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

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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 (>4.9 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 of zinc) and drank water with 35 μg/l 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) and four levels of dietary zinc (30, 60, 90, 120 mg/kg) 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 bound zinc to phytate.

Keywords: Zinc Requirement, Broiler Breeders, Phytase, Late-stage of Production, nonlinear models

INTRODUCTION

Zinc is a trace mineral involved in several biological activities in poultry (Abbasi *et al.*, 2021; 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 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 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 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 FTU (56.4%) and 0.225 mg/kg 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. While the effect of releasing bonded zinc from phytate on the requirement of obese broiler breeder hens has not been studied yet. Therefore, this study is designed to evaluate the zinc requirement of broiler breeder hens in the late stage of the production cycle fed a practical corn-soybean 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 4900 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, 13.7% crude protein, and 2750 kcal/kg AMEn was fed to hens for two weeks to deplete the retained zinc in their bodies. Table 1 displays 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) (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^2/bird). 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, 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 the experiment started 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 2000× g for 15 min 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 Kidding's method (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. Shell-breaking strength and shell thickness were 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 then weighed and frozen instantly for further analysis. Samples of bone, skin, feather, and egg yolk were dried for 24 h at 100°C and zinc content was determined on the dry samples after being digested in nitric acid and hydrogen peroxide by atomic absorption spectrophotometry 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 × 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 broken-line 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 results, by increasing zinc content from 30 to 120 mg/kg, 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 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, 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 respectively.

Carcass characteristics

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

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, respectively (Figures 1 and 2). It was determined that 81.3 and 78.2 mg/kg 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 leads to an increase in egg weight. The highest egg weight was detected in hens given a phytase-supplemented diet and 90 mg Zn/kg. Increasing yolk weight may explain the increase in the weight of eggs in the current study. In the present study, 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, and malate dehydrogenase.

Egg production and egg mass were not significantly affected by dietary treatment but increased numerically when zinc levels were increased. Contrary to our results, Kucuk *et al.* (2008) reported that 30 mg Zn/kg plus 8 mg/kg pyridoxine supplementation improved the productive performance of the laying hens. It seems that the basal diet with 46 mg Zn/kg 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 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 chicken embryo growth. 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; 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. 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 have 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; Scholey and Burton, 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) recommends 4.5 mg/hen/day of zinc for breeders, but this was based on a few research reports. According to Mayer *et al.* (2019), Adding 85.6 mg/kg 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. According to research conducted by Mayer *et al.* (2019), it was 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 requirement amount of zinc. In this experiment, the zinc requirement of aged broiler breeder hens was estimated at 96.05 mg/kg, however, considering the mentioned estimated value in hens receiving dietary phytase, it became 79.75 mg/kg. The estimated value for the egg yolk zinc content is near the value of 92.34 mg zinc/kg 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 of supplemental zinc without phytase and 7.4 mg/kg of supplemental zinc with phytase were required for the optimal weight gain of chicks.

CONCLUSION

As per the experiment conducted, it was estimated that the average zinc requirement for aged broiler breeder is 96.05 mg/kg. However, considering the estimated value of hens that received dietary phytase, the requirement was reduced to 79.75 mg/kg (which is about a 16.9% decrease). It can therefore be concluded that phytase reduced the zinc requirement of obese broiler breeders at the late stage of production by releasing 16.9% (16.3 mg zinc/kg) 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.

Reference

1. Abbasi, M., Dastar, B., Afzali, N., Shargh, M. S. and Hashemi, S. 2021. The Effects of Nano and Micro Particle Size of Zinc Oxide on Performance, Fertility, Hatchability, and Egg Quality Characteristics in Laying Japanese Quail. *Biol. Trace Elem. Res.*, 1-11.

2. Abbasi, M., Zaghari, M., Ganjkhanlo, M. and Khalaji, S. 2015. Is dietary iron requirement of broiler breeder hens at the late stage of production cycle influenced by phytase supplementation? *J. Appl. Anim. Res*. **43**: 166-176.

3. Amen, M. H. and Al-Daraji, H. J. 2011. Effect of dietary supplementation with different level of zinc on sperm egg penetration and fertility traits of broiler breeder chicken. *Pak. J. Nutr.*, **10**: 1083-1088.

4. Ao, T., Pierce, J., Pescatore, A., Cantor, A., Dawson, K., Ford, M. and Shafer, B. 2007. Effects of organic zinc and phytase supplementation in a maize–soybean meal diet on the performance and tissue zinc content of broiler chicks. *Br. Poult. Sci*. **48**: 690-695.

5. AOAC. 2000. Official Methods of Analysis. *16th Edition, Association of Official Analytical Chemists, Arlington, VA.*,

6. Aviagen. 2019. Ross 408 Parent Stock Nutrition Specifications. *Aviagen, Newbridge, Midlothian EH28 8SZ, Scotland, UK.*,

7. Cobb-Vantress. 2016. Cobb Breeder Management Guide. *Vantress Inc., Siloam Springs, AR.*,

8. Eder, K. and Kirchgessner, M. 1995. Zinc deficiency and activities of lipogenic and glycolytic enzymes in liver of rats fed coconut oil or linseed oil. *Lipids*. **30**: 63.

9. Fatholahi, A., Khalaji, S., Hosseini, F. and Abbasi, M. 2021. Nano-Bio Zinc Synthesized by Bacillus subtilis Modulates Broiler Performance, Intestinal Morphology and Microbiota, and Expression of Tight Junction's Proteins. *Livest. Sci.*, **251**: 104660.

10. Guo, Y., Yang, R., Yuan, J., Ward, T. and Fakler, T. 2002. Effect of Availa Zn and ZnSO4 on laying hen performance and egg quality. *Poult. Sci.*, **81**: 40.

11. Huang, L., Li, X., Wang, W., Yang, L. and Zhu, Y. 2019. The role of zinc in poultry breeder and hen nutrition: an update. *Biol. Trace Elem. Res.*, **192**: 308-318.

12. Kambe, T., Tsuji, T., Hashimoto, A. and Itsumura, N. 2015. The physiological, biochemical, and molecular roles of zinc transporters in zinc homeostasis and metabolism. *Physiol. Rev.*, **95**: 749-784.

13. Keiding, R., Hörder, M., Denmark, W. G., Pitkänen, E., Tenhunen, R., Strömme, J., Theodorsen, L., Waldenström, J., Tryding, N. and Westlund, L. 1974. Recommended methods for the determination of four enzymes in blood. *Scand. J. Clin. Lab.*, **33**: 291-306.

14. Kucuk, O., Kahraman, A., Kurt, I., Yildiz, N. and Onmaz, A. 2008. 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.

15. Li, L., Abouelezz, K., Gou, Z., Lin, X., Wang, Y., Fan, Q., Cheng, Z., Ding, F., Jiang, S. and Jiang, Z. 2019. Optimization of dietary zinc requirement for broiler breeder hens of chinese yellow-feathered chicken. *Animal.*, **9**: 472-486.

16. Liao, X., Li, W., Zhu, Y., Zhang, L., Lu, L., Lin, X. and Luo, X. 2018. Effects of environmental temperature and dietary zinc on egg production performance, egg quality and antioxidant status and expression of heat-shock proteins in tissues of broiler breeders. *Br. J. Nutr.*, **120**: 3-12.

17. Lim, H., Namkung, H. and Paik, I. 2003. Effects of phytase supplementation on the performance, egg quality, and phosphorous excretion of laying hens fed different levels of dietary calcium and nonphytate phosphorous. *Poult. Sci.*, **82**: 92-99.

18. Liu, Z., Lu, L., Wang, R., Lei, H., Li, S., Zhang, L. and Luo, X. 2015. Effects of supplemental zinc source and level on antioxidant ability and fat metabolism-related enzymes of broilers. *Poult. Sci.*, **94**: 2686-2694.

19. Mayer, A., Vieira, S., Berwanger, E., Angel, C., Kindlein, L., França, I. and Noetzold, T. 2019. Zinc requirements of broiler breeder hens. *Poult. Sci*. **98**: 1288-1301.

20. Mayer, A., Vieira, S., Berwanger, E., Angel, C., Kindlein, L., França, I. and Noetzold, T. 2019. Zinc requirements of broiler breeder hens. *Poult. Sci.*, **98**: 1288-1301.

21. Meyer, E. and Parsons, C. 2011. The efficacy of a phytase enzyme fed to Hy-Line W-36 laying hens from 32 to 62 weeks of age. *J. Appl. Poult. Res.*, **20**: 136-142.

22. 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. *Br. Poult. Sci*. **40**: 108-114.

23. Morgan, N., Scholey, D. and Burton, E. 2017. Use of Zn concentration in the gastrointestinal tract as a measure of phytate susceptibility to the effect of phytase supplementation in broilers. *Poult. Sci.*, **96**: 1298-1305.

24. Navidshad, B., Jabbari, S. and Mirzaei Aghjeh Gheshlagh, F. 2016. The new progresses in Zn requirements of poultry. *Iran. J. Appl. Anim. Sci.*, **6**: 763-767.

25. NRC. 1994. Nutrient requirements of poultry. *9th Revision Edition, National Academy Press, Washington, DC.*,

26. O'dell, B., Yohe, J. and Savage, J. 1964. Zinc availability in the chick as affected by phytate, calcium and ethylenediaminetetraacetate. *Poult. Sci.*, **43**: 415-419.

27. Robbins, K., Saxton, A. and Southern, L. 2006. Estimation of nutrient requirements using broken-line regression analysis. *Anim. Sci. J.*, **84**: E155-E165.

28. Roberson, K. D. and Edwards Jr, H. M. 1994. Effects of 1, 25-dihydroxycholecalciferol and phytase on zinc utilization in broiler chicks. *Poult. Sci.*, **73**: 1312-1326.

29. Rostagno, H., Albino, L., Hannas, M., Donzele, J., Sakomura, N., Perazzo, F., Saraiva, A., Teixeira, M., Rodrigues, P. and Oliveira, R. 2017. Brazilian tables for Poultry and swine: Composition of foods and nutritional requirements. *UFV, Viçosa*.

30. 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. *Animal.*, **4**: 200-209.

31. Shelton, J. and Southern, L. 2006. Effects of phytase addition with or without a trace mineral premix on growth performance, bone response variables, and tissue mineral concentrations in commercial broilers. *J. Appl. Poult. Res.*, **15**: 94-102.

32. Srinivasa, S. and Manjunath, M. 2014. Serum zinc levels in children with febrile seizures. *J. Evol. Med. Dent. Sci.*, **3**: 2983-2989.

33. Sunder, G. S., Panda, A., Gopinath, N., Rao, S. R., Raju, M., Reddy, M. and Kumar, C. V. 2008. Effects of higher levels of zinc supplementation on performance, mineral availability, and immune competence in broiler chickens. *J. Appl. Poult. Res.*, **17**: 79-86.

34. Trindade Neto, M. A. d., Pacheco, B. H. C., Albuquerque, R. d. and Schammass, E. A. 2011. Lysine and zinc chelate in diets for brown laying hens: effects on egg production and composition. *Rev. Bras. Zootec.*, **40**: 377-384.

35. Urbano, G., Aranda, P., Gómez-Villalva, E., Frejnagel, S., Porres, J. M., Frías, J., Vidal-Valverde, C. and López-Jurado, M. 2003. Nutritional evaluation of pea (Pisum sativum L.) protein diets after mild hydrothermal treatment and with and without added phytase. *J. Agric. Food Chem.*, **51**: 2415-2420.

36. Viveros, A., Brenes, A., Arija, I. and Centeno, C. 2002. Effects of microbial phytase supplementation on mineral utilization and serum enzyme activities in broiler chicks fed different levels of phosphorus. *Poult. Sci.*, **81**: 1172-1183.

37. Wilson, H. 1997. Effects of maternal nutrition on hatchability. *Poult. Sci.*, **76**: 134-143.

38. Yi, Z., Kornegay, E. and Denbow, D. 1996. Supplemental microbial phytase improves zinc utilization in broilers. *Poult. Sci.*, **75**: 540-546.

39. Zaghari, M., Avazkhanllo, M. and Ganjkhanlou, M. 2018. Reevaluation of male broiler zinc requirement by dose-response trial using practical diet with added exogenous phytase. *J. Agric. Sci. Technol.*, 333-343.

40. Zarghi, H., Golian, A., Hassanabadi, A. and Khaligh, F. 2022. Effect of zinc and phytase supplementation on performance, immune response, digestibility and intestinal features in broilers fed a wheat-soybean meal diet. *Ital. J. Anim. Sci.*, **21**: 430-444.

41. Zhang, L., Wang, Y.-X., Xiao, X., Wang, J.-S., Wang, Q., Li, K.-X., Guo, T.-Y. and Zhan, X.-A. 2017. Effects of zinc glycinate on productive and reproductive performance, zinc concentration and antioxidant status in broiler breeders. *Biol. Trace Elem. Res.*, **178**: 320-326.

42. Zhang, L., Wang, Y.-X., Xiao, X., Wang, J.-S., Wang, Q., Li, K.-X., Guo, T.-Y. and Zhan, X.-A. 2017. Effects of zinc Glycinate on productive and reproductive performance, zinc concentration and antioxidant status in broiler breeders. *Biol. Trace Elem. Res.*, **178**: 320-326.

43. Zhu, Y., Li, W., Lu, L., Zhang, L., Ji, C., Lin, X., Liu, H., Odle, J. and Luo, X. 2017. Impact of maternal heat stress in conjunction with dietary zinc supplementation on hatchability, embryonic development, and growth performance in offspring broilers. *Poult. Sci.*, **96**: 2351-2359.

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

Ingredients	Depletion 58 – 60 w	Basal 60 – 65 w
	------------- gr/kg	----------------
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
$CaCO_3$	77.8	65.2
NaCl	2.00	1.70
$NaHCO_3$	2.80	2.20
KSO_4 (K=44.6%)	9.40	-
H_3PO_4 (P=27.5 %)	10.90	-
Mineral-vitamin premix[1]	5.00	5.00
DL-Methionine	3.00	1.10
L-Lysine hydrochloride	2.60	0.800
L-Theronine	3.00	-
Experimental diet[2]	-	0.252
Calculated Nutrients (g/kg)		
MEn (Kcal/kg)[3]	2750	2750
Crude Protein[4]	145	144
Calcium[4]	30.0	30.0
Available Phosphorus	3.50	3.80
Na	1.60	1.50
Dig Lys[5]	6.40	6.50
Dig Met[5]	5.80	3.20
Dig M+C[5]	8.10	5.30
Dig Thr[5]	7.80	4.60
Dig Arg[5]	6.20	7.40
Zn (mg/kg)	9.50	30.0
Zn (mg/kg)[4]	11.0	46.0

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

2. Table 2

3. apparent metabolizable energy in kilocalories per kilogram

4. Analyzed value.

5. Calculated amino acid composition is reported on a standardized ileal digestible amino acid basis.

Table 2: Experimental design[1]

Ingredients				Zinc levels	
Phytase[2] levels	Zinc oxide[3]	Sand[4]	Phytase levels	Zinc addition	Zinc addition plus to zinc content in basal diet
(gr/kg)	(gr/kg)	(gr/kg)	(unit/kg)	(mg/kg diet)	(mg/kg diet)
0	0.000	0.252	0	0	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

1. All diets were identical to the basal diet except for phytase and zinc content.
2. Quantum® is an *E. coli* derived 6-phytase. AB Agri Ltd Woodstock Court, Blenheim Road Marlborough Business Park, UK.
3. Zinc oxide content 74.5 percent zinc.
4. Inert filler.

Table3: Effect of different zinc and phytase levels on production & egg quality of broiler breeders[1].

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

$^{a-c}$ Values with different superscripts within a column are significantly different at $P < 0.05$.
[1] 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	R^2	P- value	Requirement (mg/kg)
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	$Y=34.50-0.005\times (Z1)2 -0.104\times (Z2)$	0.54	0.062	85
	without phytase	$Y=30.82-0.093\times (Z1)-0.0026\times (Z2)^2$	0.13	0.79	88

Table 5: Effect of different zinc and phytase levels on carcass characteristics of broiler breeders[1].

treatments		Live weight	Carcass weight	Liver weight		Abdominal Fat weight	
Phytase	Zinc						
U/Kg	Mg/kg	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
Significance (P-value)							
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×Phytase		0.7922	0.2625	0.5777	0.5350	0.5856	0.5971

[1] Values are means of 4 replicates.

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[1].

treatments						
Phytase U/Kg	Zinc Mg/kg	Bone Mg/kg	Skin Mg/kg	Egg yolk Mg/kg	Plasma Mg/L	ALP U/L
	30	362	65.1	75.4^{b}	166^{c}	69.4
	60	521	99.4	87.8^{a}	205^{b}	57.6
	90	446	73.4	79.6^{ab}	222^{a}	63.5
	120	478	118	85.5^{ab}	225^{a}	74.8
SEM		42.9	18.5	3.72	4.17	8.15
0		420	91.7	83.8	205	56.9^{b}
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×Phytase		0.6267	0.9414	0.2569	0.1752	0.4348

[a-b] Values with different superscripts within a column are significantly different at $P < 0.05$.
[c] Values with different superscripts within a column are significantly different at $P < 0.01$.
[1] Values are means of 4 replicates.

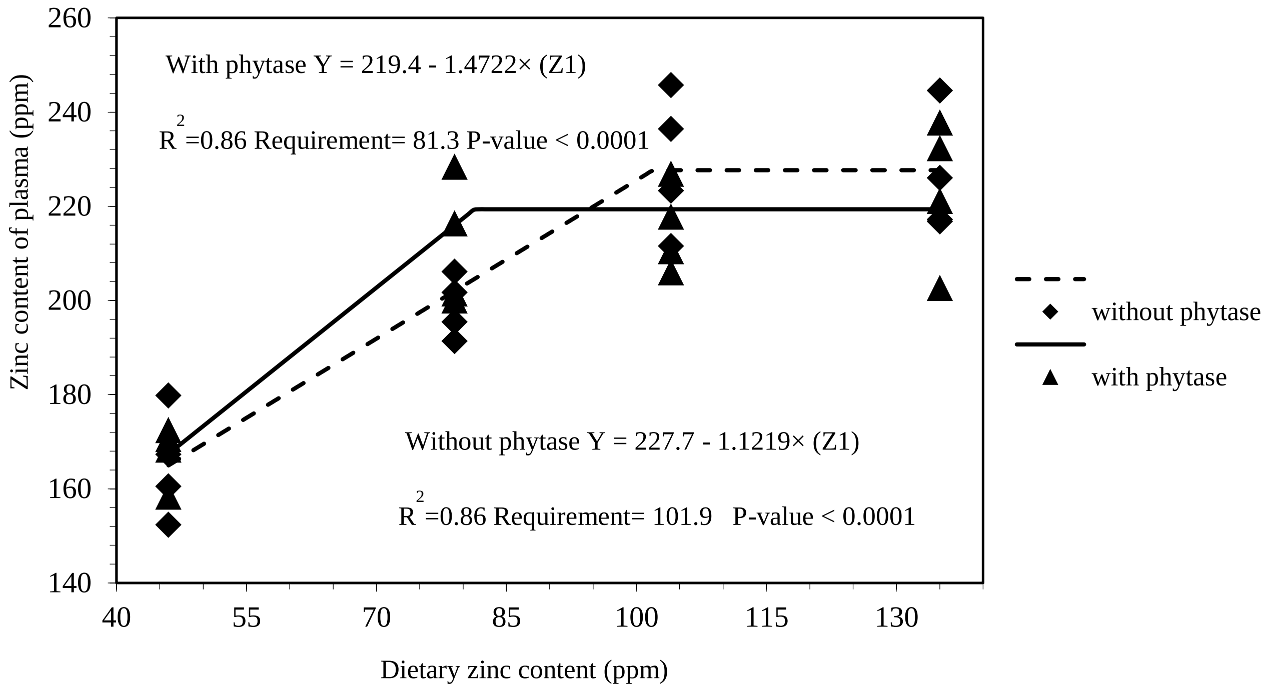

Figure (1): Zinc content of plasma response to consumption of zinc with and without phytase. Each dot (•) represents data collected over 5 weeks from 1 replicate.

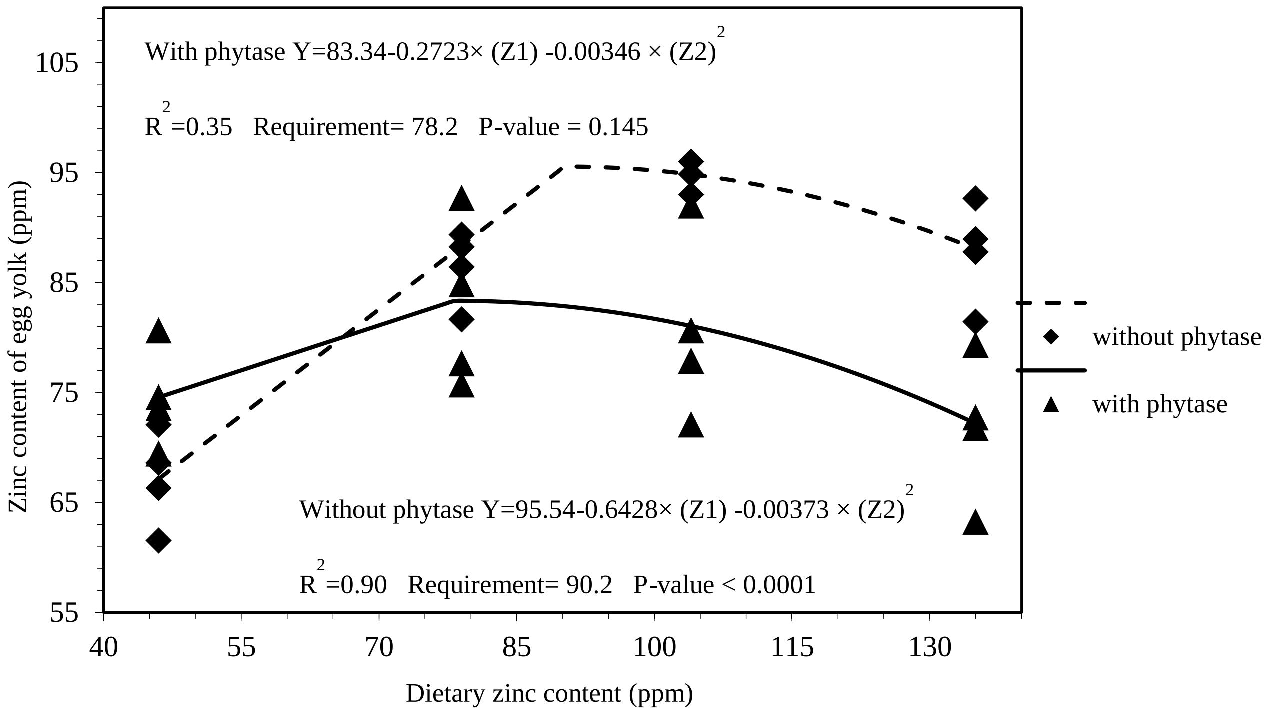

Figure (2): Zinc content of egg yolk response to consumption of zinc with and without phytase. Each dot (•) represents data collected over 5 weeks from 1 replicate.

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

چکیده

این آزمایش جهت برآورد تاثیر آنزیم 6-فیتاز در مرحله پایانی چرخه تولید مرغ های مادر بر نیاز روی، عملکرد، مقدار روی ذخیره شده در بافت ها انجام شد. 128 مرغ مادر چاق (بیشتر از 4/9 کیلوگرم وزن زنده) از نژاد کاب-500 در سن 58 هفتگی جهت اختصاص به تیمارهای آزمایشی مختلف وزن شدند. در ابتدا جهت تخلیه روی ذخیره شده در بدن، پرندگان به مدت 2 هفته جیره های نیمه خالص با فقر روی (9/5 میلیگرم روی در کیلوگرم خوراک) و آب آشامیدنی با سطح روی 35 میکروگرم در لیتر دریافت کردند. سپس مرغ ها به صورت تصادفی به 8 تیمار با دو سطح فیتاز (0 و 300 واحد در کیلوگرم)، 4 سطح روی (30، 60، 90 و 120 میلی گرم در کیلوگرم) با 4 تکرار و در هر تکرار 4 مرغ در قالب طرح فاکتوریل تقسیم شدند. وزن بدن، میزان تولید تخم مرغ، وزن تخم مرغ و کیفیت تخم مرغ در طول 5 هفته آزمایش ثبت شد. افزودن روی به شکل معنی داری سبب افزایش وزن زرده و مقدار روی زرده ($P<0.05$) و غلظت روی پلاسما ($P<0.0001$) شد. وزن تخم مرغ با افزودن فیتاز به صورت معنی دار ($P<0.05$) افزایش یافت. نتایج نشان داد افزودن فیتاز می‌تواند با آزاد کردن 16/9 درصد روی باند شده با فیتات، نیاز روی در مرغ های مادر چاق در سنین پایانی چرخه تولید را کاهش دهد.