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Bacterial or fungal origin phytase enzyme affects the performance and mineralization of calcium and phosphorus differently in broiler chickens fed deficient calcium and phosphorous diets

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ABSTRACT

Three Ca-P deficient diets viz., Diet-1 (0.85%, 0.35%), Diet-2 (0.75%, 0.30%) and Diet-3 (0.65%, 0.25%) were prepared and supplemented with either bacterial or fungal origin phytase enzymes and were compared with a control diet (1.0% and 0.45%). A total of 280 broiler (Ross 308) chicks were randomly allocated into 7 treatments having 4 replicates of 10 chicks using a completely randomized design. The effect of bacterial phytase on the growth, FCR and carcass weight was significantly ($P < 0.05$) higher in chicks on Diet-1 compared to diet-3 (28 days). Bacterial phytase increased serum minerals (Ca and P) and tibia ash in diet-1 compared to Diet-3. Tibia Ca was significantly ($P < 0.05$) higher in the control and the three diets under bacterial phytase compared to other treatments. On Diet-3, more Ca and P were reflected in tibial by bacterial phytase but did not support the optimum growth performance of broiler chicks. In conclusion, the same phytase but of different origins might have specific individual effects on the growth performance, serum and tibia Ca and P in broilers.

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Broiler performance; bacterial and fungal phytase; bone mineralization; Ca & P

Introduction

Phosphorus (P) is a key nutrient needed in significant amounts in poultry diets. It ranks second in terms of dietary importance. Plants serve as one of the sources of P, specifically in the form of phytate P. However, this form of P is largely inaccessible to birds due to the absence of phytase, an enzyme required for its digestion. To fulfil the P dietary needs, typically inorganic P supplements are added to the diets. This practice adds an extra financial burden to the overall cost of feed. For instance, dicalcium phosphate, a common source of P, is relatively expensive (Anawati and Azimi 2020; Walk et al. 2021). The combination of biological demands on the birds, the increased cost due to supplementation and the growing environmental impact caused by P in poultry waste highlights the importance of exploring methods to enhance the availability of phytate P (Saleh 2017).

Phytate P is poorly utilized by monogastric animals especially chickens due to the lack of micro-flora required to significantly hydrolyze the phytate molecule. In recent years, growing public concern over the excessive phosphorus content in poultry manure has prompted research into strategies to enhance the accessibility of phytate phosphorus. Phytase, chemically known as myo-inositol-hexaphosphate phosphohydrolase, is an enzyme that is produced by microorganisms or naturally present in certain plant ingredients. Phytase can be obtained from a variety of sources (Tanruean et al. 2021). Phytases either of microbial or fungal origin added to the feed that cleaves P moieties from phytate and thus increases the hydrolysis of the phytate molecule (de Farias et al. 2020; Saleh et al. 2021).

Phosphorus plays a vital role in almost all physiological functions of the body and supports growth and skeletal development (Bhadada and Rao 2021). Some skeletal disorders, such as rickets, osteomalacia and tibial dyschondroplasia, have been linked to a reduction in bone mineralization and decreased feed intake of containing deficient P. It revealed that not only adequate supply of dietary phosphorus is essential but the biological availability in the diet is of utmost importance (Pierce 2000). The availability of phytate P is heightened in the broiler diet after phytase addition. The optimum requirement of non-phytate P for broiler chickens is 0.45% as recommended by the NRC (1994). Driver et al. (2005) reported that a deficient diet in P compensates for the deficiency by increasing their ability to utilize phytate P when enriched with phytase of broiler diet. Usually, most of the phytases dephosphorylate phytate complexes at either the 3- or the 6- position. Both bacterial *Escherichia coli* (*E. coli*) and fungal *Pichia pastoris* (*P. pastoris*) phytases are enzymatically active at the 6-position. These phytases have been employed in commercial settings to enhance the utilization of phytate phosphorus by chicks.

Numerous studies demonstrate the effect of phytase either of fungal or bacterial origin. Some studies (Luciana et al. 2012; Attia et al. 2020; Saleh et al. 2021) showed that fungal phytase could improve the overall performance of broiler chickens

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while others (Bilal and Eracg 2003; Dilger et al. 2004; Ravindran et al. 2006; Jamal et al. 2009; Huyghebaert et al. 2009; Rouhollah et al. 2011) revealed efficacy of bacterial phytase on the growth performance, phytate digestion and relative phosphorus availability. It can be concluded that both bacterial and fungal origin phytases are efficient sources of phytases; however, scanty literature is available on their comparative efficacy in determining the relative P replacement value and its ultimate effect on the growth performance in broiler chickens. This study investigates the comparative efficacy of bacterial (*E. coli*) and fungal (*P. pastoris*) origin phytase enzymes on the growth performance, availability of Ca and P in the serum and their accumulation in the tibia bones in broiler chickens fed Ca- and P-deficient diets.

Materials and methods

A standard corn soybean meal diet that served as a control formulated met all the needs of the National Research Council (NRC 1994) for the broiler chickens (Table 1). Three deficient levels of Ca (0.85, 0.75 and 0.65%) and P (0.35, 0.30 and 0.25%) were used to formulate the Ca-P-deficient diets (Diet-1: 0.85%, 0.35%; Diet-2: 0.75%, 0.30%; Diet-3: 0.65%, 0.25%). These deficient diets were fed with the recommended doses of either a bacterial (*E. coli*) or fungal (*P. pastoris*) origin phytase and were compared with the corn-soybean meal basal diet (control). The bacterial phytase was dosed at the rate of 1000 g/ton (*E. coli,* Phyzyme XP-TPT, Danisco Animal Nutrition, Marlborough, Wiltshire, UK) while the fungal phytase (*P. pastoris,* PHY5C, Sinobios Industry Co Ltd, China) was added at the rate of 100 g/ton to get 1000 FTU/kg in the diets. The term 'FTU' stands for 'Phytase Units,' and it signifies the enzyme amount needed to generate 1 µmol of P per minute from a sodium phytate solution with a concentration of 5.1 mmol/L, at a temperature of 37°C and a pH of 5.5.

Table 1. Composition of the basal and Calcium-Phosphorus-deficient diets[1].

		Ca-*P* deficient		
Ingredients	Control	Diet-1	Diet-2	Diet-3
Yellow corn	54.40	55.86	56.73	57.55
Soybean meal (48%)	30.39	30.02	29.79	29.62
Canola meal (36%)	3.30	3.30	3.30	3.30
Corn Gluten meal (60%)	2.20	2.20	2.20	2.20
Fish meal (60%)	2.50	2.50	2.50	2.50
Vegetable oil	3.00	2.55	2.28	2.01
Molasses	1.00	1.00	1.00	1.00
Limestone	1.27	1.18	1.07	0.96
Dicalcium phosphate	1.26	0.73	0.46	0.19
Common Salt	0.43	0.43	0.43	0.43
DL-Methionine	0.12	0.12	0.12	0.12
Vit:Min premix[2]	0.12	0.12	0.12	0.12
Analysed composition				
CP, %	23.41	23.36	23.32	23.31
ME, kcal/g	3.07	3.07	3.07	3.07
Calcium, %	1.0	0.85	0.75	0.65
Available *P*, %	0.45	0.35	0.30	0.25
Lys, %	1.26	1.26	1.25	1.25
Met, %	0.53	0.53	0.53	0.53
Met + Cys, %	0.90	0.90	0.90	0.90
Thr, %	0.89	0.89	0.88	0.88

[1]Control = 1.0% Ca and 0.45% non-phytate phosphorus (NPP); Diet-1 = 0.85% Ca, 0.35% with bacterial/fungal phytase; Diet-2 = 0.75% Ca, 0.30% NPP with bacterial/fungal phytase; Diet-3 = 0.65% Ca, 0.25% NPP with bacterial/fungal phytase.

[2]Contained the following (per kg of diet) nicotinic acid, 45 mg; thiamine mononitrate, 2.5 mg; vitamin B12 (cobalamin), 13.0 µg; d-calcium pantothenate, 13 mg; d-biotin, 0.12 mg; pyridoxine hydrochloride,4.8 mg; menadione sodium bisulphate complex, 3.4 mg; folic acid, 5.52 mg; cholecalciferol, 25.7 µg; choline chloride, 222 mg; all-*raca*-tocopheryl acetate, 12 mg; *trans*-retinyl acetate, 1,991 µg; manganese (MnSO4·H2O), 64 mg; ethoxyquin, 130 mg; zinc (ZnO), 50 mg; iron (FeSO4·7H2O), 35 mg; iodine (ethylene diaminedihydroiodide), 0.16 mg; copper (CuSO4·5H2O), 7 mg; selenium (NaSeO3), 0.4 mg.

A total of 300 day-old male broiler (Ross 308) chicks were purchased from the local market. The chicks were brooded for 7 days on a commercial starter diet in an electrically heated battery brooder. After brooding, 280 chicks, having equal body weights, were selected and randomly allocated into 7 treatment groups with 4 replicates of 10 chicks each. The experimental design was complete randomized. The chicks underwent a preconditioning period for 2 days, spanning from 5 to 7 days, after which they were provided with the experimental rations *ad libitum*. The chicks were raised in an open-sided house with adequate ventilation and the floor was covered with sawdust as litter. The house was appropriately illuminated using electric bulbs. Each pen was equipped with feeders, drinkers and other necessary supplies to ensure consistent management and environmental conditions. The experiment spanned 4 weeks, during which the chicks were fed the experimental diets from 7 to 28 days of age. Vaccination procedures followed the routine schedule.

The initial average body weight was documented at the commencement of the experiment, followed by weekly recordings of average body weight, feed intake and feed conversion ratio (FCR) throughout the study.

After the trial, the average body weight and remaining feed were measured. Subsequently, a minimum of 2 birds per replicate were randomly chosen and processed for slaughter. The weights of the carcasses and livers were duly recorded. Blood samples were obtained in dry, sterile test tubes. These blood samples were then subjected to centrifugation at 3000 rpm for 2 min. Following centrifugation, the serum was carefully extracted into designated serum cups, labelled and stored for subsequent analysis. The samples were analysed to determine their Ca and P content.

The left tibia from two birds per replicate was selected. The tibia samples were collected, cleaned to remove any traces of meat and blood and then meticulously placed in appropriately labelled polythene bags. These bags were then stored in a freezer until they were prepared for analysis. The tibia samples were then analysed for tibia ash content, calculated on a fat-free dry-weight basis following the procedure outlined by AOAC International (2005). Additionally, the Ca and P contents per gram of tibia ash were determined using the AOAC (2005) method.

The dry matter content of both the feed and excreta samples was assessed by oven drying at 60°C. Subsequently, the samples were ground to a particle size of 1 mm and then stored in labelled, clean bottles at room temperature. Metabolic energy was measured using a bomb calorimeter, while the Kjeldahl method was employed to determine Nitrogen (N) levels.

Statistical analysis

The concluding data were documented in Microsoft Excel Worksheets to facilitate organization and basic statistical calculations. The data underwent the analysis of variance (ANOVA)

utilizing the general linear model (GLM) procedure of the Statistical Analysis System (SAS 2006). Subsequently, Duncan's multiple-range tests were employed to differentiate the means. A probability (P) value equal to or less than 0.05 was deemed statistically significant.

Results

The effect of supplementing different origin phytases either bacterial or fungal was compared to investigate their efficacy on the growth performance, retention of Ca and P content in the blood serum and their accumulation in the tibia bone ash of broiler chicks.

Weekly and average growth performance

The differences during the first week of the experiment among the diets did not result in significant changes. During the second week of the experiment, the bacterial-origin phytase supplementation was effective in supporting the optimum growth performance and FCR until Ca and P levels of 0.75 and 0.30%, respectively. The effectiveness of fungal phytase was lower in Diet-2. Phytases did not demonstrate their effects in Diet-3 to enhance performance (Table 2). In the third week, almost clear differences were observed in the body weight gain and FCR when enriched with phytases. The improved weight gain and FCR were obtained on Diet-1 enriched with bacterial phytase. In the other Ca- and P-deficient diets, bacterial origin phytase revealed similar growth performance as the control. Among the phytases, bacterial phytase at each deficient level of Ca and P showed enhanced growth than fungal phytase. The fungal phytase below Ca and P levels of 0.85 and 0.35%, respectively was not efficient to support optimum growth performance. The performance was gradually declined and was lowest on Diet-3 fungal supplemented phytase where the Ca and P levels were reduced up to 0.65 and 0.25, respectively.

The average weight gain and FCR were improved in chicks on Diet-1 enriched with bacterial origin phase. The supplementation of bacterial-origin phytase up to 0.75% Ca and 0.30% P in the diets showed improved weight gain and better FCR in chicks than fungal-origin phytase. When the Ca and P levels were reduced to 0.75 and 0.30%, respectively in Diet-2, the response in weight gain and FCR by supplementing bacterial origin phytase was similar to the control. The fungal-origin phytase in this diet (Diet-2) did not support the improved growth performance. Among the two enzymes used in Diet-2, the body weight gain was 6.32% higher for the microbial than fungal-based phytase supplementation. Further decrease in the levels of Ca and P to 0.65 and 0.25% (Diet-3), respectively significantly decreased weight gain and supplementation of both phytases either bacterial or fungal origin was not effective in ameliorating the growth performance.

The carcass weight showed variations as a function of different levels of Ca and P with either bacterial or fungal origin phytase enzyme (Table 3). The R^2 of the model showed that 65% of the variations in the carcass weight were caused by the diets. The broiler chicks fed Diet-1 irrespective of enzyme origin revealed similar carcass weight as the control. Microbial phytase supplementation in Diet-2 helped to get enhanced the carcass weight. Whilst the fungal based phytase supplementation at Diet-2 did not ameliorate the carcass weight. Its effect on the carcass weight of broiler chicks was pronounced only in Diet-1. There was 4.65% higher carcass weight for chicks fed microbial than fungal supplemented Diet-2 containing 75% Ca and 67% P of NRC recommended. The carcass weight was significantly decreased with the decreasing level of Ca and P in diets. It was lowest ($P < 0.05$) when the level of Ca and P in the diets were reduced below 75% and 67, respectively, of NRC. Fungal based phytase supplementation in diets having Ca and P level below 0.85 and 0.35, respectively, was less effective in improving the carcass weight of broiler chicks. No significant change was observed in liver weight between the control and three other diets supplemented with bacterial and fungal phytase.

Retention of Ca and P in the blood serum and its accumulation in the tibia bone ash

The chicks fed Diet-1 supplemented with B phytase revealed the highest Ca and P content of 12.48 and 3.76 mg per 100 ml of blood serum, respectively, while the chicks fed Diets-3 had the lowest blood serum Ca content of 10.05 and 9.89 mg/100 ml of blood serum. The accumulation of Ca and P in the blood serum was decreased with the gradual decline of these mineral in the diets. The differences between enzyme supplementation were pronounced until the Ca and P in diets were fed of 85 and 77.8%, respectively of NRC (1994). Below this level, the enzyme supplementation failed to show significant differences in the blood serum Ca and P content.

The enzyme supplementation in different Ca and P level diets had a significant ($P < 0.01$) effect on tibia ash, tibia Ca and tibia P content of broiler chicks. The models adapted had a strong coefficient of determination for the tibia ash ($R^2 = 0.81$) and tibia P ($R^2 = 0.80$) indicating strong fit of the model. However, the tibia Ca content accounted for 57% of the observed variations. The tibia ash contentdecreased with the decreasing level of Ca and P in the diets and was lowest in chicks on Diet-3. However, the tibia ash content was similar as the control in chicks on Diet-1 when enriched with microbial phytase. A similar effect of microbial phytase was determined in chicks on Diet-2 but was not enough to make it similar with the control.

The bacterial phytase was more efficient in increasing the tibia P content than fungal phytase. The tibia P content was enhanced in Diet-1 and Diet-2 when enriched with bacterial rather than fungal phytase. The supplementation of fungal phytase showed a gradual decline in P content with decreasing levels of Ca and P in the diets. However, the differences between the two phytase enzymes on the accumulation of P content disappeared in chicks on Diet-3 containing 65% Ca and 56% P of NRC (1994) recommended.

The effect of two different phytase enzymes from bacterial and fungal origin on blood and tibia calcium and phosphorous is presented in Table 4. The results showed that blood Ca and P were significantly ($P < 0.05$) higher in Diet-1 supplemented with

Table 2. The effect of different origins of phytase supplementation in calcium and phosphorus deficient diets on the body weight, feed intake and feed conversion ratio of broiler chicks during the first, second and third week of the experiment.

		First Week			Second Week			Third Week		
		Weight gain	Feed intake		Weight gain	Feed intake		Weight gain	Feed intake	
Diets[1]	Phytase[2]	g		FCR	g		FCR	g		FCR
Control	–	255.0±4.08	416.50±15.00	1.63±0.08	357.50^{ab}±13.23	547.50±9.57	1.45^{bc}±0.07	464.17^{bc}±8.67	967.59±14.92	2.09^{b}±0.05
Diet-1	B	261.25±24.28	412.75±7.50	1.59±0.15	348.75^{abc}±23.32	548.25±9.60	1.58^{abc}±0.11	527.10^{a}±24.18	972.50±11.23	1.85^{c}±0.10
	F	256.25±13.77	411.50±11.90	1.61±0.08	341.25^{abc}±11.09	547.50±8.10	1.61^{ab}±0.03	471.39^{bc}±22.27	966.20±5.24	2.05^{b}±0.11
Diet-2	B	255.00±10.80	412.75±11.09	1.62±0.09	361.25^{a}±24.96	527.50±17.08	1.47^{c}±0.14	477.50^{b}±10.41	953.00±21.98	2.00^{bc}±0.08
	F	250.00±13.54	405.25±6.29	1.63±0.09	332.50^{bc}±14.84	536.25±16.01	1.62^{ab}±0.05	446.25^{c}±20.08	960.50±9.57	2.16^{b}±0.08
Diet-3	B	242.50±14.43	400.25±16.01	1.65±0.08	328.75^{c}±8.54	526.25±20.56	1.60^{ab}±0.04	452.22^{bc}±26.72	952.45±24.61	2.11^{b}±0.17
	F	256.25±11.09	411.50±11.90	1.61±0.06	326.25^{c}±4.79	546.25±7.89	1.68^{a}±0.03	410.00^{d}±10.80	968.00±7.07	2.36^{a}±0.08
P-value		0.65	0.54	0.98	0.02	0.95	0.04	<0.01	<0.11	<0.01

Means with the same letter in each column are not significantly different at 0.05.
[1]Control diet contains 1.0% Ca and 0.45% P; Diet-1 contains 0.85% Ca and 0.35% P; Diet-2 contains 0.75% Ca and 0.30% P; Diet-3 contains 0.65% Ca and 0.25% P.
[2]B = Bacterial phytase; F = Fungal phytase.

Table 3. The effect of different origin of phytase supplementation in Ca- and *P*-deficient diets on the average weight gain, feed intake, feed conversion ratio and weights of carcass and liver of broiler chickens from 7 to 28 days of the experiment.

Diets[1]	Phytase[2]	Weight gain	Feed intake	FCR	Carcass weight	Liver weight
		g	g		g	
Control	–	1076.67^{b}±8.94	1931.59^{a}±8.84	1.80^{bc}±0.02	883.00^{ab}±13.98	40.75±7.04
Diet-1	B	1137.10^{a}±29.45	1933.50^{a}±16.23	1.70^{d}±0.05	893.63^{a}±23.86	39.88±4.97
	F	1068.89^{bc}±35.42	1925.20^{ab}±14.52	1.80^{bc}±0.05	870.13^{abc}±20.65	35.75±4.65
Diet-2	B	1093.75^{b}±38.16	1893.25^{ab}±41.10	1.73^{cd}±0.09	891.75^{a}±20.27	36.50±3.79
	F	1028.75^{cd}±36.37	1902.00^{ab}±29.15	1.85^{b}±0.04	852.13^{cd}±14.74	34.13±1.31
Diet-3	B	1023.47^{d}±26.67	1878.95^{b}±52.90	1.84^{b}±0.08	858.25^{bcd}±10.97	38.25±6.02
	F	992.50^{d}±13.23	1925.75^{ab}±24.34	1.94^{a}±0.04	835.25^{d}±11.06	35.75±4.65
P-value		<0.01	<0.01	<0.01	<0.01	0.47

Means with the same letter in each column are not significantly different at 0.05.
[1]Control diet contains 1.0% Ca and 0.45% P; Diet-1 contains 0.85% Ca and 0.35% P; Diet-2 contains 0.75% Ca and 0.30% P; Diet-3 contains 0.65% Ca and 0.25% P.
[2]B = Bacterial phytase; F = Fungal phytase.

phytase enzyme from bacterial origin. Similarly, the concentration of Ca and P in tibia was also significantly ($P < 0.05$) higher in Diet-1 fed with phytase enzyme from bacterial origin compared to Diet-2 and Diet-3.

Discussion

Dietary feed enzymes, as a supplement, have rapidly expanded and the poultry industry is becoming increasingly receptive to its use. Dietary feed enzymes may support digestive processes for efficient feed utilization (Tahir et al. 2005, 2006, 2008; Woyengo et al. 2010). This approach may also contribute to reducing the environmental footprint of poultry production by minimizing nutrient excretion, especially phosphorus and nitrogen in the excreta (Selle and Ravindran 2007). However, the poultry digestive tract lacks or deficient in some of the enzyme activities necessary to break down specific compounds in feed. Phytase is mostly absent in the birds, thus dietary phytases of either bacterial or fungal origin are added to the diets of chickens to liberate P from the phytate molecule (Aksakal and Bilal 2002). Therefore, phytase is the first successful feed enzyme developed to improve phytate P utilization and is now widely used. However, a difficulty of commercialization exists due to its origin derivation that usually results in inconsistent effects of these enzymes.

Bacterial phytase is relatively more active and stable to hydrolysis in the digestive tract than fungal-based phytase (Augspurger et al. 2003; Onyango et al. 2005). Onyango et al. (2005) found higher residual phytase activity in chicks on bacteria than fungal-based supplemented phytase. This could be one of the reasons why microbial phytases have more influence on the response than fungal-derived phytases (Igbasan et al. 2000; Selle and Ravindran 2007). These findings favoured the previous research (Onyango et al. 2005) where the improved performance of broiler chickens was attributed to the increased resistance of bacterial phytase to degradation in the digestive tract. These results were also supported by Rodriguez et al. (1999) who had shown significantly more P released from soybean meal using *E. coli* (bacterial) than a fungal phytase from Aspergillus niger.

Phytase supplementation increased phytate P utilization in the diet. Most of the phytases target the higher molecular weight phytate ester, dephosphorylating the inositol ring either at the 3- or the 6-position. The phytases used in this study are 6-position active and are used commercially to improve phytate P utilization by chicks. The effect of bacterial phytase on the growth and FCR was pronounced in chicks when fed Ca and P up to 0.75 and 0.30%, respectively. There were 6.39% more body weight gains for microbial than fungal phytase in chicks fed Diet-1. When the levels of Ca and P in the diets were decreased by 75 and 67% of NRC (1994), respectively, the body weight gain was 6.32% higher for the microbial than fungal-based phytase supplementation. Below this level, both phytases were not effective in ameliorating the growth performance although the accumulation of P in tibia ash increased. It can be concluded that the increasing ability of the birds to utilize the phytate P reflected in the blood serum and tibia ash (Paik 2003; Mondal et al. 2007) could not compensate for the response deficiency.

Data from this study indicated that the effects of phytase were different at each deficient level of Ca and P. Lowering the level of Ca and P in the broiler diets gradually decreased weight gain and FCR (Tables 2 and 3). The chick response to added microbial phytases usually depends on the concentration of Ca and P in the diet (Driver et al. 2005). Broiler diets deficient in non-phytate P are associated with decreased gain and feed intake (Taheri and Mirisakhani 2020). The gradual decrease here in the body weight gain and FCR of broiler chicks with decreasing levels of Ca and P in the diets could be the result of this phenomenon.

The exact mode of action exhibiting the effect of microbial phytases on the carcass weight is not clearly understood. Phytic acid may form binary protein-phytate complexes in the gut, thereby impairing their digestibility through changes in protein solubility or by altering the activity of endogenous proteases. Phytase supplementation may improve amino acid utilization, as indicated by several researchers (Gagne et al. 2002; Rutherfurd et al. 2002; Abd-Elsamee 2002) where amino acid digestibility was significantly greater in the presence of microbial phytases. The improvement in carcass weight observed in broiler chickens fed microbial phytase was associated with improved FCR, which might be due to the increased utilization of protein and amino acids (Ravindran et al. 2000) from the phytate protein complex. It was further revealed from the obtained results that the bacterial phytase (*E. coli*) might have alleviated the Ca and P deficiency through the liberation of the phytate complex in diets containing 75% Ca and 67% non-phytate P of NRC (1994). While the chicks fed fungal phytase met their Ca and P requirements when the diets contained 85% Ca and 78% non-phytate P of NRC (1994)

Table 4. The effect of different origin of phytase supplementation in Ca-and P-deficient diets on the blood serum and tibia bone ash Ca and P contents of broiler chickens.

Diets[1]	Phytase[2]	Blood serum Ca	Blood serum *P*	Tibia ash	Tibia Ca	Tibia *P*
		mg/100 ml			%	
Control	–	11.75^{ab}±0.82	3.40^{ab}±0.11	43.98^{a}±0.82	24.16^{a}±0.75	16.98^{ab}±0.49
Diet-1	B	12.48^{a}±0.2	3.76^{a}±0.80	43.05^{ab}±0.90	24.36^{a}±0.78	17.40^{a}±0.48
	F	11.56^{abc}±0.97	2.87^{bcd}±0.68	42.02^{dc}±0.74	23.40^{ab}±0.89	15.86^{d}±0.48
Diet-2	B	11.23^{bcd}±0.90	2.99^{abc}±0.60	42.77^{bc}±0.78	24.12^{a}±0.56	16.51^{bcd}±0.42
	F	10.45^{cde}±0.98	2.48^{bcd}±0.33	41.32^{d}±0.46	22.64^{b}±0.67	14.69^{e}±0.42
Diet-3	B	10.05^{e}±0.51	2.46^{dc}±0.19	41.51^{d}±0.23	24.04^{a}±0.68	16.80^{abc}±0.39
	F	9.89^{e}±0.61	2.11^{d}±0.55	41.31^{d}±0.31	22.44^{b}±0.66	16.16^{cd}±0.34
P-value		<0.01	<0.01	<0.01	<0.01	<0.01

Means with the same letter in each column are not significantly different at 0.05.
[1]Control diet contains 1.0% Ca and 0.45% P; Diet-1 contains 0.85% Ca and 0.35% P; Diet-2 contains 0.75% Ca and 0.30% P; Diet-3 contains 0.65% Ca and 0.25% P.
[2]B = Bacterial phytase; F = Fungal phytase.

recommended. Fungal phytase below this level of Ca and P in diets did not help to improve the carcass weight of broiler chicks. There was 4.65% more carcass weight for bacterial than fungal phytase in chicks fed diets containing 75% Ca and 67% non-phytate P of NRC (1994) recommended. It has been suggested that changes in nutrition by phytases may change the gastrointestinal tract anatomy and physiology of broilers (Camden et al. 2001). Broiler producers should know and consider how feeding dietary phytases of different origins influences broiler performance.

The accumulation of P in tibia ash by phytases was more pronounced with chicks consuming diets containing the lowest level of Ca and P (Diet-3; Table 4). It suggests that phytases at low levels of Ca and P were more efficient in liberating P from the phytate P molecule. It further supports the hypothesis that the same enzymes but of different origins may have specific individual effects on the response fed low Ca and P diets. The enhanced growth performance observed in chickens fed with phytase is typically attributed to the liberation of minerals from the phytate mineral complex, as well as the bird's utilization of inositol (Attia et al. 2020) or increased starch digestibility. However, this situation was not observed in the present study fed diets containing very low Ca and P levels. The increase in bone mineralization of chicks fed 65% Ca and 56% P of NRC (1994) did not support the optimum growth performances. It is postulated that some unknown factors other than the increased phytase liberated Ca and P from the phytate complex might have negatively influenced the absorption of proteins and amino acids in chicks fed diets containing 65% Ca and 56% non-phytate P of NRC (1994). It seems that increased bone mineralization by phytases at this level of Ca and P in the diet is not a good indicator of improved growth performance in broiler chicks. Other than Ca and protein, phytic acid also makes complexes with other minerals such as Na, K, Mg, Zn, Cu and Fe (Leeson and Summers 2001; Costa et al. 2008). These minerals are expected to increase during the hydrolysis of the phytate mineral complex by phytases. Further research of how the different origin phytases supplemented at low level of Ca and P (65 and 56%, respectively) influences the utilization of protein and amino acids and its relationship with the increased minerals content liberated from the phytate mineral complex should be investigated.

The results here indicated that the Ca and P concentrations were significantly higher in the presence of microbial than fungal phytases for the blood serum and tibia ash examined. Increased mineral (Ca and P) concentration in the blood serum and tibia ash of broilers fed phytase-supplemented diet gives evidence of phytate P utilization (Ahmad et al. 2000; Ravindran et al. 2000; Lim et al. 2001). Mondal et al. (2007) demonstrated that the addition of microbial phytases to a low P broiler diet increased tibia ash, Ca and P % significantly. Bone ash and serum P have been suggested as one of the most sensitive and dependable methods for assessing the phosphorus requirement of poultry. An elevation in calcium content in both tibia ash and blood serum was anticipated during the hydrolysis of the Ca-phytate complex by phytase that liberates Ca. To put it differently, with the heightened availability of P, there was a concurrent increase in Ca availability, resulting in deposits in the bones. These two minerals, calcium and phosphorus, collectively contribute to over 50% of the bone ash content. Interestingly, the differences between microbial and fungal phytases on bone mineralization disappeared in chicks fed diets containing 65% Ca and 56% P of NRC requirements (NRC 1994).

Numerous studies (Dilger et al. 2004; Ravindran et al. 2006; Zhang et al. 2000; Luciana et al. 2012) demonstrate the effect of microbial and/or fungal phytase supplementation in Ca and P-deficient poultry diets but with inconsistent reported results. Generally, phytase supplementation in poultry diets is believed to liberate P from the phytate P molecule during its hydrolysis, which is largely unavailable for utilization by chicks. Typically, the focus is on diets with a low level of non-phytate phosphorus but a normal level of calcium, indicating an imbalance between calcium and phosphorus. However, the chick's response to added phytase is strongly influenced by the levels of both calcium and phosphorus in the diet. It is worldwide believed that phytase supplementation during the hydrolysis of phytate P molecule increased the availability of minerals including Ca for broiler chicks (Jlali et al. 2020; Majeed et al. 2020), which indicates that Ca needs of the broiler chicks may also be reduced by phytase supplementation. Imbalanced level of Ca and P antagonizes each other in the gut of broiler chickens. When the Ca level was reduced the performance of broiler chicks improved and bone diseases were minimized. Therefore, it was envisaged in the present study that the effect of phytase may be more fruitful in supporting the optimum growth in a reduced balanced Ca and P (a ratio that precluded the antagonism of each by the other) than an imbalanced diet. The improved performance and increased bone mineralization revealed in this study could be one reason of this phenomenon. This study suggests the use

of balanced reduced levels of Ca and P in broiler diets receiving phytase supplementation. Furthermore, the enzyme producer usually recommends the reduction of non-phytate P up to 0.35% (by 0.1%) which accounts for 78% of NRC (0.35% vs. 0.45%) without reducing the level of Ca in the diets. However, the present results demonstrated that non-phytate P in the diet could be reduced up to 0.30% (by 0.15%) accounts for 67% of NRC recommended (0.30% vs 0.45%), if the level of Ca was also proportionally reduced up to 75% (by 0.25%) of NRC recommended (0.75% vs. 1.0%).

Conclusion

It was concluded that a diet deficient in calcium and phosphorous at the level of 0.75% and 0.30%, respectively may be supplemented with phytase enzyme from bacterial origin for enhanced growth performance, carcass weight and tibial calcium in broilers.

Disclosure statement

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Ethics statement

The poultry handling procedures were approved by the institutional ethical committee (AN/12/2021).

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