

# The Effects of Organic or Inorganic 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 <sup>[1]</sup>

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## Summary

This study examined the effects of zinc (Zn) from different sources and microbial phytase on the broiler performance, biochemical parameters and digestibility of nutrients when they were added to broiler diets containing low available phosphorus. A total of 875, 1-day-old male broilers of the Ross 308 strain were randomly separated into two control groups (positive and negative) and five treatment groups each containing 125 birds; each group was divided into 5 replicates of 25 birds. The positive control (PC) group was fed a diet containing adequate concentration (0.45 %) of available phosphorus 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 due to mineral premix (except zinc) and feeds. The basal diet was supplemented with 0.30% phosphorus and 500 FTU phytase (PH); 0.30% phosphorus and organic zinc (OZ; 75 mg/kg of Zn from Zn-proteinat); 0.30% phosphorus and inorganic zinc (IZ; 75 mg/kg of Zn from ZnSO<sub>4</sub>); 0.30% phosphorus, organic zinc and 500 FTU phytase (OZ + PH); and 0.30% phosphorus, inorganic zinc and 500 FTU phytase (IZ + PH) in the treatment groups 1, 2, 3, 4 and 5, respectively. The lowest value for mean body weight was in the negative control group on a diet containing low available phosphorus. The use of supplementation with organic and inorganic zinc alone or in combination with microbial phytase significantly ( $P<0.05$ ) increased the digestibility of Zn in the male broilers. Supplementation of those diets with OZ + PH or IZ + PH was very effective for increasing the body weight, body weight gain and the feed conversion ratio. In conclusion, the effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic zinc compounds in combination with microbial phytase.

**Keywords:** Broiler, Performance, Phytase, Phosphorus, Zinc

## Düşük Fosforlu Diyetlere Organik ve İnorganik Çinko İle Mikrobiyal Fitaz İlavesinin Broilerlerde Performans, Biyokimyasal Parametreler ve Besin Madde Kullanımı Üzerine Etkisi

### Özet

Bu araştırma, düşük fosforlu diyetlere organik ve inorganik çinko ile mikrobiyal fitaz ilavesinin broilerlerde performans, biyokimyasal parametreler ve besin madde kullanımı üzerine etkisini araştırmak amacıyla yapılmıştır. Araştırmada hayvan materyali olarak toplam 875 adet günlük civciv denemeye alınmıştır. Her grupta 125 adet hayvan olacak şekilde, iki kontrol (pozitif ve negatif) ve beş deneme grubu oluşturulmuştur. Her grup 5 alt gruptan, her alt grupta ise 25 civcivden 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. Temel rasyona %0.30 fosfor ve 500 FTU fitaz ilave edilmiştir (PH). Deneme grupları 1, 2, 3, 4 ve 5 ise sırasıyla %0.30 fosfor ve organik çinko (OZ; 75 mg/kg Zn-proteinat); 0.30% fosfor ve inorganik çinko (IZ; 75 mg/kg ZnSO<sub>4</sub>); 0.30% fosfor, organik çinko ve 500 FTU fitaz (OZ + PH); ve 0.30% fosfor, inorganik çinko ve 500 FTU fitaz (IZ + PH) oluşturmuştur. Düşük fosfor içeren negatif kontrol grubunda en düşük canlı ağırlık belirlenmiştir. Organik çinko ve inorganik çinko ile mikrobiyal fitaz kombinasyonunda çinkonun sindirilebilirliği artmıştır. Rasyonlarına OZ + PH ve IZ + PH ilave edilen gruplarda canlı ağırlık ve canlı ağırlık artışı artarken, yemden yararlanma oranı olumlu yönde etkilenmiştir. Sonuç olarak, düşük fosforlu diyetlerle beslenen broilerlerde organik ve inorganik çinkonun mikrobiyal fitaz ile kombinasyonlarının yeterli olacağı kanısına varılmıştır.

**Anahtar sözcükler:** Broiler, Performans, Fitaz, Fosfor, Çinko



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## INTRODUCTION

An important consideration for phosphorus in most feedstuffs used in broiler diets is that in the phytate form it is unavailable [1]. Phytic acid is a polyanionic molecule with six phosphate groups. Phytic acid forms insoluble complexes with the divalent cations of zinc, calcium, magnesium and iron in weak acidic to neutral pH conditions. It reduces their availability in chickens [2]. The reduction in Zn availability due to binding by phytate decreases the growth rate of chickens [3]. Conversely, Lonnerdahl *et al.* [4] demonstrated that dephytinization of soybean meal increased Zn availability to chickens.

Zinc is an essential trace mineral for broiler growth. It plays a role in the immune system, reproduction, maintaining correct insulin levels, thyroid function and enzyme systems. Zinc also plays an important role in DNA, RNA and protein production. Zinc 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 [5-7]. The NRC [8] estimated the Zn requirement for broiler chickens to be 40 mg kg<sup>-1</sup> in the diet. Burrell *et al.* [9] reported improved performance when broilers consumed diets formulated to contain 110 mg Zn kg<sup>-1</sup>. Furthermore, it is common practice in the U.S. broiler industry to formulate diets that contain 100-120 mg supplemental Zn kg<sup>-1</sup> [9].

Phytase is an enzyme that hydrolyzes phytate to inositol and inorganic phosphate. That enzyme is at a low level in the chicken gastro-intestinal tract. Hydrolyzing phytate also liberates Zn and thus, adding microbial phytase to diets increases Zn availability to chicks [10,11]. Animal nutritionists have long regarded phytate as both indigestible and an anti-nutritional factor in non-ruminant animals [12]. An organically complexed mineral is linked to protein/peptide/amino acids and has a higher bioavailability than those in inorganic salts [13] and lower manure loading [14]. The lack of phytase activity within the digestive tract results in phytate phosphorus and other minerals that are bound to it to be poorly digested. The phytase enzyme can be added to the diet of broilers to hydrolyze phytate within the digestive tract, resulting in phytate phosphorus and bound minerals being available for use by the animal and decreasing the need for inorganic phosphorus supplementation [15]. In addition to, replacing inorganic P with exogenous phytase has some additional benefits - it is more environmentally helpful due to reduced P excretion, and in the long run, may be more cost effective. Exogenous microbial phytase is a common ingredient added to broiler diets to improve availability of phytate P and thus reduce the P outflow into the environment in animal waste [16].

Cabahug *et al.* [17] reported that there were remarkably little differences in responses to 400 or 800 FTU kg<sup>-1</sup> phytase over a wide range of parameters in broilers. Further-

more, the addition of seven levels of phytase activity (0-1000 FTU kg<sup>-1</sup>) to broiler diets containing 7.5 and 3.0 g total P kg<sup>-1</sup> were investigated by Ravindran *et al.* [18]. While increasing phytase from 750 to 1000 FTU kg<sup>-1</sup> slightly benefited amino acid digestibility; on the contrary, weight gain, feed efficiency and apparent metabolisable energy responses to phytase reached a plateau at 750 FTU kg<sup>-1</sup>.

Therefore, in recent years, organic sources for trace minerals have been used increasingly in poultry diets [19-22]. However, only a few publications in the poultry field have reported the effects of Zn when it is supplied from organic sources. The supplementation of diets with microbial phytase increases the availability of phytate P in chicks [10,11]. Likewise, supplementation of phytase was more effective in a diet with a low concentration of available phosphorus [23]. Therefore, it would be highly desirable to supplement the low-AP diet with phytase with a high efficiency in releasing phytate P. Therefore, the objectives of the present study were to analyse the effects of the interaction between Zn source and microbial phytase on performance, some blood parameters and digestibility of nutrients in broilers.

## MATERIAL and METHODS

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

### *Poultry, Diets and Design of Experiment*

The present study was performed at the research farm of the Mudurnu Sureyya Astarçı Vocational School of Higher Education of the Abant İzzet 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 d. The initial room temperature of 31°C was gradually reduced to 21°C at 42 d. A commercial standard lighting regimen (23L:1D, 1 to 42 d) was provided by incandescent lights with intensities of 30 lx during days 1 to 7, 15 lx during days 8 to 28, and 5 lx for the remaining period. Eight hundred and seventy five 1 day old male broilers of the Ross 308 strain were obtained from a commercial hatchery (Pak Tavuk Gıda ve San. AS, Bolu, Turkey). Organic Zn (Bioplex, Alltech, Inc., Nicholasville, KY) and inorganic Zn (zinc sulphate-ZnSO<sub>4</sub>·7H<sub>2</sub>O) and microbial phytase (Karyzyme® P 500, Kartal Kimya Inc, Istanbul, Turkey) were obtained from commercial suppliers. Broiler chicks were randomly allocated to PC and NC groups and five treatment groups each containing 125 birds; each group was then divided into 5 replicate groups. The duration of the experiment was 42 days. All groups were fed broiler starter diets from days 1 to 21 and finishing diets from days 22 to 42. Chicks were given *ad libitum* access to feed and tap water containing no detectable Zn. The levels of all the essential nutrients contained in the basal diet met the requirements suggested by the NRC [8]. The PC group was

fed a diet containing adequate concentration (0.45%) of available phosphorus due to mineral premix (except zinc) and feeds. The NC group was fed a basal diet including low concentration (0.30%) of available phosphorus due to mineral premix (except zinc) and feeds. This level of available phosphorus was selected to maintain the dietary available P of current NRC [8] recommendations and to ensure responses with phytase additions. The basal diet was supplemented with 0.30% phosphorus and 500 FTU phytase; 0.30% phosphorus and organic zinc (75 mg/kg of Zn from Zn-proteinat); 0.30% phosphorus and inorganic zinc (75 mg/kg of Zn from ZnSO<sub>4</sub>); 0.30% phosphorus, organic zinc and 500 FTU phytase; or 0.30% phosphorus, inorganic zinc and 500 FTU phytase, for the treatment groups 1, 2, 3, 4 and 5, respectively. 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. The ingredients and the nutrient composition of the basal diets are shown in Table 1.

### Determination of Body Weight and Feed Intake

Broilers were weighed during the study period to determine body weight (BW) and body weight gain (BWG) at weekly intervals. Feed consumption (FC) was observed weekly. Feed conversion ratio (FCR) was calculated as feed-to-gain ratio on day 7, 14, 21, 28, 35 and 42 and over days 1 to 42 of the experiment.

### Sample Collection and Analysis

For determination of the digestibility of nutrients at 28 days of age, clean stainless steel collection trays were placed under each cage (12 per treatment) and excreta from each group were collected for 72 h [25]. A sub-sample of excreta was collected in polyethylene bags, weighed and dried. Excreta were mixed thoroughly, frozen at -20°C and freeze-dried. The feed and dried excreta samples were ground to pass through a 0.5 mm screen and then mixed thoroughly before analysis. The components of the samples were determined according to the standard

**Table 1.** Ingredient and chemical composition (as-fed basis) of the basal diet (%)

**Tablo 1.** Temel rasyonun yem ham maddeleri ve besin madde bileşimi

Ingredient	Starter (days 1-21)		Grower (days 22-42)	
	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 <sup>1</sup>	1.85	1.00	1.85	1.00
Vitamin premix <sup>2</sup>	0.10	0.10	0.10	0.10
Zn-free mineral premix <sup>3</sup>	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
<b>Analysed nutrient content</b>				
Dry matter	90.37	90.42	90.65	90.28
Metabolizable energy <sup>4</sup> , kcal/kg	3035	3063	3179	3185
Crude protein, %	22.80	23.20	19.86	20.15
Crude fat, %	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 <sub>Available</sub> <sup>4</sup> , %	0.43	0.31	0.47	0.28

<sup>1</sup> Contains 23% Ca and 18.10% available P; <sup>2</sup> Supplied per kilogram of diet: Vitamin A, 15.000 IU; cholecalciferol, 1.500 ICU; vitamin E, 30.0 IU (dl- $\alpha$ -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<sub>12</sub>, 15 mcg; <sup>3</sup> Supplied per kilogram of diet: 80 mg of iron as FeSO<sub>4</sub>·7H<sub>2</sub>O, 6 mg of copper as CuSO<sub>4</sub>·5H<sub>2</sub>O, 60 mg of manganese as MnSO<sub>4</sub>·H<sub>2</sub>O, 0.35 mg of iodine as KIO<sub>3</sub>, and 0.15 mg of selenium as sodium selenite; <sup>4</sup> Metabolizable energy was calculated using the equation of Carpenter and Clegg [24]

AOAC methods [26] for crude protein, ether extract and Zn. Digestibility of any given nutrient can be calculated as follows:

$$\text{Nutrient digestibility (\%)} = \frac{[(\text{Nutrient intake} - \text{Nutrient in feces}) / \text{Nutrient intake}] \times 100}{100}$$

At 42 days, 30 broilers from each group (6 chicks from each replicate) were randomly selected and bled from the brachial vein. Blood samples were transferred to vacutainer tubes with no anticoagulant. After sampling, tubes were centrifuged at 3000 rpm for 10 min and then left at 37°C for 30 min. Serum samples were then transferred to 2-ml eppendorf microcentrifuge tubes and stored at -20°C prior to analysis. Serum triglyceride, cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and glucose levels were determined using an autoanalyser (AMS Vegasys Chemistry Analyzer) and commercial kits (Audit Diagnostics, Ireland). Serum insulin values were determined using a plate reader (Das, Italy) and its commercial kits. Serum, feed and feces levels of Zn were measured with an atomic absorption spectrophotometer (Perkin Elmer Analyst 100). The levels of phosphorus in serum were measured with a spectrophotometer (Shimadzu UV-1700 Pharma Spec, Japan) by using commercial kit (RANDOX-Co., UK) at 340 nm absorbance.

### Statistical Analysis

Data were processed with analysis of variance (ANOVA) using the Least Square Method of the SAS GLM procedure [27]. The differences among the means of groups were determined by using Duncan's multiple-range test ( $P < 0.05$ ). All results were summarized as mean  $\pm$  standard error of means.

## RESULTS

The effects of Zn supplementation from either an organic or inorganic source and microbial phytase on broiler body weight, body weight gain, feed consumption

and feed conversion are presented in Table 2 and 3. The means of a number of serum parameters, survival rates and digestibility of diet components are presented in Table 4, 5 and 6, respectively.

## DISCUSSION

The average live weights obtained in the PC, NC, PH, OZ, IZ, OZ + PH and IZ + PH groups were 2638.2, 2497.5, 2647.4, 2577.5, 2562.1, 2757.0 and 2733.9g, respectively. At the end of the research, birds fed the OZ + PH and IZ + PH diets had higher ( $P < 0.05$ ) body weight and body weight gain than the other groups (Table 2 and 3). The present study indicated that phytase supplementation of broiler diets low in phosphorus increased body weight, which was in agreement with previous reports [28,29]. Davies and Nightingale [3] reported that the reduction in Zn availability due to its binding by phytate decreased chicken growth rate. Roberson and Edwards [30] reported that 15 to 30 mg/kg of Zn supplementation increased growth rate in broilers. Burrell *et al.* [9] showed that during a 45-d study, addition of Zn to the basal diet (0, 20, 40, and 80 mg/kg of Zn from ZnSO<sub>4</sub>) significantly increased body weight gain in broilers reared under thermoneutral (TN) conditions. This is supported by the results of the present study. Better feed conversion efficiencies ( $P < 0.05$ ) were obtained with diets containing OZ + PH and IZ + PH (Table 3). These results match those of Ao *et al.* [31], Broz *et al.* [32] and Sebastian *et al.* [33]. There were no significant differences ( $P > 0.05$ ) between the control and treatment groups with regard to mean feed intake (Table 3). The results of the present study also indicated that survival rates were not significantly different ( $P > 0.05$ ) between control and treatment groups (Table 5). Burrell *et al.* [9] also reported that addition of Zn to the basal diet did not affect mortality.

In poultry nutrition, it is difficult to exclude phytates as they are the main storage forms of phosphorus in seeds. Diets based on corn and soybean meal generally contain between 2.0 and 2.5 g phytic phosphorus per kilogram.

**Table 2.** Body weight mean of groups during experimental period, g (mean  $\pm$  S.E.M)

**Tablo 2.** Deneme süresince grupların ortalama canlı ağırlıkları, g (ortalama  $\pm$  S.E.M)

Weeks	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
0	45.0 $\pm$ 0.33	45.1 $\pm$ 0.29	45.0 $\pm$ 0.31	45.8 $\pm$ 0.32	44.9 $\pm$ 0.30	45.5 $\pm$ 0.31	45.1 $\pm$ 0.32	NS
1	150.1 $\pm$ 1.37b	143.2 $\pm$ 1.28c	152.0 $\pm$ 1.56ab	148.8 $\pm$ 1.53b	147.7 $\pm$ 1.24b	154.8 $\pm$ 1.66a	154.4 $\pm$ 1.57a	*
2	411.4 $\pm$ 5.09bc	375.2 $\pm$ 5.44e	417.4 $\pm$ 6.53abc	403.48 $\pm$ 6.65dc	392.0 $\pm$ 5.24d	431.2 $\pm$ 5.92a	422.1 $\pm$ 5.96ab	*
3	797.4 $\pm$ 11.60abc	753.3 $\pm$ 10.07d	810.2 $\pm$ 10.61a	774.8 $\pm$ 9.63bcd	769.6 $\pm$ 10.16cd	822.7 $\pm$ 11.52a	805.6 $\pm$ 10.45ab	*
4	1390 $\pm$ 18.10b	1305 $\pm$ 17.72c	1412 $\pm$ 20.66b	1369 $\pm$ 18.77b	1365 $\pm$ 17.40b	1469 $\pm$ 19.09a	1466 $\pm$ 17.29a	*
5	1997 $\pm$ 24.14bc	1855 $\pm$ 23.91d	2005 $\pm$ 26.20b	1946 $\pm$ 22.20bc	1930 $\pm$ 28.87c	2108 $\pm$ 22.05a	2092 $\pm$ 22.97a	*
6	2638 $\pm$ 29.90bc	2498 $\pm$ 32.14d	2647 $\pm$ 27.44b	2578 $\pm$ 22.86bc	2562 $\pm$ 27.40cd	2757 $\pm$ 27.39a	2734 $\pm$ 23.81a	*

NS: Non significant; \*  $P < 0.05$ ; a,b,c,d,e: 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

**Table 3.** Means of the body weight gain, feed intake and feed:gain ratio by groups (mean  $\pm$  S.E.M)**Tablo 3.** Deneme gruplarının ortalama canlı ağırlık artışı, yem tüketimi ve yemden yararlanma oranı (ortalama  $\pm$  S.E.M)

Weeks	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
<b>Body weight gain (g/bird)</b>								
1	105.1 $\pm$ 1.19bc	98.1 $\pm$ 1.05d	107.1 $\pm$ 1.30 ab	103.1 $\pm$ 1.32c	102.8 $\pm$ 0.98c	109.3 $\pm$ 1.39a	109.3 $\pm$ 1.30a	*
2	261.7 $\pm$ 4.50b	231.9 $\pm$ 4.23d	265.1 $\pm$ 5.05ab	254.6 $\pm$ 5.33bc	244.1 $\pm$ 4.1dc	276.4 $\pm$ 4.46a	267.7 $\pm$ 4.63ab	*
3	382.5 $\pm$ 7.40ab	376.3 $\pm$ 5.30ab	391.1 $\pm$ 6.84a	370.5 $\pm$ 4.10b	377.6 $\pm$ 7.04ab	390.4 $\pm$ 6.30ab	383.5 $\pm$ 7.42ab	*
4	592.3 $\pm$ 8.46b	550.00 $\pm$ 8.34c	602.0 $\pm$ 12.55b	592.1 $\pm$ 11.97b	594.2 $\pm$ 12.47b	643.1 $\pm$ 8.47a	660.7 $\pm$ 12.34a	*
5	603.3 $\pm$ 11.96ab	544.7 $\pm$ 7.73e	589.9 $\pm$ 7.80bcd	577.9 $\pm$ 12.80cde	561.4 $\pm$ 17.6ed	638.5 $\pm$ 12.48a	625.3 $\pm$ 13.82ab	*
6	634.6 $\pm$ 10.80	625.1 $\pm$ 11.04	642.5 $\pm$ 8.89	627.9 $\pm$ 6.15	629.6 $\pm$ 13.47	644.9 $\pm$ 12.76	642.3 $\pm$ 6.93	NS
0-6	2593 $\pm$ 29.60bc	2452 $\pm$ 31.90d	2602 $\pm$ 27.16b	2532 $\pm$ 22.63bc	2517 $\pm$ 27.16cd	2711 $\pm$ 27.11a	2689 $\pm$ 23.56a	*
<b>Feed intake (g/bird, as-fed basis)</b>								
1	122.0 $\pm$ 2.08	119.7 $\pm$ 1.83	124.0 $\pm$ 2.75	125.8 $\pm$ 2.85	124.4 $\pm$ 0.91	125.7 $\pm$ 2.13	124.6 $\pm$ 2.70	NS
2	325.1 $\pm$ 9.49	306.0 $\pm$ 2.68	329.0 $\pm$ 11.13	330.2 $\pm$ 10.35	312.6 $\pm$ 4.95	337.1 $\pm$ 3.88	324.0 $\pm$ 16.08	NS
3	33.3 $\pm$ 19.24	548.4 $\pm$ 19.80	536.3 $\pm$ 36.24	522.3 $\pm$ 15.25	530.8 $\pm$ 45.25	516.3 $\pm$ 16.30	497.28 $\pm$ 27.98	NS
4	88.8 $\pm$ 19.36	871.1 $\pm$ 14.59	896.8 $\pm$ 36.10	909.4 $\pm$ 67.49	903.6 $\pm$ 60.13	925.6 $\pm$ 12.80	958.6 $\pm$ 75.52	NS
5	95.7 $\pm$ 52.52	951.9 $\pm$ 30.24	962.1 $\pm$ 21.56	970.5 $\pm$ 84.35	949.18 $\pm$ 145.23	1009.5 $\pm$ 86.83	972.52 $\pm$ 79.79	NS
6	1136 $\pm$ 39.17	1200 $\pm$ 48.23	1127 $\pm$ 45.05	1158 $\pm$ 16.65	1137 $\pm$ 103.82	1104 $\pm$ 60.77	1096 $\pm$ 26.27	NS
0-6	4001 $\pm$ 24.89	3997 $\pm$ 70.90	3975 $\pm$ 59.18	4016 $\pm$ 41.61	3958 $\pm$ 93.69	4018 $\pm$ 35.34	3973 $\pm$ 53.81	NS
<b>Feed:Gain ratio</b>								
1	1.16 $\pm$ 0.008b	1.22 $\pm$ 0.007a	1.16 $\pm$ 0.006b	1.22 $\pm$ 0.010a	1.21 $\pm$ 0.007a	1.15 $\pm$ 0.007b	1.14 $\pm$ 0.007b	*
2	1.24 $\pm$ 0.011c	1.32 $\pm$ 0.011a	1.24 $\pm$ 0.007c	1.29 $\pm$ 0.009b	1.28 $\pm$ 0.007b	1.22 $\pm$ 0.010dc	1.21 $\pm$ 0.007d	*
3	1.38 $\pm$ 0.007dc	1.45 $\pm$ 0.007a	1.36 $\pm$ 0.015d	1.41 $\pm$ 0.007b	1.40 $\pm$ 0.012bc	1.32 $\pm$ 0.011e	1.30 $\pm$ 0.007e	*
4	1.50 $\pm$ 0.011b	1.58 $\pm$ 0.013a	1.49 $\pm$ 0.011b	1.53 $\pm$ 0.013b	1.52 $\pm$ 0.016b	1.43 $\pm$ 0.013c	1.45 $\pm$ 0.016c	*
5	1.64 $\pm$ 0.011cd	1.73 $\pm$ 0.016a	1.62 $\pm$ 0.012d	1.68 $\pm$ 0.015b	1.67 $\pm$ 0.007bc	1.58 $\pm$ 0.011e	1.56 $\pm$ 0.012e	*
6	1.77 $\pm$ 0.011cd	1.88 $\pm$ 0.014a	1.75 $\pm$ 0.007d	1.83 $\pm$ 0.011b	1.80 $\pm$ 0.016bc	1.70 $\pm$ 0.011e	1.68 $\pm$ 0.011e	*
0-6	1.54 $\pm$ 0.011cd	1.63 $\pm$ 0.010a	1.53 $\pm$ 0.010d	1.58 $\pm$ 0.010b	1.57 $\pm$ 0.013bc	1.48 $\pm$ 0.011e	1.47 $\pm$ 0.009e	*

NS: Non significant; \*  $P < 0.05$ ; a,b,c,d,e: 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

**Table 4.** Means of the some blood serum parameters by groups<sup>§</sup> (mean  $\pm$  S.E.M)**Tablo 4.** Grupların ortalama bazı kan parametreleri (ortalama  $\pm$  S.E.M)

Parameters	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Triglyceride, mg/dl	323.7 $\pm$ 12.6a	224.9 $\pm$ 11.3cd	265.4 $\pm$ 17.3bc	305.0 $\pm$ 18.5ab	236.9 $\pm$ 17.2cd	247.1 $\pm$ 15.4cd	212.3 $\pm$ 8.4d	*
Cholesterol, mg/dl	187.5 $\pm$ 2.5a	190.4 $\pm$ 3.4a	190.9 $\pm$ 2.6a	177.9 $\pm$ 1.8b	186.8 $\pm$ 2.3a	171.4 $\pm$ 1.8c	177.4 $\pm$ 1.7bc	*
LDL C, mg/dl	78.2 $\pm$ 4.6a	76.6 $\pm$ 2.9a	75.8 $\pm$ 4.3ab	72.2 $\pm$ 9.5ab	70.3 $\pm$ 6.2ab	60.2 $\pm$ 1.9b	68.6 $\pm$ 4.8ab	*
HDL-C, mg/dl	102.0 $\pm$ 3.6ab	84.6 $\pm$ 5.8b	106.5 $\pm$ 7.1a	101.6 $\pm$ 9.7ab	102.7 $\pm$ 6.0ab	98.5 $\pm$ 7.4ab	105.9 $\pm$ 3.0a	*
Glucose, mg/dl	217.3 $\pm$ 14a	181.3 $\pm$ 6.7b	192.2 $\pm$ 9.5ab	177.7 $\pm$ 7.5b	196.1 $\pm$ 8.6ab	187.9 $\pm$ 6.4b	200.9 $\pm$ 8.3ab	*
Insulin $\mu$ U/ml	3.6 $\pm$ 0.01a	3.6 $\pm$ 0.02a	3.6 $\pm$ 0.03a	3.5 $\pm$ 0.02ab	3.5 $\pm$ 0.01b	3.5 $\pm$ 0.01b	3.45 $\pm$ 0.01b	*
P, mg/dl	4.46 $\pm$ 0.07ab	4.06 $\pm$ 0.11d	4.29 $\pm$ 0.06bc	4.14 $\pm$ 0.10cd	4.01 $\pm$ 0.04d	4.54 $\pm$ 0.09a	4.62 $\pm$ 0.10a	*
Zn, $\mu$ g/dl	40.5 $\pm$ 6.5c	34.6 $\pm$ 4.7c	89.07 $\pm$ 7.9b	107.2 $\pm$ 10.6b	184.7 $\pm$ 10.8a	172.2 $\pm$ 11.1a	165.9 $\pm$ 8.8a	*

\*  $P < 0.05$ ; a,b,c,d,e: 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, § Blood samples were collected to assess biochemical variables related to lipid and mineral metabolism on d 28 of study

**Table 5.** Means of survival rates by groups (mean  $\pm$  S.E.M)**Tablo 5.** Deneme boyunca grupların yaşama gücü (ortalama  $\pm$  S.E.M)

Days	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
0	100	100	100	100	100	100	100	NS
7	100	100	100	100	100	100	100	NS
14	99.20	99.20	99.20	100	99.20	100	100	NS
21	96.00	99.20	98.40	99.20	100	99.20	99.20	NS
28	99.20	99.20	97.60	99.20	99.20	98.40	98.40	NS
35	99.20	98.40	99.20	100	99.20	100	100	NS
42	97.60	96.00	100	99.20	99.20	99.20	99.20	NS
0-42	98.74	98.86	99.20	99.66	99.54	99.54	99.54	NS

NS: Non significant, PC: Positive control, NC: Negative control, PH: Phytase, OZ: Organic zinc, IZ: Inorganic zinc, OZ+PH: Organic zinc + phytase, IZ+PH: Inorganic zinc + phytas

**Table 6.** Means of nutrient digestibility in groups, % (mean  $\pm$  S.E.M)**Tablo 6.** Gruplarda ortama besin madde sindirilebilirliği, % (ortalama  $\pm$  S.E.M)

Parameters	Control		Treatment Groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Dry matter	73.6 $\pm$ 1.13ab	75.2 $\pm$ 1.51ab	76.7 $\pm$ 0.74 a	72.7 $\pm$ 1.56b	77.3 $\pm$ 1.41a	77.2 $\pm$ 1.06a	75.3 $\pm$ 0.31ab	*
Crude ash	41.8 $\pm$ 3.47ab	44.4 $\pm$ 2.03ab	44.3 $\pm$ 2.89ab	29.4 $\pm$ 1.18c	48.2 $\pm$ 3.9a	36.7 $\pm$ 2.42bc	39.7 $\pm$ 1.49b	*
Crude protein	64.5 $\pm$ 2.29c	70.3 $\pm$ 2.79abc	70.3 $\pm$ 1.18abc	66.0 $\pm$ 2.34bc	71.8 $\pm$ 1.98ab	74.1 $\pm$ 2.23a	67.8 $\pm$ 0.72abc	*
Zn	12.9 $\pm$ 0.86c	12.6 $\pm$ 0.68c	12.6 $\pm$ 0.44c	30.4 $\pm$ 2.03a	28.6 $\pm$ 1.92ab	27.3 $\pm$ 2.06ab	25.1 $\pm$ 1.64b	*

NS: Non significant, \*  $P < 0.05$ ; a,b,c,d,e: 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 + phytas

Zinc content in feed components of plant origin is positively correlated with the phytic phosphorus content, with ~10 mg of Zn to 1 g phytic phosphorus [34]. However, zinc absorption is reduced whenever diets are high in phytate. The present study indicated that the use of organic and inorganic Zn alone or in combination with microbial phytase significantly increased ( $P < 0.05$ ) the digestibility of Zn. Furthermore, because of the poor availability of Zn in plant feed ingredients due to the binding of Zn by phytate, the present study also revealed that the use of organic and inorganic Zn alone or in combination with microbial phytase are necessary in the diet of broilers. Sahin and Kucuk [35] reported that increasing supplemental Zn from 0 mg/kg to 30 mg/kg and 60 mg/kg linearly increased digestibility of dry matter, crude protein and ether extracts. The present study also indicated that better digestibility of dry matter, crude ash and ether extracts were obtained in the group given a diet supplemented with inorganic Zn (Table 6).

In the present study, supplementation with PH, OZ, IZ, OZ + PH and IZ + PH significantly increased ( $P < 0.05$ ) serum concentrations of Zn in broilers (Table 4). The results indicate that phytate, which is present in plant feed ingredients, has a strong negative effect on zinc absorption from composite meals, because the level of

zinc in NC and PC is found very low rather than treatment groups. Therefore, the present study proves that OZ + PH and IZ + PH should be added to diets of broilers. That proposal is supported by the results of Ao *et al.* [31] who reported that dietary Zn supplementation linearly increased plasma Zn concentrations in broilers kept under TN conditions. Furthermore, Zhou *et al.* [36] determined that supplementation of broiler diets with phytase increased Zn content in the plasma at 42 d, which also concurs with the results of the present study.

Zinc positively affects feed utilization through participating in the metabolism of carbohydrates, lipids and proteins [37]. However, it must be supplemented to most diets of poultry [5-7]. The digestibility of Zn by male broilers was lower in the microbial phytase, NC and PC than in the organic and inorganic Zn alone or in combination with microbial phytase (Table 6). Spears [38] and Wedekind *et al.* [20] reported greater bioefficacy for organic Zn sources than that observed for inorganic forms, including Zn oxide and Zn sulfate; consequently, organic forms of the trace element have been used with increasing frequency by the feed industry. The negative effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic Zn compounds in combination with microbial phytase. Accordingly these

results, the use of OZ + PH and IZ + PH in broiler ration exhibit a similar effect. So, it may be preferred because IZ + PH is economically more proper than OZ + PH.

Zinc is required for normal protein synthesis and metabolism, and it is also a component of insulin, which has a role in carbohydrate metabolism. Zinc is necessary for the proper functioning of many enzymatic systems, and the insulin system is probably the most important one. Because zinc plays so many important metabolic roles in the body, it is an essential element in the diet of poultry. Supplementation of the diet with OZ + PH and IZ + PH decreased cholesterol and insulin levels ( $P < 0.05$ ) compared to control groups. Furthermore, supplementation of diets with only organic or inorganic Zn did not increase insulin levels. On the contrary, they decreased insulin levels.

Zinc is also involved in lipid metabolism. The present study indicated that higher levels of serum Zn decreased the serum cholesterol levels of the OZ + PH and IZ + PH groups. Triglyceride values were highest in the PC group and lowest in the IZ + PH group (Table 5). Herzig *et al.*<sup>[39]</sup> demonstrated that there was a significant decrease of plasma cholesterol when high amounts of Zn were administered to broiler chickens. Aksu *et al.*<sup>[40]</sup> also reported the decrease of total cholesterol and LDL cholesterol, combined with the increase in HDL cholesterol, in the blood plasma of chickens when the feed mixtures were supplemented with organic complexes of Zn. In contrast, Kucuk *et al.*<sup>[41]</sup> did not report any significant changes in the concentrations of total cholesterol, triglycerides and glucose when a feed mixture was supplemented with 30 mg of Zn per kg. Furthermore, Lu and Combs<sup>[42]</sup> reported that inorganic Zn did not affect the serum cholesterol level. Their finding was in agreement with that of the present study. However, Boukaiba *et al.*<sup>[43]</sup> and Uyanık *et al.*<sup>[44]</sup> reported that dietary supplementation with inorganic Zn decreased the serum cholesterol level.

The results of the current study indicate that dietary Zn supplementation increases serum Zn levels in male broilers. The low availability of Zn in plant feed ingredients caused by the binding of Zn by phytate means that the use of organic and inorganic Zn alone or in combination with microbial phytase is necessary in their diet. Moreover, higher serum Zn levels in the OZ + PH and IZ + PH groups decreased serum cholesterol levels. Supplementation of low phosphorus broiler diets with OZ + PH or IZ + PH was very effective for increasing the body weight, body weight gain and the feed conversion ratio. To summarize, the negative effects on broilers of diets with low phosphorus levels may be overcome by the addition of inorganic or organic Zn compounds in combination with microbial phytase.

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