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INFLUENCE OF ZINC CHELATED METHIONINE ADMINISTRATION ON SOME HEMATOLOGICAL, BIOCHEMICAL AND IMMUNOLOGICAL PARAMETERS CHANGES IN LAYING HENS

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ABSTRACT

Seventy-five layers of 37 weeks old, hens were housed in a completely enclosed, ventilated, conventional caged-layer house in which the lighting program was 16 hours' light: 8 hours dark (16L: 8D). The hens were fed on basal diet free from zinc additive for 3 weeks. The hens were divided into 3 groups at 40 weeks of age. The control group was fed with basal diet. The second group was fed basal diet containing 0.5 g/kg zinc chelated methionine (Zn-MHA) and the third group was fed over dose (2g Zn-MHA /kg) for 8 weeks. Five blood samples were collected from five hens of each group at the end of experiment. Each blood sample divided into three portions. The **1st** part of blood sample was collected with anticoagulant for RBCs, Hb, and differential leucocytic count. The 2nd part of blood sample was collected with anticoagulant to estimate antioxidative stress melanoldehyde, glutathione peroxidase, superoxide dismutase and carbonic anhydrase (lipid peroxide, melanolyhehyde (MDA), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and carbonic anhydrase (CA) respectively) in plasma. The 3rd part of blood sample was collected without anticoagulant for serum separation for measuring ALT, AST and alkaline phosphatase, IgA, calcium, phosphorus, cholesterol, LDL and HDL The present results revealed non-significant changes in hematological profiles including RBCs count, Hb content, WBCS count and differential leucocytic count**.** Liver enzymes showed significant decrease in ALT, AST and ALP in group 2, 3. Total protein, albumin, globulin, A/G ratio and Ig A revealed non-significant changes in all groups**.** Also serum Ca, P and Cholesterol, showed non-significant changes in all groups but LDL showed significant decrease in groups 2, 3, HDL showed significant increase in groups 2, 3 Vit A level showed significant increase in groups 2 and 3. In the same table oxidative enzymes revealed non-significant changes in MDA (melanolyhehyde) level but there were significant increase in GPx and SOD in groups 2, 3**.**

Exterior egg quality including Egg weight, Egg width, Egg length, Egg index, Shell weight, Shell thickness, Shell percentage showed non-significant changes except egg width revealed significant increase in group 2**.** Interior egg quality including Yolk weight, Yolk width, Yolk, Yolk index, Albumen weight, Albumen height, and Albumen width showed non-significant changes but yolk height and albumen percentage revealed significant increase in group 3. in the other hand yolk percentage and yolk cholesterol revealed significant decrease in group 3 and in groups 2,3 respectively. Weekly egg production showed significant increase in group 2 from the 2nd week till the end of treatment but group 3 showed significant increases from the 1st week to the 7th week then decrease in the 8th week. In conclusion, trace minerals (Zn) in the form of Zn-MHA chelates showed higher bioavailability and beneficial effects in compared with inorganic form when included in laying hens' diet.

INTRODUCTION

Zinc is a trace element that is necessary for normal growth, maintenance, bone development, feathering, and enzyme structure and function and appetite regulation for all avian species **(Batal *et al.,* 2001).** Zinc is the most common metal constituent of cellular enzymes and plays essential roles in cell proliferation, immune development and response, reproduction, gene regulation, and defense against oxidative stress and damage. Likely reflecting its role in gene regulation, zinc is required for the synthesis of two key structural proteins: keratin (the major structural protein of feathers, skin, beaks and claws) and collagen (the major structural protein of the extracellular matrix and connective tissues), **(Richards and Dibner, 2005).** Zinc is recognized as an essential mineral in erythropoiesis. Zinc plays particular catalystic role in the activity of alfa-aminolevunilic acid dehydratase which is responsible for hem synthesis **(Arcasoy 2002)**. Zinc deficiency has adverse effect on erythropoiesis in marrow **(Hughes *et al.,* 2006)** and a reduction of T and B lymphocyte production **(Haddad *et al.,* 2008)**. Zinc is a cofactor of the main antioxidative enzyme Cu Zn-superoxide dismutase; it may play a key role in suppressing free radicals and inhibiting NADPH-dependent lipid peroxidation **(Prasad, 1997)** as well as in preventing lipid peroxidation via inhibition of glutathione depletion. **(Gibbs *et al.,* 1985).** Zinc also plays an important role in egg shell and shell membrane formation because it is a co-factor and (or) structural products of enzyme systems responsible for carbonate formation and mucopolysaccharide synthesis. In recent years' organic minerals have begun to gain importance **(Aksu *et al.,* 2011, Trindade *et al.,* 2011).** 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 **(Torres 2013)**. Differences between organic and inorganic (chelate) sources of zinc in availability and effectiveness on poultry performance. The present work was conducted to investigate the potential effects of zinc chelated methionine on some hematological, biochemical and immunological changes in laying hens.

MATERIAL AND METHODS

Drug: Biofert is feed additive produced by sentovit company, USA and distributed in Egypt by Arab Trade Company, Benha. Biofert contains

Organic zinc chelates	Zn 15%
Methionine	MET 40%
Vitamin H	100 mg

Experimental design:

Chickens:-Seventy five layers of 37 weeks old, hens were housed in a completely enclosed, ventilated, conventional caged-layer house in which the lighting program was 16L: 8D. The hens were fed on basal diet free from zinc additives for 3weeks. The hens were divided into 3 groups at 40 weeks of age. The first group was kept as control group and fed with basal diet. The second group was fed basal diet containing 0.5 g/kg zinc chelated methionine (Zn-MHA) and the third group was fed over dose (2 g/kg) for 8 weeks.

Sampling:

Five blood samples were collected from wing vein of five birds from each group at the end of experiment. Each blood sample was divided into three portions. The 1st blood sample was collected with anticoagulant for RBCs, Hb, differential leucocytes count and phagocytosis, the 2nd blood sample was collected on anticoagulant for collection of plasma for estimation of antioxidant stress, malondialdehyde (MDA), Glutathione peroxidase (GPx), Superoxide dismutase (SOD) and Carbonic anhydrase CA and Vit A. The 3rd blood sample was collected without anticoagulant for serum separation for measuring ALT,AST and alkaline phosphatase, IgA, calcium, phosphorus, cholesterol, LDL and HDL, Total protein, and globulin.

Laboratory Examinations:

Hematological examinations: Blood samples were collected from all groups for RBCs count according to **(Coles, 1986).** Hb was calculated according to **(Polo *et al.* 1992)** Blood smear was stained with wright'stain for differential leucocytic count and absolute values were

calculated according to **(Campbell 1995)**.Lipid peroxidation was estimated in terms of thiobar-bituric acid reactive substances (TBARS), using malondialdehyde (MDA) as standard by the method of **(Beuge and Aust 1978).** Glutathione peroxidase (GPx) concentration was determined in samples according to the method of **(Mates *et al.* 2000)** and CA was estimated by method of **(Armstrong *et al*. 1966)**. Zn-SOD was determined by method described by **(Cavanagh *et al.* 1995)**. Blood was collected in. heparinized tubes; separated plasma was protected from light and stored at − 20°C, for Vit A estimation **(Nierenberg and Lester 1985)**. **Immunological parameter:**phagocytic assay according to **(Woldenhiwet and Rowan 1990).**

Biochemical analysis: Serum was obtained by centrifugation for Aspartate and alanine amino transferase (ALT and AST) activities were determined colorimetrically according to **(Reitman and Frankel 1957)**. Serum alkaline phosphatase activity was determined according to **(Kind and King 1954).** Serum calcium was measured spectrophotometrically at 565nm according to **(Baginski *et al.,* 1973)**. Serum inorganic phosphorus was measured spectrophotometrically at 625nm, according to the method described by **(Plummer 1978).** The IgA was measured using chicken IgA ELISA Quantitation (IgA ELISA Quantitation Kits, Bethyl Laboratories Inc., Montgomery, TX.

Egg quality determination:

Egg quality was assessed at the end of last 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, and 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 **(Bilgehan *et al.,* 2015)**. 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 measured 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 **(Solomon 1991 and Sarıca and Erensayin 2009) .** Cholesterol content of extracted yolk was determined by spectrophotometric method (Libermann-Burchard reaction) as described by **(Kenny, 1952 and Kaya**, ***et al*** **2001).**

Statistical Analysis:

The parametric were analyzed by using PROC GLM procedure of statistical analysis software **(SAS v9.4 2013).**

RESULTS

The present results revealed non-significant changes in hematological profiles including RBCs count and differential leucocytic count (Table1, 2). Phagocytosis showed significant increase in group 3 only (Table 3). Liver enzymes showed significant decrease in ALT, AST and ALP in group 2 and 3 (Table 4)**.** Total protein, albumin, globulin, albumin globulin ratio and Ig A revealed non-significant changes in all groups (Table 5)**.** Also serum ca, p, Cholesterol, LDL showed non-significant changes in all groups, LDL revealed significant decrease in groups 2 and 3 but HDL showed significant increase in groups 2 and 3 (Table 6). Vit A level showed significant increase in groups 2 and 3, in the same table oxidative enzymes revealed non-significant changes in MDA level but there was significant increase in GPx, CA and SOD in groups 2 and 3 (Table 7)**.** Exterior egg quality including Egg weight, Egg width, Egg length, Egg index, Shell weight, Shell thickness, Shell percentage showed non-significant changes except egg width revealed significant increase in group 2 (Table 8)**.** Interior egg quality including Yolk weight, Yolk width, Yolk, Yolk index, Albumin weight, Albumin height, and Albumin width showed non-significant changes but yolk height and albumin percentage revealed significant increase in group 3. In the other hand yolk percentage and yolk cholesterol revealed significant decrease in group 3 and in groups 2, 3 respectively (Table 9). Egg production showed significant increase in group 2 from the 2nd week till the end of treatment but group 3 showed significant increases from the 1st week to the 7th week then decrease in the 8th week (Table 10).

Table (1): Effect of zinc chelated methionine on RBCs and Hb in laying hens

parameters / Groups	RBCs 10^6 /µl	Hb (g/dl)
Control	1.43±0.06a	9.31±0.48a
2	1.35±0.05a	8.64±0.71a
3	1.43±0.05a	9.55±0.91a

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (2): Effect of zinc chelated methionine on total and differential leucocytic count in laying hens

Parameters / Groups	WBCs 10^3/ µl	Neutroph %	Esino %	Baso %	Lympho %	Monocyt %
Control	19.68± 2.61a	65.06±2.20a	4.56±1.01a	2.98±0.55a	24.26±1.58a	3.18±0.91a
2	22.72± 1.25a	63.54±3.41a	5.86±1.27a	2.66±0.26a	24.40±2.48a	4.20±0.71a
3	20.32± 0.97a	63.38±2.29a	7.38±1.81a	3.28±.23a	19.28±4.31a	2.64±0.77a

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (3): Effect of zinc chelated methionine on Phagocytosis in laying hens

Parameters / Groups	Phagocytic activity%	Phagocytic index
Control	37.86±2.32b	2.28±0.57
2	40.54±3.08b	3.20±0.36
3	44.20±0.56a	2.85±0.35

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (4): Effect of zinc chelated methionine on some liver enzymes in laying hens

Parameters / Groups	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Control	88.00±0.91a	161.50±6.34a	1011.0±8.03a
2	76.60±3.26b	137.60±5.77b	925.25±3.20b
3	79.80±3.67b	136.60±3.84b	871.75±3.88b

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (5): Effect of zinc chelated methionine on Serum Proteins Profile and IgA in laying hens

Parameters / Groups	Total Protein(g/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G	IgA mg/ml
Control	5.15±0.14a	4.62±0.14a	0.53±0.18a	32.73±24.31a	1.05±0.03a
2	5.56±0.23a	4.99±0.23a	0.57±0.27a	62.92±52.69a	1.09±0.08a
3	5.67±0.12a	5.07±0.17a	0.59±0.16a	27.54±20.09a	1.05±0.08a

a, b: Different letter superscripts in the same colum indicate significant differences (P < 0.05) (Mean±SE).

Table (6): Effect of zinc chelated methionine on ca, phosphorus and lipid profiles in laying hens.

Parameters / Groups	Ca mg/dL	P mg/dL	Cholest mg/dl	LDL mg/dL	HDL mg/dL
Control	10.23±0.29a	6.08±0.08a	214.78±0.67a	164.52±2.20a	38.97±2.89b
2	10.18±0.28a	6.03±0.07a	210.83±8.32a	149.72±7.81a	52.75±1.53a
3	12.20±1.57a	6.11±0.04a	218.65±0.45a	153.77±1.54a	55.65±2.25a

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (7): Effect of zinc chelated methionine on Vit A and oxidative enzymes in laying hens.

Parameters / Groups	Vit A Plasma retinol (µmol/liter)	MDA mol/ml	GPx mol/ml	CA mol/ml	SOD mol/ml
Control	84.25± 2.17b	9.45± 0.20a	1009.20± 91.73b	101.34± 3.44 b	211.77± 23.63b
2	124.75± 6.22a	10.00± 0.15a	1323.05± 112.01a	109.70± 4.02 a	274.80± 15.56a
3	128.75± 7.19a	9.80± 0.17a	1341.78± 68.01a	114.23± 3.68 a	278.08± 12.54a

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (8): Effect of zinc chelated methionine on exterior egg quality in laying hens.

Parameters / Groups	Egg weight gm	Egg width	Egg length	Egg index	Shell weight Gm	Shell thickness μm	Shell percentage %
control	60.51± 0.78	3.74± 0.36b	5.00± 0.35	0.74± 0.02	8.88± 0.15	0.15± 0.02b	0.15± 0.00
2	63.53± 2.29	4.50± 0.04a	5.68± 0.09	0.79± 0.01	9.79± 0.70	0.21± 0.02a	0.15± 0.01
3	61.33± 2.25	4.43± 0.03ab	5.70± 0.11	0.78± 0.01	9.87± 0.30	0.23± 0.01a	0.15± 0.01

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (9): Effect of zinc chelated methionine on enterior egg quality in laying hens.

Parameter / Groups	Yolk weight (gm)	Yolk height	Yolk width	Yolk %	Yolk index %	Albumen weight (gm)	Albumen height (Mm)	Albumen width	Albumen %	Cholesterol Yolk (mg/g)
Control	20.69± 2.10	1.32± 0.19b	3.22± 0.43	0.34± 0.03a	0.44± 0.07	30.93± 1.53	0.68±0 .08	7.02± 0.43	0.51± 0.03b	365.51± 22.11a
2	17.84± 0.67	1.64± 0.14ab	3.82± 0.09	0.28± 0.02ab	0.43± 0.04	35.90± 2.35	0.76±0 .09	7.08± 0.23	0.56± 0.02ab	295.87± 15.39b
3	16.30± 0.53	1.80± 0.04a	3.78± 0.03	0.27± 0.00b	0.48± 0.01	36.16± 1.74	0.80±0 .04	7.45± 0.38	0.59± 0.01a	268.70± 7.68b

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

Table (10): Effect of zinc chelated methionine on egg production in laying hens

Parameters / Groups	1st week %	2nd week %	3rd week %	4th week %	5th week %	6th week %	7th week %	8th week %
Control	82.1 ±2.92b	89.3 ±3.31	83.9 ±2.34b	78.6 ±2.34c	82.1± 2.58b	76.8± 4.32b	80.4± 4.69b	83.9± 2.34b
2	85.6 ±5.52b	85.7 ±5.76	97.6 ±2.42a	100.0± 0.0a	88.1± 7.11a	90.5± 5.03a	95.2± 3.13a	97.6± 2.42a
3	97.6 ±2.42a	92.8 ±5.03	90.5± 7.27a	90.5± 3.42b	88.9± 6.11a	92.8± 3.42a	92.8± 3.42a	83.3± 5.23b

a, b: Different letter superscripts in the same column indicate significant differences (P < 0.05) (Mean±SE).

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 **(Zhao *et al.*, 2005, and Favero *et al.*, 2013)**. 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 **(Cao *et al.,* 2002).** However mineral amino acid chelate should be supplemented to diet of layer hens for the optimal performance, in the present study supplementation of Zn- MHA chelate on diet did not significantly effect of RBC_S, Hb, Total leucocytic count and differential leucocytic count. Phagocytosis revealed significant increase in group 3, these results may be attributed to zinc amino acid might have increased thymulin activity; therefore, enhancing immune response through increased maturation of T-lymphocyte and activation of B lymphocytes by T-helper cells **(Hudson *et al.,* 2004).** Moreover, zinc has been shown to directly influence the immune system **(Kirchgessner 1993).** This element is required for normal immune function **(Kidd *et al.,* 1996).** Liver enzymes (ALT, AST and ALP) showed significant decrease in group 2 and 3 could be supported by the view of the beneficial effects of the use of antioxidants as hepatoprotective, with improvement in serum lipid profile decreasing the elevated liver enzyme and increasing the anti-oxidant enzyme levels **(Hala *et al.,* 2014)**,Decline was noticed in the value of alkaline phosphatase. This has been associated with increase or adequacy in dietary Zn **(Idowu *et al.,* 2011**). This was due to Zn binding capacity of alkaline phosphatase has been used as good indicator of Zn status. In the current study inorganic zinc did not affect the serum cholesterol level but decrease LDL and increase in HDL-cholesterol. This finding was also in agreement with previous experiment **(Uyanmk *et al.,* 2001),** indicated that a higher levels of serum Zn decreased the serum total cholesterol. Thus, **(Miller, and Miller 1975)** reported that plasma cholesterol level is inversely proportional to the plasma HDL level. HDL facilitates the transport of cholesterol from peripheral tissues to the liver for subsequent catabolism and excretion. Antioxidant is a substance that hinders a free radical reaction. A free radical is any species that contains one or more unpaired electrons. Zinc has never been shown to interact directly with an oxidant species but rather prefers to exert its effects in an indirect manner. In biochemical systems, the antioxidant properties of zinc have been clearly demonstrated and, for the most part, appear to be independent of zinc metalloenzymatic activity. Mechanism of autoxidation has been suggested that Zn increases the synthesis of metallothionein,a cystine-rich protein that acts as a free radical scavenger **(Oteiza *et al.,* 1996)**. The exposure of an organism to zinc on a long-term basis, resulting in an induction in some other substance that is the ultimate antioxidant. SODs are the first and most important line of antioxidant enzyme defense systems against reactive oxygen species **(Zelko *et al.,***

2002). Increased hepatic Zn-SOD activity indicated an increase in antioxidant activity by supplementation with Zn-MHA. CA showed significant increase in groups 2 and 3, increased CA activity by Zn-MHA supplementation could contribute to the conversion of carbon dioxide to bicarbonate and maintenance of acid-base balance in cells and tissues **(Bakst and Holm, 2003)**. The enzyme GPx is a major antioxidant enzyme that converts hydrogen peroxide to water using the coenzyme glutathione. Glutathione peroxidase is located primarily in the cytosol and has a general specificity in the detoxification of both lipid hydroperoxides and organic hydroperoxides **(Guemouri *et al.,* 1991)**. Results of the present study showed that, the plasma GPx activity was increased in groups 2, 3 treated with zinc methionine because it prevents lipid peroxidation via inhibiting glutathione depletion as well **(Prasad, 1997**). Due to the ability of zinc to replace Fe and Cu from binding sites, Zn can compete with these transition metals to bind to the cell membrane and decrease the production of free radicals and thus exert a direct antioxidant action **(Powell, 2000 and Prasad and Kucuk 2002)**. Vitamin A is required for normal growth, reproduction and maintenance of epithelial cells in good condition (skin and the linings epithelium of the digestive, reproductive, and respiratory tracts)**.** Supplementation with Zn-MHA increase Vit A level in group 2, 3 Zinc status influences several aspects of vitamin A metabolism, including its absorption, transport, and utilization. Two common mechanisms postulated to explain this dependence relate to 1) the regulatory role of zinc in vitamin A transport mediated through protein synthesis, Zinc is a component of retinol-binding protein, a protein necessary for transporting vitamin A in the blood and 2) the oxidative conversion of retinol to retinal that requires the action of a zinc-dependent retinol dehydrogenase enzyme **(Boron *et al.,* 1988 and Christian and West 1998)**. Supplementation with Zn-MHA was also found to improve eggshell thickness**.** Trace minerals may affect mechanical properties of eggshell either by their catalytic properties as key enzymes involved in eggshell formation or by interacting with calcite crystal formation and modifying crystallographic structure of eggshell **(Mabe *et al*., 2003)**. Zn- is a component of the carbonic anhydrase enzyme, which supplies the carbonate ions during eggshell formation **(Bakst and Holm, 2003, Innocenti *et al.,* 2004),** so as to benefit to eggshell quality. Eggs are rich source of dietary cholesterol and consumption of high level of dietary cholesterol increases the risk of coronary heart disease **(Kritchevsky, 2004).** Producing eggs low in cholesterol will be of great interest to egg consumers and this can be better achieved by supplementing the diets of laying birds with Zn-MHA. Egg yolk

cholesterol has been reported to be synthesized in the liver of laying hens and transported to the developing follicles via plasma very low density lipoprotein (VLDL) where it is deposited by receptor mediated endocytosis **(Nimpt and Shneider, 1991).** In the present study, supplementation of Zn-MHA on diet significantly affects hen day egg production in group 2. It has been reported that, the egg production was increased with supplementation of layers diets with organic form of Zn replacing inorganic forms, depend on higher bioavability of organic form of minerals **.**On the other hand, the similar results in group 3 till the 8th week , the egg production begin to decline . This result depended on 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.,* 2005 and Bilgehan *et al.,* 2015)** observed that high level Zn supplementation to diets causing lesions in the pancreas and gizzard of laying hens affecting feed intake and absorption of nutrients causing decrease egg production . Animals and poultry 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 and poultry feeds. The results of the current study showed that, supplementation of 0.5 g/kg mineral amino acid chelate (Zn-MHA) to diet increased egg production and had favorable effects on interior and exterior egg quality of laying hens**.** In conclusion, trace minerals (Zn) in the form of MHA chelates showed higher bioavailability and beneficial effects compared with inorganic form when included in laying hens' diet

REFERENCES

Aksu T, Özsoy B, Sarıpınar Aksu D, Yörük MA and Gül M (2011): The effects of lower levels of organically complexed zinc, copper and manganese in broiler diets on performance, mineral concentration of tibia and mineral excretion. *Kafkas Univ Vet Fak Derg*, 17, 141-146,

Arcasoy A, (2002): Çinko ve çinko eksikliği, 2. Baskl, s. 1-3. Ankara Talasemi Derneği Yaylnlarl.

Armstrong JM, Myers DV, Verpoorte JA and Edsall JT (1966): Purification and properties of human erythrocyte carbonic anhydrases. Journal of Biological Chemistry, 241: 5137-5149.

Baginski ES, Marie SS, Clark WL and Zak B. (1973): Direct microdetermination of serum calcium. Clin. Chim Acta. 46:49-54

Bakst M R and Holm L (2003): Impact of egg storage on carbonic anhydrase activity during early embryogenes. Poultry Science, 82: 1193-1197.

Batal, A.B., T.M. Parr and D.H. Baker, (2001): Zinc bioavailability in tetrabasic zinc chloride and the dietary zinc requirement of young chicks fed a soy concentrate diet. Poult. Sci., 80: 87-90.

Beuge JA and Aust SD (1978): Microsomal lipid peroxidation. Methods Enzymol. 52: 302-305.

Bilgehan Yilmaz Dikmen 1 Arda Sözcü 1 **Aydın İPEK 1and Ümran Şahan (2015):** 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. Kafkas Univ Vet Fak Derg 21 (2): 155-162.

Boron B, Hupert J, Barch DH (1988): Effect of zinc deficiency on hepatic enzymes regulating vitamin A status. J Nutr. 1988; 118 (8):995-1001.

Campbell TW (1995): Avian hematology and cytology. 2nd ed., pp. 3-20, Iowa State Press, 1995.

Cao J, Henry PR, Davis SR, Cousins RJ, Miles RD, Little RC and Ammerman CB (2002): Relative bioavailabity of organic zinc sources based on tissue zinc and metallothionein in chicks fed conventional dietary zinc concentrations. *Anim Feed Sci Technol,* 101, 161-170.

Cavanagh EMW, Inserra F, Ferder L, Romano L, Ercole L and Fraga CG. (**1995):** Superoxide dismutase and glutathione peroxidase activities are increased by enalapril and captopril in mouse liver. FEBS Letters, 361: 22-24.

Christian P, and West KP, Jr (1998): Interactions between zinc and vitamin A: an update. Am J Clin Nutr.; 68 (2 Supple):435S - 441S.

Coles, E.H. (1986): "Veterinary clinical pathology " 4th Ed. W.B. Sounders company , Philadelphia London, Toronto, Mexico, Sydney, Tokyo, Hong Kong.

Favero A, Vieira SL, Angel CR, Bos-Mikich A, Lothhammer N, Taschetto D, Cruz RFA, and Ward TL (2013): Development of bone in chick embryos from Cobb 500 breeder hens fed diets supplemented with zinc, manganese, and copper from inorganic and amino acid-complexed sources. *Poult Sci*, 92, 402-411.

Gibbs P. N. S., Gore M. G., and Jordan P. M. (1985): Investigation of the effect of metal ions on the reactivity of thiolgroups in human 5-aminolaevulinate dehydrtase. Biochem. J.,225:573-580.

Guemouri, L., Y. Artur, B., Herbeth, C., Jeandel, G. Cuny, and Siest. **G (1991):** Biological variability of superoxide dismutase, glutathione peroxidase, and catalase in blood. Clin. Chem. 37:1932-1937.

Haddad AS, Subbiah V and Lichtin AE (2008): Hypocupremia and bone marrow failure. Haematologica 93: e1-e5. DOI: 10.3324/haematol.12121.

Hala A.A. Abou-Zeina, Soad M. Nasr, Somia A, Nassar, Mohamed A.F. Genedy and Mamdouh I. Mohamed (2014) : Influence of Dietary Supplementation with Antioxidants on the Growth Performance, Hematological and Serum Biochemical Alterations in Goat Kids . Global Veterinaria 13 (5): 926-937.

Hudson B. P., Dozier W. A., Wilson J. L., Sander J. E. and Ward T. L (2004): "Reproductive Performance and Immune Status of Caged Broiler Breeder Hens Provided Diets Supplemented with Either Inorganic or Organic Sources of Zinc from Hatchery to 65 wk of Age," Journal of Ap- plied Poultry Science, Vol. 13, No. 2, pp. 49-59.

Hudson B.P., Dozier III W.A and Wilson J.L (2005): Broiler live performance response to dietary zinc source and the influence of zinc supplementation in broiler breeder diets. *Anim Feed Sci Technol,* 118, 329-335.

Hughes S, NutrDiet M and Samman S, (2006): The effect of zinc supplementation in humans on plasma lipids, antioxidant status and thrombogenesis. J Am Coll Nutr, 25, 285-291.

Idowu1 O.M.O.,Ajuwon R.O.,Oso1A.O.andAkinloye O.A.(2011): Effects of Zinc Supplementation on Laying Performance, Serum Chemistry and Zn Residue in Tibia Bone, Liver, Excreta and Egg Shell of Laying Hens. International Journal of Poultry Science 10 (3): 225-230, 2011.

Innocenti, A., S. Zimmerman, J.G. Ferry, A. Scozzafava and C.T. Supuran, **(2004):** Carbonic anhydrase inhibitors. Inhibition of the zinc and cobalt gamma class enzyme from the archaeon *Methanosarcina thermophila* with anions. Bioorg. Med. Chem. Lett., 14: 3327-3331.

Kaya, S., T. Keçeci, and S. Haliloğlu. (2001): Effects of zinc and vitamin A supplements on plasma levels of thyroid hormones, cholesterol, glucose and egg yolk cholesterol of laying hens. Res. Vet. Sci. 71:135-139.

Kenny A.P, (1952): The determination of cholesterol by the Liebermann-Burchard reaction. Biochem.J. 52,611-619.

Kidd M. T., Ferket P. R. and Qureshi M. A. (1996) : "Zinc Me- tabolism with Especial Reference to Its Role in Immu- nity," World Poultry Science Journal, Vol. 52, No. 3, pp. 309 -324.

Kind P and King E. (1954): Colorimetric determination of alkaline phosphataseJ. of clinical pathol

Kirchgessner M. (1993): "Underwood Memorial Lecture. Ho- meostasis and Homeorhesis in Trace Element Metabo- lism," In: M. Anke, D. Meissner and C. F. Mills, Eds., *Trace Elements in Man and Animals*, Vol. 8, Verlag Me- dia Touristik Dresden, Germany, pp. 4-21.

Kritchevsky, S.B. (2004): A review of scientific research and recommendations regarding eggs. Journal of American College of Nutrition, vol. 23 (6), 2004, p. 596S-600S.

Mabe I, Rapp C, Bain MM and Nys Y (2003): Supplementation of a cornsoybean meal diet with manganese, copper and zinc from organic or inorganic sources improves eggshell quality in aged laying hens. Poultry Science, 82: 1903-1913.

Mates, JM, Aledo JC, Perez-Gomez A and Segura M (2000): Interrelationship between oxidative damage and antioxidant enzyme activities: an easy and rapid experimental approach. Biochemical Education, 28: 93-95.

Miller GJ and Miller NE (1975): Plasma high density lipoprotein concentration and development of ischaemic heart disease. Lancet, 1, 16-19.

Nierenberg DW and Lester DC (1985): Determination of vitamin A and E in serum and plasma using a simplified clarification method and high-performance liquid chromatography. J. Chromatogr.345:275-284.

Nimpt.J., and Schneider,W.J., (1991) : Receptor –mediated lipoprotein transport in laying hens. *Journal of Nutrition*, vol. 121, p. 1471–1474.

Oteiza, P. L., Olin K. L., Fraga C. G., and Keen. **C. L. (1996):** Oxidant defense systems in testes from Zn deficient rats. Proc. Soc. Exp. Biol. Med. 213:85-91.

Plummer DT. (1978): An Introduction to practical biochemistry. McGraw Hill Ltd. Mardenhead Berkshire, England. Pp. 109-185.

Powell S. R. (2000): The antioxidant properties of zinc. J. Nutr. 130:1447S-1454S.

Prasad A. S. and Kucuk O. (2002): Zinc in cancer prevention. Cancer Metastasis Rev.21:291-295.

Prasad A. S. (1997): The role of zinc in brain and nerve function in metals and oxidative Damage in Neurological Disorders (Connor, A. ed.): 95-11. Plenum Press, New York, NY.

Polo FJ, Celdran JF, Peinado VI, Viscor G and Palomeque J (1992): Hematological values for four species of birds of prey. The Condor, 94, 1007-1013.

Retiman and Frankel. (1957): Colorimetric method for determination of serum transaminase activity .American j .of clini. pathol. 28, 65-68.

Richards J. and Dibner J. (2005): Organic trace minerals are not all equally effective. World Poultry, 21(9):17-19.

SAS (2013): SAS® v9.4 User's Guide. SAS Inst., Inc., Cary, NC.

Sarıca M and Erensayın C (2009): Tavukçuluk ürünleri. In, Türkoğlu M, Sarıca M (Eds): Tavukçuluk Bilimi Yetiştirme, Besleme, and Hastalıklar. 3rd ed., 103 -109, Bey Ofset Matbacılık, Ankara,

Solomon SE (1991): Egg and eggshell quality. London: Wolfe publishing; p.365.

Trindade Neto MA, Pacheco BHC, Albuquerque R and Schammass EA (2011): Lysine and zinc chelate in diets for brown laying hens: Effects on egg production and composition. R Bras Zootec, 40, 377-384.

Torres CA (2013): Influence of maternal flock age, maternal trace mineral nutrition and incubation temperature on bone development of embryos and chicks. *PhD Thesis* of University of Alberta, Edmenton, Alberta, Canada.

Uyanmk F, Eren M and Tuncoku G (2001): Effects of supplemental zinc on growth, serum glucose, cholesterol, enzymes and minerals in broilers. Pakistan J Bio Sci, 4 (6): 745-747,

Woldeniwet, Z. and Rowan ,T.G.(1990) : Some observation on phagocytosis and killing of staphylococcus aureus by polymorphnuclear leukocytes .Br. Vet .J. 146 – 163.

Zhao B, Ding XM, Zhang KYand Chen DW (2005): Effects of amino acid trace mineral chelates on egg quality and physiological function of laying hens. *J Sichuan Agric Univty*, 2, 238-243.

Zelko IN., Mariani TJ and Folz RJ. (2002): Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2) and EC-SOD (SOD3) gene structures, evolution and expression. Free Radical Biology and Medicine, 33: 337-349.