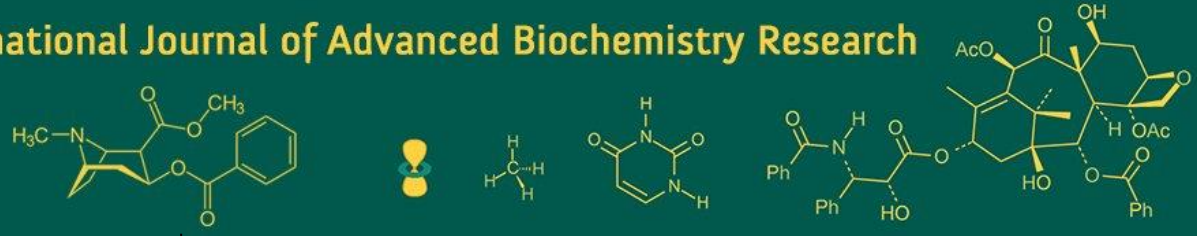
International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; SP-8(4): 525-528
www.biochemjournal.com
Received: 05-01-2024
Accepted: 11-02-2024

N Prabhuraja
M.V.Sc Scholar, Department of Poultry Science, Veterinary College Hebbal, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

BU Umesh
Associate Professor, Department of Livestock Farm Complex, Veterinary College Shivamogga, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

HC Indresh
Assistant Professor, Department of Poultry Science, Veterinary College Hebbal, Bengaluru, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

BG Veeranna Gowda
Assistant Professor, Department of Livestock Production and Management, Veterinary College Shivamogga, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

Corresponding Author:
N Prabhuraja
M.V.Sc Scholar, Department of Poultry Science, Veterinary College Hebbal, Karnataka Veterinary Animal and Fisheries Sciences University, Bidar, Karnataka, India

Effect of phytase supplementation on mineral composition in blood and bone and economy in broilers

N Prabhuraja, BU Umesh, HC Indresh and BG Veeranna Gowda

DOI: https://doi.org/10.33545/26174693.2024.v8.i4Sg.1046

Abstract
The experimental study was conducted to evaluate phytase enzyme supplementation on mineral composition in blood and bone and economy in 120 Cobb broilers up to 42 days of age. The study includes four different types of dietary treatments, they are T_1 control group (0.45% available phosphorus) as per Bureau of Indian standards 2007, T_2 low phosphorous diet (0.35% available phosphorus + 0.01% Phytase 5,000 FTU), T_3 low phosphorus diet (0.30% available phosphorus + 0.02% Phytase 5,000 FTU) and T_4 low phosphorus diet (0.25% available phosphorus + 0.03% Phytase 5,000 FTU). The result showed there is no significant difference ($p>0.05$) in calcium and phosphorous mineral composition of both blood serum and tibia bone between control group and phytase supplemented low phosphorus groups. There is significant difference ($p\leq0.05$) in economics in terms of cost of production between control group and phytase supplemented low phosphorus groups. In conclusion, the supplementation of phytase in low phosphorous diet may decrease the production cost and gives higher returns over investment but it could not effect on mineral composition of blood and bone.

Keywords: Broilers, phytase, available phosphorous, serum, tibia, economy

Introduction
Feed costs are a significant determinant of the economic viability of the poultry industry, constituting more than 70 percent of the total production expenses (Sharifi *et al*., 2012) [17]. This financial burden poses a major obstacle to the progress of the poultry sector, particularly in developing countries. Crafting poultry feed requires careful selection and utilization of diverse ingredients to ensure the provision of adequate quantities and well-balanced proportions of essential nutrients.

The primary components of poultry feed are derived from plant materials, which include cereals, grain legumes, and oilseed meals (Cowieson, 2006) [8]. Poultry diets typically comprise 50-70% grains, with seeds being a predominant source of plant-based ingredients (Amer, 2014) [3]. Corn and soybean meal are particularly essential in broiler diets within the poultry industry, as they effectively meet the bird's energy and protein requirements, thus contributing significantly to the industry's overall nutritional strategy (Butani and Parnerker, 2015) [7].

Phytate serves as the primary phosphorus form in poultry feed, constituting approximately 61–70% of the phosphorus content in feed ingredients (Aureli *et al*., 2011) [5]. Poultry birds, similar to monogastric animals, face challenges in effectively utilizing phytate phosphorus due to the absence of endogenous phytase enzyme, thus necessitating the supplementation of inorganic phosphates in poultry diets to meet phosphorus requirements (Yu *et al*., 2004) [23].

Phytic acid, known for its stability and high phosphate content, carries a significant negative charge across a wide pH range. Its presence in the diet adversely affects the bioavailability of mineral ions such as Zn^{2+}, Fe^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, and Cu^{2+}. Moreover, phytate forms complexes with proteins at both low and high pH values, leading to alterations in protein structure. These changes can result in decreased protein solubility, enzymatic activity, and proteolytic digestibility (Kumar *et al*., 2010) [12].

To counteract the negative effect of phytate, microbial phytase is added to broiler diets. This addition facilitates the hydrolysis of phytate, releasing bound phosphorus, amino acids and

starch in plant-based diets. Consequently, this process enhances the availability of these nutrients for broilers, leading to increased bioavailability of various cations and an improvement in the nutritional value of the meal (Mukhtar, 2013) [14]. Moreover, phytate is recognized as an anti-nutrient for broilers (Cowieson *et al*., 2011) [9], reduced mineral absorption (Plumstead *et al*., 2008) [16].

Utilizing phytase can mitigate the need for additional phosphorus supplementation in feed. Incorporating phytase enzyme into broiler diets can effectively decrease the reliance on costly commercial inorganic phosphate, such as dicalcium phosphate (DCP), thereby reducing the overall expense of broiler feed (Delezie, *et al*., 2015) [10].

Singh *et al.* (2003) [19] investigated the effects of phytase supplementation on the performance of broiler chickens fed diets based on maize and wheat with varying amounts of non-phytate phosphorus. They found that broiler serum mineral content (Ca and P) was improved by microbial phytase supplementation (500 units/kg diet) to low non-phytate phosphorous (0.30%) diets based on maize or wheat. Since phytate is a strong acid, it can combine with other minerals to produce salts, which will decrease the solubility and ultimately the absorption of calcium and phosphorous. Microbial phytase has the ability to hydrolyze phytate, releasing all of its constituent minerals, myo-inositol and inorganic phosphate. Lalpanmawia *et al.* (2014) [13] found that adding 500 FTU/kg of commercial phytase enzyme to low non phytate phosphorus meals (0.32% during the starting phase and 0.28% during the ending phase) increased the concentration of phosphorus in the plasma without affecting the plasma concentration of calcium.

Ghosh *et al.* (2016) [11] conducted the effects of supplementation of manganese with or without phytase on muscle and tibia composition and immunity in broiler chickens. Revealed that the manganese and phytase supplementation did not affect ash, calcium and phosphorus concentrations in tibial bone. Sobhi *et al.* (2023) [21] assessed the effect of phytase and β-xylanase enzymes in broiler chickens. Concluded that dietary supplementation with phytase 5,000 at 100 g/ton diet or phytase 10,000 at 50 g/ton diet plus β-xylanase enzyme significantly increased the tibia ash, phosphorus and calcium contents. Therefore, using higher concentration of phytase might augment the calcium and phosphorous release from the phytate molecule and contribute to improving tibia mineralization.

Ahmed *et al.* (2004) [1] examined the effect of different levels of phytase on soybean meal diet at end of 42 days of age. Concluded that profit was significantly highest (p<0.01) on supplementation of 1.50 g phytase per kg soybean meal-based diet compared to other groups (0.0 g/kg, 0.5 g/kg and 1.0 g/kg phytase). Feed cost was reduced due to supplementation of phytase, which increased the profitability of broiler rearing and Ali S. A. (2021) [2] experimented on the efficacy of supplementation of phytase enzyme (1,500 FTU/kg) with or without dicalcium phosphate in broilers and revealed that there is a significant (p<0.05) decreases in feed cost in phytase supplemented groups compared to control group.

Materials and Methods

A research investigation was carried out on one-hundred-and-twenty-day old cobb broiler chicks were obtained. The selection of cobb birds aimed to enhance feed quality and harness the bird genetic potential to minimize phosphorus excretion. The chicks were weighed and randomly distributed into four experimental treatments, each treatment with three replicates and each replicate with ten chicks. Until six weeks of age, the chicks were reared under a deep litter system, provided with ad-libitum feed and water, and adhering to standard management practices. The study was approved in the Institutional Animal Ethics Committee of KVAFSU, Bidar, Karnataka. The phytase enzyme (Zymo-phytase) sample contains 5,000 Fungal Thermal Unit (FTU) per kg which is extracted from *Aspergillus niger* was procured from STS Biotech Pvt Ltd, Mysuru.

Following the BIS-2007 [6] recommendations, standard broiler pre-starter, starter and finisher rations were formulated and prepared by using readily available feed ingredients. The control group T_1 was fed with 0.45% available phosphorus. The treatment groups T_2, T_3 and T_4 was fed with low phosphorus diet (0.35% available phosphorus + 0.01% Phytase 5,000 FTU), low phosphorus diet (0.30% available phosphorus + 0.02% Phytase 5,000 FTU) and low phosphorus diet (0.25% available phosphorus + 0.03% Phytase 5,000 FTU), respectively.

Mineral composition in blood and bone

Assessment of calcium and phosphorus in blood

Upon completion of the 42nd day of the experimental period the blood samples were collected from six birds per treatment (two birds per replicate) without the addition of an anti-coagulant. These samples were placed in sterile glass test tubes and maintained at room temperature in a slanted position for half an hour to facilitate serum separation. Subsequently, serum separation was achieved through centrifugation at 3,000 rpm for 10 minutes. The separated serum was then transferred to plastic vials and stored at – 20 °C for the assessment of calcium and phosphorus content. The mineral levels were later determined using a biochemical analyzer.

Evaluation of calcium and phosphorus in bone

To investigate bone mineralization, left tibia bones were collected from two birds (two birds per replicate) at 42nd days of age to study the Calcium and Phosphorus content. The procedure involved acid insoluble ash (AIA) for Calcium and Phosphorus determination. The collected bone sample was weighed and subjected to ashing in a muffle furnace for five hours at 550 °C. Following ashing, the sample was washed with 25 ml of concentrated Hydrochloric acid (HCl) and boiled for five minutes. The resulting solution was then filtered through Watsman filter paper with the aid of hot water and the volume was adjusted to 250 ml.

Calcium estimation procedure

The solution containing calcium underwent treatment with Ammonium oxalate until all calcium precipitated as calcium oxalate. The precipitate was further treated with Sulfuric acid, dissolving it into calcium sulphate. This process liberated free Oxalic acid which was quantitatively estimated by titration against N/10 Potassium permanganate solution to determine the calcium content in the solution (where 1 ml of N/10 potassium permanganate is equivalent to 0.002 g of calcium) (Talapatra *et al*., 1940) [22].

Phosphorus estimation procedure

The phosphorus present in the ash solution was precipitated

as ammonium phosphomolybdate and phosphorus was indirectly estimated by titration of the molybdate portion of the compound with an alkali (N/7 sodium hydroxide) (AOAC, 1990) [4].

$$\text{Mineral retention ratio} = \frac{(\text{WFI} \times \text{EF}) - (\text{WEV} \times \text{EE})}{(\text{WFI} \times \text{EF})} \times 100$$

WFI = Weight of total feed intake
EF = Concentration of calcium and phosphorous in feed
WEV = Weight of total excreta voided
EE = concentration of calcium and phosphorous in faeces

Economics
The viability of incorporating phytase supplementation in low phosphorus diets for broiler birds was assessed using the formula the calculation for feed cost per kilogram of body weight gain is determined by dividing the total feed consumed in kilogram by the body weight gain in kilogram, and then multiplying by the cost of feed per kilogram.

Statistical evaluation
The experimental design employed in this study was completely randomized design (CRD) with one - way analysis. Data related to different parameters of the biological trial were analysed using the standard procedure outlined by Snedecor and Cochran (1994) [20] and SPSS 20 statistical software was utilized. Mean differences were examined through Tukey's Range Test at a significance level of ($p \leq 0.05$).

Results

Blood calcium and phosphorus composition (mg/dl)
At the end of experiment (42nd day), the mean blood calcium was observed in T_1, T_2, T_3 and T_4 were 21.22, 21.08, 21.11 and 21.34, respectively. The statistical analysis revealed no significant ($p>0.05$) difference in blood calcium among treatments compared to control group.
At the end of experiment (42nd day), the mean blood phosphorus was observed in T_1, T_2, T_3 and T_4 were 5.83, 5.85, 5.93 and 5.76, respectively. The statistical analysis revealed no significant ($p>0.05$) difference in blood phosphorus among treatments.

Tibial bone calcium and phosphorus composition (%)
At the end of experiment (42nd day), the mean bone calcium (%) observed in T_1, T_2, T_3 and T_4 were 34.52, 34.43, 34.70 and 34.65, respectively. The statistical analysis revealed no significant ($p>0.05$) difference in bone calcium composition of tibial bone among treatments.
At the end of experiment (42nd day), the mean bone phosphorus (%) observed in T_1, T_2, T_3 and T_4 were 17.49, 17.51, 17.31 and 17.28, respectively. The statistical analysis revealed no significant ($p>0.05$) difference in bone phosphorus composition of tibial bone among treatments compared to control group.

Economics
The cost of production (Rs) at the end of experiment in different treatment groups observed was 65.45 (T_1), 60.36 (T_2), 60.48 (T_3) and 60.38 (T_4). Highest cost of production was observed in control group. The statistical analysis revealed a significant ($p \leq 0.05$) difference in cost of production among all the groups compared to the control group and there is no significant difference ($p>0.05$) in cost of production among treatment groups T_2, T_3 and T_4.

Discussion

The phytase supplemented groups had no significant difference ($p>0.05$) on blood calcium and phosphorous levels compared to control group at the end of the experiment (42nd day). The findings of the present results were in agreement with Ghosh *et al.* (2016) [11] investigated growth performance and serum biochemical profile by 50, 75 and 100 mg/kg manganese supplementation with or without 500 U/kg phytase on 350 Ven cobb 400 broilers containing 0.50%, 0.48% and 0.45% available phosphorous in starter, grower and finisher, respectively. They revealed that the serum concentrations of glucose, total cholesterol, calcium and phosphorous were not affected by supplemental manganese or phytase level or their interaction compared to control. The findings of the present results were in agreement with Shi *et al.* (2022) [18] conducted experiment on 624 female Ross 308 broilers with supplementation of high doses (0, 500, 1,000, 2,500, 5,000, and 10,000 FTU/kg phytase in the basal diet containing 0.25% non-phytate phosphorus) of phytase enzyme with 0.5% and 1% of calcium revealed phytase did not affect plasma calcium and phosphorus concentrations in 42-day-old broilers ($p>0.05$). Phytase supplementation does not disrupt the balance of calcium and phosphorous minerals, indicating its compatibility with mineral metabolism in broilers.
The phytase supplemented groups had no significant difference ($p>0.05$) on bone (tibia) calcium and phosphorous composition compared to control group at the end of the experiment (42nd day). The findings of the present results were in agreement with Pintar *et al.* (2005) [15] carried out 21-day experiment with 245-day old Ross broilers to evaluate the impact of phytase supplementation (500 and 1,000 PU/kg) to cereal-soybean meal-based diets containing 0.40% available phosphorous and two levels of calcium (0.6 and 1.0%) on the mineral content in tibia (Ca, P, Fe, Mg, Cd, Zn). They found that the levels of Iron ($p<0.024$) and Magnesium ($p<0.024$) in tibia were significantly increased by the supplementation of phytase, however, no difference was observed in the contents of Calcium, Phosphorous, Cadmium and Zinc in different treatments. The findings of the present results were in agreement with Ghosh *et al.* (2016) [11] conducted the effects of supplementation of 50, 75 and 100 mg/kg manganese with or without 500 U/kg phytase on muscle and tibia composition and immunity in 350 Ven cobb 400 broiler chickens containing 0.50%, 0.48% and 0.45% available phosphorous in starter, grower and finisher, respectively. Revealed that the manganese and phytase supplementation did not affect ash, calcium and phosphorus concentrations in tibial bone among different treatments. Phytase supplementation may not adversely affect the deposition or utilization of calcium and phosphorous in bone tissue. This implies that phytase inclusion does not compromise skeletal health or mineralization in broilers.
The economics of the birds was significantly differed ($p \leq 0.05$) by diet supplemented with phytase enzyme compared to control group at the end of the experiment (42nd day). The findings of the present results were in agreement with Singh *et al.* (2003) [19] concluded that phytase (500 FTU/kg) with varying amounts of non-phytate phosphorus (0.5% and 0.3%) completely replaces dicalcium phosphate,

so that starter and finisher diets were cheaper by Rs. 7.00 and Rs. 8.00, respectively in phytase supplemented groups compared to control in 480 Hubbard broilers. The present study results were in agreement with Ali S. A. (2021) [2] experimented on the efficacy of phytase enzyme (1,500 FTU/kg) supplementation containing different levels of available phosphorous (Starter diet with 0.39, 0.29 and 0.19% available phosphorous, finisher diet with 0.39, 0.28 and 0.17% available phosphorous) with or without dicalcium phosphate in 180 male Ross 308 broilers. Revealed that there is a significant (p<0.05) decreases in feed cost in phytase supplemented groups compared to control group. The decreased production cost may be due to microbial phytase improves growth efficiency, retained calcium and phosphorous present in the feed will result in decreased production costs.

Conclusion

Based on results it was concluded that supplementation of phytase enzyme in low phosphorous diet at different levels (0.01%, 0.02% and 0.03% Phytase 5,000 FTU) will improve higher returns on investments. However, phytase level 0.01% (5,000 FTU) showed slightly economical than other two levels but supplementation of phytase enzyme may not improve or adversely affect the both serum and tibial bone calcium and phosphorous composition.

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