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Influence of microbial phytase on nutrient digestibility, growth performance, geometric bone characteristics, and expression of calcium and phosphate homeostasis genes in broiler chickens

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ABSTRACT

Phytases are commonly used in broiler feed to break down phytic acid and release nutrients. This study evaluated the effects of microbial phytase on nutrient digestibility, growth performance, bone characteristics, and calcium/phosphate homeostasis in broilers. Six treatment groups of 576 Cobb 500 chicks were fed a control diet (T1) and diets supplemented with 200–600 FTU/kg of phytase (T2–T6). Diet phases included starter, grower, and finisher diets over five weeks. Results showed no significant growth differences in the starter phase, but birds receiving phytase showed improved feed intake, body weight, and feed conversion in the finisher phase ($P < 0.001$). Nutrient digestibility, including protein, calcium, and phosphorus, significantly improved ($P < 0.001$) with increased phytase levels. Tibia bone weight, length, and strength increased ($P < 0.01$), while medullary canal diameter was larger in control birds ($P < 0.001$). Gene expression related to calcium/phosphate homeostasis did not differ in starters, but FGF23 and VDR genes significantly varied in finishers ($P < 0.05$). Tibia mineral composition was significantly enhanced in phytase-supplemented groups ($P < 0.001$). Among treatments, 400 FTU/kg provided optimal performance and cost-effectiveness. In conclusion, microbial phytase enhances nutrient availability, improves bone mineralization, and may reduce environmental phosphorus excretion.

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Introduction

Microbial phytase is a widely utilized exogenous enzyme in animal feed, specifically for monogastric animals like poultry and fish. Its primary function is to enhance nutrient digestibility, particularly by breaking down phytate (inositol hexakisphosphate or InsP6), an antinutritional factor that limits the bioavailability of key nutrients such as phosphorus, calcium, and amino acids in plant-based diets (Selle and Ravindran 2007). Phytase is crucial for improving phosphorus absorption, thereby reducing the need for inorganic phosphorus supplementation, which in turn decreases the environmental impact of phosphorus excretion (Dersjant-Li et al. 2014). This reduction in inorganic phosphorus excretion has been linked to mitigating environmental issues such as eutrophication, which is a significant concern in aquatic ecosystems (Svetlana et al. 2023). Moreover, the enzyme's ability to increase the bioavailability of energy and amino acids enhances growth performance and overall feed utilization in animals (Dersjant-Li et al. 2020b).

Phytase supplementation in animal feed has garnered significant attention for its role in reducing feed costs while simultaneously improving feed efficiency, particularly in poultry diets. According to Rizwanuddin et al. (2023), phytase improves the digestibility of minerals and trace elements, making nutrients more readily available for absorption. The enzyme has proven effective in monogastric species such as chickens and fish, where endogenous phytase activity is insufficient to break down phytate completely (Novotny et al. 2023). As a result, exogenous phytase is typically included into animal diets to hydrolyse phytate-bound phosphorus, releasing not only phosphorus but also other bound nutrients, thus enhancing overall nutrient availability (Gautier et al. 2018; Babatunde et al. 2020). The effectiveness of phytase in promoting nutrient absorption and reducing antinutritional effects of phytates is well-documented. In poultry, for example, broilers exhibit significant pre-caecal phytate degradation (up to 76% disappearance) when phytase is included in the diet (Zeller et al. 2015; Ingelmann et al. 2019). The ileum is the final section of the small intestine and serves as the primary site for nutrient absorption in broilers (Adeola and Cowieson 2011). As a result of this, ileal digesta are used to measure nutrient digestibility which provides several advantages, including greater accuracy, avoidance of hindgut fermentation effects, and improved relevance to monogastric digestion because it is

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here that most enzymatic digestion and absorption of amino acids, peptides, fatty acids, vitamins, and minerals occur (Ravindran and Bryden 1999; Selle and Ravindran 2007). These benefits make ileal digestibility the preferred method for evaluating the nutritional value of feed ingredients and optimizing broiler diets.

Phytase also improves growth metrics such as feed conversion ratio, body weight, and carcass characteristics in broilers (Akhtar et al. 2020). The enzyme's activity is particularly effective in the upper gastrointestinal tract, where it operates optimally at pH levels between 3.0 and 5.0, conditions found in the stomachs of poultry (Svetlana et al. 2023). Furthermore, the action of phytase extends beyond phosphorus release. The enzyme's ability to cleave the carbon-phosphate bonds in phytate not only frees phosphorus but also reduces phytate's antinutritional effects, which include its binding to minerals like calcium, zinc, and iron, and limiting their absorption (Ayres et al. 2021). This makes phytase a versatile enzyme that addresses multiple nutritional challenges in monogastric animals. Commercial phytases have been developed with varying enzymatic characteristics, including substrate specificity, catalytic efficiency, and resistance to proteolysis, enhancing their efficacy in different dietary contexts (Ayres et al. 2021; Rizwanuddin et al. 2023).

The genetic and molecular mechanisms underlying nutrient absorption in response to dietary phosphorus levels were explored. The solute carrier family 34 member 2 (SLC34A2) gene, responsible for phosphate transport into epithelial cells, is regulated by dietary phosphate levels, calcitriol (active vitamin D), and parathyroid hormone (PTH) (Giral et al. 2009). In low-phosphate diets, calcitriol increases the expression of SLC34A2 to enhance phosphate absorption, while high phosphate diets downregulate this expression to prevent excess absorption. An essential function of fibroblast growth factor 23 (FGF23) is to control phosphate homeostasis by reducing phosphate reabsorption in the kidneys and inhibiting calcitriol production, which in turn reduces intestinal phosphate absorption (Shimada et al. 2001; Bai et al. 2019). These regulatory mechanisms illustrate the complex interplay between dietary nutrients and genetic expression, highlighting the importance of phytase in modulating phosphorus availability and promoting efficient nutrient absorption. The absorption of calcium is similarly regulated by complex molecular mechanisms involving the transient receptor potential vanilloid channel type 6 (TRPV6), which enables calcium uptake into epithelial cells (Deng et al. 2021). The expression of TRPV6 is influenced by vitamin D levels, underscoring the interdependence of calcium and phosphorus metabolism. Phytase supplementation has been shown to improve calcium absorption by reducing phytate's binding to calcium and other minerals, enhancing their bioavailability. In poultry production, the optimal use of phytase can lead to improved bone mineralization, as evidenced by studies showing enhanced tibia bone characteristics in broilers fed phytase-supplemented diets (Kaur et al. 2022). Moreover, phytase inclusion in broiler diets has been associated with improved feed conversion efficiency, growth performance, and overall nutrient utilization, making it a valuable tool for poultry farmers aiming to minimize the cost of feed while sustaining optimum animal growth and health (Babatunde et al. 2019; Akhtar et al. 2020).

This investigation focuses on the role of a locally produced phytase from a bacterial microbial source (*Escherichia coli)* in enhancing nutrient digestibility, growth performance, and the expression of key genes involved in calcium and phosphorus metabolism in broilers. The chosen dosages of phytase in this experiment (200–600 FTU/kg) fall within the range commonly studied and utilized in poultry nutrition research (Angel et al. 2002; Onyango et al. 2005; Cowieson et al. 2006; Pirgozliev et al. 2013). The findings will provide insights into the appropriate phytase dosages required to optimize growth performance while minimizing environmental impact. Furthermore, the study aims to encourage the broader adoption of phytase in animal feed formulations, promoting both economic efficiency and environmental sustainability in poultry production.

Materials and methods

Experimental animals and housing

The experiment was carried out based on ethical approval by the Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia (UPM/IACUC/AUP-R027/2023). The investigation involved 576 d-old Cobb 500 male chicks with an initial weight (44.41 ± 0.62). They were kept in a closed-house system after arriving. Each chick was weighed individually, had their wings banded, and were assigned into six treatment diets using a completely randomized design (CRD). Twelve chicks comprised each of the eight replicates for each treatment group. All the chicks were administered vaccinations intraocularly (i/o) against Newcastle disease (ND) and infectious bronchitis (IB) on days 7 and 21 of the rearing period using the IB-ND live vaccine. On the 14th day, the IBD vaccination against infectious bursal disease (IBD) was administered intraocularly. The birds freely accessed the experimental period's feed and water from d1–35d. The chicks were administered multivitamins via drinking water as an anti-stress solution for the first 3 days. The house was kept at 33 ± 1°C on the first day, while from day 15 onward, it was progressively lowered to roughly 25 ± 1°C until the experiment's conclusion. There was a 60–75% relative humidity range on average.

Microbial phytase production

The microbial phytase used in this study was produced locally by Asian Nutri-chemical (M) Berhad, Serdang, Malaysia.

Gene cloning and transformation

The phytase gene was obtained from a microbial source and amplified via PCR. The gene was placed within a plasmid vector containing a suitable promoter (e.g. T7 RNA polymerase) and an antibiotic resistance marker (Ampicillin). The recombinant plasmid was introduced into *Escherichia coli* cells (strain BL21 DE3) through heat shock. On LB agar plates with the proper antibiotic, successfully transformed cells were chosen.

Expression of phytase

A single *E. coli* colony harbouring the plasmid was inoculated into LB broth with the antibiotic and culture overnight at 37°

C. For large-scale production, the culture was transferred to a bioreactor and grown under controlled conditions (37°C, pH 7.0, 200 rpm). Phytase expression was induced by adding 1 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG) when the OD600 reached 0.6–0.8.

Fermentation and harvesting

After induction, the culture was grown for an extra 4–6 hours. Cells were extracted by centrifugation (4,000 × g, 10 min), and the cell pellet was stored at −20°C.

Enzyme extraction and purification

A lysis buffer (50 mM Tris-HCl, 150 mM NaCl, pH 7.5) was used to suspend the cell pellet and disrupted by sonication (3 cycles of 30 sec on/30 sec off at 20 kHz). The lysate was centrifuged (10,000 × g, 20 min), and the supernatant containing crude phytase was collected. Purification was carried out using nickel affinity chromatography, as the phytase was tagged with a His-tag (Histidine tag) for easy purification. After binding and washing steps, the enzyme was eluted with 250 mM imidazole.

Enzyme activity assay

Phytase activity was measured by determining the release of inorganic phosphate from phytic acid. The reaction mixture, containing 10 mM phytic acid and the purified enzyme, was incubated at 37°C for 30 minutes, and the released phosphate was quantified using a molybdenum blue method.

Optimization and application

To optimize production, variables such as induction time, temperature, and IPTG concentration were systematically varied. The final phytase preparation was tested for its ability to improve phosphorus bioavailability in animal feed through in vitro assays. It was later validated through in vivo studies to confirm its relevance in real-world biological systems.

Experimental diets and treatments

Three pelleted diets were developed: starter, grower, and finisher. The starter and grower diets were given for two weeks each, (0–14 days) and (15–28 days) respectively, while the finisher diet was offered for one week (29–35 days). Tables 1–3 display the feed compositions for the starter, grower, and finisher diets. The treatments consisted of: T1 = control diet (basal diet with no phytase enzyme), T2 = basal diet + phytase (200 FTU/kg), T3 = basal diet + phytase (300 FTU/kg), T4 = basal diet + phytase (400 FTU/kg), T5 = basal diet + phytase (500 FTU/kg), and T6 = basal diet + phytase (600 FTU/kg).

Growth performance measurement

The chicks were carefully weighed before the experiment began and thereafter weekly to determine the body weight gain (BWG). A measured quantity of feed was supplied ad libitum to the chicks in each replicate, and the remnants were weighed weekly to ascertain the quantity of feed consumed. The amount of feed consumed was divided by weight gain to get the feed conversion ratio.

Apparent nutrient digestibility determination

Titanium dioxide (an indigestible marker) was included in each cage's feed at a 5 g/kg dosage in the final three days of the finisher phase (32–35 d). After slaughtering, the ileal digesta were collected from each bird within every replicate (pen) in a plastic container and kept at −20°C until further laboratory examinations were performed. The proximate analysis of the digesta and feed was done using the AOAC (2007) method. Calcium (Ca) levels were measured through atomic absorption spectrometry (Perkin Elmer Analyst 400). The ether extract (EE), crude protein (CP), dry matter (DM), apparent ileal digestibility, and calcium content were quantified in both the diet and digesta using titanium marker ratios, following the procedure outlined by Tancharoenrat and Ravindran (2014).

$$AID = 100 - \left[100 \times \frac{\%\ TiO_2\ \text{feed}}{\%\ TiO_2\ \text{digesta}} \times \frac{\%\ \text{nutrient digesta}}{\%\ \text{nutrient feed}}\right]$$

Tibiotarsal physical characterization and mineral composition

After the bird's slaughter, the right tibia drumsticks were cut off, tagged, and frozen. They were defrosted later and submerged in 100°C boiling water for 10 minutes. The meat, patella, and bone cap were removed from the drumsticks after they had cooled to room temperature, while the tibia bones were left to air dry at ambient temperature for 24 hours. The tibia length was measured by an electronic digital calliper device (Mitutoyo M-500 Series) from the distal end to the proximal end, and a precision measuring balance was used to weigh the bones. Additionally, the diameter of the distal and proximal tibia epiphyses was measured (Zhang and Coon 1997). Every bone was marked in the middle before it broke, and measurements were taken of its outside diameters. A three-point bending test and a universal testing device (Model 1000R, equipped with a 5000N load cell) was used to evaluate the bone-breaking strength at the Faculty of Engineering, Department of Mechanical Engineering (UPM), which keeps track of the force needed to fracture the bone at a distance of 50 mm between supports and a speed of 10 mm/min. Using a dial calliper, the midpoint was used to quantify the medial and lateral wall thickness and the geometric properties (seedor, robusticity, and tibiotarsal indexes) of the bones. The diameter at the diaphysis was used to subtract the thickness of the lateral and medial walls to get the diameter of the medullary canal. While the tibiotarsal index is calculated as:

$$\frac{[\text{diaphysis diameter} - \text{medullary canal diameter}]}{\text{diaphysis diameter}} \times 100$$

(Barnet and Nordin 1960)

The robustness index is computed as; $\frac{\text{bone lenght}}{\sqrt[3]{\text{bone weight}}}$ (Reisenfeld 1972)

Table 1. Starter diet composition for broiler chickens fed microbial phytase supplements.

			Dietary treatment			
Ingredient (%)	T1	T2	T3	T4	T5	T6
Corn	51.80	51.80	51.80	51.80	51.80	51.90
Soybean Meal	36.40	36.40	36.40	36.40	36.40	36.40
Wheat Pollard	5.12	5.10	5.09	5.08	5.07	4.95
Crude Palm Oil	1.21	1.21	1.21	1.21	1.21	1.21
L-Lysine	0.27	0.27	0.27	0.27	0.27	0.27
Mono-dicalcium phosphate	2.47	2.47	2.47	2.47	2.47	2.48
Calcium carbonate	1.36	1.36	1.36	1.36	1.36	1.36
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.25	0.25	0.25	0.25	0.25	0.25
Vitamin Mix[$]	0.15	0.15	0.15	0.15	0.15	0.15
Mineral Mix*	0.15	0.15	0.15	0.15	0.15	0.15
Toxin binder[%]	0.10	0.10	0.10	0.10	0.10	0.10
Antioxidant[#]	0.10	0.10	0.10	0.10	0.10	0.10
Threonine	0.16	0.16	0.16	0.16	0.16	0.16
L-Arginine	0.00	0.00	0.00	0.00	0.00	0.00
DL-Methionine	0.36	0.36	0.36	0.36	0.36	0.36
Phytase	0.00	0.02	0.03	0.04	0.05	0.06
Total	100.00	100.00	100.00	100.00	100.00	100.00
ME. for Poultry	3050.09	3050.36	3050.15	3050.63	3050.42	3050.22
Protein	21.04	21.04	21.04	21.04	21.04	21.04
Fat	6.27	6.28	6.28	6.29	6.29	6.29
Fibre	4.34	4.33	4.33	4.33	4.33	4.33
Calcium	0.75	0.75	0.75	0.75	0.75	0.75
Total Phosphorus	0.73	0.73	0.72	0.72	0.72	0.72
Avail. *P* for Poultry	0.38	0.38	0.38	0.38	0.38	0.38
Salt	0.36	0.36	0.36	0.36	0.36	0.36
Arginine	1.17	1.17	1.17	1.17	1.17	1.17
Lysine	1.15	1.15	1.15	1.15	1.15	1.15
SID Lys	1.26	1.26	1.26	1.26	1.26	1.26
SID Met	0.66	0.66	0.66	0.66	0.66	0.66
SID Met + Cys	0.94	0.94	0.94	0.94	0.94	0.94
SID Thr	0.86	0.86	0.86	0.86	0.86	0.86
SID Tryp	0.24	0.24	0.24	0.24	0.24	0.24
SID Arg	1.38	1.38	1.38	1.38	1.38	1.38

*mineral mix comprises Fe (100 mg), Zn (100 mg), I (2 mg), Mn (110 mg), Cu (20 mg), Se (0.2 mg), and Co (0.6 mg). [$]Vitamin premix contains 2 mg of retinol, 0.03 mg of cholecalciferol, 0.02 mg of α-tocopherol, 0.83 mg of thiamine, 2 mg of riboflavin, 0.33 mg of folic acid, 1.33 mg of menadione, 0.03 mg of cobalamin, 0.03 mg of biotin, 3.75 mg of pantothenic acid, 23.3 mg of niacin, and 1.33 mg of pyridoxine. [#]Antioxidants include butylated hydroxyanisole (BHA). [%]Toxin binder is comprised of naturally hydrated sodium, calcium, and aluminium silicates.

The seedor index measures bone density, which is calculated as;

$$\frac{\text{tibia bone weight}}{\text{tibia bone lenght}}$$

(Seedor 1993)

The bones were dried for an entire twenty-four-hour period in an oven at 105°C. After samples were dried (DM), they were weighed again, placed in a pre-weight crucible, burned for six hours at 600°C, and weighed again (ASH, g/100 g DM). The amount of calcium (Ca) in both the feed and bone was measured by an atomic absorption spectrophotometer (Perkin Elmer Analyst 400).

Calcium and phosphate homeostasis genes

After the starter and finisher phases, eight birds from each of the six treatment diets were randomly chosen and slaughtered (neck, throat, and jugular veins severed). EDTA (ethylene diamine tetra-acetic acid) vacuum-sealed tubes were used to collect blood samples. Before centrifugation, the sample-filled tubes were briefly mixed, then carefully flipped and put inside an ice cube. It was then centrifuged at 3,000 rpm for 30 minutes at 4°C in 2 mL microcentrifuge tubes. The plasma was then collected and frozen at −80°C, followed by thawing the samples when needed. The quantities of phosphorus and calcium homeostasis's gene were measured using ELISA kits validated for avian plasma that were purchased from 'Qayeeb-Bio for Life Science' according to the manufacturer's specifications. According to Trott et al. (2009), the kits were utilized to assess the levels of FGF23, SLC34A2, TRPV6, and vitamin D receptor antibodies in the plasma of 14 and 35-day-old chicks, using microplate reader (Model 3550-UV, Bio-Rad), while the spectrophotometric analysis of ELISA reactions was measured at 450 nm wavelength.

Statistical analysis

A completely randomized design (CRD) methodology was employed. The Statistical Analysis System, version 9.4 computer software, was utilized to analyse the data using the general linear model (PROC GLM) (SAS Institute Inc. 2024). The Duncan multiple range test was used to differentiate means at a significance level of $P < 0.05$. The response to incremental microbial phytase supplementation was tested for linear and quadratic character using orthogonal polynomial contrast.

Results

Growth performance for broiler chickens fed microbial phytase supplements

Table 4 presents the growth performance indices of birds fed diets with various concentrations of microbial phytase. There

Table 2. Grower diet composition for broiler chickens fed microbial phytase supplements.

			Dietary treatment			
Ingredient (%)	T1	T2	T3	T4	T5	T6
Corn	52.9	52.9	52.9	52.9	52.9	52.9
Soybean Meal	29.55	29.57	29.57	29.57	29.6	29.58
Wheat Pollard	10.92	10.88	10.87	10.86	10.8	10.83
CPO	2	2	2	2	2	2
L-Lysine	0.34	0.34	0.34	0.34	0.34	0.34
Mono-dicalcium phosphate	1.36	1.36	1.36	1.36	1.36	1.36
Calcium carbonate	1.4	1.4	1.4	1.4	1.4	1.4
Choline chloride	0.1	0.1	0.01	0.1	0.1	0.1
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Mineral Mix*	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Mix[$]	0.15	0.15	0.15	0.15	0.15	0.15
Toxin binder[%]	0.1	0.1	0.1	0.1	0.1	0.1
Antioxidant[#]	0.1	0.1	0.1	0.1	0.1	0.1
Threonine	0.16	0.16	0.16	0.16	0.16	0.16
L-Arginine	0.07	0.07	0.07	0.07	0.07	0.07
DL-Methionine	0.35	0.35	0.35	0.35	0.35	0.35
Phytase	0	0.02	0.03	0.04	0.05	0.06
Total	100	100	100	100	100	100
ME. for Poultry	2956.13	2955.81	2955.6	2955	2955	2955.03
Protein	19	19	19	19	19	19
Fat	4.16	4.16	4.16	4.16	4.16	4.16
Fibre	4.23	4.23	4.23	4.23	4.23	4.23
Calcium	0.81	0.81	0.81	0.81	0.81	0.81
Total Phosphorus	0.75	0.75	0.75	0.75	0.75	0.75
Avail.*P* for Poultry	0.4	0.4	0.4	0.4	0.4	0.4
Salt	0.36	0.36	0.36	0.36	0.36	0.36
Arginine	1.26	1.26	1.26	1.26	1.26	1.26
Lysine	1.27	1.27	1.27	1.27	1.27	1.27
SID Lys	1.16	1.16	1.16	1.16	1.16	1.16
SID Met	0.62	0.62	0.62	0.62	0.62	0.62
SID Met + Cys	0.88	0.88	0.88	0.88	0.88	0.88
SID Thr	0.79	0.79	0.79	0.79	0.79	0.79
SID Tryp	0.21	0.21	0.21	0.21	0.21	0.21
SID Arg	1.29	1.29	1.29	1.29	1.29	1.29

*mineral mix comprises Fe (100 mg), Zn (100 mg), I (2 mg), Mn (110 mg), Cu (20 mg), Se (0.2 mg), and Co (0.6 mg). [$]Vitamin premix contains 2 mg of retinol, 0.03 mg of cholecalciferol, 0.02 mg of α-tocopherol, 0.83 mg of thiamine, 2 mg of riboflavin, 0.33 mg of folic acid, 1.33 mg of menadione, 0.03 mg of cobalamin, 0.03 mg of biotin, 3.75 mg of pantothenic acid, 23.3 mg of niacin, and 1.33 mg of pyridoxine. [#]Antioxidants include butylated hydroxyanisole (BHA). [%]Toxin binder is comprised of naturally hydrated sodium, calcium, and aluminium silicates.

was no discernible impact on body weight, and weight gain during the starter and grower periods, but the feed conversion ratio (FCR) in starter and feed intake in grower were significantly($p < 0.05$) affected. There was a significant ($p < 0.0001$) improvement in feed conversion ratio, body weight, weight gain, and feed intake (FI) at the finisher and overall phases for the birds fed phytase diets compared to the control group except for FCR in finisher which was not significantly affected. However, diets T4, T5, and T6 performed better ($p < 0.05$) in all the parameters. The overall performance of the treatment diets containing the phytase supplements had good feed efficiency (1.57–1.63) relative to the control diet. The results also, indicate that there is a consistent linear trend in the starter, grower, and finisher phases where increasing microbial phytase levels generally improve performance. While the quadratic *p*-values across the board are not significant.

Nutrient and mineral digestibility

Figure 1 displays the impact of varying dosages of microbial phytase on broiler chickens' nutritional digestibility. Phytase supplements in the diet showed no impact on dry matter's digestibility. The digestibility of ash, phosphate, calcium, and crude protein, however, varied significantly ($p < 0.001$). The digestibility of the nutrients increased linearly with higher levels of phytase, with birds fed diets T4, T5, and T6 showing better digestibility.

Tibia bone geometric characteristics

The results of the impacts of phytase on the geometric characteristics of tibia bones in broiler chickens are displayed in Table 5. With increased levels of microbial phytase supplementation, the weight of the tibia bones improved ($p < 0.01$).

Similarly, the tibia length, robusticity, diaphysis diameter, tibiotarsal index, flexure load, and the proximal and distal head width were all significantly ($p < 0.01$) higher compared to that of the control diet, with values linearly increasing as the levels of microbial phytase increased. However, the medullary canal diameter linearly decreases as the value of the phytase increases in the diet, where the control had the larger diameter ($p < 0.001$) among the treatments, and there was no significant variation in the seedor index values ($p > 0.05$). A highly significant linear trend ($p < 0.0001$) shows that increasing phytase levels consistently enhances bone weight, length, robusticity, tibiotarsal index, as well as proximal and distal head widths, and flexure load. However, both the linear and quadratic trends ($p < 0.0001$) observed in diaphysis and medullary canal diameters suggest that higher phytase levels have a notable impact on bone thickness and canal diameter,

Table 3. Finisher diet composition for broiler chickens fed microbial phytase supplements.

	Dietary treatment					
Ingredient (%)	T1	T2	T3	T4	T5	T6
Corn	48.41	48.41	48.41	48.41	48.41	48.41
Soybean Meal	26.75	26.75	26.75	26.75	26.75	26.75
Wheat Pollard	16.26	16.23	16.22	16.2	16.19	16.18
Crude Palm Oil	4.35	4.36	4.36	4.37	4.37	4.37
L-Lysine	0.28	0.28	0.28	0.28	0.28	0.28
Mono-dicalcium phosphate	1.15	1.15	1.15	1.15	1.15	1.15
Calcium carbonate	1.33	1.33	1.33	1.33	1.33	1.33
Choline chloride	0.10	0.10	0.10	0.10	0.10	0.10
Salt	0.35	0.35	0.35	0.35	0.35	0.35
Mineral Mix*	0.15	0.15	0.15	0.15	0.15	0.15
Vitamin Mix[$]	0.15	0.15	0.15	0.15	0.15	0.15
Antioxidant[#]	0.10	0.10	0.10	0.10	0.10	0.10
Toxin binder[%]	0.10	0.10	0.10	0.10	0.10	0.10
Threonine	0.11	0.11	0.11	0.11	0.11	0.11
L-Arginine	0.09	0.09	0.09	0.09	0.09	0.09
DL-Methionine	0.32	0.32	0.32	0.32	0.32	0.32
Phytase	0.00	0.02	0.03	0.04	0.05	0.06
Total	100.00	100.00	100.00	100.00	100.00	100.00
ME. for Poultry	3050.09	3050.36	3050.2	3050.63	3050.42	3050.22
Protein	18.02	18.02	18.02	18.01	18.01	18.01
Fat	6.27	6.28	6.28	6.29	6.29	6.29
Fibre	4.34	4.33	4.33	4.33	4.33	4.33
Calcium	0.75	0.75	0.75	0.75	0.75	0.75
Total Phosphorus	0.73	0.73	0.72	0.72	0.72	0.72
Avail. *P* for Poultry	0.38	0.38	0.38	0.38	0.38	0.38
Salt	0.36	0.36	0.36	0.36	0.36	0.36
Arginine	1.17	1.17	1.17	1.17	1.17	1.17
Lysine	1.15	1.15	1.15	1.15	1.15	1.15
SID Lys	1.06	1.06	1.06	1.06	1.06	1.06
SID Met	0.57	0.57	0.57	0.57	0.57	0.57
SID Met + Cys	0.83	0.83	0.83	0.83	0.83	0.83
SID Thr	0.70	0.70	0.70	0.70	0.70	0.70
SID Tryp	0.21	0.21	0.21	0.21	0.21	0.21
SID Arg	1.25	1.25	1.25	1.25	1.25	1.25

*mineral mix comprises Fe (100 mg), Zn (100 mg), I (2 mg), Mn (110 mg), Cu (20 mg), Se (0.2 mg), and Co (0.6 mg). [$]Vitamin premix contains 2 mg of retinol, 0.03 mg of cholecalciferol, 0.02 mg of α-tocopherol, 0.83 mg of thiamine, 2 mg of riboflavin, 0.33 mg of folic acid, 1.33 mg of menadione, 0.03 mg of cobalamin, 0.03 mg of biotin, 3.75 mg of pantothenic acid, 23.3 mg of niacin, and 1.33 mg of pyridoxine. [#]Antioxidants include butylated hydroxyanisole (BHA).[%]Toxin binder is comprised of naturally hydrated sodium, calcium, and aluminium silicates.

Table 4. Growth performance of broiler chickens fed diets containing microbial phytase.

	Dietary treatment								Contrast *p*-value	
Parameter	T1	T2	T3	T4	T5	T6	SEM	*p*-value	Linear[1]	Quadratic[2]
Starter:0–2weeks										
Initial BW	44.15	44.23	44.95	44.28	44.63	44.23	0.1347	0.4839	0.734	0.1982
BW (g)	380.02	383.67	382.35	376.55	390.97	394.98	2.3036	0.1822	0.053	0.2131
BWG (g)	335.79	339.44	337.41	332.27	346.34	350.75	2.2831	0.1756	0.0534	0.1827
FI (g)	436.42	437.79	441.37	439.21	432.1	440.73	1.0185	0.0988	0.911	0.7757
FCR (g:g)	1.35[ab]	1.34[ab]	1.33[ab]	1.38 [a]	1.27 [b]	1.28 [b]	0.0101	0.0494	0.0311	0.2019
Grower:3–4weeks										
BW (g)	1564.48	1582.76	1591.23	1600.26	1606.64	1616.81	7.0657	0.3763	0.0255	0.7865
BWG (g)	1182.05	1198.92	1209.34	1223.43	1207.78	1223.89	7.4437	0.5867	0.1023	0.518
FI (g)	2128.94[b]	2146.32[a]	2139.57[ab]	2127.66[b]	2146.21[a]	2144.85 [a]	1.7932	0.0016	0.0638	0.8499
FCR (g:g)	1.85	1.83	1.8	1.76	1.81	1.79	0.0117	0.2934	0.0695	0.2626
Finisher: week 5										
BW	2459.42[c]	2512.52[bc]	2536.34[b]	2543.81[b]	2554.72[b]	2638.85 [a]	8.2133	<0.0001	<0.0001	0.5598
BWG	894.94[b]	929.76[b]	945.11[b]	943.55[b]	954.08[b]	1022.05 [a]	9.7828	<0.01	<0.0001	0.5005
FI	1403.45[d]	1410.57[d]	1430.84[c]	1431.22[c]	1444.79[b]	1467.79[a]	2.1012	<0.0001	<0.0001	0.2162
FCR	1.69	1.6	1.6	1.7	1.69	1.51	0.02518	0.2263	0.3306	0.4067
Overall: 0–5 weeks										
BW	2457.15[b]	2512.52[ab]	2532.62[ab]	2543.81[a]	2555.64[a]	2608.69 [a]	8.2133	<0.0001	<0.0001	0.5598
BWG	2415.24[c]	2468.20[bc]	2491.22[b]	2499.75[b]	2510.10[b]	2594.63[a]	8.2171	<0.0001	<0.0001	0.5464
FI	3975.70[d]	3985.51[cd]	3995.39[c]	4018.66[b]	4027.13[b]	4053.37[a]	2.8848	<0.0001	<0.0001	0.2239
FCR	1.65[a]	1.63[a]	1.61[a]	1.62[a]	1.62[a]	1.57[b]	0.0056	<0.0001	<0.0001	0.6346

[abcd]Means ± SEM. The means in the same row that have different superscripts are substantially different ($P < 0.05$). BW = body weight. BWG = body weight gain. FI = feed intake. FCR = feed conversion ratio, $p < 0.05$ = *, $p < 0.01$ = **, $p < 0.001$ = ***

Linear[1] or quadratic[2] response estimated using orthogonal polynomial contrasts.

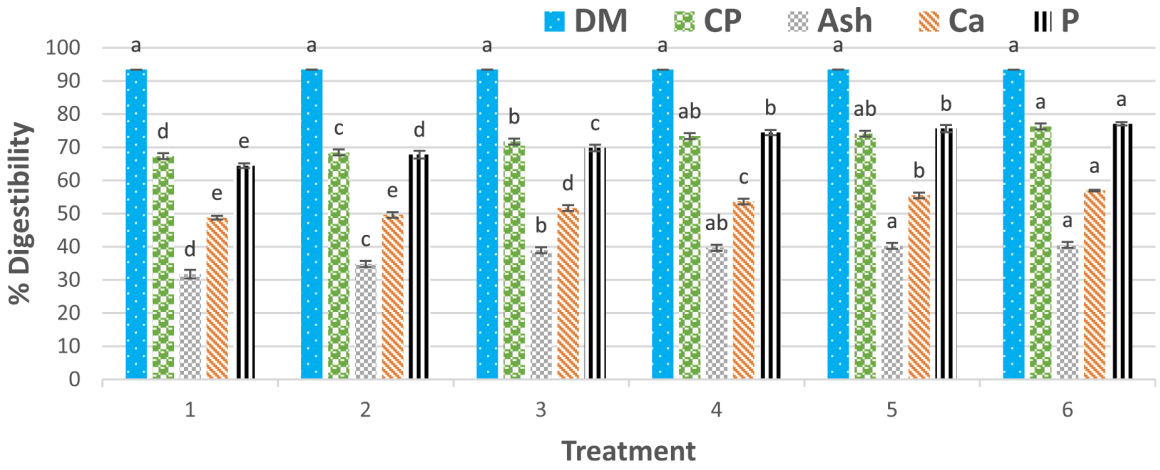

Figure 1. Apparent ileal digestibility broiler chicken fed microbial phytase supplement. [abcd]Means ± SEM. Significant differences exist between the means of the same row with different superscripts. ($P < 0.05$).

Table 5. Tibia bone geometric characteristics of broiler chickens fed microbial phytase supplements.

	Dietary treatments								Contrast *p*-value	
Parameters	T1	T2	T3	T4	T5	T6	SEM	*p*-Value	Linear[1]	Quadratic[2]
Weight (g)	9.33[b]	9.67[ab]	9.68[ab]	10.00[a]	10.16[a]	10.19[a]	0.0854	0.0146	<0.0001	0.5992
Length (cm)	8.88[d]	9.01[cd]	9.08[bcd]	9.18[abc]	9.26[ab]	9.36[a]	0.0356	<0.0001	<0.0001	0.9384
Robusticity	4.22[b]	4.23[b]	4.26[ab]	4.30[ab]	4.31[ab]	4.36[a]	0.0151	0.05	<0.01	0.7069
Diaphysis diameter (mm)	9.29[f]	10.15[e]	10.46[d]	10.53[c]	10.64[b]	10.72[a]	0.0712	<0.0001	<0.0001	<0.0001
Medullary canal diameter (mm)	7.58[a]	7.55[a]	7.50[b]	7.46[c]	7.26[d]	6.66[e]	0.0464	<0.0001	<0.0001	<0.0001
Tibiotarsal index	28.24[d]	28.46[cd]	28.69[c]	28.78[bc]	29.10[ab]	29.35[a]	0.0707	<0.0001	<0.0001	0.6209
Proximal head width (cm)	2.28[b]	2.31[b]	2.34[ab]	2.34[ab]	2.39[a]	2.40[a]	0.0105	0.0029	<0.0001	0.869
Distal head width (cm)	1.78[c]	1.81[bc]	1.82[bc]	1.86[ab]	1.88[ab]	1.92[a]	0.0116	0.0026	<0.0001	0.5309
Seedor index (g/cm)	1.05	1.07	1.07	1.09	1.1	1.09	0.0079	0.4942	0.0657	0.671
Flexure load (N)	326.16[c]	351.01[b]	364.88[b]	368.41[b]	418.19[a]	428.48[a]	5.7715	<0.0001	<0.0001	0.2133

[abcd]Means ± SEM. The means in the same row with distinct superscripts differ significantly ($P < 0.05$).Linear[1] or quadratic[2] response estimated using orthogonal polynomial contrasts.

with the quadratic effect indicating a peak effect at elevated phytase doses.

Tibia bone mineral composition

The mineral compositions of the tibia bone in broiler chickens are indicated in Figure 2. Tibia dry matter, ash, phosphorus, and calcium concentrations of birds fed microbial phytase are considerably ($p < 0.001$) higher than those of the control. The results indicate improvements in all parameters as the quantity of phytase inclusion increased. However, T5 and T6 exhibited significantly higher values ($p < 0.001$) compared to other diets, while T1 had the least significant effect among the treatments.

Calcium and phosphate homeostasis genes

The results of the concentration of FGF23, TRPV6, SLC34A2, and VDR genes are displayed in Table 6. The outcome shows that the dietary means of the birds did not significantly ($p > 0.05$) differ among the parameters in the starter diets and SLC34A2 and TRPV6 in the finisher diets. However, a significant ($p < 0.05$) difference was shown in the finisher diet for the FGF23 and vitamin D receptor genes ($p < 0.01$). There is a significant positive linear increase ($p < 0.05$) in FGF23 and TRPV6 expression, while VDR and SLC34A2 expression show a negative linear decline ($p < 0.05$) with increasing phytase levels. No quadratic effects were observed in any of the phases.

Pearson correlation result

Table 7, presents the Pearson correlation coefficients between body weight, nutrient digestibility, carcass weight, and geometric bone traits of broiler chickens fed microbial phytase. The results indicate a strong positive association between nutrient digestibility (CP, Ash, Ca, and P) ($r = 0.984, 0.993, 0.998, 0.999$, $p < 0.01$) and body weight. Additionally, there is a strong positive correlation between phytase-enhanced nutrient digestibility and geometric bone traits (tibia weight, length, robusticity index, and load, $p < 0.01$). Moreover, a strong positive correlation exists among body weight, geometric bone traits, and carcass ($p < 0.01$). These results demonstrate that all variables exhibit strong positive correlations, suggesting that they tend to increase as the other increases ($p < 0.01$).

Discussion

Growth performance

Numerous studies have shown many benefits for growth performance from the inclusion of phytase in broiler diets

Figure 2. Tibia Bone Mineral Composition of Broiler Chickens Fed Microbial Phytase Supplements. [abcd] Means ± SEM. Means within the same row with distinct superscripts significantly differ ($P < 0.05$).

Table 6. Calcium and phosphate homeostasis genes concentration in broiler chickens fed dietary microbial phytase.

			Dietary treatments						Contrast *p*-value	
Parameters (pg/mL)	T1	T2	T3	T4	T5	T6	SEM	*p*-Value	Linear[1]	Quadratic[2]
Starter (2 weeks)										
FGF23	110.00	113.33	113.33	118.33	124.58	131.46	2.7848	0.2108	<0.01	0.4841
TRPV6	74.17	75.00	78.33	80.00	81.67	84.17	2.4488	0.8601	0.1830	0.9771
VDR	29.58	27.5	27.08	26.87	26.45	25.83	0.5874	0.5742	0.0811	0.5904
SLC34A2	9.29	8.96	8.92	8.79	8.17	7.92	0.2659	0.6945	0.1051	0.7573
Finisher (5 weeks)										
FGF23	120.83^{c}	132.50^{bc}	145.42^{abc}	140.00^{abc}	155.83^{ab}	165.83^{a}	4.2036	<0.05	<0.0001	0.9679
TRPV6	74.17	85.00	89.17	90.83	94.17	95.00	2.7406	0.256	<0.05	0.802
VDR	33.33^{a}	32.92^{a}	32.29^{a}	30.00^{ab}	28.13^{bc}	25.83^{c}	0.6901	<0.0001	<0.0001	0.2571
SLC34A2	10.87	10.88	10.71	10.04	9.38	9.13	0.3164	0.4300	<0.05	0.6504

[abcd]Means ± SEM. Means with distinct superscripts in the same row vary significantly ($P < 0.05$) Fibroblast Growth Factor 23 (FGF23). Transient Receptor Potential Vanillloid channel type 6 (TRPV6), Solute Carrier Family 34 member 2 (SLC34A2), and Vitamins D. Receptor (VDR). Linear[1] or quadratic[2] response estimated using orthogonal polynomial contrasts. Picograms per millilitre (pg/mL).

(Selle and Ravindran 2007; Ravindran et al. 2008; Powell et al. 2011). This research demonstrates that performance was enhanced when dietary microbial phytase was added to broiler feed. It is noteworthy that performance increased linearly as the phytase levels increased. This could be due to phytase activity in the intestinal tract, which helps break down InsP6 and other phytate esters increasing the availability of nutrients bound to phytates (Sens et al. 2021). Many studies have revealed that varying levels of phytase have a beneficial impact on FI, growth performance, FCR, and live body weight at higher and lower doses (Sommerfeld et al. 2018; Dersjant-Li et al. 2020a). The FCR of broilers fed a supplemented phytase diet improved by 10% in research by Wang et al. (2021). The release of inositol due to the extra-phosphoric impacts of phytase may also have a vital impact on modifying the growth rate of broilers (Walk et al. 2014). According to Baradaran et al. (2021) the growth rate ($p < 0.001$) and overall FCR both improved linearly at 7% with increasing phytase dosages in the diet.

The current result shows that feed intake increases with phytase levels, suggesting that phytase enhances nutrient utilization and induces a feed intake response with higher inclusion levels. The advantages of phytase's extra-phosphoric effects might come from increased feed intake (Dersjant-Li et al. 2014). However, the phytate content effect may be the cause of the birds fed a control diet's decreased feed consumption, where phytase is not available to enhance its degradation because phytate can reduce appetite and impede the availability of nutrients, thereby affecting feed intake (Fatufe et al. 2019) and ultimately depressing growth (Selle and Ravindran 2007; Cowieson et al. 2011). Therefore, it was proposed that phytase's ability to increase the intake of digestible nutrients may be related to its ability to degrade phytates (Dos Santos et al. 2014; Kriseldi et al. 2021). This study discovered that dosage T4 (400 FTU/kg) of phytase showed more economical enhancement in the digestibility of minerals, amino acids, and feed intake than lower dosages, efficiently resulting in higher body weights and improved feed efficiency. The linear trends suggest that as the phytase levels increase, the growth performance of broiler chickens improves consistently and there is little evidence of quadratic effects, indicating that there is no sign of diminishing returns or a reversal in trends at higher phytase levels.

Table 7. Pearson's correlation coefficient ('r') between body weights, carcass weight, nutrients digestibility, and geometric bone traits of broiler chickens fed microbial phytase supplement.

Variable	BW	CP	Ash	Ca	*P*	Carcass	TW	TL	Robusticity	Load
BW	1									
CP	0.984	1								
Ash	0.993	0.977	1							
Ca	0.998	0.976	0.994	1						
P	0.999	0.971	0.995	0.996	1					
Carcass	0.986	0.972	0.993	0.995	0.997	1				
TW	0.999	0.990	0.988	0.997	0.999	0.994	1			
TL	0.999	0.984	0.988	0.996	0.999	0.993	0.999	1		
Robusticity	0.998	0.985	0.988	0.996	0.999	0.993	0.999	0.999	1	
Load	0.992	0.972	0.994	0.996	0.999	0.999	0.999	0.999	0.999	1

BW = body weight, DM – dry matter, CP – crude protein, Ca – Calcium, *P* – Phosphorus TW – Tibia weight, TL – Tibia length.

Nutrients and mineral ileal digestibility

The improvement in the digestibility ($p < 0.001$) of the nutrients from this study attests to the conclusions made by Gautier et al. (2018) who discovered that supplementation of phytase in the diets of broiler chickens led to higher levels of InsP6 breakdown and ileal inositol concentration in the birds. In addition to that, phytase increases the bioavailability of P, Ca, and amino acids (Rizwanuddin et al. 2023). This is also confirmed by an experimental study carried out by Sibahu and Umar (2009) on broiler chickens, whose results show that phytase increases the apparent digestibility of crude protein, crude ash, and dry matter ($p < 0.05$). Exogenous phytase is frequently employed in poultry feeds to hydrolyse phytates phosphorus (PP) and liberate phosphorus (P) and other minerals for birds (Gautier et al. 2018; Babatunde et al. 2021a, 2021b). The reason behind this is that endogenous phytase activity in non-ruminants is considered insufficient, which results in restricted InsP6 breakdown in the digestive tract (Novotny et al. 2023). Phytase exhibits possibilities to facilitate the digestion of amino acids, the bioavailability of cation bonded with phytates, such as zinc, iron, magnesium, calcium, and sulphur, and the release of P from IP6 (Rodehutscord et al. 2022). The breakdown and absorption of starch, amino acids, other nutrients, and minerals can be enhanced by phytase (Dersjant-Li and Kwakernaak 2019). This derived advantage of improved digestibility may be the cause of the birds' increased body weights when fed phytase diets found in this study (Kozlowski et al. 2019; Sens et al. 2021).

Tibia bone geometric characteristics

The tibia bone geometric characteristics of broilers fed different levels of microbial phytase showed highly significant effects in most parameters. This is likely due to the substantial ($p < 0.05$) increase in phosphorus and calcium in the tibia bones of broilers given microbial phytase compared to the control group. The higher tibia weight corroborates the outcomes of Amin et al. (2020) who discovered that phytase enhanced the weight of the tibia bone. The higher tibiotarsal index of the T6, T5, and T4 birds indicates a better mineralization level of the bones (Ziaie et al. 2011). This is because mineral deposition in broiler chickens has been associated with the incorporation of higher quantities of phytase in their diet (Fernandes et al. 2019). A high robusticity index (a measure of bone strength) in the phytase diets is an indication of strong bone structure because breaking strength and P content in the tibia were both enhanced ($p < 0.05$) by phytase supplementation (Moita et al. 2021). By improving phosphorus utilization, phytase contributes to more robust bone structure and mineralization. The flexion load (the maximum load the bone can withstand before it breaks) suggests that phytase supplementation can help to make the tibia bones stronger and more resistant to breaking. The higher diaphysis diameter (the width of the bone shaft) suggests that phytase supplementation can help enhance bone tissue thickness in broiler chickens. The larger medullary canal diameter (the hollow space in the centre of the bone) in the control, suggests that phytase supplementation can help reduce bone tissue's porosity in broiler chickens. This conclusion corroborates Sharif et al. (2019) who found that supplementing broiler chickens with phytase increased the diaphysis diameter, tibiotarsal index, and breaking strength of the tibia bones. The tibia seedor index, a metric used to assess bone density and quality in avian species (Almeida Paz et al. 2008; Mabelebele et al. 2017; Muszyński et al. 2018), was not significantly impacted by the treatments, while birds fed on phytase supplement treatments had greater numerical values ($p > 0.005$), indicating their heavier weights (Seedor 1993; Almeida 2006). The results clearly indicate that microbial phytase supplementation significantly enhances the geometric characteristics and strength of broiler tibia bones while a consistent linear increase in parameters like bone weight, length, robustness, diaphysis diameter, tibiotarsal index, head widths, and flexure load suggests that phytase improves bone mineralization and strength. The quadratic effects observed for diaphysis and medullary canal diameters imply that beyond a certain level of phytase, the effects may level off or diminish, particularly in terms of bone thickness. Overall, the results demonstrate the beneficial impact of phytase on bone health in broiler chickens, making it a valuable supplement for improving skeletal development.

Tibia bone mineral composition

Tibia bone ash is regarded as the best criterion for assessing the phytase impacts on mineral deposition and utilization in broilers. Studies conducted by Tang et al. (2012). and Lalpanmawia et al. (2014) support using tibia bone ash as a reliable marker in assessing the implications of phytase on mineralization within the bones of broiler chickens. Cardoso Júnior et al. (2010) stated that bone mineralization is a good indicator of bone quality. This is important because bone quality is linked to

positive outcomes for broilers, such as improved growth and development. Bone mineralization is a frequently used metric to assess phytase efficacy for quantifying the amount of phosphorus available to broilers released from diets based on meals made of corn and soybeans (Pereira et al. 2012; Dersjant-Li et al. 2014). The beneficial effect of phytase on bone mineralization in this study is likely due to its capacity to enhance the amount of available P in the diet. This is corroborated by the discoveries of Walk et al. (2014), which showed that diets supplemented with phytase improved the quantity of available phosphorus (aP) in the tibia of broilers. It is noteworthy that calcium and phosphorus are intimately associated with this result of bone mineralization since both are stored in bone, with Calcium being virtually totally deposited in the form of hydroxyapatite crystals of Ca phosphate (Bougouin et al. 2014). It can be deduced from this that phytase promotes bone mineralization, which raises aP levels (Sens et al. 2021). Moreover, these results corroborate the data gathered for growth performance assessments implying that adding phytase to broiler feeds is advantageous for bone mineralization.

Calcium and phosphate homeostasis genes

Fibroblast growth factor-23 (FGF23)

The results of this investigation on fibroblast growth factor-23 (120.83–165.83 pg/mL) were found to be compatible with that of Liu et al. (2019), who stated that the usual range for FGF23 concentration in broiler chicken blood plasma is between 100 and 200 pg/mL The insignificant difference ($p > 0.05$) found in the starter phase corroborates the findings of Horvat-Gordon et al. (2019), who found non-significant expression of plasma FGF-23 levels in young chicks. Conversely, Sitara et al. (2008) discovered that in finisher chicks, FGF-23 plasma levels increased in response to dietary nPP (non-phytate phosphorus) elevated from 0.13 to 0.45%. Ren et al. (2017) also discovered that in a compared study of chickens fed a conventional diet (0.40% aP) and a high-P diet (0.80% readily aP), FGF-23 has been shown to have elevated levels in the blood of hens that were fed a high-P diet. This indicated that phosphorus was more readily absorbed into the bloodstream and made accessible in the intestine due to the activity of phytase, which causes an increase in FGF23 synthesis to regulate excess phosphorus in the body system. On the other hand, consistent data from studies on experimental animals indicate that the level of FGF-23 in the bloodstream is controlled by the amount of phosphorus in the diet (Perwad et al. 2005; Yu et al. 2005). The levels of blood FGF-23 in normal rats and mice are directly influenced by both serum phosphorus concentrations and dietary phosphorus intake (Perwad et al. 2005; Saito et al. 2005). An oral phosphate load caused the serum FGF-23 levels to rise after being markedly decreased by the low-phosphate diet. Antoniucci et al. (2006) found that phosphorus supplementation significantly raised serum FGF-23 concentrations in healthy men. In normal mice and rats, the levels of serum FGF-23 are directly influenced by both dietary and serum phosphorus (Perwad et al. 2005; Saito et al. 2005). The low-phosphate diet caused a considerable drop in serum FGF-23 levels, which later increased in response to an oral phosphate load. Antoniucci et al. (2006) found that serum FGF-23 concentrations in healthy men increased significantly during phosphorus supplementation. The expression of FGF23 in the colon of gilts and barrows increased with phytase supplementation compared to the control diet (Julia et al. 2023). Additionally, it was discovered that increasing dietary phosphorus levels causes FGF23 to be secreted, which in turn causes the excretion of phosphorus to prevent its toxicity (Ren et al. 2017). In contrast, the body increases phosphorus retention and inhibits FGF23 secretion in response to lower dietary phosphorus levels, preventing deficiency of phosphorus (Levi et al. 2019). According to this study, the amount of phytase in the diet had a substantial impact on the amount of FGF23 in the blood plasma of broiler chickens. The chickens fed a diet with higher phytase levels had higher FGF23 concentrations in their plasma because of the phytase's effect on improving phosphorus digestibility. This supports the work of Ren et al. (2017), who discovered that providing phytase to chicks with 0.2% nPP increases the levels of plasma FGF23 by 22%. The linear increase in FGF23 suggests that as dietary phytase improves phosphorus availability, FGF23 responds by regulating phosphorus levels to prevent hyperphosphatemia.

TRPV6 (Transient receptor potential cation channel, subfamily V member 6)

The results for the TRPV6 concentration in the blood plasma of broiler chickens (74.1–95 pg/mL) are similar to the concentrations (70 and 90 pg/mL) reported by Deng et al. (2021). It shows a non-significant ($p > 0.05$) trend of increased values in the plasma concentration as the phytase level increases both at the starter and finisher stages. So, while phytase itself doesn't directly interact with TRPV6, it indirectly facilitates calcium absorption by releasing calcium ions from phytate, which can then be taken up by TRPV6. An increased calcium intake can result in TRPV6 upregulation, while a decreased calcium intake can cause TRPV6 downregulation (Deng et al. 2021). Huber et al. (2015) reported that supplemental monocalcium phosphate (MCP) was found to enhance TRPV6 expression in the ileum of broiler chickens. The higher expression of TRPV6 was induced by a high dietary Ca2 + consumption (Hoenderop et al. 2005). In addition to that, phytase supplementation increased the duodenal expression of TRPV6 when compared to the control diet in the gilts and barrows (Julia et al. 2023). The results of Cui et al. (2012) support the idea that this rise in accessible calcium can activate TRPV6, increasing calcium absorption through this channel into the circulation and subsequent elevation in its plasma levels. In contrast, an experiment carried out by Woudenberg-Vrenken et al. (2012) discovered that the levels of TRPV6 expression in the intestine increased in response to a low-Ca_2^+ diet in both wild-type and TRPV6D541A/D541A mice. The apical inflow of the active Ca transport is mediated by the intestinal TRPV6 channels, whose regulation depends on the level of concentration of 1,25-$OH_2^-D_3$ in the serum (Van de Graaf et al. 2004). Yang et al. (2011) examined TRPV6 expression and distribution in the kidney and all sections of laying chickens' guts and

discovered that TRPV6 was restricted to the brush border of the ileum, caecum, jejunum, and rectum, with varying expression levels across different intestinal segments. However, there is limited evidence supporting the expression of TRPV6 in the blood plasma of poultry birds. The significant linear contrast at the finisher phase suggests that the addition of microbial phytase enhances calcium availability, leading to upregulation of TRPV6 calcium transport mechanisms in the intestines.

Solute carrier family 34, member 2 (SLC34A2)

The result of SLC34A2, which is a Sodium-Phosphate Cotransporter Type II, doesn't show any significant ($p > 0.05$) difference both at the starter (2 weeks) and finisher (5 weeks) stages. This may be due to the gene's low concentration in the plasma because SLC34A2 is primarily located in the intestinal epithelial cells' apical membrane, where it is essential for the absorption of phosphate from the intestinal mucosa into the bloodstream (Hilfiker et al. 1998; Giral et al. 2012). Also, a study conducted on laying hens discovered that the gene SLC34A2 is an important component of the gut's transcellular phosphate transport system and that hens consuming a low-phosphate diet generally had higher expression of this gene (Sommerfeld et al. 2019). Additionally, increased jejunal expression of SLC34A2 was observed in broiler birds in response to low-phosphorus diets (Adewunmi et al. 2023). These findings imply that the manifestation of SLC34A2 in the intestines of poultry birds is possible to a significant level, but a lower concentration was obtained in the blood plasma of this study. The linear decrease in SLC34A2 expression with increasing phytase levels suggests that enhanced phosphorus digestibility from microbial phytase reduces the need for active phosphate transport.

Vitamin D receptor genes (VDR)

The result of this investigation shows a significant ($p < 0.01$) decrease in the plasma values of VDR in birds fed microbial phytase diets relative to the phytase inclusion levels in their diets. This may be due to the greater bioavailability of the chelated calcium and phosphorus released by the phytase from phytate (Rodehutscord et al. 2022). The significant reduction in VDR expression at higher phytase levels may be a compensatory response to decrease calcium and phosphorus absorption, reducing the need for VDR-mediated calcium uptake. When there is sufficient or high calcium availability in the intestine, the body's requirement for the active form of vitamin D (calcitriol) to promote calcium absorption decreases. This can result in reduced VDR (vitamin D receptor) expression in various tissues, including the plasma. Research has indicated that a high intake of dietary calcium can lower VDR mRNA expression in tissues like the intestine and kidneys. For instance, Panda et al. (2006) found that elevated calcium levels diminished the need for calcitriol activation of VDR to absorb calcium in the intestine. In contrast, when intestinal calcium levels are low, the body compensates by increasing VDR expression, making tissues more sensitive to calcitriol. This boosts calcium absorption efficiency in the intestine. Russell et al. (1993) also observed that in birds, low calcium diets caused a rise in VDR mRNA expression in the parathyroid glands, suggesting a compensatory response to enhance calcium absorption during calcium deficiency. Additionally, Zanu et al. (2020) found that birds fed low calcium and low phytase diets showed increased VDR expression, likely due to the reduced calcium concentration in the intestine and blood. This suggests that the heightened VDR expression is a compensatory mechanism aimed at optimizing calcium absorption. The study implies that higher phytase levels releases more calcium in the intestine, resulting in lower VDR expression, as there was less need for enhanced calcium uptake.

Pearson correlation impacts

The strong Pearson correlation coefficients between nutrient digestibility and body weight indicate that as nutrient digestibility increases, body weight also increases. This is likely because the digestibility of zinc, calcium, phosphorus, and other minerals can be enhanced up to 50% by phytase, as well as other nutrients (González-Vega et al. 2021). The strong positive correlation between nutrient digestibility and geometric bone traits ($p < 0.01$) indicates that geometric bone traits also increase as nutrient digestibility increases. This is possible because the phytase improves the digestibility and absorption of phosphorus and calcium, which are vital for bone development (Hui et al. 2018). Furthermore, the strong positive correlation between body weight and geometric bone traits ($p < 0.01$), indicates that geometric bone traits also increase as body weight increases. This is likely because larger birds have a larger bone mass and therefore have more bone to support their body weight (Amin et al. 2020). Overall, the findings imply that broiler chickens' geometric bone characteristics, body weight, carcass weight, and nutrient digestibility are all improved by microbial phytase supplementation.

Conclusion

Incorporating the locally produced phytase into the broiler chicken diets provides significant benefits for growth and bone development. Phytase supplementation offers 'extra-phosphoric' advantages by breaking down phytate more effectively, reducing its antinutritive effects, and potentially producing more soluble myo-inositol and phytate esters. By mitigating phytate's antinutritional properties, phytase enhances nutrient digestibility, promotes bone mineralization, and improves feed efficiency and weight gain in broilers. Additionally, phytase influences the regulation of calcium and phosphate homeostasis genes, notably affecting FGF23 and VDR genes, while reducing phosphorus excretion, which contributes to environmental sustainability. Therefore, phytase supplementation is a promising strategy for improving the nutritional quality of broiler diets. Among the diets studied, T4 (400 FTU) showed optimal performance indices and cost-effectiveness. More investigation is recommended to explore the utilization of this microbial phytase in adjusting calcium and phosphorus levels to minimize phosphorus excretion without compromising performance.

Consent to publish

All authors have agreed to publish in this journal.

Ethical approval

Ethical approval from Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia, approval number UPM/IACUC/AUP-R027/2023 was obtained.

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Disclosure statement

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

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