

## Reevaluation of Male Broiler Zinc Requirement by Dose-Response Trial Using Practical Diet with Added Exogenous Phytase

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### ABSTRACT

Some reports indicate a wide range for Zn requirements for broiler chickens i.e. from 10.6 to 105 mg kg<sup>-1</sup>. A number of factors other than dietary Zn concentration determine the need for supplementation, principally dietary phytate. Therefore, the objective of the present investigation was reevaluation of the zinc requirement for broiler, fed practical diet supplemented with phytase in a dose-response trial. A total of 768 male Ross 308 broiler chicks were used. Basal corn-soy diet deficient in Zn was supplemented with experimental diets for making 16 dietary treatments. Experimental design was a completely randomized design in a 4×4 factorial arrangement. Factors included four levels of dietary zinc (24, 54, 84 and 114 mg kg<sup>-1</sup>) and phytase (0, 100, 200, 300 FTU kg<sup>-1</sup>). Treatments were replicated four times and each had 12 birds. Linear and nonlinear functions were derived for graded levels of zinc and phytase. Results indicated that effect of dietary zinc on body weight at 42 days of age was significant (P < 0.01). The fitted quadratic model estimated 66.7, 64.8, and 60.1 mg kg<sup>-1</sup> zinc requirement for body weight at 28, 35, and 42 days of age, respectively (P < 0.001), while the fitted two slope broken line estimated 53.5, 53.8 and 57.4 mg kg<sup>-1</sup> zinc requirement for body weight at the same ages, respectively (P < 0.002). Zinc equivalence value of phytase was estimated to be 0.225 mg kg<sup>-1</sup> FTU<sup>-1</sup> and added phytase increased liver zinc storage too (P < 0.01). Estimated zinc requirement for body weight by using practical high phytate diet, low availability source of Zn, and exogenous phytase was lower than Ross 308 recommendation (60 vs 100 mg kg<sup>-1</sup>).

**Keywords:** Broiler chickens, Phytase, Practical diet, Requirement, Zinc.

### INTRODUCTION

Zinc must be present in the diets of all animals and must be supplied almost continuously, because animals have only small amounts of readily available stored body Zn (Bao *et al.*, 2009). Zinc participates as a cofactor or component of more than 300 enzymes, being important for protein and carbohydrate metabolism, growth, and reproduction (Keith *et al.*, 2000).

Table 1 shows some studies that evaluated zinc requirement of broiler chickens. There is a wide range i.e. from 10.6 to 105 mg kg<sup>-1</sup>, for Zn requirements for different traits. Batal *et al.* (2001) fed a semi purified-dextrose diet

to chicks from 8 to 22 days post-hatching, the total dietary Zn requirement was 27.1 mg Zn kg<sup>-1</sup>. Zeigler *et al.* (1961) found a similar total dietary Zn requirement of 28 mg Zn kg<sup>-1</sup> when feeding a glucose-purified soybean protein diet. Wedekind and Baker (1990) reported a total dietary Zn requirement of 33 mg Zn kg<sup>-1</sup> when a dextrose-soy protein isolate diet was fed. Dewar and Downie (1984) reported a lower Zn requirement of 18 mg Zn kg<sup>-1</sup> for chicks 0 to 3 weeks of age that were fed a starch and low sodium egg albumin diet. Zeigler *et al.* (1961) fed a casein-glucose diet, the Zn requirement was determined to be only 12 to 14 mg Zn kg<sup>-1</sup>. Emmert and Baker (1995) determined a Zn

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**Table 1.** Some studies showing the zinc requirement of broiler chickens.

References	Year	Sex	Age (Day)	Diet type	Traits evaluated	Estimated requirement (mg kg <sup>-1</sup> )
Rossi, <i>et al.</i>	2007	M <sup>a</sup>	0-42	Corn-soy	Skin tearing	105
Vieira <i>et al.</i>	2013	M	0-42	Corn-soy	Footpad integrity	100
Gomez	2008	M and F	8-21	Practical	Tibia Zn	86
Huang <i>et al.</i>	2007	M	0-21	Corn-soy	Weight gain	84
Mohanna and Nys	1999	-	5-21	Corn-soy	Tibia and plasma Zn	75
Bao <i>et al.</i>	2009	-	14-35	-	Weight gain	68
Xiudong Liao <i>et al.</i>	2013	-	22-42	Corn-soy	Tibia Zn	62
Ao <i>et al.</i>	2007	M	0-21	Corn-soy	Weight gain	37
Wedekind and Baker	1990	M	8-12	Semi purified	Weight gain	33
Ao <i>et al.</i>	2006	M	0-21	Corn-soy	Weight gain	32.8
Steinruck and Kirchgessner	1993	-	72-107	Semi purified	Weight gain	32
Zeigler <i>et al.</i>	1961	-	-	Semi purified	Weight gain	28
Batal <i>et al.</i>	2001	F <sup>b</sup>	1-3	Semi purified	Weight gain	27.1
Dewar and Downie	1984	M and F	0-3	Purified	Live weight	18
Emmert and Baker	1995	-	8-22	Purified	Weight gain	10.6

<sup>a</sup> Male, <sup>b</sup> Female.

requirement of 10.6 mg Zn kg<sup>-1</sup> for chicks 8 to 22 days post-hatching fed with a purified amino acid-cornstarch diet when weight gain was regressed on dietary Zn concentration. These requirement estimates are lower than the NRC (1994) and Ross 308 broiler management manual (Aviagen Group Ltd., 2007) Zn recommendation of 40 and 100 mg Zn kg<sup>-1</sup>, respectively. On the other hand, some reports indicated Zn requirement higher than the above-mentioned values (Table 1).

A number of factors other than dietary Zn concentration determine the need for supplementation, including dietary phytate. Phytate consists of a phosphorylated myo-inositol ring and has high chelating capacity for multivalent cations such as zinc, calcium, copper, iron, magnesium, and aluminum (Cheryan, 1980). Zinc is probably the mineral that is most susceptible to phytate complexation (Kornegay, 2001). In research with humans, chicks, swine, and rats it was observed that dietary Zn requirement was increased by dietary phytate (O'Dell and Savage, 1960; Lease, 1966; Atwal *et al.*, 1980; Morris and Ellis, 1980; Lo *et al.*, 1981; Lonnerdal *et al.*, 1989; Ao *et al.*, 2007;

Augsburger *et al.*, 2004). Morris (1986) and Oberleas and Harland (1996) reported results clearly showing that phytate was a significant factor in the development of Zn deficiency in rats and chicks.

Zinc had a potent inhibitory effect on phytase. The reason mainly is that the Zn binding caused a conformational change in the phytate, thereby rendering it less accessible to phytase (Maenz *et al.*, 1999). Additionally, it appeared that one Zn<sup>2+</sup> ion might bridge two phytate molecules over time (Champagne and Fisher, 1990). Researchers have revealed the interaction between phytase and phytate on Zn bioavailability (Schlegel *et al.*, 2013; Schlegel *et al.*, 2010; Yu *et al.*, 2010; Huang *et al.* 2013), but relatively few studies have evaluated the quantitative effect of releasing bonded zinc to phytate on broiler requirement. Therefore, this study aimed at reevaluating the zinc requirement of broiler chicks, fed practical corn soybean meal diet supplemented with exogenous phytase in a precision dose-response trial. Zinc oxide and zinc sulfate are two prevalent inorganic zinc sources for poultry feed supplementation. ZnO is highly stable but less bioavailable for

poultry than reagent-grade or feed-grade Zn sulfate (Edwards and Baker, 1999). The sulfates are highly water soluble, allowing reactive metal ions to promote free radical formation. This reaction can lead to the breakdown of vitamins and ultimately to the degradation of fats and oils, decreasing the nutrient value of the diet. Therefore, in some countries, using ZnO as an inorganic source of Zn is preferred by industry. In the present study, for following the practical condition we used ZnO for making dietary Zn graded levels.

## MATERIALS AND METHODS

An experiment was carried out to estimate zinc requirement and its equivalency value of phytase for broiler chickens. A total of 768 male Ross 308 broiler chicks were used to evaluate the response of broiler chicks to zinc deficient basal diet supplemented with phytase, and or zinc oxide. Basal corn soybean meal diets formulated for starter, grower, and finisher periods and contained 24 mg kg<sup>-1</sup> zinc (Table 2). Basal diets supplemented with 0.24 gr kg<sup>-1</sup> experimental

**Table 2.** Composition of basal diets <sup>a</sup>.

Ingredients	Starter	Grower	Finisher
	0–10 d	11–23 d	24–42 d
	gr kg <sup>-1</sup>		
Corn grain	574.50	617.90	670.33
Soybean meal (44% CP)	361.53	326.76	276.03
Corn oil	20	20	20
Dicalcium phosphate	19.98	16.90	15.94
Oyster shell	11.10	8.90	8.78
NaCl	3.51	3.40	3.26
Vit. and Min. supplement <sup>b</sup>	1.50	1.50	1.50
DL-methionine	3.50	2.50	2.20
L-lysine hydrochloride	2.90	1.40	1.31
L-threonine	1.24	0.5	0.41
Experimental diet <sup>c</sup>	0.24	0.24	0.24
Calculated nutrients	%		
AMEn (kcal kg <sup>-1</sup> )	2937	2991	3053
Crude protein	21.36	19.94	18.12
Calcium	1.019	0.85	0.81
Available phosphorus	0.48	0.42	0.40
Na	0.15	0.15	0.15
Dig Lys <sup>d</sup>	1.23	1.04	0.92
Dig Met	0.63	0.52	0.47
Dig M+C	0.91	0.79	0.722
Dig Thr	0.80	0.69	0.63
Dig Arg	1.35	1.25	1.11
Zn (mg kg <sup>-1</sup> )	24	24	24
Zn (mg kg <sup>-1</sup> ) <sup>e</sup>	30.19	32.14	34.15
Phytate (g 100 g <sup>-1</sup> ) <sup>e</sup>	0.84	0.77	0.75

<sup>a</sup> As-fed basis; <sup>b</sup> Vitamin and mineral premix provided the following per kilogram of diet: Vitamin A: 11,000 IU; Cholecalciferol: 5,000 IU; Vitamin E: 75 IU; Vitamin k3: 3 mg; Vitamin B12: 0.016 mg; Biotin: 0.15 mg; Folicin: 2 mg; Niacin: 2 mg; Pantothenic acid: 15 mg; Pyridoxine: 4 mg; Riboflavine: 8 mg; Thiamine: 3 mg. Copper (as cupric sulfate 5H<sub>2</sub>O): 16 mg; Iodin (as calciumiodate): 1.2 mg; Iron (as ferrous sulfate 4H<sub>2</sub>O): 40 mg; Manganese (as manganese oxide): 120 mg; Selenium (as sodium selenite): 0.3 m, and No added Zinc; <sup>c</sup> Table 3; <sup>d</sup> Calculated amino acid composition is reported on a standardized ileal digestible amino acid basis (AminoDat 4.0, 2010); <sup>e</sup> Analyzed value.



diets for making 16 dietary treatments (Table 3). Phytase and zinc oxide were added to the experimental diets at the expense of sand, for making different levels of phytase and zinc.

Experimental design was a completely randomized design in a 4×4 factorial arrangement. Factors included four levels of dietary zinc content (24, 54, 84, and 114 mg kg<sup>-1</sup>) and four levels of added phytase (0, 100, 200, 300 FTU kg<sup>-1</sup>). Treatments were replicated four times, each had 12 birds. One-day-old broilers were randomly distributed to 64 floor pens (100×100 cm; 0.083 m<sup>2</sup> bird<sup>-1</sup>). Each pen was equipped with 1 plastic pan feeder, 1 bell drinker, and covered by 5 cm wood shaving material. Birds received mash diet from 1 to 42 days of age and had free access to water and feed, and a 24-hour photo schedule was applied. Weekly weight gain, feed intake, and livability of chicks were measured. At the end of the experimental period, two birds near the pen mean-body-weight were selected and, after slaughter, the carcass weight (including thighs, breast, back, wings, and neck) was measured.

Basal diets were extracted in 0.66 M HCl (> 4 hours/overnight, ratio of liquid to sample of 20:1) of inositol phosphates followed by treatment with a phytase that is specific for phytic acid (IP6) and the lower myo-inositol phosphate forms (IP2, IP3, IP4, IP5). Subsequent treatment with alkaline phosphatase ensures the release of the final phosphate from myo-inositol phosphate (IP1), which is relatively resistant to the action of phytase. The total phosphate released was measured using a modified colorimetric method and the results were recorded as grams of phosphorus per 100 g of sample material from which the phytic acid content was calculated.

Zinc content of the basal diets was measured by following the procedure of AOAC (method no: CAS-7440-66-6zinc, 1995). Livers were homogenized and dried at 60°C for 72 hours, then, microwave digested with HNO<sub>3</sub> (AOAC, 1995) before undergoing the inductively coupled plasma

**Table 3.** Composition of experimental diets. <sup>a, b</sup>

Ingredients	gr kg <sup>-1</sup>															
	Zinc (mg kg <sup>-1</sup> )				Phytase (FTU kg <sup>-1</sup> )				Zinc oxide				Sand			
	24	54	84	114	0	100	200	300	0	100	200	300	0	100	200	300
Phytase <sup>c</sup>	0	0	0	0	0.04	0.04	0.04	0.04	0.08	0.08	0.08	0.08	0.12	0.12	0.12	0.12
Zinc oxide <sup>d</sup>	0	0.04	0.08	0.12	0	0.04	0.08	0.12	0	0.04	0.08	0.12	0	0.04	0.08	0.12
Sand	0.24	0.20	0.16	0.12	0.20	0.16	0.12	0.08	0.16	0.12	0.08	0.04	0.12	0.08	0.04	0

<sup>a</sup> As-fed basis; <sup>b</sup> All diets were identical to the basal diet except for phytase and zinc content. <sup>c</sup> Quantum<sup>®</sup> (QPT2; 5,000G, Batch no: c110922-25) is an *Escherichia coli* 6-phytase. AB Agri Ltd Woodstock Court, Blenheim Road Marlborough Business Park, UK.; <sup>d</sup> Zinc oxide content 74.5 percent zinc.

optical emission spectrometry analysis. At the end of the experiment, blood samples were taken from ulnar vein of two birds per pen and Alkaline phosphatase activity (ALP) was measured with an automatic biochemical analyzer (Hitachi 717, Boehringer Mannheim, Ingelheim am Rhein, Germany) using an Elitech Diagnostic kit (catalog no. A.110537).

The data were analyzed by the general linear models procedure of the SAS (2002) software with pen means as the experimental unit. Linear and nonlinear functions were derived for graded levels of zinc and for phytase concentration. Fitted linear and nonlinear models (Schutte and Pack, 1995) and broken line regression as described by Robbins *et al.* (2006) were used for estimating the zinc requirement. The derived regression equations for zinc were set equal with those obtained for phytase and were solved; zinc equivalency was calculated by subtracting the obtained values from the zinc content of basal diet. Significant treatment effects were separated by Duncan's multiple

range tests.

## RESULTS

Effects of dietary zinc and phytase on broiler performance are shown in Table 4. Birds that received the diet containing 54 mg kg<sup>-1</sup> zinc had higher body weight than the birds that received diet containing 24 and 114 mg kg<sup>-1</sup> zinc ( $P < 0.05$ ,  $P < 0.01$ ) at 28 and 42 days of age. Effect of dietary zinc content on feed conversion ratio and feed intake were not statistically significant (Table 4). Phytase had no significant effect on body weight, feed conversion ratio, and feed intake. There were no significant interactions between dietary zinc and phytase on performance of male broiler chicken.

Effects of dietary zinc level and phytase on carcass yield, liver Zn content, and blood alkaline phosphatase activity are shown in Table 5 and Figure 1. Supplemental zinc decreased carcass percentage ( $P < 0.01$ ), but

**Table 4.** Effect of dietary zinc levels and phytase on male broiler chicken performance.<sup>a</sup>

Attribute	28 d			35 d			42 d		
	BW (g)	FCR	FI (g d <sup>-1</sup> )	BW (g)	FCR	FI (g d <sup>-1</sup> )	BW (g)	FCR	FI (g d <sup>-1</sup> )
Zinc (mg kg <sup>-1</sup> )									
24	1349.39 <sup>b</sup>	1.59	114.1	2049.84	1.86	184.2	2608.66 <sup>bc</sup>	2.26	178.8
54	1429.24 <sup>a</sup>	1.48	118.1	2129.66	1.95	196.8	2711.90 <sup>a</sup>	2.18	179.8
84	1381.18 <sup>ab</sup>	1.51	111.4	2078.57	1.98	185.8	2635.14 <sup>ab</sup>	2.38	185.4
114	1343.73 <sup>b</sup>	1.54	109.1	2030.46	1.95	191.9	2535.31 <sup>c</sup>	2.50	178.8
Phytase (FTU kg <sup>-1</sup> )									
0	1370.44	1.45	110.2	2023.55	2.06	188.4	2575.01	2.19	178.2
100	1375.88	1.55	113.0	2088.20	1.14	188.9	2615.01	2.40	175.9
200	1390.14	1.58	117.7	2113.16	1.94	192.3	2672.09	2.44	188.6
300	1367.09	1.53	112.2	2063.62	1.91	189.2	2622.50	2.30	179.9
P value									
Zinc	0.014	0.754	0.220	0.11	0.87	0.163	0.003	0.143	0.554
Phytase	0.850	0.699	0.935	0.20	0.54	0.920	0.146	0.349	0.967
Zinc×Phytase	0.602	0.402	0.450	0.90	0.91	0.863	0.834	0.673	0.344

<sup>a</sup> Values with different superscripts within a column are significantly different at  $P < 0.05$  and values are means of 4 replicates. Fitted quadratic regression:  $Y_{28d} = 1272.4 + 4.27Zn - 0.032Zn^2$ ; Estimated zinc requirements = 66.7 (mg kg<sup>-1</sup>);  $Y_{35d} = 1968 + 4.54Zn - 0.035Zn^2$ ; Estimated zinc requirements = 64.8 (mg kg<sup>-1</sup>);  $Y_{42d} = 2486.8 + 6.739Zn - 0.056Zn^2$ ; Estimated zinc requirements = 60.1 (mg kg<sup>-1</sup>). Where  $y$  = Body weight and  $Zn$  = Zinc content of diet (mg kg<sup>-1</sup>). Fitted two slope broken line:  $Y_{28d} = 1428.1 - 2.66(53.5 - Zn) - 1.42(Zn - 53.5)$ ; Estimated zinc requirements = 53.5 (mg kg<sup>-1</sup>);  $Y_{35d} = 2129.4 - 2.66(53.8 - Zn) - 1.65(Zn - 53.8)$ ; Estimated zinc requirements = 53.8 (mg kg<sup>-1</sup>);  $Y_{42d} = 2723.7 - 3.44(57.3 - Zn) - 3.327(Zn - 57.3)$ ; Estimated zinc requirements = 57.4 (mg kg<sup>-1</sup>). Where  $y$  = Body weight and  $Zn$  = Zinc content of diet (mg kg<sup>-1</sup>).

**Table 5.** Effect of dietary zinc level and phytase on carcass weight and percentage, liver Zn content and blood alkaline phosphatase activity. <sup>a</sup>

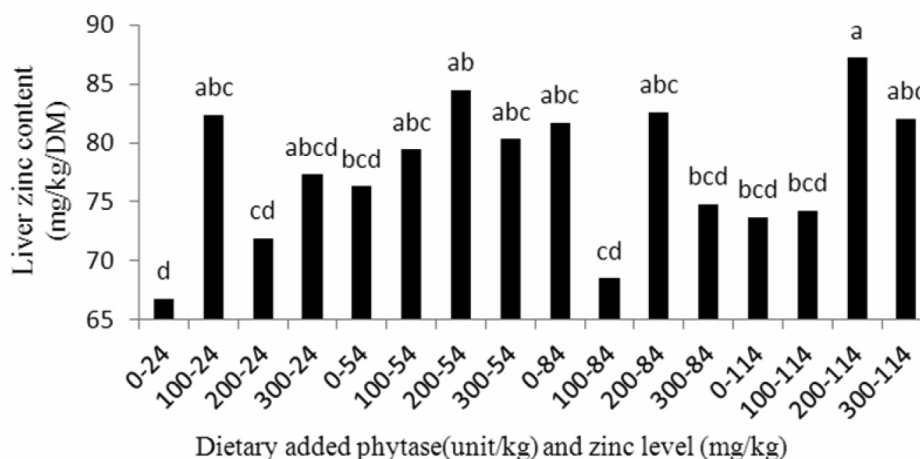
	Carcass weight	Carcass percentage	Liver Zn (mg kg <sup>-1</sup> )	ALP (Unit)
Zinc (mg kg <sup>-1</sup> )				
24	2013.8 <sup>a</sup>	77.6 <sup>a</sup>	74.63 <sup>b</sup>	3759
54	2052.6 <sup>a</sup>	75.9 <sup>ab</sup>	80.16 <sup>a</sup>	3889
84	1961.9 <sup>ab</sup>	74.9 <sup>b</sup>	76.95 <sup>ab</sup>	4654
114	1916.8 <sup>b</sup>	74.9 <sup>b</sup>	79.34 <sup>ab</sup>	4241
Phytase (FTU kg <sup>-1</sup> )				
0	1966.3	75.7	74.66 <sup>b</sup>	4571
100	1978.5	75.5	76.18 <sup>b</sup>	4292
200	2019.6	76.1	81.60 <sup>a</sup>	3823
300	1980.8	76.0	78.63 <sup>ab</sup>	3934
P value				
Zinc	0.02	0.008	0.076	0.662
Phytase	0.66	0.929	0.019	0.731
Zinc×Phytase	0.77	0.421	0.002	0.780

<sup>a</sup> Values with different superscripts within a column are significantly different at  $P < 0.05$  and values are means of 4 replicates.

phytase had no significant effect on carcass yield. Added phytase had significant effect on liver Zn content ( $P < 0.05$ ), whereas the added zinc numerically increased liver Zn content ( $P < 0.07$ ). Interaction between the dietary added zinc and phytase on liver Zn content was significant ( $P < 0.01$ ). The highest amount of liver zinc storage was observed at 114 mg kg<sup>-1</sup> dietary zinc level and 200 units of phytase, while the lowest was at 24 mg kg<sup>-1</sup> dietary zinc level and 0 unit supplemented phytase (Figure 1).

Dietary treatments had no significant effect on blood alkaline phosphatase activity.

Zinc requirements for optimum body weight gain during 0 to 42 days of age were estimated by different models (Table 4). Fitted quadratic model estimated 66.7, 64.8, and 60.1 mg kg<sup>-1</sup> zinc requirement for body weight at 28, 35, and 42 days of age, respectively ( $P < 0.001$  for all). However, the fitted two slope broken line estimated 53.5, 53.8, and 57.4 mg kg<sup>-1</sup> zinc requirement for body weight at 28, 35, and 42 days of age,

**Figure 1.** Interaction between dietary added zinc and phytase on liver Zn content.

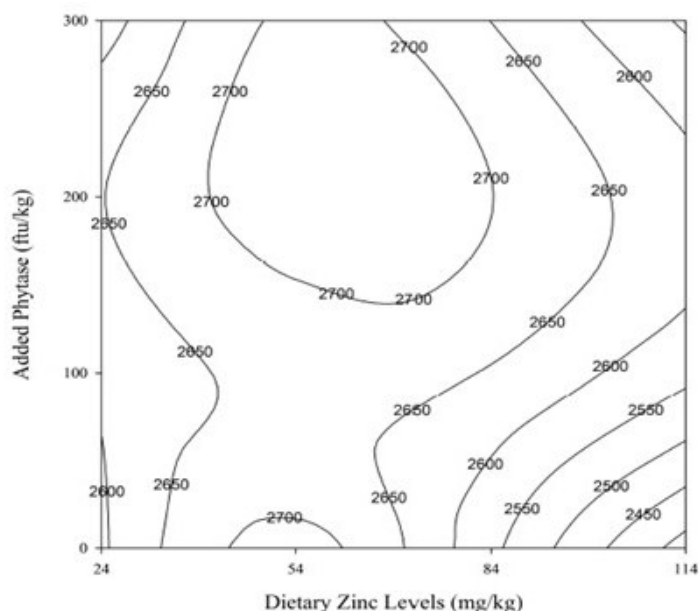
respectively ( $P < 0.002$ ).

Estimated zinc equivalency values of enzyme are shown in Table 6. Based on the fitted quadratic models, zinc equivalence value of phytase at 35 and 42 days of age in the range of phytase included, was estimated to be  $0.224 \text{ mg kg}^{-1} \text{ FTU}^{-1}$  (56.4%) and  $0.225 \text{ mg kg}^{-1} \text{ FTU}^{-1}$  (56.2%), respectively. Quadratic regression of body weight at 28 days of age on phytase supplementation was not statistically significant ( $P > 0.05$ ).

## DISCUSSION

Results showed that supplementation of basal diet, which contained  $24 \text{ mg kg}^{-1}$  Zn with  $30 \text{ mg kg}^{-1}$  zinc, increased final body weight of male broiler chicken (Table 4). This finding indicated that  $24 \text{ mg kg}^{-1}$  zinc could not meet the male broiler chicken requirement for maximum body weight. Fitted quadratic model and two slope broken line indicated that final body weight reached the maximum at  $60.1$  and  $57.4 \text{ mg kg}^{-1}$  dietary zinc. These results are inconsistent with other reports (Table 1). Also, the estimated values are higher than NRC

(1994) recommendation ( $40 \text{ mg kg}^{-1}$ ) and lower than Ross 308 broiler management manual (Aviagen Group Ltd. 2007) recommendation ( $100 \text{ mg kg}^{-1}$ ). The requirement recommended by NRC is based on research results that used a semi-purified diet as basal diet (O'Dell and Savage, 1957; Roberson and Schaible, 1958). Furthermore, variations in Zn requirement estimates are likely due to many factors such as fiber, protein source, ascorbic acid, source of supplemental Zn used, and presence of other minerals in the diet that have been shown to influence Zn absorption (Ammerman *et al.*, 1995). It is possible that, the lower Zn requirements in some of the studies mentioned in Table 1 are due to lack of soluble fiber and phytate in the purified diets. On the other hand, the source of supplemental Zn used in a Zn requirement assay can have just as much effect on the determined Zn requirement as the type of basal diet used. For example, feed-grade ZnO bioavailability relative to analytical-grade  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$  is about 37% in chicks (Edwards and Baker, 1999). Using practical corn soybean diet (high phytate), ZnO (low



**Figure 2.** Counter plot of male broiler chicken final body weight at different dietary zinc and phytase levels.



availability) as a routine source of Zn, and exogenous phytase (as a common procedure in industry) give some degree of confidence to the estimated values by the present investigation.

Results presented in Table 4 show that addition of phytase to the basal diet increased the final body weight numerically, but this effect was not statistically significant ( $P > 0.14$ ). Counter plot of chicken final body weight at different dietary zinc and phytase levels indicated that heaviest birds were observed with combination of  $54 \text{ mg kg}^{-1}$  added zinc and  $200 \text{ FTU kg}^{-1}$  phytase (Figure 2). Zinc equivalence value of phytase, or the amount of released Zn, in a practical corn soybean diet was estimated to be 56.2 percent for the final body weight (Table 5). Figure 1 indicates that the birds that received  $24 \text{ mg kg}^{-1}$  zinc had lower liver Zn content ( $P < 0.07$ ). Furthermore, addition of phytase to the basal diet increased liver zinc content ( $P < 0.01$ ). Regardless of the weak  $R^2$  of the fitted equation (Table 6), these findings further confirmed that addition of phytase to high phytate diet, balanced for all nutrients except zinc (Table 2) could release Zn bonded with myoinositol molecule. Other investigators have explained the efficacy of phytase in improving Zn availability, but none of them has estimated the dietary phytase replacement value for zinc (Schlegel *et al.*, 2013; Schlegel *et al.*, 2010; Yu *et al.*, 2010). Ao *et al.* (2007) reported that  $12 \text{ mg kg}^{-1}$  supplemental Zn without phytase and  $7.4 \text{ mg kg}^{-1}$  supplemental Zn with phytase were required for the optimal weight gain of chicks. The respective values for total dietary Zn were  $37.0$  and  $32.4 \text{ mg kg}^{-1}$  when the Zn content in basal diet was taken into consideration.

Alkaline phosphatase is a zinc dependent enzyme, whereas in the present investigation supplementation of balanced broiler chicken's diet with zinc and phytase had no significant effect on blood alkaline phosphatase activity. It seems that the amount of zinc ( $24 \text{ mg kg}^{-1}$ ) in the balanced

**Table 6.** Regression equations and estimated zinc equivalency values of phytase.

Trait	Regression equation between responses and dietary zinc levels		Regression equation between responses and dietary phytase levels		Estimated zinc equivalency of phytase	
	$R^2$	P value	$R^2$	P value	$R^2$	P value
Body weight @ 42d	$Y = 2486.8 + 6.739Zn - 0.056Zn^2$	0.21	$Y = 2569 + 0.879P - 0.0022P^2$	0.001	0.06	56.2%
Body weight @ 35d	$Y = 1968 + 4.54Zn - 0.0356Zn^2$	0.08	$Y = 2021.8 + 1.001P - 0.0028P^2$	0.023	0.06	56.4%



basal diet (esp. for phosphorus) was enough for alkaline phosphatase activity.

Precise estimated requirement and zinc equivalence of phytase can be used as a useful tool for better adjustment of dietary Zn to chick requirements considerably reduces the safety margin, risk of over consumption, reduction of Zn concentration of broiler manure and soil phytotoxicity (Mohanna and Nys, 1999).

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### REFERENCES

1. AminoDat 4.0 In: "50 Years Amino Acid Analysis". 2010. Evonik Industries, Germany, PP. 1-566.
2. Ammerman, B., Baker, D. H. and Austin, J. L. 1995. In: "Bioavailability of Nutrients for Animals Amino Acids, Minerals, and Vitamins". Academic Press, PP. 1-441.
3. Ao, T., Pierce, J. L., Pescatore, A. J., Cantor, A. H., Dawson, K. A., Ford, M. J. and Shafer, B. L. 2007. Effects of Organic Zinc and Phytase Supplementation in a Maize-soybean Meal Diet on the Performance and Tissue Zinc Content of Broiler Chicks. *British Poul. Sci.*, **48**: 690-695.
4. Ao, T., Pierce, J. L., Power, R., Dawson, K. A., Pescatore, A. J., Cantor, A. H. and Ford, M. J. 2006. Evaluation of Bioplex Zn as an Organic Zinc Source for Chicks. *Intl. J. Poul. Sci.*, **5**: 808-811.
5. AOAC. 1995. *Official Methods of Analysis*. 16<sup>th</sup> Edition, Association of Official Analytical Chemists, Arlington, VA.
6. Atwal, A. S., Eskin, N. A. M., McDonald, B. E. and Vasey-Genser, M. 1980. The Effects of Phytate on Nitrogen Utilization and Zinc Metabolism in Young Rats. *Nutr. Rep. Int.*, **21**: 257-267.
7. Aviagen Group Ltd. 2007. *Ross 308: Broiler Management Manual*. Aviagen, Newbridge, Midlothian EH28 8SZ, Scotland, UK.
8. Augspurger, N. R., Spencer, J. D., Webel, D. M. and Baker D. H. 2004. Pharmacological Zinc Levels Reduce the Phosphorus-releasing Efficacy of Phytase in Young Pigs and Chickens. *J. Anim. Sci.*, **82**: 1732-1739.
9. Batal, A. B., Parr, T. M. and Baker, D. H. 2001. Zinc Bioavailability in Tetrabasic Zinc Chloride and the Dietary Zinc Requirement of Young Chicks Fed a Soybean Concentrate Diet. *Poult. Sci.*, **80**: 87-90.
10. Bao, Y. M., Choct, M., Iji, P. A. and Bruerton, K. 2009. Optimal Dietary Inclusion of Organically Complexed Zinc for Broiler Chickens. *British Poul. Sci.*, **50**: 59-102.
11. Champagne, E. T. and Fisher, M. S. 1990. Binding Differences of Zn (II) and Cu (II) Ions with Phytate. *J. Inorg. Biochem.*, **38**: 217-223.
12. Cheryan, M. 1980. Phytic Acid Interactions in Food Systems. *Crit. Rev. Food Sci. Nutr.*, **13**: 297-335.
13. Dewar, W. A. and Downie, J. N. 1984. The Zinc Requirements of Broiler Chicks and Turkey Poulets Fed on Purified Diets. *Br. J. Nutr.*, **51**: 467-477.
14. Edwards, H. III, M. and Baker, D. H. 1999. Bioavailability of Zinc in Several Sources of Zinc Oxide, Zinc Sulfate, and Zinc Metal. *J. Anim. Sci.*, **77**: 2730-2735.
15. Emmert, J. L. and Baker, D. H. 1995. Zinc Stores in Chickens Delay the Onset of Zinc Deficiency Symptoms. *Poult. Sci.*, **74**: 1011-1021.
16. Gomes, P. C., Rigueira, D. C. M., Rostagno, H. S., Albino, L. F. T., Brumano, G. and Schmidt, T. M. 2008. Zinc Requirements for Male and Female Broilers in the Initial Phase. *R. Bras. Zootec.*, **37**: 79-83.
17. Huang, Y. L., Lu, L., Xie, J. J., Li, S. F., Li, X. L., Liu, S. B., Zhang, L. Y., Xi, L. and Luo, X. G. 2013. Relative Bioavailabilities of Organic Zinc Sources with Different Chelation Strengths for Broilers Fed Diets with Low or High Phytate Content. *Anim. Feed Sci. Technol.*, **179**: 144-148.
18. Formun Üstü
19. Huang, Y. L., Lu, L., Luo, X. G. and Liu, B. 2007. An Optimal Dietary Zinc Level of Broiler Chicks Fed a Corn-soybean Meal Diet. *Poult. Sci.*, **86**: 2582-2589.
20. Keith, A. M., Huang, C. C. and Fierke, C. A. 2000. Function and Mechanism of Zinc Metalloenzymes. *J. Nutr.*, **130**: 14375-14465.
21. Kornegay, E. T. 2001. Digestion of Phosphorus and Other Nutrients: The Role



- of Phytases and Factors Influencing Their Activity. In: "Enzymes in Farm Animal Nutrition". CABI Publishing, New York, NY, PP. 237-272
22. Lease, J. G. 1966. The Effect of Autoclaving Sesame Meal on Its Phytic Acid Content and on the Availability of Its Zinc to the Chick. *Poult. Sci.*, **45**: 237-241.
  23. Lo, G. S., Settle, S. L., Steinke, F. H. and Hopkins, D. T. 1981. Effect of Phytate:Zinc Molar Ratio and Isolated Soybean Protein on Zinc Bioavailability. *J. Nutr.*, **11**: 2223-2235.
  24. Lonnerdal, B., Sandberg, A. S., Sandstrom, B. and Kunz, C. 1989. Inhibitory Effects of Phytic Acid and Other Inositol Phosphates on Zinc and Calcium Absorption in Suckling Rats. *J. Nutr.*, **119**: 211-214.
  25. Maenz, D. D., Engele-Schaan, C. M., Newkirk, R. W. and Classen, H. L. 1999. The Effect of Minerals and Mineral Chelators on the Formation of Phytase-resistant and Phytase-susceptible Forms of Phytic Acid in Solution and in a Slurry of Canola Meal. *Anim. Feed Sci. Tech.*, **81**: 177-192.
  26. Mohanna, C. and Nys, Y. 1999. Effect of Dietary Zinc Content and Sources on the Growth, Body Zinc Deposition and Retention, Zinc Excretion and Immune Response in Chickens. *British Poult. Sci.*, **40**: 108-114.
  27. Morris, E. R. 1986. Phytate and Dietary Mineral Bioavailability. In: "Phytic Acid Chemistry and Application". Pilatus, Minneapolis, MN, PP. 57-76.
  28. Morris, E. R. and Ellis, R. 1980. Effect of Dietary Phytate/Zinc Molar Ratio on Growth and Bone Zinc Response of Rats Fed Semipurified Diets. *J. Nutr.*, **110**: 1037-1045.
  29. National Research Council (NRC). 1994. *Committee on Animal Nutrition. Subcommittee on Poultry Nutrition: Nutrient Requirements of Poultry*. 9<sup>th</sup> Edition, National Academy of Sciences, Washington, DC.
  30. Oberleas, D. and Harland, B. F. 1996. Impact of Phytic Acid on Nutrient Availability. In: "Phytase in Animal Nutrition and Waste Management". BASF Corp., Mount Olive, NJ, PP. 77-84.
  31. O'Dell, B. L. and Savage, J. E. 1960. Effect of Phytic Acid on Zinc Availability. *Proc. Soc. Exp. Biol. Med.*, **103**: 304-306.
  32. O'Dell, B. L. and Savage, J. E. 1957. Symptoms of Zinc Deficiency in the Chick. *Proceed. Federation Soc.*, **16**: 394.
  33. Roberson, R. and Schaible, P. J. 1958. The Zinc Requirement of the Chick. *Poult. Sci.*, **37**: 1321-1323.
  34. Robbins, K. R., Saxton, A. M. and Southern, L. L. 2006. Estimation of Nutrient Requirements Using Broken-line Regression Analysis. *J. Anim. Sci.*, **84**: E155-E165.
  35. Rossi, P., Rutz, F., Ancuti, M. A., Rech, J. L. and Zauk, N. H. F. 2007. Influence of graded Levels of Organic Zinc on Growth Performance and Carcass Traits of Broilers. *J. Appl. Poult. Res.*, **16**: 219-225.
  36. SAS Institute. 2002. *SAS/STAT User's Guide: Statistics*. SAS Institute Inc., Cary, NC, USA.
  37. Schlegel, P., Sauvant, D. and Jondreville, C. 2013. Bioavailability of Zinc Sources and Their Interaction with Phytates in Broilers and Piglets. *Anim.*, **7(1)**: 47-59.
  38. Schlegel, P., Nys, Y. and Jondreville, C. 2010. Zinc Availability and Digestive Zinc Solubility in Piglets and Broilers Fed Diets Varying in Their Phytate Contents, Phytase Activity and Supplemented Zinc Source. *Anim.*, **4(2)**: 200-9.
  39. Schutte, J. B. and Pack, M. 1995. Sulfur Amino Acid Requirement of Broiler Chicks from 14 to 38 Days of Age. I. Performance and Carcass Yield. *Poult. Sci.*, **74**: 480-487.
  40. Steinruck, U. and Kirchgessner, M. 1993. Estimation of the Zinc Requirement for Broilers Using Their Ability for Selective Zinc Absorption and by Dose-response Relations. *Arch. Tierenahr.*, **43**: 27-43.
  41. Vieira, M. M., Ribeiro, A. M. L., Kessler, A. M., Moraes, M. L., Kunrath, M. A. and Ledur, V. S. 2013. Different Sources of Dietary Zinc for Broilers Submitted to Immunological Nutritional and Environmental Challenge. *Appl. Poult. Res.*, **22**: 855-861.
  42. Wedekind, K. J. and Baker, D. H. 1990. Zinc Bioavailability in Feed-grade Sources of Zinc. *J. Anim. Sci.*, **68**: 684-689.
  43. Xiudong, L., Ang, L. I., Lin L., Songbai, L., Sufen, L., Liyang, Z., Guangying, W. and Xugang, L. 2013. Optimal Dietary Zinc Levels of Broiler Chicks Fed a Corn-soybean Meal Diet from 22 to 42 Days of Age. *Anim. Production Sci.*, **53**: 388-394.
  44. Yu, Y., Lu, L., Wang, R. L., Xi, L., Luo, X. G. and Liu, B. 2010. Effects of Zinc Source

and Phytate on Zinc Absorption by *In situ* Ligated Intestinal Loops of Broilers. *Poult. Sci.*, **89**: 2157-65.

45. Zeigler, T. R., Leach, R. M., Norris, L. C. and Scott, M. L. 1961. Zinc Requirement of the Chick: Factors Affecting Requirement. *Poult. Sci.*, **40**: 1584-1593.

## ارزیابی مجدد نیاز روی در جوجه‌های گوشتی نر با استفاده از جیره‌های کاربردی حاوی آنزیم فیتاز با منشاء خارجی و روش پاسخ به سطوح افزایشی

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### چکیده

نتایج تحقیقات حاکی از دامنه ۱۰/۸ تا ۱۰۵ (میلی گرم/کیلوگرم) در نیاز عنصر روی برای جوجه‌های گوشتی است. عوامل متعددی به غیر از میزان روی موجود در خوراک به ویژه فیتات در میزان نیاز روی موثر هستند. لذا هدف اصلی این پژوهش ارزیابی مجدد نیاز روی در جوجه گوشتی با استفاده از جیره‌های کاربردی حاوی آنزیم فیتاز با منشاء خارجی و روش پاسخ پرنده به سطوح افزایشی بود. آزمایش روی تعداد ۷۶۸ قطعه جوجه یکروزه نر (راس ۳۰۸) انجام شد. جیره پایه (ذرت-سویا) فقیر از لحاظ روی با جیره‌های آزمایشی مخلوط و ۱۶ تیمار تغذیه‌ای ایجاد شد. آزمایش در قالب طرح کامل تصادفی به صورت فاکتوریل (۴×۴) اجرا شد. عوامل شامل ۴ سطح روی (۲۴، ۵۴، ۸۴ و ۱۱۴ میلی-گرم/کیلوگرم) و ۴ سطح فیتاز (۰، ۱۰۰، ۲۰۰ و ۳۰۰ واحد/کیلوگرم) بود. تیمارها دارای ۴ تکرار و ۱۲ پرنده در هر تکرار بودند. توابع خطی و غیر خطی به متغیرهای مستقل در برابر سطوح مختلف روی و فیتاز برازنده شد. اثر روی بر وزن بدن در سن ۴۲ روزگی معنی‌دار بود ( $P<0.01$ ). نیاز روی در سن ۲۸، ۳۵ و ۴۲ روزگی توسط تابع درجه دو به ترتیب ۶۶/۷، ۶۴/۸ و ۶۰/۱ میلی گرم/کیلوگرم برآورد شد ( $P<0.001$ ). نیاز روی در سن ۲۸، ۳۵ و ۴۲ روزگی توسط تابع خط شکسته با دو شیب به ترتیب ۵۳/۵، ۵۳/۸ و ۵۷/۴ میلی گرم/کیلوگرم برآورد شد ( $P<0.001$ ). معادل روی آنزیم فیتاز، ۰/۲۲۵ میلی-گرم در کیلوگرم خوراک به ازای هر واحد فیتاز برآورد شد. آنزیم فیتاز ذخیره روی در کبد را افزایش داد ( $P<0.01$ ). برآورد نیاز روی برای افزایش وزن بدن با استفاده از جیره‌های کاربردی حاوی فیتات زیاد، منبع روی با قابلیت استفاده کم و فیتاز با منشاء خارجی، کمتر از پیشنهاد راهنمای مدیریت راس ۳۰۸ (۶۰ در مقابل ۱۰۰ میلی گرم/کیلوگرم) بود.