Kafkas Univ Vet Fak Derg
21 (4): 607-614, 2015
DOI: 10.9775/kvfd.2015.13001

KAFKAS UNIVERSITESI VETERINER FAKULTESI DERGISI
JOURNAL HOME-PAGE: http://vetdergi.kafkas.edu.tr
ONLINE SUBMISSION: http://vetdergikafkas.org

Research Article

The Effects of Different Zinc Sources and Microbial Phytase Supplementation on the Tibial Bone Properties, Strength and Zn Mineralization Broilers Fed with Diet Low Phosphorus [1]

Mustafa MİDİLLİ [1] Mustafa SALMAN [2] Ömer Hakan MUĞLALI [2] Sena ÇENESİZ [3]
Neslihan ORMANCI [4] Murat PAKDİL [5] İ. Safa GÜRCAN [6]

[1] *This study was supported by The Scientific Research Projects Unit of Abant Izzet Baysal University (Project no. 2010.24.24.347)*

[1] Department of Poultry Breeding, Faculty of Agriculture and Natural Sciences, Abant Izzet Baysal University, TR-14800 Bolu - TURKEY; [2] Department of Animal Nutrition and Nutritional Diseases, Faculty of Veterinary Medicine, Ondokuz Mayıs University, TR-55139 Samsun - TURKEY; [3] Department of Biochemistry, Faculty of Veterinary Medicine, Ondokuz Mayıs University, TR-55139 Samsun - TURKEY; [4] Samsun Veterinary Control and Research Instıtute, TR- 55200 Samsun - TURKEY; [5] Department of Mechanical Engineering, Faculty of Engineering & Architecture, Abant Izzet Baysal University, TR-14030 Bolu - TURKEY; [6] Department of Biostatistics, Division of Animal Husbandry and Animal Nutrition, Faculty of Veterinary Medicine, Ankara University, TR-06110 Ankara - TURKEY

Article Code: KVFD-2015-13001 Received: 16.01.2015 Accepted: 19.03.2015 Published Online: 19.03.2015

Abstract

This experiment was conducted to evaluate the effects of different Zn sources and microbial phytase supplemented to low available P (aP) corn-soy diets on morphometric indices of tibiotarsi, bone strength and Zn accumulation. The experimental period lasted 42 days. A total of 875 one-day-old Ross 308 male broiler chicks were randomly assigned to seven treatment groups (positive, negative and five experimental), each with five identical subgroups of 25 birds. The positive control (PC) group was fed a diet containing adequate concentration (0.45%) of available phosphorus (aP) due to mineral premix (except zinc) and feeds; the negative control (NC) group was fed a basal diet including low concentration (0.30%) of available phosphorus (aP) due to mineral premix (except zinc) and feeds; 0.30% aP and 500 FTU phytase kg-1; 0.30% aP and 75 mg Zn-proteinate kg-1; 0.30% aP and 75 mg ZnSO4 kg-1; 75 mg Zn-proteinate and 500 FTU phytase kg-1; 75 mg ZnSO4 and 500 FTU phytase kg-1 were added to the experimental groups of phytase (PH), organic zinc (OZ), inorganic zinc (IZ), organic zinc + phytase (OZ+PH) and inorganic zinc + phytase (IZ+PH) respectively. There were no significant differences among the groups in mean tibiotarsal diaphysis diameter, thickness of the medial wall, tibiotarsal index, medullary canal diameter, modulus of elasticity or breaking stress. However, diet with OZ + PH and IZ + PH supplementation had a greater influence on tibiotarsal weight and tibiotarsal weight/length index when compared to those measurements in broilers in both control groups ($P<0.001$). In addition, feed additives were seen to have a significant effect on tibiotarsal length ($P<0.01$), robusticity index ($P<0.001$), thickness of the lateral wall ($P<0.05$) and Zn content ($P<0.05$). In conclusion, the study indicated that the use of organic and inorganic Zn alone or in combination with microbial phytase improved tibial bone traits and Zn content.

Keywords: *Phytase, Zinc,Tibia, Morphometric indices, Bone strength, Broiler*

Değişik Çinko Kaynakları ve Mikrobial Fitaz Katkısının Düşük Düzeyde Fosfor İçeren Etlik Piliçlerin Tibia Kemik Özellikleri, Dayanıklılığı ve Çinko Mineralizasyonu Üzerine Etkileri

Özet

Bu araştırma, düşük yararlanılabilir fosfor (Py) içeren mısır-soya temeline dayanan diyetlere farklı çinko kaynakları ile mikrobiyal fitaz ilavesinin broylerlerde tibia morfometrik indeksler, kemik direnci ve çinko birikimi üzerine etkisini değerlendirmek amacıyla yapılmıştır. Araştırma kırk iki gün sürmüştür. Toplam 875 adet günlük Ross 308 civciv, yedi farklı deneme grupları ve her grup 25 adet erkek hayvan içeren beş alt gruptan oluşturulmuştur. Pozitif kontrol grubu, çinko içermeyen mineral ön karması ve yeterli miktarda yararlanılabilir fosfor (%0.45) içeren rasyonla beslenmiştir. Negatif kontrol grubu ise, çinko içermeyen mineral ön karması ve düşük fosfor (%0.30) içeren temel bir rasyonla beslenmiştir. Deneme grupları; Fitaz, organik çinko, inorganik çinko, organik çinko + fitaz ve inorganik çinko + fitaz rasyonlarına sırasıyla %0.30 Py ve 500 FTU fitaz/kg; %0.30 Py ve 75 mg/kg Zn-proteinat; %0.30 aP ve 75 mg/kg ZnSO4; 75 mg/kg Zn-proteinat ve 500 FTU fitaz/kg; 75 mg/kg ZnSO4 ve 500 FTU fitaz/kg katılmıştır. Tibiotarsal diafiz çapı, medial duvar kalınlığı, tibiotarsal indeksi, medullar kanal çapı, esnekliği ve kırılma direnci bakımından gruplar arasında bir farklılık bulunmamıştır. Bununla beraber, OZ + PH and IZ + PH rasyon grubuna ait tibiotarsal ağırlık, tibiotarsal ağırlık/uzunluk indeksi değerleri her iki kontrol grubundan önemli düzeyde farklılık ($P<0.001$) bulunmuştur. Ayrıca, yem ilavelerinin tibiatarsi uzunluğu ($P<0.01$), sağlamlık indeksi ($P<0.001$), lateral duvar kalınlığı ($P<0.05$) ve çinko içeriği ($P<0.05$) üzerine önemli etkisi olduğunu göstermiştir. Sonuç olarak, bu çalışma, organik ve inorganik çinkonun tek başına ya da mikrobiyal fitaz ile kombinasyon halinde kullanılması, tibia kemik özellikleri ve çinko içeriğini geliştirmede etkili olduğunu göstermiştir.

Anahtar sözcükler: *Fitaz, Çinko, Tibia, Morfometrik indeksler, Kemik direnci, Broyler*

İletişim (Correspondence)
+90 374 2534345
midilli_m@ibu.edu.tr

INTRODUCTION

Zn is an essential trace mineral that is required for growth, bone development, feathering, enzyme systems, reproduction, maintaining correct insulin levels, thyroid function and the immune system, appetite, DNA, RNA and protein production in all avian species. Zn must be added to most poultry diets to meet requirements because of the poor availability of Zn in plant feed ingredients caused by the binding of Zn by phytate [1]. The National Research Council (NRC) [2] has estimated the Zn requirement for broiler chickens to be 40 mg kg^{-1} in the diet. Burrell *et al.*[3] reported improved performance when broilers consumed diets formulated to contain 110 mg Zn kg^{-1}. Furthermore, it is common practice in the U.S. broiler industry to formulate diets that contain 100-120 mg supplemental Zn kg^{-1} [3]. Zinc is usually added to broiler diets as inorganic feed grade zinc sulfate, zinc chloride, zinc oxide, or one of the organic forms complexed to amino acids, proteins, or carbohydrates. In recent years, organic zinc sources have been increasingly used, due to their potentially higher zinc bioavailability [4] and lower manure loading [5]. However, some studies have indicated little or no difference in the bioavailability of Zn between organic and inorganic sources [6,7].

The major portion of phosphorus in plant-derived ingredients is primarily present in the form of phytate, which is a mixed salt of phytic acid and which is unavailable to poultry because they have a limited capability to utilize phytate phosphorus. In addition, phytate is capable of binding di- and trivalent cations such as Ca, Zn, Cu and Mn in very stable complexes [8], thus reducing the availability of these minerals to the animal [9]. Supplemental microbial phytase has been shown to be a very effective and practical method for improving dietary phytate P bioavailability [10]. Studies have shown that the inclusion of phytase in chicken diets improved the bioavailability of dietary Zn [11,12].

Normal bone development in birds is also influenced by nutritional factors, genetics, gender, aging and the absolute growth rate, but the most relevant factor for poultry bone strength is nutrition. Calcium and phosphorus are the primary inorganic nutrients in the bone that may be important for bone health and strength [13]. Reichmann and Connor [14] showed that the extent of bone mineralization affects bone strength, and poor mineralization has been associated with increased risk of fractures. Weak bones result in breaking during processing and lower meat grade. Also, weak legs often result in reduced feed intake, thus affecting weight gain and feed conversion ratio [15]. Thus, bone problems in poultry can have an economic cost of several hundred million dollars a year.

Bone condition is commonly used as an indicator of mineral adequacy in poultry diet [13]. A number of invasive (bone ash, breaking strength, weight, and bone volume), and noninvasive methods (ultrasound) exist to determine bone mineralization in poultry [16]. The cortical index (tibiotarsal index) was first proposed by Barnet and Nordin [17] to indicate bone mineralization by morphometric measurements. Virtama and Telkka [18] showed a significant positive correlation between this method and bone mineral content in bone mineralization. Reisenfeld [19] and Seedor *et al.*[20] similarly used the robusticity index and the bone weight/bone length index to describe bone mineralization. Tests of bone strength in the literature [21] typically report the kilograms of force required to break various bones. However, Patterson *et al.*[22] reported that stress and modulus of elasticity are better terms to use than force in making bone strength comparisons between groups of birds that may differ in body size and bone dimension.

As far as we know, there is very little information available concerning the effects of the addition of dietary organic or inorganic zinc and microbial phytase on the bone robusticity index, bone weight/bone length index and tibiotarsal index, or the comparison of these results with the mechanical properties of tibiotarsi in broilers. Therefore, the aim of this study was to determine the effects of the interaction between Zn sources and microbial phytase on the morphometric and mechanical properties and Zn content of the tibia of broiler chickens fed low-P diets.

MATERIAL and METHODS

The study was approved by the Local Ethics Committee on Animal Experiments of Abant Izzet Baysal University (AİBÜ, 01.07.2009, HADYEK/23).

Housing, Birds and Diets

This study was conducted in the poultry research farm of the Mudurnu Sureyya Astarci Vocational School of Higher Education of Abant Izzet Baysal University in Bolu, Turkey. The poultry were housed in an environmentally controlled room with 35 floor pens of 2 m × 2 m for 42 days. The temperature of the animal facility was maintained at 31-33°C on the first day of life and lowered by 2-3°C every week to 22-23°C in the final week. The lighting program was 23L: 1D during the entire trial, and the duration of the experiment was 42 days.

A total of 875 one-day old male broiler chickens (ROSS 308) were purchased from a local commercial hatchery, Organic Zn (Bioplex, Alltech, Inc., Nicholasville, KY), inorganic Zn (zinc sulphate-$ZnS0_4.7H0_2$) and microbial phytase (Karyzyme® P 500, Kartal Kimya AS, Istanbul, Turkey) were obtained from commercial suppliers. The chicks were randomly assigned to seven groups of 125 birds each, each group being further separated into five replicates of 25 birds each. The feeding program consisted of a starter diet until 21 days of age and a finisher diet until 42 days of age. Chicks were given *ad libitum* access to feed and tap water containing no detectable Zn. Diets were formulated to meet or exceed the requirements of the NRC [2] for broilers of this age. The PC group was fed a diet containing an adequate concentration (0.45%) of aP in a mineral premix without zinc. The NC group was fed a basal diet including a low concentration (0.30%) of aP in a mineral premix without zinc. This level of aP was selected to maintain the dietary available P of current NRC [2] recommendations and to ensure responses with phytase addition. For treatment groups PH, OZ, IZ, OZ+PH and IZ+PH, the basal diet was supplemented respectively with 0.30% aP and 500 FTU phytase, 0.30% aP and organic zinc (75 mg/kg of Zn from Zn-proteinate), 0.30% aP and inorganic zinc (75 mg/kg of Zn from ZnSO4), 0.30% aP, organic zinc and 500 FTU phytase, and 0.30% aP, inorganic zinc and 500 FTU phytase. Dietary treatments included the basic diet or basic diet supplemented with 75 mg/kg of Zn as feed-grade Zn sulfate from conventional inorganic sources or Zn-methionine inorganic Zn compounds.

Performance, some blood parameters, nutrient digestibility for broilers, and experimental treatments were described previously by Midilli *et al.*[23]. Organic and inorganic zn and phytase levels of diets used in the experiment are presented in *Table 1*. Chemical composition and Ingredients of the diets are shown in *Table 2*.

Sample Collection and Analysis

In this trial, 70 right tibiotarsi of Ross 308 male broilers were used to investigate the effects of Organic or inorganic Zn and microbial phytase supplement in corn-soybean meal based diets on bone characteristics. At 42 days, 10 broilers from each group (two chicks from each of the five replicates) were randomly selected and slaughtered by cut-ting the carotid arteries with subsequent exsanguination.

The right tibia of each bird was removed as a drumstick with the flesh intact. The drumsticks were labeled and immersed in boiling water (100°C) for 10 min. After cooling to room temperature, the drumsticks were defleshed by hand and the patella was removed. They were then air-dried for 24 h at room temperature.

The tibiotarsal length was measured with a dial caliper and the tibia bones were weighed on a precision balance. The thickness of the medial and lateral walls was measured as close as possible to the midpoint using a dial caliper. The diameter of the medullary canal was computed from the difference between internal and external diameter of the diaphysis. The bone weight/length index was obtained by dividing the tibia weight by its length [20]. The tibiotarsal and the robusticity indexes were determined using the following formulae:

Tibiotarsal index = diaphysis diameter - medullary canal diameter/diaphysis diameter x 100 [17] robusticity index = bone length/cube root of bone weight [19].

Following morphometric measurements, the modulus of elasticity and breaking stress and were determined by the method described by Timoshenko and Goodier [25]. The three-point bending test was applied to determine the elasticity moduli of the tibia bones. Prior to testing, the outer diameter and length of each bone were measured. During the test, the maximum load carrying capacities of the bonds were determined by applying an increasing load in conditions of 3-point bending on a universal testing machine operating with a load cell of 10 kN and a platter speed of 2.0 mm/min. Breaking stress was determined automatically by the device and the deflections of the bones were also measured. The elasticity modulus of each bone was then calculated by using the following force-deformation relationship [25]:

$$E = \frac{\left(\frac{P}{y}\right)L^3}{48I}\left(1 + 2.85\left(\frac{h}{L}\right)^2 - 0.84\left(\frac{h}{L}\right)^3\right)$$

Where E = modulus of elasticity; P/y = initial slope of load-displacement curve; L = length; h = height of specimen; I = area moment of inertia. The inner diameters of the bones were also measured from the cross-sections of the fractured bones in addition to the outer diameters to determine their area moment of inertia (I).

The broken tibias were later used for other measurements. They underwent a 48-h defatting process under the action of finally evaporating hexane. The bones were dried at 105°C for 24 h, placed in a desiccator, and weighed to determine their fat-free dry weight. The bones were then placed in a muffle furnace at 600°C for 24 h and cooled in a desiccator, and the ash weight was recorded. The resultant

Table 1. *Organic and inorganic Zn and phytase levels of diets used in the experiment, mg/kg*
Tablo 1. *Denemede kullanılan yemlerin organik ve inorganik çinko ve fitaz düzeyleri, mg/kg*

Supplemental	Positive Control (0.45% aP)	Negative Control (0.30% aP)	Phytase (0.30% aP)	Organic Zn (0.30% aP)	Inorganic Zn (0.30% aP)	Organic Zn + Phytase (0.30% aP)	Inorganic Zn + Phytase (0.30% aP)
Phytase, FTU/kg	-	-	500 FTU	-	-	-	-
Zn-proteinate	-	-	-	75	-	75 + 500 FTU	
$ZnSO_4$	-	-	-	-	75		75 + 500 FTU

FTU = phytase unit, Available Phosphorus = aP

***Table 2.** Chemical composition and ingredients of the diets (%)*
***Tablo 2.** Yemlerin bileşimi ve kimyasal kompozisyonu (%)*

Ingredients	Starter (1-21 d)		Grower (22-42 d)	
	PC	NC (Low P)	PC	NC (Low P)
Maize	48.70	48.50	53.00	54.00
Wheat	1.20	2.10	2.00	2.00
Soybean meal (46.50% CP)	41.20	41.00	34.40	34.00
Soybean oil	5.30	5.10	7.00	6.70
Limestone	1.00	1.55	1.00	1.55
Dicalcium phosphate[1]	1.85	1.00	1.85	1.00
Vitamin premix[2]	0.10	0.10	0.10	0.10
Zn-free mineral premix[3]	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25
DL-Methionine	0.15	0.15	0.15	0.15
Total	100.00	100.00	100.00	100.00
Chemical composition (Analysed)				
Dry matter	90.37	90.42	90.65	90.28
Metabolizable energy[4], kcal/kg	3035	3063	3179	3185
Crude protein, %	22.80	23.20	19.86	20.15
Ether extract, %	7.80	7.50	9.82	9.35
Starch, %	29.00	30.05	31.00	32.00
Sugar, %	5.93	5.80	5.90	5.75
Crude fiber, %	3.79	3.84	3.48	3.25
Ash, %	5.94	5.58	5.61	5.34
Ca, %	0.87	0.89	0.92	0.85
$P_{Available}$, %	0.43	0.31	0.47	0.28

[1] Contains 23% Ca and 18.10% available P; [2] Supplied per kilogram of diet: Vitamin A, 15.000 IU; cholecalciferol, 1500 ICU; vitamin E, 30.0 IU (dl-a-tocopheryl acetate); menadione, 5.0 mg; thiamine, 3.0 mg; riboflavin, 6.0 mg; niacin, 20.0 mg; panthotenic acid, 8.0 mg; pyridoxine, 5.0 mg; folic acid, 1.0 mg; vitamin B_{12}, 15 mcg; [3] Supplied per kilogram of diet : 80 mg of iron as $FeSO_4 7H_2O$, 6 mg of copper as $CuSO_4 5H_2O$, 60 mg of manganese as $MnSO_4 H_2O$, 0.35 mg of iodine as KIO_3, and 0.15 mg of selenium as sodium selenite; [4] Metabolizable energy was calculated using the equation of Carpenter and Clegg [24]

ash was dissolved on a sand heater (300VC 15 min) in 10 ml 6 N HCl and 30 ml demineralized water. The solution was transferred after filtration (ashless filters) into a 100 ml volumetric flask. The tibia Zn concentrations were measured by Perkin Elmer AAnalyst 100 Atomic Absorption spectrophotometry (Perkin Elmer Inc., Waltham, MA, USA). The components of the samples were analyzed according to the standard procedures of AOAC [26].

Statistical Analysis

All statistical analyses were performed using SPSS® (Version 14.0 for Windows, SPSS Inc., Chicago, IL, USA). Data are given as means ± standard error (SE). The normality and homogeneity of variances were checked for all variables tested by means of a Shapiro-Wilks test and Bartlett-Box test. For normally-distributed data, differences between groups were compared using one-way ANOVA [27] and means were separated using a Duncan's post hoc test [28]. All statements of significance are based on P<0.05.

RESULTS

Morphometric Parameters of Tibia Bone

The effects of feed additives on the morphometric parameters of tibia bone at 6 weeks of age are shown in Table 3. The use of organic and inorganic Zn alone or in combination with microbial phytase significantly increased (P<0.001) tibiotarsal weight and length (P<0.01) in comparison to the control groups. The tibiotarsal weight/length index was found to be significantly higher (P<0.001) in the five experimental groups than in the control groups, with the highest readings coming from the organic zinc and microbial phytase diet group. The robusticity index was lowest in the organic zinc and microbial phytase diet group (P<0.001). The diaphysis diameter data revealed no significant difference between the groups. There were no differences between the groups as to the thickness of the tibia medial wall. In contrast, the greatest thickness of the lateral wall was found in the inorganic zinc and microbial phytase diet-supplemented group (P<0.05). No significant differences between experimental treatments were observed in the medullary canal diameter or in the tibiotarsal index.

Mechanical Measurements and Zn Content of Tibia Bone

The effects of organic or inorganic Zinc and phytase, alone or in combination on the mechanical measurements and tibia Zn content of broilers fed diets low in available phosphorus are presented in *Table 4*. The tibia bone moduli of elasticity and breaking stress were not affected by dietary treatments. Dietary treatments significantly increased the percentage of Zn in tibias in comparison with the control groups (P<0.05).

DISCUSSION

The present study demonstrated that morphometric properties and Zn content of the tibia of broilers are affected when they are fed organic or inorganic Zn with or without microbial phytase supplementation.

Tibia Bone Morphometric Measures

Dietary PH, OZ, IZ, OZ +PH and IZ +PH supplementation significantly increased tibiotarsal weight (P<0.001) and length (P<0.01) when compared with the control groups in the current study *(Table 3)*. In agreement with this, Qian *et al.*[29] reported that the tibias of broilers fed with supplemental phytase (400, 600 and 800 U of phytase/kg) and inorganic P were longer and wider than those of broilers fed P-deficient diets. However, Sahraei *et al.*[30] reported that tibiotarsal weight and length were not influenced by adding 100, 150 or 200 mg/kg of either zinc oxide (72% Zn) or Bioplex Zn (15% Zn). The index of weight to length of bone was first introduced by Seedor *et al.*[20] to show significant variations in bone mineralization. In fact, this index indicates bone density, so that the higher the index the denser the bone [31]. The tibiotarsal weight/length index was found to be significantly higher (P<0.001) in the five experimental groups than in the control groups. The reason for the increased index with the supplemented diet compared to the control diet may be attributed to a significant increase in tibia weight. Also, Kocabagli [32] demonstrated that tibiotarsal weight/length index was increased when microbial phytase was supplemented at levels of 300, 500 and 700 U of phytase/kg in the diet. The result of this study contrasts with the results of Sahraei *et al.*[30], who found that a diet supplemented with 100, 150 or 200 mg/kg of either zinc oxide (72%Zn) or Bioplex Zn (15% Zn) had no significant effect on the tibiotarsal weight/length index.

There was no difference between the groups with regard to the thickness of the medial wall of the tibia. In contrast, the thickness of the lateral wall was different and the highest values for that were found in the IZ + PH and OZ + PH supplemented groups (P<0.05) *(Table 3)*. The lowest robusticity indices were found in the OZ + PH and IZ + PH diet groups (P<0.001). A low robusticity index indicates a strong bone structure [19]. A high value of the tibiotarsal index shows a high mineralization level of the bone [29]. The present study indicated that there were no significant differences (P>0.05) between the control and treatment groups with regard to mean tibiotarsal index *(Table 3)*. In contrast to these findings, Sahraei *et al.*[30] and Kocabagli [32] reported that phytase or zinc supplementation to diet significantly increased tibiotarsal index (P<0.05). The results of the present study indicated that diaphysis diameter and medullary canal diameter were not significantly different (P>0.05) between the control and treatment groups *(Table 3)*.

Mechanical Measurements and Zn Content of Tibia Bone

The skeletal integrity in poultry is affected by numerous factors, including nutritional regime, genetic factors, sex, age, management conditions and production system [30]. The stress at yield reflects the rigidity of bones as a whole, whereas the slope of the linear region of the stress versus strain curve (Young's modulus or elastic modulus) reflects the intrinsic stiffness or rigidity and material properties of bone. A high modulus values indicate the bone to be more rigid, whereas a low modulus could mean the bone is more ductile [15].

Although some small improvement in tibia breaking stress and the modulus of elasticity were observed due to supplemental organic and inorganic Zn alone or in combination with microbial phytase, these differences were not significantly different *(Table 4)*. These results are in agreement with the findings of Kocabagli [32], Perney *et al.*[33] and Sohail *et al.*[34] who reported that the inclusion of phytase in the diet improves bone strength. On the other hand, Patterson *et al.*[22] showed that the bone strength and modulus of elasticity were reduced in the tibiotarsi of broilers when they were fed a low Ca and low P diet. Further, our results agree with those of Shelton and Southern [35] and Scrimgeour *et al.*[36] who reported an influence of Zn on the mechanical properties of bones. These authors described that lower zinc content in animals' feed caused a reduction of bone integrity, bone density, and bone length, deterioration of compact bone formation, changes in the biomechanical competency of bone tissue and a decrease in the density of bones. These observations were supported by tibia Zn content data.

Table 3. *The effect of feed additives on morphometric parameters of tibia bone at 6 wk of age (mean ± SE)*
Tablo 3. *Altı haftalık yaşta tibia kemiklerinin morfometrik parametreler üzerine yem ilavelerinin etkisi (ortalama ± SE)*

Parameters	PC	NC	PH	OZ	IZ	OZ + PH	IZ + PH	P
Tibia weight mg	6754.10±355.18^{a}	7546.30±245.76^{a}	7811.20±201.81^{a}	7733.00±2.13^{a}	7681.50±287.98^{a}	10786.20±333.81^{b}	10717.40±532.88^{b}	***
Tibia length, mm	96.56±2.13^{a}	101.01±1.13^{ab}	102.00±0.57^{b}	103.06±2.93^{b}	102.13±1.03^{b}	102.99±0.92^{b}	103.08±0.89^{b}	**
Tibiotarsi Weight / Lenght ındex mg / mm	69.75±2.93^{a}	74.54±1.80^{a}	76.49±1.73^{a}	74.58±0.08^{a}	75.82±2.29^{a}	104.72±3.24^{b}	103.91±5.12^{b}	***
Robusticity İndex	5.10±0.08^{a}	5.14±0.04^{a}	5.14±0.04^{a}	5.22±0.31^{a}	5.20±0.04^{a}	4.65±0.06^{b}	4.70±0.08^{b}	***
Diaphysis diameter, mm	10.21±0.31	10.51±0.22	10.65±0.17	10.11±0.10	10.06±0.21	9.98±0.24	10.12±0.12	NS
Thickness of the medial wall, mm	1.60±0.10	1.84±0.09	1.74±0.14	1.88±0.08	1.77±0.16	1.68±0.13	1.56±0.11	NS
Thickness of the lateral wall, mm	2.23±0.08^{ab}	1.92± 0,14^{ab}	1.89±0.18^{b}	1.81± 0.37^{b}	1.79±0.19^{b}	2.24±0.12^{ab}	2.37±0.13^{a}	*
Medullary canal diameter mm	6.34±0.37	6.68±0.29	6.73±0.19	6.32±2.44	6.77±0.33	6.03±0.26	6.35±0.20	NS
Tibiotarsal index	38.20±2.44	36.42±2.58	36.70±1,85	38.43±1.56	32.00±2.49	39.66±1.84	37.25±1.67	NS

NS: *Non significant; * P<0.05;* ***ab:*** *The mean values within the same row with different superscript differ significantly *(P<0.05); **(P<0.01); ***(P<0.001)* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytase*

Table 4. *The effects of organic or inorganic Zinc and phytase, alone or in combination on the mechanical measurements and tibia Zn content of broilers fed diets low in available phosphorus (mean ± SE)*
Tablo 4. *Düşük fosforlu diyetlere organik ve inorganik çinko ile fitaz ilavesinin broylerlerde tibia kemiği mekanik özellikleri ve çinko içeriği üzerine etkileri (ortalama ± SE)*

Parameters	PC	NC	PH	OZ	IZ	OZ + PH	IZ + PH	P
Modulus of elasticity, kg /cm^2	3420±368	2454±225	2639±253	2894±276	2817±308	3095±160	2799±171	NS
Breaking stress, kg / cm^2	170±16	139±8	168±12	156±12	150±9	174±10	164±9	NS
Zn, mg/kg	279.20±9.13^{a}	277.20±18.91^{a}	329.90±14.28^{b}	291.10±10,83^{ab}	325.20±5.99^{b}	317.90±1.70^{b}	300.50±16.40^{ab}	*

NS: *Non significant; * P<0.05;* ***a,b:*** *The mean values within the same row with different superscript differ significantly (P<0.05);* ***PC:*** *Positive control,* ***NC:*** *Negative control,* ***PH:*** *Phytase,* ***OZ:*** *Organic zinc,* ***IZ:*** *Inorganic zinc,* ***OZ+PH:*** *Organic zinc + phytase,* ***IZ+PH:*** *Inorganic zinc + phytase*

According to Jongbloed *et al.*[37], bone and pancreatic zinc are the best response criteria to assess the biological value of zinc sources in monogastrics. Underwood and Suttle [38] found a gradual decrease in Zn concentration in wool, feathers and bones caused by lower zinc content in the feed. Scrimgeour *et al.*[36] found a positive correlation between dietary and bone zinc level.

The tibia Zn content was found to be significantly higher (P<0.05) in three of the experimental groups than in the control groups. The highest values for tibia Zn content were found in the PH (329.90), IZ (325.20), OZ + PH (317.90), and IZ + PH (300.50) groups. These results are consistent with the data published by Roberson and Edwards [39], Yi *et al.*[11], Mohanna and Nys [40], Ao *et al.*[41], Aksu *et al.*[42], Shelton and Southern [35]. Roberson and Edwards [39] showed that adding 600 U of phytase/kg of diet to the basal diet significantly increased bone Zn concentration (21 to 23 vs 23 to 25 mg/g), and Yi *et al.*[11] reported that the concentration and amount of Zn in tibias were linearly increased by the dietary addition of phytase and Zn. Results reported by Mohanna and Nys [40] indicated that tibia and plasma Zn concentrations increased linearly with increasing levels of dietary Zn up to 75 mg/kg. Ao *et al.*[41] demonstrated that the total tibia Zn content was linearly increased by dietary supplementing from both inorganic (ZnSO4.7H2O,) and organic Zn (a chelated zinc proteinate) sources. Aksu *et al.*[42], who reported that tibia Zn concentrations decreased with decreasing levels of dietary Zn.

Moreover, data from Shelton and Southern [35] determined that dietary inclusion of microbial phytase increased the availability of Zn and tibia Zn concentrations in broiler chicks. These beneficial effects of phytase could possibly be due to the release of minerals such as Zn from phytate complexes. As a conclusion, the results of this study indicate that microbial phytase (500 FTU/kg^{-1}) is effective in improving bone morphometric traits and Zn content in broilers fed with diets based on corn-soybean meal and containing 75 mg/kg from organic or inorganic (not deficiency) sources and low available phosphorus.

Acknowledgements

This study was supported by the Scientific Research Projects Unit of Abant Izzet Baysal University in Turkey (Project no. 2010.24.24.347) and this support is gratefully acknowledged by the authors.

REFERENCES

1. Fordyce EJ, Forbes RM, Robbins KR, Erdman Jr JW: Phytate, calcium/zinc molar ratios: Are they predictive of zinc bioavailability? *J Food Sci*, 52, 440–444, 1987. DOI: 10.1111/j.1365-2621.1987.tb06634.x

2. NRC: Nutrient Requirements of Poultry. 9th ed., Natl. Acad. Press, Washington, DC, 1994.

3. Burrell AL, Dozier WA, Davis AJ, Compton MM, Freeman ME, Vendrell PF, Ward TL: Responses of broilers to dietary zinc concentrations and sources in relation to environmental implications. *Br Poult Sci*, 45, 255-263, 2004. DOI: 10.1080/00071660410001715867

4. Salim HM, Lee HR, Jo C, Lee SK, Lee BD: Effect of sources and levels of zinc on the tissue mineral concentration and carcass quality of broilers. *Avian Biol Res*, 3, 23-29, 2010. DOI: 10.3184/175815510X12636595095213

5. Mañón A, Cantor A, Pescatore, A, Ford M, Gillespie H, Daley M: Influence of dietary supplementation of organic minerals and phytase on mineral concentration in manure of replacement pullets. *Poult Sci Assoc Ann Meeting Abstr*, 84-85, 2005.

6. Ammerman CB, Baker DH, Lewis AJ: Bioavailability of Nutrients for Animals: Amino Acids, Minerals and Vitamins. Academic Press, San Diego, CA, 1995.

7. Pimental JL, Cook ME, Greger JL: Bioavailability of zinc methionine for chicks. *Poult Sci*, 70, 1637-1639, 1991. DOI: 10.3382/ps.0701637

8. Wise A: Dietary factors determining the biological activities of phytase. *Nutr Abstr Rev*, 53, 791-806, 1993.

9. Pallauf J, Rimbach G: Nutritional significance of phytic acid and phytase. *Arch Anim Nutr*, 50, 301-319, 1997. DOI: 10.1080/17450399709386141

10. Denbow DM, Ravindran V, Kornegay ET, Yi Z, Hulet RM: Improving phosphorus availability in soybean meal for broilers by supplemental phytase. *Poult Sci*, 74, 1831-1842, 1995. DOI: 10.3382/ps.0741831

11. Yi Z, Kornegay ET, D.M, Denbow DM: Supplemental microbial phytase improves zinc utilization in broilers. *Poult Sci*, 75, 540-546, 1996. DOI: 10.3382/ps.0750540

12. Shelton JL, Southern LL: Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentration in commercial broilers. *J Appl Poult Res*, 15, 94-102, 2006. DOI: 10.1093/japr/15.1.94

13. Rath NC, Huff GR, Huff WE, Balog JM: Factors regulating bone maturity and strength in poultry. *Poult Sci*, 79, 1024-1032, 2000. DOI: 10.1093/ps/79.7.1024

14. Reichmann KG, Connor JK: Influence of dietary calcium and phosphorus on metabolism and production in laying hens. *Br Poult Sci*, 18, 633-640, 1977. DOI: 10.1080/00071667708416414

15. Rowland LOJr, Harms RH, Wilson, HR, Ross, IJ, Fry JL: Breaking strength of chick bones as an indication of dietary calcium and phosphorus adequacy. *Proc Soc Exp Biol Med*, 126, 399-401, 1967. DOI: 10.3181/00379727-126-32458

16. Onyango EM, Hester PY, Stroshine R, Adeola O: Bone densitometry as an indicator of percentage tibia ash in broiler chicks fed varying dietary calcium and phosphorus levels. *Poult Sci*, 82, 1787-1791, 2003. DOI: 10.1093/ps/82.11.1787

17. Barnet E, Nordin B: The radiological diagnosis of osteoporosis: A new approach. *Clin Radiol*, 11, 166-169, 1960. DOI: 10.1016/S0009-9260(60)80012-8

18. Virtama P, Telkka A: Cortical thickness as an estimate of mineral content of human humerus and femur. *Bt J Radiol*, 35, 623-625, 1962. DOI: 10.1259/0007-1285-35-417-632

19. Reisenfeld A: Metatarsal robusticity in bipedal rats. *Am J Phys Anthropol*, 40, 229-234, 1972. DOI: 10.1002/ajpa.1330360211

20. Seedor JG, Quarruccio HA, Thompson DD: The bisphosphonate alendronate (MK-217) inhibits bone loss due to ovariectomy in rats. *J Bone Miner Res*, 6, 339-346, 1991. DOI: 10.1359/jbmr.2005.20.2.354

21. Lott BD, Reece FN, Drott JH: Effect of preconditioning on bone breaking strength. *Poult Sci*, 59, 724-725, 1980. DOI: 10.3382/ps.0590724

22. Patterson PH, Cook ME, Crenshaw TD, Sunde ML: Mechanical properties of the tibiotarsus of broilers and poults loaded with artificial weight and fed various dietary protein levels. *Poultry Sci*, 65, 1357-1364, 1986. DOI: 10.3382/ps.0651357

23. Midilli M, Salman M, Muğlalı ÖH, Öğretmen T, Çenesiz S, Ormancı N: The effects of organic or ınorganic zinc and microbial phytase, alone or in combination, on the performance, biochemical parameters and nutrient utilization of broilers fed a diet low in available phosphorus.

Kafkas Univ Vet Fak Derg, 20, 89-96, 2014. DOI: 10.9775/kvfd.2013.9497

24. Carpenter KJ, Clegg KM: The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *J Sci Food Agric,* 7, 45-51, 1956. DOI: 10.1002/jsfa.2740070109

25. Timoshenko SP, Goodier JN: The Theory of Elasticity. McGraw-Hill. New York, 113-122, 1987.

26. AOAC: Official Methods of Analysis. 17th ed., Association of Official Analytical Chemists, Arlington, VA. 2003.

27. SPSS: SPSS for Windows, Standard Version 10.0.1, SPSS Inc., Headquarters, Chicago, Illinois. 2005.

28. Duncan DB: Multiple Range and Multiple F Tests. *Biometrics,* 11, 1-42, 1955.

29. Qian H, Veit HP, Kornegay ET, Ravindran V, Denbow DM: Effects of supplemental phytase and phosphorus on histological and other tibial bone characteristic and performances of broilers fed semi-purified diets. *Poult Sci*, 75, 618-626, 1996. DOI: 10.3382/ps.0750618

30. Sahraei M, Janmmohamdi H, Taghizadeh A, Cheraghi S: Effect of different zinc sources on tibia bone morphology and ash content of broiler chickens. *Adv Biol Res,* 6, 128-132, 2012.

31. Monteagudo MD, Hernandz ER, Seco C, Gonzales Riola J, Revilla M, Villa LF, Rico H: Comparison of the bone robusticity index and bone weight/bone length index with the results of bone densitometry and bone histomorphometry in experimental studies. *Acta Anat*, 160, 195-199, 1997. DOI: 10.1159/000148011

32. Kocabagli N: The effect of dietary phytase supplementation at different levels on tibial bone characteristics and strength in broilers. *Turk J Vet Anim Sci,* 25, 797-802, 2001.

33. Perney KM, Cantor AH, Straw ML, Herkelman KL: The effect of dietary phytase on growth performance and phosphorus utilization of broiler chicks. *Poult Sci*, 72, 2106-2114, 1993. DOI: 10.3382/ps.0722106

34. Sohail SS, Roland DA: Influence of supplemental phytase on performance of broilers four to six weeks of age. *Poult Sci*, 78, 550-555, 1999. DOI: 10.1093/ps/78.4.550

35. Shelton JL, Southern LL: Interactive effect of zinc, copper and manganese in diets for broilers. *Int J Poult Sci*, 6, 466-469, 2007. DOI: 10.3923/ijps.2007.466.469

36. Scrimgeour AG, Stahl CHH, McClung JP, Marchitelli LJ, Young AJ: Moderate zinc deficiency negatively affects biomechanical properties of tibiae independently of body composition. *J Nutr Bioch*, 18, 813-819, 2007. DOI: 10.1016/j.jnutbio.2006.12.018

37. Jongbloed A, Kemme P, De Grootte G, Lippens M and Meschy F: Bioavailability of major and trace elements, EMFEMA *(International Association of the European Manufacturers of Major, Trace and Specific Feed Mineral Materials)*, Brussels, pp.118, 2002.

38. Underwood EJ, Suttle NF: The Mineral Nutrition of Livestock. 3rd ed., CABI Publishing, Biddles Ltd., Guilford and King's Lynn, UK, 477-512, 1999.

39. Roberson KD, Edwards Jr HM: Effects of 1,25-dihydroxycholecalciferol and phytase on zinc utilization in broiler diets. *Poult Sci,* 73, 1312-1326, 1994. DOI: 10.3382/ps.0731312

40. Mohanna C, Carré B, Nys Y: Incidence of dietary viscosity on growth performance and zinc and manganese bioavailability in broilers. *Anim Feed Sci Technol*, 77, 255-266, 1999. DOI: 10.1016/S0377-8401(98)00256-9

41. Ao T, Pierce JL, Power R, Dawson KA, Pescatore AJ, Cantor AH, Ford MJ: Evaluation of bioplex Zn as an organic zinc source for chicks. *Int J Poult Sci,* 5, 808-811, 2006. DOI: 10.3923/ijps.2006.808.811

42. Aksu T, Özsoy B, Aksu DS, Yörük MA, Gül M: The effects of lower levels of organically complexed zinc,copper and manganese in broiler diets on performance, mineralconcentration of tibia and mineral excretion. *Kafkas Univ Vet Fak Derg,*17, 141-146, 2011. DOI: 10.9775/kvfd.2010.2735