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RESEARCH ARTICLE

The Effects of Lower Supplementation Levels of Organically Complexed Minerals (Zinc, Copper and Manganese) Versus Inorganic Forms on Hematological and Biochemical Parameters in Broilers [1]

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Summary

The present study was carried out to investigate the effects of replacing inorganic with at lower level of organically complexed minerals (Zn, Cu and Mn) on hematological and biochemical parameters in broilers. A total of two hundred Ross-308 one-day-old broiler chickens were used. Chicks were randomized into 1 control and 3 treated groups each containing 50 chicks and each experimental group comprised 5 of subgroups including 10 chicks. Mineral content of the control diet was prepared according to National Research Council (NRC) as inorganic salts. In treated groups, organically complexed Zn, Cu and Mn were separately added into the basal diet at 1/3 (group 1), 2/3 (group 2) and 3/3 (group 3) proportions as Bioplex™, instead of inorganic levels of those minerals recommended by NRC, respectively. The plasma Zn level significantly increased as the serum Cu level significantly decreased ($P<0.05$) in chickens fed at 2/3 and 3/3 levels of organic minerals. The hemoglobin concentration and packed cell volume were significantly higher in group L1. Total leukocyte count and peripheral blood leukocyte type were in the normal range reported in both the control and organic mineral supplemented groups. As the high density lipoprotein (HDL)-cholesterol level increased, low density lipoprotein (LDL)-cholesterol and total cholesterol levels decreased in chickens fed organically complexed minerals. Results showed that using at much lower level organically complexed minerals (Cu, Zn and Mn) in broiler diets instead of inorganic forms of those minerals has not created a negative impact on hematological and biochemical parameters.

Keywords: *Organically complexed mineral, Inorganic mineral, Hematological parameters, Biochemical parameters, Broiler*

İnorganik Formları Yerine Daha Düşük Seviyelerde Organik Mineral (Çinko, Bakır ve Mangan) İlavesinin Etçi Piliçlerde Bazı Hematolojik ve Biyokimyasal Parametreler Üzerine Etkisi

Özet

Bu araştırma inorganik formları yerine daha düşük seviyelerde organik mineral tüketiminin etçi piliçlerin hematolojik ve biyokimyasal parametreleri üzerine etkilerini belirlemek amacıyla yapıldı. Çalışmada 200 adet, bir günlük yaşta, etçi civciv (Ross-308) kullanıldı. Civcivler, 10'ar civciv bulunan 5 alt gruptan oluşan, biri kontrol diğer üçü deneme grubu olmak üzere 4 ana gruba tesadüfi olarak dağıtıldı. Kontrol grubunun diyeti, NRC tarafından belirtilen ticari etçi piliç yemi normlarına göre kg'da 40 mg Zn ($ZnSO_4$), 8 mg Cu ($CuSO_4$) ve 60 mg Mn (MnO) olacak şekilde hazırlandı. Üç deneme grubunun diyetlerine ise inorganik formdaki Zn, Cu ve Mn mineralleri yerine sırasıyla 1/3 (1. Grup-L1), 2/3 (2. Grup-L2) ve 3/3 (3. grup-L3) oranında organik Zn, Cu ve Mn (Bioplex™) ilavesi yapıldı. Araştırma sonunda, NRC normlarının 2/3'ü ve 3/3'ü oranlarında organik mineral tüketen etçi piliçlerin plazma çinko seviyesi önemli oranda artarken, plazma bakır seviyesi önemli oranda düştü ($P<0.05$). Hemoglobin konsantrasyonu ve hematokrit değeri 1/3 oranında organik mineral tüketen etçi piliçlerde önemli oranda arttı. Kontrol ve deneme gruplarına ait akyuvar sayısı ve akyuvar yüzde dağılımının normal referens sınırlarında olduğu belirlendi. İnorganik mineral tüketen piliçlere kıyasla organik minerallerim tüm seviyelerinde yüksek yoğunluklu kolesterol (HDL-kolesterol) seviyesi artarken, düşük yoğunluklu kolesterol (LDL-kolesterol) seviyesi ile total kolesterol seviyeleri azaldı. Araştırma sonunda, etçi piliç rasyonlarına inorganik formları yerine çok daha düşük miktarlarda organik mineral (çinko, bakır ve mangan) ilavesinin hematolojik ve bazı biyokimyasal parametrelerde herhangi bir olumsuz etki oluşturmadığı sonucuna varıldı.

Anahtar sözcükler: *Organik mineral, İnorganik mineral, Hematolojik parametreler, Biyokimyasal parametreler, Etçi piliç*

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INTRODUCTION

Copper, zinc and manganese are trace minerals which plays vital role in many physiological and biochemical processes in the organism. Copper is an important component of many enzymes which are critical to the maturation of hematopoietic cells and copper deficiency can be cause to inadequate iron utilization in organism [1]. 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 hem synthesis [2]. Zinc deficiency can be cause to adverse effect on erythropoiesis in marrow [3], and a reduction of T and B lymphocyte production [4,5]. Manganese and iron are recognized two main trace minerals have many similar physico-chemical properties but those minerals show antagonistic effect on each other's. Manganese and iron are absorbed by binding to the same divalent metal ion transporters. Therefore, a high level of manganese causes to decrease the iron absorption, thus anemia occurs [6]. On the other hand, that high level of manganese prevents iron metabolism by pressuring the synthesis of aminolevulinat which has special role in the hem synthesis [7,8]. In previous experiment, carried out in broiler by adding alone inorganic forms of the present minerals, it was reported that present minerals were also related with serum cholesterol and lipid metabolism. Some of researchers [9,10] indicated that inclusion of these minerals increased serum cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL); others of them reported that no effect was observed [11,12].

In commercial poultry diets, majority of trace mineral are provided in inorganic forms such as oxide or sulphate salts. The levels of supplementation are mostly based on National Research Council (NRC) recommendations [13]. Nowadays, livestock is generally fed highly concentrated diets that are formulated to provide an excess of nutrients to maximize performance [14]. In commercial poultry production, trace minerals are commonly added in the form of a premix to diets and used to supply from two to ten times more of these minerals than NRC recommendations [15]. Excessive use of inorganic salts causes to the damage in nutrients absorption and the low mineral bioavailability. In addition, current excessive mineral intake causes environmental pollution by higher heavy mineral excretion. Due to increasing concerns about potential mineral pollution, nutritionists have been focused on how to reduce mineral excretion without any negative affect on production performance.

Organically complexed trace minerals may provide alternative pathways for absorption, by decreasing mineral excretion [14,16]. Organically complexed mineral is a type of mineral linked to protein/peptide/amino acids that has a higher bioavailability than those inorganic salts [17]. These types of minerals are more easily absorbed compare to inorganic forms as peptide or amino acid forms. Therefore, organically complexed minerals are supposed to be more effective than the inorganic minerals [18].

Recently, it was enounced that organic minerals may be added at a much lower levels in the broilers diet than the current recommendations for inorganic minerals, without any negative affect on body weight gain and feed intake [19,20]. As far as author's knowledge, up to now there is no data about on hematological and biochemical parameters of broilers fed combined at a much lower levels of organic minerals instead of its inorganic forms. For this reason, the present study was investigated to determine the effect of reducing of organically complexed minerals levels (Zn, Cu and Mn) instead of inorganic forms of those minerals on hematological and biochemical parameters in broilers.

MATERIAL and METHODS

Animals, Diets and Experimental Design

The experiment was in accordance with Animal welfare, and was conducted under protocols by the Veterinary Faculty in Hatay-Turkey. A total of two hundred, one-day-old, broiler chickens (Ross-308) were used in the feeding trail that lasted until the chickens reached 42 d of age. In total, 20 floor pens (surface area 1 m^2) were used, each containing 10 male broilers, to give 5 pen replicates and total of 50 chickens per treatment. The chickens were given ad libitum access to feed and water. A lighting schedule of 23:1 (23 Light: 1 Dark) was imposed throughout the experimental period. The basal diet *(Table1)* was formulated according to NRC [13] and analyzed by the AOAC [21].

Two phases were applied during the experiment: A starter (0-21 d) and finisher (21-42 d). Mineral content of the control diet was prepared using standard inorganic mineral premix (containing per kilogram of 40 mg Zn as $ZnSO_4$, 8 mg Cu as $CuSO_4$ and 60 mg Mn as MnO, and) that reflects the NRC recommendation of trace minerals for commercial broiler diet. In the experimental diets, mineral premix was also prepared as inorganic forms except of Zn, Cu and Mn. Organically complexed Zn, Cu, and Mn were separately added into basal diet at 1/3 (L1), 2/3 (L2) and 3/3 (L3) proportions of NRC recommendation levels *(Table 2)*.

Bioplex™ is an amino acid-hydrate complex, bonded with Zn, Cu and Mn. Amino acid produced from

Table 1. *Ingredient composition of the basal diets*
Tablo 1. *Temel diyetin bileşimi, %*

Raw Materials (%)	Starter (0 to 21d)	Finisher (21 to 42 d)
Maize	51.5	55.2
Wheat	7	7
Bran	4.5	4.5
Soybean meal	27.5	24
Fish meal	5.5	4.3
Vegetable oil	1.5	2.5
Limestone	1	1
DCP	0.75	0.75
Salt	0.25	0.25
Vit-Min. premix *	0.5	0.5
Calculated nutrients		
ME, MJ/kg	12.6	13
Crude protein, %	22.1	20
Ca, %	0.9	0.8
P, %	0.6	0.7
Lysine, %	1.1	0.8
Analyzed nutrients		
Mn, mg/kg	38.84	36.63
Zn, mg/kg	35.65	30.23
Cu, mg/kg	9.63	8.71

***:** *Supplied per kilogram of diet: Vitamin A 15.000 IU; cholecalciferol 1.500 ICU; Vitamin E, 30 IU; Menadion, 5.0 mg; Thiamin, 3.0 mg; Riboflavin, 6.0 mg; Nniacin, 20.0 mg; Pantothenic acid, 8.0 mg, Pyridoxine, 5.0 mg; Folic acid, 1.0 mg; Vitamin B_{12}, 15 µg; Mn, 60.0 mg; Zn, 40 mg; Fe, 30.0 mg; Cu, 8.0 mg; I, 2.0 mg; Se, 0.15 mg*

Table 2. *Source and amounts of trace minerals*
Tablo 2. *İz minerallerin kaynak ve miktarları*

Diet	Added Zn (mg/kg)	Added Cu (mg/kg)	Added Mn (mg/kg)
Inorganic (control) *	40	8	60
1/3 organic (L-1) **	13	2.5	20
2/3 organic (L-2) **	26	5	40
3/3 organic (L-3) **	40	8	60

** Supplied per kilogram of diet: Vitamin A 15.000 IU; Cholecalciferol 1.500 ICU; Vitamin E, 30 IU; Menadion, 5.0 mg; Thiamin, 3.0 mg; Riboflavin, 6.0 mg; Niacin, 20.0 mg; Pantothenic acid, 8.0 mg, Pyridoxine, 5.0 mg; Folic acid, 1.0 mg; Vitamin B_{12}, 15 µg; Mn, 60.0 mg; Zn, 40 mg; Fe, 30.0 mg; Cu, 8.0 mg; I, 2.0 mg; Se, 0.15 mg*
******** *Organically complexed Mn, Zn and Cu were provided as Bioplex-Mn, Bioplex-Zn and Bioplex-Cu*

hydrolized-soy protein. Bioplex Zn™, Bioplex Cu™ and Bioplex Mn™ contain 1.000.000 mg/kg of Zn, 150.000 mg/kg of Cu, and 150.000 mg/kg of Mn, respectively. The organically complexed Zn, Cu and Mn were provided as Bioplex-Zn™, Bioplex-Cu™, and Bioplex-Mn™ (Alltech Biotechnology-TR).

Hematological Examinations

At the end of the experiment the blood samples were collected from the ulnar vein of 2 broilers randomly chosen from each treatment. The blood is collected into tube containing EDTA. Red blood cell (RBC) and white blood cell counts (WBC) and packed cell volume (PCV) were determined by the manual method using a hemocytometer [22]. Hemoglobin concentrations were determined by the cyanmethemoglobine method using Drabkin's reagent [23]. The hematimetric indices, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) were calculated by using the formulas guided by Campbell [22]. Peripheral blood leukocyte percentages determined blood smears stained with May Grunwald-Giemsa. Differential counts were counted on each smear and identified according to Campbell [22].

Plasmas were harvested by centrifuging the whole blood samples at 3.000 rpm for 15 min. Plasma Total Cholesterol (TC), High Density Lipoprotein Cholesterol (HDL-C), Trigliserid (TG), Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Amino-transferase (AST), g-Glutamyl Transferase (GGT) were analyzed in the Automatic Blood Analyzer (Olimpus AU 600) using commercial kits. Low density lipoprotein cholesterol (LDL-C) was calculated using the Friedewald equation: LDL= TC-HDL-(TG/5). For measurement of trace minerals in plasma, 4mL of plasma sample was wet-ashed in a tube by adding 10 ml of nitric acid and heated to minimal volume (the solution was never allowed to dry). After the solution was cooled, it was filtered into 25 mL flask and diluted to 25 mL with deionized water for and mixed well for trace mineral content using Inductively Coupled Plasma Emission Spectroscopy (ICP) by a laboratory specializing in these assays.

Statistical Methods

The appropriate Sum of Squares Method was selected for ANOVA [24]. The data were analyzed by one-factor ANOVA using the general linear models procedure of SAS [25] software for the main effect of treatments. Differences between means were determined by Duncan's multiple range test at a significance level of P<0.05.

RESULTS

Plasma mineral levels of chickens fed different diets are given in *Fig. 1.* The plasma Mn and Fe levels were not different among experimental groups. On the other hand, the plasma zinc level was higher in chickens fed at 1/3 and 2/3 levels of organically complexed minerals although these groups received lower levels of minerals than control group. Moreover, the plasma Mn and Fe levels in chickens fed at 1/3 levels of organically complexed minerals were tend to increase while the plasma Fe levels was tend to decrease in chickens fed at 2/3 levels of organically complexed minerals (P>0.05). The plasma Zn level significantly increased as the serum Cu level significantly decreased (P<0.05) in chickens fed at 2/3 and 3/3 levels of organically complexed minerals. No differences were observed among the plasma copper level of chickens fed at 1/3 level organically complexed minerals and the plasma copper level of chickens fed at 3/3 inorganic minerals. On the other hand, a decrease in plasma copper level was observed in the chickens fed at 2/3 and 3/3 levels of organically complexed minerals (P<0.01)

Red blood cell counts, hemoglobin, packed cell volume, MCH, MCV and MCHC are presented in *Table 3*, white blood cell counts and differential leukocyte counts are presented in *Table 4*, and some biochemical values in chickens fed different diets are presented in *Table 5*.

Table 3. *Red blood cell counts, haemoglobin, packed cell volume, MCV, MCH and MCHC values in chickens fed different diets*
Tablo 3. *Farklı diyetleri tüketen piliçlerin alyuvar sayısı, hemoglobin, hematokrit, MCV, MCH ve MCHC değerleri*

Parameters	**Diets** [1]				**SEM**	**P**
	Control	**L1**	**L2**	**L3**		
RBC ($x10^6/mm^3$)	2.50	2.57	2.26	2.40	0.049	NS
Hb (g/dl)	9.58 ^{b}	10.90 ^{a}	9.75 ^{b}	9.60 ^{b}	0.163	*
PCV (%)	28.60 ^{ab}	29.40 ^{a}	27.60 ^{b}	27.30 ^{b}	0.283	*
MCV (fl)	115	109	122	118	0.239	NS
MCH (pg)	38	44	42	43	1.366	NS
MCHC (%)	33	37	35	35	0.550	NS

Means represent from 10 chickens per treatment ^{a,b} *Means values within a row having differing superscripts are significantly different by least significant differences test (P<0.05).* ***NS:*** *non-significant, * P<0.05*
[1] ***Control:*** *Inorganic Cu, Zn and Mn at NRC recommendation levels as sulfate.* ***L1, L2, and L3:*** *Organically complexed Cu, Zn and Mn (Bioplex™) at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals recommend levels by NRC, respectively*

Table 4. *White blood cell counts and differential leukocyte counts in chickens fed different diets*
Tablo 4. *Farklı diyetleri tüketen piliçlerin akyuvar sayısı ve akyuvar yüzde oranları*

Parameters	**Diets** [1]				**SEM**	**P**
	Control	**L1**	**L2**	**L3**		
WBC ($x10^3/mm^3$)	16.9	17.5	18.79	17.39	0.27	NS
Heterophils (%)	33	35	35	39	1.07	NS
Lymphocytes (%)	52	48	51	49	1.16	NS
Eosinophiles (%)	5	6	4	3	0.39	NS
Monocytes (%)	3	3	4	2	0.40	NS
Basophiles (%)	7	8	6	7	0.49	NS
H/L	0.65	0.74	0.73	0.83	0.05	NS

Means represent from 10 chickens per treatment ^{a,b} *Means values within a row having differing superscripts are significantly different by least significant differences test (P<0.05).* ***NS:*** *non-significant, * P<0.05*
[1] ***Control:*** *Inorganic Cu, Zn and Mn at NRC recommendation levels as sulfate.* ***L1, L2, and L3:*** *Organically complexed Cu, Zn and Mn (Bioplex™) at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals recommend levels by NRC, respectively*

Table 5. *Some biochemical values of chickens fed different diets*
Tablo 5. *Farklı diyetleri tüketen piliçlerin bazı biyokimyasal değerleri*

Parameters	**Diets** [1]				**SEM**	**P**
	Control	**L1**	**L2**	**L3**		
TC (mg/dl)	117.2 ^{a}	111.2 ^{ab}	108.2 ^{b}	107.4 ^{b}	1.450	*
TG (mg/dl)	34.72	33.26	30.98	31.81	0.800	NS
HDL (mg/dl)	79.4 ^{b}	81,6 ^{ab}	85.12 ^{a}	86.42 ^{a}	1.027	*
LDL (mg/dl)	30.86 ^{a}	22.94 ^{b}	16.88 ^{bc}	14.62 ^{c}	1.825	*
ALT (U/L)	5.8	5.6	5.2	5.6	0.222	NS
AST (U/L)	252	247	232	231	4.424	NS
ALP (U/L)	1862	1781	1887	1823	71.46	NS
GGT (U/L)	19.08	18.54	18.24	18.76	0,625	NS

Means represent from 10 chickens per treatment ^{a,b} *Means values within a row having differing superscripts are significantly different by least significant differences test (P<0.05).* ***NS:*** *non-significant, * P<0.05*
[1] ***Control:*** *Inorganic Cu, Zn and Mn at NRC recommendation levels as sulfate.* ***L1, L2,*** *and* ***L3:*** *Organically complexed Cu, Zn and Mn (Bioplex™) at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals recommend levels by NRC, respectively*

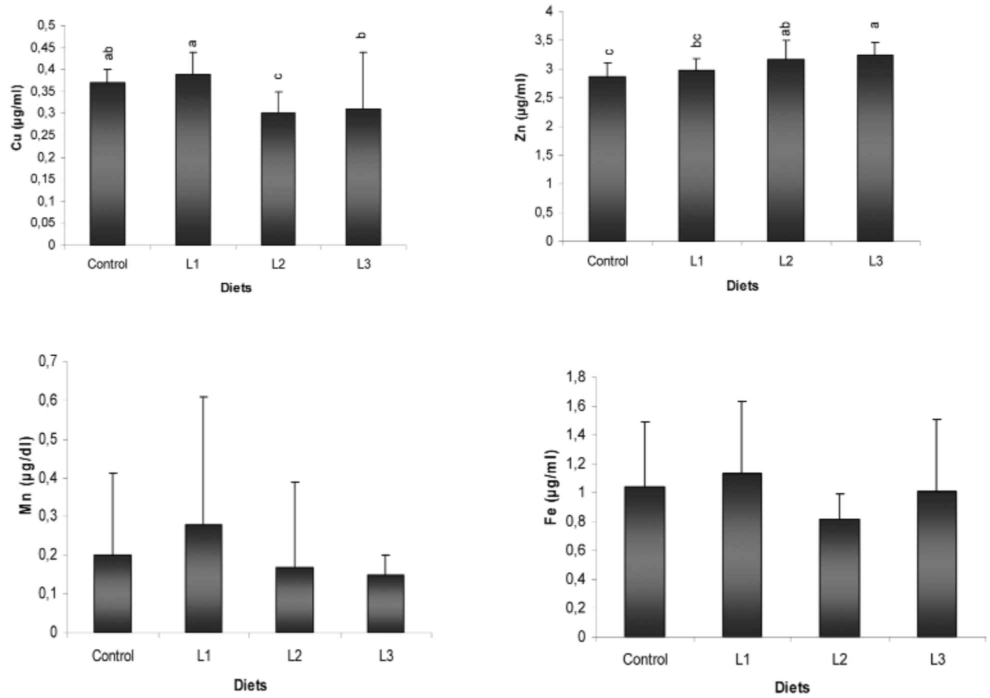

Fig 1. Concentration of Cu, Zn, Mn and Fe in the plasma of chickens fed different diets

Şekil 1. Farklı diyetleri tüketen piliçlerin plazma Zn, Cu, Mn ve Fe konsantrasyonları

Hemoglobin and packed cell volume were significantly higher in the chickens fed at 1/3 level of organically complexed minerals (P<0.01). In all groups, HDL-cholesterol level increased as the LDL-cholesterol and total cholesterol levels decreased (P<0.05). Using at lower level organically complexed minerals instead of inorganic forms did not affect the other parameters examined.

DISCUSSION

Copper, zinc and manganese are important trace elements for development of the red blood cells and immune system. Those are also related with cholesterol and lipid metabolism.

As shown in *Fig. 1*, the plasma Zn level significantly increased as the serum Cu level significantly decreased (P<0.05) in chickens fed at 2/3 and 3/3 levels of organically complexed minerals. The increase in plasma zinc level could be linked to the higher resolution and lower interaction with other's of organically complexed minerals in digestive tract [26]. Compared to inorganic minerals, these give to increase in bioavailability of organically complexed minerals. Organically complexed minerals (copper, zinc and manganese) are obtained by binding to the amino acids/proteins and therefore easily absorbed because of these structures [18,27].

On the other hand, the decrease in plasma copper levels of chickens fed at 2/3 and 3/3 levels of organically complexed minerals could be due to the interaction between copper and zinc. Zinc competes with copper for binding to metallothionein and consequently at higher dietary zinc levels, less copper is absorbed 28. Because, a high level of zinc stimulates the synthesis of metallothionein which is synthesized from enterocytes and binding the metal ions in the blood. Copper has a higher affinity which causes to binding the metallothionein by taking place of Zn, thus leads to the accumulation of copper in the enterocytes. The enterocytes are shed into the gastrointestinal tract hereby copper absorption decreases in this way [29].

The normal range of red blood cell count is 2.5-3.5x10^6/mm^3, packed cell volume is 22-35%, hemoglobin is 7-13 g/dL, MCV is 90-143 μ^3, MCH is 33-47 pg and MCHC is 26-35% in broilers [30]. These parameters were within normal range both of control and the experimental groups *(Table 3)*.

The increase in amount of Hb and PCV of chicken fed 1/3 organically complexed minerals might be increasing in plasma iron level of this group. It was reported that copper deficiency in broiler caused to reduction in the packed cell volume [31], red blood cell count and hemoglobin concentrations [32]; and also caused to anemia which happens with make changing the shape of red blood cells in Turkey [33]. In the current study, the decrease of the plasma copper levels in both L2 and L3 groups (P<0.05) did not affect the red blood cell count, hemoglobin concentration and erythrocyte indices in comparison to those control and L1 group *(Table 3)*. This could be due to the increase of plasma zinc level which stabilizes the cell membranes and regulates its functions [34]. The protective function of organically complexed zinc (Zn-proteinat) in the cell membrane against the lipid peroxidation is reported to be more effective than that of inorganic form of zinc [35].

Total leukocyte count and peripheral blood leukocyte type in both inorganic (control) and organically complexed minerals supplemented groups were in the normal range reported for poultry [30]. In the present study, the reduction of inclusion levels of minerals in organically complexed forms did not alter these parameters *(Table 4)*. This finding was supported by Donmez et al.[36], who reported that Zn supplementation did not affected leukocyte count and peripheral blood leukocyte type.

Copper, zinc and manganese are related to lipid metabolism. Lu and Combs [37] reported that inorganic zinc did not affect the serum cholesterol level. On contrary, Boukaiba et al.[38] and Uyanık et al.[39] indicated that inorganic zinc decreased the serum cholesterol level. In the current study, the decrease in total cholesterol level was connected the increase in plasma HDL-cholesterol *(Table 5)*. This finding was also in agreement with previous experiment [37,38], indicated that a higher levels of serum Zn decreased the serum total cholesterol. Thus, Miller et al.[40] 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.

In conclusion, using at much lower level organically complexed minerals (Cu, Zn and Mn) in broiler diets instead of inorganic forms of those minerals has not created a negative impact on hematological and bio-chemical blood parameters. These results therefore indicate the copper, zinc, and manganese from organic sources (Bioplex TM) can be added to broilers diets at much lower levels instead of inorganic forms of those minerals recommend levels by NRC.

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