Original Research

Journal of Advanced Veterinary Research
(2024) Volume 14, Issue 6, 1004-1011

Dose-dependent effects of phytase supplementation in the diets of Hubbard Broiler Chicks on production performance, economic efficiency, physical meat quality, and intestinal histomorphometry

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ARTICLE INFO

Recieved: 15 April 2024

Accepted: 29 May 2024

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Keywords:

Broilers chicken, Economics appraisal, Meat quality, Phytase, Villus height.

ABSTRACT

The addition of microbial phytase is crucial for lowering the reliance on inorganic forms of phosphorus and enhancing the absorption of various minerals. The present investigation sets out to ascertain the optimal outcomes of dose-dependent phytase supplementation on production performance, economic efficiency, physical meat quality, and intestinal histomorphometry in Hubbard broiler chickens. A total of 270-days-old Hubbard broilers were distributed randomly among six groups. The experimental setup consisted of: Group 1 (G1) served as the control and was provided with standard basal diets, while G2, G3, G4, G5, and G6 were supplemented with standard basal diets containing 50, 75, 100, 150, and 200gm/ton of phytase, respectively. Weekly recordings of productive performance were conducted throughout the experimental duration. At the end of the study, specimens of both intestine and meat were collected from every pen. The best outcomes regarding body weight, weight gain, and feed conversion ratio were noted for G5 and G6. The economic appraisal revealed that G6 had the lowest feed costs per kilogram of body weight and the highest revenue and economic efficiency. Intestinal morphological examination supported these findings, showed that phytase supplementation significantly improved villi length and crypt depth, with the greatest enhancement seen in groups G5 and G6. Water holding capacity and shear force exhibited notable increases in G5 and G6. In conclusion, incorporating a higher dosage of phytase (200 g/ton) in broiler diets has the potential to decrease overall feed costs per kilogram of body weight gain, enhance growth performance, economic efficiency, and intestinal histomorphometry, and have no adverse effects on meat quality.

Introduction

The production of livestock and poultry has seen a global trend in recent years towards reducing production costs and mitigating environmental effects (Jing *et al*., 2021). The poultry industry is experiencing an increasing demand for poultry products, which coincides with a rise in the costs of raw materials and feed ingredients used in production. As a result, efforts are being made to optimize the utilization of nutrients in broiler diets and reduce feeding costs. One effective strategy utilized in broiler production is the inclusion of phytase as a dietary supplement. Phytase is an enzyme that helps break down phytate, leading to improved absorption of nutrients by birds (Hernandez *et al*., 2022). According to Tahir *et al*. (2012), around 60% of the phosphorus (P) in corn-SBM-based diets is sequestered by phytate and not easily utilized by broiler chickens. Phytate, also referred to as phytic acid salt or ester, possesses the capacity to form insoluble compounds with a variety of micronutrients, proteins, calcium (Ca), zinc, and iron. Consequently, this interaction impedes the assimilation and uptake of such nutrients (Woyengo and Nyachoti, 2013). Furthermore, the availability of bound P in phytate is limited in monogastric animals, such as poultry, because there is either insufficient or no endogenous phytase to efficiently break down phytate. As a result, animal performance and health are supported by dietary inorganic P supplements such as monocalcium phosphate or dicalcium phosphate (Selle and Ravindran, 2007). The most successful strategy for boosting phosphorus availability and utilization involved supplementing the diet with phytase (Selim *et al*., 2022). For many years, exogenous phytase supplementation has been employed to lessen the requirement for inorganic phosphorus and calcium in poultry feed. This is achieved by enhancing phosphorus availability through the liberation of phosphorus from phytate present in plant-based feed components. Consequently, this practice has shown enhancements in the feed conversion ratio (FCR) and enhanced digestibility of amino acids (Zanu *et al*., 2020), along with the liberation of other minerals such as calcium, zinc, iron, and copper, while also mitigating environmental phosphorus pollution (Beeson *et al*., 2017). Commercial phytases currently available can substitute 0.3 to 1.7 g/kg of inorganic phosphorus derived from either mono- or dicalcium phosphate when administered within a dosage range of 500 - 1000 FTU/kg in broiler diets. This substitution is assessed based on parameters such as tibia ash and performance, and it concurrently lowers total phosphorus (Dersjant-Li *et al*., 2015). The current study was conducted to assess the optimal effects of dose-dependent phytase supplementation on production performance, economic efficiency, physical meat quality, and intestinal histomorphometry in Hubbard broiler chickens.

Materials and methods

Ethical approval

The current study was carried out in compliance with the Institutional Animals Ethical Committee's regulations as well as Approval Protocol Number: BUFVTM 06-12-2022 from Benha University in Egypt.

Phytase enzyme

The phytase enzyme used in this study was an innovative commercial bacterial 6-phytase variant (EC 3.1.3.26) manufactured in Trichoderma reesei with a declared activity of 30,000 FTU/g, known as Axtra PHY GOLD [Danisco Animal Nutrition, International Flavors and Fragrance (IFF) Inc., NY, US].

ISSN: 2090-6277/2090-6269/

Experimental animals

One-day-old Hubbard broiler chicks, a mix of males and females and averaging 43.76±0.86 g body weight, were purchased from El Ahram Company, Giza, Egypt. All birds were kept on fresh wood shavings, which served as bedding material, and were provided with clean food and water. The birds were accommodated in a broiler house where environmental conditions aligned closely with the specifications outlined in the Broiler Management Guide of Hubbard (2022). The lighting regimen began with 24 hours of light for the first three days, followed by 23 hours of light and one hour of darkness throughout the remaining duration of the study. The temperature control started at 35˚C and steadily decreased to 25˚C by the 37th day. Humidity was kept at a minimum of 50% using mechanical ventilation. The vaccination schedule adhered to breeder standards. Birds were allowed unrestricted access to both water and diets, which were supplied in mashed form.

Experimental design

A total of 270 unsexed Hubbard broiler chicks, one day old, were distributed among 6 experimental groups, with each group consisting of 45 birds divided into 3 equal replicates of 15 birds each. The first group (G1) served as the normal control and was fed a standard basal diet. The second, third, fourth, fifth, and sixth groups were given the standard basal diet, supplemented with 50, 75, 100, 150, and 200g/ton of phytase. All diets were formulated with a base of corn and soybean meal and tailored for three stages: starter (0 to 10 days), grower (11 to 24 days), and finisher (25 to 35 days). Diets were designed to satisfy the suggested nutrient requirements of the birds based on requirement of Hubbard (2022). The components of the feed and the chemical makeup of the experimental diets are detailed in Table 1.

Production Performance

Weekly records of body weight (BW) and feed intake (FI) were maintained for each replicate, enabling the calculation of body weight gain (BWG) and feed conversion ratio (FCR), which expressed as grams of feed per gram of gain. The daily rates of mortality for every experimental group were documented. Average daily gain (ADG) and average daily feed intake (ADFI) were computed by dividing the cumulative BW and FI by the total number of birds within each enclosure. These metrics were then employed to compute the daily FCR. The European broiler index (EBI) was determined using the approach outlined by Marcu *et al*. (2013), and expressed as follows: EBI = (livability (%) x ADG /chick/day) / (FCR x 10).

Economic efficiency

Economic efficiency encompasses production costs and return metrics. Production costs involve total costs (TC), comprising both total fixed costs (TFC) and total variable costs (TVC). The TVC comprises expenses such as feed consumption, chick prices, veterinary expenses, water and electricity charges, labor costs, and litter costs. These costs were estimated as an average value per bird in each group in local currency (LE) throughout the experimental period. Total fixed costs, including depreciation, building, and equipment costs. Total return (TR) was computed according to the procedures described by Omar (2014), which entails the following formula: TR = bird selling return per gram + litter selling price. Net return (NR) was determined using the approach outlined by Tareen *et al*. (2017), employing the formula: NR = TR – TC. Economic efficiency (EE) and relative economic efficiency (REE) were computed according to Mohammed *et al*. (2021) with consideration of the prices in the local market at the time of the study: EE = NR/TC*100, REE= (EE of each experimental group)/ (EE of the control group) x 10.

Table 1. Ingredient and nutrient composition of different experimental groups in the starter, grower, and finisher phases.

	G1	G2	G3	G4	G5	G6
Starter, 0-10 d						
Ingredients %						
yellow corn	52.54	54.18	54.59	54.64	55.03	55.55
SBM46	34.1	35	35	35	34.9	34.7
Wheat bran	3.3	3.3	3.5	3.5	3.5	3.3
vegetable oil	2.5	2.5	2.5	2.5	2.5	2.5
Corn gluten meal	2.4	0.8	0.3	0.25	-	-
Mono calcium phosphate	1.73	0.96	0.87	0.8	0.73	0.69
Limestone	1.58	1.53	1.52	1.54	1.55	1.55
Sodium bicarbonate	0.33	0.2	0.18	0.23	0.24	0.15
DL -Methionine	0.31	0.33	0.34	0.34	0.35	0.35
L- Lysine	0.31	0.26	0.26	0.25	0.24	0.24
Vit & min premix	0.3	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.23	0.24	0.24	0.24	0.24	0.24
L -Threonine	0.12	0.14	0.15	0.15	0.15	0.16
Choline chloride	0.11	0.11	0.11	0.11	0.11	0.11
Anti-mycotoxin	0.1	0.1	0.1	0.1	0.1	0.1
Anticoccidial	0.03	0.03	0.03	0.03	0.03	0.03
Antioxidant	0.01	0.01	0.01	0.01	0.01	0.01
Energy enzymes	0.01	0.01	0.01	0.01	0.01	0.01
Anti clostridial	0.01	0.01	0.01	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01	0.01	0.01	0.01
Axtraphy Gold	-	0.01	0.01	0.01	0.02	0.02
Chemical composition (%)						
ME (Kcal /Kg diet)	3,002	3,059	3,067	3,048	3,073	3,084
CP	23.14	23.1	23.05	23.1	23.09	23.12
Calcium	1.03	1.05	1.06	1.05	1.05	1.05
Available phosphorus	0.5	0.5	0.51	0.5	0.5	0.5
Phytate	0.26	0.26	0.26	0.26	0.26	0.26
Grower, 11-24 d						
Ingredients %						
yellow corn	56.72	58.21	58.62	59.19	59.72	59.97
SBM46	29.4	31.1	30.7	30.4	29.9	29.6
Wheat bran	3.5	3.4	3.5	3.3	3.4	3.5
vegetable oil	3.5	3.5	3.5	3.5	3.4	3.4
Corn gluten meal	2.1	-	-	-	-	-
Mono calcium phosphate	1.63	0.86	0.77	0.7	0.63	0.59
Limestone	1.45	1.34	1.34	1.35	1.37	1.37
Sodium bicarbonate	0.27	0.21	0.19	0.17	0.19	0.17
DL -Methionine	0.27	0.3	0.3	0.3	0.3	0.3
L- Lysine	0.3	0.23	0.23	0.23	0.23	0.23
Vit & min premix	0.3	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.21	0.23	0.23	0.23	0.23	0.23
L -Threonine	0.09	0.07	0.07	0.07	0.07	0.06
Choline chloride	0.1	0.1	0.1	0.1	0.1	0.1
Anti-mycotoxin	0.1	0.1	0.1	0.1	0.1	0.1
Anticoccidial	0.03	0.03	0.03	0.03	0.03	0.03
Antioxidant	0.01	0.01	0.01	0.01	0.01	0.01
Energy enzymes	0.01	0.01	0.01	0.01	0.01	0.01
Anti clostridial	0.01	0.01	0.01	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01	0.01	0.01	0.01
Axtraphy Gold	-	0.01	0.01	0.01	0.02	0.02
Chemical composition						
ME (Kcal /Kg diet)	3,103	3,152	3,167	3,157	3,180	3,185
CP (%)	21.12	21.1	21.16	21.12	21.12	21.12
Calcium (%)	0.95	0.95	0.96	0.95	0.95	0.95
Available phosphorus (%)	0.47	0.47	0.48	0.47	0.47	0.47
phytate (%)	0.24	0.25	0.25	0.25	0.25	0.25

Table 1 (Continue). Ingredient and nutrient composition of different experimental groups in the starter, grower, and finisher phases.

	G1	G2	G3	G4	G5	G6
Finisher, 25-35 d						
Ingredients %						
yellow corn	59.42	60.16	60.78	61.23	61.66	62.01
SBM46	25.3	28.6	28.2	27.8	27.4	27.1
Wheat bran	3.4	3.5	3.5	3.5	3.5	3.5
vegetable oil	4.4	4.5	4.4	4.4	4.4	4.4
Corn gluten meal	3.2	-	-	-	-	-
Mono calcium phosphate	1.43	0.65	0.55	0.5	0.43	0.39
Limestone	1.3	1.2	1.2	1.2	1.22	1.22
Sodium bicarbonate	0.26	0.17	0.16	0.14	0.16	0.14
DL -Methionine	0.2	0.23	0.24	0.24	0.25	0.25
L- Lysine	0.25	0.15	0.15	0.15	0.15	0.15
Vit & min premix	0.3	0.3	0.3	0.3	0.3	0.3
Sodium chloride	0.22	0.26	0.26	0.25	0.25	0.25
L -Threonine	0.07	0.03	0.03	0.03	0.03	0.02
Choline chloride	0.1	0.09	0.09	0.09	0.1	0.1
Anti-mycotoxin	0.1	0.1	0.1	0.1	0.1	0.1
Anticoccidial	0.03	0.03	0.03	0.03	0.03	0.03
Antioxidant	0.01	0.01	0.01	0.01	0.01	0.01
Energy enzymes	0.01	0.01	0.01	0.01	0.01	0.01
Anti clostridial	0.01	0.01	0.01	0.01	0.01	0.01
Protease enzyme	0.01	0.01	0.01	0.01	0.01	0.01
Axtraphy Gold	-	0.01	0.01	0.01	0.02	0.02
Chemical composition						
ME (Kcal / Kg diet)	3,204	3,236	3,247	3,235	3,264	3,270
CP (%)	20.01	20.01	20.07	20.01	20.03	20.02
Calcium (%)	0.85	0.85	0.86	0.85	0.85	0.85
Available phosphorus (%)	0.42	0.42	0.43	0.42	0.42	0.42
Phytate (%)	0.24	0.24	0.24	0.24	0.24	0.24

Physical Meat Quality

A total of 30 right breast muscles (5 per experimental group) were used for the analysis of physical meat properties (i.e., pH, water holding capacity (WHC%), shear force, drip loss%, cooking loss%, and color measurements). After 24 hours postmortem, the ultimate pH level was evaluated inside the major pectoralis muscle, roughly one centimeter down, using a transportable pH monitor (pH-meter Jenway 3510, manufactured by Cole-Parmer, Staffordshire, UK) (Albrecht *et al*., 2019). The CIELAB color measurements, including lightness (L*), redness (a*), and yellowness (b*), were obtained by averaging three readings taken from three spots of breast muscles employing a Konica Minolta data processor (Tokyo, Japan) with a Chroma meter CR-300 connected, following the procedures outlined by Xie *et al.* (2021). Subsequently, breast fillets were placed into labeled bags and stored at 4°C for further assessment. WHC% and drip loss% were assessed following the methodology outlined by Klupsaite *et al.* (2020). The evaluation of WHC% involved measuring the weight change following the application of the filter paper press technique. A 2g sample was positioned on filter paper and compressed between two plexiglass sheets sized 10×10 cm. A 2.5kg weight was applied for 10 minutes to put pressure on the sample. To analyze drip loss, meat samples weighing about 80g from the cranial aspect within the major pectoralis muscle were positioned in clear containers on racks made of sieved plastic. These were stored at 4°C for 48 hours. Afterward, the samples were reweighed following the removal of extra surface fluids, and the proportion of the weight loss over this chilled storage period was used to compute the drip loss. All the samples utilized in the drip loss evaluation procedure were sealed in a vacuum and cooked in a bath of water until the inner core reached 80°C. After cooling to room temperature, the samples were weighed to determine cooking loss (Adeyemi, 2021). Finally, the cooked samples were employed for shear force analysis utilizing an Instron Universal Testing Device (Model 2519-105, US). According to Rozanski *et al.* (2017), six tests were conducted for each sample, setting the crosshead speed of the shearing machine to 200 mm/min.

Histomorphometric examination

At the end of the experiment, 30 broilers were randomly selected for measurement of villus length (VL) and crypt depth (CD), with 5 broilers chosen from each group based on their average body weight. After the small intestine was dissected and longitudinally opened, segments from the midsection of the duodenum, the jejunum (located halfway between the entry point of the bile duct and the Meckel's diverticulum), and the ileum (approximately 0.5 cm in length) were flushed with buffer saline. Subsequently, the samples were promptly immersed in a 10% neutral buffered formalin solution for fixation. Following this, the specimens underwent rinsing in running water, dehydration using different concentrations of ethyl alcohol, clarification in xylene, embedding in paraffin, blocking, and slicing into sections with a thickness of 5 µm. These sections were then subjected to microscopic examination after staining with hematoxylin and eosin (H&E) as described by Xu *et al.* (2003). Five stained sections per bird were examined to measure the VL and the CD.

Image analysis

The image analyzer underwent automatic calibration to translate the pixel-based measurements generated by the image analysis software into a real-world micrometer's units. With a final magnification of 40×, the photographs of every slice were taken for every sample using a Leica Camera Quin 500 analyzer computer system (Leica Microsystems, Switzerland).

Statistical analysis

The data was expressed as mean±standard deviation (mean±SD). To assess the statistical significance of variances among experimental groups, we performed a one-way analysis of variance (ANOVA) followed by Tukey's range test, utilizing SPSS software (IBM Corp. 2007) version 16.0 for Windows.

Results

Production performance

The effects of phytase supplementation on BW, BWG, RGR, FI, FCR, and EBI were illustrated in Table 2. Significant differences in BW were observed on days 7, 14, 21, 28, and 35 among the various experimental groups, with the highest BW value recorded in G6. Groups G5 and G6 exhibited notably increased BW on days 7 and 14 compared to other groups, while G4 had the lowest BW values. On days 21 and 35 of the trial, G6 demonstrated a significantly higher level of BWG than the other experimental groups. Cumulative BWG was markedly elevated in G6 compared to the other experimental groups. However, on days 7, 14, and 28, no significant variances were detected in the BWG among the various experimental groups. Throughout the experimental period, there were no statistical differences observed in RGR among the different experimental groups.

During the experiment's second week, G6 exhibited a significant FI increase, whereas G2 and G4 experienced significant decreases. In the third week, there was a significant increase in FI noted in G1 and G6 compared to the other experimental groups. Cumulative FI indicated a significant increase in G1 and G6, followed by G3, compared to the other experimental groups. However, during the first, fourth, and fifth weeks of the experiment, there were no significant variations observed in FI between

the various experimental groups. Throughout the cumulative period, there was a trend toward decreased FCR in groups supplemented with phytase relative to the group acting as a control, with notably improved FCR recorded in G5 and G6. The European broiler index was significantly increased in G6 compared to different experimental groups.

Economic efficiency

The effect of phytase supplementation on economic efficiency measures of Hubbard broiler chickens were illustrated in Table 3. Although phytase supplementation did not significantly affect most financial parameters, there was a notable increase in total return, net revenue, relative economic efficiency, and economic efficiency in relation to the control groups. Total revenue and net return exhibited significant increases in G3, G5, and G6 relative to the other experimental groups, with the best revenues achieved in G6. The results demonstrated a notable improvement in both economic efficiency and relative economic efficiency in the group supplemented with phytase when contrasted with the control group, and the highest economic efficiency was recorded in G6, followed by G5, and then G3. The maximum feed cost incurred per unit weight and per unit weight gain was observed in G1 compared to the supplemented groups, with the lowest cost recorded in G6, followed by G5, and then G3.

Physical meat quality

The effects of phytase supplementation on the physical meat quality of Hubbard broiler chicken were illustrated in Table 4. The result revealed that there were no significant variations observed in pH, drip loss %, cooking loss%, or L* value of the broiler meat color between different experimental groups. However, WHC% and shear force exhibited a notable increase in G5 and G6 in contrast to the other experimental groups, with the highest values observed in G6. In addition, the a* value of G2 showed a notable increase when compared to the other experimental groups, whereas the b* value was significantly increased in G1, G2, and G3 compared to G4, G5 and G6.

Table 2. Effect of phytase supplementation on body weight (BW), body weight gain (BWG), relative growth rate (RGR), feed intake (FI) and feed conversion ratio (FCR) of Hubberd broiler chickens during the period of the experiment (n.=12).

	Parameters	G1	G2	G3	G4	G5	G6
BW	0 d	42.50±2.24^{a}	42.98±2.99^{a}	43.30±3.10^{a}	43.83±3.85^{a}	44.26±3.56^{a}	44.98±2.90^{a}
	7 d	150.17±7.46^{b}	155.83±7.90^{b}	161.52±7.43^{b}	149.67±12.40^{b}	175±10.05^{a}	183.67±11.93^{a}
	14 d	436.92±24.70^{b}	424.00±41.15^{b}	462.38±28.46^{b}	418.5±31.10^{b}	505.08±35.33^{a}	520.25±32.45^{a}
	21 d	900.67±47.83^{b}	860.25±45.42^{b}	958.18±54.11^{b}	880.25±50.63^{b}	1015.42±45.15^{b}	1103.25±68.02^{a}
	28 d	1333.00±63.44^{c}	1351.50±27.75^{c}	1456.32±67.88^{b}	1320.27±66.44^{c}	1515.58±62.16^{b}	1660.75±95.23^{a}
	35 d	1882.37±101.55^{c}	1960.03±83.78^{c}	2125.97±49.84^{b}	1875.47±81.27^{c}	2138.69±52.82^{b}	2424.61±75.03^{a}
BWG	0-7 d	107.67±7.29^{a}	112.85±6.33^{a}	118.21±6.80^{a}	105.83±9.30^{a}	130.74±7.07^{a}	138.69±10.26^{a}
	8-14 d	286.75±21.14^{a}	268.17±35.06^{a}	300.87±23.88^{a}	268.83±25.23^{a}	330.08±33.79^{a}	336.58±25.60^{a}
	15-21 d	463.75±33.31^{b}	436.25±51.69^{b}	495.8±28.19^{b}	461.75±30.66^{b}	510.33±32.50^{b}	583±41.70^{a}
	22-28 d	432.33±51.32^{a}	491.25±38.45^{a}	498.13±51.79^{a}	440.02±54.10^{a}	500.17±46.02^{a}	557.50±54.72^{a}
	29-35 d	549.37±99.93^{b}	608.53±73.04^{b}	669.65±57.79^{b}	555.21±86.78^{b}	623.11±82.88^{b}	763.86±57.08^{a}
	Total Gain	1839.87±101.50^{c}	1917.05±82.77^{c}	2082.66±48.89^{b}	1831.64±81.64^{c}	2094.43±51.62^{b}	2379.64±74.59^{a}
	Daily BWG	52.57±2.90^{c}	54.77±2.36^{c}	59.50±1.40^{b}	52.33±2.33^{c}	59.84±1.47^{b}	67.99±2.13^{a}
RGR	1st week	111.70±4.42^{a}	113.55±3.62^{a}	115.45±4.66^{a}	109.39±3.20^{a}	119.32±2.51^{a}	121.27±3.33^{a}
	2nd week	97.62±3.79^{a}	92.14±5.44^{a}	96.35±3.48^{a}	94.60±5.21^{a}	96.91±5.99^{a}	95.60±3.80^{a}
	3rd week	69.34±3.36^{a}	68.09±8.93^{a}	69.82±1.61^{a}	71.17±3.71^{a}	67.21±4.48^{a}	71.81±2.51^{a}
	4th week	38.72±4.32^{a}	44.50±4.23^{a}	41.29±4.11^{a}	40.00±4.61^{a}	39.53±3.31^{a}	40.35±3.33^{a}
	5th week	34.12±5.72^{a}	36.68±3.54^{a}	37.45±3.74^{a}	34.75±5.36^{a}	34.13±4.72^{a}	37.49±3.68^{a}
	cumulative RGR	191.15±0.62^{a}	191.41±0.59^{a}	192.02±0.55^{a}	190.85±0.91^{a}	191.89±0.59^{a}	192.71±0.48^{a}
FI	1st week	132.83± 5.69^{a}	133.64±7.86^{a}	135.24±9.30^{a}	129.96±10.21^{a}	141.56±4.63^{a}	141.92±5.67^{a}
	2nd week	372.00±1.00^{b}	358.33±9.29^{c}	373.67±3.21^{b}	352.00±6.56^{c}	371.00±15.52^{b}	390.67±6.81^{a}
	3rd week	703.14±11.09^{a}	668.97±18.88^{b}	678.69±24.34^{b}	674.62±0.73^{b}	680.85±15.21^{b}	718.43±6.49^{a}
	4th week	812.57±29.86^{a}	828.08±13.81^{a}	773.61±66.21^{a}	726.24±15.43^{a}	822.80±66.33^{a}	861.30±0.95^{a}
	5th week	962.00±36.86^{a}	933.67±38.28^{a}	975.67±10.69^{a}	936.67±12.34^{a}	892.67±53.52^{a}	927.67±30.75^{a}
	cumulative FI	3013.36±38.63^{a}	2886.56±41.34^{c}	2962.73±28.08^{b}	2811.7±27.34^{d}	2822.46±65.32^{d}	3036.38±10.03^{a}
	Daily FI	86.10±1.10^{a}	82.47±1.18^{c}	84.65±0.8^{b}	80.33±0.78^{d}	80.64±1.87^{d}	86.75±0.29^{a}
FCR	1st week	1.23±0.06^{a}	1.19±0.10^{a}	1.15±0.09^{a}	1.24±0.16^{a}	1.09±0.06^{a}	1.03±0.06^{a}
	2nd week	1.3±0.10^{a}	1.36±0.22^{a}	1.25±0.10^{a}	1.32±0.12^{a}	1.14±0.13^{a}	1.17±0.09^{a}
	3rd week	1.52±0.11^{a}	1.55±0.18^{a}	1.37±0.07^{a}	1.47±0.1^{a}	1.34±0.08^{a}	1.24±0.09^{a}
	4th week	1.9±0.23^{a}	1.7±0.13^{a}	1.57±0.21^{a}	1.67±0.21^{a}	1.66±0.17^{a}	1.56±0.15^{a}
	5th week	1.8±0.32^{a}	1.56±0.21^{a}	1.47±0.11^{a}	1.73±0.28^{a}	1.46±0.22^{a}	1.22±0.08^{a}
	cumulative FCR	1.64±0.08^{a}	1.51±0.06^{b}	1.42±0.03^{c}	1.54±0.07^{b}	1.35±0.05^{d}	1.28±0.04^{d}
	Daily FCR	1.64±0.08^{a}	1.51±0.06^{b}	1.42±0.03^{c}	1.54±0.07^{b}	1.35±0.05^{d}	1.28±0.04^{d}
European broiler index		321.17±23.04^{b}	363.77±6.02^{b}	409.91±13.42^{b}	333.85±9.08^{b}	444.52±25.17^{b}	533.02±22.50^{a}

Values are expressed as Mean ±SD. Means with different superscripts in the same row are significantly different (P≤0.05).
G1, Control group (basal diet); G2, basal Diet + 50g/ton phytase; G3, basal Diet + 75g/ton phytase; G4, basal Diet + 100g/ton phytase; G5, basal Diet + 150g/ton phytase; G6, basal Diet + 200g/ton phytase.

Intestinal histomorphometry

The effect of phytase supplementation on histomorphometry of the small intestine of Hubbard broiler chickens were illustrated in Table 5. The results indicated a normal histological structure of the small intestine across all groups. Furthermore, phytase supplementation significantly influenced the intestinal histomorphometry in comparison to the control group, as depicted in figures 1, 2, and 3. Specifically, there was a marked increase in villi lengths with higher levels of phytase supplementation when relative to the control group, with G6 exhibiting the greatest villi length. The crypt depth was significantly increased in G6 compared to the other experimental groups. The duodenal villus crypt ratio increased significantly in G3, G4, G5, and G6 in comparison to the control group. On the other hand, G4 and G5 revealed an increase in the ileal villus crypt ratio in contrast to the other experimental groups.

Discussion

However, incorporating phytase into the diet extends beyond economic benefits. Its utilization is linked to enhanced animal efficiency, improved nutrient utilization, and better growth performance (Cowieson *et al*., 2011), resulting in decreased nutrient excretion and thereby mitigating environmental pollution (Kumar *et al*., 2016). The objective of the present research was to assess the optimal effects of dose-dependent phytase supplementation on production performance, economic efficiency, physical meat quality, and intestinal histomorphometry in Hubbard broiler chickens. The results revealed improvements in BW, BWG, and EBI in phytase-supplemented groups, particularly at higher doses (75, 150, 200g/ton). These outcomes align with those reported by Nari and Ghasemi, (2020), who concluded that dietary phytase supplementation positively impacted BW and ADG, with broilers fed phytase-supplemented diets exhibiting the highest BW (at 42 days) and ADG throughout the experimental period compared to other experimental groups. This indicates that employing phytase with a suitable dosing regimen could positively impact bird performance (Hossain *et al*., 2022). The enhancements observed in the performance of broilers as a result of phytase supplementation can mainly be credited to the P liberation from diets

Table 3. Effect of phytase supplementation on Economic efficiency measures of Hubberd broiler chicks at the end of the experiment (n.=3).

Items	G1	G2	G3	G4	G5	G6
Starter cost	5.49±0.18^{a}	4.99±0.47^{a}	5.21±0.22^{a}	4.90±0.22^{a}	5.28±0.16^{a}	5.19±0.16^{a}
Grower cost	22.71±0.23^{a}	21.17±0.46^{a}	21.01±0.85^{a}	20.11±0.33^{a}	20.53±0.94^{a}	21.85±0.25^{a}
Finisher cost	34.99±1.12^{a}	33.16±0.69^{a}	33.37±0.98^{a}	31.44±0.56^{a}	31.26±1.33^{a}	33.97±0.64^{a}
Total feed cost	63.19±1.43^{a}	59.31±1.17^{a}	59.59±1.56^{a}	56.45±1.05^{a}	57.07±1.97^{a}	61.01±0.53^{a}
Light	0.56	0.56	0.56	0.56	0.56	0.56
Labor	1.85	1.85	1.85	1.85	1.85	1.85
Chick price (LE)	1	1	1	1	1	1
Litter (LE)	1.48	1.48	1.48	1.48	1.48	1.48
TVM	1.66	1.66	1.66	1.66	1.66	1.66
Building rent	1.25	1.25	1.25	1.25	1.25	1.25
Equipment	0.08	0.08	0.08	0.08	0.08	0.08
TFC/Cost	1.34	1.34	1.34	1.34	1.34	1.34
TVC	71.08±1.43^{a}	67.21±1.17^{a}	67.48±1.56^{a}	64.34±1.04^{a}	64.96±1.97^{a}	68.91±0.53^{a}
Bird selling price (LE)	109.18±4.3^{c}	113.68±1.66^{c}	123.31±0.61^{b}	108.78±3.06^{c}	124.04±2.26^{b}	140.63±2.73^{a}
Litter selling price (LE)	2.4	2.4	2.4	2.4	2.4	2.4
Total return	111.58±4.30^{c}	116.09±1.66^{c}	125.71±0.61^{b}	111.18±3.06^{c}	126.45±2.26^{b}	143.03±2.73^{a}
Net return	40.5±3.52^{c}	48.88±0.56^{c}	58.23±1.23^{b}	46.84±2.47^{c}	61.49±4.14^{b}	74.13±3.24^{a}
Economic Efficiency	0.57±0.05^{b}	0.73±0.01^{a}	0.86±0.04^{a}	0.73±0.03^{a}	0.95±0.09^{a}	1.08±0.05^{a}
Relative Economic Efficiency %	100.00±0.00^{c}	127.68±1.33^{b}	151.59±6.67^{a}	127.80±6.14^{b}	166.49±15.88^{a}	188.91±9.62^{a}
Feed cost BW(LE/BW)	34.37±1.02^{a}	30.94±0.21^{b}	28.61±0.66^{b}	30.83±0.65^{b}	27.26±1.42^{b}	25.65±0.72^{b}
Feed cost BWG(LE/BWG)	33.59±0.98^{a}	30.26±0.18^{b}	28.03±0.65^{b}	30.11±0.62^{b}	26.7±1.39^{b}	25.17±0.70^{b}

Values are expressed as Mean ±SD. Means with different superscripts in the same row are significantly different (P≤0.05).
G1, Control group (basal diet); G2, basal Diet + 50g/ton phytase; G3, basal Diet + 75g/ton phytase; G4, basal Diet + 100g/ton phytase; G5, basal Diet + 150g/ton phytase; G6, basal Diet + 200g/ton phytase.

Table 4. Effect of phytase supplementation on physical meat quality of Hubberd broiler chickens at the end of the experiment (n.=3).

Items	G1	G2	G3	G4	G5	G6
pH	5.82±0.07^{a}	6.10±0.64^{a}	5.73±0.07^{a}	5.68±0.05^{a}	5.61±0.02^{a}	5.60±0.04^{a}
WHC (%)	71.00±2.65^{b}	74.67±1.53^{b}	77.33±2.08^{b}	81.00±3.61^{b}	90.50±1.50^{a}	91.33±2.52^{a}
Drip loss (%)	1.33±0.15^{a}	1.17±0.15^{a}	0.95±0.05^{a}	1.27±0.12^{a}	1.13±0.15^{a}	0.97±0.06^{a}
Cooking loss (%)	22.63±0.55^{a}	23.33±0.45^{a}	21.87±0.40^{a}	21.27±0.40^{a}	20.30±0.20^{a}	20.23±0.35^{a}
Shear force (kg f/cm3)	5.07±0.25^{b}	4.53±0.25^{b}	6.03±0.35^{b}	5.67±0.21^{b}	7.00±0.30^{a}	7.03±0.32^{a}
Color measurements						
L*	55.12±0.37^{a}	55.66±0.06^{a}	56.15±0.50^{a}	57.24±0.05^{a}	57.86±0.32^{a}	59.27±1.26^{a}
a*	9.57±0.39^{b}	10.76±0.04^{a}	8.27±0.16^{c}	8.06±0.12^{c}	8.5±0.38^{c}	8.4±0.56^{c}
b*	11.22±0.38^{a}	11.74±0.53^{a}	12.71±0.31^{a}	8.35±0.38^{b}	9.20±0.15^{b}	9.84±0.50^{b}

Values are expressed as Mean ±SD. Means with different superscripts in the same row are significantly different (P≤0.05).
G1, Control group (basal diet); G2, basal Diet + 50g/ton phytase; G3, basal Diet + 75g/ton phytase; G4, basal Diet + 100g/ton phytase; G5, basal Diet + 150g/ton phytase; G6, basal Diet + 200g/ton phytase.

Table 5. Effect of phytase supplementation on histomorphometry of small intestine of broiler chickens at the end of the experiment (n.=5).

	Parameters	G1	G2	G3	G4	G5	G6
Duodenum	Villus length, um	1529.40±55.14^{e}	1736.69±22.91^{d}	1804.80±32.37^{d}	1995.89±79.34^{c}	2142.24±26.19^{b}	2354.71±84.57^{a}
	crypt depth, um	166.31±8.32^{b}	183.86±6.49^{b}	164.12±8.14^{b}	181.94±3.01^{b}	179.13±7.66^{b}	210.10±13.02^{a}
	Ratio	9.21±0.36^{c}	9.45±0.26^{c}	11.01±0.41^{b}	10.97±0.28^{b}	11.97±0.48^{a}	11.22±0.32^{b}
Jejunum	Villus length, um	777.25±48.67^{c}	903.5±34.66^{b}	982.23±8.30^{a}	1013.69±65.95^{a}	1084.8±4.52^{a}	1100.91±16.49^{a}
	crypt depth, um	73.54±1.74^{c}	127.74±10.20^{b}	113.76±12.86^{b}	110.65±14.48^{b}	138.09±14^{b}	167.74±6.66^{a}
	Ratio	10.57±0.64^{a}	7.12±0.71^{a}	8.71±0.87^{a}	9.23±0.69^{a}	7.92±0.82^{a}	6.57±0.23^{a}
Ilium	Villus length, um	721.14±12.12^{d}	771.34±21.01^{c}	848.57±8.25^{b}	839.51±11.39^{b}	950.48±1.44^{a}	963.52±24.50^{a}
	crypt depth, um	113.36±6.28^{c}	109.21±7.33^{c}	126±7.21^{b}	104.27±6.69^{c}	111.69±2.62^{c}	140.99±4.78^{a}
	Ratio	6.37±0.27^{b}	7.08±0.31^{b}	6.75±0.34^{b}	8.08±0.55^{a}	8.52±0.20^{a}	6.84±0.19^{b}

Values are expressed as Mean ±SD. Means with different superscripts in the same row are significantly different (P≤0.05).
G1, Control group (basal diet); G2, basal Diet + 50g/ton phytase; G3, basal Diet + 75g/ton phytase; G4, basal Diet + 100g/ton phytase; G5, basal Diet + 150g/ton phytase; G6, basal Diet + 200g/ton phytase.

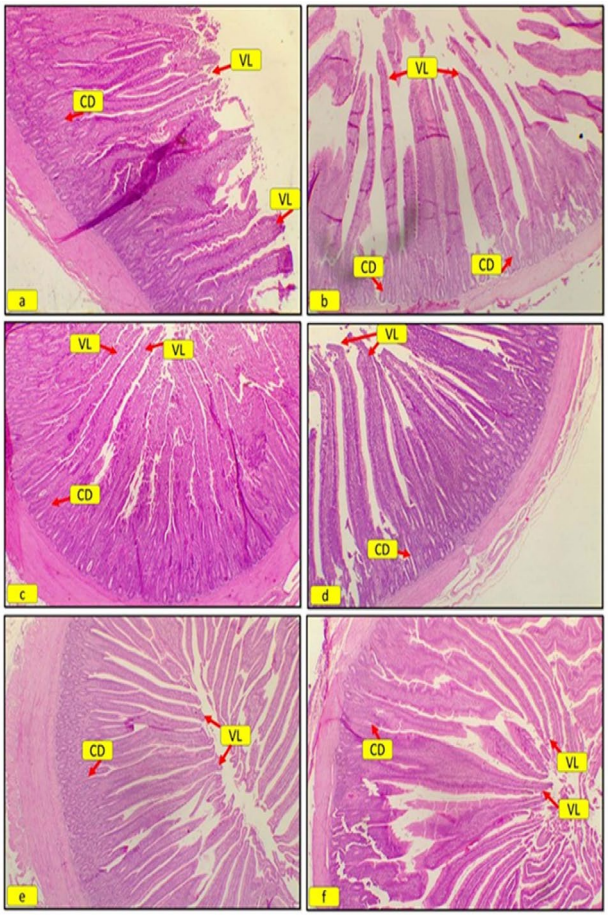

Fig. 1. Histological sections from the small intestine of the poultry duodenal part show normal histological structure of the tunica mucosa, propria submucosa, muscularis, and tunica serosa. Notice: intestinal villi and basic tubular intestinal crypts, both of which are covered with absorptive columnar epithelial cells. It's notable how the length of the villi and the depth of the crypts directly increase as more phytase is added. VL: Villus length. CD: Crypt depth. (H&E, ×100) (Scale bar represents 200 µm).

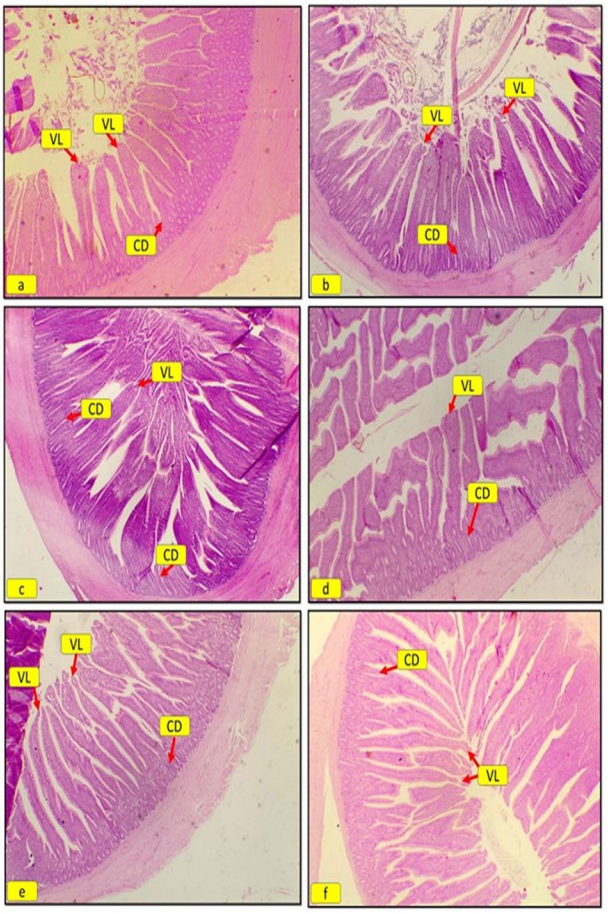

Fig. 2. Histological sections from the small intestine of the poultry jejunal part show normal histological structures of the tunica mucosa, propria submucosa, muscularis, and tunica serosa. Notice: intestinal villi and basic tubular intestinal crypts, both of which are covered with absorptive columnar epithelial cells. It's notable how the length of the villi and the depth of the crypts directly increase as more phytase is added. VL: Villus length. CD: Crypt depth. (H&E,×100) (Scale bar represents 200 µm).

deficient in P and the mitigation of phytate's detrimental effects on the digestion of diverse nutrients, including calcium, zinc, iron, starch, amino acids, and lipids (Walk *et al.*, 2014). These results are in line with Metwally *et al.* (2020), who determined that birds supplemented with phytase (at a dosage of 1500 FTU/kg) exhibited notably higher BW and BWG at the second and fifth weeks of age when compared to the non-supplemented control group. Nonetheless, Ajith *et al.* (2018) did not observe a substantial difference in broiler performance (BW, BWG, and FI) throughout the entire phase. This could potentially be attributed to the low levels of enzyme supplementation and the purification of the enzyme.

Increasing the phytase level up to 200g/ton in the current study also showed a tendency to enhance FI in broiler chickens compared to other groups receiving lower doses of phytase. Männer *et al.* (2022) conducted a comparable study, wherein they noted that birds administered the highest phytase dose exhibited the maximum FI and achieved the best BW by the conclusion of the trial. Additionally, Walters *et al.* (2019) supported the findings from the present study and demonstrated a linear relationship between phytase dose and FI. They also observed a substantial rise in FI with doses ranging from 2000 to 3000 FTU/kg even when used in a low-phosphorus diet, surpassing that of the control group. In contrast, lower levels of phytase supplementation resulted in a reduction in FI. These findings align with those of Cowieson *et al.* (2011), who suggested that phytate could suppress the bird's appetite due to phosphorus deficiency, which can be compensated by phytase supplementation. Once the birds' phosphorus requirement is met, FI becomes stagnant until further increases in phytase levels in the diet. Phytase overdosing can enhance FI by releasing more phosphorus and nutrients from the remaining lower phytate esters not initially dephosphorylated by phytase.

In the current investigation, superior FCR was observed among birds in G5 and G6 compared to the other experimental groups. These results coincided with Walk *et al.* (2013), who observed notable improvements in FCR among broilers aged 49 days when provided with phytase-sup-

plemented diets compared to those fed diets meeting their nutritional needs. Hofmann *et al.* (2022) demonstrated that supplementing phytase at a concentration of 1,500 FTU/kg into a diet low in phosphorus, protein, and amino acids resulted in enhanced weight gain and improved FCR. Likewise, Smith *et al.* (2019) proposed that phytase addition at 3,000 FTU/kg resulted in better FCR as compared with inclusion at 500 FTU/kg, while phytase level at 1,500 FTU/kg showed intermediate effects.

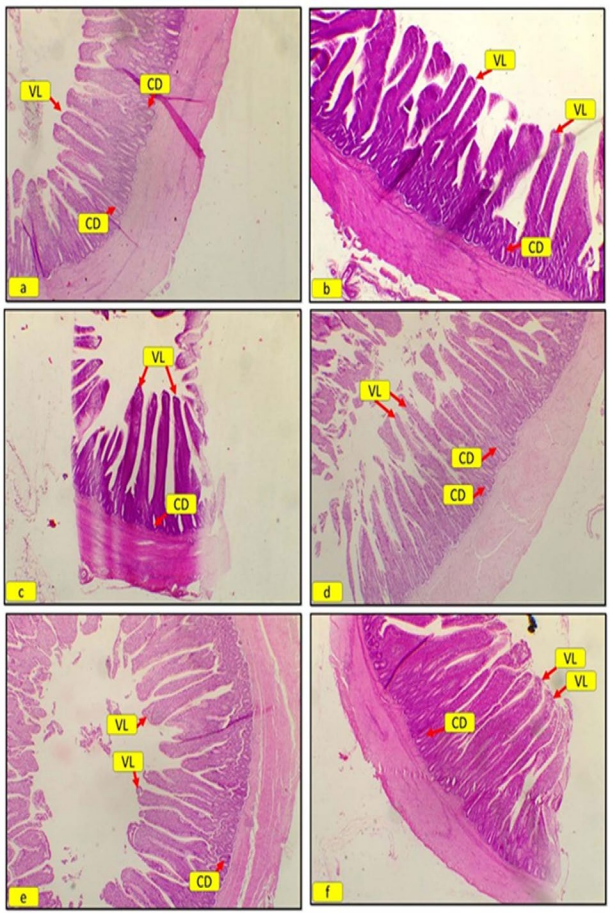

Fig. 3. Histological sections from the small intestine of the poultry ilium part show normal histological structures of the tunica mucosa, propria submucosa, muscularis, and tunica serosa. Notice: intestinal villi and basic tubular intestinal crypts, both of which are covered with absorptive columnar epithelial cells. It's notable how the length of the villi and the depth of the crypts directly increase as more phytase is added. VL: Villus length. CD: Crypt depth. (H&E,×100) (Scale bar represents 200 μm).

The economic assessment indicated a positive outcome with the incorporation of the phytase enzyme in broiler diets. The result of the present study revealed that, as the concentrations of phytase enzyme increased, the net profit also increased, and the maximum profit was achieved in G6, with G5 following closely behind. Al-Harthi *et al.* (2020) found that adding phytase at a concentration of 500 FTU/kg significantly boosted overall revenue, net profit, and economic efficiency in contrast to the control group. Additionally, Scholey *et al.* (2018) noted that phytase can fulfill the P needs of broilers during the finishing phase without relying on inorganic phosphorus, presenting an economical solution for meeting these requirements. Alshamiri *et al.* (2021) affirmed the significant impact of supplementing broiler diets with phytase enzyme on economic efficiency, highlighting that those provided with diets containing 1500 FTU/kg exhibited the lowest cost and highest return. Similarly, Rezaei *et al.* (2007) demonstrated that incorporating phytase into broiler diets boosts economic efficiency by improving productive performance, increasing retention of calcium and phosphorus, and minimizing production costs.

In the present study, increasing the phytase level in broiler diets led to a significant decrease in feed cost incurred per unit weight and unit weight gain, with the lowest cost observed in G6 which received (200g/ton phytase), in contrast to the control group, which recorded the highest cost. These results align with those of Bello *et al.* (2022), who discovered that total feed expenditures per kilogram of body weight gain were reduced when phytase was introduced to diets without additional inorganic phosphate at doses of 1,000 FTU/kg, 2,000 FTU/kg, and 3,000 FTU/kg.. Similarly, Iqbal *et al.* (2023) demonstrated that adding phytase at a level of 1000 FTU/kg to dietary accessible phosphorus at 0.35% led to a 9.17% reduction in the costs incurred per unit weight gain, while also positively impacting growth performance. Furthermore, Anjum *et al.* (2018) observed that broilers fed diets containing 50% less digestible crude protein with added phytase incurred numerically lower total feed costs per unit weight gain contrasted with those received phytase-free diets and the positive control diet.

In the current study, adding phytase at different doses to broiler diets did not affect the pH, cooking loss%, drip loss%, or L* value of broiler meat color. However, there was a significant increase in WHC% and shear force in G5, and G6 compared to other experimental groups. These findings affirmed with those of Srikanthithasan *et al.* (2020), who reported that adding phytase to the diet did not significantly affect meat quality indices when compared to diets lacking phytase. Similarly, Hao *et al.* (2017) concluded that dietary phytase supplementation did not influence breast muscle color, drip loss, or PH. Regarding the a* and b* values of broiler meat, there were significant decreases observed in birds fed high doses of phytase (G4, G5, and G6) compared to G1, G2 and G3. These findings are corroborated by Hakami *et al.* (2022), who noted a reduction in the a* value of breast meat color in the groups that received phytase supplements at both 15 minutes and 24 hours postmortem, suggesting improved meat color. Maynard *et al.* (2023) observed that phytase supplementation had a dietary impact on the b* values of broiler meat, with the negative control diet showing more yellowness in breast fillets than the phytase-supplemented group at both 4 hours and 24 hours postmortem.

These results were supported by the enhancements observed in the histomorphological images of the intestines of broilers treated with phytase when compared to those in the control group. The study demonstrated that phytase application positively impacted intestinal histomorphology, leading to favorable changes in VL, CD, and the VL:CD ratio. An optimal intestinal morphology is characterized by longer and wider intestinal villi, which indicates a larger surface area for absorption and increased activity of digestive enzymes, facilitating better nutrient uptake (Laudadio *et al.*, 2012). The results obtained were in accordance with those of Suryani *et al.* (2022), who discovered that supplementing broiler diets with L. plantarum phytase at a level (500 FTU/kg) led to significantly higher VL and VL:CD ratios in contrast to the control group. Karami *et al.* (2020) also observed consistent results, showed that supplementing the diet with phytase notably elevated the VL in broiler chickens and enhanced the VL:CD ratio. Another study by Guler *et al.* (2022) demonstrated that phytase supplementation in broiler diets significantly enhanced VL, VL:CD ratio, intestinal mucosal thickness, and area of villus absorption relative to the non-supplemented control group. Sajadi Hezaveh *et al.* (2020) concluded that incorporating phytase (500 FTU /kg) into low non-phytate phosphorus diets had more favorable effects on enhancing both growth performance and intestinal morphology. They observed significant improvements in the VL in both the jejunum and ileum. The present findings agree with Smulikowska *et al.* (2010), who also noted greater villus length in the epithelium of the small intestine in 28-days-old broilers that received diets fortified with phytase (1000 FTU/kg).

Conclusion

The current study demonstrated that incorporating high doses (150 and 200 gm/ton) of phytase in broiler diets positively impacts BW, BWG, and FCR while also significantly enhanced intestinal morphometry. In addition, a high dose of phytase supplementation significantly decreased feed cost per unit weight gain, consequently improving overall profit. This suggests that the dosage-dependent application of phytase positively influences the economic efficiency of broilers.

Conflict of interest

The authors declare no competing interests.

References

Adeyemi, K.D., 2021. Comparative effect of dietary Morinda lucida leaf and butylated hydroxyanisole (BHA) on carcass traits, meat quality, and oxidative stability of broiler chickens. J. Food Sci. Technol. 58, 4359–4369. https://doi.org/10.1007/s13197-020-04916-2

Ajith, S., Shet, D., Ghosh, J., Awachat, V.B., Bhat, K., Pal, D., Elangovan, A.V., 2018. Effect of immobilized fungal phytase on growth performance and bone traits of broilers fed with low dietary calcium and phosphorus. Vet. World. 11, 758–764. https://doi.org/10.14202/vetworld.2018.758-764

Al-Harthi, M.A., Attia, Y.A., El-Shafey, A.S., Elgandy, M.F., 2020. Impact of phytase on improving the utilisation of pelleted broiler diets containing olive by-products. Ital. J. Anim. Sci. 19, 310–318. https://doi.org/10.1080/1828051X.2020.1740896

Albrecht, A., Hebel, M., Heinemann, C., Herbert, U., Miskel, D., Saremi, B., Kreyenschmidt, J., 2019. Assessment of meat quality and shelf life from broilers fed with different sources and concentrations of methionine. J. Food Qual. 2019. 1–10. https://doi.org/10.1155/2019/6182580

Alshamiri, M.M.A., Ali, S.A.M., Abdalla, H ., Elkheir, H.B.A., 2021. Efficacy of supplementation of phytase enzyme to broiler diets with or without di-calcium phosphate on performance and economic appraisal. Egypt. Poult. Sci. J. 41, 317–331. https://doi.org/10.21608/epsj.2021.182510

Anjum, M.I., Javaid, S., Nadeem, M.A., 2018. Effect of supplementing microbial phytase on broiler chicks fed low di-calcium phosphate diets. Pak. J. Zool. 50, 347–351. https://doi.org/10.17582/journal.pjz/2018.50.1.347.351

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

Bello, A., Dersjant-Li, Y., van Eerden, E., Kwakernaak, C., Marchal, L., 2022. Supplementation of an all-plant-based inorganic phosphate-free diet with a novel phytase maintained tibia ash and performance in broilers under a commercial production setting. J. Appl. Poult. 31, 100253. https://doi.org/10.1016/j.japr.2022.100253

Cowieson, A.J., Wilcock, P., Bedford, M.R., 2011. Super-dosing effects of phytase in poultry and other monogastrics. Worlds. Poult. Sci. J. 67, 225–235. https://doi.org/10.1017/S0043933911000250

Dersjant-Li, Y., Awati, A., Schulze, H., Partridge, G., 2015. Phytase in non-ruminant animal nutrition: A critical review on phytase activities in the gastrointestinal tract and influencing factors. J. Sci. Food Agric. 95, 878–896. https://doi.org/10.1002/jsfa.6998

Guler, S., Asmaz, E.D., Kayapunar, N.V., Isbilir, I., Cengiz, S.S., Yesilbag, D., Sanli, A.B., Gultepe, E.E., 2022. Effects of dietary calcium, phosphorus and microbial phytase on intestinal morphology in laying hens. Turkish J. Vet. Anim. Sci. 46, 293–303. https://doi.org/10.55730/1300-0128.4177

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

Hao, X., Yoo, J., Kim, I., 2017. Effect of phytase supplementation on growth performance, nutrient digestibility, and meat quality in broiler. Korean J. Agric. Sci. 45, 401–409. https://doi.org/10.7744/kjoas.20170041

Hernandez, J.R., Gulizia, J.P., Adkins, J.B., Rueda, M.S., Haruna, S.I., Pacheco, W.J., Downs, K.M., 2022. Effect of phytase level and form on broiler performance, tibia characteristics, and residual fecal phytate phosphorus in broilers from 1 to 21 days of age. Animals. 12, 1952. https://doi.org/10.3390/ani12151952

Hofmann, P., Krieg, J., Francesch, M., Feuerstein, D., Rodehutscord, M., 2022. Effects of added phytase on growth performance, carcass traits, and tibia ash of broiler chickens fed diets with reduced amino acid, crude protein, and phosphorus concentration. J. Appl. Poult. 31, 100258. https://doi.org/10.1016/j.japr.2022.100258

Hossain, S., Hossain, M.A., Akter, N., Akter, M., 2022. Effects of phytase super dosing on performance, plasma mineral contents and bone mineralization in broiler chicken. Int. J. Poult. Sci. 21, 1–9. https://doi.org/10.3923/ijps.2022.1.9

Hubbard, 2022. Hubbard efficiency plus Management guide broiler.' Available at broiler_guide_efficiency_plus_en_imp_20220120_ld.pdf (hubbardbreeders.com).

Iqbal, W., Yaseen, M., Rahman, M., Bhatti, S., Rahman, M., Yaqoob, M., Ahmad, F., Zahid, M., Shoaib, M., 2023. Effect of phytase supplementation on growth performance, mineral digestibility, and tibia calcium and phosphorous in broilers fed low phosphorus diets. Braz. J. Poult. Sci. 25. https://doi.org/10.1590/1806-9061-2023-1773

Jing, M., Zhao, S., Rogiewicz, A., Slominski, B.A., House, J.D., 2021. Effects of phytase supplementation on production performance, egg and bone quality, plasma biochemistry and mineral excretion of layers fed varying levels of phosphorus. Animal. 15, 100010. https://doi.org/10.1016/j.animal.2020.100010

Karami, M., Karimi, A., Sadeghi, A., Zentek, J., Goodarzi Boroojeni, F., 2020. Evaluation of interactive effects of phytase and benzoic acid supplementation on performance, nutrients digestibility, tibia mineralisation, gut morphology and serum traits in male broiler chickens. Ital. J. Anim. Sci. 19, 1428–1438. https://doi.org/10.1080/1828051X.2020.1846468

Klupsaite, D., Buckiuniene, V., Sidlauskiene, S., Lele, V., Sakiene, V., Zavistanaviciute, P., Klementaviciute, J., Viskontaite, E., Bartkiene, E., 2020. Comparison studies of the chemical, physical, technological, and microbiological characteristics of the European roe deer, boar, red deer, and beaver hunted wild game meat. Anim. Sci. J. 91, 1–11. https://doi.org/10.1111/asj.13346

Kumar, A., Chanderman, A., Makolomakwa, M., Perumal, K., Singh, S., 2016. Microbial production of phytases for combating environmental phosphate pollution and other diverse applications. Crit. Rev. Environ. Sci. Technol. 46, 556–591. https://doi.org/10.1080/10643389.2015.1131562

Laudadio, V., Passantino, L., Perillo, A., Lopresti, G., Passantino, A., Khan, R.U., Tufarelli, V., 2012 Productive performance and histological features of intestinal mucosa of broiler chickens fed different dietary protein levels. Poult. Sci. 91, 265–270. https://doi.org/10.3382/PS.2011-01675

Männer, B. , Ader, K., Zentek, P., Nczyk, S., Bienko, M., Radzki, R.P., Walk, C.L., Martínez-Vallespín, B., Männer, K., Ader, P., Zentek, J., 2022. Evaluation of high doses of phytase in a low-phosphorus diet in comparison to a phytate-free diet on performance, apparent ileal digestibility of nutrients, bone mineralization, intestinal morphology, and immune traits in 21-day-old broiler chickens. Animals. 12, 1955. https://doi.org/10.3390/ANI12151955

Marcu, A., Vacaru-Opriş, I., Dumitrescu, G., Ciochina, L.P., Marcu, A., Nicula, M., Peţ, I., Dronca, D., Kelciov, B., 2013. The influence of the genotype on economic efficiency of broiler chickens growth. Anim. Sci. Biotechnol. 46, 339–346.

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

Metwally, M.A., Farghly, M.F.A., Ismail, Z.S.H., Ghonime, M.E., Mohamed, I.A., 2020. The effect of different levels of optizyme and phytase enzymes and their interactions on the performance of broiler chickens fed corn/soybean meal: 1-broiler performance, carcass traits, blood constituents and nitrogen retention efficiency. Egypt. J. Nutr. Feed. 23, 123–136. https://doi.org/10.21608/EJNF.2020.95831

Mohammed, L.S., Sallam, E.A., Edris, S.N., Khalifa, O.A., Soliman, M.M., Shehata, S.F., 2021. Growth performance, economic efficiency, meat quality, and gene expression in two broiler breeds fed different levels of tomato pomace. Vet. Res. Commun. 45, 381–397. https://doi.org/10.1007/s11259-021-09819-x

Nari, N., Ghasemi, H.A., 2020. Growth performance, nutrient digestibility, bone mineralization, and hormone profile in broilers fed with phosphorus-deficient diets supplemented with butyric acid and Saccharomyces boulardii. Poult. Sci. 99, 926–935. https://doi.org/10.1016/j.psj.2019.10.046

Omar, M.A.E., 2014. Economic evaluation of probiotic (*Lactobacillus acidophilus*) using in different broiler breeds within egypt. Benha Vet. Med. J. 2, 52–60.

Rezaei, M., Borbor, S., Zaghari, M., Teimouri, A., 2007. Effect of phytase supplementation on nutrients availability and performance of broiler chicks. Int. J. Poult. Sci. 6, 55–58. https://doi.org/10.3923/ijps.2007.55.58

Rozanski, S., Vivian, D.R., Kowalski, L.H., Prado, O.R., Fernandes, S.R., Souza, J.C., de Freitas, J.A., 2017. Carcass and meat traits, and non-carcass components of lambs fed ration containing increasing levels of urea. Semin.Ciências Agrárias. 38, 1587. https://doi.org/10.5433/1679-0359.2017v38n3p1577

Sajadi Hezaveh, M.S., Ghasemi, H.A., Hajkhodadadi, I., Moradi, M.H., 2020. Single and combined effects of phytase and citric acid on growth performance, nutrient digestibility, bone characteristics, intestinal morphology, and blood components in meat-type quails fed low-phosphorous diets. Anim. Feed Sci. Technol. 269, 114677. https://doi.org/10.1016/j.anifeedsci.2020.114677

Scholey, D.V., Morgan, N.K., Riemensperger, A., Hardy, R., Burton, E.J., 2018. Effect of supplementation of phytase to diets low in inorganic phosphorus on growth performance and mineralization of broilers. Poult. Scie. 97, 2435–2440. https://doi.org/10.3382/ps/pey088

Selim, S., Abdel-Megeid, N.S., Khalifa, H.K., Fakiha, K.G., Majrashi, K.A., Hussein, E., 2022. Efficacy of various feed additives on performance, nutrient digestibility, bone quality, blood constituents, and phosphorus absorption and utilization of broiler chickens fed low phosphorus diet. Animals. 12, 1742. https://doi.org/10.3390/ani12141742

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

Smith, A.K., Wyatt, C.L., Lee, J.T., 2019. Evaluation of increasing levels of phytase in diets containing variable levels of amino acids on male broiler performance and processing yields. J. Appl. Poult. Res. 28, 253–262. https://doi.org/10.3382/JAPR/PFY065

Smulikowska, S., Czerwiński, J., Mieczkowska, A., 2010. Effect of an organic acid blend and phytase added to a rapeseed cake-containing diet on performance, intestinal morphology, caecal microflora activity, and thyroid status of broiler chickens. J. Anim. Physiol. Anim. Nutr. (Berl). 94, 15–23. https://doi.org/10.1111/j.1439-0396.2008.00876.x

Srikanthithasan, K., Macelline, S.P., Wickramasuriya, S.S., Tharangani, H., Li-Ang, Jayasena, D.D., Heo, J.M., 2020. Effects of adding phytase from aspergillus Niger to a low phosphorus diet on growth performance, tibia characteristics, phosphorus excretion, and meat quality of broilers 35 days after hatching. J. Poult. Sci. 57, 28–36. https://doi.org/10.2141/jpsa.0180143

Spss, I., 2007. SPSS version 16.0. Chicago, IL: SPSS Incorporated.

Suryani, A.E., Anggraeni, A., Karimy, M., Herdian, H., Istiqomah, L., 2022. Evaluation of phytase supplementation on performance , metabolic energy , ileal histomorphology , meat and bone mineralization of broiler chickens. 1–19. https://doi.org/10.21203/rs.3.rs-1503110/v1

Tahir, M., Shim, M.Y., Ward, N.E., Smith, C., Foster, E., Guney, A.C., Pesti, G.M., 2012. Phytate and other nutrient components of feed ingredients for poultry. Poult. Sci. 91, 928–935. https://doi.org/10.3382/ps.2011-01893

Tareen, M.H., Wagan, R., Siyal, F.A., Babazadeh, D., Bhutto, Z.A., Arain, M.A., Saeed, M., 2017. Effect of various levels of date palm kernel on growth performance of broilers. Vet. World 10, 227–232. https://doi.org/10.14202/vetworld.2017.227-232

Walk, C.L., Bedford, M.R., Santos, T.S., Paiva, D., Bradley, J.R., 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

Walk, C.L., Santos, T.T., Bedford, M.R., 2014. Influence of superdoses of a novel microbial phytase on growth performance, tibia ash, and gizzard phytate and inositol in young broilers. Poult. Sci. 93, 1172–1177. https://doi.org/10.3382/ps.2013-03571

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. 28, 1210–1225. https://doi.org/10.3382/JAPR/PFZ087

Woyengo, T.A., Nyachoti, C.M., 2013. Review: Anti-nutritional effects of phytic acid in diets for pigs and poultry. Can. J. Anim. Sci. 93, 9–21. https://doi.org/10.4141/cjas2012-017

Xie, M., Wang, R., Wang, Y., Liu, N., Qi, J., 2021. Effects of dietary supplementation with fermented chenopodium album l. on growth, nutrient digestibility, immunity, carcase characteristics, and meat quality of broilers. Ital. J. Anim. Sci. 20, 2063–2074. https://doi.org/10.1080/1828051X.2021.1996289

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. DOI: 10.1093/ps/82.6.1030

Zanu, H.., Keerqin, C., Kheravii, S.K., Morgan, N., 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