with 1 (L1) g/kg, 2 (L2) g/kg and 4 (L3) g/kg mineral amino acid chelate (5.000 mg/kg Zn Amino acid - ZnAA, 3.500 mg/kg Mn Amino acid-MnAA and 85% CaCO₃ as carriers). During to trial, feed and water were offered adlibitum to all hens. The hens were placed into the cages and kept under 16L:8D light. The environmental conditions were the same for all groups.

Data

The laying hens were weighed individually with a digital scale (± 1 g) at the beginning and at the end of the experiment. Eggs from each cage were collected 1 times a day. Egg production as hen day (EP), number of damaged eggs (cracked and soft-shelled eggs), mortality were monitored daily. Feed intake (FI) and egg weight (EW) were recorded on weekly basis. Based on the collected data, the basic production parameters (egg production, feed conversion ratio, and feed intake) were calculated. Egg mass (EM) was calculated with formula as $EM = (EP * EW) / 100$. Feed conversion ratio (FCR) was calculated with

Table 1. Chemical composition of diet used in the experiment**Tablo 1.** Denemede kullanılan rasyonun kimyasal kompozisyonu

Ingredient,%	Layer Diet
Ground corn	64.74
Soybean meal (46% CP)	25.03
Dicalcium phosphate	1.42
Calcium carbonate	8.12
DL-methionine	0.02
NaCl	0.37
Vitamin and Trace mineral premix*	0.30
Chemical Composition	
Crude protein,%	16.0
ME, kcal/ kg	2700
Ca,%	3.00
Phosphorus,%	0.70
Metionin,%	0.33
Lysine,%	0.70
Cystine,%	0.31
* Ingredients in 1 kg of premix: Vitamin A 8.000 IU; Vitamin D ₃ 2.000 IU; Vitamin B ₂ 4 mg; Vitamin B ₁₂ 10 mg; Vitamin E 15 mg; Vitamin K ₃ 2 mg; Vitamin B ₁ 3 mg; Niacin 30 mg; Cal-D-pantothenic acid 10 mg; Vitamin B ₆ 5 mg; Folic acid 1 mg; D-biotin 0.05 mg; Vitamin C 50 mg; Choline Chloride 300 mg; Mn 60 mg; Zn 50 mg; Fe 60 mg; Cu 5 mg; Co 0.5 mg; Iodine 2 mg; Se 0.15 mg	

formula as $FCR = FI/EM$. The FI and FCR were determined for each replicate weekly.

Egg quality was assessed at 2, 4, 8, and 12th week of the experiment, eighty eggs from each treatment group were randomly collected to determine the egg interior and exterior quality parameters. Subsequently the eggs were weighed then broken, and the yolks were separated from the albumen. The chalazae were carefully removed from the yolk, using forceps, prior to weighing the yolk. The shells were carefully washed and dried 24 h in a drying oven at 105°C then weighed. Albumen weight was calculated by subtracting yolk and shell weights from the original egg weight. Eggshell thickness was measured at three different egg points (air cell, sharp end, and any side of the equator) using a caliper. An average of three different thickness measurements from each egg was used to estimate the eggshell thickness. The weights of egg components and shell thickness were measured to the precision of 0.01 g or 0.01 mm, respectively. The data of egg weight, yolk weight, shell weight (g) were recorded using digital scale.

The egg shape index (%) was determined by equipment developed by Rauch and egg shell strength (kg/cm²) was measured by special equipment. Egg yolk diameter, albumen length, albumen width (mm) were measured with digital caliper.

The albumen and yolk height (mm) were measure using tripod micrometer. The proportion of eggshell,

albumen and yolk were calculated as (shell or albumen or yolk weight/egg weight) x 100). Egg yolk index was calculated as (yolk height/yolk diameter) x100. Albumen index was calculated as (albumen height/(albumen length + albumen width)/2)) x 100. The Haugh unit (HU) was calculated by the formula $HU = 100 \text{ Log } (H + 7.57 - 1.7W^{0.37})$, where W refers to measurements of egg weight (g) and H refers to albumen height (mm). The egg yolk color was measure using color fans [21].

At the end of the treatment blood samples were taken from nine hen from each treatment group for determination of serum total protein, glucose, total cholesterol, Ca, P and Zn level. Blood samples were taken from the wing vein and immediately centrifuged at 3.000 rpm for 15 min and the serum was removed in vacutainer tubes [2]. Serum levels of total protein, glucose, total cholesterol, Ca and P were determined by use of an Roche autoanalyser (Cobas 6000 series C501 module, Roche Diagnostic, Indianapolis, IN, USA) and Roche kits used. The serum Zn level was determined by use of atomic absorption spectrophotometer (Atomic Absorption Spectrophotometer AAnalyst 300, PerkinElmer, Shelton, CT, USA).

At the end of the study randomly selected 40 hens from each treatment group was observed for plumage condition and scored. Plumage condition was scored using a 4 point scoring system [22] for 6 different areas of the body (neck, breast, back, wings, tail, and vent). A score of 4 indicated very good feathering with few worn or otherwise deformed feathers. Score 3 was used when feathers showed deterioration but when complete feather coverage was observed. Score 2 indicated areas of the body that showed marked deterioration with some part being denuded. Score 1 indicated areas with little or no feather coverage and when feathers were present they were severely damaged.

The average plumage condition for each bird was calculated by adding over all 6 areas, to yield a total score ranging from 6 to 24 points. Also same person did a macroscopical subjective scoring of the melanin coloration of feathers each individual hen at the end of the trial, giving a score between 3 - point scoring system for colored feathers of the body (back). A score 3 was used when feathers intensive coloured with light grey-dirty white colour. Score 2 was used when feathers few coloured with light grey-dirty white colour. Score 1 indicated no any coloured feather on hen body.

Statistical Analysis

The parametric data (live weight, feed intake, egg production, egg weight, egg mass, damaged egg ratio, FCR, egg quality traits, blood plasma values) were analysed by using PROC GLM procedure of statistical analysis software (SAS v9.4) [23]. The non-parametric (Plumage score) data were analyzed with Wilcoxon scores (Rank Sums) test

with using PROC NPAR1WAY procedure of SAS 9.4 and Kruskal Wallis test was used to determine the differences among groups [24]. Differences were considered significant at $P \leq 0.05$. Mortality data were analyzed with using PROC FREQ procedure and Chi-Square test was used to determine the differences among groups. All statistical analyzes were done with using SAS v9.4 [23].

RESULTS

The mean live weight, feed intake, hen day egg production, egg mass, feed conversion, damaged egg ratio and mortality ratio of treatment groups were shown

in Table 2. There were no statistically differences among the groups in live weight, feed intake, FCR, damaged egg ratio, and mortality ($P > 0.05$). The difference in hen day egg production, egg weight and egg mass were statistically significant ($P < 0.01$).

The mean egg interior and exterior quality of treatment groups were given in Table 3. The highest yolk index was found in control group ($P < 0.01$). The egg weight ($P < 0.01$), albumen weight ($P < 0.01$), shell weight ($P < 0.05$), yolk ratio ($P < 0.01$), albumen ratio ($P < 0.01$), and shell ratio ($P < 0.05$) were found significant in the treatment groups.

The mean blood plasma total protein, glucose, total

Table 2. Mean live weight, egg production, feed intake, egg mass, FCR and mortality values of groups (n=80 hen)

Tablo 2. Grupların ortalama canlı ağırlık, yumurta üretimi, yem tüketimi, yumurta kütlesi, YYO ve ölüm oranı değerleri (n=80 tavuk)

Traits	Diets ¹				P
	Control	L1	L2	L3	
Initial live weight, kg	1.74±0.04	1.81±0.05	1.77±0.03	1.83±0.04	n.s.
Final live weight, kg	1.88±0.04	1.86±0.04	1.82±0.03	1.87±0.05	n.s.
FI, g/d/hen	131.81±5.14	126.99±3.52	126.18±3.69	126.47±3.47	n.s.
EP, %	70.71±1.13 ^a	71.42±0.63 ^a	66.47±1.01 ^b	66.64±0.98 ^b	**
EM, g/d/hen	48.41±0.92 ^{ab}	51.00±0.46 ^a	45.35±0.73 ^c	46.49±0.72 ^{bc}	**
FCR	2.75±0.13	2.50±0.08	2.80±0.10	2.73±0.09	n.s.
Damaged Egg Ratio, %	3.02±0.67	2.66±0.12	3.20±0.74	2.32±0.31	n.s.
Mortality, %	5	5	0	2.5	n.s.

^{a-c} within column, values with different superscript letters differ significantly ($P < 0.01$); ** $P < 0.01$; n.s.: not significant; ¹ Control: Fed with basal diet, L1, L2, and L3: Fed with basal diet containing 1, 2 and 4 g/kg mineral amino acid chelate (Zn Amino acid - Mn Amino acid; ZnAA - MnAA), respectively; EP: Heday egg production, FI: Feed intake, EM: Egg mass, FCR: Feed conversion ratio, FCR= FI/EM

Table 3. Mean interior and exterior egg quality trait values of groups (n=80 egg)

Tablo 3. Grupların ortalama yumurta iç ve dış kalite özellik değerleri (n=80 yumurta)

Traits	Diets ¹				P
	Control	L1	L2	L3	
Egg weight, g	68.71±0.43 ^b	70.96±0.61 ^a	68.61±0.56 ^b	69.38±0.59 ^{ab}	**
Yolk weight, g	19.16±0.16	18.92±0.16	18.69±0.14	19.07±0.18	n.s.
Albumen weight, g	43.45±0.38 ^b	45.78±0.48 ^a	43.82±0.46 ^b	44.35±0.47 ^{ab}	**
Shell weight, g	6.09±0.08 ^{ab}	6.26±0.07 ^a	6.10±0.06 ^{ab}	5.97±0.07 ^b	*
Shell Thickness, mm	0.3774±0.00	0.3776±0.00	0.3763±0.00	0.3706±0.01	n.s.
Shell breaking strength, kg/cm ²	0.85±0.09	0.78±0.07	0.88±0.07	0.71±0.08	n.s.
Shape index, %	75.38±0.25	75.08±0.28	74.44±0.27	74.81±0.28	n.s.
Albumen Index, %	7.61±0.15	7.58±0.19	7.66±0.17	7.54±0.16	n.s.
Yolk Index, %	45.07±0.29 ^a	43.82±0.36 ^b	43.48±0.34 ^b	43.56±0.31 ^b	**
Haugh Unit	75.80±0.78	75.55±1.04	76.74±0.89	76.27±0.88	n.s.
Yolk Color	12.08±0.17	11.65±0.15	11.91±0.13	11.66±0.16	n.s.
Yolk Ratio, %	27.94±0.25 ^a	26.72±0.21 ^b	27.30±0.18 ^{ab}	27.53±0.21 ^a	**
Albumen Ratio, %	63.19±0.26 ^b	64.45±0.22 ^a	63.80±0.20 ^{ab}	63.85±0.23 ^{ab}	**
Shell Ratio, %	8.87±0.10 ^{ab}	8.83±0.06 ^{ab}	8.91±0.07 ^a	8.61±0.08 ^b	*

^{a-c} within column, values with different superscript letters differ significantly ($P < 0.05$, $P < 0.01$); * $P < 0.05$; ** $P < 0.01$; n.s.: not significant; ¹ Control: Fed with basal diet, L1, L2, and L3: Fed with basal diet containing 1, 2 and 4 g/kg mineral amino acid chelate (Zn Amino acid - Mn Amino acid; ZnAA - MnAA), respectively

cholesterol, Serum Ca, P and Zn values of groups were given in *Table 4*. The dietary supplementation of amino acid chelate (ZnAA - MnAA) were not significantly affected plasma total protein, glucose, total cholesterol, Ca, P, Zn ($P>0.05$).

The mean plumage score values of six regions of hen body and plumage coloring were given in *Table 5*. The only back region plumage score of hens was found significant ($P<0.05$). Plumage coloring was greater in all treatment groups and this difference was statistically significant ($P<0.01$). But numerically highest plumage coloring was found in 4 gr/kg mineral amino acid chelate (ZnAA-Mn AA) supplemented group (L3) and there was not any plumage coloring in control group.

DISCUSSION

Recently, organic trace minerals and especially mineral amino acid complex or chelate have become the focus of attention for the role in the high quality egg production of layers and breeders [11,25,26]. The organic trace minerals have a greater bioavailability compared with inorganic trace minerals. This availability caused increased solubility and decreased interaction with other nutrients during absorption in gastrointestinal tract [27]. However mineral amino acid chelate should be supplemented to diet of

layer hens for the optimal performance, in the present study supplementation of ZnAA - Mn-AA chelate on diet did not significantly affect of groups live weight, feed intake, FCR, damaged egg ratio, and mortality ($P>0.05$). Rossi *et al.* [28] reported that increased level of organic Zn (0, 15, 30, 45 and 60 ppm) supplementation had no effect on live weight, feed intake, FCR and mortality in broilers which was similar to the results of our study. According to those authors, lack of consistent effects of dietary Zn on performance of birds may be due to the amount of Zn present in the basal diet or to the amount and sources such as phytate, which forms insoluble complexes with Zn and prevents its absorption, added. On the other hand, Ferket *et al.* [29] reported that 20 and 40 ppm Zn and Manganese-methionine supplementation to turkey diets improved FCR and also reduced mortality when compared to 80 ppm zinc and 120 ppm manganese as sulfates. The percentage of cracked egg was reduced by supplementation of Zn and manganese chelate to the layer diets depend on the significant effects of Zn and Manganese on carbonic anhydrase activity levels which is essential for eggshell formation, in the shell gland of laying hens [11].

In the present study, supplementation of ZnAA - MnAA on diet significantly affect hen day egg production and egg mass of treatment groups ($P<0.01$). It has been reported that the egg production [30] and egg weight [31]

Table 4. Blood plasma values of groups (n=9 hen)

Tablo 4. Grupların kan plazma değerleri (n=9 tavuk)

Traits	Diets ¹				P
	Control	L1	L2	L3	
Total Protein, g/dL	5.12±0.16	5.53±0.15	5.03±0.07	5.50±0.27	n.s.
Glucose, mg/dL	208.70±7.97	230.27±8.55	237.67±13.30	233.87±3.53	n.s.
Total Cholesterol, mg/dL	131.03±13.05	173.23±21.92	102.10±16.86	181.73±31.30	n.s.
Ca, mg/dL	25.83±0.62	28.73±0.77	23.80±1.73	28.30±1.74	n.s.
P, mg/dL	4.03±0.17	5.77±0.03	5.27±0.82	5.60±0.76	n.s.
Zn, µg/dL	421.67±41.47	423.33±13.64	496.67±22.31	398.33±14.53	n.s.

n.s.: not significant; ¹ Control: Fed with basal diet, L1, L2, and L3: Fed with basal diet containing 1, 2 and 4 g/kg mineral amino acid chelate (Zn Amino acid - Mn Amino acid; ZnAA - MnAA), respectively

Table 5. Mean plumage score and plumage coloring values of groups (n=40 hen)

Tablo 5. Grupların ortalama tüy scoru ve tüy renklenme değerleri (n=40 tavuk)

Diets ¹	Body Region						Total	Plumage Coloring
	Neck	Breast	Vent	Back	Wings	Tail		
Control	2.30±0.15	2.40±0.18	2.25±0.16	2.30±0.31 ^b	2.80±0.17	2.10±0.12	14.15±0.71	1.00±0.00 ^b
L1	2.40±0.15	2.50±0.18	2.30±0.15	2.90±0.18 ^{ab}	2.60±0.11	1.90±0.07	14.60±0.60	2.50±0.11 ^a
L2	2.40±0.15	2.50±0.15	2.40±0.18	3.50±0.19 ^a	2.40±0.11	1.90±0.07	15.10±0.54	2.50±0.15 ^a
L3	2.60±0.21	2.80±0.17	2.60±0.18	3.20±0.23 ^{ab}	2.70±0.15	2.20±0.14	16.10±0.75	2.70±0.12 ^a
P	n.s.	n.s.	n.s.	*	n.s.	n.s.	n.s.	**

^{a,b} within row, values with different superscript letters differ significantly ($P<0.05$, $P<0.01$); * $P<0.05$; ** $P<0.01$; n.s.: not significant; Plumage scoring: 1 no feather - 4 feathered; Plumage coloring: 1 no color- 3 colored; ¹ Control: Fed with basal diet, L1, L2, and L3: Fed with basal diet containing 1, 2 and 4 g/kg mineral amino acid chelate (Zn Amino acid - Mn Amino acid; ZnAA - MnAA), respectively

were increased with supplementation of layers diets with organic form of Mn and Zn replacing inorganic forms of minerals, depend on higher bioavailability of organic form of minerals. And also, Xavier *et al.*^[32] verified beneficial effects on the performance and egg quality of brown layers during the second laying cycle with the use of organic selenium, Zn, and manganese combinations than inorganic forms. In contrast to our findings some researchers have found that supplementation of eggshell-49 (contained organic Mn and Zn chelate) did not affect performance of layers^[14,33]. Mineral depended results were also reported by Lim and Paik^[2] who have been found that egg production was increased by Cu-Met chelate supplementation but decreased by Zn-Met chelate supplementation. According to authors it might be due to negative interactions between minerals, such as; Zn has a strong antagonism with Cu. Also Bülbül *et al.*^[34] found that both organic and inorganic sources of Zn and Mn decreased the oxidative stress in the laying hens, whereas organic Cu source increased it.

Park *et al.*^[15] observed that at high dietary concentrations, Zn can reduce the use of calcium by the hen, because this element would be the first limiting factor for ovulation. In the present study better results in 1 mg/kg ZnAA - MnAA chelate group (L1) might be a result of sufficient requirement need may be met to the hens at this level. On the other hand, the similar results at higher levels of Zn in mineral amino acid chelate might be a result of disruption of feed ingredients in the digestive organs. Thus, Hudson *et al.*^[16] observed that high level Zn supplementation to diets had detrimental effects on egg production affecting absorption of nutrients and causing lesions in the pancreas and gizzard of laying hens.

Deficiency of manganese increases incidence of thin shelled eggs^[10]. The use of organic complexes of Zn and Mn could alleviate the negative effect of hen age on eggshell breaking strength^[35]. However improvement of eggshell quality was expected by supplementation of Zn or Mn in diets. In the present study, the difference in egg weight ($P < 0.01$), albumen weight ($P < 0.01$), shell weight ($P < 0.05$), yolk ratio ($P < 0.01$), albumen ratio ($P < 0.01$), and shell ratio ($P < 0.05$) were found significant in the treatment groups. But egg shell thickness and shell breaking strength were similar to the control. A similar result have been reported by Nys *et al.*^[36] and Mabe *et al.*^[37] who have been observed that organic or inorganic Mn, Cu and Zn combinations supplemented with diets, did not affect egg shell quality. Zhao *et al.*^[25] found that basal diet supplemented with methionine trace mineral chelates improved egg quality and physiological function of laying hens, also yolk weight and proportion increased significantly. Ceylan and Scheideler^[11] found that Mn and Zn chelate supplementation to layer diets increased egg shell quality after 40 wks of age but, egg production and FCR did not affected by supplementation of Mn and Zn chelate. Lima *et al.*^[14] reported that supplementation of Zn-Met chelate in single

or in combination with Mn-Met chelate increased eggshell strength and eggshell thickness. However Lim and Paik^[2] found that supplementation of Zn-Met or its combination with other mineral chelates (Cu-Met, Mn-Met) had no beneficial effects on laying performance, but Zn-Mn-Met treatment showed significantly better eggshell strength than the control. Also Hudson *et al.*^[38] found that dietary Zn in high concentration reduced shell thickness of eggs of hens at 66 weeks of age.

The abnormal accumulation of Zn in the liver causes decrease in plasma Zn concentration^[17]. The plasma total protein was increased with Zn-Gly dietary supplementation in broilers^[39] and Zn proteinat in laying hens^[40]. Uyanik *et al.*^[41] indicated that Zn supplementation decreased serum cholesterol concentration of broilers and Abd-El-Samee^[42] of quails. However in the present study dietary supplementation of ZnAA - MnAA chelate were not significantly affected plasma total protein, glucose, total cholesterol, Ca, P, Zn level ($P > 0.05$). Our results are in agreement with the findings of Güçlü and İşcan^[43] who have been reported that organic Mn and Zn chelate supplementation did not affect live weight, feed intake, FCR, haugh unit and plasma Ca and P level. But in contrast to our findings organic Zn supplementation to broiler diets consistently increased plasma Ca levels, that show that interactive effects between Zn and Ca metabolism^[39,44]. Thus Dobrzanski *et al.*^[45] found that organic form of Fe and Mn supplementation to diet did not cause any significant changes in the content of Fe, Cu, Mn and Zn in blood. Also Aksu Sarıpinar *et al.*^[46] found that using at much lower level organic forms of minerals (Cu, Zn and Mn) in broiler diets instead of inorganic forms of those minerals did not created a negative impact on blood parameters.

Since feathers are 89-97% protein, dietary amino acids play a critical role in feather development. Severe feather pecking has been demonstrated in birds that were fed with low mineral, protein or amino acid levels in diet^[18]. In the present study only back region plumage score of hens was found significant ($P < 0.05$). The better results in treatment groups might be an effect of Zn and amino acid on feather development. However, increasing the dietary protein and amino acid contents resulted in improved plumage condition^[19]. Also supplementation of Zn had an effect on improved the feather score from poor to good^[47]. In contrast to these results, it has been reported that dietary amino acid or trace mineral treatment did not show any significant effects on feather scores^[12].

Studies have shown that there is a high level of correlation between the level of metal concentration in bird's diet and the level found in its feathers (melanic pigmentation)^[48,49]. Dobrzanski *et al.*^[45] found that organic form of Fe and Mn supplementation to diet resulted with significantly higher level of Mn concentration in feathers of hen than control group. Birds can eliminate heavy metals in their feathers. The darker portions of the

feather were enriched with Zn and Fe, which supports the suggestion that feather melanins are efficient ligands of some metals found in the environment, sequestering potentially harmful particles away from the body^[50]. In consistent with Chatelain *et al.*^[51] showed that darker individuals had higher Zn in their feathers and effectiveness of detoxification via melanic feathers may depend on level of metal intake. During to present study we have been observed that the hens white feather color was changed to grey-dirty white color. In the present study plumage coloring was greater in all treatment groups and this difference was statistically significant ($P < 0.01$). But numerically highest plumage coloring was found in L3 group while there was not any plumage coloring in control group. Indeed, high levels of mineral amino acid chelate (ZnAA - MnAA) supplementation may have been resulted toxic effects and performance loss and reduction in egg quality could be considered due to this situation.

Animals absorb, digest and use mineral chelate better than inorganic form of minerals. This means that lower concentrations of organic trace minerals can be used in animal feeds. In addition, animals fed chelated sources of essential trace minerals excrete lower amounts in their feces and so there is less environmental contamination. The results of the current study showed that, the supplementation of 1 g/kg mineral amino acid chelate (ZnAA - MnAA) to diet increased egg weight, egg production and had favourable effects on interior and exterior egg quality of laying hens in the late laying period.

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