Effects of Phytase Enzyme Supplementation on Protein Digestibility, Efficiency of Feed Utilization, Growth and Carcass Protein Content of Tambaqui (*Colossoma macropomum*)

Abstract

The success of intensive cultivation of tambaqui (*Colossoma macropomum*) is largely determined by the availability of high-quality feed. High-quality fish diets consist of fish meal produced from animal-based proteins in order to provide fish with the protein requirement. In intensive farming systems, feed costs account for 60–70% of total production costs. Due to fish meal's increasing cost; hence, less expensive ingredients are required to substitute fish meal. Soybean meal can be used as an alternative source of protein to substitute for fish meal due to its lower price and high protein content in fish diet. Phytate acid, one of the antinutritional materials contained within soybean meal, may decrease the nutrient content of feed by affecting with the fish's ability to absorb nutrients. The research aimed to examine the effect of adding the phytase enzyme to diet feed on increasing protein digestibility, efficiency of feed utilization and growth of tambaqui fish. The fingerlings of tambaqui used as test fish had an average weight of 3.85 ± 0.08 g/fish. The diet treatments in this study were phytase enzyme addition in diet with different doses, namely: A (0 FTU/kg feed), B (250 FTU/kg feed), C (500 FTU/kg feed), D (750 FTU/kg feed) and E (1000 FTU/kg feed). The results showed that phytase enzyme addition in diet significantly increased protein digestibility, efficiency of feed utilization, growth, and carcass protein content of tambaqui fish. The most effectiveness phytase enzyme dose for tambaqui fish was 1000 FTU/kg feed, which resulted in highest values of ADCp, PER, FCR, and RGR at 68.46±0.28%, 2.27±0.05, 1.53±0.04, 74.59±0.45%, and 6.01±0.19%/day, respectively.

Keywords: digestibility, efficiency, feed, growth, protein

Introduction

Tambaqui (*Colossoma macropomum*) is one variety of freshwater fish that is widely cultivated due to its high demand in Indonesia. During 2016–2020, Indonesia produced 215,000 tonnes of tambaqui fish, valued at 3.5 trillion rupiah, according to KKP (2022). Tambaqui has considerable business potential because it is a valuable fishery commodity that is used for both ornamental and consumption. It is also easy to cultivate, relatively fast-growing, omnivorous and adaptable (Rachmawati and Samidjan, 2018).

The success of intensive cultivation of tambaqui (*C. macropomum*) is largely determined by the availability of high-quality feed. High-quality fish diets consist of fish meal produced from animal-based proteins in order to provide fish with the protein requirement. In intensive farming systems, feed costs account for 60–70% of production costs (Rachmawati and Samidjan, 2018). The cost of fish meal as an ingredient for artificial fish feed is increasing, so a lower-cost

alternative source of ingredients is required to substitute fish meal (Shapawi *et al*., 2013). Soybean meal can be used as an alternative protein source to fish meal because it is cost-effective and suitable for fish diet (Hussain *et al*., 2011; 2021). However, soybean meal contains anti-nutrients such phytate acid, which could cause problems with the fish's ability to digest and absorb nutrients, hence reducing the feed's nutritional value (Kumar *et al*., 2011).

Phytate acid from plant-based feed ingredients has the role of an anti-nutritional factor. (Kishore *et al*., 2020). The presence of phytate acid affected the digestion of the cultivant, where the digestibility and utilization of the feed were reduced (Kumar *et al*., 2011). Phytate acid negatively influences the morphology and physiology of the digestive system, which in turn affects the growth of cultivants. Phytate acid is able to bind protein and phospor together into insoluble complex compounds that may reduce efficiency of feed utilization and digestibility (Cao *et al*., 2007). One alternative to overcome this problem is the addition of phytase enzyme to the diet. The addition of phytase enzyme in diet fish enhances nutrient absorption, controls the excreted nutrients of cultivant such as phosphorus, nitrogen, minerals, hydrolyses phytate acid into inositol and phosphoric acid, and releases minerals that bind to phytate acid (Hussain *et al*., 2021). Phytate acid, previously a storage form of phosphorus, will be converted by the phytase enzyme into available phosphorus that can be used directly by the fish. Furthermore, the phytase enzyme also prevents the degradation of amino acids, reduces the possibility of loss of water-soluble components, and enhances the bioavailability of some minerals (Cao *et al*., 2007). Phytase enzymes may also afford the release of inositol (myo-inositol) through the process of phytate dephosphorylation. Inositol deficiency will cause increased liver lipid, haematological changes, pathological organ transformations, lethargy, and decreased appetite (NRC, 2011). Through the addition of phytase enzymes, nutrients such as phosphorus, amino acids and energy that were previously bound in the form of indigestible phytate acid would be converted into nutrients that could be digested by the fish. This will enhance the fish's ability to assimilate nutrients and encourage optimum growth.

Some studies on the utilization of phytase enzymes in feed to promote growth and feed efficiency had been conducted on *Pagrus major* (Biswas *et al*., 2019), *Trachinotus blochii* (Siburian *et al*., 2019), *Oreochromis niloticus* (Pontes *et al.,* 2019), *Clarias gariepinus* (Rachmawati *et al.,* 2023a) and *Cyprinus carpio* (Rachmawati *et al*., 2023b). However, there is not much information on the addition of phytase enzyme in dietary of tambaqui (*C.*

macropomum). Therefore, this study is necessary to examine the effect of phytase enzyme addition to artificial feed on increasing protein digestibility, feed utilisation efficiency and growth of tambaqui (*C. macropomum*).

Materials and Methods

Design of Experiment and Test Fish Preparation

This study used an experimental methodology with a completely randomised design (CRD), five treatments, and three replications at the Freshwater Fisheries Aquaculture Centre (FFAC), Muntilan, Central Java, Indonesia. The test fish applied were tambaqui fingerlings (*C. macropomum*), weighing an average of 3.85±0.08 g and having an average length of 7±0.18 cm. There were 1,695 fish in total. Fish were obtained from the research centre and reared for 56 days. Fingerlings of tambaqui had a week-long adaptation process before being used as test fish. Fish fingerlings were determined by selecting fish that were considered healthy, had the same size and weight, swim actively, and were not malformed (Rachmawati e*t al*., 2023a). The adaptation process aims to allow the test fish to adapt to the culture environment and feed. Furthermore, the test fish were starved the day before the study to eliminate the remaining feed provided previously.

Preparation of treatment diet

The treatment diet used was an artificial feed in the form of pellets, which contained 31% protein (Rachmawati dan Samidjan, 2018), was added with the phytase enzyme Natuphos E 10,000 G according to the treatment, namely doses of 0 FTU/kg (A), 250 FTU/kg (B), 500 FTU/kg (C), 750 FTU/kg (D) and 1,000 FTU/kg. The phytase enzyme applied in this study was granule-shaped and marketed under the brand name Natuphos E 10,000 G by PT BASF Indonesia. Natuphos E 10,000 G is a phytase enzyme that has higher enzyme activity, more resistance to acidic pH, and temperatures up to 95°C (Bavaresco *et al*., 2020). The treatment diet formulation is presented in Table 1.

Table 1. Feed formulation and chemical composition of treatment diet for tambaqui fingerlings

Ingredient (g)	Treatment Diet				
	A	B	C	D	E
Fish meal	299.39	299.39	299.39	299.39	299.39
Soybean meal	404.84	404.84	404.85	404.85	404.85
Corn meal	150.00	150.00	150.00	150.00	150.00
Bran	99.77	99.745	99.72	99.695	99.67
Tapioca flour	6.00	6.00	6.00	6.00	6.00
Fish oil	10.00	10.00	10.00	10.00	10.00
Corn oil	10.00	10.00	10.00	10.00	10.00
Min. Vit[1)]	10.00	10.00	10.00	10.00	10.00
Phytase Enzymes (FTU)	0.00	0.025	0.05	0.075	0.100
Cr_2O_3	10.00	10.00	10.00	10.00	10.00
Total	1000	1000	1000	1000	1000
Proximate Analysis Results					
Protein (%)*	31.24	31.33	31.04	31.12	31.28
Lipid (%)*	8.87	8.40	8.99	8.73	8.40
BETN (%)*	28.06	28.13	28.41	28.19	28.36
Energy (Kkal)[2)]	18.47	18.52	18.38	18.32	18.63

Notes :

[1)]Vitamin and Mineral mix kg-1: sodium (Na) 117 mg, selenium (se) 150 mg, Vit. B1 52 mg, magnesium (Mg) 1.900 mg, Vit. B2 97 mg, Vit. B6 46 mg, potassium (K) 150 mg, calcium (Ca) 219 mg, copper (Cu) 9 mg, iron (Fe) 90 mg, Vit. C (coated) 68,800 mg activity, Zinc (Zn) 90 mg, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, Vit. B12 60 mg, Vit. A 36,000 I.U., Vit. D3 9,000 I.U., manganese (Mn) 105 mg, Panthothenic acid 93 mg, Inositol 225 mg, Biotin 450 mg, Vit. E 187 mg, Vit. K3 19 mg, Niacin 130 mg., Folic acid 10 mg.

[2)] It was calculated based on Digestible Energy according to NRC (2011) for 1 g protein is 3.5 kcal/g, 1 g fat is 8.1 kcal/g, and 1 g carbohydrate is 2.5 kcal/g.

[3)] According to NRC (2011), the E/P value for optimal fish growth ranges from 8-12 kcal/g.

* Results of proximate analysis of Animal Food Science Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University (2023).

The process of production of the treatment diet was initiated by measuring the ingredients according to the feed formulation, then mixing the ingredients of the diet starting from a small amount of ingredients to a large amount stirred until homogeneous using a mixer machine stirring until homogeneous, then fish oil, corn oil and water were added to the dough, the ingredients were mixed until homogeneous. Next, the dough is added to the extruder floating pellet moulding machine. After that, the treatment diet in the form of floating pellets was dried at temperature 26°C, followed by packaging in airtight plastic and stored until the time to be used.

Preparation of Research Container

The research container utilised consisted of fifteen 1x1x1 m^3 fibre tanks, each equipped with a recirculation system that maintained the water quality within the optimal range. The rearing medium water in this research was precipitated in a reservoir prior to use.

The study was initiated by weighing the test fish, followed by entering the fish into the research container with a density of 113 fish/fibre tanks. Fish stocking density was 50 fish/m^2 referring to Rachmawati et al. (2023a). During the study, the treatment diet was administered at satiation (fish until satiated) with a frequency of feeding three times a day, at 07.00, 12.00 and 17.00 (Western Indonesian Time/WIB). The weight of the test fish was weighed every week whereas siphoning was carried out 2 hours after feeding. Siphoning was conducted to remove residual feed and faeces from the cultivation media to maintain water quality for fish. Water quality parameters observed according to Boyd, (2003) include pH 6.5-8.6 (Jenway 3510), DO ≥3 mg/L (Jenway 970), temperature 25°C-30°C (Water quality checker) and ammonia (HANNA: HI. 8633).

Proximate Analysis

Proximate analyses were conducted on the treatment diet and carcasses of the test fish fingerling according to the method of Jayant *et al*. (2018), whereas protein content was determined using a semi-automatic Kjeldahl system (FOSS Kjeltec 2300). Lipid content was determined using an ether extraction method based on the Soxhlet method (FOSS Soxtec 2043). Ash content was determined by burning the treatment diet and fish samples in a furnace at 550°C for 24 hours.

Analysis of Protein Digestibility

In accordance with Pérez-Jiménez *et al*. (2014), the treatment diet's protein digestibility was determined indirectly with the addition of 1% of Cr_2O_3 as an indication. Fish faeces were collected every morning, afternoon and evening after the fish were fed for 56 days of rearing. Faeces collection was done using a small pipe, the end of which is tied to a wooden stick to make it more flexible for collecting faeces. Following this, the collected faeces were transferred into a bucket. Then the faeces were filtered using a plankton cloth net, stored in a small plastic bottle

and cooled at 4°C. In the next step, the faeces were analysed and dried in an oven (Gravity Oven) at 6°C for 24 hours before analysis. Then the protein and Cr_2O_3 content in the faeces were analysed using an SSA spectrophotometer with a wavelength of 350 nm.

Observed Parameters

The parameters observed consisted of protein digestibility (ADCp) followed by Pérez-Jiménez *et al*. (2014), efficiency of feed utilization (EFU), feed corversion ratio (FCR), protein efisiensi rasio (PER), relative growth rate (RGR), survival rate (SR) followed by NRC (2011) and, respectively, were determined based on the following formula:

ADCp (%) = 100 - {100 x Cr2O3 in the feed /% Cr2O3 in the feces x % protein in the feces/ % protein in the feed}

EFU (%) = Final weight – Initial Weight/the weight of feed consumed x 100
RGR (%) =100 × (final weight - initial weight)/(times of experiment x initial weight)
FCR) = Feed intake (g)/body weight gain (g).
PER = 100 × (Final weight – Initial weight)/The amount of diet consumed x Protein content of diet
SR (%) = 100 (final count/initial count)

Statistics for Research Data

Data on parameter examined were analysed using analysis of variance (ANOVA) to determine the effect of treatment. If the ANOVA results had a significant effect ($p<0.05$) or a highly significant effect ($p<0.01$), there was a Duncan's multiple range test to determine the difference in mean values between treatments (Steel *et al*., 1997).

Result

The results of the study on protein digestibility (ADCp), protein efficiency ratio (PER), feed conversion ratio (FCR), efficiency of feed utilization (EFU), relative growth rate (RGR), and survival rate (SR) of tambaqui fingerlings were presented in Table 2.

Table 2. The mean values of Protein Digestibility (ADCp), Protein Efficiency Ratio (PER), Feed Conversion Ratio (FCR), Efficiency of Feed Utilization (EFU), Relative Growth Rate (RGR), and Survival (SR) of tambaqui fingerlings in the study.

Parameters	Treatments				
	A	B	C	D	E
ADCp (%)	52.32 ± 0.22^{e}	56.52 ± 0.29^{d}	60.46 ± 0.28^{c}	68.46 ± 0.28^{b}	75.24 ± 0.21^{a}
PER	1.51 ± 0.03^{e}	1.76 ± 0.07^{a}	$1.86\pm0,05^{c}$	$2.27\pm0,05^{b}$	3.37 ± 0.05^{a}

FCR	1.83±0.02[e]	1.76±0.04[d]	1.61±0.04[c]	1.53±0.04[b]	1.29±0.04[a]
EFU (%)	53.27±0.89[e]	60.83±0.26[d]	65.59±0.45[c]	74.59±0.45[b]	81.95±0.40[a]
RGR (%/hari)	3.69±0.42[e]	4.48±0.12[d]	5.74±0.19[c]	6.01±0.19[b]	6.85±0,06[a]
SR (%)	93.34±6.67[a]	88.89±6.18[a]	100±0.0[a]	100±0.0[a]	88.89±6.18[a]

Notes: Values with the same superscript in the column indicated no significant difference

Table 2 showed that supplementation of phytase enzyme in the diet resulted in a significant enhancement of ADCp, PER, EFU, ADCp and RGR, but had no effect on SR of tambaqui fingerlings. Tambaqui fingerlings fed phytase enzyme supplementation had higher values of ADCp, PER, EFU, ADCp, and RGR than those fed no supplementation. The addition of phytase enzyme at 1000 FTU/kg feed is the best dose for tambaqui fingerlings because it resulted in the highest ADCp, PER, FCR, EFU, ADCp and RGR values compared to other treatments.

The effect of phytase enzyme supplementation in the diet on the carcass chemical composition of tambaqui fingerling is presented in Table 3. Crude protein content and ash content of tambaqui fish fingerlings carcass were higher in the diet that contained phytase enzyme than without enzyme. There was no significant difference in crude lipid and dry matter content in each treatment.

Table 3. The body chemical composition (%) of tambaqui fingerling

Treatments	Dry matter	Protein	Lipid	Ash
A	25.28±0.57[a]	52.36±0.27[b]	24.60±0.20[a]	18.17±0.14[b]
B	25.67±0.38[a]	55.29±0.43[a]	24.68±0.29[a]	19.43±0.32[a]
C	25.54±0.25[a]	55.48±0.29[a]	24.83±0.22[a]	19.51±0.10[a]
D	25.73±0.40[a]	55.19±0.42[a]	24.47±0.35[a]	19.82±0.28[a]
E	25.73±0.25[a]	56.38±0.39[a]	24.22±0.56[a]	19.62±0.32[a]

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

Discussion

In accordance with Table 2, the study's result showed that administering supplements with phytase enzymes to the diet had a significant ($P<0.05$) influence on ADCp, PER, FCR, EFU and RGR, but had no significant effect ($P>0.05$) on the survival of tambaqui. Phytase enzyme

supplementation has a beneficial role in the growth performance of tambaqui compared to diets without supplementation. This indicated that the addition of the phytase enzyme in the diet was able to hydrolyze the phytate complex compound of protein into digestible amino acids for fish growth (Haghbayan and Mehrgan, 2015). Similar research results were reported by Hussain *et al*. (2011), Shahzad *et al*. (2022), Salem *et al*. (2022), Rachmawati *et al*. (2023a,b).

Phytase enzyme supplementation of 1000 FTU/kg feed (treatment E) had the highest ADCp, PER, EFU, RGR, and lowest FCR compared to the other treatments. Phytase enzyme 1000 FTU/kg feed is assumed to be effective in hydrolysing anti-nutritional factors (phytate acid) in the diet (Cao *et al.,* 2007). The same research results were also reported by Salem *et al*. (2022) on *Sparus aurata*, Hussain *et al*. (2011) on *Labeo rohita*, Shahzad *et al*. (2022) on *O. niloticus*, Shahzad *et al.* (2021) on *C. carpio*. Tambaqui fed with phytase enzyme supplementation of 1000 FTU/kg feed had the highest increase in protein digestibility (ADCp) followed by the highest efficiency of feed utilization (EFU) and growth (RGR) values when compared to other treatment diets. Dietary of Phytase enzyme enhances protein digestibility, straightforwardly related to increased efficiency of feed utilization and growth in fish (Biswas *et al*., 2019). Similar research results were reported by Rachmawati *et al*. (2023b), Shahzad *et al*. (2022) and Salem *et al*. (2022).

Treatment E had the highest efficiency of feed utilisation value at 81.95 ± 0.40%, whereas treatment A had the lowest value at 53.27 ± 0.89% as well (Table 2). These results indicate that phytase, which has the highest protein digestibility value, may be able to improve efficiency of feed utilization when administered to the diet at a dose of 1000 FTU/kg feed. The enhanced efficiency of feed utilization is assumed to result from the protein's conversion into amino acids, which are required for fish growth. As a result, the fish grow more efficiently, decrease the feed conversion ratio, and increase the protein efficiency ratio in order to maximise growth. Studies on the addition of phytase enzymes to other spesies also validated the results of this study, whereas phytase enzymes in feed increased efficiency of feed utilization in *Procambarus clarkii* (Yang *et al*., 2022), *T. blochii* (Siburian *et al*., 2019), and *Pangasius hypothalamus* (Rachmawati *et al.*, 2018), *C. gariepinus* (Rachmawati *et al*., 2023a) and *C. carpio* (Rachmawati *et al*., 2023b).

Feed conversion ratio (FCR) represents the amount of feed consumed by the fish to convert 1 kg of biomass. Based on the observations in Table 3, the addition of phytase enzyme as much

as 1000 FTU/kg feed decreased the FCR because it was assumed that the absorption rate of feed nutrients was maximised, the digestibility value of protein increased and the efficiency of feed utilisation enhanced. The process of phytate acid hydrolysis by the phytase enzyme will release minerals and other nutrients which are bound in phytate acid, so that these nutrients become available and can be utilised by the fish. As a result, the absorption of important macro and micro nutrients is increased. This has a positive effect on improved fish growth, increased feed utilisation efficiency, and reduced feed quantity requirements to achieve optimal biomass. The decreased FCR value with the addition of phytase enzyme in the diet were also reported by Dian and Ester (2023) on *Dicentrarchus labrax*, *C. gariepinus* (Rachmawati *et al*., 2023a) and *C. carpio* (Rachmawati *et al*., 2023b), Karabulut *et al*. (2021) on *Acipenser baerii*, and Rachmawati and Istiyanto (2018) on *P. hypophtalmus*.

The highest relative growth rate (RGR) value was obtained in treatment E (dose of phytase enzyme 1000 FTU/kg feed) at 6.85 ± 0.06%/day and the lowest average value was found in treatment A at 3.69 ± 0.42%/day. These results indicate that the supplementation of phytase enzyme dose of 1000 FTU/kg feed was supposed to increase the value of protein digestibility, feed utilisation efficiency and protein utilisation ratio so that the relative growth rate increased.

The results showed that the addition of enzymes in the feed diet had no significant effect ($P>0.05$) on the survival rate of tambaqui fingerlings, this was thought to be caused by the reason that there was no direct effect of the addition of phytase enzyme in the feed on the survival rate of tambaqui fingerlings during the rearing period. The highest mean survival rate was reported in treatment B at 100±0.00% and the lowest mean value was obtained in treatment D at 88.89±10.18%. The mortality of the test fish during the study was suspected to be due to fish stress during sampling handling. Fish that are stressed frequently show lethargy, stay at the bottom of the tank or surface often, have damaged or shrinkage fins, and have mucous skin (Akram *et al.*, 2021). Both of internal and external factors affected the survival of fish during rearing. Fish health, age, genetics, and adaptation are examples of internal influences. The management of aquaculture, environmental factors, competition and predators, and water quality are the other factors.

The addition of phytase enzyme in the diet affected the carcass protein content of tambaqui fingerlings. Based on Table 3, it is known that the carcass of tambaqui fingerlings fed with phytase enzyme supplementation had a higher body protein content than without the addition.

This might be due to the ability of the phytase enzyme to enhance protein bioavailability and neutralise the negative influence of phytate on proteins by hydrolyzing the phytate-protein complex in the fish intestine (Liebert and Portz, 2005).

Based on the results in Table 2 and Table 3, the carcasses of tambaqui fish fingerlings fed with phytase enzyme supplementation not only increased the protein content of the fish body but also increased the PER. According to the results, test diet E, which was fed to tambaqui fingerling supplemented with phytase enzyme at a concentration of 1000 FTU/kg feed, provided the most significant enhancement of feed utilisation efficiency and the highest increase in protein digestibility compared to the other treatments. Phytase enzymes enhance efficiency of feed utilisation in fish and increase the digestibility of protein. (Biswas *et al*., 2019). Similarly studies have been done on Sparus aurata (Salem et al., 2022) and Marsupenaeus japonicas (Bulbul et al., 2015). The tambaqui fingerling carcasses fed with phytase enzyme supplementation had a higher ash content than the carcasses without supplementation. Bulbul *et al.* (2015) stated that the addition of phytase enzyme in feed increased the content of bone ash and phosphorus in Marsupenaeus japonicas. Liebert and Portz. (2005) also reported that the ash content of scales and vertebrae in *O. niloticus* increased significantly after being fed a diet containing the enzyme phytase.

Conclusion

The conclusion of the study was that phytase enzyme addition in diet had a significant effect ($P<0.05$) on protein digestibility, efficiency of feed utilization and growth of tambaqui (C. *macropomum*). Protein digestibility, feed utilisation efficiency and growth of tambaqui (C. *macropomum*) fed with phytase enzyme supplementation significantly increased compared to diet without supplementation. Phytase enzyme supplementation of 1000 FTU/kg feed was the best dose for tambaqui fingerling.

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