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ORIGINAL PAPER

Effects of two levels of phytase supplementation on growth performance, nutrient retention, and tibia mineralisation in broiler ducks fed a diet deficient in available phosphorus

Ł. Szymański[1], P. Konieczka[1,*], B. Fotschki[2], A. Drażbo[1], L. Nollet[3] and K. Kozlowski[1]

[1] University of Warmia and Mazury in Olsztyn, Faculty of Animal Bioengineering, Department of Poultry Science and Apiculture, Oczapowskiego 5, 10-719 Olsztyn, Poland
[2] Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Tylińskiego 18, 10-683 Olsztyn, Poland
[3] Huvepharma N.V., Uitbreidingsstraat 80, 2600 Antwerpen, Belgium

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* Corresponding author:
e-mail: p.konieczka@uwm.edu.pl

ABSTRACT. The aim of the study was to assess the efficacy of two dietary levels of 6-phytase in male ducks fed diets with reduced available phosphorus (P) over a 42-day period. A total of 320 ducks (Cherry Valley SM3) were divided into 4 groups, each consisting of 16 floor pens (replicates) of 5 birds. Birds were offered diets during the starter (days 1–14) and grower (days 15–42) phases that contained standard available P content (0.50 and 0.38%, respectively; control, T1), reduced available P content (0.30 and 0.20%, respectively; negative control, T2), or diets as in T2 supplemented with phytase at 1000 (T3) or 2000 (T4) phytase units (FTU)/kg diet. During the experiment, birds' performance was recorded, and excreta were collected for nutrient digestibility determination. At the end of the trial, carcass traits, breast muscle quality, and tibia mineralisation were determined. Data (n = 16 per treatment) were analysed using one-way ANOVA, with $P < 0.05$ considered significant. Birds fed the T2 diet showed reduced body weight (907 vs. 947 g; $P = 0.005$) and daily gain (60.7 vs. 64.5 g; $P = 0.043$) during the starter phase. Supplementation with 6-phytase significantly improved nitrogen, calcium, P, and ash retention (2.5, 7.4, 11.1, 5.2%, respectively) with a dose-dependent effect on mineral utilisation. Increased tibia ash content in phytase-supplemented ducks further confirmed improved P bioavailability ($P = 0.005$). Carcass yield was unaffected; however, phytase supplementation reduced breast muscle redness and yellowness, potentially linked to decreased oxidative stress. Overall, phytase effectively mitigates the negative effects of dietary P deficiency in ducks, improving growth performance, nutrient utilisation, and selected aspects of meat quality, particularly in the early growth phase.

Introduction

Conventional duck diets are frequently deficient in available phosphorus (P), as plant-based feed ingredients store P predominantly in the form of phytate (Jiang et al., 2020). Ducks have a limited capacity to hydrolyse this compound due to insufficient endogenous phytase activity (Adeola, 2018). Consequently, P present in plant-based feedstuffs is poorly utilised, necessitating careful diet formulation with the inclusion of inorganic P sources or exogenous phytase to increase its availability (Zhang et al., 2022). Broiler ducks are particularly sensitive to reduced levels of non-phytate P (nPP), also referred to as available P, which can adversely affect their growth, bone development, and overall health. Inadequate P intake can result in disturbed bone mineralisation, reduced growth, lower carcass and muscle weights, as well as impaired intestinal function. Therefore, maintaining adequate P levels is essential for optimal duck development and health. As in other poultry species, dietary phytase is used to improve P utilisation. This enzyme hydrolyses phytate present in plant-based feeds, improving growth and bone mineralisation, while reducing the demand for inorganic P and minerals in the diet. In addition, phytase supplementation lowers mineral excretion into the environment and reduces feed cost. By releasing bound P and other essential minerals, such as zinc (Zn) and calcium (Ca), dietary phytase increases their digestibility and bioavailability. The scientific literature regarding the use of phytase in duck nutrition is currently limited, with relatively few studies addressing its effects on nutrient digestibility, growth performance, or P utilisation compared to other poultry species (Fan et al., 2019; Liu et al., 2020; Wu et al., 2021). Numerous scientific opinions issued by the European Food Safety Authority (EFSA) Panels have evaluated the safety and efficacy of feed additives containing 6-phytase for poultry. However, most assessments concern chickens for fattening, laying hens, or turkeys, and the resulting data are extrapolated to other minor poultry species, including ducks.

Therefore, the objective of the experiment was to assess the efficacy of two dietary levels of 6-phytase produced by a genetically modified strain of *Komagataella phaffi* on growth performance and mineral bioavailability parameters in broiler ducks fed diets with a reduced level of available P during a 42-day period. Cherry Valley ducks are a highly efficient Pekintype breed, specifically developed for meat production. They are characterised by rapid growth, high feed efficiency, and favourable carcass quality, including a high breast meat yield (Yang et al., 2025). This breed is widely used in commercial production worldwide and is typically reared for slaughter at approximately 42 days of age, consistent with the experimental timeframe of the present study.

Material and methods

Birds and experimental treatments

All procedures involving animals were conducted in accordance with the ARRIVE guidelines, EU Regulations (Directive 2010/63/EU), and Polish Law. This study was conducted by qualified veterinarians responsible for all procedures that involved the handling of birds. No procedures causing pain or distress were applied. The experimental protocol did not require approval from the Local Ethics Committee for Animal Experimentation in Olsztyn. All animal procedures strictly adhered to the requirements of the Polish Act of 15 January 2015 on the Protection of Animals Used for Scientific or Educational Purposes.

The study was carried out at the experimental farm in Bałdy near Olsztyn, belonging to the University of Warmia and Mazury (UWM) in Olsztyn, Poland. The trial lasted 42 days and involved one-day-old male Cherry Valley (SM3) broiler ducks obtained from a commercial hatchery. Upon arrival, birds were randomly allocated to experimental groups and replicates based on equal average weight per pen. Ducks were housed in floor pens with netting walls to prevent movement between pens. The experiment was carried out in a windowed poultry house equipped with programmable artificial lighting and climate control systems, including automated electric heating and forced ventilation. The heating program followed the breeder's recommendations. A total of 320 birds were used (4 × 80), housed in 64 pens (0.7 m^2 each), with five birds per pen. Each treatment group consisted of 16 pens (replicates). Drinking water was provided *ad libitum* via nipple drinkers. The birds were assigned to the following groups:

- T1 – positive control – diet with standard P content (Table 1)
- T2 – negative control – diet with reduced P content (Table 2)
- T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg
- T4 – negative control + phytase at 2000 FTU/kg.

The feed mixtures, based on maize and soybean meal, were prepared by Research Diet Services (Wijk bij Duurstede, The Netherlands). Two feeding periods were applied: starter and grower (0–14 days and 15–42 days, respectively). Phytase was added to the feed according to the minimum declared specification. Feed was produced in crumbled form for the starter phase and pelleted form for the grower phase, with pelleting temperatures maintained below 80 °C. The basal diets were analysed for crude protein (AOAC International, 2006; 990.03), crude fibre (978.10), crude fat (920.39), dry matter (930.15), ash (942.05), phosphorus (965.17), and Ca (927.02).

Measurements

Body weight was measured on a pen basis on days 1, 14, 28, and 42. Feed intake was recorded

Table 1. Composition and nutrient content of positive control diet (PC), %

Item	Feeding period	
	1 to 14 days	15 to 42 days
Ingredients		
maize	58.62	66.00
soybean meal	34.83	28.31
soybean oil	1.48	1.41
limestone	1.27	1.34
monocalcium phosphate	2.14	1.52
sodium bicarbonate	0.23	0.20
sodium chloride	0.31	0.31
magnesium oxide	0.03	0.03
choline chloride	0.12	0.13
L-lysine hydrochloride	0.25	0.18
DL-methionine	0.32	0.22
L-threonine	0.10	0.05
toxin binder	0.10	0.10
vitamin-mineral premix	0.20	0.20
Nutritional value		
metabolizable energy, kcal/kg	2875	2950
crude protein	21.10	18.49
crude fat	4.67	4.66
crude fibre	3.40	3.19
lysine	1.33	1.11
methionine	0.64	0.51
methionine + cysteine	1.00	0.84
threonine	0.88	0.74
tryptophan	0.25	0.21
Ca	1.00	0.90
available P	0.50	0.38
Na	0.19	0.18
Cl	0.27	0.26

Table 2. Composition and nutrient content of negative control diet (NC), %

Item	Feeding period	
	1 to 14 days	15 to 42 days
Ingredients		
maize	60.70	68.06
soybean meal	34.69	28.08
soybean oil	0.78	0.73
limestone	1.20	1.21
monocalcium phosphate	1.08	0.59
sodium bicarbonate	0.15	0.11
sodium chloride	0.31	0.31
magnesium oxide	0.03	0.04
choline chloride	0.12	0.13
L-lysine hydrochloride	0.24	0.18
DL-methionine	0.31	0.21
L-threonine	0.10	0.05
toxin binder	0.10	0.10
vitamin-mineral premix	0.20	0.20
Nutritional value		
metabolizable energy kcal/kg	2875	2950
crude protein	21.19	18.54
crude fat	4.03	4.05
crude fibre	3.44	3.22
lysine	1.33	1.11
methionine	0.64	0.51
methionine + cysteine	1.00	0.84
threonine	0.88	0.74
tryptophan	0.25	0.21
Ca	0.80	0.70
available P	0.30	0.20
Na	0.17	0.16
Cl	0.27	0.26

and calculated for the following experimental periods: days 1–14, 15–28, 29–42, and 1–42. Mortality was recorded throughout the trial; birds that died or were removed after weighing were included in the feed intake and feed conversion ratio (FCR) calculations. FCR was calculated for all experimental periods.

Total tract apparent nutrient digestibility and retention coefficients (dry matter, ash, nitrogen, Ca, and P), as well as apparent metabolisable energy corrected to zero nitrogen retention (AME_N), were determined using yttrium oxide (Y_2O_3) as an indigestible dietary marker at a final concentration of 1.0 g of Y_2O_3/kg diet. At week 6, excreta were collected over three consecutive days from each pen in each group. Samples were stored in plastic bags at 20 °C, pooled per pen on the final day of collection to form one replicate, and subsequently freeze-dried for further analyses. Total tract apparent digestibility coefficients and nutrient retention were calculated using the following equation:

$$\text{digestibility (\%)} = [1 - (Y_2O_3 \text{ diet} / Y_2O_3 \text{ excreta}) \times (\text{excreta nutrient content} / \text{dietary nutrient content})] \times 100.$$

The AME_N of the experimental diets was calculated using the following formula:

$$AME_N \text{ (MJ/kg)} = \text{GE diet} - [\text{GE excreta} \times (Y_2O_3 \text{ diet}/ Y_2O_3 \text{excreta})] - 8{,}22 \times \{\text{N diet} - [\text{N excreta} \times (Y_2O_3 \text{ diet} / Y_2O_3 \text{ excreta})]\},$$

where: GE – gross energy, N – nitrogen, Y_2O_3 – yttrium oxide, and 8.22 – the energy equivalent of uric acid nitrogen (i.e. 8.22 kcal/kg uric acid nitrogen).

At the end of the experiment (day 42), 16 birds (one per pen) representing the average body weight (BW) of each treatment were slaughtered in the departmental processing facility. Birds were electrically stunned (400 mA; 350 Hz), suspended on a shackle line, and exsanguinated by a unilateral neck cut severing the right carotid artery and jugular vein. Subsequently, carcasses were defeathered and manually eviscerated. Non-edible viscera (intestines, proventriculus, gall bladder, spleen, oesophagus, and crop), head, legs, edible giblets (heart, gizzard, and liver), and the wing portion distal to the first joint were removed to obtain ready-to-cook carcass. After 24 h of chilling, carcass, abdominal fat and breast muscle weights were recorded. Organ and muscle weights were calculated relative to live BW and expressed as a percentage. Chilled breast muscles were subjected to physicochemical analysis, including pH_{24} value and colour assessment. Ultimate pH (24 h postmortem) was measured in duplicate using a Testo 206-pH2 pH-meter equipped with a piercing probe head for semisolid substances (Testo GmbH & Co., Lenzkirch, Germany). Meat colour parameters L* – (lightness), a* – (redness), and b* – (yellowness) were measured in the CIE LAB system (CIE, 1978) using the reflectance method. Measurements were taken in triplicate with a HunterLab MiniScan XE Plus spectrocolorimeter (Hunter Associates Laboratory Inc., Reston, VA, USA) at different points on the muscle cross-section area, using illuminant D65 and a 10° standard observer angle. The instrument was calibrated against standard black and white reference plates before each measurement session.

Tibiae were collected from the same birds, cleaned of adhering muscles and cartilage, and stored at −25 °C until analysis. The bones were assayed for dry matter and ash content. Tibiae were weighed, and their volume was determined by displacement in distilled water. Samples (25–500 mg) were digested with 4 ml H_2SO_4 (SUPRAPUR, Merck, Darmstadt, Germany) and 2 ml H_2O_2 (SUPRAPUR, Merck, Darmstadt, Germany) in closed 50 ml quartz vessels using a Multiwave® microwave digestion system (Anton Paar, Graz, Austria). After cooling, 5.0 ml of internal standard reagent (yttrium; 10 mg Y/L) = was added, and the digest was diluted to a final volume of 1000 ml with deionised (DI) water. To avoid acid interference, acid concentration in all solutions was adjusted to match that in the digested samples.

Statistics

Data were tested for normality using Shapiro-Wilk test prior to statistical analysis. All data were analysed by one-way ANOVA, and differences were considered significant at $P \leq 0.05$, while values between $0.05 < P \leq 0.10$ considered a near-significant trend (x,y). The pen served as the experimental unit for all parameters (n = 16 per treatment), with the exception of tibia dry matter and ash content (n = 12 per treatment). Results are presented as treatment means with pooled standard error of the mean (SEM), calculated as the standard deviation divided by the square root of the number of replicates.

Results

The analysed phytase activity was as follow: starter phase – T1 – 120 FTU/kg, T2 – 110 FTU/kg, T3 – 1230 FTU/kg, and T4 – 1200 FTU/kg; grower feed: T1 – 90 FTU/kg, T2 – 90 FTU/kg, T3 – 1245 FTU/kg, and T4 – 1290 FTU/kg. BW of ducks fed the reduced-P diet (T2) on days 14 and day 28 was significantly lower as compared to the remaining treatments i.e., positive control (T1) and phytase-supplemented groups (T3 and T4) (Tables 3–5). Average daily weight gain (ADWG) during days 1–14 was lowest in T2 ($P < 0.05$ vs. all other groups). For the 15–28 and 1–28 day periods, ADWG remained lowest in the negative control (group T2) but differed significantly only from group T1 ($P < 0.05$). Average daily feed intake (ADFI), calculated for days 1–14, was significantly lower in T2 compared to T1 birds ($P < 0.05$). A suggestive trend ($P = 0.053$) was noted towards reduced ADFI values during days 1–28 in both

Table 3. Growth performance of broiler ducks for the 1–14-day period

	BW, 1 days, kg	BW, 14 days, kg	ADWG, g	ADFI, g	FCR, kg/kg
Treatment					
T1	0.058 ± 0.001	0.953[a] ± 0.033	63.9[a] ± 2.4	77.6[a] ± 3.4	1.223 ± 0.043
T2	0.058 ± 0.001	0.907[b] ± 0.038	60.7[b] ± 2.7	73.7[b] ± 4.0	1.218 ± 0.043
T3	0.058 ± 0.001	0.945[a] ± 0.041	63.3[a] ± 3.0	75.3[ab] ± 3.5	1.190 ± 0.137
T4	0.058 ± 0.001	0.943[a] ± 0.032	63.2[a] ± 2.3	75.9[ab] ± 3.5	1.202 ± 0.046
SEM	<0.001	0.005	0.356	0.484	0.005
P-value	0.912	0.005	0.005	0.043	0.131

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, BW – body weigh, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio; values in same columns with no common superscript (ab) are significantly different at $P \leq 0.05$

Table 4. Growth performance of broiler ducks for the 15–28-day period

	BW, 14 days, kg	BW, 28 days, kg	ADWG, g	ADFI, g	FCR, kg/kg
Treatment					
T1	0.953[a] ± 0.033	2.612[a] ± 0.084	118.5[a] ± 4.2	203.6 ± 8.5	1.750 ± 0.095
T2	0.907[b] ± 0.038	2.448[b] ± 0.113	112.1[b] ± 7.6	203.5 ± 10.2	1.799 ± 0.133
T3	0.945[a] ± 0.041	2.572[a] ± 0.110	116.2[ab] ± 5.5	200.0 ± 5.9	1.726 ± 0.066
T4	0.943[a] ± 0.032	2.544[a] ± 0.067	114.6[ab] ± 3.9	198.5 ± 5.1	1.722 ± 0.071
SEM	0.005	0.014	0.749	0.991	0.012
P-value	0.005	<0.001	0.017	0.171	0.113

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet, T2 –negative cntrol – with a reduced P content of a die, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error mean, BW – body weight, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio; values in same columns with no common superscript [(ab)] are significantly different at $P \leq 0.05$

Table 5. Growth performance of broiler ducks for the 1–28-day period

	BW, 1 day, kg	BW, 28 days, kg	ADWG, g	ADFI, g	FCR, kg/kg
Treatment					
T1	0.058 ± 0.001	2.612[a] ± 0.084	91.2[a] ± 3.0	141.0[x] ± 4.4	1.563[ab] ± 0.061
T2	0.058 ± 0.001	2.448[b] ± 0.113	85.4[b] ± 4.0	138.3[xy] ± 4.2	1.598[b] ± 0.092
T3	0.058 ± 0.001	2.572[a] ± 0.110	89.8[ab] ± 3.9	137.5[y] ± 3.7	1.537[a] ± 0.030
T4	0.058 ± 0.001	2.544[a] ± 0.067	88.8[ab] ± 2.4	137.5[y] ± 3.1	1.541[a] ± 0.040
SEM	<0.001	0.014	0.508	0.520	0.008
P-value	0.912	<0.001	<0.001	0.053	0.034

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet;T2 – negative control – with a reduced P content of a die, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, BW – body weight, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio, values in same columns with no common superscript [(ab)] are significantly different ($P \leq 0.05$) and values between $0.05 < P \leq 0.10$ considered a near-significant trend[(xy)]

phytase-supplemented groups relative to T1 ($P = 0.053$; Table 5). As a consequence, FCR for days 1–28 was significantly higher in T2 compared to T3 and T4 ($P < 0.05$; Table 5). BW on day 42 did not differ between treatments ($P > 0.05$; Table 6). ADFI during days 29–42 days was highest in T2 and T4 and lowest in the positive control T1 ($P < 0.05$ vs. T2 and T4). Considering the entire feeding period (days 1–42), ADWG, ADFI, and FCR were comparable in all treatments ($P > 0.05$; Table 7). Livability among groups was also similar between groups, ranging from 96.3 to 100% ($P > 0.05$).

Dietary treatments had no significant effect on carcass yield and breast meat percentage ($P > 0.05$; Table 8). However, the relative weight of abdominal fat was significantly lower in T2 and T4 compared to group T1 ($P < 0.05$). Additionally, a near-significant trend was observed towards increased relative gizzard weight of ducks in T2 compared to the group receiving 2000 FTU/kg 6-phytase supplementation ($P = 0.068$). Breast muscle pH_{24} was significantly lower in all three groups receiving diets with reduced available P, irrespective of 6-phytase supplementation ($P < 0.05$ vs. T1).

Table 6. Growth performance of broiler ducks for the 29–42-day period

	BW, 28 days, kg	BW, 42 days, kg	ADWG, g	ADFI, g	FCR, kg/kg
Treatment					
T1	2.612[a] ± 0.084	4.029 ± 0.161	102.3 ± 9.1	228.0[b] ± 7.4	2.243 ± 0.142
T2	2.448[b] ± 0.113	3.923 ± 0.160	105.7 ± 9.1	238.6[a] ± 9.9	2.226 ± 0.137
T3	2.572[a] ± 0.110	3.965 ± 0.143	101.1 ± 5.1	233.9[ab] ± 6.4	2.331 ± 0.209
T4	2.544[a] ± 0.067	3.964 ± 0.170	99.9 ± 11.7	236.4[a] ± 8.2	2.387 ± 0.273
SEM	0.014	0.020	0.167	1.154	0.027
P-value	<0.001	0.336	0.315	0.005	0.105

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, BW – body weight, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio; values in same columns with no common superscript [(a,b)] are significantly different at $P \leq 0.05$

Table 7. Growth performance of broiler ducks for the 1–42-day period

	BW, 1 days, kg	BW, 42 days, kg	ADWG, g	ADFI, g	FCR, kg/kg	Liveability, %
Treatment						
T1	0.058 ± 0.001	4.029 ± 0.161	94.5 ± 3.8	170.9 ± 2.8	1.815 ± 0.055	98.8 ± 5.0
T2	0.058 ± 0.001	3.923 ± 0.160	92.6 ± 4.3	172.2 ± 4.1	1.839 ± 0.060	97.5 ± 6.8
T3	0.058 ± 0.001	3.965 ± 0.143	93.0 ± 3.4	171.6 ± 9.0	1.818 ± 0.070	96.3 ± 8.1
T4	0.058 ± 0.001	3.964 ± 0.170	93.0 ± 4.1	171.8 ± 1.3	1.846 ± 0.067	100.0 ± 0.0
SEM	<0.001	0.020	0.494	0.664	0.006	0.734
P-value	0.912	0.336	0.527	0.925	0.473	0.310

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, BW – body weight, ADWG – average daily weight gain, ADFI – average daily feed intake, FCR – feed conversion ratio; $P > 0.05$ (no statistically significant)

Table 8. Carcass traits and breast quality of broiler ducks, %

Treatment	T1	T2	T3	T4	SEM	*P*-value
BWbs, kg	4.100 ± 0.175	3.950 ± 0.168	3.985 ± 0.158	3.980 ± 0.123	0.026	0.172
Carcass yield	67.3 ± 1.1	67.4 ± 1.4	68.0 ± 1.6	68.3 ± 0.9	0.214	0.331
Breast	15.2 ± 1.6	14.2 ± 0.7	14.4 ± 1.3	14.7 ± 1.2	0.204	0.379
Abdominal fat	0.447^{a} ± 0.211	0.267^{b} ± 0.066	0.376^{ab} ± 0.173	0.269^{b} ± 0.075	0.026	0.030
Heart	0.429 ± 0.035	0.463 ± 0.051	0.428 ± 0.044	0.420 ± 0.033	0.007	0.116
Liver	2.819 ± 0.333	2.926 ± 0.397	2.772 ± 0.438	3.102 ± 0.451	0.066	0.302
Gizzard	2.302^{xy} ± 0.243	2.520^{x} ± 0.192	2.288^{xy} ± 0.164	2.233^{y} ± 0.313	0.041	0.068
pH_{24}	5.057^{a} ± 0.039	4.994^{b} ± 0.031	4.993^{b} ± 0.023	5.005^{b} ± 0.056	0.007	0.003
Color						
L*	38.3 ± 2.8	39.0 ± 1.6	39.5 ± 1.7	40.8 ± 2.8	0.383	0.108
a*	15.3^{ab} ± 1.1	16.0^{a} ± 1.2	15.3^{ab} ± 1.0	14.3^{b} ± 1.2	0.196	0.012
b*	12.2^{y} ± 1.2	13.5^{x} ± 1.3	13.1^{xy} ± 0.9	12.7^{xy} ± 1.0	0.184	0.092

number of replicates = 64 (16 replicates/birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, BWbs – body weight before slaughter (100%); pH_{24} = pH measured 24 h post mortem; L*, a*, b* – meat color was measured in to the CIE L*a*b* system; values in same rows with no common superscript (ab) are significantly different ($P \leq 0.05$) and $0.05 < P \leq 0.10$ considered a near-significant trend(xy)

The colour a* (redness) value was highest in T2 and lowest in T4 ($P < 0.05$ vs. T2). A tendency towards increased b* (yellowness) was observed in group T2 vs. positive control T1 ($P = 0.092$). The AME_N of phytase-supplemented diets was significantly lower in comparison to the positive control ($P < 0.05$; Table 9). Digestibility results showed higher nitrogen (N) retention in both phytase-supplemented groups (T3, T4) compared to T2 ($P < 0.05$). Regarding Ca and P retention, all groups differed significantly among all treatments, with the highest values observed in the group fed 2000 FTU/kg phytase (T4) and the lowest in the group fed a lower P diet without phytase supplementation (T2). The relationship between treatments was in the following order: $T4^{a}>T3^{b}>T1^{c}>T2^{d}$ ($P < 0.05$). Ash retention was significantly higher in both phytase-supplemented groups than in the positive and negative controls

Table 9. AME_N (kcal) and nutrients retention in broiler ducks fed feed mixtures, %

	AME_N	N retention	Ca retention	P retention	Ash retention	DM retention
Treatment						
T1	3314^{a} ± 33.3	69.3^{ab} ± 2.2	38.0^{c} ± 2.6	49.1^{c} ± 2.3	36.5^{b} ± 3.6	94.8 ± 0.5
T2	3291^{ab} ± 42.4	66.2^{b} ± 5.6	34.8^{d} ± 3.9	43.5^{d} ± 6.3	35.6^{b} ± 4.0	94.5 ± 0.8
T3	3264^{b} ± 66.4	72.8^{a} ± 3.2	42.3^{b} ± 4.8	53.0^{b} ± 2.7	43.4^{a} ± 3.2	95.1 ± 0.7
T4	3246^{b} ± 56.3	70.7^{a} ± 5.1	46.4^{a} ± 2.5	61.7^{a} ± 1.6	42.6^{a} ± 3.3	94.9 ± 0.6
SEM	8.56	0.718	0.942	0.664	0.742	0.104
P-value	0.020	0.006	<0.001	<0.001	<0.001	0.257

number of replicates = 64 (16 replicates of 5 birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative control + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; SEM – standard error of the mean, AME_N – apparent metabolizable energy corrected to zero-nitrogen retention, DM – dry matter; values in same columns with no common superscript (a–d) are significantly different at $P \leq 0.05$

Table 10. Dry matter and ash content in tibia bones of broiler ducks, %

	DM	Ash
Treatment		
T1	7.43[x] ± 0.8	50.9[a] ± 3.3
T2	6.36[y] ± 1.1	46.5[b] ± 4.5
T3	6.27[y] ± 1.1	51.9[a] ± 3.8
T4	6.76[xy] ± 0.8	51.6[a] ± 2.5
SEM	0.167	0.651
P-value	0.051	0.005

number of replicates = 48 (12 replicates/birds/treatment); T1 – positive control – with a standard P content of a diet, T2 – negative control – with a reduced P content of a diet, T3 – negative ontrol + phytase (OptiPhos Plus, Huvepharma) at 1000 FTU/kg, T4 – negative control + phytase at 2000 FTU/kg; DM – dry matter, .SEM – standard error of the mean; values in same columns with no common superscript [(ab)] are significantly different at $P \leq 0.05$ and values between $0.05 < P \leq 0.10$ considered a near-significant trend [(xy)]

($P < 0.05$; Table 10). Dry matter content in the tibia was lower in treatments T2 and T3 compared with ducks in T1 ($P < 0.05$). Tibia ash content was lowest in the negative control group (T2; $P < 0.05$ vs. other treatments; Table 10).

Discussion

Phytases are widely employed in animal nutrition to hydrolyse phytate-bound P in feedstuffs, thereby increasing its availability and digestibility. However, despite the inclusion of phytase in feed formulations, P deficiencies may still occur, particularly due to the enzyme's susceptibility to thermal degradation during high-temperature processing, such as pelleting. To address this limitation, the present study utilised a heat-stable phytase preparation with confirmed thermostability up to at least 85 °C and pelleting maintained below 80 °C. The formulated starter and grower diets contained 0.3 and 0.2% available P, respectively, levels insufficient to meet the requirements of rapidly growing meat ducks. This was reflected in significantly lower body weight observed on days 14 and 28, as well as reduced ADWG during the early (days 1–14) and later (days 15–28) growth phases in the negative control group (T2). FCR during days 1–28 was significantly better in both phytase-supplemented groups than in the P-deficient group without phytase (T2). No such differences were observed in the later stages of rearing, indicating that broiler ducks are more sensitive to available P deficiency in the first half of the fattening period. Liu et al. (2022) reported a similar pattern in Cherry Valley ducks over a 42-day trial, with significant differences in weight gain between standard and P-deficient diets recorded during days 0–21 but not during days 22–42. Zhang et al. (2022) emphasised that the starter phase in ducks is critically important for the development of body tissues, internal organs, and the skeletal system, and that adequate nutrition during this period significantly affects subsequent growth performance and overall productivity. Although published data support the benefits of phytase supplementation in P-deficient diets throughout the rearing period, present results suggest that these benefits may plateau in the second phase, as ducks tolerated available P deficiencies quite well during this period. This hypothesis should be thoroughly verified in future studies, as the available literature has focused mainly on the negative effects of P-deficient diets and the associated benefits of phytase supplementation. In broiler chickens, young birds are considerably more sensitive to P deficiency, whereas reduced P intake during the late rearing/early finishing phases is better tolerated and may allow for compensatory growth with limited impact on performance if dietary P is reduced appropriately (Kozłowski et al., 2009, 2010a; Omotoso et al., 2023).

In the present study, the diet with lower available P content caused a near-significant increase in relative gizzard weight in ducks. Although no published data directly link low dietary P to increased gizzard mass in ducks or broiler chickens, reduced available P in poultry diets has been associated with impaired nutrient digestibility and adverse changes in intestinal morphology, including reductions in villus height and crypt depth (Lee et al., 2024). It is well established that less digestible feed is retained longer in the gastrointestinal tract of monogastric animals (Svihus, 2011), and prolonged retention in the proventriculus-gizzard complex may increase functional load on the gizzard, contributing to its hypertrophy. In the present experiment, a marked reduction in abdominal fat percentage was observed in ducks fed diets with reduced available P, as well as in birds receiving higher levels of 6-phytase supplementation. The underlying mechanism remains unclear; however Li et al. (2016) reported that both deficient and excessive dietary P inclusion levels may negatively affect meat quality in broiler chickens, including a reduction in intramuscular fat content and alterations in the fatty acid profile of breast muscles compared with birds fed a diet with adequate P levels. In line with these findings, the present results indicate that reducing the dietary P and concomitant 6-phytase supplementation significantly decreased the a* (redness) colour parameter of duck breast meat relative to the non-supplemented P-deficient group. These results are consistent with observations in broiler chickens reported by Hakami et al. (2022), who observed decreased a* values in phytase-supplemented broiler chicken breast meat at both 15 min and 24 h postmortem, indicating improved meat colour stability. Similarly, Maynard et al. (2023) found that phytase supplementation influenced the b* (yellowness) parameter of broiler breast meat, with higher b* values observed in the negative control group compared with phytase-treated birds at both 4 and 24 h postmortem. In the present study, the P-deficient group likewise showed increased breast meat yellowness compared with the positive control. The mechanisms underlying increased yellowness are not clearly explained in the cited studies. One possible explanation is increased formation of reactive oxygen species (ROS) under conditions of reduced dietary available P content. Studies in rodents fed a phosphate-deficient diet have shown impaired mitochondrial respiration and oxidative phosphorylation, leading to excessive ROS generation (Brautbar et al., 1983; Andres-Hernando et al., 2023). Viana et al. (2017) reported that broiler breast meat showed higher b* values in broiler breast meat in parallel with increased lipid oxidation (measured by TBARS), suggesting that oxidative processes may contribute to reduced colour stability and increased yellowness.

In the present experiment, phytase supplementation significantly improved the retention of nitrogen, Ca, P and ash, with Ca and P retention showing a clear dose-dependent response. Moreover, both phytase-supplemented groups showed higher Ca and P retention not only relative to the negative (P-deficient) group, but also to the positive control group. This relationship is not new, as numerous studies have reported reduced nutrient digestibility and absorption caused by low P availability (Kozłowski et al., 2010b; Czerwiński et al., 2012; Bougouin et al., 2014; Wu et al., 2021; Pirzado et al., 2024). However, confirming this mechanism in ducks is scientifically important, given that available data for this species are more limited than for broiler chickens (Venter et al., 2024). Furthermore, phytase supplementation allows lowering Ca, inorganic P, and protein levels in rations, while decreasing P and N excretion, as demonstrated in broiler chickens, thereby contributing to reduced environmental impact (Konieczka et al., 2020). The present results also indicate that dietary supplementation with 6-phytase affected AME_N. Dietary levels of available P can indirectly affect apparent metabolizable energy (AME) through their impact on overall nutrient digestibility and growth performance. This effect is particularly evident under P-deficient conditions, where supplementation with available P, either directly or via exogenous phytase, can partially restore performance parameters such as FCR and nutrient utilisation efficiency (Ravindran et al., 2004; Cozannet et al., 2021). In addition to improvements in growth performance, bone mineralisation, and P utilisation in response to phytase supplementation, Wu et al. (2021) emphasised that growth performance and tibia ash content were more sensitive indicators of P bioavailability in ducks than other response criteria. The current findings support this assertion, as tibia ash content in ducks receiving diets supplemented with 6-phytase was comparable to that observed in the positive control group. At the same time, ash retention was significantly higher in both phytase-supplemented groups compared to the positive and negative controls, which is consistent with the findings reported by Biesek et al. (2021).

Conclusions

This study confirms that early dietary phosphorus (P) deficiency negatively affects growth performance, feed efficiency, and nutrient retention in meat ducks. Supplementation with a thermostable 6-phytase effectively improved performance and N, Ca, P, and ash retention, with Ca and P utilisation exceeding the levels observed in the P-adequate control. Increased tibia ash content further supported improved P bioavailability following phytase supplementation. Low dietary P also influenced meat quality traits, increasing both redness and yellowness of breast muscle, the latter potentially associated with elevated oxidative stress. Although carcass yield was not significantly affected, reduced abdominal fat and a trend toward increased gizzard weight in P-deficient ducks indicate physiological adaptations. Overall, phytase supplementation effectively counteracted the adverse effects of diets low in available P, particularly during the early rearing phase in ducks.

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Conflict of interest

The Authors declare that there is no conflict of interest.

References

Adeola O., 2018. Phytase in starter and grower diets of White Pekin ducks. Poult. Sci. 97, 592−598, https://doi.org/10.3382/ps/pex352

Andres-Hernando A., Cicerchi C., Garcia G.E., Orlicky D.J., Stenvinkel P., Johnson R.J., Lanaspa M.A., 2023. Phosphate depletion in insulin-insensitive skeletal muscle drives AMPD activation and sarcopenia in chronic kidney disease. Science 26, 106355, https://doi.org/10.1016/j.isci.2023.106355

AOAC International, 2006. Official methods of analysis of AOAC International, 18th Edition, Revision 1, Chapter 4, pp. 30-31. AOAC, Arlington, VA

Biesek J., Banaszak M., Adamski M., 2021. Ducks' growth, meat quality, bone strength, and jejunum strength depend on zeolite in feed and long-term factors. Animals 11, 1015, https://doi.org/10.3390/ani11041015

Bougouin A., Appuhamy J.A., Kebreab E., Dijkstra J., Kwakkel R.P., France J., 2014. Effects of phytase supplementation on phosphorus retention in broilers and layers: a meta-analysis. Poult. Sci. 93, 1981−1992, https://doi.org/10.3382/ps.2013-03820

Brautbar N., Carpenter C., Baczynski R., Kohan R., Massry S.G., 1983. Impaired energy metabolism in skeletal muscle during phosphate depletion. Kidney Int. 24, 53−57, https://doi.org/10.1038/ki.1983.125

Cozannet P., Davin R., Jlali M., Jachacz J., Preynat A., Molist F., 2021. Dietary metabolizable energy, digestible lysine, available phosphorus levels and exogenous enzymes affect broiler chicken performance. Animal 15, 100206, https://doi.org/10.1016/j.animal.2021.100206

Czerwiński J., Smulikowska S., Mieczkowska A., Konieczka P., Piotrowska A., Bartkowiak-Broda I., 2012. The nutritive value and phosphorus availability of yellow-and dark-seeded rapeseed cakes and the effects of phytase supplementation in broilers. J. Anim. Feed Sci. 21, 677−695, https://doi.org/10.22358/jafs/66140/2012

Fan L., He Z.Z., Ao X., Sun W.L., Xiao X., Zeng F.K., Wang Y.C., He J., 2019. Effects of residual superdoses of phytase on growth performance, tibia mineralization, and relative organ weight in ducks fed phosphorus-deficient diets. Poult. Sci. 98, 3926−3936, https://doi.org/10.3382/ps.0681118

Hakami Z., Sulaiman A.R.A., Alharthi A.S., Casserly R., Bouwhuis M.A., Abudabos A.M., 2022. Growth performance, carcass and meat quality, bone strength, and immune response of broilers fed low-calcium diets supplemented with marine mineral complex and phytase. Poult. Sci. 101, 101849, https://doi.org/10.1016/j.psj.2022.101849

Jiang J., Wu H., Zhu D., Yang J., Huang J., Gao S., Lv G., 2020. Dietary supplementation with phytase and protease improves growth performance, serum metabolism status, and intestinal digestive enzyme activities in meat ducks. Animals 10, 268, https://doi.org/10.3390/ani10020268

Konieczka P., Kaczmarek S. A., Hejdysz M., Kinsner M., Szkopek D., Smulikowska S., 2020. Effects of faba bean extrusion and phytase supplementation on performance, phosphorus and nitrogen retention, and gut microbiota activity in broilers. J.. Sci. Food Agric. 100, 4217−4225, https://doi.org/10.1002/jsfa.10461

Kozłowski K., Jankowski J., Jeroch H., 2009. Efficacy of different phytase preparations in broiler rations. Pol. J. Vet. Sci. 12, 389−393

Kozłowski K., Jankowski J., Jeroch H., 2010a. Efficacy of different levels of *Escherichia coli* phytase in broiler diets with a reduced P content. Pol. J. Vet. Sci. 13, 431−436

Kozłowski K., Jankowski J., Jeroch H., 2010b. Efficacy of *Escherichia coli*-derived phytase on performance, bone mineralization and nutrient digestibility in meat-type turkeys. Vet. Med. Zoot. 55, 59−66

Lee C., Kim H.W., Kwon C.H., Han G.P., Lee J.H., Kil D.Y., 2024. Effects of decreasing phosphorus concentrations in diets and phytase supplementation on growth performance, stress response, and intestinal health in broiler chickens. Poult. Sci. 103, 104418, https://doi.org/10.1016/j.psj.2024.104418

Li X.K., Wang J.Z., Wang C.Q., Zhang C.H., Li X., Tang C.H., Wei X.L., 2016. Effect of dietary phosphorus levels on meat quality and lipid metabolism in broiler chickens. Food Chem. 205, 289−296, https://doi.org/10.1016/j.foodchem.2016.02.133

Liu H., Walk C.L., Sorbara J.O., Stamatopoulos K., Zhang J.C., Wu J.L., 2022. Effects of graded levels of phytase supplementation on growth performance, plasma myo-inositol, tibia mineralization and nutrient digestibility of meat ducks fed phosphorus-deficient diets. Anim. Feed Sci. Technol. 290, 115364, https://doi.org/10.1016/j.anifeedsci.2022.115364

Liu Y.F., Zhang K.Y., Zhang Y., Bai S.P., Ding X.M., Wang J.P., Peng H.W., Xuan Y., Su Z.W., Zeng Q.F., 2020. Effects of graded levels of phytase supplementation on growth performance, serum biochemistry, tibia mineralization, and nutrient utilization in Pekin ducks. Poult. Sci. 99, 4845−4852, https://doi.org/10.1016/j.psj.2020.06.047

Maynard C.J., Maynard C.W., Mullenix G.J., Ramser A., Greene E.S., Bedford M.R., Dridi S., 2023. Impact of phytase supplementation on meat quality of heat-stressed broilers. Animals 13, 2043, https://doi.org/10.3390/ani13122043

Omotoso A.O., Reyer H., Oster M., Maak S., Ponsuksili S., Wimmers K., 2023. Broiler physiological response to low phosphorus diets at different stages of production. Poult. Sci. 102, 102351, https://doi.org/10.1016/j.psj.2022.102351

Pirzado S.A., Liu G., Purba M.A., Cai H., 2024. Enhancing the production performance and nutrient utilization of laying hens by augmenting energy, phosphorous and calcium deficient diets with fungal phytase (*Trichoderma reesei*) supplementation. Animals 14, 376, https://doi.org/10.3390/ani14030376

Ravindran V., Wu Y.B., Hendriks W.H., 2004. Effects of sex and dietary phosphorus level on the apparent metabolizable energy and nutrient digestibility in broiler chickens. Arch. Anim. Nutr. 58, 405−411, https://doi.org/10.1080/00039420400008582

Svihus B., 2011. The gizzard: Function, influence of diet structure and effects on nutrient availability. Worlds Poult. Sci. J. 67, 207−224, https://doi.org/10.1017/S0043933911000249

Venter K., Li W., Angel R., Plumstead P.W., Proszkowiec-Weglarz M., Enting H., Ellestad L.E., 2024. Calcium and phosphorus digestibility in broilers as affected by varying phytate concentrations from corn. Poult. Sci. 103, 104191, https://doi.org/10.1016/j.psj.2024.104191

Viana F.M., Canto A.C., Costa-Lima B.R., Salim A.P., Conte-Junior C.A., 2017. Color stability and lipid oxidation of broiler breast meat from animals raised on organic versus non-organic production systems. Poult. Sci. 96, 747−753, https://doi.org/10.3382/ps/pew331

Wu Y., Xu S., Wang X., Xu H., Liu P., Xing X., Qi Z., 2021. Phosphorus equivalency of phytase with various evaluation indicators of meat duck. Poult. Sci. 100, 101216, https://doi.org/10.1016/j.psj.2021.101216

Yang C., Li Y., Liu B., Chen A., Bai H., Jiang Y., Chang G., Chen G., Wang Z., 2025. Comparative analysis of duck meat quality in different breeds and age. Food Chem. X. 29, 102651, https://doi.org/10.1016/j.fochx.2025.102651

Zhang D., Xu S., Xu H., Wang X., Liu P., Xu H., Qi Z., 2022. Phosphorus equivalency of phytase with various evaluation indicators of duck in starter. J. Anim. Physiol. Anim. Nutr. 106, 1345−1355, https://doi.org/10.1111/jpn.13661