Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(1): 58-62

Received: 15-11-2018
Accepted: 18-12-2018

Vidyasagar,
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

Jayanaik
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

V Malathi
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

BC Umashankar
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

C Kotresh Prasad
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

Irfan Ayub Sayyad
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

Correspondence
Vidyasagar Jayanaik
Veterinary College Hebbal, Bengaluru Karnataka Veterinary, Animal and Fisheries Science University, Bidar, Karnataka, India

Influence of phytase supplementation on mineral retention, blood and bone minerals content in low phosphorus diet fed in Giriraja birds

Vidyasagar, Jayanaik, V Malathi, BC Umashankar, C Kotresh Prasad and Irfan Ayub Sayyad

Abstract
The present experiment was conducted to evaluate the effect of phytase on mineral retention, blood and bone mineral content of low phosphorus diet fed in Giriraja birds supplied with *ad libitum* feed and water. Weighed and wing banded 420, day old chicks were randomly divided into seven groups(T_1- T_7) with four replicates (R_1-R_4) in each group containing 15 birds in each replicate (60 chicks per treatment) and reared under deep litter system till 6 weeks of age and fed as per the recommendations of ICAR (2013). The diet in T_1 (0.45% available P), T_2 (0.35% aP), T_3 (0.3% aP), T_4 (0.35% aP) + Phytase (P) 500 FTU/kg, T_5 (0.3% aP) + P 500 FTU/ kg, T_6 (0.35% aP) + P1000 FTU/kg, T_7 (0.3% aP) + P1000 FTU/kg. The data revealed that the addition of phytase enzyme in low phosphorus diet had significant ($P \leq 0.05$) effect on calcium and phosphorus retention and in bone Ca and P but there was no significant difference found in T_4, T_5, T_6 and T_7. Low blood Ca was observed in phytase fed birds. Whereas, the significant difference ($P \leq 0.05$) was observed in blood P concentration.

Keywords: Griraja, phytase, growth, enzyme, mineral retention

1. Introduction
Poultry feed consist major portion of plant based feed ingredients and the availability of Phosphorous in commonly used feedstuffs of plant origin is approximately 30-40% (Nelson *et al.*, 1968) [11]. Phytic acid is a potent anti-nutritional factor present in plant-derived feeds. The phytic acid contains phosphorous in bond form, which reduces the availability of phosphorus. Concentration of the enzyme in the small intestine of the chicken is too low to degrade phytate, effectively (Maenz *et al.*, 1997) [9].

The phytate- phosphorus (PP) presents in the diet but not available to the birds and is excreted in the faeces. Environmental pollution from P in poultry wastes is becoming a concern in areas of intensive poultry production because of the considerable quantities of poultry manure being applied to pastures and croplands. The P which is not utilized by the plants accumulates in the root zone. The leaching, runoff and erosion in that area can lead to pollution of surface water causing eutrophication (Waldroup, 1999) [20]. The high levels of P in surface waters increases the growth of algae and bacteria that in turn consumes a greater proportion of the oxygen in water resulting in death of many aquatic species (Corell, 1999) [5]. Thus, it is desirable to find a method for reducing the P in the manure of poultry without affecting the performance of the birds. The dietary supplementation of microbial phytase in maize-soyabean based broiler diets improved the availability or retention of P and calcium (Ca) (Singh *et al.*, 2003) [17]. There has been many research conducted to study the mineral availability in commercial broilers but Giriraja is a backyard pultry breed and it is necessary to reduce the pollution of the environment by retention of phosphorous. Hence, the present study was conducted to observe the effect of phytase on mineral retention in Giriraja birds.

2. Materials and Methods
2.1 Treatment details
Study was conducted in Giriraja birds from 1 to 6 weeks of age. A total of 420, day old straight run Giriraja chicks were procured and the experiment was conducted at the Department of Poultry Science, Veterinary College, KVAFSU, Hebbal, Bengaluru of Karnataka. Chicks were wing banded, weighed and randomly assigned to seven groups (T_1-

T_7) with four replicates (R1-R4) in each group containing 15 birds in each replicate (60 chicks per treatment). The diet T_1 (0.45% available P), T_2 (0.35% aP), T_3 (0.3% aP), T_4 (0.35% aP) + Phytase (P) 500 FTU/kg, T_5 (0.3% aP) + P 500 FTU/ kg, T_6 (0.35% aP) + P1000 FTU/kg, T_7 (0.3% aP) + P1000 FTU/kg. The chicks were reared in deep litter system with all standard manage mental practices till sixth week of age. Standard vaccination schedule was followed for immunizing the chicks. Feed ingredients required for the formulation of the experimental diets were procured from the feed mill unit of the Department of Poultry Science, Veterinary College, KVAFSU and prepared as per the recommendations of ICAR (2013). The enzyme phytase sample (Cibenza phytoverse 10000 FTU/g), which is extracted from *Pseudomonas fluorescens*, required for the trial was obtained from Novas international Pvt. Ltd.

2.2 Mineral Retention (Retention of Ca and P)

A three day metabolic trial was conducted from 35th-38th days of age during which the feed consumed by each replicate in the respective dietary group was recorded and dropping voided by each replicate over same period were collected quantitively.

After completing 35 days of feeding trial all the birds were starved for the period of six hours. During which preliminary preparation such as cleaning of feeders, waters and feacal trays and keeping of feed and drinking water was done. The feacal materials voided in the feacal trays by each replicate were cleaned. The respective feeder and feacal tray were introduced into cage housing and weighed quantity of feed was offered to all the birds. The dropping of respective replicate was collected separately once daily and transferred into pre weighed aluminium dishes, weighed again and placed into the forced draft hot air oven at 60±5 ^{0}C for during all the three days of collection.

On the last day, the feeders were removed to determine the net feed intake and feacal trays were removed to collect the faeces in aluminium dishes. The droppings collected were dried for four to five days in oven at 60±5 ^{0}C till a constant weight was attained which represented the net dried feacal output. Then the dried excreta samples were ground and stored in air tight plastic container. The collected sample was weighed and kept in muffle furnace for ashing up to five hours at 550 ^{0}C. (AIA) and the Ca and P was estimated as per the standard procedure

2.3 Analysis of bone Ca and P Content

In order to study the bone mineralization, the left tibia bones were collected from three birds (three birds from each replicate) at 42nd day of age to study the bone mineral content (Ca and P). The bones were dried and collected sample was weighed and kept in muffle furnace for ashing up to five hours at 550 ^{0}C. (AIA). Ca and P were estimated as per the standard procedure (Talapatra, 1940) [18].

2.3.1 Procedure for estimation of Ca and P (Retention of Ca, P and bone Ca and P Content)

2.3.1.1 Acid insoluble ash (AIA) for Ca and P

The collected sample was weighed and kept in muffle furnace for ashing up to five hours at 550 ^{0}C. The sample is washed with 25 ml of conc. HCl and boiled it for five minutes, later it was filtered through watsman filter paper with the help of hot water. Then the volume is made up to 250 ml.

2.3.1.2 Determination of calcium

The solution containing Ca is treated with ammonium oxalate till all the calcium is precipitated as calcium oxalate. The precipitate is treated with sulphuric acid which further dissolves it into calcium sulphate, liberating free oxalic acid which is quantitavely estimated by titration against N/10 potassium permanganate solution to arrive at the calcium content in the given solution (1 ml of N/10 potassium permanganate is equivalent to 0.002g of calcium) (Talpatra *et al.*, 1940) [18].

2.3.1.3 Determination of phosphorus

The phosphorus present in the ash solution is precipitated as ammonium- phosphomolybdate and phosphorus is estimated indirectly by titration of the molybdate portion of the compound with an alkali (N/7 sodium hydroxide). (AOAC, 1990).

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

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

2.4 Analysis of blood Ca and P content

At the end of 42nd d of experimental period, blood samples from 12 birds/treatment (3birds/replicate) were collected into sterile glass test tube without addition of anti-coagulant. Test tubes containing the blood were kept in a slanted position at a room temperature for an half an hour to facilitate separation of serum. Serum was separated by centrifugation at 3000 rpm for 10 minutes and serum was decanted into plastic vials and then stored at -20 ^{0}C for estimation of serum calcium and phosphorus content. Later the minerals are estimated by using biochemical analyser.

Table 1: Percent ingredient and nutrient composition of basal experimental diet (ICAR, 2013)

Ingredients (Kg)	T_1	T_2	T_3	T_4	T_5	T_6	T_7
Yellow maize	56	56	56	56	56	56	56
Soya bean meal	35	35	35	35	35	35	35
DORB	4.95	5.3	5.36	5.3	5.36	5.3	5.36
Mineral mixture*	2	1	0.7	1	0.7	1	0.7
Shell grit	0.4	1.25	1.6	1.25	1.6	1.25	1.6
DCP	0.3	0.2	0.09	0.2	0.09	0.2	0.09
Vitamin premix **	0.2	0.2	0.2	0.2	0.2	0.2	0.2
B complex***	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Coccistat	0.1	0.1	0.1	0.1	0.1	0.1	0.1

Liver tonic	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Mycotoxin Binder	0.1	0.1	0.1	0.1	0.1	0.1	0.1
DL-Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Antibiotic	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Total	100	100	100	100	100	100	100
Phytase****	0	0	0	5g	5g	10g	10g
Nutrient composition							
ME (Kcal/kg) [b]	2833	2839.6	2844	2839.6	2844	2839.6	2844
Crude protein (%) [b]	21.09	21.12	21.15	21.12	21.15	21.12	21.15
Calcium (%) [a]	1.10	1.10	1.12	1.10	1.12	1.10	1.12
Phosphorous (%) [a]	0.47	0.36	0.32	0.36	0.32	0.36	0.32
Lysine (%) [a]	1.27	1.27	1.27	1.27	1.27	1.27	1.27
Methionine (%) [a]	0.45	0.45	0.45	0.45	0.45	0.45	0.45

* Mineral mixture: Each 100 g contains Magnesium oxide- 1.48g, Ferrous sulphate- 6.0 g, copper sulphate- 0.05g, Manganese Sulphate-0.04 g, Potassium Iodide- 0.001g, Potassium Chloride-17.09g and Sodium selenite- 0.001g.

** Vitamin- Premix: Each 100g contains Vitamin AD3 (Vitamin A-10, 00, 000 IU/g, Vitamin D-200000 IU/g)- 0.165g, Vitamin K3-0.103g,

***B complex and vitamin E:Vitamin E- 2.4g, Thiamine Mononitrate- 0.206 g, Riboflavin- 0.513g, Pyridoxine hydrochloride- 0.309g, Cyanocobalamine- 0.00031g, Folic acid- 0.103g, Niacin-4.124 g, Ca-D-Pantothenate- 1.031g, Biotin- 1.5g, Maltodextrine- 89.545g.

**** Each gram of phytase contain 10000FTU/g

[a] calculated values; [b] analyzed values

2.5 Statistical analysis

Data pertaining to various parameters obtained during the trial were analyzed statistically by ANOVA using SPSS 20.0 statistical software. Differences between the means were tested using Duncans Multiple Range Test (Duncan, 1995) [6] at $P \leq 0.05$.

3. Results and Discussion

3.1 Mineral Retention

The addition of phytase enzyme in low phosphorus diet had significant ($P \leq 0.05$) effect on calcium and phosphorus retention but no significant difference was found in T4, T_5, T_6 andT_7 (Table 2.). The result are in agreement with Onyango *et al.* (2005) [12] who added *Escherichia coli*-derived phytase @500 FTU/kg+ 0.75g/kg P and 1000 FTU/kg +1.5 g /kg P in P deficient diet and concluded that *Escherichia coli* phytase helps in improving the retention of P, Ca ($P<0.01$). The present results are also in agreement with Saima *et al*. (2009) [15] Viveros *et al.* (2002), Ahmad *et al.* (2000) [1], Singh *et al.* (2003) [17] and Zhou *et al.* (2008) [21], who reported that phytase supplementation significantly ($P<0.05$) improved the availability of P and Ca in the chicks fed on rations with different levels of Phytase as compared to the control diet. This improvement in Ca retention was due to the phytase that liberated Ca from the Ca-phytate complex and P retention was due to hydrolysis of phytate by microbial phytase, which may release all constituents minerals, myo-inositol and inorganic phosphate which in turn increases the P retention and its concentration (Viveros *et al.* 2002).

Table 2: Effect of phytase on mineral retention (Calcium and Phosphorus)

Experimental group	Description of the treatment	Calcium %	Phosphorus %
T_1	Control(0.45% available P)	34.46±0.66[a]	65.72±0.60[ab]
T_2	Low P diet (0.35% available P)	33.07±1.11 [a]	63.83±0.59 [a]
T_3	Low P diet (0.3% available P)	32.40±1.43 [a]	62.76±0.63 [a]
T_4	Low P diet (0.35% available P + Phytase 500 FTU/kg)	38.35±0.75[b]	70.83±0.64 [c]
T_5	Low P diet (0.3% available P + phytase 500 FTU/ kg)	38.10±1.04 [b]	69.01±1.61 [bc]
T_6	Low P diet (0.35% available P + Phytase 1000 FTU/kg)	39.99±0.48 [b]	70.89±1.71 [c]
T_7	Low P diet (0.3% available P + Phytase 1000 FTU/kg)	39.12±0.74 [b]	69.98±2.46 [c]

[a,b,c,] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

3.2 Bone Ca and P

From the results in table 3 it was observed that the phytase enzyme in low phosphorus diet had significant ($P \leq 0.05$) effect on bone calcium and phosphorus percentage (increased concentration of Ca and P). No significant difference was observed between T_4, T_5 and T_6, T_7 in calcium as well as phosphorus bone mineral concentration. The present results are in correlation with the result of Chung *et al.* (2013) [4] who studied the effect of two microbial phytases on bone mineral density in low-phosphorus diets for broilers and observed that phytase supplementation (1500 or 3000 FTU/kg in low-P diet) improved bone P and calcium retention. Similarly Rezaei *et al*. (2007) [14] also found that supplementation of phytase @ 500 FTU/ kg of diet has increased the Ca and P concentration in tibial ash ($P>0.05$), Brenes *et al.* (2003) [3], Bozkurt *et al.* (2006) [2], Mondal *et al.* (2007) [10] and Lalpanmawia *et al.* (2014) who also observed that phytase supplementation at different level had improved in bone phosphorus and calcium retention and there was no significant difference observed between mineral concentration supplemented with phytase in low-P diet.

Table 3: Effect of phytase on bone calcium and phosphorus

Experimental group	Description of the treatment	Calcium (%)	Phosphorus (%)
T_1	Control(0.45% available P)	28.02±1.26[a]	13.26±0.84[b]
T_2	Low P diet (0.35% available P)	27.46±0.92 [a]	11.01±0.53 [a]
T_3	Low P diet (0.3% available P)	27.34±0.78 [a]	10.92±0.38 [a]
T_4	Low P diet (0.35% available P + Phytase 500 FTU/kg)	32.08±0.69[b]	15.13±0.43 [c]
T_5	Low P diet (0.3% available P + phytase 500 FTU/ kg)	31.47±1.18 [b]	15.01±0.52 [c]
T_6	Low P diet (0.35% available P + Phytase 1000 FTU/kg)	32.91±0.61 [b]	15.60±0.35 [c]
T_7	Low P diet (0.3% available P + Phytase 1000 FTU/kg)	31.98±0.69 [b]	15.18±0.42 [c]

[a,b,c,] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

3.3 Blood Ca and P

The addition of phytase enzyme in low phosphorus diet had significantly increased ($P \leq 0.05$) blood phosphorus, whereas it had non-significant ($P > 0.05$) effect on blood Ca (Table 4.). The result of calcium and phosphorus supplement are in agreement with Sebastian *et al*. (1996) [16], they conducted a trial to study the efficacy of microbial phytase (Natuphos 1000) on relative retention of P, Ca, Cu, and Zn, and mineral contents of plasma and concluded that the microbial phytase increased the plasma P by 15.7% and reduced ($P < 0.05$) the Ca concentration by 34.1%. Viveros *et al*. (2002) also reported that phytase increased the ($P < 0.0001$) plasma P level by 8% and reduced the plasma Ca ($P < 0.0001$) level by 2.3% in broilers, along with them Brenes *et al.* (2003) [3], Panda *et al.* (2007) [10, 13] and Lan *et al.* (2012) [8] who supplied the phytase enzyme at different concentration and observed similar results.

Table 4: Effect of phytase on blood Calcium and Phosphorus

Experimental group	Description of the treatment	Calcium (mg/dl)	Phosphorus (mg/dl)
T_1	Control(0.45% available P)	11.98±0.48[b]	6.61±0.45 [a]
T_2	Low P diet (0.35% available P)	11.61±0.51 [b]	4.67±0.33 [a]
T_3	Low P diet (0.3% available P)	11.84±0.77[b]	4.35±0.20 [b]
T_4	Low P diet (0.35% available P + Phytase 500 FTU/kg)	9.78±0.32[a]	9.30±0.43 [c]
T_5	Low P diet (0.3% available P + phytase 500 FTU/ kg)	9.96±0.52 [a]	9.01±0.28 [c]
T_6	Low P diet (0.35% available P + Phytase 1000 FTU/kg)	9.85±0.26 [a]	10.84±0.53 [d]
T_7	Low P diet (0.3% available P + Phytase 1000 FTU/kg)	9.71±0.43 [a]	10.52±0.28 [d]

[a,b,c,d] Means in the same column with no common superscript differ significantly ($P \leq 0.05$)

4. Conclusion

The Giriraja birds supplemented with phytase showed a significant improvement in mineral retention, bone mineral retention, blood phosphorus when compared to birds with low phosphorus diet and controlled diet but non-significant difference was observed in blood calcium level among the various groups of birds. From the present study it can be concluded that the supplementation of phytase will helps in mineral utilization and thus reduces the environmental pollution by minimizing the excretion of unutilized minerals by birds.

5. Acknowledgments

The authors thankful to Karnataka Veterinary, Animal and Fisheries Sciences University, Bidar, Karnataka for financial support.

6. References

1. Ahmad T, Rasool S, Sarwar M, Haq A, Hasan Z. Effect of microbial phytase produced from a fungus *Aspergillus niger* on bioavailability of phosphorus and calcium in broiler chickens, Anim. Feed Sci. Tech. 2000; 83:103-114.
2. Bozkurt MM, Cabuk, Alcicek A. The effect of microbial phytase in broiler grower diets containing low phosphorous, energy and protein. J Poult. Sci. 2006; 43:29-34.
3. Brenes A, Viveros A, Arija I, Centeno C, Pizarro M, Bravo C. The effect of citric acid and microbial phytase on mineral utilization in broiler chicks. Anim. Feed. Sci. Technol. 2003; 110:201-219.
4. Chung TK, Rutherfurd SM, Thomus DV, Moughan PJ. Effect of two microbial phytases on mineral availability and retention and bone mineral density in low phosphorous diet for broilers. Br. Poult. Sci. 2013; 54(3):362-373.
5. Correll DL. Phosphorus: A rate limiting nutrient in surface waters. Poult. Sci. 1999; 78:674-682.
6. Duncan DB. Multiple range and multiple F-tests. Biometrics. 1995; 11:1-42.
7. Lalpanmawia H, Elangovan AV, Sridhar M, Shet D, Ajith S, Pal DT. Efficacy of phytase on growth performance, nutrient utilization and bone mineralization in broiler chicken. Anim. Feed Sci. Technol. 2014; 192:81-89.
8. Lan G, Abdullah N, Jalaludin S, Hoa YW. Effects of freeze-dried *Mitsuokella jalaludinii* culture and Natuphos phytase supplementation on the performance and nutrient utilisation of broiler chickens. J Sci. Food Agri. 2012; 92:266-273.
9. Maenz DD, Engele-Schaan CM, Classen HL. Endogenous phytase activity in the small intestinal brush border membrane of broiler chicks and laying hens. Poult. Sci. 1997; 76:71.
10. Mondal MK, Panda S, Biswas P. Effect of microbial phytase in soybean meal based broiler diets containing low phosphorous. Int. J. Poult. Sci. 2007; 6(3):201-206.
11. Nelson TS, Sheih TR, Wodzinski RJ, Ware JH. The availability of phytate phosphorus in soybean meal before and after treatment with a mold phytase. Poult. Sci. 1968; 47:1842-1848.
12. Onyango EM, Bedford MR, Adeola O. Efficacy of an evolved *Escherichia coli* Phytase in Diets of Broiler Chicks. Poult. Sci. 2005; 84:248-255.

13. Panda AK, Savaram V, Rao R, Mantena VLN, Gajula SS, Bhanja SS. Performance of broiler chicken fed low non phytate phosphorus diets with microbial phytase. The J Poult. Sci. 2007; 44:258-264.
14. Rezaei M, Borbor S, Zaghari M, Teimouri A. Effect of phytase supplementation on nutrients availability and performance of broiler chicks. Int. J of Poultry Sci. 2007; 6(1):55-58.
15. Saima MZU, Khan MA, Jabbar M, Qadee MA. Efficacy of microbial phytase at different levels on growth performance and mineral availability in broiler chicken. J Anim. Plant Sci. 2009; 19(2):58-62.
16. Sebastian S, Touchburn SP, Chavez ER, Lague PC. Efficacy of supplemental microbial phytase at different dietary calcium levels on growth performance and mineral utilization of broiler chickens. Poult. Sci. 1996; 75:1516-1523.
17. Singh PK, Khatta VK, Thakur RS, Dey S, Sangwan ML. Effects of phytase supplementation on the performance of broiler chickens fed maize and wheat based diets with different levels of non-phytate phosphorus. Asian-Aust. J Anim. Sci. 2003; 16(11):1642-1649.
18. Talpatra SK, Ray SC, Sen KC. Estimation of phosphorus, cholin, calcium, magnesium, sodium, potassium in feeding stuffs. J Vet. Sci. Anim. Husb. 1940; 10:243-245.
19. Viveros A, Centeno C, Brenes A, Canales R, Lozano A. Phytase and acid phosphatase activities in plant feedstuffs. J Agric. Food Chem. 2000; 48:4009-4013.
20. Waldroup PW. Nutritional approaches to reducing phosphorus excretion by poultry. Poult. Sci. 1999. 78:683-691.
21. Zhou JP, Yang ZB, Yang WR, Wang XY, Jiang SZ, Zhang GG. Effects of a new recombinant phytase on the performance and mineral utilization of broilers fed phosphorus-deficient diets. J Appl. Poult. Res. 2008. 17:331-339.