Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola

ISSN 1516-635X 2023 / v.25 / n.4 / 001-010

http://dx.doi.org/10.1590/1806-9061-2023-1773

Original Article

Effect of Phytase Supplementation on Growth Performance, Mineral Digestibility, and Tibia Calcium and Phosphorous in Broilers Fed Low Phosphorus Diets

■Author(s)

Iqbal W[I] https://orcid.org/0000-0001-7721-2034
Yaseen MA[I,II] https://orcid.org/0000-0002-5518-1027
Rahman MA[I] https://orcid.org/0000-0002-6894-1128
Bhatti SA[I] https://orcid.org/0000-0002-5626-3809
Rahman MS[I] https://orcid.org/0000-0001-7767-6007
Yaqoob MU[III,IV] https://orcid.org/0000-0003-4072-2182
Ahmad F[V] https://orcid.org/0000-0002-7185-6610
Zahid MU[VI] https://orcid.org/0000-0003-4413-912X
Shoaib M[I] https://orcid.org/0000-0002-6408-7737

[I] Institute of Animal and Dairy Sciences, University of Agriculture, Faisalabad 38000, Pakistan.
[II] Faculty of Veterinary Medicine, University of Teramo, Loc. Piano D'Accio, Teramo 64100, Italy.
[III] College of Animal Science, Zhejiang University, Hangzhou 310058, China.
[IV] Provincial Key Agricultural Enterprise Research Institute of King Techina, Hangzhou King Techina Feed Co., Ltd., Hangzhou 311107, China.
[V] Nizami Feeds Pvt. Ltd., Lahore, Pakistan.
[VI] Department of Animal Nutrition, University of Veterinary and Animal Science, Lahore, Pakistan.

■Mail Address

Corresponding author e-mail address
Muhammad Umar Yaqoob
College of Animal Science, Zhejiang University, Hangzhou 310058, China and Provincial Key Agricultural Enterprise Research Institute of King Techina, Hangzhou King Techina Feed Co., Ltd., Hangzhou 311107, China.
Phone: +86 135 16852571
Email: 11817048@zju.edu.cn

■Keywords

Bone mineralization, carcass characteristics, growth performance, mineral digestibility, phytase, phosphorous.

Submitted: 11/February/2023
Approved: 02/JuLY/2023

ABSTRACT

The aim of present study was to investigate the effect of phytase supplementation on growth performance, mineral digestibility, and tibia calcium and phosphorous in broilers fed low phosphorus diets. Three hundred broiler chicks were allotted to six different treatments with five replicates each (10 chicks/replicate). T1: Control, 0.5% available phosphorus (Av.P); T2: 0.35P, 0.35% Av.P; T3: 0.20P, 0.20% Av.P; T4: 0.35P-1Phy, diet 0.35P + 1000FTU/kg phytase; T5: 0.20P-2Phy, diet 0.20P + 2000 ftu/kg phytase; T6: 0.20P-3Phy, diet 0.20P + 3000 ftu/kg phytase. Feed intake and weight gain were higher (p=0.001) in broilers fed phytase supplemented diets. Feed conversion ratio was better (p<0.01) in the 0.35P-1Phy than in the others. Calcium and P digestibility was higher (p<0.05) in the 0.35P-1Phy than the 0.35P, 0.20P and 0.20P-2Phy, groups. The highest (p<0.05) tibia ash was observed in the 0.35P and 0.35P-1Phy groups rather than the 0.20P at 21st day, while at 33rd day it was higher (p<0.05) in the control than in 0.20P. At 21st day, tibia Ca content was higher (p<0.05) in the 0.35P-1Phy group as compared to 0.20P and 0.35P, while at 33rd day, tibia Ca content in the control and 0.20P-2Phy groups was higher (p<0.05) than that of 0.20P and 0.35P. Furthermore, tibia P content was higher (p<0.05) in all phytase supplemented groups. It could be concluded that dietary available phosphorus at 0.35% with phytase addition at 1000 FTU/kg reduces the cost per unit weight gain by 9.17%, with positive effects on growth performance.

INTRODUCTION

Calcium (Ca) and phosphorus (P) play a significant role in bone development and chicken well-being, as well as providing compressive strength and rigidity to the bones (Tizziani *et al.,* 2016). Most of the ingredients of plant origin used for poultry feed formulation contain P as a phytate complex, which affects the availability of P (Tahir *et al.*, 2012). Wheat and corn both contain 0.18% phytate phosphorus, but the bioavailability of phosphorus from both sources is different, i.e. 61.5 and 47.06 %, respectively (Ahmed *et al.*, 2003; Mossa *et al.*, 2018). Phytate also binds many minerals like Ca, P, iron, zinc and makes them unavailable to birds, which results in weak bones and stunted growth (Hakami *et al.*, 2022). Phosphorus deficiency causes bone abnormalities like osteopenia, osteoporosis, and osteodystrophy (Applegate *et al.*, 2003; Manangi & Coon, 2008), leading to bone breakage or defects. To fulfill P requirements, inorganic phosphorus (mostly dicalcium phosphate "DCP") is supplemented in poultry feed, but it increases feeding costs (Makiyama *et al.*, 2012). This problem could be resolved by supplementing phytase in poultry rations to increase the bioavailability of P already present as phytate complex in feed ingredients, which ultimately decreases the feeding cost (Gehring *et al.*, 2013). Dietary supplementation of phytase enhances the bioavailability of P in broiler chicks. Phytase supplementation of 5000 FTU/kg hydrolyzes 99.45 % of the phosphorus present in cereals (Manangi & Coon, 2008) and could replace DCP in broiler feed (Scholey *et al.*, 2018). The phytase enzyme releases phosphorus by breaking the phytic acid and helps in the synthesis of myoinositol, giving better results in growth and development (Cowieson *et al.*, 2011; Baloch *et al.*, 2021). Phytase catalyzes the inorganic phosphate that contains chelated minerals and improves their availability to birds (Al-Harthi *et al.*, 2020). Keeping in view the importance of phytase in poultry diets, the present study was designed to investigate the effect of phytase supplementation on growth performance, mineral digestibility, and tibia calcium and phosphorous in broilers fed low phosphorus diets. It was hypothesized that phytase supplementation could increase the availability of phosphorus to broilers and dietary phosphorus levels could be reduced without compromising the growth performance in broilers. Therefore, this experiment was designed to study the effect of phytase supplementation on growth performance, mineral digestibility, and tibia calcium and phosphorous in broilers fed low phosphorus diets.

MATERIAL AND METHODS

This study was carried out after the animal experiment was approved by the Office of Graduate Studies, University of Agriculture, Faisalabad (Decision number: DGS/18981-84; Dated: 05/08/2020).

House preparation: Feeding trial was conducted at Research Farms of University of Agriculture, Faisalabad, Pakistan. Cleaning, washing, fumigating, and sealing the house prior to the arrival of chicks helped reduce the number of bacteria and other microorganisms in the environment. All proper sanitary procedures were followed during the experimental trial. The chickens were vaccinated on day 1, day 8, day 18, and day 25 with ND + IB, IBD, and IBD vaccines, respectively.

Experimental design and birds: Three hundred day-old broiler chicks (Ross-308) with average weight of 40.05 grams were divided into 6 different treatments, following a completely randomized design (Table 1). Treatments were as following: T1: Control, 0.5% available phosphorus (Av.P); T2: 0.35P, 0.35% Av.P; T3: 0.20P, 0.20% Av.P; T4: 0.35P-1Phy, diet 0.35P + 1000 FTU/kg phytase; T5: 0.20P-2Phy, diet 0.20P + 2000 FTU/kg phytase; T6: 0.20P-3Phy, diet 0.20P + 3000 FTU/kg phytase, each for starter and finisher phase. The basal diet was formulated according to the breed-specific guide (Ross-308) as shown in Table 2 and 3. Faczyme-10HG (heat stable phytase with 10.000 U/g, Tangshan Finely Animal Care Co., Ltd. China) was used as a source of phytase. Birds were provided with free access to feed and water throughout the feeding trial and an optimal environment was provided. The experiment lasted for 33 days and was divided into two phases (starter phase from day 1 to 21 and finisher phase from day 22 to 33). During the first week, temperature was maintained at 35 °C, being reduced by 5 °C per week and maintained at 21 °C during the rest of the experimental period. Relative humidity was adjusted to 65%, and a stocking density of 0.06 m^2 per bird was used.

Table 1 – Experimental plan.

Treatments	Description
Control	0.50% Available Phosphorus (Av.P)
0.35P	0.35% Av.P
0.20P	0.20% Av.P
0.35P-1Phy	0.35% Av.P + 1000 FTU/kg Faczyme
0.20P-2Phy	0.20% Av.P + 2000 FTU/kg Faczyme
0.20P-3Phy	0.20% Av.P + 3000 FTU/kg Faczyme

Samples collection and measurements

Growth Performance: Initial weight of chicks was recorded on arrival at the shed. The weight of feed offered and refusal were recorded to determine feed intake. Body weight of chicks was recorded weekly to determine the weight gain. Feed conversion ratio (FCR) was calculated from data on feed intake and weight gain,.

Carcass Characteristics: At the end of the feeding trial, two birds were collected randomly from each replicate. Those birds were weighted and slaughtered through halal neck cut method as explained by (Ali *et al.*, 2011). After slaughtering, visceral organs and giblets were removed and carcass weight was measured to calculate dressing percentage. After evisceration, the absolute weight of various internal organs such as heart, liver and gizzard of the slaughtered birds were recorded. The weight of internal organs were expressed as a percentage of carcass weight.

Minerals Digestibility: Calcium and phosphorus digestibility were determined at the end of each phase by total collection method. Celite® was used as an external marker and added in the last five days of feed at the rate of 1% of feed, and fecal samples were collected during the last two days. Composited samples for each replicate were dried at 65 °C and stored till further processing. Feed and fecal material were analyzed (AOAC, 2000) for Ca, P and celite present in it to calculate digestibility of Ca and P.

Table 2 – Ingredients and nutrient composition of basal diets (Starter phase).

Ingredients	Treatments					
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy
Corn	50	50	50	50	50	50
Soybean meal	25.5	25.5	25.5	25.5	25.5	25.5
Canola meal	6.00	6.00	6.00	6.00	6.00	6.00
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00
Rice broken	4.00	4.00	4.00	4.00	4.00	4.00
Rice polish	3.00	3.00	3.00	3.00	3.00	3.00
Poultry Fat	3.00	3.00	3.00	3.00	3.00	3.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium Phosphate	1.63	0.87	0	0.87	0	0
Limestone	0.7	1.2	1.8	1.2	1.8	1.8
Salt	0.4	0.4	0.4	0.4	0.4	0.4
DL- Methionine	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine sulphate	0.4	0.4	0.4	0.4	0.4	0.4
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06
Phytase	0	0	0	0.01	0.02	0.03
Vit. Min. premix*	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Nutrient composition (Calculated)						
Crude Protein	22.0	22.0	22.0	22.0	22.0	22.0
Metabolic Energy	3000	3000	3000	3000	3000	3000
Calcium	0.98	0.98	0.98	0.98	0.98	0.98
Available Phosphorus	0.50	0.35	0.20	0.35	0.20	0.20
Ether Extract	6.1	6.1	6.1	6.1	6.1	6.1
Fiber	3.2	3.2	3.2	3.2	3.2	3.2
Ash	2.5	2.5	2.5	2.5	2.5	2.5
Dietary Electrolyte Balance	245	245	245	245	245	245
Dig. Methionine	0.53	0.53	0.53	0.53	0.53	0.53
Dig. Lysine	1.2	1.2	1.2	1.2	1.2	1.2
Dig. Threonine	0.8	0.8	0.8	0.8	0.8	0.8
Dig. Tryptophan	0.22	0.22	0.22	0.22	0.22	0.22
Dig. Met+Cys	0.75	0.75	0.75	0.75	0.75	0.75
Nutrient composition (Analyzed)						
Dry matter	90.23	89.87	90.1	90.4	90.54	89.45
Crude Protein	21.98	21.88	21.93	22.04	21.96	21.75
Ether Extract	6.12	5.98	6.1	6.13	5.98	6.15
Calcium	0.96	0.97	0.99	1.00	0.97	0.98
Total Phosphorus	0.64	0.46	0.27	0.45	0.28	0.27

* Vit. A 15000 IU/kg, E 60 IU/kg, D_3 3000 IU/kg, B_1 2 mg/kg, B2 8 mg/kg, K3 3 mg/kg, niacin 45 mg/kg, B6 4 mg/kg, pantothenic acid 15 mg/kg, B12 1 mg/kg, folic acid 1 mg/kg, choline chloride (60%) 500 mg/kg, manganese sulphate 18.5 mg/kg, magnesium sulphate 53 mg/kg, zinc sulphate 2 mg/kg, copper sulphate 45 mg/kg and ferrous sulphate 35 mg/kg.

Digestibility (%) = 100 – (100 × maker in feed (%) / marker in feces (%) × mineral in feces (%) / mineral in feed (%))

Tibia Ash Determination: The tibiae were dried at 110 °C for 12 hours, then extracted with ether, dried once more, and finally weighed. The dry, fat-free bones were ashed at 550 °C in a muffle furnace. The percentage of dry, fat-free bone mass that was transferred to ash weight was determined as explained by (Catalá-Gregori *et al.,* 2006).

Tibia Calcium and Phosphorous: Tibia samples were also collected from slaughtered birds and analyzed for the concentration of Ca and P present in them (AOAC, 2000).

Complete Blood Count: On the last day of trial, 2 birds from each replicate were randomly picked and blood was collected through slaughtering and stored in EDTA tubes for determination of complete blood count (Shoaib *et al.*, 2021).

Economic analysis: The experiment was conducted in 2020, but the latest cost of feed ingredients, chick cost, as well as miscellaneous expenses has been used. Economic parameters of the flock were determined by calculating the cost of chicks, feed ingredients (including phytase enzyme), and miscellaneous costs for flock raising.

Statistical Analysis: The observed data were analyzed using a statistical software. Analysis of

Table 3 – Ingredients and nutrient composition of basal diets (Finisher phase).

Ingredients (%)	Treatments					
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy
Corn	53.00	53	53	53	53	53
Soybean meal	21.5	21.5	21.5	21.5	21.5	21.5
Canola meal	6.00	6.00	6.00	6.00	6.00	6.00
Rapeseed meal	2.00	2.00	2.00	2.00	2.00	2.00
Rice broken	5.00	5.00	5.00	5.00	5.00	5.00
Rice polish	3.00	3.00	3.00	3.00	3.00	3.00
Poultry Fat	3.00	3.00	3.00	3.00	3.00	3.00
Fish meal	3.00	3.00	3.00	3.00	3.00	3.00
Dicalcium Phosphate	1.63	0.87	0	0.87	0	0
Limestone	0.7	1.2	1.8	1.2	1.8	1.8
Salt	0.4	0.4	0.4	0.4	0.4	0.4
DL- Methionine	0.2	0.2	0.2	0.2	0.2	0.2
L-Lysine sulphate	0.4	0.4	0.4	0.4	0.4	0.4
L-Threonine	0.06	0.06	0.06	0.06	0.06	0.06
Phytase	0	0	0	0.01	0.02	0.03
Vit. Min. Premix*	0.5	0.5	0.5	0.5	0.5	0.5
Total	100	100	100	100	100	100
Nutrient composition (Calculated)						
Crude Protein	20	20	20	20	20	20
Metabolic Energy	3200	3200	3200	3200	3200	3200
Calcium	0.98	0.98	0.98	0.98	0.98	0.98
Available Phosphorus	0.5	0.35	0.2	0.35	0.2	0.2
Ether Extract	6.1	6.1	6.1	6.1	6.1	6.1
Fiber	3.2	3.2	3.2	3.2	3.2	3.2
Ash	2.5	2.5	2.5	2.5	2.5	2.5
Dietary Electrolyte Balance	205	205	205	205	205	205
Dig. Methionine	0.53	0.53	0.53	0.53	0.53	0.53
Dig. Lysine	1.2	1.2	1.2	1.2	1.2	1.2
Dig. Threonine	0.8	0.8	0.8	0.8	0.8	0.8
Dig. Tryptophan	0.22	0.22	0.22	0.22	0.22	0.22
Dig. Met+Cys	0.75	0.75	0.75	0.75	0.75	0.75
Nutrient composition (Analyzed)						
Dry matter	90.32	90.55	90.12	90.54	90.22	90.10
Crude Protein	20.10	20.00	19.95	20.11	20.05	19.93
Ether Extract	5.98	5.93	6.12	6.06	6.12	5.93
Calcium	0.95	0.97	0.94	1.05	0.95	0.94
Phosphorus	0.65	0.47	0.27	0.45	0.28	0.27

* Vit. A 15000 IU/kg, E 60 IU/kg, D_3 3000 IU/kg, B_1 2 mg/kg, B_2 8 mg/kg, K_3 3 mg/kg, niacin 45 mg/kg, B_6 4 mg/kg, pantothenic acid 15 mg/kg, B_{12} 1 mg/kg, folic acid 1 mg/kg, choline chloride (60%) 500 mg/kg, manganese sulphate 18.5 mg/kg, magnesium sulphate 53 mg/kg, zinc sulphate 2 mg/kg, copper sulphate 45 mg/kg and ferrous sulphate 35 mg/kg.

variance were conducted through a completely randomized design using Minitab 17, and means of treatments were compared using Tukey's Test (Steel *et al.*, 1997; Minitab, 2010).

RESULTS

During the starter phase, feed intake and weight gain were higher in phytase supplemented groups than non-supplemented groups. Better FCR was recorded in birds that received 0.35P-1Phy than in other treatments. Similarly, during the finisher phase, birds receiving enzymes in a low phosphorus diet had higher feed intake and weight gain. Improved FCR was recorded in birds receiving the 0.35P-1Phy and 0.20P-3Phy treatments. During the overall period, feed intake and weight gain were higher in birds of phytase supplemented groups than non-supplemented groups. Better FCR was recorded in birds receiving the 0.35P-1Phy treatment than other groups (Table 4).

Data regarding carcass characteristics at day 21 and 33 are given in Table 5. At day 21, dressing percentage and relative organ weights were not affected (p>0.05) by different phosphorus levels and phytase supplementation. Similarly, at day 33, all parameters of carcass characteristics were similar (p>0.05), except

Table 4 – Effect of phytase supplementation on growth performance of broilers fed low phosphorus diets.

Parameters	Treatments						SEM	*p*-value
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy		
Starter phase (1-21 days)								
Feed intake (g)	1073.65^{bc}	1143.00^{ab}	1005.00^{c}	1196.67^{a}	1211.50^{a}	1181.17^{a}	18.50	0.001
Weight gain (g)	749.30^{bc}	813.27^{ab}	718.90^{c}	883.70^{a}	877.23^{a}	851.30^{a}	16.50	0.001
FCR (g/g)	1.43^{a}	1.41^{ab}	1.40^{ab}	1.35^{b}	1.38^{ab}	1.39^{ab}	0.01	0.015
Finisher phase (22-33 days)								
Feed intake (g)	1019.09^{c}	1193.64^{b}	1116.36^{bc}	1370.91^{a}	1382.73^{a}	1423.64^{a}	36.40	0.001
Weight gain (g)	557.95^{c}	671.97^{b}	615.23^{bc}	789.32^{a}	787.80^{a}	814.85^{a}	21.90	0.001
FCR (g/g)	1.83^{a}	1.78^{b}	1.82^{a}	1.74^{c}	1.76^{bc}	1.75^{c}	0.00	0.001
Overall (1-33 days)								
Feed intake (g)	2092.74^{c}	2336.64^{b}	2121.36^{c}	2567.58^{a}	2594.23^{a}	2604.80^{a}	38.60	0.001
Weight gain (g)	1307.25^{c}	1485.24^{b}	1334.13^{c}	1673.02^{a}	1665.04^{a}	1666.15^{a}	22.20	0.001
FCR (g/g)	1.60^{a}	1.57^{ab}	1.59^{a}	1.53^{c}	1.56^{ab}	1.56^{ab}	0.00	0.001

Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg phytase. Means with different superscript differ significantly ($p<0.05$).

for heart and gizzard weight. Birds in the control group had higher ($p<0.05$) relative heart and gizzard weight than other groups.

A significant ($p<0.05$) effect of dietary treatments was found on Ca and P digestibility at the 21st and 35th days of experiment (Figure 1). At the 21st day, Ca digestibility was higher ($p<0.05$) in the control, 0.35P-1Phy and 0.20P-3Phy groups. At the 33rd day, higher ($p<0.05$) Ca digestibility was observed for the 0.35P-1Phy group as compared to other treatments. The highest ($p<0.05$) P digestibility was found in the control group and in birds of the 0.35P-1Phy group at the 21st and 33rd day, respectively.

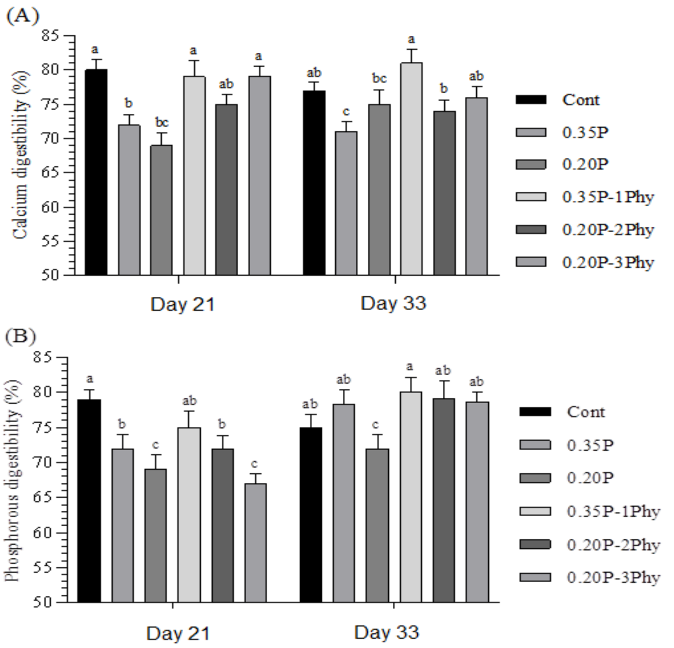

Figure 1 – Effect of phytase supplementation on calcium (A), and phosphorous (B) digestibility (%) in broilers fed low phosphorus diets. Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg phytase. Columns with different superscripts differ significantly ($p<0.05$).

Tibia ash contents were also significantly ($p<0.05$) affected by dietary treatments (Figure 2). The highest ash content was found for 0.35P and 0.35P-1Phy on the 21st day. Meanwhile, on day 33, tibia ash content of the control was higher than other groups.

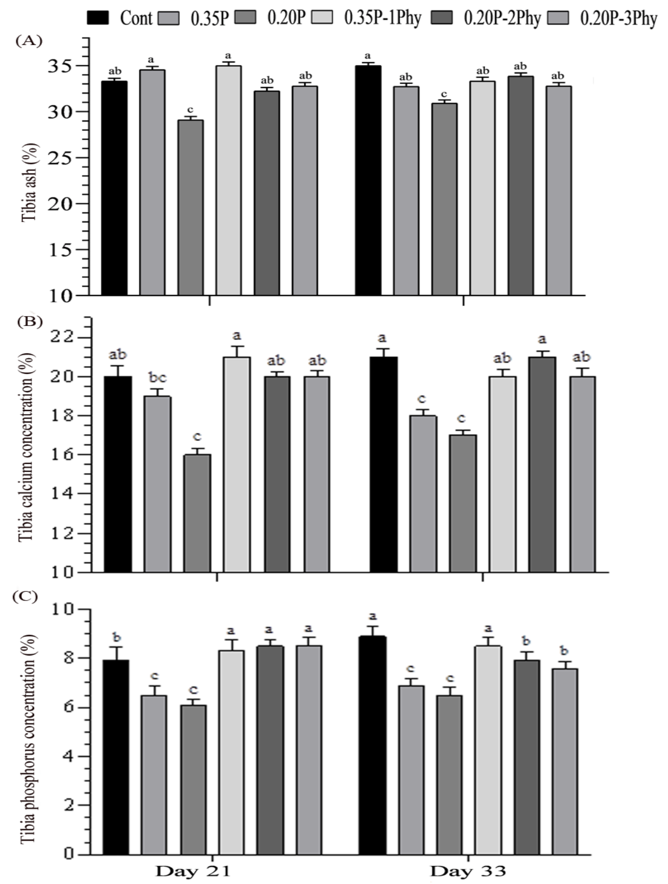

Figure 2 – Effect of phytase supplementation on tibia ash (A), tibia calcium (B), and tibia phosphorus (C) concentration of broilers fed low phosphorus diets. Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg phytase. Columns with different superscripts differ significantly ($p<0.05$).

Table 5 – Effect of phytase supplementation on carcass characteristics of broilers fed low phosphorus diets.

Parameters (%)	Treatments						SEM	*p*-value
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy		
At day 21								
Live weight (g)	760[b]	810[ab]	740[b]	890[a]	810[ab]	870[a]	22.90	0.001
Carcass weight (g)	413.62[b]	455[ab]	417.2[b]	499.2[a]	461.2[ab]	499.8[a]	15.20	0.001
Dressing %	54.42	56.19	56.46	56.04	56.96	57.45	0.42	0.505
Thigh yield	21.99	22.53	22.30	22.68	23.63	22.26	0.23	0.790
Breast yield	32.43	33.65	34.17	33.36	33.33	35.19	0.38	0.442
Heart weight	0.87	0.74	0.71	0.66	0.79	0.74	0.03	0.643
Gizzard weight	2.46	2.29	2.49	2.37	2.49	2.19	0.05	0.547
Liver weight	3.05	2.70	3.27	2.83	3.06	2.83	0.09	0.516
At day 33								
Live weight (g)	1270[b]	1560[a]	1420[ab]	1600[a]	1582[a]	1630[a]	56.10	0.001
Carcass weight (g)	742	878	827	915	909	883	55.10	0.197
Dressing %	58.19	56.22	58.43	57.27	57.26	54.18	0.64	0.844
Thigh yield	24.51	22.03	20.60	21.88	22.19	20.93	0.56	0.564
Breast yield	33.68	34.18	37.83	35.39	35.07	33.26	0.67	0.238
Heart weight	0.61[a]	0.49[ab]	0.61[a]	0.43[b]	0.50[ab]	0.42[b]	0.03	0.001
Gizzard weight	2.12[a]	1.69[ab]	1.92[ab]	1.57[ab]	1.70[ab]	1.29[b]	0.12	0.048
Liver weight	1.95	1.93	2.27	2.26	2.23	2.20	0.06	0.627

Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg phytase. Means with different superscript differ significantly ($p<0.05$).

Tibia Ca and P contents were also significantly ($p<0.05$) affected by dietary treatments (Figure 2). The highest tibia Ca content was found in the 0.35P-1Phy group at the 21st day. At day 33, tibia Ca contents of the control and 0.20P-2Phy groups were higher than in other groups. On the other hand, tibia P content of all phytase supplemented groups was higher than other groups at day 21. At day 35, the tibia P content of the control and 0.35P-1Phy groups were higher than other groups.

Blood hematology parameters (WBC, RBC, HGB, HCT, MCV, MCH, MCHC and PLT) were not affected ($p>0.05$) by phytase addition in broilers fed a low phosphorus diet (Table 6).

Table 6 – Effect of phytase supplementation on complete blood count of broilers fed low phosphorus diets.

Parameters	Treatments						SEM	*p*-Value
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy		
WBC 103/ul	28.53	26.98	28.48	27.64	28.20	28.48	0.647	0.485
RBC 103 / ul	2.33	2.07	2.37	2.18	2.90	2.37	0.212	0.161
HGB g/dl	10.03	8.90	10.38	9.23	12.50	10.38	0.940	0.161
HCT (%)	29.73	25.95	23.43	25.30	35.63	23.43	5.16	0.540
MCV (+FL)	127.70	126.03	123.98	118.58	122.65	123.98	2.51	0.223
MCH (+pg)	43.10	43.30	41.23	39.84	42.70	41.23	1.06	0.197
MCHC (g/dl)	33.73	34.35	32.83	33.52	34.83	32.83	0.624	0.193
PLT 103 /ul	2.25	1.75	1.88	2.75	3.00	1.88	0.751	0.789

Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg Phytase.

The economical appraisal of the experiment on the basis of dietary treatments is shown in (Table 7). The highest total cost of production (PKR 641.00) was observed in the 0.20P-3Phy group. Cost per kg weight gain was lower than the control in all phytase supplemented groups. Cost per unit weight gain in the 0.35P-1Phy group was PKR 38.45 less than that of control.

DISCUSSION

Results showed that boilers could grow better at low dietary available P with supplementation of phytase. They are supported by Hofmann *et al.* (2022), who showed that addition of phytase at 1,500 FTU/kg in reduced protein, amino acid and P diet had improved weight gain and FCR. Babatunde *et al.* (2022) observed

Table 7 – Effect of phytase supplementation on economics of broilers fed low phosphorus diets.

Cost in PKR	Treatments					
	Control	0.35P	0.20P	0.35P-1Phy	0.20P-2Phy	0.20P-3Phy
Broiler Chick	90.00	90.00	90.00	90.00	90.00	90.00
Feed cost/kg	194.96	193.44	191.86	193.57	192.35	192.34
Total Feed intake (kg)	2.09	2.34	2.12	2.57	2.59	2.60
Total feeding cost	408.00	452.00	407.00	497.00	499.00	501.00
Miscellaneous	50.00	50.00	50.00	50.00	50.00	50.00
Production cost / bird	548.00	592.00	547.00	637.00	639.00	641.00
Average weight gain/bird (kg)	1.31	1.49	1.33	1.67	1.67	1.67
Cost per kg weight gain	419.20	398.59	410.01	380.75	383.77	384.72

Control: 0.5% Av. P; 0.35P: low phosphorus 0.35% Av. P; 0.20P: low phosphorus 0.20% Av. P; 0.35P-1Phy: 0.35P+1000 ftu/kg phytase; 0.20P-2Phy: 0.20P+2000 ftu/kg phytase; 0.20P-3Phy: 0.20P+3000 ftu/kg Phytase.

that birds on a low P diet had lower weight gain and feed efficiency, but these parameters were improved with addition of phytase. Zarghi *et al.* (2022) revealed that phytase addition in wheat-based diets improved weight gain. In addition, Zanu *et al.* (2020) proposed that phytase supplementation improved intestinal morphology, and broilers could grow better with a healthy intestine. Scholey *et al.* (2018) stated that phytase could be used to provide the required level of P to broilers during finisher phase without using inorganic P, and that it is also an economical approach to fulfilling the P requirement of broilers. Furthermore, inorganic P sources like DCP are more expensive than phytase, so supplementation of phytase as an alternative to DCP ultimately reduces feed cost and is also environmentally friendly. Phytase supplementation also reduced the fecal phytate P excretion to the environment and reduced pollution (Pieniazek *et al.*, 2017). Truong *et al.* (2017) stated that phytase also enhanced the sodium reabsorption from the small intestine, which is indirectly linked to improving the absorption of nutrients. Phytate enhances the availability and efficiency of nutrients, which results in better production and lowers anti-nutritional effects in broilers (Beeson *et al.*, 2017). Phytase supplementation has been reported to increase the digestibility of amino acids and proteins in broilers (Gehring *et al.*, 2013), which might be the reason for improved growth performance. Phytase supplementation also regulates the endogenous enzyme secretions in broilers (Liu *et al.*, 2009). Pirgozliev *et al.* (2008) stated that broiler diets containing phytase at the levels of 250, 500 and 2500 FTU/kg increase BWG to 39.6, 41.1 and 44.0 gm per day, respectively.

A significant effect of dietary treatments was found on Ca and P digestibility. Ca and P digestibility was improved by phytase supplementation. Phytic acid reduces the availability and absorption of amino acids and protein by making them into insoluble complexes and lowering their digestibility (De Sousa *et al.*, 2015). Results are in line with Babatunde *et al.* (2022), who observed that Ca and P digestibility were improved with the addition of phytase. Zarghi *et al.* (2022) showed that phytase addition in a wheat-based diet improved dry matter digestibility. Supplementation of phytase is the best way for releasing chelated minerals from phytates. However, contrasting results were found by Mossa *et al.* (2018), who stated that supplemental phytase did not affect mineral digestibility. Dave & Modi (2019) reported a lower Ca digestibility for limestone when a purified assay diet was used and compared to a maize-based diet. Conflicting results might be due to different dosage rate of phytase, bird species, durations of the experiment, or source of phytase (fungal or bacterial) (Abd El-Hack *et al.*, 2018).

Reduced Ca and P levels in broiler ash contents ($p<0.05\%$ DM) given the diet with phytase supplementation was expected, as phytase enhances the release of phosphorus and other minerals from feedstuffs. According to various scientists, bone ash content is the most effective way to determine the amount of phosphorus produced by phytase in diets including maize and soybean meal Pereira *et al.* (2012), Nelson and Walker (1964).

Tibia Ca and P deposition during the starter and finisher phases were significantly improved by dietary supplementation of phytase in broiler diets. Results supported by Hofmann *et al.* (2022) showed that the addition of phytase at 1500 FTU/kg in reduced protein, amino acid, and P diets had improved tibia ash weight. Yan *et al.* (2001) stated that P availability could be increased by maintaining the Ca and P balance through phytase supplementation. Leeson *et al.* (2000) reported that tibias rich in ash results in better bone mineralization. High tibia ash percentage relates to more Ca and P retention in the tibia. Rutherfurd *et al.* (2004) concluded that phytase supplementation at the level of 1000 and 2000 FTU/kg in low-P diets enhanced both tibial mineral concentration and density by 35% and 24%, respectively. Phytase supplementations improved Ca and P digestibility (Ravindran *et al.*, 2006), which ultimately enhanced the availability of these minerals and might be the reason for enhanced Ca and P deposition in the tibia.

Blood hematology parameters were not affected by phytase addition in broilers fed a low phosphorus diet. Results are in line with the findings of Baloch *et al.* (2021), who stated that different levels of phytase had no effect on blood hematology parameters. Al-Harthi *et al.* (2020) reported that phytase addition in broiler diets had no effect on blood hematology parameters.

Improvement in the economic appraisal was observed with the supplementation of phytase enzyme in broiler diets. These findings agree with those of Rezaei *et al.* (2007), who concluded that phytase supplementation could improve production efficiency by enhancing growth performance, and Ca and P retention, and reducing production costs.

CONCLUSION

It could be concluded that dietary available phosphorus could be reduced by up to 0.35% with supplementation of phytase at the level of 1000 FTU/kg in broiler diets. Phytase supplementation improved calcium and phosphorus availability by increasing their digestibility, and also improved growth performance, and tibia calcium and phosphorus contents, without compromising carcass traits in broilers. Moreover, phytase supplementation at a reduced dietary available phosphorus level could reduce the cost per unit weight gain up to 9.17%.

ACKNOWLEDGMENTS

The authors are very grateful to Dr. Farhan Ahmad, General Manager, Nizami Feeds Pvt. Ltd., Lahore for providing the necessary facilities to conduct the research work hereby presented.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

REFRENCES

Abd El-Hack ME, Alagawany M, Arif M, *et al.* The uses of microbial phytase as a feed additive in poultry nutrition–A review. Annals of Animal Science 2018;18:639-58. https://doi.org/10.2478/aoas-2018-0009

Ahmed I, Javed K, Sattar A. Effect of phytate contents of cereals on bioavailability of total phosphorus in poultry. Pakistan Veterinary Journal 2003;14:23-6.

Al-Harthi MA, Attia YA, El-Shafey AS, *et al.* Impact of phytase on improving the utilisation of pelleted broiler diets containing olive by-products. Italian Journal of Animal Science 2020;19:310–8. https://doi.org/10.1080/1828051X.2020.1740896

Ali SA, Abdalla HO, Mahgoub IM, *et al.* Effect of slaughtering method on the keeping quality of broiler chickens' meat. Egypt Poultry Science. 2011;31(5):727-36.

AOAC- Association of Official Analytical Chemists. Official Methods of Analysis. 17th ed. Gaithersburg; 2000.

Applegate TJ, Angel R, Classen HL. Effect of dietary calcium, 25-hydroxycholecalciferol, or bird strain on small intestinal phytase activity in broiler chickens. Poultry Science 2003;82:1140–8. https://doi.org/10.1093/ps/82.7.1140

Babatunde OO, Bello A, Dersjant-Li Y, *et al.* Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. II. Grower phase (day 12–23 post hatching). Poultry Science 2022;101:101616. https://doi.org/10.1016/j.psj.2021.101616

Baloch FH, Baloch HN, Khan AU, *et al.* Effect of phytase enzyme on organs growth performance and blood profile of broiler. Advances in Enzyme Research 2021;9:37-49. https://doi.org/10.4236/aer.2021.93004

Beeson LA, Walk CL, Bedford MR, *et al.* Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. Poultry Science 2017;96:2243–53. https://doi.org/10.3382/ps/pex012

Catalá-Gregori P, García V, Madrid J, *et al.* Response of broilers to feeding low-calcium and total phosphorus wheat-soybean based diets plus phytase: performance, digestibility, mineral retention and tibiotarsus mineralization. Canadian journal of animal science. 2007: 87(4):563-9. https://doi.org/10.4141/CJAS07059

Cowieson AJ, Wilcock P, Bedford MR. Super-dosing effects of phytase in poultry and other monogastric. World's Poultry Science Journal 2011;67:225-36. https://doi.org/10.1017/S0043933911000250

Dave G, Modi H. Phytase-Fe3O4 nanoparticles-loaded microcosms of silica for catalytic remediation of phytate-phosphorous from eutrophic water bodies. Environmental Science and Pollution Research 2019;26:14988-15000. https://doi.org/10.1007/s11356-019-04794-y

Gehring C, Bedford M, Dozier W. Extra-phosphoric effects of phytase with and without xylanase in corn-soybean meal-based diets fed to broilers. Poultry Science 2013;92:979-91. https://doi.org/10.3382/ps.2012-02769

Hakami Z, Al Sulaiman AR, Alharthi AS, *et al.* Growth performance, carcass and meat quality, bone strength, and immune response of broilers fed low-calcium diets supplemented with marine mineral complex and phytase. Poultry Science. 2022;101(6):101849. https://doi.org/10.1016/j.psj.2022.101849

Hofmann P, Krieg J, Francesch M, *et al.* Effects of added phytase on growth performance, carcass traits, and tibia ash of broiler chickens fed diets with reduced amino acid, crude protein, and phosphorus concentration. Journal of Applied Poultry Research 2022;31:100258. https://doi.org/10.1016/j.japr.2022.100258

Leeson S, Namkung H, Cottrill M, *et al.* Efficacy of new bacterial phytase in poultry diets. Canadian Journal of Animal Science 2000;80:527-8. https://doi.org/10.4141/A99-123

Liu N, Ru YJ, Li FD. Effect of dietary phytate and phytase on metabolic change of blood and intestinal mucosa in chickens. Journal of Animal Physiology and Animal Nutrition 2009;94:368-74. https://doi.org/10.1111/j.1439-0396.2009.00917.x

Makiyama L, Alvarenga RR, Rodrigues PB, *et al.* Energetic and nutrient metabolizability values of corn obtained with nutritional corrections for broilers. Brazilian Journal of Animal Science 2012;41:1308-12. https://doi.org/10.1590/S1516-35982012000500031

Manangi MK, Coon CN. Phytate phosphorus hydrolysis in broilers in response to dietary phytase, calcium, and phosphorus concentration. Poultry Science 2008;87:1577-86. https://doi.org/10.3382/ps.2007-00336

Minitab I. Minitab statistical software (Release 17). State College; 2010.

Mossa AF, Chrystalb PV, Dersjant-Lic Y, *et al.* Responses in digestibilities of macro-minerals, trace minerals and amino acids generated by exogenous phytase and xylanase in canola meal diets offered to broiler chickens. Animal Feed Science and Technology 2018;2018:22-30. https://doi.org/10.1016/j.anifeedsci.2018.03.011

Nelson TS, Walker AC. The biological evaluation of phosphorus compounds: A summary. Poultry Science 1964;43:94-8. https://doi.org/10.3382/ps.0430094

Pereira R, Menten JFM, Romano GG, *et al.* Efficiency of a bacterial phytase to release phytate phosphorus in broiler chicken diets. Brazilian Archive of Veterinary Medicine and Animal Science 2012;64:137-44.

Pieniazek J, Smith KA, Williams MP, *et al.* Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poultry Science 2017;96:370-82. https://doi.org/10.3382/ps/pew225

Pirgozliev V, Oduguwa O, Acamovic T, *et al.* Effects of dietary phytase on performance and nutrient metabolism in chickens. British Poultry Science 2008;49:144-54. https://doi.org/10.1080/00071660801961447

Ravindran V, Morel PCH, Partridge GG, *et al.* Influence of an Escherichia coli-derived phytase on nutrient utilization in broiler starters fed diets containing varying concentrations of phytic acid. Poultry Science 2006;85:82-9. https://doi.org/10.1093/ps/85.1.82

Rezaei M, Borbor S, Zaghari M, *et al.* Effect of phytase supplementation on nutrients availability and performance of broiler chicks. International Journal of Poultry Science. 2007;6:55-8. https://doi.org/10.3923/ijps.2007.55.58

Rutherfurd SM, Chung TK, Morel PC, *et al.* Effect of microbial phytase on ileal digestibility of phytate phosphorus, total phosphorus, and amino acids in a low-phosphorus diet for broilers. Poultry Science 2004;83:61-8. https://doi.org/10.1093/ps/83.1.61

Scholey DV, Morgan NK, Riemensperger A, *et al.* Effect of supplementation of phytase to diets low in inorganic phosphorus on growth performance and mineralization of broilers. Poultry Science 2018;97: 2435-40. http://doi.org/10.3382/ps/pey088

Shoaib M, Bhatti SA, Nawaz H, *et al.* Effect of different emulsifiers on growth performance, intestinal histology and serum biochemistry in broilers reared on different fat sources. Pakistan Veterinary Journal 2021;41:185-190. https://doi.org/10.2478/aoas-2022-0083

Sousa JPL de, Albino LFT, Vaz RGM, *et al.* The effect of dietary phytase on broiler performance and digestive, bone and blood biochemistry characteristics. Brazilian Journal of Poultry Science 2015;17:69-76. https://doi.org/10.1590/1516-635x170169-76

Steel RGD, Torrie JH, Dickie DA. Principles and procedures of statistics. A biometric approach. 3rd. Toronto: McGraw-Hill; 1997.

Tahir M, Shim MY, Ward NE, *et al.* Phytate and other nutrient components of feed ingredients for poultry. Poultry Science. 2012 91(4):928-35. https://doi.org/10.3382/ps.2011-01893

Tizziani T, Donzele MO, Donzele JL, *et al.* Available phosphorus levels in diets supplemented with phytase for male broilers aged 22 to 42 days kept in a high-temperature environment. Brazilian Journal of Animal Science 2016;45:48-55. http://doi.org/10.1590/S1806-92902016000200002

Truong HH, Yu S, Mossa AF, *et al.* Phytase inclusions of 500 and 2000 FTU/kg in maize-based broiler diets impact on growth performance, nutrient utilisation, digestive dynamics of starch, protein (N), sodium and IP6 phytate degradation in the gizzard and four small intestinal segments. Animal Feed Science and Technology 2017;223:13-22. https://doi.org/10.1016/j.anifeedsci.2016.10.018

Yan F, Kersey J, Waldroup P. Phosphorus requirements of broiler chicks three to six weeks of age as influenced by phytase supplementation. Poultry Science 2001;80:455-9. https://doi.org/10.1093/ps/80.4.455

Zanu HK, Keerqin C, Kheravii SK, *et al.* Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 2. intestinal permeability, organ weights, hematology, intestinal morphology and jejunal gene expression. Poultry Science 2020;99:2581-94. https://doi.org/10.1016/j.psj.2019.12.049

Zarghi H, Golian A, Hassanabadi A, *et al.* Effect of zinc and phytase supplementation on performance, immune response, digestibility and intestinal features in broilers fed a wheat-soybean meal diet. Italian Journal of Animal Science 2022;21:430–44. https://doi.org/10.1080/1828051X.2022.2034061