# Is Dietary Zinc Requirement of Broiler Breeder Hens at the Late Stage of Production Cycle Influenced by Phytase Supplementation?

S. P. Mirfakhraei<sup>1</sup>, M. Zaghari<sup>1\*</sup>, F. Ghaziani<sup>1</sup>, and M. Abbasi<sup>2</sup>

## ABSTRACT

This experiment was conducted to determine whether 6-phytase has a positive effect on zinc requirements, production performance, and zinc content of tissues in broiler breeders at the end of their production cycle. One hundred and twenty-eight obese Cobb-500 broiler breeder hens (>v4.9 kg) were weighed at 58 weeks of age and assigned to various treatment groups. To deplete the zinc reserves in hens, they were given a zinc-deficient diet (9.5 mg kg<sup>-1</sup> of zinc) and drank water with 35 µg L<sup>-1</sup> zinc for two weeks. Then, hens were randomly allocated to 8 dietary treatments in a factorial arrangement of two levels of phytase (0, 300 FTU kg<sup>-1</sup>) and four levels of dietary zinc (30, 60, 90, 120 mg kg<sup>-1</sup>) with four replicates of 4 hens in each. Bodyweight, egg production, egg weight, and egg quality were measured during the five-week experimental period. Added zinc significantly increased yolk weight and zinc content of yolk (P< 0.05) and plasma (P< 0.0001). Egg weight was significantly increased by adding phytase (P< 0.05). As the results of this experiment show, adding exogenous phytase can decrease the zinc requirement of broiler breeder hens by releasing 16.9% of the zinc bound to phytate.

Keywords: Dietary zinc, Exogenous phytase, Nonlinear models, Zinc bound to phytate.

## **INTRODUCTION**

Zinc is a trace mineral involved in several biological activities in poultry (Abbasi *et al.*, 2022; Fatholahi *et al.*, 2021). The results of previous studies have shown that zinc supplementation is essential in broiler breeders to achieve optimal productive and reproductive performance, as well as egg quality (Amen and Al-Daraji, 2011; Liao *et al.*, 2018; Zhang *et al.*, 2017; Zhu *et al.*, 2017). This is due to regulating reproductive hormones during sexual maturation and protein synthesis in the epithelium during egg formation (Huang *et al.*, 2019).

According to Zhang *et al.* (2017), supplementing a basal diet containing 24 mg

Zn/kg with 80 mg supplemental zinc (104 mg/kg as a final concentration) improved the performance of broiler breeders from 38 to 57 weeks of age in terms of FCR, egg-laying rate, and fertility. According to Mayer *et al.* (2019), feeding Cobb 500 broiler breeder hens with diets containing 50.3 to 170.6 mg Zn kg<sup>-1</sup> between the ages of 37 and 40 weeks resulted in higher egg production than hens in the control group. Zarghi *et al.* (2022) reported that broiler fed diets containing more than 70 mg Zn kg<sup>-1</sup> had a greater live body weight and feed intake than those fed a non-Zn supplemented diet.

The concentration of zinc in feedstuffs is low (NRC, 1994). Moreover, the utilization of zinc from plant feedstuffs is poorly utilized by chickens due to its chelation to

<sup>&</sup>lt;sup>1</sup>Department of Animal Science, College of Agriculture and Natural Resources, University of Tehran, Karaj, Islamic Republic of Iran.

<sup>&</sup>lt;sup>2</sup> Department of Animal & Poultry Nutrition, Gorgan University of Agricultural Sciences and Natural Resources, Gorgan, Islamic Republic of Iran.

<sup>\*</sup>Corresponding author; e-mail: mzaghari@ut.ac.ir

phytic acid (O'dell *et al.*, 1964). Phytate can bind minerals and protein preventing their absorption in the digestive (Urbano *et al.*, 2003). Zinc-protein-phytate complexes are formed when phytate binds to positively charged zinc and cannot be absorbed in the digestive tract (Schlegel *et al.*, 2010).

The addition of exogenous phytase to the diet of monogastric animals can hydrolyze phytate and release bound nutrients. Researchers have found that phytase improves zinc absorption and retention. According to Yi et al. (1996), over the range of 150 to 600 FTU of phytase, 0.9 mg of Zn was released for each 100 FTU of phytase. As reported by Zaghari et al. (2018) on broiler chicken, zinc equivalence values of phytase were 0.224 mg kg<sup>-1</sup> FTU (56.4%) and 0.225 mg kg<sup>-1</sup> FTU (56.2%) at 35 and 42 days of age. Morgan et al. (2017) found that, as phytase activity increased, more phytate was hydrolyzed and more zinc was released. Then, fewer ingredients are necessary to meet the exact nutrient requirement (Abbasi et al., 2015).

It is hypothesized that the hydrolysis of phytate by phytase can increase the zinc availability in broiler breeder hens. However, the effect of releasing bonded zinc from phytate on the requirement of obese broiler breeder hens has not been studied, yet. Therefore, this study was designed to evaluate the zinc requirement of broiler breeder hens in the late stage of the production cycle and fed a practical cornsoybean meal diet, supplemented with and without exogenous phytase.

### MATERIALS AND METHODS

The experiment was performed in the Poultry Research Facility of the College of Agriculture, Tehran University, Karaj, Iran.

#### Birds, Design, and Management

A total of 128 obese cobb-500 broiler breeders at 58 weeks of age were selected to

evaluate the response of birds to the experimental diets. The selection was based on egg production ability and body weight over 4,900 grams (Approximately one kilogram above the weight recommended by the Cobb-500 Broiler Breeders Nutrition Guide). A semi-purified zinc-deficient diet containing 9.5 mg Zn kg<sup>-1</sup>, 13.7% crude protein, and 2750 kcal kg<sup>-1</sup> AMEn was fed to hens for two weeks to deplete the retained zinc in their bodies. Table 1 shows the dietary composition.

After the depletion period, the hens were weighed once again. Then, they were randomly divided into eight different dietary treatments based on their average weight and production ability, using a factorial arrangement of two levels of phytase (0 and 300 FTU kg<sup>-1</sup>) (Quantum Blue, AB Vista, UK) and four levels of dietary zinc (30, 60, 90 and 120 mg/kg, calculated) with four replicates of four hens in each. Birds received a diet from 60 to 65 weeks of age. Dietary treatments were made with the addition of ZnO to the basal diet. The experimental diets are presented in Table 2. Birds were reared in floor pens (100×100 cm; 0.25 m<sup>2</sup> bird<sup>-1</sup>). Each pen was furnished with a plastic pan feeder and bell drinker, and covered with 5 cm of wood shavings. The hens followed a regular schedule of 16 hours of light, starting at 6 am. The water that was consumed had approximately 35 µg of Zn L<sup>-1</sup>, which was measured using polarography (Model VA 797 Metrohm). Egg production and egg weight were recorded daily, and the egg mass was calculated at the end of the experiment (egg weight×egg production).

## Chemical Analysis, Sampling, and Measurements

Blood plasma zinc concentration was measured by collecting blood before and at the end of the experiment. Samples of blood were collected from the brachial vein in heparin-coated tubes. Blood samples were instantly centrifuged at  $2,000 \times g$  for 15

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	Depletion	Basal
Ingredients	58-60  w	$60-65 \mathrm{w}$
-	gr k	g <sup>-1</sup>
Corn grain	-	645.948
Corn Starch	483.8	-
Soybean meal (CP= 44%)	110.8	162.6
Gluten Meal	151	2.70
Alfalfa Meal	-	87.7
Cellulose	115.3	-
Corn oil	22.6	10.0
Dicalcum phosphate (P= 18%, Ca= 22%)	-	14.8
CaCO <sub>3</sub>	77.8	65.2
NaCl	2.00	1.70
NaHCO <sub>3</sub>	2.80	2.20
$KSO_4 (K = 44.6\%)$	9.40	-
$H_3PO_4$ (P= 27.5 %)	10.90	-
Mineral-vitamin premix <sup><i>a</i></sup>	5.00	5.00
DL-Methionine	3.00	1.10
L-Lysine hydrochloride	2.60	0.800
L-Theronine	3.00	-
Experimental diet <sup>b</sup>	-	0.252
Calculated Nutrients (g kg <sup>-1</sup> )		
MEn (Kcal kg <sup>-1</sup> ) <sup><math>c</math></sup>	2750	2750
Crude Protein <sup>d</sup>	145	144
Calcium <sup>d</sup>	30.0	30.0
Available Phosphorus	3.50	3.80
Na	1.60	1.50
Dig Lys <sup>e</sup>	6.40	6.50
Dig Met <sup>e</sup>	5.80	3.20
Dig M+C <sup>e</sup>	8.10	5.30
Dig Thr <sup>e</sup>	7.80	4.60
Dig Arg <sup>e</sup>	6.20	7.40
$Zn (mg kg^{-1})$	9.50	30.0
$Zn (mg kg^{-1})^e$	11.0	46.0

Table1. Composition of basal diet (As-fed basis).

<sup>*a*</sup> Vitamin and mineral premix provided the following per kilogram of diet: Vitamin A, 12000IU; Cholecalciferol, 3000IU; Vitamin E ,50IU; Vitamin k3, 6mg; Vitamin B12, 0.35 mg; Biotin, 0.3 mg; FolicAcid , 4 mg; Niacin, 40 mg; Pantothenic acid, 25 mg; Pyridoxine, 6 mg; Riboflavine, 10 mg; Thiamine 2.5 mg. Copper (as copper sulphate), 10 mg; Iodin (as calcium iodate), 0.2 mg; Iron (as ferrous sulfate), 40 mg; Manganese (as manganese oxide), 120 mg; Selenium (as sodium selenite), 0.3 m; No added Zinc; Corn starch as carrier;

<sup>b</sup> Table 2;

<sup>c</sup> Apparent metabolizable energy in kilocalories per kilogram;

<sup>d</sup> Analyzed value;

<sup>e</sup> Calculated amino acid composition is reported on a standardized ileal digestible amino acid basis.

minutes to collect blood plasma. Plasma samples were kept at -20°C, pending zinc concentration assays. Zinc concentration in plasma was determined by the calorimetric method (Srinivasa and Manjunath, 2014), and alkaline phosphatase activity (ALP) was measured based on Keiding *et al.* (1974) using an automated spectrophotometric analyzer (enzyme-linked immunosorbent assay plate reader model no. 259293).

Egg component, shell-quality test, and shell-breaking strength measurements were done at the end of the experiment. Shellbreaking strength and shell thickness were

	Ingredients				Zinc levels
Phytase levels <sup>b</sup>	Zinc oxide <sup><math>c</math></sup>	Sand <sup>d</sup>	Phytase levels	Zinc addition	Zinc addition plus zinc content in basal diet
$\frac{(g kg^{-1})}{0}$	$\frac{(g kg^{-1})}{0.000}$	$\frac{(g kg^{-1})}{0.252}$	$\frac{(\text{unit kg}^{-1})}{0}$	$\frac{(\text{mg kg}^{-1} \text{ diet})}{0}$	$\frac{(\text{mg kg}^{-1} \text{ diet})}{46}$
0	0.044	0.208	0	30	79
0	0.088	0.164	0	60	104
0	0.132	0.120	0	90	135
0.12	0.000	0.132	300	0	46
0.12	0.044	0.088	300	30	79
0.12	0.088	0.044	300	60	104
0.12	0.132	0.000	300	90	135

 Table 2. Experimental design.<sup>a</sup>

<sup>a</sup> All diets were identical to the basal diet except for phytase and zinc content.

<sup>b</sup> Quantum<sup>®</sup> is an *E. coli* derived 6-phytase. AB Agri Ltd Woodstock Court, Blenheim Road Marlborough Business Park, UK.

<sup>c</sup> Zinc oxide content 74.5 percent zinc.

<sup>d</sup> Inert filler.

measured using an eggshell force gauge (model no. 55R1123, Instron Corp., Canton, MA) and Karl Deutsch D-56 (Wuppertal echometer 1061), respectively.

Two hens per replicate were slaughtered using a neck cutter at the end of the experiment, and the characteristics of their carcass were measured. The bone, skin, and feather samples were extracted from the carcass and weighed and frozen instantly for further analysis. Samples of bone, skin, feather, and egg yolk were dried for 24 hours at 100°C, and zinc content was determined on the dry samples after being digested in nitric acid and hydrogen peroxide by atomic spectrophotometry absorption instrument (Shelton and Southern, 2006). The nitrogen content of the feed was analyzed by the Kjeldahl procedure (method 984.13; AOAC, 2000). The zinc and calcium content of the semi-purified diet and other experimental diets were determined by atomic absorption spectrophotometry (Shelton and Southern, 2006).

## **Statistical Analyses**

Data were evaluated in a completely randomized design with a  $4 \times 2$  factorial

arrangement considering zinc and phytase levels as the main effects. The data were analyzed by the General Linear Model (GLM) procedure of the SAS Institute (2002) with pen means as the experimental unit. Differences among means were separated using the LS-MEANS option of SAS adjusted for Duncan's test at P< 0.05. Various broken-line regression models were evaluated for estimating zinc requirements from zinc dose-response data. The analyzed zinc content of diet data was utilized to estimate the required amount of zinc. The SAS NLIN procedure was used to fit one-slope broken-line and two-slope brokenline with quadratic function (Robbins et al., 2006). The significant traits with higher  $R^2$ , including yolk zinc and plasma zinc, were selected to determine the required zinc.

#### RESULTS

#### Productive Performance and Egg Quality

The effect of different levels of zinc and phytase on egg production and egg quality are presented in Table 3. Zinc and phytase levels had no significant effect on egg production, egg mass, egg weight, shell weight, shell breaking strength, and shell thickness (P> 0.05). According to our

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Treat	ments	Egg Production	Total Egg production	Egg weight	Egg mass	Yolk weight	Shell weight	Shell thickness	Shell strength
Phytase	Zinc								
U kg <sup>-1</sup>	mg kg <sup>-1</sup>	%	HH	g	g	g	g	mm	kg cm <sup>-2</sup>
	30	33.9	12.2	70.2	24.5	22.5 <sup>ab</sup>	7.54	0.28	2.78
	60	43.1	15.1	68.1	29.4	21.1 <sup>b</sup>	7.83	0.28	2.76
	90	39.7	13.9	69.4	27.6	21.1 <sup>b</sup>	7.98	0.29	2.93
	120	43.5	15.2	69.8	30.4	23.1ª	7.98	0.28	2.76
SE	Μ	4.63	1.59	0.700	3.2	0.570	0.26	0.005	0.06
0		42.2	14.9	68.7	29.4	21.2 <sup>b</sup>	7.79	0.28	2.75
300		34.9	13.3	68.0	26.5	22.7 <sup>a</sup>	7.87	0.29	2.86
SE	Μ	3.27	1.12	0.530	2.30	0.400	0.180	0.003	0.040
	30	36.1	13.4	69.4 <sup>ab</sup>	26.6	$21.7^{ab}$	7.73	$0.27^{bc}$	2.71
0	60	42.8	15.0	69.4 <sup>ab</sup>	29.6	$21.6^{ab}$	7.97	$0.27^{bc}$	2.69
	90	42.3	14.8	66.8 <sup>b</sup>	28.3	20.1 <sup>b</sup>	7.73	0.31 <sup>a</sup>	2.97
	120	47.5	16.6	69.4 <sup>ab</sup>	33.1	21.3 <sup>ab</sup>	7.76	0.26 <sup>c</sup>	2.63
	30	31.8	11.1	$71.0^{a}$	22.4	23.4 <sup>ab</sup>	7.35	$0.29^{ab}$	2.85
300	60	43.4	15.2	66.9 <sup>b</sup>	29.1	20.6 <sup>b</sup>	7.70	$0.28^{bc}$	2.82
	90	37.1	13.0	71.9 <sup>a</sup>	26.9	22.1 <sup>ab</sup>	8.23	$0.28^{bc}$	2.89
	120	39.5	13.8	70.2 <sup>a</sup>	27.7	24.9 <sup>a</sup>	8.21	$0.29^{ab}$	2.89
SE	Μ	6.55	2.25	1.06	4.60	0.800	0.370	0.007	0.080
Significance (P-value)									
Zi	nc	0.4491	0.5385	0.2632	0.6076	0.0439	0.6060	0.0658	0.1414
Phy	tase	0.3690	0.3036	0.1122	0.3889	0.0117	0.7710	0.1873	0.0713
Zinc×F	hytase	0.9300	0.9199	0.0132	0.9485	0.0572	0.5261	0.0173	0.2515

Table3. Effect of different zinc and phytase levels on production and egg quality of broiler breeders.<sup>a</sup>

<sup>*a*</sup> (a-c): Values with different superscripts within a column are significantly different at P< 0.05. Values are means of 4 replicates.

Table 4. The estimated zinc requirement of broiler breeder hens with and without phytase.

Response	With or without phytase	Equation	$\mathbb{R}^2$	P- value	Requirement (mg kg <sup>-1</sup> )
Egg production	With phytase	$Y = 44.4-0.387 \times (Z1) - 0.0002 \times (Z2)^{2}$	0.28	0.277	79
(%)	without phytase	$Y = 42.33 - 0.339 \times (Z1) - 0.0001 \times (Z2)^2$	0.42	0.157	92
Egg mass (g)	With phytase	Y= 34.50-0.005× (Z1)2 -0.104× (Z2)	0.54	0.062	85
255 mass (8)	without phytase	$Y = 30.82 - 0.093 \times (Z1) - 0.0026 \times (Z2)^{2}$	0.13	0.79	88

results, by increasing zinc content from 30 to 120 mg mg kg<sup>-1</sup>, yolk weight grew by 10% (P< 0.05). Furthermore, by adding 300 units of phytase, yolk weight increased significantly by about 7% (P< 0.01). Zinc and phytase exhibited significant interactions on egg weight (P< 0.01), yolk weight (P< 0.05), and shell thickness (P< 0.01). A diet supplemented with 300 phytase

units and zinc increased the weight of the eggs. Adding phytase to the diet with a zinc level of 90 mg kg<sup>-1</sup> increased the egg weight by almost 5 grams from 66.80 to 71.94. Table 4 summarizes the requirements, equations, P-values, and  $R^2$  of equations for egg production and egg mass responses by using the broken-line regression models. Based on the results, the estimated zinc

requirement without phytase for egg production and egg mass were 89 and 100 mg Zn kg<sup>-1</sup>, respectively. According to the broken line models, estimated zinc requirement with phytase for egg production and egg mass were 80 and 79 mg Zn kg<sup>-1</sup>, respectively.

#### **Carcass Characteristics**

The effects of different levels of zinc and phytase on carcass characteristics of broiler breeders are listed in Table 5. With varying zinc and phytase levels (P > 0.05), there was no significant effect on the weight of carcass, liver, and abdominal fat in the late stages of production.

#### **Zinc in Tissues and Blood Parameters**

Effects of dietary zinc and phytase levels on the zinc content of tissues are shown in Table 6. The zinc content of blood plasma (P< 0.0001) and egg yolk (P< 0.05) were significantly affected by the zinc levels. Zinc levels had no significant effect on the zinc content of bone, skin, and alkaline phosphatase activity (P> 0.05). Birds fed diets supplemented with phytase showed significantly higher alkaline phosphatase activity (P< 0.05) compared to the birds fed non-supplemented, however, it was not significant for zinc content of bone, blood plasma, skin, and egg yolk.

Based on the results of the broken line models, the estimated zinc requirement without phytase for zinc content of plasma and egg yolk were 101.9 and 90.2 mg kg<sup>-1</sup>,

Table 5. Effect of different zinc and phytase levels on carcass characteristics of broiler breeders.a

Treatm	nents	<b>.</b>	Carcass			Al	odominal Fat	
Phytase	Zinc	Live weight	weight	Liver	weight		weight	
U kg <sup>-1</sup>	mg kg <sup>-1</sup>	g	g	%	g	%	g	
	30	4581	3604	1.12	52.4	2.93	135	
	60	4532	3530	1.12	50.7	2.81	127	
	90	4602	3624	1.10	50.9	2.92	135	
	120	4491	3599	1.15	52.6	3.34	155	
SEM		71.9	60.1	0.05	2.48	0.420	20.3	
0		4552	3556	1.10	50.5	2.94	134	
300		4626	3623	1.14	52.8	3.05	142	
SEM		50.8	42.5	0.030	1.75	0.29	14.3	
	30	4581	3540	1.09	50.4	2.79	127	
0	60	4532	3540	1.14	52.0	3.26	147	
	90	4602	3662	1.03	47.3	2.62	121	
	120	4491	3480	1.16	52.5	3.10	142	
	30	4727	3667	1.15	54.4	3.06	143	
300	60	4515	3520	1.09	49.4	2.37	107	
	90	4625	3586	1.18	54.6	3.21	148	
	120	4637	3719	1.14	52.7	3.58	169	
SEM		101	85.1	0.750	3.51	0.590	28.6	
Significand	e (P-val	ue)						
Zinc		0.6033	0.7103	0.9398	0.9258	0.8202	0.7884	
Phytase		0.3111	0.2741	0.4884	0.3734	0.7905	0.7119	
Zinc×Ph	ytase	0.7922	0.2625	0.5777	0.5350	0.5856	0.5971	

<sup>*a*</sup> Values are means of 4 replicates.

Phytase	Zinc	D		Effects		
	Zinc		~1.1			
TT1 -		Bone	Skin	Egg yolk	Plasma	ALP
U kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	mg kg <sup>-1</sup>	$mg L^{-1}$	U L <sup>-1</sup>
	30	362	65.1	75.4 <sup>b</sup>	166 <sup>c</sup>	69.4
	60	521	99.4	$87.8^{\mathrm{a}}$	205 <sup>b</sup>	57.6
	90	446	73.4	79.6 <sup>ab</sup>	222 <sup>a</sup>	63.5
	120	478	118	85.5 <sup>ab</sup>	225 <sup>a</sup>	74.8
SEM		42.9	18.5	3.72	4.17	8.15
0		420	91.7	83.8	205	56.9 <sup>b</sup>
300		484	86.5	80.3	204	$75.8^{a}$
SEM		30.3	13.1	2.63	2.95	5.76
	30	368	59.3	72.0	165	60.4
0	60	574	107	88.0	199	58.3
	90	365	69.9	82.7	229	45.0
	120	371	130	92.6	226	63.8
	30	356	70.9	78.8	167	78.4
300	60	469	91.3	87.7	211	56.9
	90	527	77.0	76.4	215	81.9
	120	585	107	78.4	223	85.9
SEM		61.6	26.1	5.27	5.90	11.9
Significance	(P-value)					
Zinc		0.4944	0.4301	0.0545	0.0001	0.4358
Phytase		0.9726	0.2562	0.3583	0.9398	0.0354
Zinc×Phytas	e	0.6267	0.9414	0.2569	0.1752	0.4348

**Table 6.** Effect of different zinc and phytase levels on zinc content of bone, skin, egg yolk, plasma and the alkaline phosphatase activity of broiler breeders.<sup>*a*</sup>

" (a-b): Values with different superscripts within a column are significantly different at P< 0.05. Values with different superscripts within a column are significantly different at P< 0.01. Values are means of 4 replicates.

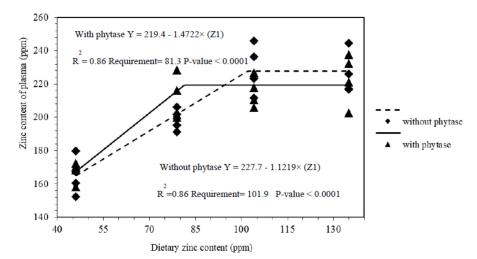
respectively (Figures 1 and 2). It was determined that 81.3 and 78.2 mg kg<sup>-1</sup> of supplemental zinc with phytase were needed by using broken-line regression to assess plasma and egg yolk zinc content, respectively.

## DISCUSSION

According to the study, increasing the amount of zinc while adding phytase led to an increase in egg weight. The highest egg weight was detected in hens given a phytase-supplemented diet and 90 mg Zn kg<sup>-1</sup>. Increasing yolk weight may explain the increase in the weight of eggs in the current study, where diets supplemented with zinc

and phytase had a significant impact on the yolk weight. Zinc may increase yolk weight by stimulating lipogenesis and fat synthesis in the liver and the subsequent transfer to the yolk during the laying process (Eder and Kirchgessner, 1995). Fatty acid synthase, lipoprotein lipase, and malate dehydrogenase are key enzymes in the metabolism of fats (Kambe et al., 2015). Liu et al. (2015) discovered that adding zinc to diets increased the activity of lipogenic enzymes such as fatty acid synthetase, lipoprotein lipase, malate and dehydrogenase.

Egg production and egg mass were not significantly affected by dietary treatment, but increased numerically when zinc levels



**Figure 1.** Zinc content of plasma response to consumption of zinc with and without phytase. Each dot (•) represents data collected over 5 weeks from one replicate.

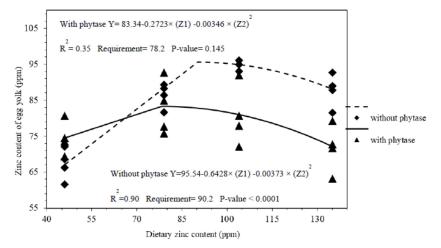


Figure 2. Zinc content of egg yolk response to consumption of zinc with and without phytase. Each dot (•) represents data collected over 5 weeks from one replicate.

were increased. Contrary to our results, Kucuk et al. (2008) reported that 30 mg Zn kg<sup>-1</sup>  $kg^{-1}$ plus 8 mg pyridoxine supplementation improved the productive performance of the laying hens. It seems that the basal diet with 46 mg Zn kg<sup>-1</sup> was sufficient to prevent significant decrease in egg production in aged broiler breeders. Consistent with our results, Lim et al. (2003) reported that dietary supplementation with phytase did not affect egg production. Meyer and Parsons (2011) showed that there was no significant difference in laying hen

production performance when they were fed diets supplemented with either 150, 250, or 15,000 FTU kg<sup>-1</sup> of phytase enzyme.

For the embryo to develop correctly, the egg needs to receive nutrients from the hen's diet. Therefore, the hen must have a good nutritional status (Wilson, 1997). An increase in the zinc content of egg yolks leads to a higher Zn availability for growth of chicken embryo. According to our research, zinc content in the egg yolk and blood plasma is linearly correlated with dietary zinc levels. There is a clear

correlation between the level of zinc in the diet and the amount of zinc absorbed into the bloodstream and, ultimately, into the egg yolk (Trindade Neto *et al.*, 2011). Similar results were found in studies by Ao *et al.* (2007) and Sunder *et al.* (2008). Guo *et al.* (2002) found that supplementing laying hen diets with zinc amino acid complex increased zinc concentration in egg yolks. The result obtained here is consistent with the previous finding of Mohanna and Nys (1999) who reported that plasma zinc concentrations increased linearly with increasing levels of dietary zinc.

Based on the results, including 300 units of phytase enzyme in the diet of broiler breeders resulted in a significant increase in the alkaline phosphatase activity levels in their blood plasma, rising from 56.9 to 75.8 U L<sup>-1</sup>. There may be an association between this increase and either zinc retention or low marginal phosphorus level in the diet. Viveros *et al.* (2002) observed a 9.1% upsurge in serum ALP activity as dietary nPP decreased. Contrary to our results, Roberson and Edwards Jr (1994) found that phytase did not affect plasma ALP activity in broiler chicks.

Consuming either excess or inadequate zinc can negatively affect feed intake, growth rate, and feed conversion ratio. It can also cause problems with protein and carbohydrate metabolism, as well as abnormalities in immune responses and reproductive performance (Morgan et al., 2017; Navidshad et al., 2016). The recommended supplemental zinc levels for broiler breeder hens range from 65 to 110 mg/kg, as per widely used tables (Aviagen, 2019; Cobb-Vantress, 2016; Rostagno et al., 2017). The NRC (1994) recommended 4.5 mg hen<sup>-1</sup> d<sup>-1</sup> of zinc for breeders, but this was based on a few research reports. According to Mayer et al. (2019), Adding 85.6 mg kg<sup>-1</sup> of zinc to broiler breeders diet between 33 and 44 weeks increased their total egg production. The variation in zinc requirement across studies may be attributed to genetic factors, bird age, diet, rearing conditions, and the statistical models

employed in different experiments. Mayer et al. (2019) found that utilizing a broken-line quadratic model was more suitable for determining the zinc requirements of Cobb 500 broiler breeder hens. Therefore, we utilized the broken-line quadratic model to determine the required amount of zinc. In this experiment, the zinc requirement of aged broiler breeder hens was estimated at 96.05 mg kg<sup>-1</sup>; however, considering the mentioned estimated value in hens receiving dietary phytase, it became 79.75 mg kg<sup>-1</sup>. The estimated value for the egg yolk zinc content is near the value of 92.34 mg zinc kg<sup>-1</sup> estimated by Li et al. (2019) for Chinese yellow-feathered broiler breeder hens between 58 to 65 weeks old. In agreement with our result, Ao et al. (2007) suggested that 12 mg kg<sup>-1</sup> of supplemental zinc without phytase and 7.4 mg kg<sup>-1</sup> of supplemental zinc with phytase were required for the optimal weight gain of chicks.

#### CONCLUSIONS

As per the experiment conducted, it was estimated that the average zinc requirement for aged broiler breeder is 96.05 mg kg<sup>-1</sup>. However, considering the estimated value of hens that received dietary phytase, the requirement was reduced to 79.75 mg kg<sup>-1</sup>, which is about 16.9% less. Therefore, it can be concluded that phytase reduced the zinc requirement of obese broiler breeders at the late stage of production by releasing 16.9%  $(16.3 \text{ mg zinc kg}^{-1})$  of the bound zinc from phytate. Based on these results, the zinc equivalent of 300 FTU phytase was 16.3 mg, which can be considered in feed formulation.

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## آیا اضافه کردن فیتاز در مرحله پایانی چرخه تولید مرغ های مادر گوشتی، بر نیاز روی موثر است؟

س. پ. میرفخرائی، م. زاغری، ف. قاضیانی، و م. عباسی

## چکیدہ

این آزمایش برای برآورد تاثیر آنزیم ۶-فیتاز در مرحله پایانی چرخه تولید مرغ های مادر بر نیاز روی، عملکرد و مقدار روی ذخیره شده در بافت ها انجام شد. ۱۲۸ مرغ مادر چاق (بیشتر از ۴/۹ کیلوگرم وزن زنده) از نژاد کاب-۵۰۰ در سن ۵۸ هفتگی جهت اختصاص به تیمارهای آزمایشی مختلف وزن شدند. در ابتدا جهت تخلیه روی ذخیره شده در بدن، پرندگان به مدت ۲ هفته جیره های نیمه خالص با فقر روی (۵/۹ میلیگرم روی در کیلوگرم خوراک) و آب آشامیدنی با سطح روی ۳۵ میکروگرم در لیتر دریافت کردند. سپس مرغ ها به صورت تصادفی به ۸ تیمار با دو سطح فیتاز (۰ و ۳۰۰ واحد در کیلوگرم)، ۴ سطح روی (۳۰، ۰۰، ۰۰ مرغ ها به صورت تصادفی به ۸ تیمار با دو سطح فیتاز (۰ و ۳۰۰ واحد در کیلوگرم)، ۴ سطح روی (۳۰، ۰۰، ۰۰ مرغ ها به صورت تصادفی به ۸ تیمار با دو سطح فیتاز (۰ و ۳۰۰ واحد در کیلوگرم)، ۴ سطح روی (۳۰، ۰۰، ۲۰ میزان تولید تخم مرغ، وزن تخم مرغ و کیفیت تخم مرغ در طول ۵ هفته آزمایش ثبت شد. افزودن روی به شکل معنی داری سبب افزایش وزن زرده و مقدار روی زرده (۵۰۵۶) و غلظت روی پلاسما (۲۰۵۰) شد. وزن تخم مرغ با افزودن فیتاز به صورت معنی دار (۵۰۵۶) افزایش یافت. نتایج نشان داد افزودن فیتاز میتواند با آزاد کردن ۱۹/۹ درصد روی باند شده با فیتات، نیاز روی در مرغ های مادر چاق در سین پایانی چرخه تولید را کاهش دهد.