Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 29(3): 957 – 973 (2025)
www.ejabf.journals.ekb.eg

Enhancing Growth and Health in Tilapia (*Oreochromis niloticus*) Juvenile: The Role of Dietary Phytase in Digestive Enzymes, Hematobiochemical Index, and Bone Mineral Composition

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ARTICLE INFO

Article History:
Received: Jan. 30, 2025
Accepted: May 11, 2025
Online: May 21, 2025

Keywords:
Digestibility,
Feed,
Growth,
Mineral,
Phytase

ABSTRACT

Juvenile tilapia generally require higher protein for growth and health than fish in the advanced stage (enlargement). Phytase is important for juvenile tilapia fish since it can hydrolyze phytate, increase digestive enzyme activity and mineral digestibility, and improve fish growth performance. The current study aimed to investigate the effect of phytase on growth performance, digestive enzyme activity, hematological, biochemical indices, bone mineral composition, and feed efficiency of juvenile-stage tilapia. A total of 500 juvenile tilapia (6.42 ± 0.35g/ head) were used and were then fed with experimental feed containing phytase enzyme supplementation at 0, 250, 500, 750, 1000, and 1250 FTU/kg feed (treatments A-F). The digestive enzyme, hematobiochemical index, bone mineral composition, protein digestibility, weight gain, apparent digestibility coefficients (ADCp), relative growth rate (RGR), protein efficiency ratio (PER), feed intake (FI), feed conversion ratio (FCR), and survival rate (SR) of juvenile-stage tilapia were evaluated. The supplementation of phytase significantly (P<0.05) enhanced digestive enzyme activities, including chymotrypsin and trypsin, compared to those without phytase. Futhermore, it improved hematological parameters (red blood cells, hemoglobin, packed cell volume, white blood cells, and platelets) and serum biochemical indices (total protein, albumin, and globulin). Bone mineral composition, including phosphorus (P), calcium (Ca), magnesium (Mg), and zinc (Zn), was also improved. Among the tested doses, 1000 FTU/kg feed provided the highest improvements in growth performance, feed efficiency, and bone mineral composition, making it the optimal dose for juvenile tilapia.

INTRODUCTION

Tilapia (*Oreochromis niloticus*) is one of the most economically important freshwater fish spesies due to its ease of cultivation, popularity among consumers, and affordability **(Rathod *et al.*, 2018)**. It is also characterized by rapid growth rate, high acceptance of artificial feed, tolerance to high stocking densities, resistance to many diseases, and adaptability to a wide range of environmental conditions **(Kummari *et al.*,**

2018). The use of high-quality feed mainly influences the effectiveness of intensive tilapia farming. Such feed typically contains fishmeal as a primary source of animal protein, sometimes comprising up to 50% of the formulation. However, fishmeal is also the most expensive component in aquafeed, posing a significant economic challenge to sustainable aquaculture **(Kumar *et al.*, 2018)**. The aquafeed industry has increasingly turned to plant-based protein sources, such as soybean meal, due to their availability and lower cost **(Hussain *et al.*, 2020; Fiorella *et al.*, 2021)**.

Despite its advantages, soybean meal contains phytate, an antinutrient that reduces protein and mineral bioavailability. Phytate forms insoluble complexes with proteins and essential minerals, such as calcium (Ca), phosphorus (P), iron (Fe), zinc (Zn), and magnesium (Mg), thereby inhibiting digestive enzymes like pepsin and amylase **(Cao *et al.*, 2007; Liu *et al.*, 2013)**. As a result, fish experience reduced nutrient absorption and impaired growth due to limited uptake of essential macro- and microminerals **(Akpoilih *et al.*, 2016)**. Given these limitations, strategies to improve the digestibility of plant-based ingredients are essential for sustainable aquaculture.

Fish generally lack adequate endogenous phytase to hydrolyze phytate; thus, dietary phytase supplementation is a practical strategy to enhance nutrient utilization **(Adeshina *et al.*, 2023; Rachmawati *et al.*, 2023a)**. Numerous studies have demonstrated the effectiveness of dietary phytase in the diets of a variety of fish species. **Rachmawati *et al.* (2023a)** demonstrated the effectiveness of phyase in improving the growth performance of *Clarias gariepinus* juveniles by increasing nutrient digestibility. **Rachmawati *et al.* (2023b)** also found that phytase enzymes elevated the protein and amino acid digestibility of *Cyprinus carpio*. Similarly, **Salem *et al.* (2022)** found that the growth performance and body nutritional value of *Sparus aurata* were improved after being supplemented with phytase. **Shahzad *et al.* (2022)** demonstrated that dietary phytase could reduce the release of nutrients and minerals to the feces of *Oreochromis niloticus* fingerlings.

Our previous research confirmed that phytase administration enhances the growth performance, feed efficiency, and mineral content at the fingerlings stage of tilapia **(Rachmawati *et al.*, 2024)**. However, limited data exist on the use of phytase in soybean meal-based feed for different stages of tilapia development. Addressing this gap is crucial for optimizing feed formulations that support sustainable tilapia farming practices. Therefore, this study focused on examining the impact of phytase addition on growth performance, digestive enzyme activity, hematobiochemical index, bone mineral composition, and feed efficiency of juvenile-stage tilapia. The findings will contribute to advancing cost-effective and nutritionally balanced feeding strategies for tilapia aquaculture.

MATERIALS AND METHODS

1. Fish and experimental design

The present study was carried out at the laboratory of the Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Diponegoro University, Indonesia. Five hundred juvenile-stage tilapia (average weight of 6.42 ± 0.35g) were obtained from the Freshwater Fish Cultivation Center (BBIAT), Muntilan, Central Java, Indonesia. Juvenile-stage tilapia were acclimated for one week before being fed the experimental feed. Ad satiation feeding was used three times a day at 07:00, 13:00, and 19:00 to provide the fish with non-phytase food during the acclimation phase (temperature of 25-30°C, pH 6.5-8.6, and dissolved oxygen/DO ≥ 3 mg/L). Furthermore, only fish with uniform size, weight, and active swimming behavior, without deformities and in good health, were selected for the study. The day before the experiment began, these fish were fasted to ensure their digestive tracts were clear of any remaining feed **(Rachmawati *et al*., 2017)**.

2. Preparation of test feed

The test feed was formulated as pellets containing 31% protein and isoenergetic 252 kcal/g **(Shahzad *et al*., 2022)**. To assess nutrient digestibility, 1% chromic oxide (Cr_2O_3) was incorporated into each diet as an inert marker, in accordance with **NRC (2011)** guidelines. Cr_2O_3 is indigestible and does not affect the digestive process, making it a reliable indicator for estimating apparent digestibility coefficients by comparing its concentration in feed and feces **(Pérez-Jiménez *et al*., 2014)**.

Phytase enzyme was supplemented at different concentrations: 0 (A), 250 (B), 500 (C), 750 (D), 1,000 (E), and 1,250 FTU/kg feed (F). The phytase used was neutral with an enzyme activity of 10,000 U/g, produced by BASF SE, Ludwigshafen, Germany. According to the test feed formulation (Table 1), the ingredients were added gradually, except for fat and water sources, which were added after all ingredients were mixed **(NRC, 2011)**. The feed mixture was stirred using a stirring mixer until homogeneous, and then fish oil, corn oil, and water were added. The homogeneous feed mixture was put into an extruder floating pellet molding machine (H 2700, China) at 50-60°C. Next, the test feed was dried by aerating at room temperature around 26°C, and the dried test feed was packed in an airtight plastic bag and stored until used. Optimal feed storage conditions include a dry, non-humid environment protected from direct sunlight. Ideally, feed should be stored for no longer than 30 days post-production to preserve its nutritional quality and prevent spoilage or microbial growth.

Table 1. The formulation of the test feed (g/1000 g of feed)

Ingredient (g)	**Test Feed**					
	A	**B**	**C**	**D**	**E**	**F**
Fishmeal	240	240	240	240	240	240
Soybean meal	345	345	345	345	345	345
Corn flour	155	155	155	155	155	155
Bran	145	145	145	145	145	145
Tapioca flour	80	80	80	80	80	80
Fish oil	10	10	10	10	10	10
Corn oil	10	10	10	10	10	10
Mineral	10	10	10	10	10	10
Vitamin mix[1]						
Chromium (III) oxide (Cr_2O_3)	10	10	10	10	10	10
Total	1,000	1,000	1,000	1,000	1,000	1,000
Phytase levels (FTU/kg diet)	0	250	500	750	1000	1250
Proximate composition (g/kg diet)						
Dry matter*	980.0	970.0	970.0	970.0	960.0	960.0
Crude protein*	311.5	311.0	311.0	311.2	311.4	311.2
Ether extract*	87.4	86.9	87.5	86.8	87.5	87.2
Carbohydrate*	204.0	204.2	204.3	204.3	204.0	204.2
Ash*	76.2	76.5	76.2	76.5	76.3	76.4
Crude fibre*	122.2	122.3	122.0	122.2	122.3	122.3
Analysed phytase (FTU/kg diet) *	84.2	249	468	749	998	1246
Energy (kcal)*	252.34	252.82	252.89	252.98	252.99	252.98
Ratio of energy/protein (E/P) (cal/g)[3]	8.10	8.13	8.13	8.12	8.12	8.12
Mineral contents (g/kg diet)						
Calcium*	17.8	17.7	17.8	17.7	17.8	17.7
Magnesium*	13.4	13.4	13.3	13.4	13.3	13.4
Zinc*	27.6	27.7	27.8	27.8	27.8	27.6
Phosphorus*	14.2	14.3	14.3	14.4	14.4	14.3

Note.

[1)]Vitamin and Mineral mix kg-1: vitamin A (4,000,000 IU); vitamin B1 (4000 mg); vitamin B2 (3000 mg); vitamin B6 (3800 mg); vitamin B12 (3 mg); vitamin D3 (8,00,000 IU); vitamin E (40,000 IU); vitamin K3

(1600 mg); folic acid (800 mg); biotin (100 mcg); coated vitamin C (60,000 mg); inositol (10,000 mg); nicotinic acid (18,000 mg); pantothenic acid (8000 mg); choline chloride (120,000 mg); lysine (10,000 mg); iron (8000 mg); iodine (400 mg); selenium (40 mg); cobalt (150 mg); copper (800 mg); manganese (6000 mg); zinc (20,000 mg).

[2)]According to NRC (2011), the E/P value for optimal growth of fish is around 8-12 kkal/g.

*From the results of the proximate analysis of the Animal Food Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2024)

3. Fish rearing containers

Twenty-four aquariums (80×80×80cm) with a volume of 512L were used in this study as the fish-rearing containers. Recirculation techniques were set up in the aquariums to maintain the water quality within the ideal range (temperature 25-30°C, pH 6.5-8.6, and DO ≥3 mg/L). The rearing media in this study included the water from water sources that had been precipitated in reservoirs and aerated before being used as cultivation media. This study utilized a Recirculating Aquaculture System (RAS), where water is continuously circulated and reused following filtration. The system incorporates both mechanical and biological filters.

4. Experimental procedure

The selected fish were placed into aquariums containing 25L of water, at a stocking density of one fish per liter. Feed was given using the ad satiation method with a frequency of feeding three times a day, namely at 06.00, 12.00, and 18.00. To determine the weight gain of fish, weighing was carried out every week during the 56-day study. To maintain the water quality of the culture media, a small hose was used to remove feces and feed residue from the bottom of the fiber tanks. Flushing was done approximately 2h after feeding time every day. The following formula was used to determine growth and feed utilization:

$$Weight\ gain\ (\%) = \frac{(Wt2 - Wt1)}{Wt1} \quad (1)$$

$$Apparent\ Digestibility\ Protein\ (ADCp)(\%) = 100 - \left\{\frac{100 \times Cr_2O_3\ in\ the\ feed}{\%\ Cr_2O_3\ in\ the\ feces} \times \frac{\%\ protein\ in\ the\ feces}{\%\ protein\ in\ the\ feed}\right\} \quad (2)$$

$$Relative\ growth\ rate\ (RGR)(\%/day) = 100 \times \frac{(final\ weight - initial\ weight)}{(times\ of\ experiment \times initial\ weight)} \quad (3)$$

$$Feed\ intake\ (FI)(g) = the\ summation\ of\ the\ offered\ feed\ (g)\ to\ fish\ throughout\ the\ experiment \quad (4)$$

$$Feed\ converion\ ration\ (FCR) = \frac{total\ feed\ fed\ (g)}{total\ wet\ weight\ gain\ (g)} \quad (5)$$

$$Protein\ efficiency\ ratio\ (PER) = 100 \times \frac{(final\ weight - initial\ weight)}{Amount\ of\ diet\ consumed \times protein\ content\ diet} \quad (6)$$

$$Survival\ Rate\ (SR)\ (\%) = 100\left(\frac{final\ count}{initial\ count}\right) \quad (7)$$

5. Protein digestibility analysis

During the 56-day trial, fish excrement was collected in the morning, midday, and evening after being fed. The protein digestibility was determined by adding Cr_2O_3 at 1% to indicate protein digestibility in the test feed **(Pérez-Jiménez *et al.*, 2014)**. Using a plankton cloth net, fecal filtering was carried out, and then the results of the fecal filtering were put and stored in a 4°C refrigerator. Furthermore, the feces were dried in an oven (Gravity Oven) at 6°C for 24h and were then analyzed. Analysis of protein and Cr_2O_3 content in feces was conducted using an SSA spectrophotometer at 350nm.

6. Digestive enzyme analysis

6.1. Trypsin activity evaluation

A total of 43.5mg benzoyl-DL-arginine-p-nitroanilide/BAPNA (Sigma) was added with 1mL dimethylsulfoxide (DMSO) and 100mL 0.05 M Tris-HCl buffer containing 0.02 M $CaCl_2.2H_2O$, pH 7.5. To stop the reaction, 250µL of 30% acetic acid was added after 25µL of crude extract enzyme sample and 1.25mL of fresh BAPNA substrate solution were combined and allowed to incubate for 10min at 37°C. The absorbance of the mixture was then determined using a spectrophotometer at 410nm. Trypsin activity was calculated based on the release of p-nitroaniline and expressed as units per mg protein (U/mg protein), where one unit represents the amount of enzyme that releases 1 µmol of p-nitroaniline per minute under assay conditions.

6.2. Chymotrypsin activity evaluation

Briefly, the fresh substrate solution (0.59mL) was prepared by mixing 20 mM $CaCl_2$ (pH 8.5), 0.1 mM succinyl-(Ala)2-Pro-phep-nitroanilide (SAPNA) in 50 mM Tris-HCl and 10µL of the enzyme sample at 25°C. For five minutes, the absorbance level was measured at 410nm every minute with a spectrophotometer. Chymotrypsin activity was expressed as µg of nitroanilide released/min per mg protein, calculated using a standard curve of p-nitroaniline.

6.3. Amylase activity evaluation

A total of 20 mM sodium phosphate buffer (pH 6.9) and 6.0 mM NaCl were used to make the 1% starch solution. Following a 3-minute incubation at 95°C, 0.5mL of substrate solution was added to 0.5mL of crude enzyme extract. After adding 0.5ml of dinitrosalicylic acid, the mixture was once more incubated for 5min in hot water. At 540nm, the absorbance level was determined with a spectrophotometer. Amylase activity was expressed as µg of maltose released per minute per mg protein, with values calculated against a maltose standard curve.

7. Hematobiochemistry index evaluation

Before blood collection, the fish were fasted for one day. Then, four fish from each experimental unit were anesthetized using 30mg/ L buffered tricaine methane sulphonate (Sigma-Aldrich). Buffered tricaine methane was selected due to rapid induction and recovery times and minimal physiological disruption. Compared to other anesthetics, this reagent is widely accepted in fish research for its predictable action and safety profile, particularly in hematological and biochemical assessments. Fish blood was obtained from the tail vein. Fish blood was placed into anticoagulant tubes to evaluate the amount of hemoglobin (Hb), packed cell volume (PCV), platelets, red blood cells (RBC), and white blood cells (WBC). Red blood cells and white blood cells were observed by using Neubauer slides under a light microscope. Wright-Giemsa staining was used to quantify the leukocyte count, including heterocytes, eosinophils, basophils, lymphocytes, and monocytes. Next, to obtain the serum, the obtained fish blood was centrifuged at 5000g for 10 min at room temperature. Total protein, albumin, glucose, and total cholesterol levels were colorimetrically measured in the serum. Globulin was determined by subtracting total protein from albumin. While, alkaline phosphatase (ALP; IU/L), aspartate (AST; IU/L), and alanine aminotransferase (ALT; IU/L) activities were assessed colorimetrically.

8. Evaluation of mineral content

Fish spines, skulls, and fins (back, chest, and tail) from each fish were collected. All bone samples were rinsed using deionization and were dried in an oven at 110°C for 2h (Memmert UN55). A total of 1.5g of bone sample was burned at 480°C in a silencer furnace (AME-MF1100), then cooled to room temperature (20-25°C). After heating the bone sample, to enhance the solubility of all solutes, 2mL of 6 N HCl was added, and then diluted with 50mL of deionized distilled water. The Mg, Ca, Zn, and Fe content was assessed using an atomic absorption spectrophotometer (Model: Buck 205; Buck Scientific). The colorimetric technique of vanadomolybophosphoric acid was utilized to determine the phosphorus content **(Nwanna & Schwarz, 2007)**. After adding 3mL of vanadate-molybdate reagent to 3mL of diluted bone sample, the mixture was incubated for 10min. A spectrophotometer was then used to determine the absorbance level (Heidolph REAX 2000, No. 54119) at 430nm. The mineral content in the test feed was done with the same procedure **(Nwanna & Schwarz, 2007)**. All samples were anayzed in triplicate for accuracy.

9. Data analysis

Before being analyzed using one-way analysis of variance (ANOVA), additivity, homogeneity, and normality tests were conducted. Duncan's multiple area test was done to determine the best treatment with $P< 0.05$ **(Yossa & Verdegem, 2015)**. All statistical data analysis were done using the SPSS Version 20.

RESULTS AND DISCUSSION

The growth performance and feed efficiency of juvenile-stage tilapia increased significantly ($P< 0.05$) along with the increase of phytase dose in the test feed (Table 2). The feed conversion ratio exhibited a significant reduction ($P< 0.05$) as the dosage of phytase in the test feed increased. Phytase supplementation had no significant effect on survival rate ($P> 0.05$) across all treatments. The juvenile-stage tilapia showed high ADCp, PER, RGR, and low FCR following feeding with phytase at 1,000 FTU/kg feed.

Table 2. Growth performance parameters of tilapia juveniles

Parameter	Test Feed					
	A	B	C	D	E	F
Initial body weight (g)	6.42 ± 0.35	6.42 ± 0.35	6.42 ± 0.35	6.42 ± 0.35	6.42 ± 0.35	6.42 ± 0.35
Final body weight (g)	32.10 ± 0.12^{f}	48.15 ± 0.23^{e}	57.78 ± 0.18^{d}	80.96 ± 0.12^{b}	89.10± 0.15^{a}	69.20 ± 0.15^{c}
Weight gain (g)	25.68 ± 0.24^{f}	41.73 ± 0.29^{e}	51.36 ± 0.26^{d}	74.54 ± 0.24^{b}	82.68 ± 0.25^{a}	62.78 ± 0.2^{c}
FI (g feed/fish)	58.34^{f}	92.42^{e}	104.65^{d}	132.18^{b}	144.26^{a}	118.84^{c}
ADCp (%)	53.16 ± 0.21^{f}	60.33 ± 0.20^{e}	68.18 ± 0.24^{d}	78.23 ± 0.14^{b}	88.54 ± 0.15^{a}	70.26 ± 0.22^{c}
FCR (g feed/g gain)	1.58±0.23^{f}	1.50±0.18^{e}	1.42±0.22^{d}	1.29±0.19^{b}	1.21±0.20^{a}	1.36±0.18^{c}
PER	1.35±0.01^{f}	1.58±0.13^{e}	1.67±0.05^{d}	2.27±0.13^{b}	2.78±0.16^{a}	1.89±0.13^{c}
RGR (%/day)	1.86±0.18^{f}	2.01±0.15^{e}	2.32±0.20^{d}	2.74±0.12^{b}	3.54±0.14^{a}	2.58±0.12^{c}
SR (%)	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	100.00±0.00^{a}	98.83±2.18^{a}

Note. Means in the same column with different superscripts indicate significantly different (P<0.05). FI: feed intake, ADCp: apparent digestibility coefficients, FCR: feed conversion ratio, PER: protein efficiency ratio, RGR: relative growth rate, SR: survival rate. The test feed contains phytase at 0 (A), 250 (B), 500 (C), 750 (D), 1,000 (E), and 1,250 FTU/kg feed (F).

All test meal treatments did not significantly ($P> 0.05$) altered the α-amylase activity of tilapia juveniles. However, the chymotrypsin and trypsin activities of tilapia juveniles were increased ($P< 0.05$) in a phytase dose-dependent manner (Table 3). High phytase supplementation significantly increased chymotrypsin and trypsin activity. These results also observed a significant rise ($P< 0.05$) in the level of PCV, red blood cells, Hb, WBC, and platelets in juvenile-stage tilapia (Table 4). However, no effect of test feed ($P> 0.05$) was observed on monocytes (%), eosinophils (%), and basophils (%) among tilapia juveniles treated with test feed.

Table 3. Specific digestive enzyme activity of tilapia juveniles

Enzymes	**Test Feed**					
	A	**B**	**C**	**D**	**E**	**F**
Amylase (U/mg protein)	33.16±0.24^{a}	33.23±0.18^{a}	33.36±0.19^{a}	33.32±0.22^{a}	33.26±0.25^{a}	32.28±0.28^{a}
Chymotrypsin (U/mg protein)	33.28±0.14^{f}	44.47±0.23^{e}	50.62±0.21^{d}	60.75±0.25^{b}	69.86±0.18^{a}	55.29±0.20^{c}
Trypsin (U/mg protein)	62.19±0.13^{f}	70.23±0.17^{e}	75.28±0.14^{d}	83.34±0.22^{b}	89.47±0.25^{a}	79.92±0.21^{c}

Note. Means in the same column with different superscripts indicate significantly different (P< 0.05).

Table 4. Hematology profile of the blood from tilapia juveniles

Variable	Test Feed					
	A	B	C	D	E	F
Red blood cells ($\times10^{6}$/µl)	0.96^{f}	1.22^{e}	1.29^{d}	1.45^{b}	1.56^{a}	1.36^{c}
Haemoglobin (g/dl)	3.68^{f}	4.56^{e}	5.12^{d}	5.63^{b}	5.87^{a}	5.28^{c}
Packed cell volume (%)	12.3^{f}	14.9^{e}	16.2^{d}	17.4^{b}	18.2^{a}	16.9^{b}
White blood cells ($\times10^{3}$/µl)	9.35^{f}	11.30^{e}	12.12^{d}	12.90^{b}	13.02^{a}	12.52^{c}
Platelets ($\times10^{3}$/µl)	93.2^{f}	104.4^{e}	112.7^{d}	130.2^{b}	136.8^{a}	127.5^{c}
Lymphocytes (%)	46.4^{f}	48.2^{e}	50.2^{d}	56.3^{b}	59.7^{a}	52.3^{c}
Heterocytes (%)	45.2^{a}	38.4^{b}	36.7^{c}	34.2^{d}	32.7^{e}	30.4^{f}
Monocytes (%)	5.2^{a}	5.1^{a}	5.2^{a}	5.2^{a}	5.2^{a}	5.1^{a}
Eosinophils (%)	2.2^{a}	2.0^{a}	2.1^{a}	2.2^{a}	2.2^{a}	2.1^{a}
Basophils (%)	1.0^{a}	1.1^{a}	1.0^{a}	1.2^{a}	1.1^{a}	1.0^{a}

Note. Means in the same column with different superscripts indicate significantly different (P< 0.05).

The glucose and cholesterol levels of tilapia juveniles had no significant effect (P> 0.05) after being treated with phytase supplementation. Phytase could increase the total albumin, protein, and globulin of tilapia juveniles compared to those without phytase. However, a significant reduction in AST and ALT values was found in juvenile-stage tilapia fed with phytase compared to without phytase (Table 5). Furthermore, the levels of Mg, Zn, P, and Ca in the bones of tilapia juveniles were raised significantly (P< 0.05) along with the increasing dose of phytase in the test feed (Table 6). Meanwhile, increasing the dosage of phytase in the test diet did not affect the Fe content or Ca-P ratio of juvenile-stage tilapia (P> 0.05).

Table 5. Biochemical parameters of the serum from tilapia juveniles

Variable	Test Feed					
	A	**B**	**C**	**D**	**E**	**F**
Glucose (mg/dl)	254.8[a]	255.2[a]	254.9[a]	255.2[a]	254.8[a]	255.2[a]
Total cholesterol (mg/dl)	113.3[a]	114.4[a]	113.3[a]	114.1[a]	114.1[a]	114.1[a]
Total protein (g/dl)	3.42[f]	3.96[e]	4.03[d]	5.21[b]	6.50[a]	4.89[c]
Albumin (g/dl)	2.57[f]	2.98[e]	3.07[d]	3.44[b]	3.86[a]	3.20[c]
Globulin (g/dl)	1.03[f]	1.14[e]	1.49[d]	1.67[b]	1.98[a]	1.51[c]
Aspartate aminotransferase (IU/L)	244.3[a]	226.5[b]	210.5[c]	200.3[d]	184.5[e]	168.2[f]
Alanine aminotransferase (IU/L)	42.5[a]	36.4[b]	32.7[c]	28.3[d]	19.2[e]	12.1[f]

Note. Means in the same column with different superscripts indicate significantly different ($P< 0.05$).

Table 6. Bone mineral concentration in the tilapia juveniles

Variable	Test Feed					
	A	**B**	**C**	**D**	**E**	**F**
Calcium (Ca; mg/g)	66.4[e]	72.3[d]	78.8[c]	85.9[b]	89.5[a]	74.9[d]
Phosphorus (P; mg/g)	43.7[e]	48.4[e]	50.6[d]	59.8[b]	62.2[a]	53.2[c]
Ca–P ratio	1.42[a]	1.44[a]	1.44[a]	1.41[a]	1.42[a]	1.41[a]
Magnesium (Mg; mg/g)	1.13[f]	1.66[e]	1.98[d]	3.26[b]	3.69[a]	3.02[c]
Zinc (Zn; mg/g)	0.20[f]	0.25[e]	0.28[d]	0.35[b]	0.38[a]	0.30[c]
Iron (Fe; mg/g)	0.42[a]	0.41[a]	0.42[a]	0.43[a]	0.42[a]	0.42[a]

Note. Means in the same column with different superscripts indicate significantly different ($P< 0.05$).

As an antinutritional agent, phytate can form complexes with protein and minerals. Protein binding with phytate interferes with the digestion and absorption of proteins and amino acids from feed **(Morales *et al.*, 2016)**. The high level of phytate in plant materials as a feed constituent is known to limit fish growth, hence, phytase supplementation is needed in feed to hydrolyze phytate complex molecules **(Adeshina *et al.*, 2022; Salem *et al.*, 2022; Shahzad *et al.*, 2022; Rachmawati *et al.*, 2023a; Rachmawati *et al.*, 2023b)**. In this study, phytase was added to the feed using soybean meal as a vegetable protein source to boost tilapia juveniles' growth and feed efficiency. Similar growth-enhancing effects of phytase have been observed in *Carassius auratus* **(Nie *et al.*, 2017)** and *Cyprinus carpio* **(Rachmawati *et al.*, 2023b)**, supporting our findings.

Phytase supplementation significantly improved FI, ADCp, PER, and RGR while reducing FCR in juvenile tilapia. Increasing phytase supplementation in feed improved protein digestibility, which resulted in increased growth **(Bulbul *et al.*, 2015)**. **Hussain *et al.* (2017)** stated that phytase supplementation in feed can hydrolyze protein phytate complex compounds into amino acids that are easier to digest for fish growth. Increased growth was observed in tilapia juveniles fed with phytase supplementation, which is

suggested to be related to increased protein digestibility due to increased protease enzyme activity. Increasing the dosage of phytase in feed may improve the protein digestibility of juvenile-stage tilapia. Phytase enhances the activity of trypsinogen, which transforms into trypsin, an enzyme that may break down proteins into amino acids **(Hussain *et al*., 2017)**.

In tilapia juveniles, phytase supplementation in the test feed significantly boosted chymotrypsin and trypsin activity compared to the test feed without phytase. The tilapia juveniles in this investigation exhibited low FCR, high ADCp, PER, and RGR values when administered phytase at 1,000 FTU/kg feed. It is hypothesized that at this phytase dosage, the enzyme can optimally hydrolyze phytate complex compounds, thereby releasing phosphorus, protein, and minerals from soybean meal. In addition, tilapia juveniles fed with phytase supplementation of 1000 FTU/kg feed had higher chymotrypsin and trypsin digestive enzyme activities than other test feed treatments. Thus, the release of phytate complex molecules by phytase facilitates the absorption of phosphorus, protein, and minerals and can boost protein digestibility, feed efficiency, and growth **(Tahoun *et al*., 2009)**.

Table (3) shows that chymotrypsin and trypsin levels increased in juvenile-stage tilapia fed with phytase supplementation, while the amylase showed no change. This indicated that protein utilization increased in juvenile-stage tilapia fed with phytase supplementation but not carbohydrates. The observed enhancement in protein utilization in juvenile-stage tilapia fed with phytase supplementation is likely attributed to phytase's ability to hydrolyze phytate-protein complex compounds, thereby increasing protein digestibility and promoting growth. Phytase can hydrolyze phytate-mineral-protein complexes in feed, resulting in enhanced protein consumption and digestibility **(Bulbul *et al*., 2015)**. The observed increase in chymotrypsin and trypsin activity in this study is likely due to phytase's role in releasing digestive enzymes bound by phytate (Table 2). Pepsin, amylopsin, and amylase are among the enzymes that are inhibited by phytic acid before phytate-protein-mineral breakdown **(Cao *et al*., 2007)**. Phytase can simultaneously hydrolyze phytate-protein-mineral complex compounds and release digestive enzymes. **Nie *et al*. (2017)** also reported that the increased activity of digestive enzymes is partially attributed to the release of minerals bound to phytate through phytase supplementation **(Liu *et al*., 2014)**. Additionally, phytase supplementation can lead to significant activation of protease enzymes, including chymotrypsin and trypsin, by releasing Ca^{2+}, Mg^{2+}, and Zn^{2+} **(Hu & Pan, 2006; Jiang *et al*., 2016)**. This synergistic effect likely explains why tilapia juveniles fed with phytase supplementation exhibit higher chymotrypsin and trypsin activities compared to those fed without phytase.

Fawole *et al.* (2020) suggested that to determine the effect of phytase supplementation in feed on fish growth and health, one of them can is to analyze the hematobiochemical profile. The current study found a significant elevation in red blood cells, hemoglobin, packed cell volume, white blood cells, platelets, and lymphocytes of tilapia juveniles after being fed with phytase supplementation. However, heterocytes (%)

were decreased. The results of this study indicated that tilapia juveniles were in good health during the study.

Table (5) showed that blood protein in the serum of tilapia juveniles increased significantly with increasing dosage of phytase in the feed. Fish humoral immunity depends on blood protein, and a higher level of it indicates a stronger immune response **(Hamed & Abdel-Tawwab, 2021)**. Humoral immunity in fish is a defense mechanism involving antibody production and is an important mechanism for preventing disease **(Abdel-Tawwab & El-Araby, 2021)**. The observed increase in serum blood protein levels in tilapia juveniles, concurrent with increasing phytase supplementation in their feed, suggests a positive correlation between phytase dosage and fish immune response. **Adeshina *et al*. (2022)** and **Kaiza *et al*. (2022)** revealed that phytase supplementation can enhance fish immunity. Furthermore, as the dosage of phytase was increased, the study found a substantial ($P< 0.05$) decrease in the enzyme activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST). This indicates the protection of liver cell membranes against factors that cause stress responses in tilapia juveniles. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are critical liver aminotransferases that signal liver condition and function. The decline in AST and ALT enzyme activity in this study implies that there is protection against the effect of phytase on liver damage by enhancing membrane stability against toxins caused by free radicals **(Adeshina *et al*., 2022)**. Thus, it can be concluded that tilapia stadia seeds did not experience stress during the study.

Phytase addition in feed can hydrolyze phytate complex molecules that bind minerals, therefore raising mineral concentrations in cartilage, plasma, and the entire body of fish **(Nie *et al.*, 2017)**. The results of this study showed that phytase supplementation can considerably raise the amounts of calcium, phosphorus, magnesium, and zinc in juvenile-stage tilapia's bones. It is suspected that phytase hydrolyzes mineral phytate complex compounds, so it can increase mineral digestibility. The digestibility of P is higher than that of other minerals, and the addition of phytase to the diet can boost the digestibility of Mg, Mn, Zn, and P **(Hussain *et al*., 2020)**. **Shahzad *et al*. (2021)** found that fish fed with phytase supplementation showed an increased mineral digestibility (Cu, Ca, Zn, Mg, K, Fe, Na, and Mn). Another study found that fish fed with phytase had higher phosphorus, zinc, and iron mineral availability in their bodies due to a reduction in phytic acid in the gastrointestinal tract and increased mineral bioavailability **(Laining *et al*., 2012)**. Several studies reported that the addition of phytase in feed significantly increased bone P and serum Ca levels in *Carassius auratus* **(Nie *et al*., 2017)**, Ca, Zn, and Mn levels in the African catfish bones **(Adeshina *et al*., 2022)**.

This study revealed that bone mineral concentrations of tilapia juveniles after receiving phytase at 1,000 FTU/kg feed exhibited higher levels of Ca, P, Mg, and Zn than other test feeds. This is likely due to the optimal phytase dosage, which allows for the maximum hydrolysis of phytate-bound minerals, thus causing a decrease in phytic acid in

the gastrointestinal tract and increasing mineral bioavailability, resulting in the availability of Ca, P, Mg, and Zn minerals higher than other treatments. **Rachmawati *et al.* (2023b)** revealed similar research findings, demonstrating that at a level of 1,000 FTU/kg feed, phytase enhanced the concentration of Ca, P, Mg, and Zn in *Cyprinus carpio* bones. *Ictalurus punctatus* bones had higher Ca, Mg, and Mn contents when fed microbial phytase at 1000 FTU/kg feed **(Yan *et al*., 2007)**. **Adeshina *et al.* (2022)** found that the addition of phytase at 750-1000 FTU/kg feed increased the content of Ca, Mg, and Mn in the African catfish bones. **Kumar *et al*. (2011)** stated that the Ca-P ratio has an important influence on fish bone development. **Cao *et al*. (2007)** suggested that the increasing value of the Ca-P ratio had a positive influence on fish health. Moreover, the Ca-P ratio in the 1.1–1.4:1 range indicates good effectiveness for a phytase-supplemented diet **(Kumar *et al*., 2012)**. The results of this study showed that the Ca-P ratio value was in the range of 1.41-1.44, which means that the tilapia juveniles during the study were within the range recommended by **Kumar *et al.* (2012)**.

This study demonstrated that dietary supplementation of phytase significantly improved the growth performance, feed utilization, digestive enzyme activity, hematological health, and bone mineralization in juvenile tilapia, with the most pronounced effects observed at a dosage of 1,000 FTU/kg feed. Phytase enhanced protein digestibility by increasing trypsin and chymotrypsin activities, without affecting amylase, indicating a specific benefit to protein metabolism. Improvements in hematological indices such as red and white blood cell counts, hemoglobin, and serum protein levels suggest enhanced immune status and overall fish health. Additionally, decreased liver enzyme activities (AST and ALT) imply reduced physiological stress and better liver function. Bone mineral content (Ca, P, Mg, Zn) also increased significantly, highlighting improved mineral bioavailability due to phytate hydrolysis. However, the study was limited by its short duration and focus on the juvenile stage, leaving long-term effects unexplored. It also used only soybean meal as a plant protein source and did not assess gut microbiota, environmental impacts, or cost-effectiveness, all of which are important for broader application in sustainable aquaculture.

CONCLUSION

Phytase supplementation at 1,000 FTU/kg feed significantly enhanced growth performance, protein digestibility, digestive enzyme activity, hematological and biochemical parameters, and bone mineralization in juvenile tilapia. These results highlight the potential of phytase to mitigate the negative effects of phytate and support sustainable, plant-based aquafeed development. Future research should focus on elucidating the molecular and biochemical mechanisms by which phytase enhances digestive function, as well as assessing its long-term impact on immune competence, health resilience, and economic viability in commercial aquaculture settings.

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