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EFFECT OF PHYTASE SUPPLEMENTATION IN PLANT-BASED FEED ON FEED UTILIZATION AND GROWTH OF *Pangasius hypophthalmus* DURING THE GROW-OUT STAGE

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ABSTRACT

Phytic acid, found in many plant-based fish feed ingredients, is an anti-nutritional compound that binds with minerals, forming complexes that fish intestines cannot easily absorb. Adding phytase, an enzyme, to plant-based feeds has shown potential in enhancing nutrient absorption and has been effective for various aquaculture species. However, its impact on *Pangasius hypophthalmus*, a commonly farmed fish, remains underexplored. This study examines the effects of phytase on feed conversion ratio (FCR), feed utilization efficiency (EFU), protein efficiency ratio (PER), relative growth rate (RGR), and survival rate (SR) of *P. hypophthalmus*. Fish (average weight 11.55 g) were kept at a density of 40 fish m^{-3} in a fully randomized design with four treatments (0, 500, 1000, and 1500 FTU kg^{-1} feed) and three repetitions. Data on RGR, EFU, PER, FCR, SR, and water quality were analyzed. Results indicated that phytase significantly improved RGR, EFU, PER, and FCR ($P<0.05$), though SR remained unaffected. The optimal phytase dose, 738-810 FTU kg^{-1} feed, produced an EFU of 69.3% and an RGR of 4.77% per day during the grow-out stage. Water quality parameters remained stable and within optimal ranges across all treatments.

KEYWORDS: catfish; efficiency; feed; growth; phytase enzyme

ABSTRAK: ***Pengaruh Suplementasi Enzim Fitase dalam Pakan Berbasis Tanaman terhadap Pemanfaatan Pakan dan Pertumbuhan*** *Pangasius hypophthalmus* ***selama Stadia Pembesaran***

Asam fitat, yang ditemukan dalam banyak bahan pakan ikan berbasis tanaman, adalah senyawa anti-nutrisi yang mengikat mineral, membentuk kompleks yang sulit diserap oleh usus ikan. Penambahan fitase, enzim, pada pakan berbasis tanaman menunjukkan potensi dalam meningkatkan penyerapan nutrisi dan telah efektif untuk berbagai komoditas budidaya. Namun, dampaknya pada ***Pangasius hypophthalmus****, ikan yang umum dibudidayakan, masih belum banyak dieksplorasi. Penelitian ini mengkaji efek fitase pada rasio konversi pakan (RKP), efisiensi pemanfaatan pakan (EPP), rasio efisiensi protein (REP), laju pertumbuhan relatif (LPR), dan tingkat kelangsungan hidup (TKH) dari* ***P. hypophthalmus****. Ikan (berat rata-rata 11,55 g) dipelihara pada kepadatan 40 ikan* m^{-3} *dalam rancangan acak lengkap dengan empat perlakuan (0, 500, 1000, dan 1500 FTU* kg^{-1} *pakan) dan tiga ulangan. Data LPR, EPP, REP, RKH, TKH, dan kualitas air dianalisis. Hasil menunjukkan bahwa fitase secara signifikan meningkatkan LPR, EPP, REP, dan RKP (P<0,05), meskipun berpengaruh signifikan terhadap*

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TKH. Dosis fitase optimal, 738-810 FTU kg^{-1} pakan, menghasilkan EPP sebesar 69,3% dan LPR sebesar 4,77% per hari selama tahap pembesaran. Parameter kualitas air tetap stabil dan dalam rentang optimal di semua perlakuan.

KATA KUNCI: efisiensi; enzim fitase; ikan patin; pakan; pertumbuhan

INTRODUCTION

Catfish (*Pangasius hypophthalmus*) is one of the popular fish species that has been successfully farmed in Indonesia. A relatively recent statistical data released by the Ministry of Marine Affairs and Fisheries (2021) shows that the production volume of catfish in Indonesia reached 1.845.324 tons. With the price of catfish around UDS 2.3 per kg (Rifai *et al.*, 2020), the total value of the production contributes at least 4.3 billion USD annually to the Indonesian economy. In addition, catfish meat contains 20.19% protein, 2.11% fat, 1.23% carbohydrates, 75.05% water, and 0.52% ash (Chakma *et al*., 2022). Such relatively less expensive prices and nutritious content have made farmed catfish contribute to solving malnutrition and improving the welfare of local communities in Indonesia and worldwide.

One of the main issues in the grow-out stage of catfish farming is the affordability of feed. The feed cost in the grow-out stage ranges from 60% to 70% of the total production cost (Restianti *et al.*, 2016). The effects of feed cost were noticeably problematic, particularly at the end of 2020 when the feed price increased dramatically by 15%, resulting in a 15% profit loss (Pane, 2021). Therefore, various efforts have been carried out to mitigate these feed issues, particularly the increasing trend of feed cost.

Catfish is a warm-water omnivorous species (Halder & Hazrat, 2015). Its wider acceptance to feed means that catfish can be fed with alternative feed ingredients such as plant or vegetable-based feed. However, many vegetable raw materials contain an anti-nutritional substance called phytic acid. Phytic acid (myo-inositol hexaphosphate) is an organic phosphorus compound plentiful in grains and cereals (Hussain *et al.*, 2022). The content of phytic acid in soybean flour, rice bran and corn can be as high as 10-15 g kg^{-1}, 10.44 g kg^{-1}, and 18.49 g kg^{-1} of feed, respectively (Francis *et al*., 2001, Muniroh *et al*., 2021). Phytate compounds are anti-nutritional and easily bind to proteins and minerals (Zn, Ca, Mg, and Fe), causing incomplete metabolism to occur in the digestive tract of animals, especially monogastric animals (Yanuartono *et al.*, 2016). Unbroken phytic acid causes proteins and minerals to come out with fish feces, thus inhibiting the digestibility of protein and the biological availability of essential minerals, such as phosphate (P), which will be released into the waters (Pratama *et al.*, 2015). High P content in the waters will, in turn, trigger an eutrophication process which will be detrimental to the continuity of the cultivation process (Singh & Satyanarayana, 2015).

One of the solutions to handle the anti-nutritional substance issues caused by phytic acid is by adding a phytase. Phytase is an enzyme capable of hydrolyzing phytic acid into mio-inositol mono, di, tri, tetra, and pentaphosphate (Kumar *et al*., 2012). The addition of phytase in the feed can increase the proteins and mineral utilization from vegetable raw materials sources. Kosim *et al*. (2016) argued that providing phytase in feed positively impacts nutrient absorption by hydrolyzing phytic acid into inositol and phosphoric acid, thereby increasing growth. The application of phytase has been widely proven to be able to increase the growth of several fish species, such as tilapia (*Oreochromis niloticus*) (Rachmawati & Samidjan, 2014), polka dot grouper (*Cromileptes altivelis*) (Zulaeha *et al.*, 2015), milkfish (*Chanos chanos*)

(Rachmawati *et al.*, 2017), sea bass fish (*Lates calcarifer)* (Yudhiyanto *et al*., 2017), whiteleg shrimp (*Litopenaeus vannamei*) (Rachmawati & Samidjan, 2018a), African catfish (*Clarias gariepenus*) (Kemigabo *et al.*, 2018), and golden pomfret (*Trachionotus blochii*) (Siburian *et al*., 2019). Considering the potential of phytase in improving the digestibility of fish feed and its application in several farmed aquaculture species, it is quite remarkable that such applications have been limitedly studied for catfish. Therefore, this study was carried out to examine the effect of phytase and determine the optimum dose of phytase in vegetable-based feed to increase the efficiency of feed utilization and growth of catfish during the grow-out stage.

MATERIALS AND METHODS

Preparation of Test Fish

This study was conducted in the Loka Muntilan, Magelang, East Java. The test fish with an average weight of 11.55 ± 0.21 g fish^{-1} were obtained from a catfish cultivator in Muntilan. The test fish were selected based on the size, weight, completeness of organs, and physical health (no parasite and active swimming) (Zulaeha *et al.*, 2015), followed by adaptation to the rearing condition for seven days. During the adaptation period, the fish were given artificial feed without phytase ad satiation with a frequency of feeding three times a day at 08:00 am, 12:00 pm, and 04:00 pm.

Preparation of Experimental Feed

The phytase unit is commonly abbreviated as FTU, although other abbreviations, including FYT, U, and PU, have also been used. In this study, only the abbreviation FTU was used. The doses were 0 FTU kg^{-1} of feed, 500 FTU kg^{-1} of feed, 1,000 FTU kg^{-1} of feed, and 1,500 FTU kg^{-1} of feed containing 30% protein. The protein level was set at the value to follow with the SNI (Indonesian National Standard) 7548:2009 that the protein content of artificial feed for catfish is 30% (National Standardization Board, 2009b). The phytase enzyme used in this study was Natuphos E 10.000 G phytase in the form of yellow granules produced by PT. BASF Indonesia, which has a phytase activity of 10.000 FTU g^{-1}. The use of the dose refers to the research by Kemigabo *et al*. (2018), which states that the addition of the phytase 1.000 FTU kg^{-1} of feed is the best dose for feed conversion ratio and growth performance for African catfish. Converting the FTU value to grams using a reference for phytase enzyme activity in the Natuphos E 10.000 G product is 10.000 FTU g^{-1}, which means that every gram of phytase enzyme has 10.000 enzyme activities (Bavaresco *et al*., 2020). Hence, getting 500 FTU is equivalent to 0.05 g kg^{-1}, 1,000 FTU is equivalent to 0.1 g kg^{-1}, and 1,500 FTU is equivalent to 0.15 g kg^{-1}. The preparation of the test feed was started by conducting proximate tests of feed raw materials, followed by calculating the feed formulation and weighing the raw ingredients according to the calculated feed formulation (Table 1).

After all the raw materials were weighed, the raw materials were mixed evenly (Sayuti *et al*., 2022). Small volumes of raw materials to the largest were mixed gradually and sequentially until homogeneous. The feed dough was then put into the extruder machine to be pelleted. The pelleted feed were then dried to reduce the water content in the feed. The drying process was done manually by drying it in the sun or airing it (Yunaidi *et al*., 2019). After being dried, the feed was cooled down at room temperature and packaged using plastic containers labelled with the dosages according to the treatment and stored.

Preparation of the Research Rearing Units

The research rearing units were 12 pieces of hapa nets measuring 1 x 1 x 0.6 m^3 placed in an earthen pond with a size of 467 m^2. The condition of the hapa nets was inspected, cleaned with water, and dried before use or

Table 1. Formulation and proximate analysis of test feed used in the present experiment (in g kg^{-1} wet weight)

Material type feed compiler	Feed composition (g kg^{-1} feed)			
	A (0 FTU)	B (500 FTU)	C (1,000 FTU)	D (1,500 FTU)
Fish meal	343.5	343.5	343.5	343.5
Soybean flour	283.9	283.9	283.9	283.9
Corn flour [a]	132.1	132.05	132	131.95
Tapioca flour	96.8	96.8	96.8	96.8
Bran flour	113.7	113.7	113.7	113.7
Fish oil	10	10	10	10
Corn oil	10	10	10	10
Vit-min mix	10	10	10	10
Phytase [b]	0	0.05	0.1	0.15
Total (g)	1000	1000	1000	1000
Protein (%) [c]	30.33	30.20	30.27	30.19
NFE* (%) [c]	30.79	30.89	30.69	30.57
Lipid (%) [c]	10.46	10.41	10.95	10.46
En.(kkal g^{-1}) [d]	267.9	267.2	271.4	266.8
Ratio E/P[d]	8.83	8.85	8.96	8.84

Note:
*Nitrogen free extract (NFE)

a. Different quantities of corn flour were used because it has a relatively low protein content. It is used to supplement the amount of phytase used without changing the contribution of the targeted protein.
b. One g of phytase contains an enzymatic activity of 10,000 phytase units (FTU). The doses of phytase used in the treatments were 0 FTU, 500 FTU is equivalent to 0.05 g kg^{-1}, 1.000 FTU is equivalent to 0.1 g kg^{-1}, and 1,500 FTU is equivalent to 0.15 g kg^{-1} (Bavaresco *et al.,* 2020).
c. The results of proximate analysis of Chem-Mix Pratama Laboratory, Bantul, Yogyakarta.
d. Calculated based on digestible energy, according to (Wilson, 1982) 1 g of protein is 3.5 kcal g^{-1}, 1 g of fat is 8.1 kcal g^{-1}, and 1 g of carbohydrates is 2.5 kcal g^{-1}.

installation. A counterweight was placed at each inner corner of the hapa nets to prevent them from floating to the surface of the water. The hapa nets were then arranged in the pond according to the layout of the research-rearing units. The placement of treatment positions on the containers was done randomly. The positions of the hapa nets in the pond were fixed using bamboo poles.

Experimental Procedure

Before the treatment began, the test fish were fasted for one day to remove food residues and metabolism in the body of the fish to obtain pure catfish weight without any metabolic residue (Kosim *et al.,* 2016). Then, stocking was carried out on each hapa with a stocking density of 20 fish hapa^{-1}. The water volume in the hapa was maintained at 500 L or a water height of 0.5 m. This setting referred to the research of Durai *et al.* (2021), where the stocking density used was 40 fish m^3 for catfish during the grow-out stage. The treatment feed was given to test fish ad satiation with a daily feeding frequency of three times (08:00 am, 12:00 pm, and 04:00 pm). The fish maintenance was carried out for 60 days, and fish sampling was conducted every 10 days. The water quality parameters observed, including temperature, pH, and dissolved oxygen (DO), were carried out every day in the morning and evening. The study also measured ammonia concentration in rearing media by collecting water samples at the day 1, day 30, and day 60. The samples were analyzed for ammonia concentration at the Laboratory Yogyakarta Center for Environmental Health Engineering and Disease Control.

Observed Parameters

The observed data in this study included total feed consumption, efficiency value of feed utilization, protein efficiency ratio, feed conversion ratio, relative growth rate, survival rate, and water quality.

Feed consumption (FC)((Pereira *et al*.,2007)
= amount of feed on the first day + amount of feed on the second day + … + amount of feed on the n^{th}-day

Efficiency of feed utilization (EFU)(Tacon,1987)

$$= \frac{\text{(Final fish biomass – Initial fish biomass)}}{\text{Total weight of feed consumed}}$$

Protein efficiency ratio (PER)(Hepher,1989)

$$= \frac{\text{(Final fish biomass – Initial fish biomass)}}{\text{(Protein content x feed consumed by fish)}} \text{x100\%}$$

Feed conversion ratio (FCR) (Tacon,1987)

$$= \frac{\text{Total amount of feed consumed}}{\text{(Final fish biomass – Initial fish biomass) - Total weight of fish that died during the study)}}$$

Relative growth rate (RGR)(Steffens,1989)

$$= \frac{\text{(Final fish biomass – Initial fish biomass)}}{\text{(Initial biomass of fish x Length of study)}} \text{x100\%}$$

Survival rate (SR) (Effendi,1991)

$$= \frac{\text{Number of fish at the end of the study}}{\text{Number of fish at the beginning of the study}} \text{x100\%}$$

Ethical Statement

The use of fish in this work follows the ethical protocol of animal welfare for research at Diponegoro University and SNI 7551.2009 (National Standardization Board, 2009a)

Statistical Analysis

The data were then analyzed using variance (ANOVA) to determine the significant differences among the treatments ($P<0.05$). A post hoc test using the Duncan's multiple range test was performed to determine the difference in mean values between the treatments. All statistical analyses were conducted using the IBM SPSS statistics 25 (IBM Coporation, US). Furthermore, the optimum phytase dose was estimated using the orthogonal polynomial test with MapleSoftware (Waterloo Maple Inc., Ontario, Canada). Water quality data were analyzed descriptively and compared to the optimum values for catfish grow-out.

RESULTS AND DISCUSSION

The results of the efficiency of feed utilization, protein efficiency ratio, feed conversion ratio, relative growth rate, and the growth stage survival of catfish during the study are presented in Table 2.

The water quality during maintenance was relatively good and still feasible to support the life of catfish in the grow-out stage. The results of water measurements during the study are in Table 3.

Feed Consumption

Calculation of the total feed consumption aimed to determine how much feed consume during maintenance. Based on Table 2, it is shown that the addition of the phytase enzyme increased the total consumption of fish feed. The results of the analysis of variance showed that the addition of the phytase enzyme to catfish feed grow-out stage had a significant effect ($P<0,05$) on FC. The highest FC value

Table 2. Average value of feed utilization efficiency (EFU), protein efficiency ratio (PER), feed conversion ratio (FCR), relative growth rate (RGR), and survival (SR) of catfish fed with phytase-supplemented plant-based feed for 60 days

Variable	**Treatment**			
	A	**B**	**C**	**D**
FC	916.7 ± 17,99[c]	962.36 ± 6.39[a]	939.63 ± 8.71[b]	921.74 ± 6.52[bc]
EFU	51.58 ± 2.80[d]	70.57 ± 2.61[a]	64.66 ± 1.741[b]	57.54 ± 4.17[c]
PER	1.71 ± 0.09[d]	2.34 ± 0.09[a]	2.14 ± 0.06[b]	1.91 ± 0.14[c]
FCR	1.75 ± 0.06[d]	1.33 ± 0.04[a]	1.44 ± 0.06[b]	1.60 ± 0.06[c]
RGR	3.42 ± 0.26[c]	4.90 ± 0.22[a]	4.39 ± 0.15[b]	3.82 ± 0.30[c]
SR	86.67 ± 5.77[a]	88.33 ± 2.89[a]	86.67 ± 2.89[a]	85.00 ± 5.00[a]

Note: The results presented are based on data normalization. Different superscripts in the same row indicate significant differences at a confidence level of 95% (P<0.05). The treatments were the addition of phytase in artificial feed with different doses, which were A (0 FTU kg^{-1} feed), B (500 FTU kg^{-1} feed), C (1,000 FTU kg^{-1} feed), and D (1,500 FTU kg^{-1} feed).

Table 3. The results of checking the water quality of Pangasius catfish (*Pangasius* sp.) maintenance media during the study

Variable	**Unit**	**Range**		**Feasibility**
		Morning	**Evening**	
Temperature	^oC	24.7-30	25.6-29.8	25-32[a]
pH	-	7.2-8.8	7.3-8.7	6-9[b]
Dissolved Oxygen	mg L^{-1}	5-7	5-6.9	>3[c]
Ammonia	mg L^{-1}	<0.0064 – 0.0149		<0,01[c]

Note: a: (Abedin *et al*., 2017), b:(Hassan *et al*., 2021), c:(SNI, 2009b)

for catfish was 961,69 ± 7,54 g obtained in treatment 500 FTU kg^{-1} feed. The appropriate dose will increase appetite and the process of phytic acid hydrolysis and breaking of protein bonds, thus increasing protein availability. The phytase hydrolyzes phytic acid into inositol and phosphoric acid and breaks the bonds of phytic acid with proteins and minerals, thereby increasing the bioavailability of nutrients (Song *et al*., 2019).

The hydrolysis of phytic acid in the form of inositol in the feed plays a role in increasing fish appetite, affecting the level of consumption of fish feed. Inositol is a compound that is part of a polyhydroxylated cycloacanth. It is generally known as cyclitol and classified as an essential nutrient because of its role as a growth promoter (Refriana *et al*., 2021). Inositol is one of the vitamins needed to support normal body growth, maintenance, and reproduction (Pratama *et al*., 2015). Ideal protein hydrolysis will produce higher amino acids so that more is absorbed by the body, increasing the growth rate. This can be seen in Figure 1.d. that treatment 500 FTU kg^{-1} feed produced the highest growth rate. As a result of the different sizes and growth rates caused by the different doses of the phytase gave a difference in total feed consumption, causing large fish to consume more feed than small fish. This is reinforced by Rolin *et al*. (2015) that differences in fish size and growth rate allow larger fish to require and consume more feed than small fish.

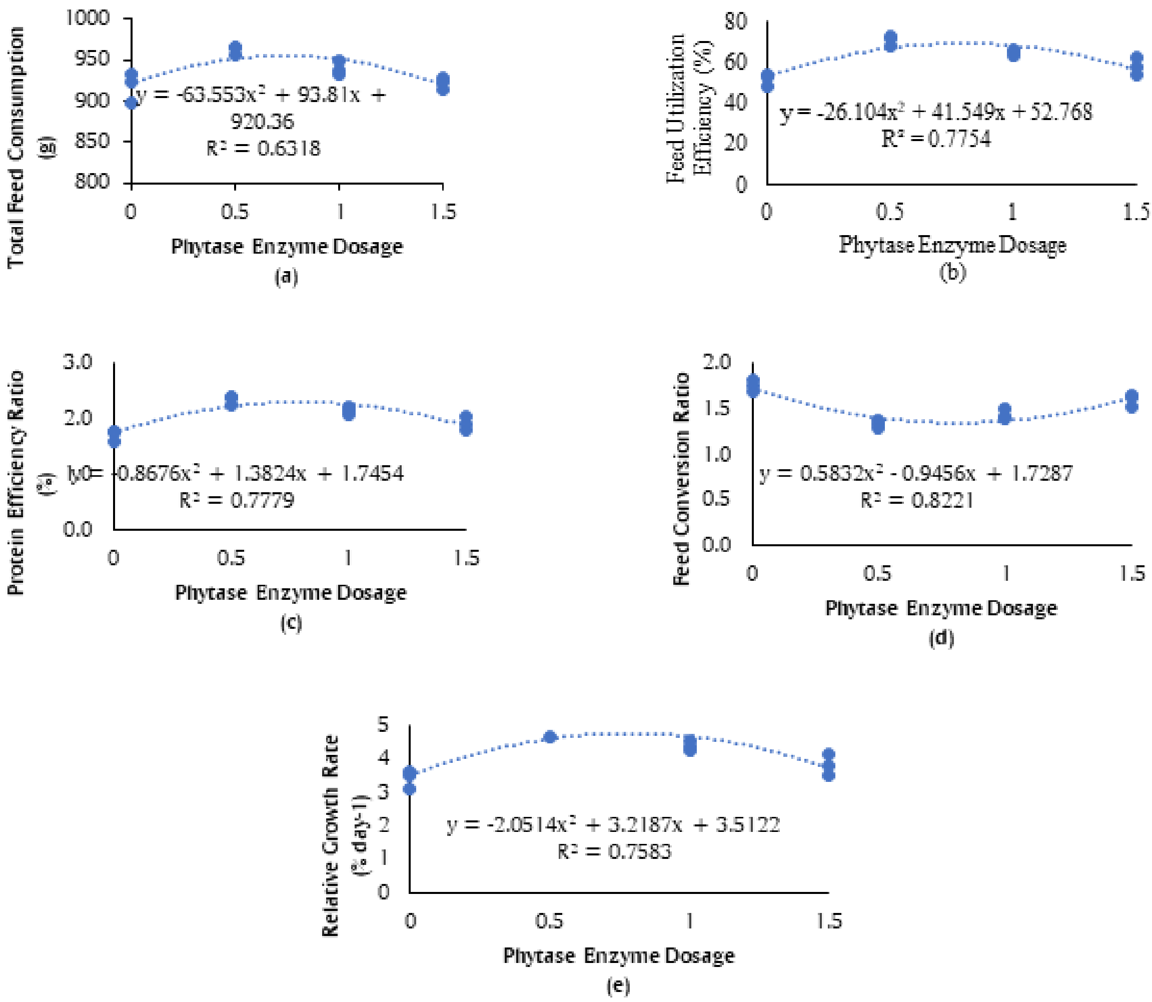

Figure 1. Orthogonal polynomial graph of total feed consumption (a), efficiency of feed utilization (b), protein efficiency ratio (c), feed conversion ratio (d), and relative growth rate (e) of catfish grow-out stage

The treatment of 0 FTU kg^{-1} (treatment A) feed had low feed consumption, presumably due to a lack of inositol in the body of the fish, resulting in reduced appetite. Similarly, Sintia *et al*. (2020) pointed out several symptoms of inositol deficiency, such as reduced appetite, slow gastric emptying, anemia, and slowed growth. Excessive doses of phytase can cause the phytic acid content in the feed to decompose, leading too much protein and P bound to phytic acid to decompose, resulting in decreased fish growth (Rachmawati & Samidjan, 2014; Rochmawati *et al*., 2016). It was suspected that much phytic acid decomposes, resulting in most of the minerals to break down, causing the fish to overdose with minerals, preventing the fish from growing optimally. This process has more impact on the growth rate of small fish due to the fish consume less feed.

The highest FC value for catfish was 961,69 ± 7,54 g obtained in treatment 500 FTU kg^{-1} feed. The optimum dose of phytase in artificial feed to improve the total feed consumption of catfish in the grow-out stage was determined by carrying out the orthogonal polynomial test to obtain a quadratic pattern relationship (Y= -63.553x2 + 93.81x + 920.36 and R^2 =0.6318). From this equation, the optimum dose of phytase in artificial feed was obtained, which was 738 FTU kg^{-1} of feed; it can produce a maximum total feed consumption value of 954.97 g.

Efficiency of Feed Utilization

The results of the analysis of variance showed that the addition of the phytase enzyme to catfish feed in the grow-out stage had a significant effect ($P<0.05$) on the efficiency value of feed utilization. The highest EFU value was obtained by treatment B of 70.57±2.61%, adding phytase to the artificial feed of 500 FTU kg^{-1}. According to Ginting *et al.* (2022), feed is good if it has a feed efficiency value of more than 50% or even better if close to 100%. A high EFU value was interpreted as a high ability of fish to utilize feed and protein content in the feed to produce a low FCR value. In this regard, the nutrients from the feed can be appropriately utilized by fish for growth. It was argued here that the supplementation of phytase in the feed had improved the process of breaking bonds between phytic acid and proteins and minerals. As a result, the supplementation has successfully increased nutrient digestibility and feed absorption in the body of the test fish. This is supported by Rodrigues *et al.* (2023) where phytase supplementation has the potential to increase nutrient bioavailability and feed energy as well as feed efficiency and improve growth performance.

In addition, the byproducts of the hydrolysis process in the form of inositol, a class of antioxidant vitamins, play a role in increasing the immunity of the body. Inositol enhances hemolymph homeostasis and defends the host immune by enhancing immune and microbial barrier function in hemolymph and improves gut health status by enhancing physical, immune, and microbial barrier functions in the gut (Lu *et al*., 2023). Besides that, inositol maintains the number of erythrocytes in the blood and helps the absorption of nutrients in the gut mucosa to facilitate the digestive system (Refriana e*t al.*, 2021). As a result, the availability of sufficient nutrients and the presence of inositol further increased the absorption of nutrients in the body (protein and energy) of the fish and subsequently increased the fish's weight.

The treatment without the addition of phytase had the lowest EFU value. It was suspected that the feed given was not utilized efficiently because phytic acid still bound the nutrients and minerals in the feed. This is supported by Singh & Satyanarayana (2015), who asserted that the lack of phytase levels in the digestive tract of animals (especially monogastric or non-ruminant animals, such as poultry and fish) results in phytic acid being excreted in the feces. Phytic acid can directly or indirectly inhibit amylase activity by chelating Ca, a necessary cofactor for amylase (Yanuartono *et al.*, 2016). In contrast, excessive doses of phytase could cause phytic acid to decompose in large amounts and break the mineral bond. Such a process will increase the availability of excess minerals, thus impacting enzyme activity. High Ca levels from this process can directly reduce phytase activity and limit feed efficiency because it acts as a chelating agent for most of the nutrients released by phytase, significantly limiting benefits on nutrient digestibility and growth (Bavaresco *et al*., 2020).

The relationship between the optimum dose of phytase in the artificial feed with the feed utilization efficiency of catfish was determined by conducting an orthogonal polynomial test to obtain a quadratic relationship ($Y= -26.104x^2 + 41.549x + 52.768$ and $R^2 = 0.7754$). From this equation, it was obtained that the optimum dose of phytase in the artificial feed was 795 FTU kg^{-1} of feed, capable of producing a maximum EFU value of 69.30%. This EFU value was lower than commercial feed, which ranged from 76.67% to 77.27% for catfish in the grow-out stage (Sedana & Sumadana, 2020). However, it was higher than the study by (Rolin *et al.,* 2015) using commercial feed, which produced an EFU of 66.20%.

Protein Efficiency Ratio

The PER value is influenced by the ability of the fish to digest the feed given (Rachmawati & Samidjan, 2014). The results of the analysis of variance showed that the addition of phytase enzyme to catfish feed (*Pangasius* sp.) had a significant effect ($P<0.05$) on the value of the protein efficiency ratio. The highest PER value was 2.34 ± 0.9%, obtained by the treatment of 500 FTU kg^{-1} feed, while the lowest PER value was obtained by the treatment of 0 FTU kg^{-1} feed of 1.71 ± 0.09%. This finding suggests that the addition of phytase catalyzed the decomposition reaction of phytic acid into inositol and phosphoric acid in the feed so that the protein and minerals in the phytate complex compound are liberated. Then, the fish used the protein efficiently, increasing the value of the protein efficiency ratio. In other words, adding phytase in feed increases protein digestibility by releasing protein in complexes, thereby increasing the utilization of nutrients (Orisasona *et al.,* 2017). Myo-inositol, a product of phytic acid hydrolysis, could have roles in this process due to its ability to increase pepsin activity and protein accumulation and utilization (Yu *et al.*, 2023). Based on these findings, the study suggests that adding phytase to feed has successfully increased PER. The positive relationship between feed digestibility, PER and growth values was shown by more feed consumed by the fish indicated better weight.

Protein digestibility affects the quality of the amino acids in the feed source (Winata *et al*., 2018). Treatment A gave the lowest PER value, presumably because the phytic acid in the feed had not been hydrolyzed, resulting in minimal protein being broken down into amino acids that the body could absorb. Phytic acid binds to phosphate (P) in fish to make complex phytate-P, which renders P unavailable to fish (Hussain *et al*., 2022). P is one of the most essential mineral nutrients for fish because it is a component of nucleic acids and cell membranes and is directly involved in energy production. Phosphate also maintains the osmotic pressure of body fluids, metabolic processes of carbohydrates, fats and amino acids, and muscle and nervous tissue (Pratama *et al.,* 2015). Phosphate deficiency eventually leads to anorexia, lethargy, decreased growth, increased FCR, and bone malformations (Dias & Santigosa, 2023).

The relationship between the optimum dose of phytase in artificial feed with the PER in catfish was determined by conducting an orthogonal polynomial test and a quadratic pattern relationship ($Y= -0.8676x^2 + 1.,3824x + 1.7454$ and $R^2 = 0.7779$). From this equation, it was obtained that the optimum dose of phytase in artificial feed was 796 FTU kg^{-1} of feed, capable of producing a maximum protein efficiency ratio value of 2.3%. The optimum protein efficiency ratio value of 2.3% in this study was lower compared to commercial feed, which was 2.69% for the grow-out stage of catfish (Chowdhury & Roy, 2020).

Feed Conversion Ratio

The analysis of variance showed that adding the phytase to the feed had a significant ($P<0.05$) effect on the FCR. The lowest FCR value (1.33) was achieved by the treatment using 500 FTU kg^{-1} feed. Overall, the dataset in Table 1 showed that adding phytase in feed significantly decreases the FCR values. The addition of phytase can degrade phytate, causing phytic acid proteins and minerals to no longer be bound together. Rachmawati & Samidjan (2014) argued that, in addition to regulating the excretion of nutrients (such as P, N, and minerals), phytase can hydrolyze phytic acid (phosphate elemental reserves) in fish feed into inositol and phosphoric acid. With the degradation of this anti-nutritional phytic acid, the process of breaking down complex proteins and minerals can function optimally after decomposing phytic acid. The breakdown of phytic acid positively increases activity to change trypsinogen into trypsin, which further breaks down protein into amino acids to reduce the feed conversion ratio and increase feed efficiency (Rachmawati *et al*., 2017). The more efficient the fish is in utilizing the feed it consumes for growth, the body of the fish weight will increase, and the lower the feed conversion value (Sedana & Sumadana, 2020). A low FCR value and high EFU achieved in this study indicate that the given feed was used efficiently to meet the energy needs and growth of the fish.

The treatment of 0 FTU kg^{-1} feed gave the highest FCR results which is suspected by the presence of an anti-nutritional substance in the form of phytic acid. Phytate compounds bind proteins and minerals such as Zn, Ca, Mg, and Fe, preventing a complete metabolism in the digestive tract of the fish. This process occurs more intensely in monogastric animals due to the limitations of phytic acid-breaking enzymes in the form of phytase (Yanuartono *et al*., 2016). In the case of higher FCR values in treatments 1,000 FTU kg^{-1} feed and 1,500 FTU kg^{-1} feed, the excess dose of the phytase had caused excessive breaking of mineral bonds, resulting in loss of minerals through fish excretion, negatively impacting the FCR. The relationship between the optimum dose of phytase in artificial feed and the FCR values was determined by conducting an orthogonal polynomial test to obtain a quadratic relationship ($Y= 0.5832x^2 – 0.9456x + 1.7287$ and $R^2 = 0.8221$). From this equation, the optimum dose of phytase in the artificial feed was obtained, namely 810 FTU kg^{-1} of feed, which can produce a maximum feed conversion ratio value of 1.34.

Relative Growth Rate

The RGR is measured to determine fish growth during a certain period of maintenance (Wibowo *et al*., 2018). The highest relative growth rate value was obtained by 500 FTU kg^{-1} of feed at 4.90% day^{-1}. The high RGR value in treatment B was likely due to the highest EFU and PER values and the lowest FCR. It is argued here that phytase supplementation in feed effectively hydrolyzed phytic acid, thereby increasing protein/amino acid digestibility, which could lead to increased growth. This is reinforced by relevant studies that phytase can increase the relative growth rate of milkfish (Rachmawati *et al.*, 2017), red tilapia (Rachmawati *et al.*, 2018), and carp fish (Rachmawati & Samidjan, 2018[b]).

Adding phytase to feed can regulate the absorption rate of nutrients (such as N, P, and minerals) and hydrolyze phytic acid into inositol and phosphoric acid (Anggani *et al*., 2021). Myo-inositol produced from this process is vital in the glycerophospholipid pathway, particularly in increasing choline, the precursor of acetylcholine. Increasing acetylcholine can raise pancreatic secretion and improve digestion and absorption of proteins and lipids (Yu *et al.*, 2023). Sintia *et al*. (2020) also confirmed that phytase supplementation in feed can increase the utilization of amino acid availability, especially methionine, which is responsive to the addition of phytase in feed. The improved amino acid availability to fish due to the effect of phytase supplementation was evidently shown by the study of Rachmawati *et al*. (2023a), where the digestibility of sangkuriang catfish increased from 68.32% to 80.15%.

The treatment without the addition of phytase had the lowest RGR value. It is highly likely that the phytic acid in the feed did not decompose or, at least, did not hydrolyze completely. It is expected that the absence of positively charged mineral cations, proteins, positively charged amino acids, and fatty acids might have interfered with the fish growth performance (Akhtar *et al.,* 2020)

The relationship between the optimum dose of phytase in feed with the relative growth rate of pangasius catfish was determined by orthogonal polynomial test and a quadratic pattern ($Y= -2.0514x^2 + 3.2187x + 3.5122$ and $R^2 = 0.7583$). From this equation, it was determined that the optimum dose of phytase in artificial feed was 784 FTU kg^{-1} of feed, capable of producing a maximum relative growth rate of 4.77% day^{-1}.

Survival Rate

The addition of artificial feed phytase with different doses had no significant effect ($P>0.05$) on the survival rate (SR) of the *Pangasius hypophthalmus* grow-out stage. The SR of catfish ranged from 85-88%, which was classified in the good category (Simorangkir *et al.*, 2020). A fish SR above 50%, between 30-50% and below below 30% is classified as good, moderate, and poor, respectively (Simorangkir *et al.*, 2020). The SR result of this study is similar to that of studies on milkfish (Rachmawati *et al*., 2017), freshwater eel (Winata *et al*., 2018), and saline red tilapia (Rachmawati *et al*., 2018) concerning the addition of phytase in feed. The high survival rate of catfish during the study indicated that the test feed given during the research did not have a toxic effect on the fish. Food availability in this study was deemed sufficient to meet the needs of fish to survive.

CONCLUSION

The addition of phytase to catfish artificial feed grow-out stage had a significant effect ($P<0.05$) on FC, EFU, PER, FCR, and RGR but had no significant effect ($P>0.05$) on SR. Phytase breaks down phytic acid in plant-based feed, increasing the availability of essential minerals like phosphorus, allowing more nutrients to be absorbed by the fish, thus improving feed efficiency and growth. With more easily digestible feed, fish require less feed to achieve optimal growth, leading to better efficiency and lower feed conversion ratios. Although phytase enhances growth performance and feed efficiency, it does not affect fish survival, as the primary factors influencing survival are related to environmental quality, stress, and disease, rather than improved nutrient absorption alone.

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AUTHOR CONTRIBUTION

ESR: Conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, resources, software, supervision, validation, writing – original draft, writing – review, and editing. DR: Formal analysis, funding acquisition, project administration, supervision, validation, visualization, writing – original draft, writing – review, and editing; S: Formal analysis, funding acquisition, project administration, supervision, validation, visualization, writing – original draft, writing – review, and editing.

DECLARATION OF COMPETING INTEREST

The authors declare no conflict of interest.

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