Journal of Animal and Feed Sciences, 35, , 2026, e21, https://doi.org/ 10.22358/jafs/209876/2026
The Kielanowski Institute of Animal Physiology and Nutrition, Polish Academy of Sciences, Jabłonna

ARTICLE IN PRESS

ORIGINAL PAPER

Effects of phosphorus sources and high phytase doses on growth, nutrient digestibility, bone strength, carcass traits, and meat quality in broilers

D.X. Jin[1,2], E. Tugiyanti[1], E.A. Rimbawant[1], R. Rosidi[1], T.T. Widiyastuti[1], A. Susanto[1] and I. Ismoyowati[1,*]

[1] Jenderal Soedirman University, Faculty of Animal Science, Department of Animal Production, 53123 Purwokerto, Indonesia
[2] New Hope Liuhe Indonesia Co., Ltd, 15610 Jakarta, Indonesia

KEY WORDS: bone strength, broiler chickens, carcass traits, nutrient digestibility, phytase superdosing, phosphorus sources

Received: 30 June 2025
Revised: 18 August 2025
Accepted: 25 August 2025

* Corresponding author:
e-mail: 718992185@qq.com

ABSTRACT. This study evaluated the effects of phosphorus (P) sources (mineral, animal, or plant-based) and phytase supplementation levels (0, 500, or 2500 FTU/kg) on broiler growth performance, nutrient digestibility, bone development, carcass traits, and meat quality using a 3 × 3 factorial design. A total of 1800 male Cobb 500 broilers were reared for 35 days, fed identical basal diets varying only in P source and phytase dose. Significant interactions were observed between P source and phytase level for growth performance, tibia mineralisation, intestinal morphology, and meat quality. High phytase dose (2500 FTU/kg) in plant-based P diets fully restored body weight gain and feed efficiency to the level of mineral P diets, and markedly improved tibia strength, bone mineral content, and intestinal villus height. Carcass traits also responded to phytase supplementation, particularly leg and wing yields. In the absence of phytase, plant-based P diets resulted in the poorest outcomes for growth, nutrient utilisation, bone quality, and meat traits, while mineral and animal P sources produced comparable results. Phytase supplementation reduced drip and cooking losses, though the impact on other traits was less pronounced. For all P sources, phytase improved phosphorus and calcium digestibility, intestinal morphology, and protein status. These findings demonstrate that phytase superdosing effectively mitigate the nutritional limitations of high-phytate plant ingredients, allowing increased inclusion of alternative feedstuffs such as rice bran while reducing dependence on inorganic phosphate without affecting performance or product quality.

Introduction

In poultry, phosphorus (P) is a critical mineral involved in multiple biological processes, including energy metabolism, musculo-skeletal development, and egg production. P deficiency in poultry can impair growth and physiological function, cause skeletal disorders such as rickets, and reduce production performance (McDonald et al., 2002). It is also the third most expensive nutrient in poultry diets after energy and protein. Dietary P sources are generally classified as mineral inorganic, animal-derived inorganic, and plant-derived organic sources. Mineral inorganic P sources, mainly derived from rock phosphate in the form of calcium phosphates, are commonly add to poultry diets to compensate for the low P availability in plant ingredients. Common inorganic P supplements in poultry feeds

include calcium phosphate salts such as di-calcium phosphate (DCP, $CaHPO_4$), mono-calcium phosphate (MCP, $Ca(H_2PO_4)_2$) and tri-calcium phosphate (TCP, $Ca_3(PO_4)_2$). In broilers, the mean standardised ileal digestibility (SID) of P, estimated by regression and P-free diet methods, is approximately 0.82 for MCP, 0.77 for DCP (Woyengo et al., 2022), and 0.57 for TCP (An et al., 2020). These differences are attributed to variations in solubility and Ca:P ratios; Ca-bound phosphate is inherently less soluble than free phosphate, reducing P availability in the small intestine (Mekmene et al., 2009). Although mineral P sources provide high digestibility and consistent composition, they depend on non-renewable resources and increase feed cost. Animal-derived P sources are obtained from hydroxyapatite present in bones or eggshells that can be used directly as meat and bone meal (MBM), bone meal (BM), and fish meal (FM), or further processed into inorganic phosphate salts such as MCP, DCP, and TCP (Anon, 2003; Someus and Pugliese, 2018). These rendered products are globally utilised in poultry diets as sources of energy, amino acids, P and Ca. However, the true ileal P digestibility of MBM in poultry, averaging approximately 0.57 in both direct and regression methods (Mutucumarana et al., 2015; 2016), is substantially lower than rock-derived DCP (0.83) (van Harn et al., 2017), but similar to TCP (0.57) (An et al., 2020). The nutritional value of MBM and BM is limited by poorly digestible proteins such as collagen and keratin, heat-induced amino acid denaturation (lysine, cysteine, and threonine), and high calcium (Ca) content, which increases gut pH and promotes nutrient binding to phytic acid, reducing their bioavailability (Kim et al., 2012; Paiva et al., 2014). Additionally, high dietary inclusion of MBM may elevate the risk of necrotic enteritis due to anaerobic fermentation of aromatic amino acids (tyrosine and tryptophan) by *Clostridium perfringens* (Rinttilä and Apajalahti, 2013). Nevertheless, the use of animal by-products such as BMB/BM can be considered as nutrient cycling, and incorporation of animal-derived inorganic P in poultry diets can contribute to a more sustainable production of meat and eggs. Plant-derived P sources are primarily composed of phytate P, with 60–80% of total P bound to phytic acid (myo-inositol hexakisphosphate, IP_6) (Lei et al., 2013). This form is considered an anti-nutritional factor, occurring abundantly in cereal and legume seeds (maize, rice, soybean) and their by-products. Due to its strong chelating capacity, phytate rarely exists in a free form in nature. In addition to binding P, phytate chelates mineral ions such as Ca, Mg, Fe, K, Zn and Mn in the digestive tract, forming insoluble complexes that significantly reduce their bioavailability (Weaver and Kannan, 2002). Additionally, phytate interacts with proteins to form binary phytate-protein or ternary phytate-metal ion-protein complexes, altering protein conformation and decreasing protein digestibility (Cheryan and Rackis, 1980). Phytate can also form complexes directly with digestive enzymes, including amylase, trypsin, pepsin, and lipase, thereby inhibiting their activity and reducing the digestion and absorption efficiency of nutrients such as starch and fats (Kumar et al., 2010). Consequently, phytate is a significant anti-nutritional factor in plant-based poultry diets, and exogenous phytase is widely added to feed formulations to mitigate its effects.

Phytase, is an esterase enzyme that degrades phytic acid and its salts, catalysing the hydrolysis of phytate to release phosphoric acid, myo-inositol, and lower myo-inositol phosphate derivatives. Monogastric animals, including poultry have insufficient gastrointestinal phytase activity to fully degrade phytate into lower phosphoester forms. Although plant-based feed ingredients contain endogenous phytase that can improve P utilisation, much of this activity is lost during thermal processing (Jongbloed and Kemme, 1990). As a result, P bioavailability from plant sources is limited, typically 20–30% of total P. To address this, microbial-derived exogenous phytase is commonly incorporated into poultry diets to increase P and energy availability, overall nutrient utilisation, and ultimately production performance. Supplementation with conventional phytase doses (500 FTU/kg) can hydrolyse approximately 62% of dietary phytate, releasing at least 0.15% available P, increasing P digestibility by 6–10%, and reducing P excretion by 30–40% (Walk et al., 2013; Walters et al., 2019). Additionally, phytase supplementation improves growth performance by increasing body weight gain and feed conversion, and positively affecting bone quality parameters such as tibia strength and bone ash content (Powell et al., 2011; Leyva-Jimenez et al., 2019). The underlying mechanism involves phytase-catalysed hydrolysis (dephosphorylation) of phytate (IP_6) into intermediate inositol phosphates (IP_5, IP_4, IP_3, IP_2, IP_1), which reduces phytate's anti-nutritional effects, and enhances the digestibility of P and other bound nutrients (Selle et al., 2000; Walk et al., 2018). Conventional phytase doses (500 FTU/kg) are often insufficient to completely dephosphorylate dietary phytate. Rising feed costs, stricter P disposal regulations, and advances in enzyme production have increased interest in phytase superdosing (>500 FTU/kg). High phytase supply further

degrades intermediate inositol phosphates (IP_4 and IP_3), eliminating their anti-nutritive effects and improving nutrient availability, growth performance, and bone parameters beyond conventional levels (Zyla et al., 2013; Manobhavan et al., 2016; Beeson et al., 2017; Walters et al., 2019). Moreover, high phytase doses also improve intestinal morphology by increasing villus height and the villus height to crypt depth ratio (VH:CD) (Zanu et al., 2020; Moita et al., 2021); they also modulate gut microbiota by promoting beneficial bacteria (e.g., lactic acid species) while reducing harmful populations, thereby supporting intestinal health and nutrient absorption (Ptak et al., 2015; Mulvenna et al., 2022).

Agricultural by-products such as rice bran, which contains more than 2% total P (2.16%) (NRC, 2012), represent a valuable feed resource with P levels markedly exceeding those of conventional cereal grains like maize (0.26%) and wheat (0.35%) (NRC, 2012). Rice bran is frequently incorporated into poultry diets due to its high protein content, balanced essential amino acid profile, elevated levels of vitamins B and E, and rich mineral content (Warren and Farrell, 1990a). Recommended inclusion levels in broiler diets are approximately 10% for chicks aged 0–21 days and 20% for birds aged 22–40 days; however, exceeding 20% typically reduces growth performance and bone mineralisation due to high phytate content, enzyme inhibitors, and fibre (Warren and Farrell, 1990b; Gallinger et al., 2004). With commercial phytase supplementation, higher inclusion levels of rice bran could be feasible, serving as both a P source and a means of reducing reliance on inorganic P supplements. Phytase supplementation in poultry diets has shown consistent benefits at both conventional and super-dosing levels. Therefore, we hypothesised that P source and phytase inclusion level would interact, with diets based on plant P responding more strongly to phytase due to their high phytate content, whereas animal and mineral P sources still benefiting from phytase supplementation owing to the substantial plant-based component in typical poultry diets. The objective of the study was to comparatively analyse the effectiveness of three primary P sources (mineral inorganic, animal-derived inorganic, and plant-derived organic) in broiler diets and evaluate the effects of two phytase levels (500 and 2500 FTU/kg) in low-P diets on broiler growth performance, nutrient digestibility, bone strength, carcass traits, and meat quality. The aim was to establish a theoretical foundation for reducing exogenous P supplementation in broiler diets, decreasing dependence on non-renewable phosphate reserves, promoting the use of locally available feed resources, and ultimately lowering feed costs.

Material and methods

The experimental protocols were approved by the Animal Ethics Committee at the Jenderal Soedirman University (Approval No. 158/UN.23/14/PN.01.00/2019).

Dietary treatments and diets

Details of dietary ingredients and nutrient composition are presented in Table 1. The primary ingredients were analysed prior to formulation using near-infrared reflectance spectroscopy (NIRS; Evonik AminoProx, Frankfurt, Germany). Diets were formulated according to the recommended nutrient specifications for Cobb 500 broilers (Cobb, 2021).

P content (total and available P, AP) for the main ingredients were as follows: meat and bone meal (MBM), total P 6.70%, AP 3.85% (57.5% of total P) (Mutucumarana et al., 2015; Mutucumarana and Ravindran, 2016) dicalcium phosphate (DCP), total P 18%, AP 13.86% (77% of total P) (Woyengo et al., 2022); and rice bran, total P 2.01%, AP 0.76% (38% of total P) (Conte et al., 2002). The experimental treatments consisted of 9 diets based on three different P sources: mineral P, animal-derived P, and plant P. Each was tested without phytase supplementation (0 FTU/kg) and with two inclusion levels of phytase (500, and 2500 FTU/kg). To investigate the effect of supplementing phytase in low-P diets with different P sources, the feeds for the 500 and 2500 FTU/kg phytase groups were formulated with reductions in dietary energy (−56 kcal/kg), crude protein (−0.4%), AP (−0.15%), and Ca (−0.16%) compared to the phytase-free control diets. These low-P diets were then supplemented with exogenous phytase (commercial phytase, 5000 FTU/g; VTR Biotech Ltd, Zhuhai, GD, China) at 500 and 2500 FTU/kg diet, respectively. Nutrient levels in the low-P groups were reduced using the phytase matrix values for 500 FTU/kg, which were applied to both the 500 and 2500 FTU/kg phytase diets. Phytase-free diets had normal AP levels and no exogenous phytase, providing calculated AP levels of 0.48 and 0.4% for the starter and grower phases, respectively. Titanium dioxide (TiO_2) was added to all diets during both periods at 0.4% as an indigestible marker to determine nutrient digestibility.

Table 1. Ingredient and nutrient composition of basal diets (g/kg), as-fed basis

Ingredient composition, kg/t	Starter (days 0–14)								
	MP0	MP500	MP2500	AP0	AP500	AP2500	PP0	PP500	PP2500
Maize	414.8	445.2	444.8	491.6	493.6	493.2	325.7	353	352.6
Wheat	150	150	150	150	150	150	150	150	150
Soyabean meal, 48%	350	336	336	255	279	279	338	325	325
Di-calcium phosphate, 18%	26	15.5	15.5				20	9.8	9.8
MBM, 50%				75	44	44			
Rice bran							100	100	100
Limestone	7.5	9.5	9.5		5.3	5.3	11.2	13	13
Crude palm oil	35	27	27	10	10	10	38	32	32
Sodium bicarbonate	2	2	2	2	2	2	2	2	2
Salt	3	3	3	3	3	3	3	3	3
DL-methionine	3.6	3.7	3.7	3.9	3.7	3.7	3.7	3.7	3.7
L-lysine HCL	3.1	3.1	3.1	4.1	3.9	3.9	3.2	3.2	3.2
L-threonine	1.5	1.5	1.5	1.9	1.9	1.9	1.7	1.7	1.7
Choline chloride 60	1	1	1	1	1	1	1	1	1
Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
VTR-phytase (5000 FTU/g)		0.1	0.5		0.1	0.5		0.1	0.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated values, %									
dry matter, %	88.93	88.70	88.71	88.76	88.57	88.57	89.04	88.84	88.84
metabolizable energy, kcal/kg	2956	2898 (2955)	2898 (2955)	2958	2899 (2955)	2899 (2955)	2949	2893 (2949)	2888 (2944)
crude protein, %	22.08	21.65 (22.04)	21.65 (22.04)	22.05	21.63 (22.02)	21.62 (22.01)	22.06	21.66 (22.04)	21.66 (22.04)
calcium	0.97	0.82 (0.98)	0.82 (0.98)	0.98	0.82 (0.98)	0.82 (0.98)	0.98	0.83 (0.99)	0.83 (0.99)
total P	0.83	0.65	0.65	0.84	0.65	0.65	0.95	0.77	0.77
phytate-P	0.35	0.32	0.32	0.36	0.32	0.32	0.43	0.44	0.44
available P	0.48	0.33 (0.48)	0.33 (0.48)	0.48	0.33 (0.48)	0.33 (0.48)	0.48	0.33 (0.48)	0.33 (0.48)
SID amino acid									
lysine	1.28	1.25	1.25	1.28	1.27	1.27	1.28	1.25	1.25
methionine	0.65	0.65	0.65	0.68	0.66	0.66	0.66	0.66	0.66
met+cys	0.97	0.96	0.96	0.97	0.96	0.96	0.97	0.97	0.97
tryptophan	0.21	0.21	0.21	0.18	0.19	0.19	0.21	0.21	0.21
threonine	0.87	0.86	0.86	0.88	0.87	0.87	0.88	0.87	0.87
CF	2.73	2.78	2.78	2.77	2.81	2.81	3.17	2.21	3.21
Grower (day 15–35)									
maize	434.3	466.1	465.7	500	498	497.6	259.2	290.8	290.4
wheat	150	150	150	150	150	150	150	150	150
soyabean meal, 48%	328	314	314	252	276	276	304	290	290
Di-calcium phosphate, 18%	21	10.1	10.1				9		
MBM, 50%				60	29	29			
rice bran							200	200	200
limestone	6	8	8		5.2	5.2	13	13.8	13.8
crude palm oil	46	37	37	22	26	26	50	40	40
sodium bicarbonate	2	2	2	2	2	2	2	2	2
salt	3	3	3	3	3	3	3	3	3
DL-methionine	3	3	3	3.2	3.2	3.2	3.1	3.1	3.1
L-lysine HCL	2.2	2.2	2.2	3	2.7	2.7	2.5	2.5	2.5
L-threonine	1	1	1	1.3	1.3	1.3	1.2	1.2	1.2
choline chloride 60	1	1	1	1	1	1	1	1	1

continued on the next page

Table 1. continued

Ingredient composition, kg/t	Grower (days 15–35)								
	MP0	MP500	MP2500	AP0	AP500	AP2500	PP0	PP500	PP2500
Premix	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
VTR-phytase (5000 FTU/g)		0.1	0.5		0.1	0.5		0.1	0.5
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000
Calculated values, %									
dry matter, %	88.93	88.68	88.68	88.73	88.59	88.60	89.10	88.85	88.85
metabolizable energy, kcal/kg	3006	2950 (3006)	2950 (3006)	3009	2942 (2998)	2942 (2998)	3000	2947 (3003)	2947 (3003)
crude protein, %	21.04	20.63 (21.03)	20.63 (21.03)	21.05	20.60 (21.01)	20.60 (21.01)	21.03	20.62 (21.02)	20.61 (21.01)
calcium	0.80	0.64 (0.80)	0.64 (0.80)	0.8	0.65 (0.81)	0.65 (0.81)	0.80	0.64 (0.80)	0.64 (0.80)
total P	0.72	0.54	0.54	0.74	0.55	0.55	0.85	0.70	0.70
phytate-P	0.32	0.28	0.28	0.34	0.30	0.30	0.45	0.45	0.45
available P	0.40	0.25 (0.40)	0.25 (0.40)	0.40	0.25 (0.40)	0.25 (0.40)	0.40	0.25 (0.40)	0.25 (0.40)
SID amino acid									
lysine	1.16	1.13	1.13	1.16	1.13	1.13	1.16	1.13	1.13
methionine	0.58	0.58	0.58	0.60	0.60	0.60	0.59	0.59	0.59
met+cys	0.89	0.89	0.89	0.89	0.89	0.89	0.89	0.88	0.88
tryptophan	0.20	0.20	0.20	0.18	0.18	0.18	0.20	0.20	0.20
threonine	0.79	0.78	0.78	0.79	0.79	0.79	0.79	0.77	0.77
CF	2.72	2.77	2.77	2.77	2.79	2.79	3.61	3.66	3.66

MBM – meat and bone meal, SID – standardised ileal digestible, HCL – hydrochloric acid, FTU – phytase unit, MP0/500/2500 – mineral P source with 0/500/2500 FTU/kg phytase, AP0/500/2500 – animal-derived P source with 0/500/2500 FTU/kg phytase, PP0/500/2500 – plant P sources with 0/500/2500 FTU/kg phytase; vitamin/mineral premix supplied per kg of diet: IU: vit. A 12 000; vit. D 5 000; mg: vit. E 80, vit. K 3, riboflavin 10, nicotinic acid 55, calcium pantothenic acid 15, folic acid 2, thiamine 3, riboflavin 10, biotin 0.3, pyridoxine 5, cobalamin 20, cobalamin 25, manganese 100, iron 40, zinc 100, copper 15, iodine 1, selenium 0.35; means presented in brackets are calculated according to Guangdong VTR Bio-tech Co., Ltd

Birds and management

A total of 1800 one-day-old Cobb 500 male broiler chicks were obtained from the Newhope poultry hatchery (Cirebon, Indonesia). Upon arrival, chicks of uniform body weight (average 42 g ± 0.22) were randomly allocated to 9 dietary treatments, each consisting of 8 replicates with 25 birds per pen (2.5 m × 1.2 m, 3 m^2). Birds were reared and housed in an environmentally controlled room bedded with fresh rice hull litter (10 cm deep) and provided *ad libitum* access to feed and water throughout the trial. Starter diets were offered from day 0 to 14, and grower diets from day 15 to 35. Each pen was equipped with two tube feeders (32 cm diameter) and six nipple drinkers. Lighting and temperature schedules followed breeder guidelines (Cobb, 2021), with an initial room temperature of 33 °C on day 1 and gradually reduced to 24–26 °C by the third week, and maintained thereafter. On day 7, five birds per replicate pen (total n = 360) were randomly selected and transferred to metabolism cages (77 cm × 55 cm × 38 cm) for the digestibility trial. The remaining birds continued in the performance trial. Body weight and feed intake were measured weekly on a pen basis weekly, allowing for determination of weekly feed intake (FI), weight gain (WG), and feed conversion ratio (FCR). The latter was calculated as the ratio of feed intake per pen to average weekly weight gain per pen for the same phases.

On day 35, blood samples (0.5 ml) were collected into Falcon tubes form two randomly selected birds per replicate (n = 144) in the performance trial. Samples were centrifuged at 5000 rpm, and the serum was transferred to the 2-ml Eppendorf tube and stored at −20 °C until further analysis. Following blood collection, the same broilers were weighed and euthanised by carbon dioxide asphyxiation for carcass yield evaluation and sample collection. Carcass yield was determined for the whole carcass after removal of feathers, viscera, head, and feet. Individual cut yields (breast, leg, thigh, back and wing) were also recorded. Yields were calculated using the following formulas:

$$\text{Carcass yield, \%} = (\text{carcass weight} / \text{live weight}) \times \times 100;$$

Cut yield, % = (individual cut weight / carcass weight) × 100.

The liver, spleen, abdominal fat, kidney, bursa of Fabricius, and gizzard were carefully excised and weighed to calculate relative organ weight (g/kg) = organ weight (g) / live BW (kg). Within 20 min post-mortem, breast muscle samples were collected and transferred to the laboratory for subsequent analyses. Right tibia bones were also collected, cleaned of muscle and adhering tissues, weighed, and stored at −20 °C until analyses. Segments of the jejunum (midpoint between the bile duct entry and Meckel's diverticulum) and ileum (from Meckel's diverticulum to 40 mm proximal to the ileo-caeco-colic junction) were collected directly after euthanasia, rinsed with ice-cold saline, and cleared of luminal contents. Two-centimetre sections were then excised and fixed in 10% buffered formalin for histological examination. Intestinal histology preparation and measurements were performed following the methods described by Jin et al. (2024).

Digestibility trial

The digestibility trial began on day 7 with birds 360 housed individually in metabolism cages. Ambient temperature was initially set at 33 °C and was gradually reduced by 1 °C every two days until reaching 26 °C, while lighting and other management protocols were maintained as described for the performance trial. Birds remained on their assigned starter and grower diets with *ad libitum* access to feed and water from day 7 to 35. On days 14 and 35, two birds per replicate from the starter and grower phases were euthanised humanely by isoflurane overdose followed by cervical dislocation. Ileal digesta were collected from the terminal ileum (midpoint between Meckel's diverticulum and 2 cm proximal to the ileocecal junction), pooled by replicate, immediately frozen, and stored at −80 °C for subsequent analysis of nutrient content and TiO_2 concentration. Feed samples (approximately 500 g per diet) were collected prior to feeding, ground using a mortar and pestle, and thoroughly mixed for laboratory analysis.

Chemical analysis

Tibia length and width were measured twice using a digital calliper (to two decimal places, mm). Tibia breaking strength was assessed by a three-point bending test using a WDS-1 microcomputer-controlled universal testing machine (Shanghai Yanrun Optical Machinery Technology Co., Ltd, Shanghai, China), with a 30 mm span, a 50 kg load cell, and a crosshead speed of 10 mm/min. Each bone was loaded until fracture, and breaking strength was recorded in Newtons (N). Subsequently, tibias were defatted in petroleum ether for 8 h, oven-dried at 100 °C for 24 h, weighed, and then ashed at 600 °C overnight to determine ash content. Ash weight, Ca, and P content were analysed according to AOAC procedures (AOAC, 2005). Serum levels of total protein (TP), Ca, P, alkaline phosphatase (ALP), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) were determined using commercial assay kits (Randox Laboratories Ltd, Crumlin, UK) and a semi-automated biochemical analyser (Humalyzer 4000, Merck®, Wiesbaden, Germany).

Dry diet and ileal digesta samples were analysed to determine the apparent digestibility of dry matter (DM), crude protein (CP), gross energy (GE), ash, crude fibre (CF), fat, Ca, and P. DM was measured by oven-drying at 100 °C for 24 h, and ash content was determined by combustion in a muffle furnace (Vecstar, Chesterfield, UK) at 600 °C for 4–6 h. GE was determined by adiabatic bomb calorimetry (IKA Werke C7000, GMBH & Co., Staufen, Germany) using benzoic acid as the standard. Nitrogen (N) content was determined by combustion using a CNS-2000 auto analyser (LECO Corporation, St. Joseph, MI, USA), and CP was calculated by multiplying total N by 6.25. Crude fibre was analysed using the Ankom filter bag technique (Ankom Technology, Macedon, NY, USA), while fat by Soxhlet extraction (method 991.36). Ca was measured colorimetrically (Flexor E, Vital Scientific NV, Dieren, The Netherlands) after digestion with 6 M HCl (AOAC 968.08D), while P content was determined spectrophotometrically at 680 nm (UV mini 1240, Shimadzu Corp., Kyoto, Japan). TiO_2 concentration was determined as an indigestible marker following the method described by Short et al. (1996). Apparent ileal digestibility (AID, %) of nutrients was calculated using the following formula:

AID, % = 1 − [(ileal nutrient / ileal TiO_2) / (dietary nutrient / dietary TiO_2)].

For meat quality evaluation, breast muscle colour (lightness, redness, yellowness) was measured at three locations per sample using a portable colorimeter (Model CR-410, Konica Minolta, Osaka, Japan). Muscle pH was measured with a calibrated pH meter (using pH 4.0 and 7.0 buffers), with three repeated readings per sample. Water-holding capacity (WHC) was determined following the method of Kauffman et al. (1986). Drip loss was determined using the plastic bag method, and cooking loss was calculated following the procedure of Honikel (1998).

Statistical analysis

The experiment was conducted as a completely randomised design in 3 × 3 factorial arrangements with three P sources (mineral, animal-derived, and plant-based) without phytase or two phytase doses (500, and 2500 FTU/kg). Phytase-free groups served as the reference controls. All data were analysed using two-way ANOVA under the general linear model (GLM) procedure implemented in SPSS (v25.0, IBM Corp., Chicago, IL, USA), with interaction effects tested. When significant differences were detected ($P < 0.05$, $0.05 < P < 0.10$ was considered a trend), group means were compared using Tukey's honest significant difference (HSD) post-hoc test.

Results

Growth performance

The source of P significantly affected broiler growth performance (Table 2). During days 1–14, birds fed the plant P diet had lower body weight gain (BWG) than those on mineral or animal P diets ($P < 0.05$), with a tendency for poorer feed conversion ratio (FCR) in the plant P group ($P = 0.09$). From days 15–35 and overall (days 1–35), plant P diets resulted in significantly lower feed intake (FI) and BWG compared to mineral and animal P sources ($P < 0.05$). No significant differences in growth performance were observed in broilers fed mineral P or animal P diets. Phytase supplementation improved growth performance during the grower phase (days 15–35) and over the entire trial period. Specifically, 2500 FTU/kg phytase significantly increased BWG during days 15–35 and overall FI (days 1–35) compared to the 0 FTU/kg group ($P < 0.05$). A significant interaction ($P < 0.05$) was observed between P source and phytase supplementation for BWG (days 1–35) and FCR (days 15–35). In birds fed mineral P diets, 500 FTU/kg phytase resulted in similar BWG to the 0 FTU/kg group, with no additional benefit from 2500 FTU/kg; phytase supplementation had no effect on FCR. For animal P diets, BWG in the 2500 FTU/kg group was comparable to the 0 FTU/kg group, whereas the 500 FTU/kg group showed a significant reduction in BWG; FCR was significantly improved with phytase supplementation. In plant P diets, BWG in the 500 FTU/kg group was comparable to 0 FTU/kg, while 2500 FTU/kg significantly increased BWG relative to 0 FTU/kg and was similar to mineral and animal P diets without phytase; FCR was also significantly decreased by phytase.

Table 2. Effects of different phosphorus sources supplemented with high phytase doses on the growth performance of broiler at 35 day of age

Treatment		Days 1–14			Days 15–35			Days 1–35		
Phosphorus source	Phytase level, FTU/kg	FI, g/bird	BWG, g/bird	FCR	FI, g/bird	BWG, g/bird	FCR	FI, g/bird	BWG, g/bird	FCR
	0	571.2	478.0	1.19	2663.1	1633.6	1.62^{ab}	3234.3	2128.8^{a}	1.52
Mineral	500	551.9	482.0	1.15	2660.6	1655.5	1.61^{b}	3212.5	2137.0^{a}	1.50
	2500	580.2	486.5	1.20	2684.8	1699.3	1.58^{bc}	3265.0	2179.3^{a}	1.49
	0	538.3	470.8	1.14	2635.8	1680.5	1.57^{c}	3174.0	2151.3^{a}	1.48
Animal	500	541.8	472.3	1.15	2678.8	1600.8	1.67^{a}	3220.5	2073.0^{bc}	1.55
	2500	584.4	491.0	1.19	2779.8	1687.5	1.64^{a}	3364.3	2178.5^{a}	1.54
	0	547.6	455.8	1.21	2542.9	1574.5	1.62^{a}	3064.5	2030.2^{c}	1.52
Plant	500	544.5	444.3	1.23	2539.3	1592.8	1.59^{bc}	3045.3	2037.0^{c}	1.51
	2500	571.8	461.5	1.24	2617.4	1665.8	1.57^{c}	3175.8	2127.3^{a}	1.50
SEM		18.95	14.30	0.051	72.01	32.54	0.038	76.15	31.81	0.026
Phosphorus source										
mineral		567.8	482.2^{a}	1.18	2669.5^{a}	1662.8^{a}	1.60	3237.3^{a}	2148.5^{a}	1.51
animal		554.8	478.0^{a}	1.16	2698.1^{a}	1656.3^{a}	1.63	3252.9^{a}	2134.3^{a}	1.52
plant		554.6	453.8^{b}	1.23	2566.6^{b}	1611.0^{b}	1.59	3095.2^{b}	2064.8^{b}	1.51
Phytase level										
0		552.3^{b}	468.2	1.18	2613.9	1629.5^{b}	1.60	3157.6^{b}	2103.4^{b}	1.51
500		546.1^{b}	466.2	1.18	2626.2	1616.3^{b}	1.62	3159.4^{b}	2082.5^{b}	1.52
2500		578.8^{a}	479.7	1.21	2694.0	1684.2^{a}	1.60	3268.3^{a}	2161.7^{a}	1.51
Main effects and interactions (*P*-values)										
phosphorus source		0.398	0.003	0.094	0.008	0.023	0.256	0.002	0.002	0.563
phytase level		0.012	0.224	0.451	0.131	0.003	0.493	0.024	<0.001	0.672
phosphorus × phytase		0.721	0.870	0.886	0.816	0.081	0.011	0.671	0.019	0.147

FI – feed intake, BWG – body weight gain, FCR – feed conversion ratio, FTU – phytase unit, SEM –standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Nutrient digestibility

Diets with different P sources generated significant differences in GE, DM, EE, and Ca digestibility (Table 3). Plant P diets had lower GE and DM digestibility (mean 68–69%) compared to mineral or animal P diets (71–73%; $P < 0.05$). Ileal Ca digestibility was also significantly reduced in plant P diets ($P < 0.01$), with a tendency towards higher CF digestibility ($P = 0.087$) relative to other sources. In contrast, EE digestibility was highest in the plant P group ($P < 0.05$), while CP and ash digestibility did not differ significantly between P sources ($P > 0.05$). P utilisation was not significantly affected by P source ($P > 0.05$), although values tended to be higher in the plant P diet. Phytase supplementation markedly improved mineral digestibility (Table 3). GE, EE, and CF digestibility were not significantly affected ($P > 0.05$), whereas ileal digestibility of Ca and P increased significantly. Phytase superdosing at 2500 FTU/kg significantly elevated Ca and P digestibility compared to the 0 FTU/kg group ($P < 0.05$), whereas 500 FTU/kg did not produce a statistically significant improvement. Phytase also tended to improve DM, CP and ash digestibility ($0.05 < P < 0.1$). No significant interactions between P sources and phytase levels were observed for any nutrient digestibility parameters ($P > 0.05$).

Intestinal morphology

Significant interactions between P source and phytase supplementation were observed for jejunal VH, CD, and VH:CD, as well as ileal CD and VH:CD ($P < 0.05$; Table 4). In contrast, ileal VH was affected only by main effects, not their interactions. In birds fed mineral P diets, 500 FTU/kg significantly increased CD and reduced the VH:CD ratio compared to lack of supplementation, whereas 2500 FTU/kg increased VH and restored the VH:CD ratio to levels similar to the 0 FTU/kg group. In animal P diets, 500 FTU/kg significantly reduced VH and increased CD, while 2500 FTU/kg significantly increased VH and the VH:CD ratio relative to the control group. In plant P diets, 500 FTU/kg phytase significantly reduced CD and increased the VH:CD ratio compared to the 0 FTU/kg group, with no further improvement at the 2500 FTU/kg dose. For ileal VH, birds fed the mineral P diet had the highest average values of this parameter, and the 0 FTU/kg and 2500 FTU/kg phytase groups showed higher ileal VH than the 500 FTU/kg group, which was the lowest, as shown by significant main effects. Ileal CD and the VH:CD ratio displayed significant interactions ($P < 0.05$). In mineral P diets, the 2500 FTU/kg group had the lowest CD and highest VH:CD ratio, while in animal P diets,

Table 3. Effects of different phosphorus sources supplemented with high phytase doses on apparent ileal nutrient digestibility of broiler at 35 days of age

Treatment		Apparent ileal nutrient digestibility, %							
Phosphorus source	Phytase level, FTU/kg	GE	DM	CP	EE	CF	Ash	Ca	P
	0	73.76	68.61	75.98	82.63	14.56	48.31	49.45	53.07
Mineral	500	72.04	70.82	76.26	80.91	14.38	48.03	49.80	51.33
	2500	72.47	70.85	75.23	78.84	14.15	48.22	52.08	53.80
	0	72.26	71.58	76.12	79.87	14.40	46.71	48.94	49.29
Animal	500	68.75	70.39	74.01	78.87	14.55	48.35	50.23	51.34
	2500	72.00	72.30	80.03	80.86	15.17	51.43	52.00	54.06
	0	66.01	66.72	72.55	81.72	15.63	44.77	44.27	48.46
Plant	500	66.00	70.00	74.47	82.25	16.17	46.68	47.68	57.07
	2500	70.59	69.23	75.37	88.52	16.10	48.09	49.18	59.56
SEM		2.905	1.579	2.598	2.808	1.293	2.095	1.975	3.478
Phosphorus source									
mineral		72.76^{a}	70.10^{ab}	75.82	80.79^{b}	14.37	48.19	50.44^{a}	52.73
animal		71.00^{a}	71.42^{a}	76.72	79.87^{b}	14.71	48.83	50.39^{a}	51.56
plant		67.59^{b}	68.65^{b}	74.13	84.16^{a}	15.97	46.52	47.05^{b}	55.03
Phytase level, FTU/kg									
0		70.71	68.97	74.88	81.41	14.87	46.60	47.55^{b}	50.27^{b}
500		68.93	70.41	74.91	80.68	15.03	47.69	49.24^{ab}	53.5^{ab}
2500		71.71	70.79	76.88	82.74	15.14	49.25	51.09^{a}	55.81^{a}
Main effects and interactions (*P*-values)									
phosphorus source		0.010	0.013	0.222	0.026	0.087	0.150	0.005	0.221
phytase level		0.253	0.081	0.093	0.439	0.933	0.096	0.011	0.028
phosphorus × phytase		0.608	0.365	0.341	0.108	0.966	0.572	0.843	0.210

GE – gross energy, DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fibre, FTU – phytase unit, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 4. Effects of different phosphorus sources supplemented with high phytase doses on intestinal morphology of broilers at 35 day of age

Treatment		Jejunum			Ileum		
Phosphorus source	Phytase level, FTU/kg	VH, µm	CD, µm	VH/CD	VH, µm	CD, µm	VH/CD
	0	1014.30^{ab}	233.42^{c}	4.50^{a}	807.42	246.36^{b}	3.47^{b}
Mineral	500	907.87^{b}	293.35^{b}	2.80^{b}	676.51	217.89^{b}	3.15^{b}
	2500	1171.06^{a}	296.12^{b}	4.22^{a}	753.88	154.03^{c}	5.12^{a}
	0	931.72^{b}	229.70^{c}	2.63^{bc}	669.57	143.82^{c}	4.91^{a}
Animal	500	747.17^{c}	300.48^{b}	2.47^{b}	514.69	199.42^{bc}	2.56^{b}
	2500	1260.20^{a}	366.91^{b}	6.06^{a}	659.35	210.57^{b}	3.28^{b}
	0	687.26^{c}	367.86^{a}	1.76^{c}	797.75	296.88^{a}	2.76^{b}
Plant	500	829.57^{bc}	271.22^{bc}	4.42^{a}	570.30	171.03^{c}	3.43^{ab}
	2500	942.93^{b}	371.76^{a}	2.10^{bc}	644.25	237.19^{b}	2.78^{b}
SEM		91.411	29.013	0.980	65.206	22.364	0.453
Phosphorus source							
mineral		1031.08^{a}	273.96^{b}	3.84	745.94^{a}	206.09^{b}	3.91^{a}
animal		979.69^{a}	299.03^{b}	3.72	614.54^{b}	184.60^{b}	3.58^{a}
plant		819.92^{b}	336.95^{a}	2.76	670.77^{b}	235.04^{a}	2.99^{b}
Phytase level, FTU/kg							
0		804.08^{c}	277.00^{b}	2.96	758.25^{a}	229.02^{a}	3.71^{a}
500		828.20^{b}	288.02^{b}	3.23	587.16^{b}	196.11^{b}	3.05^{b}
2500		1124.73^{a}	344.94^{a}	4.13	685.83^{a}	200.60^{b}	3.73^{a}
Main effects and interactions (*P*-values)							
phosphorus source		<0.001	0.001	0.117	0.003	<0.001	0.002
phytase level		<0.001	<0.001	0.102	<0.001	0.024	0.013
phosphorus × phytase		0.013	<0.001	<0.001	0.540	<0.001	<0.001

VH – villus height, CD – crypt depth, FTU – phytase unit, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

phytase supplementation significantly increased CD and decreased the VH:CD ratio. Lastly, in birds fed plant P diets, phytase dosing at 500 FTU/kg resulted in the lowest CD and the highest VH:CD ratio.

Bone development

Birds fed the plant P diet had the poorest bone mineralisation and strength (Table 5). Tibia breaking strength was lower in the plant P group than in the mineral and animal P groups ($P = 0.001$). Feeding mineral and animal P diets resulted in similar tibia morphology and bone mineralisation ($P > 0.05$). Phytase supplementation improved bone development metrics (Table 5). Broilers receiving 2500 FTU/kg phytase had the highest tibia bone strength among all groups ($P < 0.05$). Significant interactions ($P < 0.05$) were observed between P source and phytase level for tibia proximal and distal epiphysis widths, with the mineral P diet + 500 FTU/kg phytase producing the lowest values for both measurements. For tibia mineralisation, superdosing phytase increased tibia P content ($P < 0.05$) relative to the non-supplemented phytase group. A significant interaction was also observed for tibia ash percentage and Ca content ($P < 0.05$). In mineral P diets, 500 FTU/kg phytase significantly reduced tibia ash and Ca compared to the non-supplemented group, whereas 2500 FTU/kg maintained control values. In animals fed P diets, tibia ash and Ca were unaffected by phytase supplementation. In birds receiving plant P diets, phytase supplementation at both 500 and 2500 FTU/kg significantly increased tibia Ca content compared to the control group, although tibia ash was unchanged. Across phytase levels, mineral and animal P diet administration generally resulted in higher tibia ash and Ca than in plant P diets.

Organ weight and meat quality

Dietary P source did not significantly affect the relative weights of the liver, spleen, kidney, bursa, or gizzard (Supplementary Table 1; $P > 0.05$). Similarly, phytase supplementation had minimal impact, with the exception of kidney, as 500 FTU/kg phytase significantly reduced ($P < 0.05$) relative kidney weight compared to 0 FTU/kg. No significant interactions between P source and phytase level were observed for organ weights ($P > 0.05$). In contrast, meat quality parameters differed significantly between dietary P sources (Table 6). Broilers fed plant P diets had significantly lower breast muscle lightness (L*) compared to those fed mineral or animal P sources ($P < 0.05$). Additionally, cooking loss and drip loss were higher in broilers fed the plant-based

Table 5. Effects of different phosphorus sources supplemented with high phytase doses on bone development of broiler at 35 day of age

Treatment		Bone morphometry						Tibia bone mineralisation, %		
Phosphorus source	Phytase level, FTU/kg	bone weight, g	bone length, cm	bone strength, n	bone width, cm proximal epiphysis	bone width, cm diaphysis	bone width, cm distal epiphysis	bone ash	Ca	P
	0	2.78	6.81	356.9	1.83^{ab}	0.923	1.70^{ab}	45.88^{a}	16.54^{a}	3.61
Mineral	500	2.52	6.58	306.1	1.77^{c}	0.889	1.63^{c}	38.99^{c}	13.72^{c}	3.70
	2500	2.84	6.79	384.4	1.85^{ab}	0.936	1.70^{ab}	42.15^{bc}	15.21^{ab}	3.79
	0	2.71	6.66	358.0	1.78^{c}	0.950	1.67^{ab}	40.50^{bc}	14.54^{b}	3.73
Animal	500	2.73	6.75	337.2	1.81^{bc}	0.947	1.73^{a}	42.52^{b}	15.29^{ab}	3.48
	2500	2.80	6.85	371.7	1.91^{a}	0.943	1.72^{a}	43.58^{b}	15.35^{ab}	3.93
	0	2.79	6.67	301.1	1.82^{bc}	0.953	1.65^{bc}	37.31^{c}	13.10^{c}	3.61
Plant	500	2.68	6.71	310.4	1.90^{a}	0.939	1.72^{a}	40.99^{bc}	14.91^{ab}	3.82
	2500	2.75	6.79	316.0	1.88^{ab}	0.947	1.63^{b}	40.86^{bc}	14.50^{ab}	4.02
SEM		0.142	0.080	18.529	0.038	0.030	0.035	2.007	0.666	0.183
Phosphorus source										
mineral		2.71	6.72	349.2^{a}	1.82	0.916	1.68	42.34^{a}	15.16^{a}	3.70
animal		2.75	6.75	355.6^{a}	1.83	0.947	1.71	42.20^{a}	15.05^{a}	3.66
plant		2.74	6.73	309.2^{b}	1.86	0.946	1.67	39.72^{b}	14.17^{b}	3.82
Phytase level, FTU/kg										
0		2.76	6.71	338.7^{b}	1.81^{b}	0.942	1.67	41.23	14.73	3.59^{b}
500		2.64	6.67	317.9^{b}	1.83^{b}	0.925	1.69	40.38	14.63	3.67^{b}
2500		2.80	6.81	357.4^{a}	1.88^{a}	0.942	1.67	42.20	15.02	3.91^{a}
Main effects and interactions (*P*-values)										
phosphorus source		0.901	0.933	0.001	0.093	0.126	0.157	0.047	0.025	0.303
phytase level		0.153	0.239	0.002	0.005	0.516	0.709	0.485	0.573	0.010
phosphorus × phytase		0.561	0.543	0.062	0.047	0.796	0.011	0.004	<0.001	0.588

FTU – phytase unit, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

P diet than those fed mineral or animal P ($P < 0.05$). Cooking loss and drip loss were higher in the plant P group ($P < 0.05$), resulting in the lowest water-holding capacity (WHC) ($P < 0.001$) and a lower ultimate pH at 24 h post-mortem ($P < 0.05$). Mineral and animal P diets produced similar organ weights and meat quality ($P > 0.05$). Phytase supplementation improved several meat quality characteristics (Table 6). Birds supplemented with phytase (particularly at 2500 FTU/kg) demonstrated significantly reduced cooking loss ($P < 0.05$) and drip loss ($P < 0.05$) compared to birds without phytase addition. Phytase exerted no significant effects on breast muscle ultimate pH or colour parameters (L*, a*, b*) ($P > 0.05$). No significant interactions between P sources and phytase were detected for any measured meat quality traits ($P > 0.05$).

Carcass characteristics

Birds fed the mineral P diet showed the highest dressing percentage, significantly greater than in the plant P group (Table 7; $P < 0.05$), with the animal P group reaching intermediate values ($P > 0.05$). The mineral P diet also produced the largest relative breast yield compared to the animal and plant P groups ($P < 0.05$). Thigh yield was lower in the plant P group than in the mineral and animal P groups ($P < 0.001$). Birds on the animal P diet accumulated more abdominal fat than those on mineral or plant P diets ($P < 0.05$). Phytase supplementation significantly improved certain carcass traits (Table 7). Birds receiving 2500 FTU/kg phytase had higher carcass yield than those without phytase or with 500 FTU/kg ($P < 0.05$). Superdosing phytase (2500 FTU/kg) also increased thigh proportion ($P < 0.05$), while phytase did not affect relative breast yield and abdominal fat ($P > 0.05$). For relative leg yield, a significant ($P < 0.05$) P sources × phytase interaction was observed, with mineral P diets containing 500 FTU/kg phytase producing the highest values, whereas animal P diets with 500 FTU/kg phytase the lowest. For relative wing yield, the interaction was also significant ($P < 0.05$), with 2500 FTU/kg phytase supplementation generally producing the highest values among most P sources. No significant differences were detected in other cut yields between treatments.

Blood biochemistry

Birds fed the plant P diet had significantly lower serum Ca than those on mineral or animal P diets (Table 8, $P < 0.001$). Serum P tended to be slightly higher in the plant P group, but differences were not

Table 6. Effects of different phosphorus sources supplemented with high phytase doses on meat quality of broilers of broiler at 35 day of age

Treatment		Breast muscle colour			pH value	cooking loss, %	WHC, %	drip loss day 7, %
Phosphorus source	Phytase level, FTU/kg	L*	a*	b*				
	0	56.94	9.62	13.97	5.76	17.70	53.59	8.09
Mineral	500	56.80	10.94	14.03	5.74	19.64	53.69	8.14
	2500	60.13	10.88	14.39	6.20	18.01	58.03	7.35
	0	56.34	11.33	14.78	6.05	19.79	57.77	8.42
Animal	500	55.66	11.19	13.99	6.07	19.90	56.89	8.83
	2500	58.64	11.18	15.60	6.07	18.49	58.29	6.79
	0	53.58	11.05	13.49	5.52	23.40	51.36	11.75
Plant	500	53.30	10.96	13.63	5.64	21.96	50.69	12.37
	2500	51.88	11.11	14.02	5.75	19.05	52.98	10.68
SEM		2.949	0.675	0.908	0.280	1.568	2.435	1.100
Phosphorus source								
mineral		57.95^{a}	10.48	14.13	5.90^{a}	19.12^{b}	55.10^{a}	7.86^{b}
animal		56.88^{a}	11.23	14.79	6.07^{a}	19.39^{b}	57.65^{a}	8.01^{b}
plant		52.92^{b}	11.04	13.72	5.64^{b}	21.47^{a}	51.68^{b}	11.60^{a}
Phytase level, FTU/kg								
0		55.62	10.67	14.08	5.78	20.97^{a}	54.24	9.42^{a}
500		55.25	11.03	13.88	5.82	20.50^{a}	53.76	9.78^{a}
2500		56.88	11.06	14.67	6.01	18.51^{b}	56.44	8.27^{b}
Main effects and interactions (*P*-values)								
phosphorus source		0.011	0.151	0.125	0.035	0.022	<0.001	<0.001
phytase level		0.607	0.588	0.300	0.313	0.021	0.136	0.030
phosphorus × phytase		0.715	0.456	0.860	0.762	0.693	0.827	0.919

WHC – water-holding capacity, L* – lightness, a* – redness, b* – yellowness, FTU – phytase unit, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 7. Effects of different phosphorus sources supplemented with high phytase doses on carcass characteristics of broilers of broiler at 35 day of age

Treatment		Carcass weight/ live body weight, %	Relative carcass weight, %				
Phosphorus source	Phytase level, FTU/kg		breast	leg	thigh	wing	abdominal fat
	0	66.64	32.26	9.32^{b}	13.01	8.86^{b}	1.22
Mineral	500	64.82	30.65	11.26^{a}	12.57	9.65^{b}	1.64
	2500	67.43	31.41	10.32^{a}	15.37	11.60^{a}	1.61
	0	63.26	27.22	9.98^{ab}	12.84	9.94^{ab}	1.51
Animal	500	65.29	27.36	8.61^{c}	11.79	7.53^{c}	2.07
	2500	68.08	29.76	9.25^{bc}	15.44	10.03^{a}	1.91
	0	64.06	29.60	9.25^{bc}	11.51	11.01^{a}	1.51
Plant	500	59.46	30.51	10.06^{b}	11.21	8.89^{bc}	1.42
	2500	65.68	28.17	9.41^{bc}	11.40	9.60^{ab}	1.29
SEM		2.239	1.594	0.580	1.087	0.988	0.250
Phosphorus source							
mineral		66.30^{a}	31.44^{a}	10.29^{a}	13.65^{a}	10.03	1.48^{b}
animal		65.54^{ab}	28.12^{b}	9.28^{b}	13.36^{a}	9.17	1.83^{a}
plant		63.07^{b}	29.43^{b}	9.57^{b}	11.37^{b}	9.83	1.41^{b}
Phytase level, FTU/kg							
0		64.65^{ab}	29.69	9.52	12.45^{b}	9.94^{b}	1.41
500		63.19^{b}	29.51	9.97	11.85^{b}	8.69^{c}	1.71
2500		67.06^{a}	29.78	9.66	14.07^{a}	10.41^{a}	1.61
Main effects and interactions (*P*-values)							
phosphorus source		0.039	0.002	0.010	<0.001	0.289	0.010
phytase level		0.014	0.957	0.385	0.002	0.011	0.118
phosphorus × phytase		0.274	0.189	0.004	0.184	0.030	0.263

FTU – phytase unit, SEM – standard error of the mean; ^{abc} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 8. Effects of different phosphorus sources supplemented with high phytase doses on blood biochemical constituents of broilers of broiler at 35 day of age

Treatment		Protein profile			Enzyme activities			Minerals	
Phosphorus source	Phytase level, FTU/kg	TP, g/dl	ALB, g/dl	GLB, g/dl	ALT, U/dl	AST, U/dl	ALP, U/dl	Ca, mg/dl	P, mg/dl
	0	3.62	2.72	2.72	64.87	49.60	255.68	12.76	6.70
Mineral	500	3.76	2.85	2.85	65.10	51.94	240.12	11.32	6.35
	2500	3.86	2.97	2.97	63.69	50.03	252.8	11.12	6.41
	0	3.91	2.93	2.93	64.37	53.17	232.54	12.40	5.98
Animal	500	3.84	2.88	2.88	61.99	49.95	260.52	12.23	6.41
	2500	4.09	3.15	3.15	63.24	49.56	243.51	10.92	6.91
	0	3.51	2.63	2.63	60.30	50.68	238.22	10.40	5.77
Plant	500	3.77	2.83	2.83	65.36	52.31	242.32	9.62	6.58
	2500	3.93	2.99	2.99	65.72	51.46	238.64	8.85	7.18
SEM		0.216	0.163	0.053	1.904	2.172	18.262	1.010	0.405
Phosphorus source									
mineral		3.75	2.85	0.90	64.56	50.54	249.56	11.73^{a}	6.49
animal		3.95	2.99	0.96	63.20	50.89	245.52	11.85^{a}	6.43
plant		3.74	2.81	0.92	63.80	51.48	239.73	9.63^{b}	6.56
Phytase level, FTU/kg									
0		3.68^{b}	2.76^{b}	0.92	63.18	51.17	242.15	11.86^{a}	6.15^{b}
500		3.79^{ab}	2.86^{ab}	0.93	64.15	51.40	247.65	11.06^{a}	6.45^{b}
2500		3.96^{a}	3.04^{a}	0.92	64.22	50.35	245.01	10.30^{b}	6.83^{a}
Main effects and interactions (*P*-values)									
phosphorus source		0.163	0.153	0.113	0.777	0.750	0.646	<0.001	0.166
phytase level		0.090	0.017	0.895	0.831	0.682	0.873	0.034	0.012
phosphorus × phytase		0.838	0.842	0.633	0.451	0.367	0.562	0.919	0.856

TP – total protein, ALB – albumin, GLB – globulin, ALT – alanine aminotransferase, AST – aspartate aminotransferase, ALP – alkaline phosphatase, FTU – phytase unit, SEM – standard error of the mean; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

significant ($P > 0.05$). No effects of dietary P source were observed for total protein, albumin, globulin, or the liver enzymes ALT and AST ($P > 0.10$). Phytase supplementation, however, significantly altered several blood parameters (Table 8). It tended to increase total protein ($P = 0.09$) and significantly elevated serum albumin levels ($P < 0.05$), with the highest concentration observed at 2500 FTU/kg. Phytase had no effect on globulin, or ALT, AST and ALP activities ($P > 0.05$). Broilers on the low-P diet with 500 FTU/kg phytase addition showed serum Ca and P concentrations statistically equivalent to those on the normal P diet without phytase ($P > 0.05$). Importantly, phytase improved mineral status in the blood: by increasing serum P ($P < 0.05$), but it also reduced serum Ca compared to the 0 and 500 FTU/kg groups ($P < 0.05$). No significant interactions were observed for any blood parameter.

Feed cost and economic efficiency

Diets formulated with plant-derived P had the lowest formulation cost (USD/kg), followed by animal and mineral P diets (Supplementary Table 2). Phytase supplementation slightly increased feed additive cost (USD/kg diet) proportionally to the enzyme inclusion level, raising expenses by 0.0003 USD/kg at 500 FTU/kg and 0.0014 USD/kg at 2500 FTU/kg. Despite this, total feed cost remained lowest in plant P diets with phytase supplementation and was significantly lower than in mineral P diets. Total feed cost per kg of meat gain varied among treatments, reflecting differences in feed conversion efficiency. The lowest value (0.6015 USD/kg) was observed in broilers fed the plant P diet with 2500 FTU/kg phytase, whereas the highest (0.6500 USD/kg) was calculated for the mineral P feed without phytase. Compared with the mineral P diet without phytase, adding this enzyme reduced production costs in all treatments. The greatest savings were obtained for plant P diets with phytase addition (500 and 2500 FTU/kg), lowering costs by 0.0478–0.0485 USD/kg of meat gain.

Discussion

Growth performance

Broilers fed diets with animal-derived P performed as well as those fed mineral P, indicating that animal P could effectively substitute inorganic phosphates. Replacing DCP with MBM had no negative

impact on body weight gain or FCR, which aligns with previous findings that bone-derived phosphates are as effective as rock-derived sources (Liu et al., 2016; Woyengo et al., 2022). In contrast, broilers on the plant P diet showed reduced growth performance despite formulating diets to provide equal available P content. Using rice bran as the main P source led to lower FBW and poorer FCR than mineral or animal P diets, likely due to an overestimation of P availability. Significant proportion of P in rice bran is bound as phytate, which limits absorption and also binds other nutrients (Ravindran et al., 1995; Attia et al., 2003). Furthermore, the high fibre and non-starch polysaccharide content in rice bran can dilute dietary energy and impair nutrient digestion (Gallardo et al., 2020). High rice bran inclusion exceeding 20% is known to reduce weight gain (Farrell, 1994; Katukurunda et al., 2017). Attia et al. (2003) observed that while broilers may increase feed intake to compensate for lower nutrient density at moderate inclusion levels, intake can decline at very high levels due to gut-fill and palatability issues. These factors likely created an actual P deficiency and overall nutrient limitation, explaining the observed growth depression.

Phytase supplementation markedly improved growth outcomes in the low-P dietary groups. Adding 500 FTU/kg phytase to plant and mineral P diets restored performance levels comparable to the normal-P group, demonstrating that standard phytase can compensate for P deficiency. Phytase hydrolyses phytate, releasing bound P and other nutrients to prevent growth retardation (Selle and Ravindran, 2007). Multiple studies have confirmed that 500–1000 FTU/kg phytase in P-deficient broiler diets recovers BWG and FCR to the level of a P-adequate diet (Cowieson et al., 2006; Han et al., 2009; Moita et al., 2021). However, in the animal P diet, 500 FTU/kg phytase was less effective, likely due to MBM's high Ca and protein content, which creates a complex nutrient matrix. Excess Ca can form phytate-calcium complexes that resist enzymatic hydrolysis, limiting phytase efficacy (Selle et al., 2000; Plumstead et al., 2008). In contrast, the defined composition of dicalcium phosphate diets likely made them less susceptible to such disturbances. These findings suggest that phytase dose must be carefully tailored not only to dietary phytate content but also to the specific P source used. In animal and plant low-P diets, 500 FTU/kg phytase supplementation resulted in substantially lower growth than in mineral P diets, indicating that standard dose was insufficient to overcome their antinutritional effects. Conversely, superdosing phytase at 2500 FTU/kg overcame these limitation in both diets, achieving BWG comparable to the mineral P diet and significantly improving FCR in the plant P group. These benefits likely reflect more complete phytate degradation and release of additional nutrients, providing 'extra-phosphoric' effects (Cowieson et al., 2011; 2017). Similar doseresponse improvements have been reported, with BWG increasing up to 3000–5000 FTU/kg phytase in P-deficient diets (Sampath et al., 2023; Jabbar et al., 2024). In line with these findings, the present 2500 FTU/kg treatment produced the best growth outcomes, indicating that the substantial phytate load in the plant P diet was most effectively neutralised at high enzyme doses.

Nutrient digestibility

Diets with animal or mineral P sources had similar apparent nutrient digestibility, indicating that broilers utilised nutrients equally well when P was supplied from MBM or DCP. Liu et al. (2016) reported that moderate substitution of DCP by MBM (60 g/kg) did not affect protein, amino acid, or starch digestibility, although higher inclusion (120 g/kg) reduced protein and amino acid digestibility. These findings indicate that animal-derived P can safely replace mineral P at moderate levels without impairing nutrient utilisation. The plant-derived P diet, however, showed significantly lower digestibility of key nutrients before phytase supplementation. P digestibility in the rice bran-based diet without phytase was poor, reflecting high phytate content and low intrinsic P availability. Dry matter and Ca digestibility were also reduced, suggesting broader nutrient binding or malabsorption effects. Phytate chelates minerals like Ca, Zn, and Fe, forms indigestible complexes with proteins and starch, and inhibits digestive enzymes such as pepsin, trypsin and chymotrypsin (Ravindran et al., 1995). The high rice bran inclusion likely exacerbated these effects by increasing intestinal viscosity and reducing nutrient accessibility to digestive enzymes (Gallardo et al., 2020; Attia et al., 2023). Consequently, birds fed the non-supplemented plant P diet had reduced nutrient digestibility, consistent with their lower growth rates and poorer feed efficiency.

The inclusion of phytase markedly improved nutrient utilisation in the low-P diets. Supplementation with 500 FTU/kg phytase significantly increased the apparent digestibility of P, Ca, and other nutrients compared to the non-supplemented plant P diet. By hydrolysing phytate, phytase releases bound P and Ca, making them available for absorption, and reduces the anti-nutrient effects of phytate on protein and energy digestibility (Selle and Ravindran, 2007; Amerah et al., 2014). The 500 FTU/kg phytase diet achieved P and Ca digestibility comparable to the normal

P diet, demonstrating effective compensation for the lack of inorganic P. These results align with previous studies showing that microbial phytase improves mineral, amino acid, and energy utilisation in high-phytate diets (Han et al., 2009; Cowieson et al., 2011; Manobhavan et al., 2016). Birds receiving 2500 FTU/kg phytase showed the highest digestibility coefficients among the treatments, significantly surpassing the 500 FTU/kg group for several nutrients. This agrees with findings of Sampath et al. (2023), who observed a linear improvements in dry matter, ash, Ca, and P utilisation up to 3000 FTU/kg. Moita et al. (2021) reported higher apparent ileal CP and P digestibility with phytase up to 4000 FTU/kg. Similarly, Mulvenna et al. (2022) observed that superdosing phytase (1500 FTU/kg) in reduced-nutrient diets improved digestibility of dry matter and minerals, and increased ileal myo-inositol, a bioactive compound contributing to 'extra-phosphoric' benefits such as enhanced growth and feed efficiency (Cowieson et al., 2015). The extra phytase in our study likely degraded residual phytate remaining after 500 FTU/kg supplementation, further reducing interference with nutrient absorption. While 500 FTU/kg of phytase restored most digestibility parameters, subtle deficits (e.g., in energy or ether extract digestibility) persisted, which were fully corrected by the 2500 FTU/kg dose. These results indicate that standard phytase doses substantially improve, but may not fully resolve phytate-related limitations in extremely high-phytate diets.

Intestinal morphology

Dietary treatments also affected intestinal morphology, reflecting changes in gut health and absorptive capacity. Broilers fed the plant P diet without phytase showed suboptimal intestinal structure compared to those receiving adequate P. In the jejunum and ileum of the plant P group, villus height was reduced and crypt depth tended to increase, leading to a lower villus height-to-crypt depth ratio. Shorter villi with deeper crypts indicate less developed intestinal mucosa and a compensatory increase in crypt cell proliferation characteristic of nutritional stress or gut irritation (Xu et al., 2003). The compromised intestinal morphology in the plant P diet was likely caused by the combination of P deficiency and high phytate levels. P deficiency can impair cellular energy metabolism, suppressing the growth of fast-turnover tissues like the intestinal epithelium. Concurrently, phytate and other anti-nutrients in rice bran can directly irritate the gut lining and alter villus development (Ravindran et al., 1999; Cowieson et al., 2004). High levels of insoluble fibre in rice bran may also increase gut fill and cause mechanical abrasion, negatively affecting villus height and crypt depth (Gonzalez-Alvarado et al., 2008; Tejeda and Kim, 2020). Overall, the low-P, high-phytate diet compromised intestinal architecture, likely contributing to reduced nutrient absorption, lower digestibility, and poorer performance. In contrast, the animal P and mineral P diets did not differ in villus morphology, consistent with their similar nutritive status and growth outcomes.

Phytase supplementation ameliorated the adverse intestinal morphological changes caused by the plant P diet. Inclusion of 500 FTU/kg phytase increased villus height and improved villus-to-crypt ratios in the small intestine compared to the non-supplemented plant P group. This effect is likely due to phytase reducing phytate-induced irritation and releasing nutrients (P, Ca, amino acids) essential for intestinal tissue growth and maintenance (Shaw et al., 2011b). Moreover, alleviating phytate's chelation of zinc and other minerals may have further supported mucosal development. Standard phytase supplementation was less effective in low-P diets based on mineral or animal P, as limited phytate substrate restricted enzymatic P release, leaving some P deficiency-induced morphological deficits uncorrected. High phytase doses overcame this limitation, producing substantial improvements in villus and crypt morphology, in some cases slightly exceeding values observed in birds fed adequate-P diets. These results are consistent with recent studies showing that phytase superdosing significantly increases villus height and villus height-to-crypt depth ratio (VH:CD) (Zanu et al., 2020; Moita et al., 2021). The underlying mechanisms likely include increased availability of phytate-bound nutrients and modulation of gut microbiota, with a rise in beneficial bacteria (e.g. lactic acid producers) and a decrease in pathogenic species (Ptak et al., 2015; Mulvenna et al., 2022). This favourable microbial changes may reduce intestinal inflammation, improve epithelial integrity, and create a more optimal environment for villus development. High phytase doses can also increase inositol phosphate breakdown products and free inositol, which may further support gut health (Cowieson et al., 2017). By increasing villus height and surface area, high phytase doses likely facilitated nutrient absorption, reinforcing gains in digestibility and growth.

Bone development

Bone mineralisation and strength were strongly influenced by P sources and phytase levels. Broilers

fed the MBM diet had bone ash percentage, bone Ca/P content, and tibia breaking strength statistically equivalent to those fed the DCP diet. This demonstrates that animal-derived P fully sustained bone development, confirming its high bioavailability. Previous studies support this finding; for example, Woyengo et al. (2022) reviewed that bone-derived calcium phosphates had digestible P values equal or higher than rock-derived phosphates in poultry, and Liu et al. (2016) reported that replacing DCP with MBM did not reduce tibia ash or strength. These results confirm that MBM can meet broiler requirements for P and Ca without risk of skeletal deficiencies. In contrast, the plant P diet without phytase led to significantly poorer bone mineralisation and strength. This functional P deficiency was likely caused by both overestimation of available P and the anti-nutritional properties of rice bran. Although all diets were formulated to contain equal P concentrations (0.48% starter; 0.40% grower), the assumed 38% availability (Conte et al., 2002) from rice bran likely overestimated its true bioavailable fraction. The non-supplemented rice bran diet therefore provided inadequate P for optimal skeletal development, as reflected by the lower tibia ash content and bone breaking strength compared to the mineral and animal P groups. Rice bran is rich in phytate, which chelates Ca and other minerals, disturbing the Ca:P balance and limiting deposition in bone (Rutherfurd et al., 2002). The reduced tibia Ca content in this group supports this interpretation. In addition, its high fibre content may have exacerbated its negative effects on bone development by diluting nutrients, accelerating digesta passage, and reducing overall nutrient absorption efficiency. Similar negative effects of rice bran on performance and tibia ash in broilers were reported by Gallinger et al. (2004), who attributed them to phytate and fibre.

Phytase exerted considerable positive effects on bone parameters in birds fed the low-P diets. In the 500 FTU/kg group, bone metrics were generally comparable to those of the normal P control, although certain parameters (e.g., bone ash and Ca content in the mineral P diet group) remained relatively lower. This is consistent with earlier studies demonstrating that standard phytase doses restore bone mineralisation in P-deficient diets (Selle and Ravindran, 2007; Shaw et al., 2011a). Augspurger and Baker (2004) observed that 500 FTU/kg phytase in a low-P maize-soybean diet restored tibia ash in broilers to the same level as in birds on P-sufficient diet. In the present trial a similar outcome was obtained, effectively restoring bone mineralisation and strength in the low-P diet to levels comparable with the normal-P treatment. Although 500 FTU/kg phytase met the birds' P requirements, 2500 FTU/kg phytase supplementation further improved bone morphometry and mineralisation, with certain parameters, such as bone strength and bone P content, exceeding those of the normal-P diet. This indicates that releasing extra phytate-bound P (and potentially other minerals) above the requirement can further increase bone deposition. The improved bone mineralisation and increased tibia breaking strength observed in birds receiving phytase supplementation are consistent with results of Han et al. (2009), who found that high phytase doses (≥2000 FTU/kg) significantly improved bone ash, Ca and P content, and tibia strength due to the higher phytate-P availability. Similarly, Jabbar et al. (2024) observed that supplementing broiler diets with 5000 FTU/kg phytase significantly increased tibia ash content, bone Ca, P deposition, and bone breaking strength under sequential calcium-P-deficient feeding regimens, further confirming the skeletal benefits of phytase superdosing, which can eliminate nearly all phytate, thereby increasing mineral retention. The latter authors noted that broilers supplemented with extremely high phytase doses (12000 FTU/kg) had higher bone ash content than those on a standard P diet, indicating that phytate, if not sufficiently degraded, can fully inhibit bone mineralisation. In the present study, tibia ash and breaking strength increased progressively as phytase inclusion rose from 0 through 500 to 2500 FTU/kg, with the highest dose correlating with the strongest bones. Such improvements in bone strength at high phytase inclusion levels may be particularly beneficial in commercial production, reducing the risk of fractures or deformities in heavy broilers.

Organ weight and meat quality

The dietary treatments had no significant impact on relative organ weights, as liver, spleen, kidney, bursa, and gizzard weights were statistically similar between groups. This agrees with previous findings that neither high rice bran (plant P) diets nor phytase supplementation alter organ indices (Atapattu and Gamage, 2007), indicating that P source and phytase level influence nutrient utilisation rather than organ development. In contrast, meat quality parameters were clearly affected. Birds fed the plant P (high rice bran) diet had a significantly lower ultimate pH and higher drip and cooking loss, corresponding to reduced WHC. The lower pH of breast muscle in this group indicates accelerated post-mortem glycolysis and acidification, promoting protein denaturation and contraction of the myofibrillar lattice, thereby increasing fluid exudation and producing pale co-

lour (Woelfel et al., 2002). These changes can be attributed to nutritional and oxidative strains associated with high dietary phytate and polyunsaturated fatty acid (PUFA)-rich rice bran oil (Al-Abdullatif et al., 2023). Phytate binds P and essential nutrients, impairing muscle ATP synthesis, buffering capacity, and antioxidant defences (Selle and Ravindran, 2007). Concurrently, the high PUFA content in rice bran predisposes muscle lipids to peroxidation, increasing oxidative stress, and protein oxidation (Mir et al., 2017; Al-Abdullatif et al., 2023). This combined nutritional-oxidative impact reduces muscle functionality and elevates drip and cooking losses. Accordingly, the decline in meat quality in broilers fed the plant P diet is a consequence of the interaction of phytate-induced metabolic impairment and PUFA-driven oxidative deterioration, underscoring the limitations of plant-based P sources in poultry nutrition.

Phytase supplementation improved meat quality for all P sources. Birds receiving 500 FTU/kg phytase had meat quality parameters comparable to broilers on the normal-P diets, whereas superdosing at 2500 FTU/kg further improved meat quality, particularly by significantly reducing cooking and drip losses, and numerically increasing WHC and pH. These improvements contributed to more controlled post-mortem acidification and reduced exudate formation. Similar findings have been reported by Sampath et al. (2023), who showed that graded phytase supplementation (up to 3000 U/kg) in low-P diets linearly increased breast muscle pH and decreased drip and cooking losses. The authors attributed these changes to improved muscle energy status and reduced protein degradation, consistent with the present results. Thus, phytase superdosing provides extra benefits in addition to its usual role in bone mineralisation and growth, representing a practical nutritional strategy to improve meat quality in broilers fed low-P diets.

Carcass traits

Carcass characteristics were significantly influenced by both P source and phytase level. Birds fed mineral P showed the highest carcass weight, breast, leg, and thigh yields, while those receiving plant P diet had the lowest. These differences correspond to the growth performance and reflect the limited bioavailability of plant-derived P. Phytate-bound P in plant ingredients is largely indigestible to broilers (Selle and Ravindran, 2007), thus non-supplemented or under-supplemented plant-based diets can induce P deficiency, impairing growth and carcass yield. Additionally, the high fibre and phytate contents of rice bran may lower dressing percentage and nutrient absorption efficiency, further suppressing carcass development (Attia et al., 2023). Insufficient non-phytic P in plant-based diets has been specifically linked to reduced carcass yields due to compromised nutrient metabolism and skeletal development (Attia et al., 2020). The animal P group showed carcass development comparable to the mineral group, consistent with previous findings, indicating that MBM inclusion at moderate levels (up to 5%) does not negatively affect carcass yield or the proportions of carcass components in broilers (Bozkurt et al., 2004). However, excessive MBM addition, or variability in its amino acid balance and Ca:P ratio, may affect nutrient utilisation and slightly reduce carcass yield under certain conditions.

Phytase supplementation exerted a clear dose-dependent effect on carcass traits. The 500 FTU/kg dose partially alleviated the negative impact of plant P but did not restore carcass yields to mineral P levels. In contrast, superdosing at 2500 FTU/kg significantly increased carcass and cut weights (thigh and wing), bringing carcass performance in plant P-fed birds closer to that of the mineral P groups. Similar trends have been reported by Dang et al. (2022), who observed a linear increase in 35-day carcass weight with dietary phytase increase from 500 up to 1500 FTU/kg. Cowieson et al. (2011) likewise showed that high phytase doses resulted in substantial increments in broiler weight gains and meat yield compared to standard supplementation. More recently, Jabbar et al. (2024) reported that very high phytase doses (≥2500 FTU/kg) significantly elevated carcass weight and dressing percentage in broilers fed Ca- and P-deficient diets. Collectively, these results confirm that high phytase supplementation can effectively restore carcass yield and stimulate the development of economically valuable cuts. Abdominal fat was also affected by P source, with the lowest values recorded in the plant P group. This reduction is likely a secondary consequence of stunted growth and lower feed intake- in severely P-deficient birds, resulting in less energy available for fat deposition. Interestingly, high phytase dosing improved lean tissue deposition (breast, thigh) without increasing fat content, suggesting improved protein utilisation and energy transfer toward muscle (Attia et al., 2020). This trend aligns with the proposed ‘lipotropic’ effect of phytate breakdown products, such as myo-inositol, generated by high phytase supplementation, which may redirect nutrients from fat to muscle accretion (Cowieson et al., 2011; Lee and Bedford, 2016). Therefore, the high

phytase dose in plant-based diets not only improved total carcass output but also preserved a favourable lean carcass profile, an important consideration for broiler meat quality and economic value.

Blood biochemical indices

Birds fed the phytase-supplemented low-P diets showed reduced serum Ca levels accompanied by an increase in serum P relative to those fed the non-supplemented P-deficient diet. This inverse relationship between Ca and P indicates an improved P status and altered mineral homeostasis. Low dietary P typically triggers compensatory bone resorption, elevating circulating Ca and reducing P (Li et al., 2020). By hydrolysing phytate and liberating bound P, phytase increases its intestinal availability and absorption. The resultant higher serum P reduced the body's need to mobilise Ca from bone, allowing more Ca to be retained in skeletal tissue, normalising plasma levels (Li et al., 2020). This mechanism explains the observed reduced blood Ca concurrent with elevated P in phytase-treated groups. Similar outcomes have been reported by Manobhavan et al. (2016), where superdosed phytase (2500–5000 FTU/kg) in low-P diets raised serum P and stimulated bone Ca deposition. Han et al. (2009) also reported increased serum P and reduced Ca levels in broilers given high-dose phytase supplements, which aligns well with the current findings and confirms that phytase improves P absorption and subsequently decreases the mobilization of Ca from skeletal reserves. Overall, phytase corrected the Ca-P imbalance induced by P deficiency, restoring mineral concentrations toward physiological range.

In addition to minerals, phytase affected protein metabolism, as evidenced by increased serum total protein and albumin levels. Birds receiving phytase showed higher concentrations of both parameters, indicating improved protein nutritional status and hepatic synthetic capacity. This effect is likely due to the reduction of phytate antinutritional effects on protein digestion and amino acid utilisation. Phytate can form complexes with dietary proteins and digestive enzymes, impairing protein hydrolysis and absorption (Nafea et al., 2020). With greater amino acid supply, the liver can synthesise more albumin and globulins, leading to elevated circulating protein levels. Rana et al. (2024) observed a comparable trend in broilers, where high phytase dosing in low Ca/P diets significantly increased serum albumin and total protein levels, concurrent with better growth performance. These results suggest that phytase supplementation not only mitigates mineral deficiencies but also supports accelerated protein anabolism, as evidenced by higher serum protein indicators in treated birds. The effects of phytase were dependent on the supplementation level. Birds receiving 2500 FTU/kg had numerically higher serum protein and albumin levels compared to both the 500 FTU/kg and positive control groups, suggesting an improved physiological status. High phytase doses can maximise the release of minor phytate-bound minerals (such as Zn and Mg) and reduce residual antinutritional inositol phosphates, which synergistically promotes better growth and overall health (Manobhavan et al., 2016; Nafea et al., 2020). High phytase provision has also been shown to improve amino acid digestibility and weight gain beyond standard supplementation and to elevate serum albumin as a marker of enhanced protein status (Pieniazek et al., 2017; Rana et al., 2024). Importantly, no adverse effects on liver function were detected in any phytase-treated group. Serum activities of hepatic enzymes (AST and ALT) were statistically similar in all treatments, including the negative control and both phytase doses, with all values remaining within normal ranges. There were no signs of hepatic stress or damage associated with phytase supplementation, consistent with previous reports that practical phytase inclusion does not compromise liver health (Derakhshan et al., 2023). The unchanged liver enzyme profiles in the present study, even at the 2500 FTU/kg dose, confirm that high phytase levels are well-tolerated. This supports the conclusion that phytase is a safe feed additive, with potent effects on nutrient utilisation without imposing metabolic stress on the liver.

Economic efficiency

Economic implications of individual treatments are crucial for practical feeding strategies. The present analysis (Supplementary Table 2) revealed clear advantages of alternative P sources and supplemental phytase. Diets with animal P source (MBM) were more economical than those with DCP, supplying highly available P and Ca, as well as protein and energy, reducing the need for additional expensive ingredients. This aligns with industry trends favouring by-product utilisation, particularly amid rising inorganic phosphate prices. The plant P diet offered the lowest ingredient cost due to rice bran inclusion, but feed efficiency was compromised without phytase. Supplementing 500 FTU/kg phytase improved economic efficiency, although digestibility and intestinal morphology remained below those of animals on the P-sufficient diet. Superdosing at 2500 FTU/kg produced the most

optimal economic benefit. Although enzyme costs were higher, improvements in growth performance and feed conversion compensated for this, maximising the value of low-cost plant ingredients. These results suggest that in high-phytate diets, standard phytase doses may be insufficient, whereas superdosing is economically justified. Considering that feed accounts for up to 70% of broiler production costs (Jin et al., 2024), even modest gains in feed cost efficiency can substantially affect profitability. Environmentally, combining plant P sources with high-dose phytase reduces reliance on mined phosphates and lowers P excretion, supporting more sustainable poultry production (Lei et al., 2013). The plant P, in combination with high phytase supplementation, proved to be the most economical and sustainable strategy, offering a practical approach where DCP/MCP costs are high or inorganic phosphate availability is limited.

Conclusions

The results demonstrate that mineral and animal-based phosphorus (P) sources are equally effective in supporting broiler growth, bone development, and meat quality. However, relying solely on plant-derived P (high rice bran) without phytase supplementation severely impair growth performance, primarily due to reduced body weight gain, underdeveloped intestinal morphology, bones, and inferior meat quality. Phytase supplementation improved broiler productivity and nutrient utilisation in all dietary treatments, with the highest dose (2500 FTU/kg) providing the greatest benefits by markedly improving P and Ca digestibility, as well as intestinal development, which translated into optimal growth performance, bone strength, carcass weight and meat quality. Even the standard phytase dose (500 FTU/kg) largely restored the growth and bone parameters in broilers fed low-P plant diets to levels comparable with plant normal-P diet, though performance remained below that of mineral and animal P diets. Importantly, phytase exerted no adverse effects on health, as all blood biochemical indices and liver enzyme activities remained within physiological ranges. Overall, replacing expensive mineral phosphates with animal by-products or phytate-rich plant sources, in combination with phytase supplementation (particularly at high doses), proved economically advantageous. This strategy maximises P release, maintains or enhances growth, bone strength, and product quality, while reducing feed costs and reliance on inorganic phosphates. It offers a cost-effective and sustainable approach to broiler nutrition without compromising performance or health.

Acknowledgments

The authors would like to thank New Hope Liuhe Indonesia Co., Ltd. Cirebon for providing the study site and funding, Guangdong VTR Bio-tech Co., Ltd for supplying the enzymes and usage guidance.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

Al-Abdullatif A., Hussein E., Suliman G., Akasha M., Al-Badwi M., Ali H., Azzam M., 2023. Evaluating rice bran oil as a dietary energy source on production performance, nutritional properties and fatty acid deposition of breast meat in broiler chickens. Foods 12, 366, https://doi.org/10.3390/foods12020366

Amerah A.M., Plumstead P.W., Barnard L.P., Kumar A., 2014. Effect of calcium level and phytase addition on ileal phytate degradation and amino acid digestibility of broilers fed corn-based diets. Poult. Sci. 93, 906–915, https://doi.org/10.3382/ps.2013-03465

An S.H., Sung J.Y., Kong C., 2020. Ileal digestibility and total tract retention of phosphorus in inorganic phosphates fed to broiler chickens using the direct method. Animals 10, 2167, https://doi.org/10.3390/ani10112167

Anon E.C., 2003. Updated opinion and report on the safety of dicalcium phosphate (DCP) and tricalcium phosphate (TCP) from bovine bones, used as an animal feed additive or as fertilizer. European Commission Health & Consumer Protection Directorate-General, https://ec.europa.eu/food/system/files/2020-12/sci-com_ssc_ out322_en.pdf

AOAC International, 2005. Official Methods of Analysis of AOAC International. 18th Edition. AOAC International, MA (USA)

Atapattu N., Gamage V.L.R., 2007. Effects of supplementation of diets containing high levels of rice bran with microbial phytase on the performance of broiler chicken. 4th Academic Sessions. Uniwersity of Ruhuna. Mapalana (Sri Lanka), http://ir.lib.ruh.ac.lk/xmlui/handle/iruor/13423

Attia Y.A., Ashour E.A., Nagadi S.A., Farag M.R., Bovera F., Alagawany M., 2023. Rice bran as an alternative feedstuff in broiler nutrition and impact of Liposorb® and vitamin E-Se on sustainability of performance, carcass traits, blood biochemistry, and antioxidant indices. Vet. Sci. 10, 299, https://doi.org/10.3390/vetsci10040299

Attia Y.A., Bovera F., Iannaccone F., Al-Harthi M.A., Alaqil A.A., Zeweil H., Mansour A.E., 2020. Microbial and fungal phytases can affect growth performance, nutrient digestibility and blood profile of broilers fed different levels of non-phytic phosphorus. Animals 10, 580, https://doi.org/10.3390/ani10040580

Attia Y.A., Qota E.M.A., Aggoor F.A.M., Kies A.K., 2003. Value of rice bran, its maximal utilisation and its upgrading by phytase and other enzymes and diet-formulation based on available amino acids in the diet for broilers. Eur. Poult. Sci. 67, 157–166, https://doi.org/10.1016/S0003-9098(25)00085-2

Augspurger N.R., Baker D.H., 2004. High dietary phytase levels maximize phytate-phosphorus utilization but do not affect protein utilization in chicks fed phosphorus- or amino acid-deficient diets. J. Anim. Sci. 82, 1100–1107, https://doi.org/10.2527/2004.8241100x

Beeson L.A., Walk C.L., Bedford M.R., Olukosi O.A., 2017. Hydrolysis of phytate to its lower esters can influence the growth performance and nutrient utilization of broilers with regular or super doses of phytase. Poult. Sci. 96, 2243–2253, https://doi.org/10.3382/ps/pex012

Bozkurt M., Basmacioğlu H., Ergül M., 2004. Effect of dietary concentration meat and bone meal on broiler chickens performance. Int. J. Poult. Sci. 3, 719–723, https://doi.org/10.3923/ijps.2004.719.723

Cheryan M., Rackis J.J., 1980. Phytic acid interactions in food systems. Crit. Rev. Food Sci. Nutr. 13, 297–335, https://doi.org/10.1080/10408398009527293

Cobb, 2021. Cobb Broiler Management Guide. https://www.cobbgenetics.com/assets/Cobb-Files/Broiler-Guide_English-2021-min.pdf

Conte A.J., Teixeira A.S., Figueirêdo A.V.D., Vitti D.M.S.S., Silva Filho J.C.D., 2002. Effect of phytase on the bioavailability of phosphorus of rice bran in broilers chickens. Pesq. Agropec. Bras., BrasÌlia 37, 547–552, https://doi.org/10.1590/S0100-204X2002000400017

Cowieson A.J., Acamovic T., Bedford M.R., 2004. The effects of phytase and phytic acid on the loss of endogenous amino acids and minerals from broiler chickens. Br. Poult. Sci. 45, 101–108, https://doi.org/10.1080/00071660410001668923

Cowieson A.J., Acamovic T., Bedford M.R., 2006. Phytic acid and phytase: implications for protein utilization by poultry. Poult. Sci. 85, 878–885, https://doi.org/10.1093/ps/85.5.878

Cowieson A.J., Aureli R., Guggenbuhl P., Fru-Nji F., 2015. Possible involvement of myo-inositol in the physiological response of broilers to high doses of microbial phytase. Anim. Prod. Sci. 55, 710–719, https://doi.org/10.1071/AN14044

Cowieson A.J., Ruckebusch J.P., Sorbara J.O.B., Wilson J.W., Guggenbuhl P., Roos F.F., 2017. A systematic view on the effect of phytase on ileal amino acid digestibility in broilers. Anim. Feed Sci. Technol. 225, 182–194, https://doi.org/10.1016/j.anifeedsci.2017.01.008

Cowieson A.J., Wilcock P., Bedford M.R., 2011. Super-dosing effects of phytase in poultry and other monogastrics. World's Poult. Sci. J. 67, 225–236, https://doi.org/10.1017/S0043933911000250

Dang H.T., Lee J.H., Kim I.H., 2022. Effects of dietary supplementation of S. pombe-expressed phytase on growth performance, nutrient digestibility, meat quality, organ indexes, and footpad lesions in broiler chicks. Anim. Biosci. 35, 1390–1399, https://doi.org/10.5713/ab.21.0462

Derakhshan M., Ghasemian S.O., Gholami-Ahangaran M., 2023. The effects of probiotic and phytase on growth performance, biochemical parameters and antioxidant capacity in broiler chickens. Vet. Med. Sci. 9, 860–866, https://doi.org/10.1002/vms3.1075

Farrell D.J., 1994. Utilization of rice bran in diets for domestic fowl and ducklings. World's Poult. Sci. J. 50, 115–131, https://doi.org/10.1079/WPS19940012

Gallardo C., Dadalt J.C., Neto M.A.T., 2020. Carbohydrases and phytase with rice bran, effects on amino acid digestibility and energy use in broiler chickens. Animal 14, 482–490, https://doi.org/10.1017/S1751731119002131

Gallinger C.I., Suárez D.M., Irazusta A., 2004. Effects of rice bran inclusion on performance and bone mineralization in broiler chicks. J. Appl. Poult. Res. 13, 183–190, https://doi.org/10.1093/japr/13.2.183

Gonzalez-Alvarado J.M., Jimenez-Moreno E., Valencia D.G., Lazaro R., Mateos G.G., 2008. Effects of fiber source and heat processing of the cereal on the development and pH of the gastrointestinal tract of broilers fed diets based on corn or rice. Poult. Sci. 87, 1779–1795, https://doi.org/10.3382/ps.2008-00070

Han J.C., Yang X.D., Qu H.X., et al., 2009. Evaluation of equivalency values of microbial phytase to inorganic phosphorus in 22- to 42-day-old broilers. J. Appl. Poult. Res. 18, 707–715, https://doi.org/10.3382/japr.2009-00029

Honikel K.O., 1998. Reference methods for the assessment of physical characteristics of meat. Meat Sci. 49, 447–457, https://doi.org/10.1016/S0309-1740(98)00034-5

Jabbar A., Tahir M., Naz S., Ahmad S., Alhidary I.A., Abdelrahman S., Tufarelli V., 2024. Enhancing calcium and phosphorus utilization in broiler chickens with phytase in sequential calcium-phosphorus-deficient diets. J. Appl. Anim. Res. 52, 2402046, https://doi.org/10.1080/09712119.2024.2402046

Jin D., Tugiyanti E., Rimbawanto E.A., Rosidi R., Widiyastuti T., Susanto A., Ismoyowati I., 2024. Effects of high-level dietary distiller dried grains with solubles supplemented with multienzymes on growth performance, nutrient utilization, intestinal morphology, and pellet quality in broiler chickens. Vet. World 17, 1943–1954, https://doi.org/10.14202/vetworld.2024.1943–1954

Jongbloed A.W., Kemme P.A., 1990. Effect of pelleting mixed feeds on phytase activity and the apparent absorbability of phosphorus and calcium in pigs. Anim. Feed Sci. Technol. 28, 233–242, https://doi.org/10.1016/0377-8401(90)90155-2

Katukurunda K., Atapattu N., Perera P.W.A., 2017. Effects of mega doses of phytase on growth performance, bone status and nutrient excretion of broilers fed diets containing high levels of rice bran. Iran. J. Appl. Anim. Sci. 7, 119-127

Kauffman R.G., Eikelenboom G., van der Wal P.G., Engel B., Zaar M., 1986. A comparison of methods to estimate water-holding capacity in post-rigor porcine muscle. Meat Sci. 18, 307–322, https://doi.org/10.1016/0309-1740(86)90020-3

Kim E.J., Utterback P.L., Parsons C.M., 2012. Comparison of amino acid digestibility coefficients for soybean meal, canola meal, fish meal, and meat and bone meal among 3 different bioassays. Poult. Sci. 91, 1350–1355, https://doi.org/10.3382/ps.2011-01861

Kumar V., Sinha A.K., Makkar H.P.S., De Boeck G., Becker K., 2010. Dietary roles of phytate and phytase in human nutrition: A review. Food Chem. 120, 945–959, https://doi.org/10.1016/j.foodchem.2009.11.052.

Lee S.A., Bedford M.R., 2016. Inositol - an effective growth promoter? World's Poult. Sci. J. 72, 743–760, https://doi.org/10.1017/S0043933916000660

Lei X.G., Weaver J.D., Mullaney E., Ullah A.H., Azain M.J., 2013. Phytase, a new life for an "old" enzyme. Annu. Rev. Anim. Biosci. 1, 283–309, https://doi.org/10.1146/annurev-animal-031412-103717

Leyva-Jimenez H., Alsadwi A.M., Gardner K., Voltura E., Bailey C.A., 2019. Evaluation of high dietary phytase supplementation on performance, bone mineralization, and apparent ileal digestible energy of growing broilers. Poult. Sci. 98, 811–819, https://doi.org/10.3382/ps/pey389

Li T., Xing G., Shao Y., Zhang L., Li S., Lu L., Liu Z., Liao X., Luo X., 2020. Dietary calcium or phosphorus deficiency impairs the bone development by regulating related calcium or phosphorus metabolic utilization parameters of broilers. Poult. Sci. 99, 3207–3214, https://doi.org/10.1016/j.psj.2020.01.028

Liu S.Y., Cowieson A.J., Selle P.H., 2016. The influence of meat-and-bone meal and exogenous phytase on growth performance, bone mineralisation and digestibility coefficients of protein (N), amino acids and starch in broiler chickens. Anim. Nutr. 2, 86–92, https://doi.org/10.1016/j.aninu.2016.03.003

Manobhavan M., Elangovan A.V., Sridhar M., Shet D., Ajith S., Pal D.T., Gowda N.K.S., 2016. Effect of super dosing of phytase on growth performance, ileal digestibility and bone characteristics in broilers fed corn-soya-based diets. J. Anim. Physiol. Anim. Nutr. 100, 93–100, https://doi.org/10.1111/jpn.12341

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A., Wilkinson R.G., 2002. Animal Nutrition. 6th Edition. Pearson Education Ltd. Harlow (UK), https://api.pageplace.de/preview/DT0400.9781408204276_A25051376/preview-9781408204276_A25051376.pdf

Mekmene O., Quillard S., Rouillon T., Bouler J.-M., Piot M., Gaucheron F., 2009. Effects of pH and Ca/P molar ratio on the quantity and crystalline structure of calcium phosphates obtained from aqueous solutions. Dairy Sci. Technol. 89, 301–316, https://doi.org/10.1051/dst/2009019

Mir N.A., Rafiq A., Kumar F., Singh V., Shukla V., 2017. Determinants of broiler chicken meat quality and factors affecting them: a review. J. Food Sci. Technol. 54, 2997–3009, https://doi.org/10.1007/s13197-017-2789-z

Moita V.H.C., Duarte M.E., Kim S.W., 2021. Supplemental effects of phytase on modulation of mucosa-associated microbiota in the jejunum and the impacts on nutrient digestibility, intestinal morphology, and bone parameters in broiler chickens. Animals 11, 3351, https://doi.org/10.3390/ani11123351

Mulvenna C.C., McCormack U.M., Magowan E. et al., 2022. The growth performance, nutrient digestibility, gut bacteria, and bone strength of broilers offered alternative, sustainable diets varying in nutrient specification and phytase dose. Animals 12, 1669, https://doi.org/10.3390/ani12131669

Mutucumarana R.K., Ravindran V., 2016. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens using the direct method. Anim. Feed Sci. Technol. 219, 249–256, https://doi.org/10.1016/j.anifeedsci.2016.06.025

Mutucumarana R.K., Ravindran V., Ravindran G., Cowieson A.J., 2015. Measurement of true ileal phosphorus digestibility in meat and bone meal for broiler chickens. Poult. Sci. 94, 1611–1618, https://doi.org/10.3382/ps/pev132

Nafea H., Hussein F., Hamid B., 2020. Effect of supplementation with high levels of phytase enzyme compared with commercial dose in corn-soybean based diets on productive and physiological performance in broiler. Indian J. Ecol. 46, 21–27

National Research Council (NRC), 2012. Nutrient Requirements of Swine. 11th Edition. National Academies Press. Washington, DC (USA), https://doi.org/10.17226/13298

Paiva D., Walk C., McElroy A., 2014. Dietary calcium, phosphorus, and phytase effects on bird performance, intestinal morphology, mineral digestibility, and bone ash during a natural necrotic enteritis episode. Poult. Sci. 93, 2752–2762, https://doi.org/10.3382/ps.2014-04148

Pieniazek J., Smith K.A., Williams M.P., Manangi M.K., Vazquez-Anon M., Solbak A., Miller M., Lee J.T., 2017. Evaluation of increasing levels of a microbial phytase in phosphorus deficient broiler diets via live broiler performance, tibia bone ash, apparent metabolizable energy, and amino acid digestibility. Poult. Sci. 96, 370-382, https://doi.org/10.3382/ps/pew225

Plumstead P.W., Leytem A.B., Maguire R.O., Spears J.W., Kwanyuen P., Brake J., 2008. Interaction of calcium and phytate in broiler diets. 1. Effects on apparent prececal digestibility and retention of phosphorus. Poult. Sci. 87, 449–458, https://doi.org/10.3382/ps.2007-00231

Powell S., Bidner T.D., Southern L.L., 2011. Phytase supplementation improved growth performance and bone characteristics in broilers fed varying levels of dietary calcium. Poult. Sci. 90, 604–608, https://doi.org/10.3382/ps.2010-01000

Ptak A., Bedford M.R., Świątkiewicz S., Żyła K., Józefiak D., 2015. Phytase modulates ileal microbiota and enhances growth performance of the broiler chicken. PLoS ONE 10, e0119750, https://doi.org/10.1371/journal.pone.0119770

Rana M.M., Sarker M.S.K., Islam M.R., Hassan M.R., Aftabuzzaman M., 2024. Effectiveness of superdosing phytase to low phosphorus and calcium diet on growth performance, nutrient digestibility, blood parameters of broiler chickens. Int. J. Poult. Sci. 23, 78–85, https://doi.org/10.3923/ijps.2024.78.85

Ravindran V., Bryden W.L., Kornegay E.T., 1995. Phytates: occurrence, bioavailability and implications in poultry nutrition. World's Poult. Sci. J. 51, 125–143

Ravindran V., Cabahug S., Ravindran G., Bryden W.L., 1999. Influence of microbial phytase on apparent ileal amino acid digestibility of feedstuffs for broilers. Poult. Sci. 78, 699–706, https://doi.org/10.1093/ps/78.5.699

Rinttilä T., Apajalahti J., 2013. Intestinal microbiota and metabolites-implications for broiler chicken health and performance. J. Appl. Poult. Res. 22, 647–658, https://doi.org/10.3382/japr.2013-00742

Rutherfurd S.M., Chung T.K., Moughan P.J., 2002. The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. Br. Poult. Sci. 43, 598–606, https://doi.org/10.1080/0007166022000004516

Sampath V., Gao S., Park J.H., Kim I.H., 2023. Exogenous phytase improves growth performance, nutrient retention, tibia mineralization, and breast meat quality in Ross-308 broilers. Agriculture 13, 1906, https://doi.org/10.3390/agriculture13101906

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

Selle P.H., Ravindran V., Caldwell A., Bryden W.L., 2000. Phytate and phytase: Consequences for protein utilisation. Nutr. Res. Rev. 13, 255–278, https://doi.org/10.1079/095442200108729098

Shaw A.L., Hess J.B., Blake J.P., Ward N.E., 2011a. Assessment of an experimental phytase enzyme product on live performance, bone mineralization, and phosphorus excretion in broiler chickens. J. Appl. Poult. Res. 20, 561–566, https://doi.org/10.3382/japr.2011-00389

Shaw A.L., van Ginkel F.W., Macklin K.S. Blake J.P., 2011b. Effects of phytase supplementation in broiler diets on a natural Eimeria challenge in naive and vaccinated birds. Poult. Sci. 90, 781-790, https://doi.org/10.3382/ps.2010-01158

Short F.J., Gorton P., Wiseman J., Boorman K.N., 1996. Determination of titanium dioxide added as an inert marker in chicken digestibility studies. Anim. Feed Sci. Technol. 59, 215–221, https://doi.org/10.1016/0377-8401(95)00916-7

Someus E., Pugliese M., 2018. Concentrated phosphorus recovery from food grade animal bones. Sustainability 10, 2349, https://doi.org/10.3390/su10072349

Tejeda O.J., Kim W.K., 2020. The effects of cellulose and soybean hulls as sources of dietary fiber on the growth performance, organ growth, gut histomorphology, and nutrient digestibility of broiler chickens. Poult. Sci. 99, 6828–6836, https://doi.org/10.1016/j.psj.2020.08.081

van Harn J., Spek J.W., van Vuure C.A., van Krimpen M.M., 2017. Determination of pre-cecal phosphorus digestibility of inorganic phosphates and bone meal products in broilers. Poult. Sci. 96, 1334–1340, https://doi.org/10.3382/ps/pew458

Walk C.L., Bedford M.R., Olukosi O.A., 2018. Effect of phytase on growth performance, phytate degradation and gene expression of myo-inositol transporters in the small intestine, liver and kidney of 21 day old broilers. Poult. Sci. 97, 1155–1162, https://doi.org/10.3382/ps/pex392

Walk C.L., Bedford M.R., Santos T.S., Paiva D., Bradley J.W., Wladecki H., Honaker C., McElroy A.P., 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92, 719–725, https://doi.org/10.3382/ps.2012-02727

Walters H.G., Coelho M., Coufal C.D., Lee J.T., 2019. Effects of increasing phytase inclusion levels on broiler performance, nutrient digestibility, and bone mineralization in low-phosphorus diets. J. Appl. Poult. Res. 28, 1210–1225, https://doi.org/10.3382/japr/pfz087

Warren B.E., Farrell D.J., 1990a. The nutritive value of full-fat and defatted Australian rice bran. I. Chemical composition. Anim. Feed Sci. Technol. 27, 219–228, https://doi.org/10.1016/0377-8401(90)90084-L

Warren B.E., Farrell D.J., 1990b. The nutritive value of full-fat and defatted Australian rice bran. II. Growth studies with chickens, rats and pigs. Anim. Feed Sci. Technol. 27, 229–246, https://doi.org/10.1016/0377-8401(90)90085-M

Weaver C.M., Kannan S., 2002. Phytate and mineral bioavailability. In: N.R. Reddy, S.K. Sathe (Editors). Food Phytates. CRC Press. Boca Raton, FL (USA), pp. 211–223, https://doi.org/10.1201/9781420014419.ch13

Woelfel R.L., Owens C.M., Hirschler E.M., Martinez-Dawson R., Sams A.R., 2002. The characterization and incidence of pale, soft, and exudative broiler meat in a commercial processing plant. Poult. Sci. 81, 579–584, https://doi.org/10.1093/ps/81.4.579

Woyengo T.A., Nørgaard J.V., van der Heide M.E., Nielsen T.S., 2022. Calcium and phosphorus digestibility in rock- and bone-derived calcium phosphates for pigs and poultry: A review. Anim. Feed Sci. Technol. 294, 115509, https://doi.org/10.1016/j.anifeedsci.2022.115509

Xu Z.R., Hu C.H., Xia M.S., Zhan X.A., Wang M.Q., 2003. Effects of dietary fructooligosaccharide on digestive enzyme activities, intestinal microflora and morphology of male broilers. Poult. Sci. 82, 1030–1036, https://doi.org/10.1093/ps/82.6.1030

Zanu H.K., Keerqin C., Kheravii S.K., Morgan N.K., Wu S.B., Bedford M.R., Swick R.A., 2020. Influence of meat and bone meal, phytase, and antibiotics on broiler chickens challenged with subclinical necrotic enteritis: 2. intestinal permeability, organ weights, hematology, intestinal morphology, and jejunal gene expression. Poult. Sci. 99, 2581–2594, https://doi.org/10.1016/j.psj.2019.12.049

Zyla K., Dulinski M., Pierzchalska M., Grabacka M., Jozefiak D., Swiatkiewicz S., 2013. Phytases and myo-inositol modulate performance, bone mineralization and alter lipid fractions in the serum of broilers. J. Anim. Feed Sci. 22, 56–62, https://doi.org/10.22358/jafs/66017/2013

Supplementary Table 1. Effects of different phosphorus sources supplemented with high phytase doses on organ weight of broilers of broiler at 35 day of age

Treatment		Relative organ weight, %				
Phosphorus source	Phytase level, FTU/kg	Liver	Spleen	Kidney	Bursa of Fabricius	Gizzard
	0	2.13	0.089	0.986	0.097	1.37
Mineral	500	2.14	0.089	0.729	0.116	1.24
	2500	2.14	0.104	0.865	0.101	1.36
	0	2.41	0.129	0.941	0.101	1.38
Animal	500	2.35	0.083	0.926	0.085	1.42
	2500	2.30	0.084	0.875	0.116	1.23
	0	2.20	0.096	0.963	0.110	1.39
Plant	500	2.41	0.095	0.876	0.120	1.37
	2500	2.23	0.110	0.943	0.119	1.39
SEM		0.184	0.020	0.083	0.018	0.098
Phosphorus source						
mineral		2.14	0.094	0.860	0.105	1.32
animal		2.35	0.098	0.914	0.101	1.34
plant		2.28	0.100	0.927	0.116	1.38
Phytase level, FTU/kg						
0		2.25	0.105	0.963[a]	0.103	1.38
500		2.30	0.089	0.844[b]	0.107	1.34
2500		2.22	0.099	0.894[ab]	0.112	1.33
Main effects and interactions (*P*-values)						
phosphorus source		0.124	0.845	0.335	0.319	0.581
phytase level		0.755	0.397	0.049	0.675	0.636
phosphorus × phytase		0.852	0.197	0.272	0.452	0.225

FTU – phytase unit, SEM – standard error of the mean; $P > 0.05$ (not statistically significant); [a,b] – means with different superscripts within a row are significantly different at $P < 0.05$

Supplementary Table 2. Analysis of formula cost and unit meat production cost of treatment diets

Phosphorus source	Mineral			Animal			Plant		
Phytase level, FTU/kg	0	500	2500	0	500	2500	0	500	2500
Formulation cost, USD/kg	0.4124	0.4026	0.4026	0.4007	0.3971	0.3971	0.3926	0.3829	0.3829
Phytase cost, USD/kg	0	0.0003	0.0014	0	0.0003	0.0014	0	0.0003	0.0014
Feed manufacturing cost, USD/kg	0.0154	0.0154	0.0154	0.0154	0.0154	0.0154	0.0154	0.0154	0.0154
Total feed cost, USD/kg	0.4278	0.4183	0.4194	0.4161	0.4128	0.4139	0.408	0.3986	0.3997
Feed consumed/kg of meat gain, kg	1.5194	1.5028	1.4936	1.4753	1.5543	1.5442	1.5215	1.5107	1.5049
Total feed cost/kg of meat gain, USD/kg	0.6500	0.6286	0.6264	0.6139	0.6416	0.6391	0.6208	0.6022	0.6015
Difference		−0.0214	−0.0236	−0.0361	−0.0084	−0.0109	−0.0292	−0.0478	−0.0485

FTU – phytase unit; main raw material prices (USD/kg): maize 0.3058, wheat, 0.3268, CPO 0.9032, soybean meal 0.4841, meat and bone meal (MBM) 0.5607, di-calcium phosphate (DCP) 0.4322, rice bran 0.2185, VTR Phytase 5000 2.7282 (Guangdong VTR Bio-tech Co., Ltd.); total feed cost/kg meat gain = total feed cost × feed consumed/kg meat gain; difference versus mineral + 0 FTU/kg phytase (USD/kg)