

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

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I. INTRODUCTION

Abstract—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; 75mg/kg of Zn from Zn-proteinate); 0.30% phosphorus and inorganic zinc (IZ; 75 mg/kg of Zn from ZnSO₄); 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.

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An important consideration for phosphorus in most feedstuffs used in broiler poultry 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], [6], [7]. The NRC [8] estimated the Zn requirement for broiler chickens to be 40mg kg⁻¹ in the diet. Burrell et al. [9] reported improved performance when broilers consumed diets formulated to contain 110mg Zn kg⁻¹. Furthermore, it is common practice in the U.S. broiler industry to formulate diets that contain 100–120mg supplemental Zn kg⁻¹ [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^{-1} phytase over a wide range of parameters in broilers. Furthermore, the addition of seven levels of phytase activity (0–1000 FTU kg^{-1}) to broiler diets containing 7.5 and 3.0g total P kg^{-1} were investigated by Ravindran et al. [18]. While increasing phytase from 750 to 1000 FTU kg^{-1} 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^{-1} .

Therefore, in recent years, organic sources for trace minerals have been used increasingly in poultry diets [19], [20], [21], [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.

II. MATERIAL AND METHODS

A. 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 2m × 2m for 42d. The initial room temperature of 31°C was gradually reduced to 21°C at 42d. A commercial standard lighting regimen (23L: 1D, 1 to 42d) 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-ZnSO₄·7H₂O) and microbial phytase (Karyzyme® P 500, Kartal KimyaInc, 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 ZnSO₄); 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 I.

B. 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.

C. 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 72h. [24]. 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 0.5mm screen and then mixed thoroughly before analysis. The components of the samples were determined according to the standard AOAC methods [25] for crude protein, ether extract and Zn. Digestibility of any given nutrient can be calculated as follows:

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

TABLE I
INGREDIENT AND CHEMICAL COMPOSITION (AS-FED BASIS) OF THE BASAL DIET (%)

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 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
Analysed nutrient content				
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
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 _{Available} , %	0.43	0.31	0.47	0.28

¹Contains 23 % Ca and 18.10 % available P; ²Supplied per kilogram of diet: Vitamin A, 15,000 IU; cholecalciferol, 1,500 ICU; vitamin E, 30.0 IU (dl- α -tocopheryl acetate); menadione, 5.0 mg; thiamine, 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 B₁₂, 15 mcg; ³Supplied per kilogram of diet : 80 mg of iron as FeSO₄·7H₂O, 6 mg of copper as CuSO₄·5H₂O, 60 mg of manganese as MnSO₄·H₂O, 0.35 mg of iodine as KIO₃, and 0.15 mg of selenium as sodium selenite; ⁴Metabolizable energy was calculated using the equation of Carpenter and Clegg [26].

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 3000rpm for 10min and then left at 37°C for 30min. 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.

D. 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.

III. RESULTS

The effects of Zn supplementation from either an organic or inorganic sources with microbial phytase on broiler body weight, body weight gain, feed consumption and feed conversion are presented in Tables II and III. The means of a number of serum parameters, survival rates and digestibility of diet components are presented in Tables IV and V, respectively.

TABLE II
MEANS OF THE BODY WEIGHT GAIN, FEED INTAKE AND FEED:GAIN RATIO BY GROUPS (MEAN ± S.E.M)

Weeks	Control		Treatment groups					P
	PC	NC	PH	OZ	IZ	OZ+PH	IZ+PH	
Body weight gain (g/bird)								
1	105.1±1.19bc	98.1±1.05d	107.1±1.30 ab	103.1±1.32c	102.8±0.98c	109.3±1.39a	109.3±1.30a	*
2	261.7±4.50b	231.9±4.23d	265.1±5.05ab	254.6±5.33bc	244.1±4.1dc	276.4±4.46a	267.7±4.63ab	*
3	382.5±7.40ab	376.3±5.30ab	391.1±6.84a	370.5±4.10b	377.6±7.04ab	390.4±6.30ab	383.5±7.42ab	*
4	592.3±8.46b	550.00±8.34c	602.0±12.55b	592.1±11.97b	594.2±12.47b	643.1±8.47a	660.7±12.34a	*
5	603.3±11.96ab	544.7±7.73e	589.9±7.80bcd	577.9±12.80cde	561.4±17.6ed	638.5±12.48a	625.3±13.82ab	*
6	634.6±10.80	625.1±11.04	642.5±8.89	627.9±6.15	629.6±13.47	644.9±12.76	642.3±6.93	NS
0-6	2593±29.60bc	2452±31.90d	2602±27.16b	2532±22.63bc	2517±27.16cd	2711±27.11a	2689±23.56a	*
Feed intake (g/bird, as-fed basis)								
1	122.0±2.08	119.7±1.83	124.0±2.75	125.8±2.85	124.4±0.91	125.7±2.13	124.6±2.70	NS
2	325.1±9.49	306.0±2.68	329.0±11.13	330.2±10.35	312.6±4.95	337.1±3.88	324.0±16.08	NS
3	533.3±19.24	548.4±19.80	536.3±36.24	522.3±15.25	530.8±45.25	516.3±16.30	497.28±27.98	NS
4	888.8±19.36	871.1±14.59	896.8±36.10	909.4±67.49	903.6±60.13	925.6±12.80	958.6±75.52	NS
5	995.7±52.52	951.9±30.24	962.1±21.56	970.5±84.35	949.18±145.23	1009.5±86.83	972.52±79.79	NS
6	1136±39.17	1200±48.23	1127±45.05	1158±16.65	1137±103.82	1104±60.77	1096±26.27	NS
0-6	4001±24.89	3997±70.90	3975±59.18	4016±41.61	3958±93.69	4018±35.34	3973±53.81	NS
Feed:Gain ratio								
1	1.16±0.008b	1.22±0.007a	1.16±0.006b	1.22±0.010a	1.21±0.007a	1.15±0.007b	1.14±0.007b	*
2	1.24±0.011c	1.32±0.011a	1.24±0.007c	1.29±0.009b	1.28±0.007b	1.22±0.010dc	1.21±0.007d	*
3	1.38±0.007dc	1.45±0.007a	1.36±0.015d	1.41±0.007b	1.40±0.012bc	1.32±0.011e	1.30±0.007e	*
4	1.50±0.011b	1.58±0.013a	1.49±0.011b	1.53±0.013b	1.52±0.016b	1.43±0.013c	1.45±0.016c	*
5	1.64±0.011cd	1.73±0.016a	1.62±0.012d	1.68±0.015b	1.67±0.007bc	1.58±0.011e	1.56±0.012e	*
6	1.77±0.011cd	1.88±0.014a	1.75±0.007d	1.83±0.011b	1.80±0.016bc	1.70±0.011e	1.68±0.011e	*
0-6	1.54±0.011cd	1.63±0.010a	1.53±0.010d	1.58±0.010b	1.57±0.013bc	1.48±0.011e	1.47±0.009e	*

NS: Non significant; *: p < 0.05; a, b, c, d and 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 III
MEANS OF THE SOME BLOOD SERUM PARAMETERS BY GROUPS[§](MEAN ± S.E.M)

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

*: p < 0.05; a, b, c and d: 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 IV
MEANS OF SURVIVAL RATES BY GROUPS (MEAN ± 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+phytase

TABLE V
MEANS OF NUTRIENT DIGESTIBILITY IN GROUPS, % (MEAN ± S.E.M)

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

*: $p < 0.05$; a, b and c: 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

IV. 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 (Tables II and III). 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 30mg/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 ZnSO₄) 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 III). 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 III). The results of the present study also indicated that survival rates were not significantly different ($P > 0.05$) between control and treatment groups (Table V). 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 2.5g phytic phosphorus per kilogram. Zinc content in feed components of plant origin is positively correlated with the phytic phosphorus content, with ~10mg of Zn to 1g 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 0mg/kg to 30mg/kg and 60mg/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 VI).

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 IV). 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 VI). 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 V). Herzig et al. [39] demonstrated that there was a significant decrease of plasma cholesterol when high amounts of Zn were administered to broiler chickens. Aksu et al. [40] 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. [41] 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 [42] 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. [43] and Uyanik et al. [44] 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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REFERENCES

- [1] Reddy NR, Sahte SK, Salunkhe DK: Phytates in legumes and cereals. *Adv In Food Res*, 28, 1-91, 1982.
- [2] Brink EJ, Dekker PR, Van Beresteijn ECH, Beynen AC: Inhibitory effect of dietary soybean protein vs. casein on magnesium absorption in rats. *J Nutr*, 121, 1374-1381, 1991.
- [3] Davies, NT, Nightingale R: The effects of phytate on intestinal absorption and secretion of zinc, and whole-body retention of zinc, copper, iron and manganese in rats. *Br J Nutr*, 34, 243-258, 1975.
- [4] Lonnerdahl B, Bell JG, Hendrickx AG, Burns RA, Keen CL: Effect of phytate removal on zinc absorption from soy formula. *Am J Clin Nutr*, 48, 1301-1306, 1988.
- [5] O'dell BL, Savage JE: Effects of phytic acid on zinc availability. *Proc Soc Exp Biol Med*, 103, 304-306, 1960.
- [6] Ellis R, Morris ER, Hill, AD, Smith JC: Phytate:zinc molar ratio, mineral and fibre content of three hospital diets. *J Am Diet Assoc*, 81, 26-29, 1960.
- [7] Fordyce EJ, Forbs, RM, Robbins, KR, Erdman JW: Phytate, calcium/zinc molar ratios. Are they predictive of zinc bioavailability? *J Food Sci*, 52, 440-444, 1987.
- [8] NRC: Nutrient requirements of poultry. 9th rev. ed. National Academy Press, Washington, DC, 1994.

- [9] 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. *Brit PoultSci*, 45, 255-263, 2004.
- [10] Simons PCM, Versteegh HAJ, Jongbloed AW, Kemme PA, Slump P, Bos KD, Wolters, MGE, Beudeker RF, Verschoor GL: Improvement of phosphorus availability by microbial phytase in broilers and pigs. *Br J Nutr*, 64, 525-540, 1990.
- [11] Yi X, Kornegay ET, Denbow DM: Effect of microbial phytase on nitrogen and amino acid digestibility and nitrogen retention of turkey poults fed corn-soybean meal diets. *PoultSci*, 75, 979-990, 1996.
- [12] Swick RA, Ivey FJ: Effects of dietary phytase addition on broiler performance in phosphorus deficient diets. *PoultSci*, 69 (Suppl. 1): 133 (Abstr), 1990.
- [13] Brown TF, Zeringue LK: Laboratory evaluations of solubility and structural integrity of complexed and chelated trace mineral supplements. *J Dairy Sci*, 77, 181-189, 1994.
- [14] Pierce JL, Shafer BL, Power R, Dawson KA: Nutritional means to lower trace mineral excretion from poultry without compromising performance. *PoultSci*, 84 (Suppl.1): 11-17, 2005.
- [15] Maenz DD: Enzymatic characteristics of phytases as they relate to their use in animal feeds. Pages 61-83 in enzymes in farm animal nutrition. MR Bedford, and GG Partridge, ed. CAB International, Wallingford, UK, 2001.
- [16] Onyango EM, Bedford MR, Adeola O: Efficacy of an evolved *Escherichia coli* phytase in diets of broiler chicks. *Poult. Sci* 84, 248-255, 2005.
- [17] Cabahug S, Ravindran V, Selle PH, Bryden WL: Response of broiler chickens to microbial phytase as influenced by dietary phytic acid and non-phytate phosphorus levels. I. Effects on bird performance and toe ash content. *Br PoultSci* 40, 660-666, 1999.
- [18] Ravindran V, Selle PH, Ravindran G, Morel PCH, Kies AK, Bryden WL: Microbial phytase improves performance, apparent metabolizable energy, and ileal amino acid digestibility of broilers fed a lysine-deficient diet. *PoultSci* 80, 338-344, 2001.
- [19] Wedekind KJ, Baker DH: Zinc bioavailability in feed-grade sources of zinc. *J AnimSci*, 68, 684-689, 1990.
- [20] Wedekind KJ, Hortin AE, Baker DH: Methodology for assessing zinc bioavailability: efficacy estimates for zinc methionine, zinc sulfate, and zinc oxide. *J AnimSci*, 70, 178-187, 1992.
- [21] Cao J, Henry PR, Guo R: Chemical characteristics and relative bioavailability of supplemental organic zinc sources for poultry and ruminants. *J AnimSci*, 78, 2039-2054, 2000.
- [22] 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. *International J PoultSci*, 5, 808-811, 2006.
- [23] Fordyce EJ, Forbs RM, Robbins KR, Erdman JW: Phytate, calcium/zinc molar ratios. Are they predictive of zinc bioavailability? *J Food Sci*, 52, 440-444, 1987.
- [24] Lessire M: Effect of feeding technique, ad libitum, dry or wet force feeding, on the metabolisable energy values of raw materials for poultry. *Br PoultSci*, 31 (4): 785-793, 1990.
- [25] AOAC: Official method of analysis. 15th ed. Association of Official Analytical Chemist. Washington DC, 1990.
- [26] Carpenter KJ, Clegg KM: The metabolizable energy of poultry feeding stuffs in relation to their chemical composition. *J Sci Food Ag*, 7, 45-51, 1956.
- [27] SAS: Statistical software: SAS Compus drive, Carry, NC 27513 USA, 2009.
- [28] Namkung H, Leeson S: Effect of phytase enzyme on dietary nitrogen-corrected apparent metabolizable energy and ileal digestibility of nitrogen and amino acids in broiler chicks. *PoultSci*, 78, 1317-1319, 1999.
- [29] Singh PK, Khatta VK, Thakur RS: Effect of phytase supplementation in maize based diet on growth performance and nutrients utilization of broiler chickens. *Indian J AnimSci*, 73, 455-458, 2003.
- [30] Roberson KD, Edwards HM: Effects of 1,25-Dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poultry Sci*, 73, 1312-1326, 1994.
- [31] Ao T, Pierce JL, Pescatore AJ, Cantor AH, Dawson KA, Ford MJ, Shafer BL: Effects of organic zinc and phytase supplementation in a maize-soybean meal diet on the performance and tissue zinc content of broiler chicks. *Br. PoultSci*, 48, 690-695, 2007.
- [32] Broz J, Oldale P, Perrin-Voltz AH, Rychen G, Schulze J, Nunes CS: Effect of supplemental phytase on performance and phosphorus

- utilization in broiler chickens fed a low phosphorus diet without addition of inorganic phosphates. *Br PoultSci*, 35, 273-280, 1994.
- [33] Sebastian S, Touchburn SP, Chavez ER, Lague PC: The effects of supplemental microbial phytase on the performance and utilization of dietary calcium, phosphorus, copper, and zinc in broiler chickens fed corn-soybean diets. *PoultSci*, 75, 729-736, 1996.
- [34] Revy PS, Jondreville C, Dourmad JY, Nys Y: Zinc in pig nutrition: The essential trace element and potential risk for the environment. *Nat. Inst. Agronomic Res, Anim Prod (Paris, France)* 16, 3-18, 2003.
- [35] Sahin K, Kucuk O: Zinc supplementation alleviates heat stress in laying Japanese quail. *J Nutr*, 133 (9): 2808-2811, 2003.
- [36] Zhou P, Yang ZB, Yang WR, Wang XY, Jiang SZ, Zhang GG: Effects of a new recombinant phytase on the performance and mineral utilization of broilers fed phosphorus deficient diets. *J ApplPoult Res*, 17, 331-339, 2008.
- [37] Macdonald RS: The role of zinc in growth and cell proliferation. *J Nutr*, 130, 1500-1508, 2000.
- [38] Spears JW: Zinc methionine for ruminants: Relative bioavailability of zinc in lambs and effects of growth and performance of growing heifers. *J AnimSci*, 67: 835-843, 1989.
- [39] Herzig I, Navratilova M, Totusek J, Suchy P, Vecerek V, Blahova J, Zraly Z: The effect of humic acid on zinc accumulation in chicken broiler tissues. *Czech J AnimSci*, 54, 121-127, 2009.
- [40] Aksu DS, Aksu T, Ozsoy B: The effects of lower supplementation levels of organically complexed minerals (zinc, copper and manganese) versus inorganic forms on hematological and biochemical parameters in broiler. *KafkasUniv Vet FakDerg*, 16, 553-559, 2010.
- [41] Kucuk O, Kahraman A, Kurt I, Yildiz N, Onmaz AC: A combination of zinc and pyridoxine supplementation to the diet of laying hens improves performance and egg quality. *Biol Trace Elem Res*, 126, 165-175, 2008.
- [42] Lu J, Combs GF: Effect of excess dietary zinc on pancreatic exocrine function in the chick. *J Nutr*, 118, 681-689, 1988.
- [43] Boukaiba N, Flament C, Acher S, Chappuis P, Piau A, Fusselier M, Dardenne M, Lemonnier D: A physiological amount of zinc supplementation: effects on nutritional, lipid, and thymic status in an elderly population. *Am J ClinNutr*, 57, 566-572, 1993.
- [44] Uyanik F, Eren M, Tuncoku G: Effect of supplemental Zn on growth, serum glucose, cholesterol, enzymes and minerals in broilers. *Pakistan J BiolSci*, 6, 745-747, 2001.