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Enhancing calcium and phosphorus utilization in broiler chickens with phytase in sequential calcium-phosphorus-deficient diets

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ABSTRACT
In this trial, a 3 × 2 factorial design was implemented with dietary treatments consisting of Diet-1 (1.00% calcium [Ca], 0.45% available phosphorus [P]), Diet-2 (0.80% Ca, 0.35% P), and Diet-3 (0.70% Ca, 0.30% P), alongside two phytase levels: 0 and 5,000 FTU/kg. A total of 540 Ross-308 broilers were used. Significant improvements ($P < 0.05$) in body weight gain, feed intake, feed conversion ratio (FCR), live weight, carcass weight, and yield were observed in Diet-1 and Diet-2 when supplemented with phytase compared to Diet-3. Gross energy (GE) increased significantly ($P < 0.05$) with phytase in Diet-1 and Diet-2. Bone ash content was significantly higher ($P < 0.01$) in Diet-1 and Diet-2, with tibia Ca levels notably improved ($P < 0.05$) in Diet-1. Phytase supplementation also resulted in significantly elevated ($P < 0.05$) serum Ca and available P in Diet-1. The application of phytase restored body weight gain, live weight, carcass weight, GE, and tibia P in Diet-2. Overall, the findings highlight the beneficial effects of phytase supplementation on growth performance, bone mineralization, and nutrient utilization.

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Phytase enzyme; calcium; phosphorous; nutrient digest ability; growth

Introduction

Phosphorus (P) holds immense significance in the context of broiler chickens due to its pivotal role in their growth and development. Typically, over half of the total P found in plants is tightly bound within a compound known as phytic acid (Kheiri and Landy 2019; Baradaran et al. 2021). However, broiler chickens possess a limited capacity to efficiently utilize P from phytate, which is the form present in phytic acid. The efficacy of phytase in enhancing the availability of P and calcium (Ca), as well as its positive impact on performance and the content of ash in bones, is widely acknowledged (Moradi et al. 2023). Multiple publications have provided substantial evidence of these beneficial effects (Selle and Ravindran 2007; Dersjant-Li et al. 2014; Abd El-Hack et al. 2018; Marchal et al. 2020).

Research has indicated that the phytase enzyme exerts effects beyond its primary phosphorus-related actions. These additional effects stem from the release of myo-inositol from phytate within the gastrointestinal tract (Walk et al. 2014; Gonzalez-Uarquin et al. 2020). Calcium stands as the most abundant mineral within the diets of broiler chickens, which is mostly bound to phytate in the gastrointestinal tract. This results in the creation of insoluble complexes that are resistant to breakdown through the action of phytase (Selle et al. 2009). As a result, this process reduces the accessibility of phytate-bound P and Ca for the birds. The ratio of Ca to P in the diet are recognized as pivotal factor that impacts the digestibility and performance of the birds, as well as the effectiveness of externally added phytase (Dersjant-Li et al. 2018; Angel et al. 2002). The broiler industry's drive to increase production effectiveness and environmental sustainability makes it imperative to develop phytases that exhibit improved abilities in terms of both phosphorus-related and non-phosphorus-related actions (Babatunde et al. 2021). Consequently, the development and testing of new-generation phytases revolve around evaluating their heightened efficacy, aiming to achieve more significant reductions in dietary nutrient requirements. This proactive approach is crucial for meeting the demand for higher performance standards while minimizing the environmental impact (Moradi et al. 2023).

While numerous studies have explored the impact of the phytase enzyme on the growth performance and bone mineralization of broiler chickens, there exists a scarcity of information regarding the ideal Ca:P ratio in diets when supplemented with phytase at different dosage levels. The main aim of this study was to assess the impact of lowering dietary Ca content in relation to P. The investigation present on the study

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investigates the performance and nutrient utilization responses of broiler chickens that were fed pelleted diets from day 1 to day 35. Additionally, the study evaluated effects on Ca and P in the tibia and serum. These measurements were conducted to offer further evidence of the phytase's ability to recover available P effectively, especially in situations where Ca was deficient. Based on a comprehensive review of the available literature, it is clear that there has been a scarcity of research regarding the potential effects of sequentially feeding broiler chickens in term of Ca and P (Landy and Toghyani 2018; Moradi et al. 2023). This research gap prompted the initiation of the current study, which aims to thoroughly examine the consequences of employing a sequential feeding approach. The primary focus of this investigation encompasses several critical aspects of broiler chicken performance and health, including growth patterns, bone characteristics, the digestibility of nutrients, and the serum levels of Ca and P.

Materials and methods

Diets and experimental treatments

The baseline diet was formulated using corn-soybean meal to fulfill the minimum nutritional requirements for the birds, including sufficient levels of P and Ca. This formulation covered both the starter phase (days 1–21) and the finisher phase (days 22–35) of the birds' growth. In contrast, two negative control diets in terms of Ca and P were prepared, each with reductions of 1.5% and 2%, respectively. The study encompassed three experimental diets: Diet-1 (normal 1.00% Ca, and 0.45% P), Diet-2 (0.80% Ca, and 0.35% P), and Diet-3 (0.70% Ca, and 0.30% P). Each of these experimental diets was tested both with and without the inclusion of the phytase enzyme. The composition and calculated nutritional analysis of the diets are presented in Table 1. To ensure uniform dispersion of the enzyme, the phytase was blended with ground corn prior to being mixed with the primary batch using a horizontal mixer. These diets were administered to the birds in pellet form. In this study, the phytase employed was produced from a third-generation modified *E. coli* phytase, which was produced through expression in *Trichoderma reesei*. It was anticipated to exhibit an activity of 5,000 FTU phytase per gram. 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 basal and calcium-phosphorus deficient diets (1–35 days).

Ingredients	Diet-1(Control)	Ca-*P* deficient	
		Diet-2	Diet-3
Corn	58.40	58.04	58.05
Corn GM-30%	3.85	3.500	3.50
Soybean Meal – 44%	11.50	11.00	11.00
Canola (Meal)	8.10	8.00	8.00
Corn gluten-60% (Meal)	3.86	3.75	3.74
Oil (Vegetable)	2.20	2.00	2.00
Poultry By-Product (Meal)	3.30	3.00	3.00
Sunflower (Meal)	5.00	6.00	6.00
Bone (Meal)	0.80	0.05	0.12
Salt	0.20	2.10	2.47
Dicalcium phosphate	1.00	0.80	0.500
Limestone	1.00	1.02	0.87
Threonine	0.11	0.07	0.07
DL-Methionine	0.12	0.15	0.15
L-Lysine HCL	0.38	0.37	0.37
Vit:Min Premix (standard level)	0.20	0.20	0.20
Calculated composition			
ME/ kcal/g	2.95	2.95	2.95
CP %	19	19	19
Available total *P*, %	0.45	0.35	0.30
Nonphytate *P*, %	0.23	0.15	0.12
Calcium %	1.00	0.80	0.70
Threonine, %	0.80	0.80	0.80
Lysine, %	1.15	1.15	1.15
Methionine + Cystine, %	0.90	0.90	0.90
Methionine, %	0.54	0.54	0.54

Broilers, housing and management

In total, six distinct experimental diets (five replicates) were provided to the one-day old male Ross 308 chicks (n = 540) under *ad-libitum* feeding conditions in 30 pens. The chicks were raised on a bedding of wood shavings. The household temperature was gradually dropped from 35°C in first week and then maintained at 25 ± 2°C, with a humidity level of 61%. Light was maintained for 20 h per day. All the diets were formulated as pellets and maintained isocaloric and isonitrogenous properties. They included levels of digestible amino acids that either fulfilled or surpassed the established requirements (NRC 1994). The study employed a 3 × 2 factorial design, incorporating three different diets as mentioned above, along with two levels of phytase enzyme (0 and 5000 FTU/kg). Throughout the entire duration of the experiment, both the availability and consumption of feed were closely monitored.

Performance traits

Throughout the entire duration of the study, the feed supply and consumption were meticulously quantified., The weight gain of the broilers for each treatment was noted weekly. The values of feed intake and weight gain were employed to calculate the feed conversion ratio (FCR). At day 35 of the experimental trial, two birds from each replicate were selected, weighed, and then slaughtered. The slaughtering process involved bleeding and subsequent feather plucking. The weight of the dressed carcass was then compared relative to the live body weight of the birds.

Total tract digestibility assay

On the 35th day of the experiment, an acid insoluble ash was used as a digestibility marker, to find total tract nitrogen (N) retention. On the 33rd day of the experiment (five birds per replicate), an acid insoluble marker was blended into the experimental diets and administered to the birds, which were individually housed in metallic cages until the conclusion of the trial. When the experiment reached its 35th day, fecal samples were cleanly collected twice daily and preserved in plastic bags at a temperature of −20°C. Subsequently, samples were milled on 0.5 mm sieve and were used in the Kjeldahl technique to calculate total tract N retention and then multiplied by a conversion factor of 6.25. Furthermore, a bomb calorimeter was used to determine the gross energy (GE) following the method illustrated by Jabbar et al. (2021a, 2021b).

Tibia measurements

On day 35 of the experiment, two birds from each pen were euthanized. The tibia were removed from the surrounding tissues and dried at a temperature of 100°C. Following this, fat content was removed in Soxhlet apparatus for 16 h using 100% ethyl ether, dried again and weighed. This process followed modified procedures in accordance with the guidelines provided by Watson et al. (2006). The tibia samples were then transported to a laboratory for the measurement of Ca, P and total ash contents. The tibia samples, which were devoid of fat and moisture, were incinerated in a muffle furnace maintained at 600○C. The resulting ashes were then weighed and employed to assess the concentrations of Ca and P. These incinerated tibia samples underwent digestion in a mixture of nitric and perchloric acids to determine their Ca and P contents, following Method as specified in AOAC (2000).

Serum calcium and phosphorous

On day 35 of the study, two birds were randomly chosen from each replicate based on their average body weight. Blood samples of about 2.5 ml were collected using syringe from the wing vein and then centrifuged at 3500 x g for 15 min to separate the serum, which was subsequently frozen at a temperature of −20°C for later analysis. The concentrations of Ca and P in the serum were determined using commercial kits from Biocheck, Inc. (UK) and Iran. These measurements were performed using a clinical chemistry auto-analyzer (IRMECO Model, U 2020, Germany).

Statistical analysis

The collected data underwent analysis of variance (ANOVA) using the general linear model (GLM) procedure within the SAS statistical analysis system (SAS 2002). This was performed to discern the primary effects of diets and enzyme supplementation, as well as their interaction, as per the factorial design. In case where a significant interaction was detected, Tukey's test was utilized to differentiate between the means. Statistical significance was established with a p-value below 0.05.

Results

The data concerning the impacts of various dietary combinations and phytase on the performance of broiler chicks are displayed in Table 2. The outcomes reveal that the body weight gain and feed intake exhibited significant reductions ($p < 0.05$) in birds fed withDiet-3. Consequently, the feed conversion ratio (FCR) showed a notable increase ($p < 0.05$) among birds fed with Diet-3. Likewise, there was a significant improvement ($p < 0.05$) in body weight gain and feed intake observed in birds that received enzyme treatment. Additionally, the FCR exhibited a notable reduction ($p < 0.05$) in enzyme supplemented groups. Furthermore, the interaction between the different diets and enzyme supplementation revealed that body weight gain and feed intake demonstrated notable increases ($p < 0.05$) in Diet-1E and Diet-2E compared to Diet-3_0 and Diet-3_E. Consequently, the FCR was significantly lower ($p < 0.05$) in Diet-2E compared to Diet-3_0 and Diet-3E.

The effects of varying diets and phytase enzyme supplementation on live weight on day 35 and carcass yield are detailed in Table 3. The findings demonstrated a substantial reduction ($P < 0.05$) in live weight, carcass weight, and carcass yield in birds fed with Diet-3. Furthermore, it's evident that the inclusion of phytase supplementation led to a notable increase ($P < 0.05$) in live weight, carcass weight and carcass yield compared to

Table 2. Weight gain, feed intake and FCR of broilers fed on phytase supplemented diets in broilers on day 35.

Item	Body Weight Gain (g)	Feed Intake (g)	FCR (g/g)
Diets[1]			
Diet-1	1019.8^{a}	1767.5^{a}	1.73^{b}
Diet-2	1022.6^{a}	1766.2^{a}	1.73^{b}
Diet-3	942.4^{b}	1695.3^{b}	1.79^{a}
Pooled SEM	56.43	93.21	0.01
Enzyme[2]			
Without enzyme	981.4^{b}	1734.7_{b}	1.77^{a}
Enzyme	1008.5^{a}	1751.4^{a}	1.74^{b}
Pooled SEM	34.55	87.66	0.12
CP × Enzyme			
Diet-1_0	1003.9^{ab}	1760.0^{ba}	1.75^{bc}
Diet-1_E	1035.6^{ab}	1775.1^{a}	1.72^{c}
Diet-2_0	1001.6^{b}	1749.9^{ab}	1.75^{bc}
Diet-2_E	1043.7^{a}	1782.4^{a}	1.71^{c}
Diet-3_0	938.6^{c}	1694.1^{b}	1.81^{a}
Diet-3_E	946.2^{c}	1696.6^{b}	1.79^{ab}
Pooled SEM	77.54	23.45	0.01
p-Value			
Diet	< 0.01	0.0081	0.0007
Enzyme	0.028	0.03773	0.0363
Diet*Enzyme	0.045	0.01010	0.0445

Means in the same column with different superscript differ ($P < 0.05$).
[1]Diets contained different levels of Ca and P wherein the Diet-1 (Control) = Ca 1.00% and P 0.45%; Diet-2 = Ca 0.80% and P 0.35%; Diet-3 = Ca 0.70% and P 0.30%, [2]Phytase 5,000 FTU.

Table 3. Live weight, carcass weight and carcass yield of broilers fed on phytase supplemented diets in broilers on day 35.

Item	Live Weight (g)	Carcass Weight (g)	Carcass yield (%)
Diets[1]			
Diet-1	1878.8^{a}	1333.3^{a}	70.959^{a}
Diet-2	1868.1^{a}	1324.2^{a}	70.870^{a}
Diet-3	1706.6^{b}	1160.8^{b}	68.017^{b}
Pooled SEM	23.45	11.78	8.54
Enzyme[2]Enzyme[2]			
Without enzyme	1773.6^{b}	1237.7^{b}	69.741^{b}
Enzyme	1862.0^{a}	1307.9^{a}	70.157^{a}
Pooled SEM	10.87	13.43	14.66
Diets × Enzyme			
Diet-1_0	1821.3^{b}	1287.3^{b}	70.690^{a}
Diet-1_E	1936.4^{a}	1379.3^{a}	71.227^{a}
Diet-2_0	1807.1^{b}	1274.5^{b}	70.523^{a}
Diet-2_E	1929.1^{a}	1373.9^{a}	71.218^{a}
Diet-3_0	1692.5^{c}	1151.2^{c}	68.010^{b}
Diet-3_E	1720.7^{c}	1170.5^{c}	68.025^{b}
Pooled SEM	1821.3^{b}	1287.3^{b}	70.690^{a}
p-Value			
Diet	0.0000	0.0000	0.0000
Enzyme	0.0000	0.0000	0.01819
Diet*Enzyme	0.0388	0.0325	0.0288

Means in the same column with different superscript differ ($P < 0.05$).
[1]Diets contained different levels of Ca and P wherein the Diet-1 (Control) = Ca 1.00% and P 0.45%; dDiet-2 = Ca 0.80% and P 0.35%; dDiet-3 = Ca 0.70% and P 0.30%, [2]Phytase 5,000 FTU.

the absence of supplementation. As a consequence, the interaction showed that live weight, carcass weight, and carcass yield experienced significant increases ($P < 0.05$) in Diet-1E and Diet-2E compared to Diet-3E and Diet-3_0.

The impacts of diets and phytase on total tract N retention and GE are illustrated in Table 4. The results unveiled that total tract N retention and GE did not exhibit significant differences ($p < 0.05$) among birds subjected to different diets in the absence of phytase. However, the GE was significantly affected ($p < 0.05$) by the phytase supplementation. Upon analyzing the interaction, it was evident that the combinations of diets and phytase did not exert a significant influence ($p < 0.05$) on total tract N retention. Nonetheless, the GE was found to be significantly lower ($p < 0.05$) in Diet-1 and Diet-2 without phytase compared to the other treatment groups.

The effects of different diets and phytase enzyme supplementation on bone ash, tibia Ca, and P levels are shown in Table 5. Notably, bone ash exhibited a significant decrease ($P < 0.05$) in Diet-3 compared to Diets 1 and 2. Tibia Ca showed a significant reduction ($P < 0.05$) in Diet-2 and Diet-3, while no differences in tibia P among diets were observed. The addition of phytase supplementation resulted in a marked improvement ($P < 0.05$) in bone ash, tibia Ca, and P levels. Upon examining the interaction outcomes, it became evident that Diet-2E and Diet-1E displayed significantly higher ($P < 0.05$) bone ash, while tibia Ca levels were notably higher ($P < 0.05$) in Diet-1E when compared to the other groups. Moreover, tibia P exhibited a significant increase ($P < 0.05$) in Diet-1E compared to Diet-3, regardless with or without enzyme supplementation.

The impact of diets and phytase enzyme supplementation on serum Ca and P levels is presented in Table 6. Serum Ca and P levels exhibited a significant decrease ($P < 0.05$) in both Diet-2 and Diet-3 when compared to Diet-1. The introduction of phytase supplementation notably improved ($P < 0.05$) serum Ca and P levels in the broiler diet. Analyzing the interaction between diet and phytase, it was evident that serum Ca and P levels experienced a significant increase ($P < 0.05$) in Diet-1 and Diet-2 when phytase was supplemented. However, it's worth noting that serum Ca and P levels in Diet-3 were not restored either with or without phytase supplementation.

Discussion

In this study, notable negative impacts were observed when birds were fed a diet deficient in P and Ca, as evidenced by reduced weight gain and deteriorated FCR in Diet-3, when compared to the nutritionally sufficient Diet-1. However, the introduction of phytase supplementation to the Ca and P deficient diet (Diet-2) resulted in a marked enhancement, reaching levels comparable to those in Diet-1. This suggests that the phytase enzyme effectively hydrolyzed sufficient phytate to meet the P requirements, thereby supporting growth performance and carcass yield in broiler chickens from days 1–35. This effect is to be expected, considering the vital roles that P plays in energy metabolism, fat transport, as well as protein synthesis (Proszkowiec-Weglarz and Angel 2013; Moradi et al. 2023). The documented growth suppression associated with low non-phytase phosphorous diets may be partially ascribed to reduced feed consumption and an imbalanced dietary Ca and P ratio (Rousseau et al. 2016). Our findings regarding growth performance are partially similar to these of Babatunde et al. (2021), Baradaran et al. (2021), Moradi et al. (2023) who reported poor growth performance in response to diet defecient in Ca and P.

The primary objective behind introducing phytase into broilers diets is to facilitate the breakdown of phytate, thereby releasing bound P as a nutrient that can be effectively retained by the birds (Selle and Ravindran 2007). The enhancements

Table 4. Ileal total tract N retention and metabolizable energy of broilers fed on phytase and protease supplemented diets at finisher phase on day 35.

Diets[1]	Total Tract N Retention (%)	GE (Kcal/kg)
Diet-1	70.813	2955.2
Diet-2	70.925	2951.8
Diet-3	70.873	2981.4
Pooled SEM	5.43	14.67
Enzyme[2]		
Without enzyme	70.586	2946.7^{b}
Enzyme	71.154	2978.9^{a}
Pooled SEM	2.38	17.65
Diet ×Enzyme		
Diet-1_0	70.498	2926.9^{b}
Diet-1_E	71.127	2983.5^{a}
dDiet-2_0	70.568	2923.8^{b}
dDiet-2_E	71.282	2979.8^{a}
dDiet-3_0	70.692	2989.3^{a}
dDiet-3_E	71.053	2973.5^{a}
Pooled SEM	6.53	14.56
P-value		
Diet	0.9476	0.1094
Enzyme	0.060	0.0145
Diet*Enzyme	0.8665	0.0348

Means in the same column with different superscript differ ($P < 0.05$).
[1]Diets contained different levels of Ca and P wherein the Diet-1 (Control) = Ca 1.00% and P 0.45%; dDiet-2 = Ca 0.80% and P 0.35%; dDiet-3 = Ca 0.70% and P 0.30%, All these diets were with and without Phytase.
[2]Phytase 5,000 FTU.

Table 5. Bone ash, Tibia Ca and Tibia P of broilers fed on phytase supplemented diets of broilers on day 35.

	Bone ash	Tibia Ca	Tibia P
Diets[1]		%	
Diet-1	41.756^{a}	30.605^{a}	14.822
Diet-2	41.335^{a}	28.828^{b}	14.522
Diet-3	38.702^{b}	28.523^{b}	14.473
Pooled SEM	3.45	7.64	2.11
Enzyme[2]			
Without enzyme	39.446^{b}	28.562^{b}	14.185^{b}
Enzyme	41.749^{a}	30.075^{a}	15.026^{a}
Pooled SEM	1.56	1.22	1.76
Interaction			
Diet-1_0	41.297^{ab}	29.512^{b}	14.108^{c}
Diet-1_E	42.215^{a}	31.699^{a}	15.537^{a}
dDiet-2_0	40.483^{b}	28.331^{b}	14.016^{c}
dDiet-2_E	42.186^{a}	29.324^{b}	15.027^{ab}
dDiet-3_0	36.559^{c}	27.843^{b}	14.432^{bc}
dDiet-3_E	40.846^{ab}	29.203^{b}	14.515^{bc}
Pooled SEM	1.12	2.34	0.76
Source of variance *P*-value			
Diet	0.0001	0.0173	0.348
Enzyme	0.0002	0.0163	0.0008
Diet*Enzyme	0.0234	0.0286	0.0431

Means in the same column with different superscript differ ($P < 0.05$).
[1]Diets contained different levels of Ca and P wherein the Diet-1 (Control) = Ca 1.00% and P 0.45%; dDiet-2 = Ca 0.80% and P 0.35%; dDiet-3 = Ca 0.70% and P 0.30%, All these diets were with and without Phytase.
[2]Enzyme used was Phytase 5,000 FTU.

Table 6. Serum Ca and P contents of broilers fed in phytase supplemented diets in broilers on day 35.

Diets[1]	Serum Ca	Serum P
	mg/100ml	
Diet-1	10.248[a]	8.25[a]
Diet-2	8.248[b]	6.63[b]
Diet-3	8.08[b]	6.25[b]
Pooled SEM	0.23	0.56
Enzyme[2]		
0	8.97[a]	7.17[b]
E	8.73[b]	6.92[a]
Pooled SEM	0.13	0.11
Interaction		
Diet-1_0	9.68[ab]	8.00[ab]
Diet-1_E	10.80[a]	8.50[a]
Diet-2_0	8.64[bc]	6.75[bc]
Diet-2_E	7.85[bc]	6.50[bc]
Diet-3_0	8.60[bc]	6.75[bc]
Diet-3_E	7.55[c]	5.75[c]
Pooled SEM	0.23	0.55
Source of Variance *P*-value		
Diet	0.0110	0.0027
Enzyme	0.0468	0.05500
Diet*Enzyme	0.02558	0.03515

Means in the same column with different superscript differ ($P < 0.05$).
[1]Diets contained different levels of Ca and P wherein the Diet-1 (Control) = Ca 1.00% and P 0.45%; dDiet-2 = Ca 0.80% and P 0.35%; dDiet-3 = Ca 0.70% and P 0.30%, All these diets were with and without Phytase.
[2]Enzyme used was Phytase 5,000 FTU.

phenomenon in terms of growth and other parameters can be predominantly attributed to the expected liberation of Ca and P in the purposely deficient diets, coupled with this effect can be mainly attributed to the predicted alleviation of the negative impact of phytate on the digestive process of vital nutrients (Walk et al. 2014). Moreover, the process of dephosphorylation of phytate leads to the formation of myo-inositol, a compound that plays a crucial role in improving the growth performance of broiler chickens (Huber et al. 2015). This suggests that the utilization of phytase contributes not only to the liberation of important nutrients but also to the production of compounds that positively influence broiler growth. To counteract the detrimental impacts of phytate, there emerges a necessity for phytase to efficiently and thoroughly hydrolyze phytate within the upper portion of the gastrointestinal tract. This process effectively reduces the anti-nutritional impact of phytate and enables the additional non-phosphoric advantages linked to phytase supplementation, as reported by Dersjant-Li et al. (2020). Phytase operates in a sequential manner, breaking down phytate within the digestive system and liberating previously bound nutrients for utilization by birds. Simultaneously, this degradation process reduces the P utilization, that would otherwise be lost through excretion and released into the environment.

In the current study, gross energy of the nutrients with inclusion of phytase in all the three diets was improved suggesting that gross energy was highly dependent on phytase enzyme irrespective of Ca and P levels. previous research has indicated that the consumption of diets lacking essential nutrients adversely affects the efficient utilization of these nutrients (Dilger et al. 2004). Notably, P deficiency has been recognized for its negative influence on energy utilization and the processing of other nutrients. This is due to the fact that P is involved in numerous biological reactions that underpin energy production and related processes. Further, the higher live weight and the carcass yield in the phytase supplemented diets could be attributed to the higher body weight gain in the respective group of birds.

In the current study, bone ash, Ca and P deficiency were restored in Diet-2 in response to phytase supplementation and were statistically similar to Diet-1. Nevertheless, the concentration of phytase employed proved inadequate to fully restore bone ash, Ca and P levels in Diet-3. Phosphorus assumes the functions that are particularly vital in bone structure, surpassing its significance in soft tissue growth. This can be attributed to the fact that P is a key part of the skeletal system in broilers (Proszkowiec-Weglarz and Angel 2013). As a result, the ash content of the tibia serves as a more precise and sensitive criterion for evaluating responses compared to the measurement of growth in broilers (Zyla et al. 2004). Consistent with the present results, Akter et al. (2016) and Baradaran et al. (2021) demonstrated that supplementing a low-non-phytate phosphorus (NPP) diet with phytase enhanced both tibia ash content and tibia strength. The detrimental effects of P deficiency on bone mineralization is well known (Dersjant-Li et al. 2018; Babatunde et al. 2020). Given that bones primarily consist of phosphorus and calcium, any deficiency in these essential minerals within the diet prompts a process of resorption from the bones themselves. This resorption is necessitated to fulfill the phosphorus or calcium requirements of the birds, ultimately affecting bone health and mineral content. This observation is consistent with earlier research findings where the addition of phytase, whether at conventional or elevated levels, significantly enhanced bone mineralization and contributed to the overall skeletal integrity of broiler chickens (Gautier et al. 2018; Babatunde et al. 2021; Moradi et al. 2023).

In the present study, diets deficient in Ca and P (Diet-2 and Diet-3) demonstrated reduced serum Ca and P levels, regardless of whether phytase was included or not. However, when phytase was introduced into the diets, serum Ca and P levels were successfully restored in Diet-2. Further, in Diet-1, where the nutrient levels were already sufficient, the inclusion of phytase resulted in an additional increase in serum Ca and P levels. Nevertheless, it's important to note that phytase supplementation failed to fully restore Ca and P levels in the serum of Diet-3. These findings align with the results presented by Baradaran et al. (2021), whose study demonstrated that feeding broiler chickens diets low in P led to decreased serum P concentrations compared to birds fed a control diet at 42 days post-hatch. Phytase enhances P availability by degrading InsP6 in diets for broiler chickens, leading to an increase in serum P concentration, is consistent with findings reported in earlier research (Taheri et al. 2015; Farhadi et al. 2017; Taheri and Mirisakhani 2020).

Conclusion

Based on the outcomes of the current study, it can be inferred that the supplementation of phytase at a dosage of 5000 FTU/kg improved growth performance, increased carcass yield, and successfully remedied Ca and P deficiencies in broilers fed a diet having 0.35% P, in comparison to a standard level of P (0.45%) in the diet. However, at this particular dosage, phytase did not yield favorable outcomes when Ca and P levels were furthermore reduced to 0.30% in the diet.

Ethical statement

The experiments were conducted according to The University of Agriculture, Animal Ethics Committee guidelines (FAHVS 123/2021).

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Authors contribution

Conceptualization and methodology, Muhammad Tahir and Abdul Jabbar; formal analysis and data curation, Muhammad Tahir; writing – original draft preparation, Ibrahim Alhidary, shabana Naz, sifa Dai, writing – review and editing, Ibrahim Alhidary, Rifat Ullah Khan; supervision and project administration, Muhammad Tahir All authors have read and agreed to the published version of the manuscript.

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References

Abd El-Hack ME, Alagawany M, Arif M, Emam M, Saeed M, Arain MA, Siyal FA, Patra A, Elnesr SS, Khan RU. 2018. The uses of microbial phytase as a feed additive in poultry nutrition–a review. Ann Anim Sci. 18(3):639–658. doi:10.2478/aoas-2018-0009.

Akter M, Graham H, Iji PA. 2016. Response of broiler chickens to different levels of calcium, non-phytate phosphorus and phytase. Br Poult Sci. *57*(6):799–809. doi:10.1080/00071668.2016.1216943.

Angel R, Tamim NM, Applegate TJ, Dhandu AS, Ellestad LE. 2002. Phytic acid chemistry: influence on phytin-phosphorus availability and phytase efficacy. J Appl Poult Res. 11:471–480. doi:10.1093/japr/11.4.471.

AOAC. 2000. Official methods of analysis. 17th ed. assoc. off. anal. chem. Gaithersburg, MD, USA; p. 2742–2744.

Babatunde OO, Bello A, Dersjant-Li Y, Adeola O. 2021. Evaluation of the responses of broiler chickens to varying concentrations of phytate phosphorus and phytase. I. starter phase (day 1–11 post hatching). Poult Sci. 100(10):101396. doi:10.1016/j.psj.2021.101396.

Babatunde OO, Jendza JA, Ader P, Xue P, Adedokun SA, Adeola O. 2020. Response of broiler chickens in the starter and finisher phases to three sources of microbial phytase. Poult Sci. 99:3997–4008. doi:10.1016/j.psj.2020.05.008.

Baradaran N, Shahir MH, Taheri HR, Bedford MR. 2021. Effect of sequential feeding of phosphorus-deficient diets and high-dose phytase on efficient phosphorus utilization in broiler chickens. Livest Sci. 243:104368. doi:10.1016/j.livsci.2020.104368.

Dersjant-Li Y, Archer G, Stiewert AM, Brown AA, Sobotik EB, Jasek A, Marchal L, Bello A, Sorg RA, Christensen T, et al. 2020. Functionality of a next generation biosynthetic bacterial 6-phytase in enhancing phosphorus availability to broilers fed a corn-soybean meal-based diet. Anim Feed Sci Tech. 264:114481. doi:10.1016/j.anifeedsci.2020.114481.

Dersjant-Li Y, Awati A, Schulze H, Partridge G. 2014. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J Sci Food Agric. 95:878–896. doi:10.1002/jsfa.6998.

Dersjant-Li Y, Evans C, Kumar A. 2018. Effect of phytase dose and reduction in dietary calcium on performance, nutrient digestibility, bone ash and mineralization in broilers fed corn-soybean meal-based diets with reduced nutrient density. Anim Feed Sci Technol. 242:95–110. doi:10.1016/j.anifeedsci.2018.05.013.

Dilger RN, Onyango EM, Sands JS, Adeola O. 2004. Evaluation of microbial phytase in broiler diets. Poult Sci. 83:962–970. doi:10.1093/ps/83.6.962.

Farhadi D, Karimi A, Sadeghi G, Rostamzadeh J, Bedford MR. 2017. Effects of a high dose of microbial phytase and myo-inositol supplementation on growth performance, tibia mineralization, nutrient digestibility, litter moisture content, and foot problems in broiler chickens fed phosphorus-deficient diets. Poult Sci. 96:3664–3675. doi:10.3382/ps/pex186.

Gautier AE, Walk CL, Dilger RN. 2018. Effects of a high level of phytase on broiler performance, bone ash, phosphorus utilization, and phytate dephosphorylation to inositol. Poult Sci. 97(1):211–218.

Gonzalez-Uarquin F, Molano E, Heinrich F, Sommerfeld V, Rodehutscord M, Huber K. 2020. Research note: jejunum phosphatases and systemic myo-inositol in broiler chickens fed without or with supplemented phytase. Poult Sci. 99:5972–5976. doi:10.1016/j.psj.2020.08.045.

Huber K, Zeller E, Rodehutscord M. 2015. Modulation of small intestinal phosphate transporter by dietary supplements of mineral phosphorus and phytase in broilers. Poult Sci. 94:1009–1017. doi:10.3382/ps/pev065.

Jabbar A, Tahir M, Alhidary MA, Abdelrahman M, Albaadani H, Khan RU, Selvaggi M, Laudadio V, Tufarelli V. 2021a. Impact of microbial protease enzyme and dietary crude protein levels on growth and nutrients digestibility in broilers over 15 to 28 days. Animals. 11(9):2499. doi:10.3390/ani11092499.

Jabbar A, Tahir M, Khan RU, Ahmad N. 2021b. Interactive effect of exogenous protease enzyme and dietary crude protein levels on growth and digestibility indices in broiler chickens during the starter phase. Trop Anim Health Prod. 53:1–5. doi:10.1007/s11250-020-02466-5.

Kheiri F, Landy N. 2019. The effects of dietary 1-alpha-hydroxycholecalciferol individually or in combination with different levels of cholecalciferol on growth performance and tibia criteria in broiler chickens. Livestock Sci. 221:172–176. doi:10.1016/j.livsci.2019.02.007.

Landy N, Toghyani M. 2018. Evaluation of one-alpha-hydroxy-cholecalciferol alone or in combination with cholecalciferol in CaP deficiency diets on development of tibial dyschondroplasia in broiler chickens. Anim Nutr. *4*(1):109–112. doi:10.1016/j.aninu.2017.11.002.

Marchal L, Bello A, Sobotik E, Archer G, Dersjant-Li Y. 2020. A novel consensus bacterial 6-phytase variant completely replaced inorganic phosphate in broiler diets, maintaining growth performance and bone quality: data from two independent trials. Poult Sci. 100(3):100962.

Moradi S, Abdollahi MR, Moradi A, Jamshidi L. 2023. Effect of bacterial phytase on growth performance, nutrient utilization, and bone mineralization in broilers fed pelleted diets. Animals. 13(9):1450. doi:10.3390/ani13091450.

National Research Council. 1994. Nutrient requirements of poultry, 9th Rev. Ed. Washington, DC: National Academy Press.

Proszkowiec-Weglarz M, Angel R. 2013. Calcium and phosphorus metabolism in broilers: effect of homeostatic mechanism on calcium and phosphorus digestibility. J Appl Poult Res. 22:609–627. doi:10.3382/japr.2012-00743.

Rousseau X, Valable A-S, Letourneau-Montminy M-P, Meme N, Godet E, Magnin M, Nys Y, Duclos MJ, Narcy A. 2016. Adaptive response of broilers to dietary phosphorus and calcium restrictions. Poult Sci. 95:2849–2860. doi:10.3382/ps/pew172.

SAS Institute Inc. 2002. Sas/STAT user's guide, 9.1 ed. Cary, NC, USA: SAS Institute Inc.

Selle PH, Cowieson AJ, Ravindran V. 2009. Consequences of calcium interactions with phytate and phytase for poultry and pigs. Livest Sci. 124:126–141. doi:10.1016/j.livsci.2009.01.006.

Selle PH, Ravindran V. 2007. Microbial phytase in poultry nutrition. Anim Feed Sci Technol. 135:1–41. doi:10.1016/j.anifeedsci.2006.06.010.

Taheri HR, Heidari A, Shahir MH. 2015. Effect of high-dose phytase supplementation in broilers from 22 to 42 days post-hatch given diets severely limited in available phosphorus. Br Poult Sci. 56:330–336. doi:10.1080/00071668.2015.1020480.

Taheri HR, Mirisakhani L. 2020. Effect of citric acid, vitamin D3, and high-dose phytase on performance of broiler chicken fed diet severely limited in non-phytate phosphorus. Livest Sci. 241:104223. doi:10.1016/j.livsci.2020.104223.

Walk CL, Santos TT, Bedford MR. 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult Sci. 93:1172–1177. doi:10.3382/ps.2013-03571.

Watson BC, Matthews JO, Southern LL, Shelton JL. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult Sci. 85:493–497. doi:10.1093/ps/85.3.493.

Zyla K, Mika M, Stodolak B, Wikiera A, Koreleski J, Swiatkiewicz S. 2004. Towards complete dephosphorylation and total conversion of phytates in poultry feeds. Poult Sci. 83:1175–1186. doi:10.1093/ps/83.7.1175.