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Phytase replacing inorganic phosphate improves broiler growth performance

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ABSTRACT. This study evaluated the effects of completely replacing dietary inorganic phosphate (Pi) with phytase in a high-phytate diet (>0.30% phytate phosphorus [phytate P]). Male Ross 308 broilers (n = 390) were distributed in a completely randomised design to one of three experimental treatments, with 10 replicate pens per treatment and 13 birds per pen in three feeding phases: starter (days 0–10), grower (days 11–23), and finisher (days 24–35). The positive control (PC) diet was based on corn, broken rice, soybean, wheat, rice bran, and rapeseed meal, supplemented with Pi from monocalcium phosphate. The two experimental diets (P1000 and P1500) were formulated to be nutritionally equivalent but contained no added Pi. Instead, they were supplemented with 1000 or 1500 phytase units (FTU)/kg, with corresponding reductions in Ca (0.17 and 0.24%) and available phosphorus (0.20 and 0.28%), respectively. A negative control was not included for animal welfare reasons. Feed conversion ratio improved during the finisher phase (days 24–35) and over the entire experimental period (days 0–35) in the P1000 and P1500 groups compared to the PC group ($P < 0.05$). Carcass yield on day 35 did not differ between the phytase-supplemented groups and the PC group. Overall, replacing Pi with 1000 or 1500 FTU/kg can compensate or even improve growth performance and carcass yield, without adversely affecting bone strength or serum P and Ca concentrations in broilers.

Introduction

Commercial exogenous phytases have been used in poultry and swine nutrition since 1990 to reduce the environmental impact of intensive animal production and to improve performance through more efficient nutrient utilisation (Simons et al., 1990). Phytase hydrolyses phytate (the salt of phytic acid, myo-inositol hexaphosphate [IP6]) present in plant-based ingredients (Konietzny and Greiner, 2002). Most commercially available phytases are of fungal or bacterial origin but are produced using yeast or fungal expression systems (Navone et al., 2021). Bacterial phytases, compared with analogous enzymes from other organisms, including fungi, possess unique properties such as resistance to proteolysis, activity across a broader pH range (from acidic to alkaline), dependence on metal ions, and high substrate specificity (Jain and Sing, 2016). There is evidence that bacterial phytases may be more effective in broiler nutrition (Selle and Ravindran, 2007). However, the feasibility of completely replacing inorganic phosphate (Pi) with phytase in modern diets remains insufficiently studied. Although existing evidence supports reductions in dietary phosphorus (P) and calcium (Ca) levels through enzyme supplementation, complete removal of Pi requires precise adjustment of phytase dosages throughout the growth cycle to maintain performance. Therefore, the present study evaluated the efficacy of high-dose bacterial phytase (1000 and 1500 FTU/kg) in Pi-free diets, as a strategy to maximise growth performance

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while reducing environmental P excretion (Marchal et al., 2021).

Phosphorus (P) is the third most expensive component of a broiler diet, after energy and protein (Dos Santos et al., 2019). It is essential for boiler growth and bone formation, and supports nervous system development and energy conversion (Li et al., 2017). Corn and soybean, the most commonly used feed ingredients in poultry diets, contain 0.24 and 0.55% total P, respectively. However, approximately 70% of this P is present as phytate (Woyengo and Nyachoti, 2011), which is largely unavailable to poultry due to their limited endogenous phytase activity (Morgan et al., 2015). To meet P requirements, Pi is routinely added to poultry feed, often in the form of mono- or dicalcium phosphate (MCP or DCP, respectively), which are produced from phosphate rock; however, it is a finite resource and global demand for Pi is high (Dersjant-Li et al., 2022). In addition, undigested P is excreted, representing inefficient use of a limited resources and contributing to environmental pollution. Excess P in manure may contaminate soil and groundwater and disrupt ecosystems (Abbasi et al., 2019). Therefore, strategies that reduce the Pi content in the broiler diets may lower the environmental impact of the poultry industry and potentially reduce feed costs, while ensuring that mineral requirements are adequately met (Lim et al., 2024).

Most feed nutritionists formulate diets by adding microbial phytase to partially or completely replace Pi supplied as MCP or DCP (Dersjant-Li et al., 2018). However, the effects of complete Pi replacement with commercial phytase throughout the broiler growth cycle have not been extensively investigated. Ribeiro et al. (2019) reported that complete replacement of Pi with a high phytase dose (4000 FTU) during the grower and finisher phases of broilers negatively affected performance and bone conformation. Total replacement of Pi during the starter phase is particularly challenging due to the high P requirement for rapid growth and bone mineralisation at this stage (Dersjant-Li et al., 2022). The latter authors demonstrated that complete replacement of Pi with microbial phytase (1000–3000 FTU/kg) combined with 2000 xylanase units (XU)/kg during the entire broiler growth cycle maintained or improved growth performance and tibia ash content. In that study, broilers were randomly allocated to pens with an equal number of males and females. However, when evaluating the capacity of phytase to fully replace Pi, it is important to analyse males and females separately.

The present study evaluated the efficiency of completely replacing Pi with bacterial phytase (1000 or 15000 FTU/kg). This design enabled a precise determination of the phytase capacity to substitute for inorganic phosphate, ensuring that any effects on growth, bone ash, and carcass traits were attributable solely to phytase activity rather than non-starch polysaccharide (NSP) degradation.

Material and methods

Animals, experimental design, and diets

All animal procedures were approved by the Kasetsart University Animal Care and Use Committee number ACKU66-AGR-008. The experiment was conducted in the experimental poultry house of the Kasetsart University, Thailand.

A total of 390 one-day-old male Ross 308 broilers were used in a completely randomised design with 3 treatments, 10 replicates, 13 birds each. Birds were reared from day 1 to 35 in a closed house equipped with an evaporative cooling system and bedded with rice husk litter. Chicks were vaccinated against Newcastle disease and infectious bursal disease on day 10. Each pen was equipped with a feeder and nipple drinkers, and birds had *ad libitum* access to feed and water throughout the experiment.

The experimental diets were formulated for 3 developmental phases: starter (days 1–10), grower (days 11–23), and finisher (days 24–35). Birds had *ad libitum* access to feed and water throughout the study. The composition of the experimental diet is presented in Table 1. The positive control (PC) diet was based on corn, broken rice, soybean, wheat, rice bran, rapeseed meal, and contained Pi from monocalcium phosphate. Two experimental diets (P1000 and P1500) were formulated to be nutritionally comparable to the PC diet but contained no added Pi. Instead, calcium (Ca) and available phosphorus (avP) levels were reduced by 0.17–0.24% and 0.20–0.28%, respectively, and the diets were supplemented with phytase at 1000 or 1500 FTU/kg for P1000 and P1500 diets, respectively.

The phytase used was a novel biosynthetic bacterial 6-phytase (Rovabio PhyPlus, Adisseo France S.A.S, Antony, France) derived from *Buttiauxella* sp. and produced in *Trichoderma reesei*.

Growth performance and sample collection

Growth performance and feed consumption were recorded by phases on days 1, 10, 23, and 35. These data were used to calculated body weight (BW) gain (BWG), feed intake (FI), and feed conversion ratio (FCR). In addition, the European Poultry Efficiency Factor (EPEF) was calculated to

Table 1. Ingredients and calculated nutritional composition of treatments, % (as fed basis)

Treatments	Starter (days 0–10)			Grower (days 11–23)			Finisher (days 24–35)		
	PC	P1000	P1500	PC	P1000	P1500	PC	P1000	P1500
Ingredient									
corn	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00	20.00
broken rice	20.04	20.04	20.04	22.12	22.12	22.12	24.50	24.50	24.50
wheat	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
soybean meal, 48% CP	20.44	20.44	20.44	20.25	20.25	20.25	20.03	20.03	20.03
full fat soybean	15.00	15.00	15.00	10.00	10.00	10.00	5.00	5.00	5.00
rice bran, extracted	6.00	6.00	6.00	6.00	6.00	6.00	8.00	8.00	8.00
rapeseed meal	5.00	5.00	5.00	6.00	6.00	6.00	6.00	6.00	6.00
palm oil	3.75	3.75	3.75	6.27	6.27	6.27	7.73	7.73	7.73
monocalcium phosphate 22	1.45	-	-	1.32	-	-	1.03	-	-
limestone	1.32	1.32	1.32	1.16	1.16	1.16	1.03	1.03	1.03
salt	0.56	0.56	0.56	0.46	0.46	0.46	0.28	0.28	0.28
sodium bicarbonate	-	-	-	0.15	0.15	0.15	0.30	0.30	0.30
DL-methionine	0.38	0.38	0.38	0.32	0.32	0.32	0.28	0.28	0.28
L-lysine	0.34	0.34	0.34	0.27	0.27	0.27	0.23	0.23	0.23
L-threonine	0.16	0.16	0.16	0.12	0.12	0.12	0.08	0.08	0.08
choline chloride 60%	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08	0.08
antioxidant and toxin binder	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16	0.16
coccidiostat	0.05	0.05	0.05	0.05	0.05	0.05	-	-	-
vitamin and mineral premix[1]	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24	0.24
corn cob	0.03	1.46	1.45	0.03	1.33	1.32	0.03	1.04	1.03
phytase 5000 FTU/g	-	0.02	0.03	-	0.02	0.03	-	0.02	0.03
Nutrient composition, %									
ME, kcal/kg	2950.00	2950.00	2950.00	3050	3050	3050	3100.00	3100.00	3100.00
crude protein	22.00	22.00	22.00	20.50	20.50	20.50	19.00	19.00	19.00
calcium	0.92	0.68	0.68	0.83	0.61	0.61	0.72	0.55	0.55
total phosphorus	0.78	0.45	0.45	0.74	0.45	0.45	0.68	0.45	0.45
available phosphorus	0.43	0.15	0.15	0.40	0.15	0.15	0.34	0.14	0.14
fibre	4.37	4.37	4.37	4.22	4.22	4.22	4.21	4.21	4.21
fat	8.21	8.21	8.21	9.76	9.76	9.76	10.27	10.27	10.27
methionine	0.72	0.72	0.72	0.65	0.65	0.65	0.59	0.59	0.59
methionine + cysteine	1.08	1.08	1.08	0.99	0.99	0.99	0.91	0.91	0.91
lysine	1.44	1.44	1.44	1.29	1.29	1.29	1.16	1.16	1.16
threonine	0.97	0.97	0.97	0.88	0.88	0.88	0.78	0.78	0.78
sodium	0.23	0.23	0.23	0.23	0.23	0.23	0.20	0.20	0.20
DEB, mEq/kg	247	247	247	242	242	242	243	243	243
phytate phosphorus	0.31	0.31	0.31	0.31	0.31	0.31	0.32	0.32	0.32

PC – positive control, P1000 – basal diet replaced total additional inorganic phosphate (Pi) with phytase supplementation at 1000 FTU/kg, P1500 – basal diet replaced total additional Pi with phytase supplementation at 1500 FTU/kg; CP – crude protein, DEB – dietary electrolyte balance; [1] provided per kg of diet: IU: vit. A (transretinyl acetate) 4 800 000, vit. D$_3$ (cholecalciferol) 1 200 000, vit. E 6 000; mg; menadione 600, thiamin 600, riboflavin 2 200, pyridoxine 800, cobalamin 4, nicotinic acid 10 000, pantothenic acid 4 800, folic acid 200, biotin 48, Fe (from ferrous sulphate) 16 000, Cu (from copper sulphate) 3 200, Mn (from manganese sulphate) 32 000, Zn (from zinc oxide) 24 000, I (from calcium iodate) 200, Se (from sodium selenite) 40, cobalt 40

provide an integrated measure of performance, incorporating FCR, liveability, and body weight.

At day 35 of age, 10 birds per treatment (1 bird per replicate) with a similar mean body weight were selected for sampling. Blood was collected from the brachial vein into serum clot activator tubes and analysed for Ca and Pi. Birds were subsequently euthanised by carbon dioxide (CO_2) and slaughtered for determination of carcass, breast muscle, leg, wing, liver, and kidney weights. Tibia bones were collected and stored at −20 °C for subsequent analysis of bone strength, and ash Ca, and P contents.

Chemical analysis

Serum concentrations of Ca and Pi were analysed using commercial dry chemistry kits (SPOTCHEMTM II, arkray®, ARKRAY, Japan) with an automated biochemical analyser (SPOTCHEMTM EZ SP-4430, ARKRAY, Japan) at the Veterinary

Technical Laboratory, Kasetsart University, Thailand. All tibiae were cleaned of fibula, muscle, and connective tissue. The left tibiae were analysed for ash, Ca, and P using in-house procedures based on AOAC methods 942.05, 927.02, and 965.17, respectively (AOAC International, 2023).

Bone strength

The right tibiae were used for bone strength measurements with a universal testing machine. Each sample was placed on beam supports, and a single load was applied at the midpoint. The compression rate was set to apply a force corresponding to approximately 3–6% of the ultimate load. Pressure was increased continuously, without interruption, at a rate of 0.9–1.2 MPa/min (9–12 kgf/cm^2/min) until fracture occurred. The maximum force at break was recorded. Measurements were conducted in the Engineering Laboratory at Kasetsart University, Thailand.

Statistical analysis

Data are presented as mean ± standard deviation (SD) and standard error of the mean (SEM). Statistical analysis was performed using one-way ANOVA with the GLM (Generalised Linear Model) procedure of SAS software (SAS Institute, 2003). Differences between treatment means were assessed using Duncan's multiple range test (Duncan, 1955). Statistical significance was set at $P < 0.05$.

Results

Growth performance

At 35 days of age, the average BW was 2 463.50 g and the feed conversion ratio (FCR) was 1.398, slightly exceeding the breed standard (Aviagen, 2022). Table 2 presents the effects of dietary treatments on growth performance during each phase and over the entire experimental period.

Table 2. Effect of total replacement of inorganic phosphate by phytase on growth performance in broiler diets

Parameters	Treatments			SEM	*P*-value
	PC	P1000	P1500		
Starter, days 0–10					
Initial BW, g/bird	42.67 ± 0.18	42.68 ± 0.19	42.69 ± 0.16	0.0307	0.9672
BWG, g/bird	279.15 ± 12.41	272.76 ± 8.97	271.32 ± 10.80	2.008	0.2446
ADG, g/bird	27.92 ±1.24	27.28 ± 0.90	27.13 ± 1.08	0.201	0.2455
Avg. FI, g/bird	287.60 ± 14.44^{a}	275.00 ± 9.96^{b}	276.30 ± 6.78^{b}	2.182	0.0284
FCR	1.029 ± 0.01	1.008 ± 0.02	1.017 ± 0.03	0.004	0.0812
Grower, day 11–23					
BWG, g/bird	977.95 ± 41.76	951.79 ± 22.03	960.22 ± 26.69	5.876	0.1816
ADG, g/bird	75.23 ± 3.21	73.21 ±1.70	73.86 ± 2.05	0.452	0.1812
Avg. FI, g/bird	1368.94 ± 43.32^{a}	1334.43 ± 28.53^{b}	1299.47 ± 21.24^{c}	7.761	0.0002
FCR	1.402 ± 0.05	1.401 ± 0.04	1.356 ± 0.04	0.010	0.0532
Starter-grower, days 0–23					
BWG, g/bird	1257.10 ± 49.29	1224.55 ± 26.62	1231.54 ± 34.36	7.171	0.1496
ADG, g/bird	54.66 ± 2.14	53.24 ± 1.16	53.55 ± 1.50	0.312	0.1496
Avg. FI, g/bird	1656.50 ± 48.56^{a}	1609.49 ± 34.21^{b}	1575.78 ± 22.63^{c}	8.915	0.0002
FCR	1.319 ± 0.04^{a}	1.315 ± 0.03^{a}	1.280 ± 0.04^{b}	0.007	0.0413
Finisher, days 24–35					
BWG, g/bird	1180.85 ± 116.51	1238.91 ± 71.64	1257.56 ± 115.41	19.186	0.2413
ADG, g/bird	98.40 ± 9.71	103.24 ± 5.97	104.80 ± 9.62	1.599	0.2414
Avg. FI, g/bird	1884.82 ± 51.47^{a}	1753.08 ± 73.67^{b}	1831.72 ± 97.80^{a}	16.838	0.0026
FCR	1.611 ± 0.17^{a}	1.417 ± 0.04^{b}	1.463 ± 0.09^{b}	0.025	0.0019
All phases, days 0–35					
BWG, g/bird	2437.94 ± 101.66	2463.46 ± 70.93	2489.09 ± 129.16	18.616	0.5495
ADG, g/bird	69.66 ± 2.90	70.38 ± 2.03	71.12 ± 3.69	0.532	0.5492
Avg. FI, g/bird	3541.32 ± 69.62^{a}	3362.57 ± 96.92^{b}	3407.50 ± 105.01^{b}	21.451	0.0005
FCR	1.455 ± 0.07^{a}	1.367 ± 0.02^{b}	1.372 ± 0.05^{b}	0.012	0.0009
FCG	23.45 ± 1.05^{a}	22.36 ± 0.95^{b}	21.96 ± 0.84^{b}	0.204	0.0049
EPEF	473.366 ± 46.74	504.170 ± 31.91	508.510 ± 51.42	8.302	0.1715

PC – positive control, P1000 – basal diet replaced total additional inorganic phosphate (Pi) with phytase supplementation at 1000 FTU/kg, P1500 – basal diet replaced total additional Pi with phytase supplementation at 1500 FTU/kg; BW – body weight, BWG – body weight gain, ADG – average daily gain, Avg. FI – average feed intake, FCR – feed conversion ratio, FCG - feed cost for 1 kg of weight gain, EPEF – European production efficiency factor = % liveability × BW (kg) × 100/FCR × trial duration (day); SEM – standard error of the mean, n = 10; values are presented as mean ± SD; ^{abc} – means in the same row with different superscripts are syignificantly different at $P < 0.05$

For the entire growth cycle, broilers fed diets in which Pi was completely replaced by phytase showed comparable or improved performance relative to the PC group. During the starter phase (days 0–10), BWG and average daily gain (ADG) were not significantly different between the experimental groups. FI in the P1000 and P1500 groups was significantly lower than in the PC group ($P < 0.05$). Although FCR did not differ significantly between the groups, the P1000 group showed an improvement trend (1.008) relative to the PC (1.029) and P1500 (1.017) treatments.

During the grower phase (days 11–23), the FI differed significantly among treatments ($P < 0.05$). The lowest FI was observed in the P1500 group (1 299.47 g/bird), followed by the PC group (1 368.94 g/bird) and the P1000 group (1 334.43 g/bird). BW did not differ significantly between the groups ($P < 0.05$) with values of 977.95, 951.79, and 960.22 g/bird for the PC, P1000, and P1500 groups, respectively. FCR was lower ($P < 0.05$) in the P1500 group (1.356) compared with the PC (1.402) and P1000 (1.401) groups.

Table 3. Effect of total replacement of added inorganic phosphate by phytase supplementation on carcass characteristics in broiler diets, %

Parameters	Treatments			SEM	*P*-value
	PC	P1000	P1500		
Day 23					
carcass yield	93.21 ± 0.85	93.24 ± 0.98	93.23 ± 0.53	0.143	0.9942
breast yield	22.51 ± 1.23	22.83 ± 1.51	22.96 ± 0.73	0.215	0.6953
leg yield	20.21 ± 5.54	22.12 ± 1.66	23.49 ± 0.75	0.643	0.1107
wing yield	8.07 ± 0.33	7.84 ± 0.38	8.19 ± 0.33	0.067	0.0919
liver yield	2.31 ± 0.24	2.60 ± 0.26	2.47 ± 0.15	0.041	0.2798
kidney yield	0.67 ± 0.18	0.68 ± 0.21	0.66 ± 0.15	0.032	0.9749
Day 35					
carcass yield	93.45 ± 0.87	93.62 ± 0.58	93.34 ± 0.79	0.135	0.7185
breast yield	25.82 ± 2.04	26.57 ± 2.13	25.89 ± 1.54	0.344	0.6385
leg yield	24.93 ± 1.21	24.40 ± 1.64	24.60 ± 1.11	0.239	0.6829
wing yield	7.94 ± 0.37	8.11 ± 0.45	8.13 ± 0.39	0.073	0.5494
liver yield	2.11 ± 0.28	1.98 ± 0.23	2.10 ± 0.22	0.045	0.4534
kidney yield	0.68 ± 0.08	0.65 ± 0.08	0.59 ± 0.13	0.019	0.1399

PC – positive control, P1000 – basal diet replaced total additional inorganic phosphate (Pi) with phytase supplementation at 1000 FTU/kg, P1500 – basal diet replaced total additional Pi with phytase supplementation at 1500 FTU/kg; SEM – standard error of the mean, n = 10; values are presented as mean ± SD; $P > 0.05$ (no statistically significant)

During the finisher phase (days 24–35), BWG and ADG did not differ significantly between the groups. However, FI was lower in the P1000 group compared to the other groups ($P < 0.05$). FCR was improved ($P < 0.05$) in both the P1000 and P1500 groups relative to the PC group.

For cumulative growth (days 0–35), BWG and ADG again did not differ significantly between the groups ($P > 0.05$). FI and FCR were lower ($P < 0.05$) in the P1000 and P1500 groups compared to the control group. The EPEF calculated as (liveability [%] × BW (kg) × 100) / (FCR × trial duration [day]) was 473.366 for the PC group, 504.170 for the P1000 group, and 508.510 for the P1500 group. Although differences were not significant ($P > 0.05$), a tendency towards higher values was observed in in the P1000 and P1500 groups. Finally, feed cost per gain (FCG) was significantly lower ($P < 0.05$) in the P1000 (22.36 baht/kg) and P1500 (21.96 baht/kg) groups compared to the PC group (23.45 baht/kg).

Carcass characteristics

Table 3 presents the effects of dietary treatments on carcass yield at the end of the grower phase (day 23) and the finisher phase (day 35). Carcass yield (%), breast yield (%), leg yield (%), wing yield (%), liver yield (%), and kidney yield (%) did not differ significantly ($P > 0.05$) among treatments.

Bone characteristics

The modulus of rupture (MPa), used to assess bone strength at fracture, differed between treatments ($P < 0.05$), with values of 23.81 MPa for the PC group, 24.33 MPa for the P1000 group, and 20.68 MPa for the P1500 group.

Tibia ash content and tibia and serum Ca and P contents

Tibia ash content was similar in all groups: 14.86% in the P1000 group, 15.22% in the P1500 group, and 14.95% in the PC group. Consistently, tibia and serum Ca and P concentrations did not differ between treatments ($P > 0.05$; Table 5).

Table 4. Effect of total replacement of added inorganic phosphate by phytase supplementation on bone strength of tibia in broiler diets

Parameters	Treatments			SEM	*P*-value
	PC	P1000	P1500		
Ultimate load, N	188.74 ± 36.20	203.46 ± 60.63	179.62 ± 51.40	9.064	0.5730
Modulus of rapture, MPa	23.81 ± 4.17	24.33 ± 8.27	20.68 ± 45.50	1.089	0.3466

PC – positive control, P1000 – basal diet replaced total additional inorganic phosphate (Pi) with phytase supplementation at 1000 FTU/kg, P1500 – basal diet replaced total additional Pi with phytase supplementation at 1500 FTU/kg; SEM – standard error of the mean, n = 10; values are presented as mean ± SD; *P* > 0.05 (no statistically significant)

Table 5. Effect of total replacement of added inorganic phosphate by phytase supplementation on tibia ash, tibia Ca, tibia P, serum Ca, and serum P in broiler diets, %

Parameters	Treatments			SEM	*P*-value
	PC	P1000	P1500		
Tibia ash	14.95 ± 0.99	14.86 ± 1.16	15.22 ± 0.88	0.232	0.8323
Tibia Ca	5.16 ± 0.42	5.44 ± 0.73	5.22 ± 0.81	0.152	0.7264
Tibia P	2.43 ± 0.33	2.50 ± 0.41	2.64 ± 0.48	0.092	0.6826
Serum Ca	8.50 ± 0.45	9.24 ± 1.75	8.98 ± 1.54	0.308	0.6313
Serum P	4.95 ± 0.45	5.10 ± 0.83	4.88 ± 0.51	0.138	0.8184

PC – positive control, P1000 – basal diet replaced total additional inorganic phosphate (Pi) with phytase supplementation at 1000 FTU/kg, P1500 – basal diet replaced total additional Pi with phytase supplementation at 1500 FTU/kg; SEM – standard error of the mean, n = 10; values are presented as mean ± SD; *P* > 0.05 (no statistically significant)

Discussion

Phytate is an anti-nutritional factor that can chelate essential minerals such as Ca, zinc (Zn), and iron (Fe), thereby reducing their bioavailability (Gibson et al., 2018; Humer et al., 2015). It contains six phosphate groups and can form insoluble complexes with other nutrients, while also suppressing pepsin and trypsin activity in the gut, leading to reduced protein digestibility (Selle et al., 2000). High dietary phytate levels, in the absence of exogenous phytase, can reduce nutrient availability below formulated requirements (Choi et al., 2025). Therefore, birds may increase feed intake to compensate for reduced nutrient availability and meet their nutritional requirements (Babatunde et al., 2022).

In this study, average feed intake in the P1000 and P1500 groups was lower than in the PC group; however, ADG and FCR were improved in both phytase-supplemented groups. Phytase reduces protein–phytate complexes, thereby increasing the susceptibility of dietary protein to pepsin in the upper gastrointestinal tract. Increased pepsin activity facilitates the breakdown of polypeptides into oligopeptides and promotes their intestinal absorption, ultimately improving amino acid digestibility (Wang et al., 2017; Fu et al., 2021). Previous studies reported that supplementation of broiler diets with 1000 FTU/kg significantly improved apparent ileal amino acid digestibility coefficients (Amerah et al., 2014; Martínez-Vallespín et al., 2022). In addition, increased apparent ileal Na digestibility was shown to enhance Na^+/K^+-ATPase activity, thereby improving intestinal uptake of amino acids and glucose and supporting growth performance (Kiela and Ghishan, 2016; Akter et al., 2019).

Hydrolysis of phytate by phytase releases myo-inositol, which regulates adenosine monophosphate-activated protein kinase (AMPK) in enteroendocrine cells. Accordingly, reduced appetite and feed intake in broilers receiving phytase-supplemented diets may be associated with the regulation of energy homoeostasis and feedback mechanisms in the central nervous system (Tachibana et al., 2012; Hu et al., 2019; Prakash et al., 2021). Bello et al. (2022) evaluated the effects of supplementing low Ca and Pi-free broiler diets with 1000, 2000, or 3000 FTU/kg and reported a significantly reduced feed intake ($P < 0.05$) alongside improved FCR. Taken together with previous findings and the present results, supplementation with bacterial phytase improves feed efficiency in broilers by reducing the anti-nutritional effects of phytate and increasing the digestibility of P and other nutrients.

On day 35, carcass yield did not differ between the three groups. Muscle development depends strongly on phosphorus for protein synthesis and energy metabolism (Jahan et al., 2025); therefore, P deficiency is typically associated with reduced feed intake and impaired muscle deposition (Cowieson et al., 2020). The comparable breast and leg yields between the PC and the high-dose phytase groups (P1000 and P1500) demonstrated that phytase effectively mitigated the risks associated with a Pi-free diet (Cowieson and Bedford, 2009). This efficient hydrolysis of phytate-bound P supported maximal muscle growth without compromising metabolic status, as evidenced by the absence of differences in liver and kidney relative weights. Similarly, previous studies have observed that a novel bacterial 6-phytase can completely replace Pi and maintain or improve carcass and breast meat yield (Marchal et al., 2021). The efficacy of high-dose phytase was further supported by bone quality, which is as a key indicator of dietary P bioavailability. In the present study, phytase supplementation did not affect tibia width, weight, and breaking strength, and these parameters were comparable to the PC. The preservation of bone breaking strength in the 1000 and 1500 FTU/kg groups suggests that P released from the phytate complex was sufficient to support hydroxyapatite crystal formation without impairing muscle development. These findings confirm that the tested phytase dosages effectively provide adequate Ca and P to maintain both physiological homeostasis and optimal skeletal mineralisation (Rath et al., 2000). Scholey et al. (2018) supplemented a Pi-free broiler diet with 1000 FTU/kg and obtained equivalent tibia strength to the control diet, although tibia ash content was reduced. In contrast, in the present study, tibia ash, Ca, and P contents did not differ among treatments. These findings are consistent with a study showing that the inclusion of 250–3000 FTU/kg in lowPi diets was sufficient to maintain bone mineralisation and preserve tibia ash, Ca, and P levels (Sampath et al., 2023).

Serum Ca and P levels in the present study remained consistent across all experimental groups, with P concentrations ranging from 4.88 to 5.10 mg/dl. These results were consistent with Lim et al. (2024), who observed that high-dose phytase supplementation (1000 and 1500 FTU/kg) maintained serum P stability even in low-mineral diets. Serological P is a tightly regulated parameter, controlled by the balance between intestinal absorption, renal excretion, and bone mobilisation (Wagner, 2024). Under P-deficient conditions, birds typically activate a hormonal response involving parathyroid hormone (PTH) and active vitamin D_3 to mobilise bone minerals (Jacquillet and Unwin, 2019). However, the stability of serum parameters in the present study, together with the absence of decline in bone ash or strength, indicated that systemic mineral homeostasis was maintained without the need for compensatory bone resorption. This confirmed that the bacterial phytase effectively hydrolysed the ester bonds of dietary phytate, supplying sufficient orthophosphate to the plasma pool to match the kinetic bioavailability of inorganic phosphate provided in the control diet. Moreover, serum Ca levels remained unaffected in all treatments, ranging from 8.50 to 9.24 mg/dl. High concentrations of free phytate (IP6) are potent chelators of cationic minerals, readily forming insoluble Ca-phytate complexes that impair intestinal calcium absorption (Selle et al., 2009). The maintenance of stable serum Ca in the phytase-supplemented groups indicated that high-dose phytase (1000 and 1500 FTU/kg) degraded these anti-nutritional complexes, thereby supporting mineral availability and absorption.

Our results show that 1000 FTU/kg phytase represents an optimal dose for the complete replacement of Pi in commercial broiler diets based on corn, broken rice, and soybean meal. While the P1500 group showed a transient improvement in FCR during the starter phase, the P1000 group achieved comparable overall efficiency by the end of the trial. No additional performance benefits were observed when phytase was increased from 1000 to 1500 FTU/kg. These findings suggest that 1000 FTU/kg provides sufficient enzymatic activity to meet the P requirements of broilers fed corn-broken rice-soybean meal-based diets.

Conclusions

In summary, complete replacement of inorganic phosphate with 1000 or 1500 FTU/kg of a novel biosynthetic bacterial 6-phytase in high-phytate diets reduced feed intake in broilers but maintained their growth performance, bone quality, and carcass characteristics. This approach may help reduce feed costs and potential environmental contamination without compromising production performance.

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Conflict of interest

The Authors declare that there is no conflict of interest.

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