The effect of phytase enzyme in feed on growth performance, protein digestibility, feed utilization efficiency, mineral content, and body nutrient composition in Nile tilapia (*Oreochromis niloticus*) fingerlings

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Abstract. Feed is the largest production cost factor, accounting for 60-70% of the total production costs. Thus, to reduce feed costs, fish farmers can produce their own fish feed using local plant-based ingredients as feed components. However, local plant-based ingredients contain anti-nutritional factors, such as phytic acid. Phytic acid can form complex compounds that bind to proteins and phosphorus, reducing the digestibility and utilization of protein in fish bodies. One way to address this issue is by adding phytase enzymes to the feed, which can potentially increase the bioavailability of nutrients and energy, as well as improve feed efficiency, mineral digestibility, and fish growth parameters. The aim of this study was to evaluate the effects of phytase enzyme supplementation in the feed on growth performance, protein digestibility, feed efficiency, and mineral and nutrient content in the body of Nile tilapia (*Oreochromis niloticus*) fingerlings. The test animals used were Nile tilapia fingerlings with an average initial weight of 9.28±0.26 g per fish. The experimental feed used was floating pellets with added phytase enzymes according to the treatments: A (0 FTU kg^{-1}), B (500 FTU kg^{-1}), C (1000 FTU kg^{-1}), and E (1500 FTU kg^{-1}). The results of the study showed that the addition of phytase enzymes in the feed significantly ($p < 0.05$) improved growth performance, protein digestibility, feed efficiency, mineral content (Ca, P, Mg, and Zn), and nutrient content (protein and ash) in the body of Nile tilapia fingerlings. A phytase enzyme dose of 1000 FTU kg^{-1} was found to be the most effective for Nile tilapia fingerlings, yielding the highest values in growth performance, feed efficiency, and mineral content variables observed during the study, compared to other feed treatments.
Key Words: anti-nutritional, fish, nutrient, plant-based, phosphorus.

Introduction. One of the key factors that determine the success of fish farming activities is the availability of feed. Feed is a crucial factor as it provides the nutrients necessary for the growth and development of fish (NRC 2011). However, the high cost of feed remains a major issue for fish farmers (de Verdal et al 2018). Feed accounts for the largest production expense, making up 60-70% of the total production costs (Abdel-Tawwab & El-Araby 2021). To mitigate feed costs, the Ministry of Marine Affairs and Fisheries (KKP) launched the "Independent Fish Feed Movement" (GERPARI) in 2019, encouraging fish farmers to produce their own feed using locally sourced plant-based ingredients.

However, local plant-based feed ingredients contain anti-nutritional factors, specifically phytic acid. Phytic acid is a form of phosphorus stored in legumes, seeds, oilseeds, and cereal grains, which is not bioavailable to most fish species (Kumar et al 2019). Phytic acid in plant-based ingredients can act as an anti-nutrient (Kishore et al 2020), forming complexes that bind proteins and phosphorus, reducing the digestibility and utilization of these nutrients in fish (Rachmawati et al 2023a). According to Hussain et al (2020), phytic acid in feed disrupts the ability of fish to digest and absorb nutrients, thereby reducing the nutritional value of the feed. This can affect the digestive system of fish, decreasing feed utilization efficiency (Cao et al 2007). Phytic acid reduces nutrient bioavailability, impacts fish growth performance and metabolism, and increases nitrogen and other nutrient excretion into the aquatic environment (Kokou & Fountoulaki 2018). Phosphorus that cannot be absorbed by fish due to its binding with phytate complexes is excreted through feces, potentially causing water eutrophication, known as algal blooms (Liu et al 2021). Moreover, phytic acid can also bind with amino acids, reducing their availability (Rachmawati et al 2023b).

Several strategies can be employed to address the anti-nutritional factors in local plant-based feed ingredients, such as grinding, soaking, fermentation, sprouting, and adding phytase enzymes (Gupta et al 2015). In this study, phytase enzyme supplementation was selected due to its ability to effectively eliminate phytic acid. According to Kaiza et al (2023), phytase supplementation is the most effective method for reducing phytic acid content in plant-based ingredients, as it maximally reduces phytic acid without diminishing mineral content. Phytase is a phosphatase enzyme that hydrolyzes phytate, releasing inorganic phosphorus and available inositol esters (Kokou & Fountoulaki 2018). The addition of phytase to fish feed can potentially enhance the bioavailability of nutrients, improve feed efficiency, and promote growth parameters (Rodrigues et al 2023). In recent years, the use of phytase in fish feed has gained attention due to its distinct physiological effects (Orisasona et al 2017). The appropriate dosage and concentration of phytase in formulated fish feed depend on the type of feed and fish species, allowing for the optimization of phytase benefits in aquaculture (Liang et al 2022). Various studies have demonstrated the positive effects of phytase supplementation on growth in several fish species, including *Clarias gariepinus* var. Sangkuriang (Rachmawati et al 2023a), *Oreochromis niloticus* (Shahzad et al 2022), *Cyprinus carpio* (Shahzad et al 2021), *Litopenaeus vannamei* (Qiu & Davis 2017), *Psetta maxima* (Von Danwitz et al 2016), and *Penaeus monodon* (Rachmawati & Samidjan 2016).

Nile tilapia (*O. niloticus*) is one of the leading freshwater fish commodities, characterized by rapid growth, high stocking density, disease resistance, and tolerance to various environmental conditions (Kummari et al 2018). In Indonesia, Nile tilapia ranks as the top aquaculture commodity in terms of production quantity, with a total production of 1.49 million tons in 2021. It also ranks second in production value, amounting to IDR 37.1 trillion, following shrimp (KKP 2022). Information regarding phytase supplementation in Nile tilapia at the fingerling stage remains limited, highlighting the need for this study. The objective of this research is to evaluate the effects of phytase supplementation in feed on growth performance, protein digestibility, feed efficiency, mineral content, and body nutrient composition of Nile tilapia fingerlings. Thus, this study is essential to provide insights into the impact of phytase addition on the growth performance, protein digestibility, feed efficiency, mineral content, and body nutrient composition of Nile tilapia fingerlings.

Material and Method

Experimental design and test fish preparation. This study was conducted from June to August 2024 at the Sido Makmur Fish Farmer Group, Tambaksari Village, Rowosari Subdistrict, Kendal Regency, Central Java, Indonesia, using an experimental method with a completely randomized design, consisting of 4 treatments and 3 replications. The test fish used were Nile tilapia juveniles with an average initial length of 11.20±0.21 cm and an average initial weight of 9.28±0.26 g per fish, obtained from the Sawangan Fish Seed Center, Magelang, Central Java, Indonesia. The preparation of the test fish began with an adaptation process, where the fish were acclimated to the environment and experimental feed for 7 days. This adaptation aimed to allow the fish to adjust to the new environment and feed, ensuring they did not experience stress during the experiment. During this period, the fish were fed without phytase enzyme at satiation, three times a day at 08:00, 12:00, and 16:00 WIB. After adaptation, the fish were selected for uniform size, physical integrity, active movement, and good health (Rachmawati et al 2023b). Prior to the experiment, the fish were fasted for 1 day to remove any residual feed and metabolic waste, ensuring accurate initial weight measurements.

Experimental feed preparation. The experimental feed was in the form of pellets with a protein content of 32% and isocaloric value of 301 kcal (Rachmawati et al 2023a), supplemented with 1% Cr_2O_3 as an indicator for protein digestibility (NRC 2011) and phytase enzyme according to the treatments: A (0 FTU kg^{-1} feed), B (500 FTU kg^{-1} feed), C (1000 FTU kg^{-1} feed), and D (1500 FTU kg^{-1} feed). The phytase enzyme used was Nathupos*E 10000 G, produced by BASF SE, Ludwigshafen, Germany, which has higher enzymatic activity compared to Nathupos*E 50000 G and is more resistant to acidic pH and temperatures up to 95°C (Bavaresco et al 2020). The experimental feed formulation is presented in Table 1. Feed preparation involved weighing the ingredients according to the formulation, then mixing the components starting with smaller amounts and gradually adding larger quantities until homogenized using a mechanical mixer. Fish oil, corn oil, and water were added, and the mixture was homogenized further. The feed was then extruded into 4 mm pellets, air-dried at room temperature (26°C), vacuum-packed, and stored until use.

Table 1

Experimental feed formulation (g/1000 g)

Ingredient (g)	*Test diet*			
	A	*B*	*C*	*D*
Fish meal	404.58	404.58	404.58	404.58
Soybean meal	159.34	159.34	159.34	159.34
Corn meal	139.79	139.79	139.79	139.79
Rice bran	156.81	156.81	156.81	156.81
Tapioca	92.44	92.44	92.44	92.44
Fish oil	9.26	9.26	9.26	9.26
Corn oil	9.26	9.26	9.26	9.26
Vitamin-mineral mix[1)]	18.52	18.52	18.52	18.52
Cr_2O_3	10.00	10.00	10.00	10.00
Total	1000	1000	1000	1000
Phytase enzyme (FTU)	0	500	1000	1500
Proximate analysis results				
Protein (%)*	32.18	32.19	32.20	32.20
Lipid (%)*	8.87	8.50	8.62	8.57
NFE (%)*	35.28	35.30	34.29	32.32
Energy (kcal)[2)]	301.29	301.45	301.32	301.35
E/P ratio (kcal/g)[3)]	9.36	9.36	9.35	9.36

Note: [1)] Vitamin and mineral mix per kg: selenium (Se) 150 mg, sodium (Na) 117 mg, vitamin B1 52 mg, magnesium (Mg) 1,900 mg, calcium (Ca) 219 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, vitamin C (coated) 68,800 mg activity, vitamin B12 60 mg, potassium (K) 150 mg, vitamin A 36,000 IU, vitamin B2 97 mg, vitamin B6 46 mg, vitamin D3 9,000 IU, manganese (Mn) 105 mg, pantothenic acid 93 mg, inositol 225 mg, biotin 450 mg, vitamin E 187 mg, vitamin K3 19 mg, niacin 130 mg, folic acid 10 mg, zinc (Zn) 90 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg; [2)] Calculated based on digestible energy according to NRC (2011): 1 g protein = 3.5 kcal g^{-1}, 1 g fat = 8.1 kcal g^{-1}, and 1 g carbohydrate = 2.5 kcal g^{-1}; [3)] According to NRC (2011), the optimal E/P ratio for fish growth ranges from 8 to 12 kcal g^{-1}; * Proximate analysis results provided by the Laboratory of Animal Feed Science, Faculty of Animal Science and Agriculture, Diponegoro University (2024).

Experimental containers. The study used 12 fiber tanks (1.0 x 1.0 x 1 m^3), each equipped with a recirculating system to maintain optimal water quality. The tanks were filled with treated groundwater, which had undergone sedimentation in a 5000-liter fiber tank equipped with a recirculating system, before being transferred to the experimental tanks. Test fish, with known initial weights, were stocked at a density of 50 fish per tank. Feed was provided at satiation three times a day (07:00, 13:00, and 19:00). Fish growth was monitored weekly by weighing the fish with a digital scale. Siphoning of feces and feed residues was performed 2 hours after feeding to maintain water quality. Water quality parameters observed included temperature (25-30°C; Water quality checker), pH

(6.5-8.6; Jenway 3510), dissolved oxygen (≥ 3 mg L^{-1}; Jenway 3510), and ammonia (0.13 mg L^{-1} (HANNA: HI. 8633), according to Boyd & Tucker (2012).

Fish mineral composition*.* The mineral composition analysis of fish samples was performed by weighing 5 g of fish samples, which were dried at a temperature of 100-105°C. Once dried, the samples were placed in a furnace at 1000°C to obtain ash. The resulting ash was digested with concentrated HNO_3 (p) until dissolved. The solution was then diluted in a 100 mL volumetric flask up to the mark. The mineral content (Mg, Ca, Zn, and Fe) was measured using an atomic absorption spectrophotometer (Model: Buck 205; Buck Scientific), while phosphorus (P) content was determined using the vanadomolybdophosphoric acid colorimetric method (Nwanna & Schwarz 2007).

Proximate analysis of test feed and fish carcass*.* Proximate analysis of feed and fish carcasses followed NRC (2011) methods. Fat content was determined by ether extraction using the Soxhlet method (FOSS Soxtec 2043), protein content was determined by the Kjeldahl method (FOSS Kjeltec 2300), and ash content was determined by combustion at 500-600°C for 24 hours.

Protein digestibility analysis*.* Protein digestibility was measured using the indirect method, with 1% Cr_2O_3 as a digestibility marker in the feed, according to Pérez-Jiménez et al (2009).

Observed variables*.* The variables measured included total feed consumption (TFC) according to Pereira et al (2007), apparent digestibility coefficient of protein (ADCp) according to Pérez-Jiménez et al (2009), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) according to NRC (2011), all calculated based on the following formulas:

TFC = Initial feed amount (g) + Remaining feed (g)+…+ Feed amount on the nth day (g)

ADCp (%) = 100 - {100 x Cr_2O_3 in feed / % Cr_2O_3 in feces x % protein in feces / % protein in feed}

FUE (%) = Final weight – Initial weight / Feed consumed x 100

FCR = Feed intake (g) / Body weight gain (g)

PER = 100 × (Final weight – Initial weight) / Feed consumed x Protein content of feed

RGR (% day^{-1}) = 100 × (Final weight - Initial weight) / (Experiment duration x Initial weight)

SR (%) = 100 x (Final fish count / Initial fish count)

Data analysis*.* The observed parameters were analyzed using analysis of variance (ANOVA) to assess treatment effects. If significant ($p < 0.05$) or highly significant ($p < 0.01$) effects were observed, Duncan's multiple range test was applied to compare treatment means. Polynomial orthogonal analysis using SPSS 26 and Maple12 (Steel et al 1997) was performed to determine the optimal phytase enzyme dosage in the feed.

Results. The observations of TFC, ADCp, FUE, FCR, PER, RGR, and SR of Nile tilapia fingerlings during the study are presented in Table 2.

The growth and feed efficiency observations presented in Table 2 indicate that the addition of phytase to the diet significantly ($p < 0.05$) increased TFC, ADCp, FCR, FUE, PER, and RGR, but had no significant effect ($p > 0.05$) on the SR of Nile tilapia fingerlings. TFC, ADCp, FCR, FUE, PER, and RGR values increased with higher doses of phytase in the diet, while FCR decreased as phytase dosage increased. The addition of 1000 FTU kg^{-1} of phytase in the diet was the optimal dosage for Nile tilapia fingerlings, as it yielded the highest growth and feed efficiency variables compared to other treatments.

The mineral composition of Nile tilapia fingerlings fed with the test diets is presented in Table 3. The table shows that the levels of Ca, P, Mg, and Zn in the fingerlings increased significantly ($p < 0.05$) with higher phytase doses in the diet. However, Fe content and the Ca-P ratio were not significantly affected ($p > 0.05$) by increasing phytase doses in the test diets.

The effect of phytase addition on the chemical composition of Nile tilapia fingerlings is presented in Table 4. The table shows that Nile tilapia fingerlings fed with phytase-supplemented diets had higher protein and ash content compared to those without phytase. There were no significant differences in lipid and dry matter content among the different test diets.

Table 2

Mean values of total feed consumption (TFC), apparent digestibility coefficient of protein (ADCp), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) of Nile tilapia fingerlings during the study

Variable	*Test diet*			
	A	*B*	*C*	*D*
TFC (g)	159.20±0.76^{d}	164.87±0.36^{c}	181.20±0.89^{a}	175.03±0.87^{b}
ADCp (%)	52.24±0.37^{d}	68.20±0.42^{c}	78.53±0.34^{a}	72.38±0.22^{b}
FCR	1.82±0.01^{d}	1.64±0.02^{c}	1.23±0.01^{a}	1.41±0.02^{b}
FUE (%)	54.94±0.30^{d}	60.99±0.79^{c}	81.29±0.46^{a}	71.32±0.95^{b}
PER	1.20±0.01^{d}	1.32±0.02^{c}	1.77±0.01^{a}	1.55±0.02^{b}
RGR (% day^{-1})	2.52±0.24^{d}	2.86±0.21^{c}	3.94±0.13^{a}	3.47±0.23^{b}
SR (%)	100^{a}	100^{a}	100^{a}	100^{a}

Note: Different superscripts in the same row indicated statistical difference ($p < 0.05$).

Table 3

Mineral composition of Nile tilapia fingerlings fed with test diets

Variable	*Test diet*			
	A	*B*	*C*	*D*
Calcium (Ca; mg g^{-1})	58.6^{d}	70.3^{c}	82.5^{a}	77.8^{b}
Phosphorus (P; mg g^{-1})	42.3^{d}	47.9^{c}	59.2^{a}	53.4^{b}
Ca-P ratio	1.41^{a}	1.41^{a}	1.42^{a}	1.41^{a}
Magnesium (Mg; mg g^{-1})	1.64^{d}	2.36^{c}	3.17^{a}	2.89^{b}
Zinc (Zn; mg g^{-1})	0.19^{d}	0.26^{c}	0.39^{a}	0.32^{b}
Iron (Fe; mg g^{-1})	0.40^{a}	0.41^{a}	0.40^{a}	0.40^{a}

Note: Different superscripts in the same row indicated statistical difference ($p < 0.05$).

Table 4

Chemical composition (%) of Nile tilapia fingerlings

Test feed	*Dry matter*	*Protein*	*Lipid*	*Ash*
A	22.17±0.32^{a}	54.37±0.13^{b}	23.76±0.24^{a}	16.52±0.13^{b}
B	22.13±0.18^{a}	56.82±0.48^{a}	23.61±0.30^{a}	18.26±0.45^{a}
C	22.15±0.23^{a}	56.62±0.35^{a}	23.58±0.28^{a}	18.51±0.37^{a}
D	22.20±0.21^{a}	56.45±0.38^{a}	23.69±0.31^{a}	18.34±0.41^{a}

Note: Different superscripts in the same row indicated statistical difference ($p < 0.05$).

Discussion. The total feed intake with the addition of phytase enzyme was higher compared to the treatment without the phytase enzyme. This is likely due to the total feed consumption being influenced by feed palatability. Increased fish feed consumption indicates that the feed has good palatability (Shahzad et al 2021). The addition of phytase enzyme to the feed can enhance palatability by breaking down phytate-protein and phytate-mineral bonds in the feed, thereby promoting growth as fish consume more feed. This is supported by Kaiza et al (2023), who stated that increased feed intake is due to the release of nutrients from the feed because of breaking the complex bonds formed by phytic acid, which may enhance feed palatability. Indirectly, palatability can affect both consumption and digestibility by making feed appealing to fish (Al-Souti et al 2019). Additionally, it is speculated that the hydrolysis product of phytic acid, inositol, in the feed can stimulate fish appetite. Adeshina et al (2019) mentioned that inositol, a product of phytic acid hydrolysis, plays a role in increasing fish appetite. Nile tilapia fingerlings fed test feed C had a higher feed intake value (181.20±2.89 g) compared to other test feed treatments. This is suspected to be due to the optimal action of the phytase enzyme in treatment C in breaking down phytic acid in the feed, thereby enabling inositol in the fish's body to meet their appetite needs. Inositol is a vitamin necessary for fish to support growth, maintenance, and appetite stimulation (Chen et al 2022). Inositol deficiency can result in reduced fish appetite (Rachmawati et al 2023b).

Protease enzyme activity in the digestive tract decreases in the presence of phytate-bound proteins. Phytates bind proteins and minerals in the digestive tract, potentially inhibiting digestive enzyme activity. The results in Table 2 show that the ADCp of Nile tilapia fingerlings increased with higher phytase doses in the feed. The addition of phytase to the feed can hydrolyze phytic acid, enabling the nutrients bound by phytic acid to be absorbed by the intestines and improving protein digestibility (Hassan et al 2022). According to Cao et al (2007), phytic acid breakdown enhances nutrient absorption due to phytase enzyme hydrolysis, which reduces phytic acid and releases protein and mineral bonds. Karabulut et al (2021) also reported that the breakdown of phytate-protein complex bonds can increase trypsinogen and trypsin enzyme activity, which helps break down proteins into amino acids. The addition of phytase to the feed improves protein digestibility, which correlates with enhanced feed utilization efficiency in fish (Biswas et al 2019). Similar results were reported by Adeshina et al (2019) in *C. gariepinus*, Chen & Gatlin (2024) in *Sciaenops ocellatus*, Rachmawati et al (2023b) in *C. carpio*, and Nie et al (2017) in *Carassius auratus*.

The best FCR was observed in Nile tilapia fingerlings fed test feed C with a value of 1.23±0.01. This is likely because the phytase enzyme dose of 1000 FTU kg^{-1} feed was sufficient to break down phytic acid in artificial feed, which can hinder nutrient absorption. The addition of phytase is believed to assist in nutrient absorption, resulting in a lower FCR. This is supported by Rachmawati et al (2023a), who found that phytase enzyme supplementation can lower FCR by maximizing nutrient absorption, improving protein digestibility, and enhancing feed utilization efficiency. Furthermore, Hussain et al (2017) suggested that the addition of phytase to the feed can minimize nutrient waste through feces, indicating that more nutrients are utilized by the fish. El-Salem et al (2022) also stated that reduced nutrient excretion through fish feces is due to the hydrolysis of phytate by phytase, thereby allowing more nutrients to be utilized. FCR is related to feed utilization; a lower FCR indicates higher feed utilization efficiency, whereas a higher FCR indicates lower feed utilization. This is evidenced by the FUE value in Table 2, where Nile tilapia fingerlings fed feed supplemented with phytase at 1000 FTU kg^{-1} feed (test feed C) showed the highest FUE value compared to other treatments. This is reinforced by Rachmawati et al (2023b), who reported that phytase enzyme usage in feed can increase FUE values, leading to a reduction in FCR. Similar findings were reported by Orisasona et al (2017) in *C. gariepinus*, Rachmawati et al (2023b) in *C. carpio*, and Karabulut et al (2021) in *Acipenser baerii*. Treatment C had the lowest FCR value, likely because the nutrients were wasted through feces. The highest FCR value was observed in treatment A, where no phytase enzyme was added to the feed. This is suspected to be due to the unbroken phytic acid in the feed, leading to inefficient feed utilization. This is supported by Shahzad et al (2021), who found that unhydrolyzed phytic acid in feed can bind to and reduce nutrient absorption needed by fish. Differences in FCRs can be attributed to variations in nutrient and mineral absorption capabilities among species, ages, and sizes of fish. The high FCR is related to the low PER in treatment A. The low PER in treatment A is likely because the fish were unable to effectively digest the feed due to the unbroken phytic acid, leading to poor feed utilization and resulting in unutilized compounds being excreted through feces.

Higher FUE values indicate more efficient feed utilization by fish. Variance analysis showed that the different phytase enzyme doses in the feed had a significant effect ($p < 0.05$) on the FUE of Nile tilapia fingerlings. The highest FUE value was observed in Nile tilapia fingerlings fed test feed C at 81.29±0.46%, followed by test feed D at 71.32±0.95%, test feed B at 60.99±0.79%, and the lowest FUE value in test feed A at

54.94±0.30%. The high FUE value in test feed C is likely due to the optimal performance of the phytase enzyme at a dose of 1000 FTU kg^{-1} feed, making it the appropriate dose to hydrolyze phytic acid and allow protein to be effectively utilized and digested by fish, thereby promoting growth and metabolic rates. This is supported by Terrey et al (2024), who reported that phytase supplementation can increase the availability of nutrients and minerals, as well as improve protein and amino acid digestibility. Chen & Gatlin (2024) stated that the addition of phytase enzyme can break down the complex bonds formed between amino acids and minerals. Phytase can assist fish in digesting feed as it is thought to hydrolyze phytic acid, allowing nutrients to be absorbed efficiently. Furthermore, Xu et al (2022) explained that adding phytase enhances the activity of converting trypsinogen into trypsin, an enzyme that breaks down proteins into amino acids, thus improving digestive enzyme activity. Similarly, Rachmawati & Samidjan (2018) reported that the addition of phytase can break down complex compounds, making them more easily absorbed by the digestive system and optimally distributed throughout the body. Chen & Gatlin (2024) also noted that the addition of phytase reduces the phytic acid content in feed ingredients, enhancing the feed utilization efficiency. A higher feed utilization efficiency indicates that fish can absorb protein more efficiently to support growth rather than utilizing it for metabolism, osmoregulation, and reproduction (Xu et al 2022). FUE is positively correlated with the fish growth rate and inversely correlated with the FCR. This is evident in the FCR and RGR values (Table 2), where treatment C had the lowest FCR and highest RGR compared to other treatments. Similar findings were reported by Hussain et al (2020) in *Cirrhinus mrigala*, El-Salem et al (2022) in *Sparus aurata*, Rachmawati & Samidjan (2018) in *Pangasius hypothalamus*, and Nie et al (2017) in *C. auratus*.

One factor influencing the PER is the fish's ability to digest the given feed. Additionally, the quality and quantity of nutrients in the feed must meet the fish's needs, as these factors affect growth. The addition of phytase to the feed can break down the phytic acid in plant-based feed ingredients, enabling fish to utilize the protein more effectively. Abo Norag et al (2018) suggested that phytase can decompose and cleave the bonds between phytic acid and protein into its constituent amino acids. The addition of phytase to feed can hydrolyze phytate-protein complexes into easily digestible amino acids, improving fish growth and PER (Morales et al 2014). Treatment C resulted in a higher PER than other treatments, likely due to the optimal phytase dose used, which maximally hydrolyzed phytic acid. The efficient action of phytase can hydrolyze phytic acid and release protein and mineral bonds with phytic acid, increasing the solubility of amino acids, which are easily digestible by fish, enhancing fish body protein, and increasing biomass (Akpoilih et al 2017). Nie et al (2017) also noted that adding phytase to feed improves body protein availability, positively influencing fish growth. The effect of phytase on improving the protein efficiency ratio has been reported by Shahzad et al (2021) in *C. carpio*, Hussain et al (2017) in *Labeo rohita*, and Morales et al (2014) in *S. aurata*.

The results of the analysis of variance (ANOVA) showed that the addition of phytase to artificial feed had a significant effect ($p < 0.05$) on the RGR of Nile tilapia fingerlings. This indicates that phytase can hydrolyze phytic acid, improving protein digestibility and enhancing growth. The highest RGR was observed in Nile tilapia fingerlings fed treatment C at 3.94±0.13% day^{-1}, while the lowest was in treatment A at 2.52±0.24% day^{-1}. Treatment C had the highest RGR due to the highest values of FUE, ADCp, and PER, and the lowest FCR compared to the other test feeds. Kumar et al (2019) noted that the addition of phytase to feed can result in optimal FUE, thereby increasing the RGR. Maas et al (2018) reported that the addition of phytase to feed can hydrolyze phytate-protein complexes into easily digestible amino acids, promoting fish growth. Similarly, Haghbayan & Mehrgan (2015) demonstrated that phytase could hydrolyze these complexes into amino acids, facilitating growth in fish. Similar findings have been reported by Maas et al (2018) in Nile tilapia, El-Salem et al (2022) in *S. aurata*, Yang et al (2022) in *Procambarus clarkii*, and Xu et al (2022) in *C. auratus*.

SR is the ratio of the number of fish still alive at the end of the maintenance period to the number of fish at the beginning (Gao et al 2022). The addition of phytase at varying doses did not significantly affect ($p > 0.05$) the SR of Nile tilapia fingerlings. This may indicate that phytase does not directly influence the survival of Nile tilapia fingerlings during the study. These findings suggest no significant differences between treatments, with all test feeds (A, B, C, and D) yielding a 100% SR, considered excellent. The SR can be used as a parameter to assess fish tolerance and ability to thrive. According to Naz et al (2023), SRs above 70% are considered good, 50-60% moderate, and below 50% poor. Fish survival is influenced by internal factors, such as adaptation ability, parasites, and population density, and external factors, such as the physical and chemical properties of the water (NRC 2011).

The concentration of bone minerals in Nile tilapia fingerlings fed the test diets (Table 2) shows that the addition of phytase enzyme to the feed significantly ($p < 0.05$) increased the levels of Ca, P, Mg, and Zn in the bones. Similar results were reported by Cheng & Hardy (2003), who found that adding phytase to the diet of rainbow trout *Oncorhynchus mykiss* improved the digestibility of Mg, Mn, Zn, and P, with P digestibility being higher than other minerals. Sugiura et al (2001) explained that different doses of phytase supplementation affect the utilization of phosphorus, minerals, and protein in rainbow trout. Laining et al (2012) reported that phytase supplementation significantly increased the concentration of minerals (P, Ca, Mg, and Zn) in the bones of *Pagrus major*. Akpoilih et al (2017) and Adeshina et al (2023) stated that the supplementation of phytase in feed significantly increased P, Ca, Zn, and Mn levels in the bones of African catfish *C. gariepinus*.

Nile tilapia fingerlings fed a diet supplemented with 1000 FTU kg^{-1} phytase had higher concentrations of Ca, P, Mg, and Zn compared to other treatments. This is likely because this phytase dosage hydrolyzed phytate-mineral complexes, increasing the availability of minerals (Ca, P, Mg, Zn) in the fish due to the reduction of gastrointestinal phytic acid and the enhanced bioavailability of minerals compared to other test diets. Similar findings were reported by Rachmawati et al (2023a, b), who found that the addition of phytase significantly increased the concentration of minerals (P, Ca, Mg, and Zn) in the bones of *C. gariepinus* var. Sangkuriang and *C. carpio*. Yan et al (2002) demonstrated that microbial phytase supplementation at a concentration of 1000 FTU kg^{-1} feed increased the levels of Ca, Mg, and Mn in the bones of *Ictalurus punctatus*. The Ca-P ratio plays a crucial role in fish bone development (Adeshina et al 2023), and an increased Ca-P ratio positively affects fish health. A Ca-P ratio of 1.1–1.4:1 suggests that the addition of phytase to the feed is highly efficient (Kumar et al 2012). The results of this study (Table 3) show that the Ca-P ratio of Nile tilapia fingerlings was within the range of 1.41–1.42, indicating that phytase addition to the feed was highly efficient for Nile tilapia fingerlings during the study, as it falls within the recommended range by Kumar et al (2012).

The addition of phytase enzyme to the feed and its impact on the nutritional content of Nile tilapia fingerlings is presented in Table 4. The table shows that Nile tilapia fingerlings fed phytase-supplemented feed had higher body protein content than those fed without supplementation. This may be due to phytase hydrolyzing phytate-protein complexes in the fish's intestines, thereby increasing body protein. The results (Table 2) also show that Nile tilapia fingerlings fed phytase-supplemented diets had higher PER values compared to those without supplementation. Rachmawati et al (2023b) reported that the addition of phytase to feed can hydrolyze phytate-protein complexes into easily digestible amino acids for fish growth, thus increasing PER. Abo Norag et al (2018) suggested that phytase can break down the bonds between phytic acid and protein into amino acids, making them easier to absorb and increasing fish body protein. Similar results have been reported for *C. gariepinus* var. Sangkuriang (Rachmawati et al 2023a), *Marsupenaeus japonicus* (Bulbul et al 2015), *S. aurata* (El-Salem et al 2022), *C. auratus* (Nie et al 2017), and *L. rohita* (Hussain et al 2017). The study also showed that the ash content in Nile tilapia fingerlings fed phytase-supplemented diets was higher than in those without supplementation. Similar findings were reported by Rachmawati et al (2023b) in *C. carpio*, Baruah et al (2007) in *L. rohita*, and Liebert & Portz (2005) in Nile tilapia.

Conclusions. Based on the results of this study, it can be concluded that the addition of phytase enzyme to the feed significantly ($p < 0.05$) improved growth performance, protein digestibility, feed efficiency, mineral content (Ca, P, Mg, and Zn), and body nutrition (protein and ash) in Nile tilapia fingerlings. A phytase enzyme dose of 1000 FTU kg^{-1} feed was the most effective for Nile tilapia fingerlings, showing the highest values for growth performance, feed efficiency, and mineral and body nutrition content observed during the study compared to other test feed treatments.

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Conflict of interest. The authors declare that there is no conflict of interest.

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