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RESEARCH ARTICLE

The Effects of Lower Levels of Organically Complexed Zinc, Copper and Manganese in Broiler Diets on Performance, Mineral Concentration of Tibia and Mineral Excretion [1]

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Summary

The effects of replacing inorganic with lower levels of organic trace minerals of Zn, Cu and Mn on growth performance, mineral excretion and mineral concentration in tibia were investigated in broilers. One-day-old Ross-308 broiler chicks were used. Chicks were divided into 4 different groups as 1 control and 3 experimental groups consisted of 50 chicks. All groups were also divided into 5 replicates containing 10 chicks. Starter (0-21 d) and finisher (21-42 d) broiler diets which not included Zn, Cu and Mn were used. Specially prepared mineral premix (containing at 40 mg Zn, 80 mg Cu and 60 mg Mn levels of inorganic minerals in each kg as sulphate form) added to control diet in the level of recommended by NRC. Mineral content of the control diet was prepared using standard inorganic mineral premix that reflects the normal supplementary levels and source of trace minerals for commercial broiler feed recommended by National Research Council. 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, 2/3 and 3/3 proportions as BioplexTM instead of inorganic forms of those mineral recommended levels by NRC. Experiment lasted 42 days. Results showed that organically complexed trace minerals can be used at a much lower concentration than the current recommended as inorganic minerals, without a negative impact on performance, while also decreasing the excess mineral excretion. Further studies are needed to determine the proper level of organic trace minerals by taking tibia mineral levels into consideration.

Keywords: *Organically complexed mineral, Poultry, Performance, Mineral Excretion*

Etlik Piliç Diyetlerinde Daha Düşük Seviyelerde Organik Çinko, Bakır ve Manganın Performans, Tibia Mineral Yoğunluğu ve Dışkı Mineral Atılımı Üzerine Etkileri

Özet

Araştırma, etlik piliç rasyonlarına inorganik formları yerine daha düşük seviyelerde organik bakır, çinko ve mangan ilavesinin etlik piliçlerde performans, tibia mineral yoğunluğu ve dışkı mineral atılımı üzerine etkilerini belirlemek amacı ile yürütüldü. Araştırmada toplam 200 adet bir günlük yaşta Ross-308 civciv kullanıldı. Civcivler biri kontrol diğer üçü deneme grubu olmak üzere her biri 10'ar civcivli beş tekrardan oluşan dört gruba tesadüfi olarak dağıtıldı. Araştırmada bakır, çinko ve mangan içermeyen standart etlik piliç büyütme (0-21 gün) ve bitirme (21-42 gün) yemleri kullanıldı. Kontrol (inorganik) grubu yemlerine kg'ında sülfat formunda 40 mg Zn, 8mg Cu ve 60 mg Mn içeren özel olarak hazırlanmış mineral karışımı NRC tarafından bildirilen seviyelerde katıldı; deneme gruplarına ise bu minerallerin sırasıyla 1/3, 2/3 ve 3/3'ü oranında organik formlarını (Bioplex TM) içeren özel olarak hazırlanmış mineral karışımı ilave edildi. Araştırma 42 gün sürdürüldü. Araştırma sonucunda, NRC tarafından etlik piliçler için bildirilen inorganik Zn, Cu ve Mn seviyeleri yerine daha düşük miktarlarda organik formlarının katılabileceği; mineral seviyelerindeki bu azaltmanın etlik piliç performansını olumsuz etkilemediği; dışkı ile atılan mineral yoğunluğunu azalttığı; etlik piliç diyetlerinde en uygun mineral seviyelerinin belirlenebilmesi için tibia mineral yoğunluğunun da göz önüne alındığı ileri çalışmalara ihtiyaç olduğu kanaatine varıldı.

Anahtar sözcükler: *Organik mineral, Kanatlı, Performans, Mineral atılımı*

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INTRODUCTION

Trace minerals such as copper, zinc and manganese play important roles in various bodily functions and are necessary to sustain life and maintain optimal health, and thus are essential nutrients for all animals. Minerals provide structure to bones and participate in many enzyme, hormone secretion pathways, and immune defense systems [1]. In commercial poultry diets, majority of trace minerals are commonly supplemented in the form of inorganic salts, such as sulfates, oxides and carbonates, to provide levels of minerals that prevent clinical deficiencies and allow the birds to reach their genetic growth potential[2]. In commercial poultry diets [3], inorganic trace minerals supplementation is much higher than those recommended by National Research Council [4]. Excessive mineral supplementation leads to wasteful and the environmental contamination. Due to increasing concerns about potential mineral pollution, there has been considerable interest in discussion on how to reduce mineral excretion without any negative effect on production performance. The use of organically complexed or chelated minerals at a much lower concentration for livestock diets have been suggested as an answer to this discussion, based on the hypothesis that such mineral complexes have a higher bioavailability than inorganic salt analogues. Organically complexed or chelated minerals have a higher bioavailability than inorganic salts. Chelates are each molecule that consists of a non-covalent bond of a mineral (either dietary essential or not) with organic ligands. Animals absorb, digest and use mineral chelates better than inorganic minerals. This means that much lower concentrations can be used in animal feeds.

In previous experiment where lower levels of organically complexed minerals (Zn, Cu and Mn) were used instead of inorganic forms of those minerals, it was reported that using at a much lower levels of organically complexed minerals did not cause to a weakness in the plasma antioxidant defence system [5] and created a negative impact on hematological and biochemical blood parameters [6]. In addition, 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 [7]. In addition, animals fed chelated sources of essential trace minerals excrete lower amounts in their feces and the risk of environmental contamination may be reduced from manure [8,9]. Therefore, the objective of the present study was to evaluate the substitution of much lower levels of organically complexed minerals (Zn, Cu and Mn) rather than inorganic forms of minerals those recommend by NRC on growth performance, mineral excretion and mineral concentration of the tibia in broilers.

MATERIAL and METHODS

Animals, Diets and Experimental Design

The experiment was conducted under protocols by The University of Mustafa Kemal, Ethical Commission Accession (No: 2009-1-1). A total of two hundred Ross-308 one-day-old broiler chickens were used in the feeding trial that lasted until the birds reached 42 d of age. The experimental animals in both sexes were divided into four groups comprising 1 control group, and three experimental groups each consisting of 50 chickens. All groups were also divided into 5 replicates containing 10 broiler chicks. The birds were given ad libitum access to feed and water. A lighting schedule of 23L: 1D was imposed throughout the experimental period. Newcastle disease vaccination was performed on d 10, whereas the Gumboro vaccination was administrated on d 18. The basal diet *(Table 1)* was formulated according to NRC [4]. Two phases were applied during the experiment: A starter (0-21 d) and finisher (21-42 d).

The control premix was also formulated to meet the requirement allowance of trace elements reported by

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

Ingredients,%	**Starter (0 to 21 d)**	**Finisher (21 to 42 d)**
Maize	51.5	55.2
Wheat	7	7
Wheat Bran	4.5	4.5
Extracted Soybean Meal	27.5	24
Fish Meal	5.5	4.3
Vegetable Ooil	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		
Cu, mg/kg	9.63	8.71
Zn, mg/kg	35.65	30.23
Mn, mg/kg	38.84	36.63

** 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; Fe, 30.0 mg; I, 2.0 mg; Se, 0.15 mg*

NRC [4], using standard inorganic mineral premix (containing 40 mg Zn as $ZnSO_4$ and 8 mg Cu as $CuSO_4$, 60 mg Mn as MnO and others trace mineral per kg) that reflects the normal supplementary levels and source of trace minerals for commercial broiler feeds. The experimental premixes were also formulated using organically complexed Zn, Cu and Mn (organic) at 1/3 (L1), 2/3 (L2) and 3/3 (L3) levels instead of inorganic forms of those minerals as Bioplex™ *(Table 2)*.

Table 2. *Source and amounts of trace minerals fed to broilers*
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, 3.0mg; 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™*

Bioplex containing amino acids from hydrolysed-soy protein is an amino acid-hydrate complex bonded with Cu, Zn, and Mn. Bioplex Cu™, Bioplex Zn™, and Bioplex Mn™ contain 100 g/kg of Cu, 150 g/kg of Zn, and 150 g/kg of Mn, respectively (Alltech UK, Standford, England).

Measurements, Sample Collection and Laboratory Analysis

Birds in each pen were individually weighed weekly for determine body weight (BW) and body weight gain (BWG). At the same days, feed intake (FI) was also recorded to determine feed conversion ratio (FCR), feed:gain (g:g). Mortality was recorded as it occurred. At the end of the experiment, the right tibia from 2 birds per each replicate was pooled and boiled for approximately 10 min in deionized water and were then cleaned of all soft tissue. At d 26, fecal samples were taken for mineral analysis. All birds per pen were placed in cages, where lighting was reduced for approximately 20 min, until sufficient droppings were produced (minimum of 50 g per 10 birds). After fecal collection, birds were returned to their original pens. Fecal samples from each pen were homogeneously mixed and frozen for analysis. Bone, basal diet and tibia samples were then ashed (550°C for 4 h). Approximately 1 g of ash samples was then dissolved in 10 ml of $HClO_4$ and boiled for 10 min, then the remainder mixed with 5 ml HNO_3. It was filtered and diluted to 50 ml flask. All samples were then analyzed using Inductively Coupled Plasma Emission Spectroscopy (ICP) by a laboratory specializing in this assay.

Statistical Analysis

The appropriate Sum of Squares Method was selected for ANOVA [10]. The data were analyzed by one-factor ANOVA using the general linear models procedure of SAS [11] 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

Broiler Performance

During the experiment, any death was not recorded. No differences in response to addition of lower levels organic Zn, Cu and Mn instead of their inorganic forms were observed among any of the performance parameters measured. There was a tendency toward improved production performance for the birds fed at 3/3 level of organic minerals (L3) in d 21, but production performance was similar among all groups the end of the experiment *(Table 3)*.

Mineral Concentration in Tibia

The mineral concentrations of tibia are shown in *Table 4*.

Trace mineral concentrations in tibia significantly decreased ($P<0.05$), when organic mineral inclusion was applied at 1/3 level of recommended inorganic levels. Except zinc concentration of tibia, this reduction in tibia mineral concentrations were restored in the birds fed at 2/3 and 3/3 level of organic minerals for Cu and Mn. The zinc concentration of tibia in the birds reached to the same value with control group only at 3/3 level of organic minerals. Not any findings were observed brings to mind the deformation of bone or articular during experiment.

Mineral Excretion

Mineral levels of basal diet and supplemented mineral levels from either the inorganic premix (control) or the organic source are shown in *Table 1* and *Table 2*. The results from mineral analysis in manure samples at d 26 are also shown in *Table 5*.

The significantly lower excretion of Zn and Mn were observed in the birds fed at 1/3 levels of organic minerals ($P<0.01$). The excretion of Zn, Cu and Mn were not significantly different between the birds fed inorganic mineral and the birds fed at 3/3 levels of organic mineral. Higher levels of organic mineral did not lead to higher mineral retention ($P<0.05$).

Table 3. *Performance of birds fed containing different levels of organic minerals*
Tablo 3. *Farklı seviyelerde organik mineral içeren diyetlerle beslenen piliçlerin performansı*

Parameters	Diets [1]					P
	Control	L1	L2	L3	SEM	
BW, g						
1 d	45.58	45.31	45.32	45.34	0.22	NS
21 d	664.40 [b]	642.76 [b]	656.96 [b]	705.60 [a]	6.23	*
42 d	1859.36	1875.24	1914	1910	14.74	NS
BWG, g/bird/week						
1 to 21 d	618.82	597.45	611.64	660.26	6.25	*
22 to 42 d	1194.96	1232.48	1243.18	1204.42	15.98	NS
1 to 42 d	1813.78	1829.93	1854.82	1864.68	14.73	NS
FI, g/birds						
1 to 21 d	993,64	911.10	970.44	953.88	9.45	NS
22 to42 d	2288.06	2282.40	2265.62	2272.44	10.19	NS
1 to 42 d	3221.70	3193.50	3236.06	3226.32	12.54	NS
FCR, feed:gain, g:g						
1 to 21 d	1.51	1.53	1.59	1.45	0.01	NS
22 to 42 d	1.93	1.86	1.82	1.89	0.02	NS
1 to 42 d	1.78	1.73	1.75	1.73	0.02	NS

[a,b] Means values within a row having differing superscripts are significantly different (P<0.05), ***NS:*** *non-significant*
[1] Control: Inorganic Zn, Cu and Mn at NRC recommended levels as sulfate, L1, L2, and L3: Organically complexed Zn, Cu and Mn at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals as Bioplex[TM], respectively

Table 4. *Trace mineral concentrations in tibia of birds fed containing different levels of organic minerals*
Tablo 4. *Farklı seviyelerde organik mineral içeren diyetlerle beslenen piliçlerin tibia iz mineral konsantrasyonları*

Trace Minerals	Diets [1]				SEM	P
	Control	L1	L2	L3		
Zn (µg/g)	124.78 [a]	111.18 [c]	116.20 [bc]	120.79 [ab]	2.24	**
Cu (µg/g)	4.50 [a]	3.08 [b]	3.47 [ab]	4.35 [a]	0.22	*
Mn (µg/g)	7.46 [a]	4.09 [b]	6.27 [a]	6.66 [a]	0.34	**

Means represent from 10 birds per treatment
[a,b,c] Means values within a row having differing superscripts are significantly different (P<0.05)
******* *P<0.05,* ******** *P<0.01*
[1] Control: Inorganic Zn, Cu and Mn at NRC recommended levels as sulfate
L1, L2, and L3: Organically complexed Zn, Cu and Mn at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals as Bioplex[TM], respectively

Table 5. *Trace mineral excretion of birds fed containing different levels of organic minerals experimental diets with different levels of organic minerals at 26 d of age*
Tablo 5. *Farklı seviyelerde organik mineral içeren diyetlerle beslenen piliçlerin 26. gündeki iz mineral atımı*

Trace Minerals	Diets [1]				SEM	P
	Control	L1	L2	L3		
Zn (µg/g/bird)	33.03 [a]	19.25 [c]	25.50 [b]	30.82 [ab]	1.47	**
Cu (µg/g/bird)	2.85	2.19	2.91	3.14	0.15	NS
Mn (µg/g/bird)	16.24 [ab]	8.91 [c]	13.92 [b]	18.86 [a]	1.07	**

Means represent from 5 birds per treatment
[a,b,c] Means values within a row having differing superscripts are significantly different (P<0.05)
NS: *non-significant,* ******** *P<0.01*
[1] Control: Inorganic Zn, Cu and Mn at NRC recommended levels as sulfate.
L1, L2, and L3: Organically complexed Zn, Cu and Mn at 1/3, 2/3 and 3/3 proportions instead of inorganic forms of those minerals as Bioplex[TM], respectively

DISCUSSION

The use of organically complexed or chelated minerals at much lower levels in premixes has been suggested as a solution to environmental pollution originated from high mineral excretion, without jeopardizing animal health and performance. In the present study, we examined the organic minerals at much lower levels instead of NRC recommended levels of those mineral as sulfate. In the present study, BWG values of birds were slightly lower than those before reported studies. Environmental temperature and relative humidity levels during the current study ranged from 31.6-32.7°C and 62.8-65.5%, respectively. These unfavorable conditions on the period of present study may be caused to low BWG. On the other hand, lower levels inclusion of organic zinc, copper, and manganese did not affect animal performance. Similarly, some researchers [2,12] reported that trace mineral at lower levels can be included the diets without any negative effect on broiler performance. Sacranie [13] indicated that the total amount of inorganic minerals in a broiler premix could be totally replaced by 20% organic minerals without affecting growth performance and, at the same time reducing the environmental contamination. The use of organic mineral sources can improve intestinal absorption of trace elements as they reduce interference from agents that form insoluble complexes with the ionic trace elements [14]. In addition, Nollet et al.[12] reported that in the starter period feed conversion rate tended to improve in broiler fed organically complexed mineral diet. However, no sigficant differences were observed in any of the growth performance parameters measured during the trial. On the other hand, Leeson [9] found that using trace minerals with greater bioavailability (Bioplex ™ trace minerals) did not affect body weight gain and had little effect on feed efficiency of broilers even when fed at 20% of the inorganic trace mineral level.

Zinc and manganese play important role in development of bone and cartilage, and Mn is essential in the prevention of perosis in chicks [15,16]. As shown in *Table 4*, mineral concentration of the tibia dramatically decreased in birds fed at 1/3 level of organic minerals. This decline may be ascribed that the interaction between minerals. This interaction could be determined and interpreted if current minerals could be individually added to the diet. Whereas, the experimental design of the current study was not appropriate to thoroughly discuss and interpret this interaction. Except zinc concentration of tibia, this reduction in tibia mineral concentrations were restored in the birds fed at 2/3 and 3/3 level of organic minerals for Cu and Mn. However, during the current study, there was no macroscopical findings related to articular damage and bone deformation such as perosis, lameness etc.

In agreement with the present study, Bao et al.[2] found that low levels inclusion of organic Zn, Cu and Mn (2 mg/kg of Cu and 20 mg/kg of diet each of Mn and Zn in each per kg of diet) into diet based mainly on sorghum and isolated soy with 20.38 mg/kg Zn, 4.20 mg/kg Cu and 14.82 mg/kg Mn from raw material, significantly decreased the concentration of Zn and Mn in tibia in comparison to the mid or high levels inclusion of those minerals. Similar to Bao et al.[2], reduction of tibia trace mineral concentration was restored when dietary levels of Zn, Mn and Cu were raise to 2/3 and 3/3 proportions of recommended inorganic levels in the current study. On the other hand, Loveridge [17] expressed that bone growth is intimately connected with overall body growth, making tibia Zn concentration a good predictor of whole-body growth. Contrary to Loveridge [17], body growth was not different among inclusion levels in the current study. These finding is an agreement with Ao et al.[18], who reported that tibia Zn concentration was lower in the birds fed 20 ppm Bioplex Zn than the birds fed 40 ppm Bioplex Zn, although weight gain was similar among these supplemental organic mineral levels.

Reduction of the amount of trace minerals in the diet would therefore reduce mineral excretion to environment. Most of studies on minerals for animal performance [19,20] which used conventional inorganic mineral mixtures, indicates that animal usually reduces the rate of growth and excretion to maintain normal tissue concentration if dietary mineral deficiency is moderate for growth**.** In the present study, reduction of the amount of Zn and Mn in manure of birds fed at 1/3 levels of recommendation in organic minerals were significant while the rate of growth was not different among inclusions levels. This could be due to the fact that organically complexed or chelated minerals have a higher bioavailability than inorganic salts [2,12,21]. On the other hand, mineral excretion was higher in manure of birds fed at 2/3 and 3/3 levels of recommendation in organic mineral than in those of birds fed at 1/3 levels of organic minerals *(Table 5)*. It is presumed that the body becomes “loaded” with these minerals [21]. Organically complexed minerals can be easily absorbed by intestines in comparison to inorganic salts but not be completely utilized in high levels. Similarly, Nollet et al.[21], indicated that the mineral excretion of birds fed at 100% and 67% portion of organic mineral premix were not significantly different from the birds fed at 100% levels of recommended levels in inorganic minerals.

As a result, using at 1/3 level of organic minerals (at 1/3 level of Zn, Cu and Mn) in broiler diet instead of recommended level of inorganic forms of those minerals did not adversely affect broiler performance and reduced the amount of mineral excreted in feces. Using at a much lower level of organic minerals (at 1/3 level of inorganic form) were decreased the tibia mineral levels but this decline was restored starting from at 2/3 level of organic minerals. Organically complexed minerals (Zn, Cu and Mn) can be supplied at a much lower levels into the broiler diets comparison to the current recommended of inorganic forms without a negative impact on performance. But further studies are needed to determine the proper levels of organic minerals by taking tibia mineral levels into consideration.

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