



The effect of phytase enzyme on protein digestibility, feed utilization efficiency, growth, and body chemical composition of Java barb (*Barbonymus gonionotus*) juvenile stage

Diana Rachmawati, Sarjito, Rosa Amalia

Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Diponegoro, Semarang, 50275, Central Java, Indonesia. Corresponding author: D. Rachmawati, dianarachmawati1964@gmail.com

Abstract. Soybean meal contains antinutritional factors, such as phytic acid, which can interfere with protein absorption and digestion in feed, resulting in decreased feed nutritional quality. Supplementation of feed with phytase enzyme can hydrolyze phytic acid complexes that bind proteins, leading to increased protein digestibility, improved feed utilization efficiency, and better fish growth. The aim of this study was to examine the effect of phytase enzyme supplementation on protein digestibility, feed utilization efficiency, growth, and body chemical composition of the Java barb (*Barbonymus gonionotus*) juvenile stage. The test fish used were Java barbs with an average weight of 125.93 ± 0.26 g fish⁻¹. The experimental feed used was artificial feed in the form of floating pellets supplemented with different doses of phytase enzyme: A (0 FTU/kg feed), B (250 FTU/kg feed), C (500 FTU/kg feed), D (750 FTU/kg feed), E (1000 FTU/kg feed), and F (1250 FTU/kg feed). The observed parameters included apparent digestibility coefficient of protein (ADC_p), feed utilization efficiency (FEU), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), survival rate (SR), and body chemical composition of the Java barb juvenile stage. The results showed that supplementation of phytase enzyme in feed significantly increased protein digestibility, feed utilization efficiency, growth, and body chemical composition of the Java barb juvenile stage. A phytase enzyme dose of 1000 FTU/kg feed was found to be the best dose for the juvenile stage of Java barb.

Key Words: dose, feed, ingredient, parameters, supplement.

Introduction. The Java barb (*Barbonymus gonionotus*) is one of Indonesia's native fish species that is easy to cultivate, has high economic value, and has significant potential for aquaculture activities (Rachmawati et al 2025). The success of intensive Java barb farming is highly dependent on artificial feed availability. Artificial feed uses up to 50% fish meal as a source of animal protein, making it the main and most expensive protein ingredient (Kumar et al 2012). Fish meal is used as an animal protein source in feed because it provides protein and essential amino acids, essential fatty acids, cholesterol, vitamins, and mineral attractants that are crucial for fish growth (NRC 2011). As aquaculture activities increase, so does the demand for fish meal over time, leading to limited fish meal availability. This raises concerns regarding escalating overfishing, which may jeopardize the sustainability of natural ecosystems (Adeshina et al 2023). The limited supply of fish meal increases the price of fish feed, causing feed costs to rise to approximately 60% of the total production cost per cultivation cycle, which has become a major problem for fish farmers (Kemigabo et al 2018). Therefore, it is necessary to seek plant-based ingredients as alternative protein sources with the potential to replace fish meal, in an effort to develop sustainable and environmentally friendly aquaculture (Hussain et al 2020). One plant-based ingredient that is available and relatively inexpensive as a protein source is soybean meal, owing to its availability, affordability, and balanced essential amino acid profile (Biswas et al 2000). However, the presence of anti-nutritional factors, such as phytic acid, limits the inclusion of soybean meal in fish feed (Bulbul et al 2015). Phytic acid can form phytate-protein and phytate-mineral-

protein complexes, which create problems in protein digestibility, thereby lowering the digestibility and utilization of protein from the feed (Cao et al 2007). Phytate-protein complexes cannot be digested by proteolytic enzymes (Shahzad et al 2022). In fact, the activities of several enzymes, such as pepsin, amylase, and lipase, are inhibited by phytic acid (Cao et al 2007).

Phytic acid also chelates other minerals, such as calcium, magnesium, zinc, iron, and copper, forming insoluble complexes that reduce the absorption and bioavailability of macro and micro minerals required by fish (Papatryphon et al 1999). The presence of phytic acid in the feed lowers the efficiency of feed utilization during aquaculture, resulting in suboptimal fish growth. An effort to address the issue of phytic acid in plant-based protein feed ingredients is the addition of phytase enzyme (Shahzad et al 2021; Salem et al 2022; Shahzad et al 2022; Rachmawati et al 2023b, 2024).

The application of phytase enzymes in feed can hydrolyze phytate, sequentially producing myo-inositol penta-, tetra-, tri-, di-, and mono-phosphate, and neutralize the negative effects of phytic acid on protein and other nutrients in the feed. In addition, the presence of phytase enzyme in feed can increase the bioavailability of phosphorus and nitrogen bound to phytate, thus reducing the release of P and N into aquatic environments (Cao et al 2007). Several studies on phytase enzyme supplementation in feed have reported improvements in growth, feed conversion ratio, and protein and mineral digestibility in *Ephinephelus fuscoguttatus* (Shapawi et al 2013), *Marsupenaeus japonicus* (Bulbul et al 2015), *Psetta maxima* (Von Danwitz et al 2016), *Cirrhinus mrigala* (Hussain et al 2020), *Sparus aurata* (Salem et al 2022), *Oreochromis niloticus* (Shahzad et al 2022; Rachmawati et al 2024), *Cyprinus carpio* (Rachmawati et al 2023b), and *Clarias gariepinus* (Rachmawati et al 2023a). Information regarding phytase enzyme supplementation in feed for the grow-out stage of Java barbs is still limited, highlighting the need for this research. The aim of this study was to assess the effect of phytase enzyme in feed on protein digestibility, feed utilization efficiency, growth, and body chemical composition at the Java barb juvenile stage.

Material and Method

Preparation of test fish and research design. This study was conducted in May until July 2025 at the Freshwater Fish Hatchery (BBIAT) in Muntilan, Central Java, Indonesia, using an experimental method with a completely randomized design, consisting of five treatments and four replications. The test fish used were Java barb juveniles with an average initial weight of 125.93 ± 0.26 g fish⁻¹. Preparation of the test fish began by selecting Java barb juveniles that were uniform in size, actively swimming, not deformed, and healthy (Rachmawati et al 2024). This was intended to ensure that the Java barb juveniles used as test subjects would have a uniform size at the start of rearing and be able to grow normally without any disease disturbances. Subsequently, the test fish were acclimated to the new environment and artificial feed for 7 days (Rachmawati et al 2023a). During the acclimation period, the test fish were fed an artificial feed without phytase. The experiment began by placing the test fish, whose average initial weight was already known, into fiber tanks with dimensions of 1 x 1 x 1 m³ filled with 30 liters of water and a stocking density of 1 fish L⁻¹. Feeding was carried out using the ad satiation method, with a frequency of three times daily at 8:00, 13:00, and 18:00. Sampling to measure weight gain of the test fish was conducted weekly throughout the 56 day study period. The water quality parameters observed refer to Boyd (2003), the parameters observed include pH, dissolved oxygen (DO), and temperature using the High Quality AZ8603 water quality tester, China, and ammonia using Hanna HI715, Indonesia.

Preparation of test feed. The test feed used was artificial feed in the form of floating pellets with a protein content of 30% (Rachmawati et al 2025), with the addition of phytase enzyme according to the treatment (A - 0 FTU/kg feed, B - 250 FTU/kg feed, C - 500 FTU/kg feed, D - 750 FTU/kg feed, E - 1000 FTU/kg feed, and F - 1250 FTU/kg feed) and 1% chromic oxide (Cr₂O₃) as the protein digestibility indicator (NRC 2011). Three

replicates were used for each treatment. The phytase enzyme used was Nathupos*E 10000 G, produced by BASF SE (Ludwigshafen, Germany). The test feed was prepared by weighing the raw feed ingredients according to the feed formulation (Table 1), followed by mixing the feed ingredients starting from the smallest quantity and gradually adding and mixing other ingredients in larger quantities, except for the fat source and water, which were added after all other ingredients were mixed (NRC 2011). The feed dough was mixed until homogeneous using a livestock feed mixer machine (Model Horizontal Mixer, China) until uniform. Finally, fish oil, corn oil, and sufficient water were added, and the dough was mixed again until evenly blended. The homogeneous feed dough was then placed in a floating pellet extruder machine (H 2700, China). After extrusion, the feed was dried by air at room temperature, approximately 26°C. Once dry, the feed was packed in an airtight plastic and stored until use.

Table 1
Formulation of test feed used in the study (1000 g) and proximate composition analysis results

<i>Ingredient (g)</i>	<i>Test feed</i>					
	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>	<i>F</i>
Fish meal	369.65	369.65	369.65	369.65	369.65	369.65
Soybean meal	427.30	427.30	427.30	427.30	427.30	427.30
Corn flour	62.72	62.72	62.72	62.72	62.72	62.72
Bran	70.62	70.62	70.62	70.62	70.62	70.62
Tapioca flour	29.71	29.71	29.71	29.71	29.71	29.71
Fish oil	10.00	10.00	10.00	10.00	10.00	10.00
Corn oil	10.00	10.00	10.00	10.00	10.00	10.00
Mineral & vitamin mix ¹	10.00	10.00	10.00	10.00	10.00	10.00
Cr ₂ O ₃	10.00	10.00	10.00	10.00	10.00	10.00
Total	1000	1000	1000	1000	1000	1000
Phytase levels (FTU/kg diet)	0	250	500	750	1000	1250
<i>Proximate composition</i>						
Dry matter*	31.18	31.18	31.22	31.17	31.18	31.24
Crude protein*	30.15	30.11	30.16	30.17	30.17	30.18
Ether extract*	9.45	9.46	9.43	9.44	9.45	9.43
Carbohydrate*	251.43	251.53	250.61	251.37	251.15	251.21
Ash*	8.43	8.39	8.44	8.42	8.41	8.44
Crude fiber*	120.3	120.2	120.4	120.4	120.2	120.2
Analysed phytase (FTU/kg diet)*	0	245	496	747	996	1242
Energy (kcal)*	251.43	251.53	251.61	251.37	251.15	251.21
Ratio of energy/protein (E/P) (cal g ⁻¹) ²	8.34	8.35	8.34	8.33	8.32	8.32

Notes: 1) Vitamin and Mineral mix kg⁻¹: magnesium (Mg) 1.900 mg, Vit. B2 97 mg, Vit. B6 46 mg, potassium (K) 150 mg, calcium (Ca) 219 mg, sodium (Na) 117 mg, selenium (Se) 150 mg, Vit. B1 52 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, copper (Cu) 9 mg, iron (Fe) 90 mg, Vit. C (coated): 68,800 mg activity, zinc (Zn) 90 mg, panthothenic acid: 93 mg, Vit. K3 19 mg, niacin 130 mg, folic acid 10 mg, inositol 225 mg, biotin 450 mg, Vit. E 187 mg; 2) According to NRC (2011) the E/P value for optimal fish growth ranges between 8 and 12 kcal g⁻¹; *)Proximate analysis results of the Animal Feed Science Laboratory, Faculty of Animal Husbandry and Agriculture, Diponegoro University (2025).

Research container. Fifteen fiber tanks measuring 1 x 1 x 1 m³ were used as research containers. Each fiber tank was equipped with a recirculation system aimed at stabilizing the water quality within the optimal range. In this study, the rearing medium used was water sourced from a water supply that had been previously settled in a reservoir to precipitate organic materials.

Protein digestibility analysis. Protein digestibility was determined using an indirect method by adding 1% Cr₂O₃ as an indicator of protein digestibility in the test feed, according to the method described by Pérez-Jiménez et al (2009).

Proximate composition analysis of test feed and fish carcasses. The proximate composition analysis was based on the NRC (2011) method. The protein content was determined using a semi-automatic Kjeldahl system (FOSS Kjeltex 2300). The fat content was determined using the ether extraction method based on the Soxhlet method (FOSS Soxtec 2043). Ash content was determined by incinerating feed and fish test samples in a furnace at 550°C for 24 h.

Observed parameters. The observed parameters consisted of apparent digestibility coefficient of protein (ADC_p) referring 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) referring to NRC (2011), each calculated based on the following formulas:

$$\text{ADC}_p (\%) = 100 - \left\{ 100 \times \frac{\text{Cr}_2\text{O}_3 \text{ in the feed}}{\% \text{ Cr}_2\text{O}_3 \text{ in the feces}} \times \frac{\% \text{ protein in the feces}}{\% \text{ protein in the feed}} \right\}$$

$$\text{FUE} (\%) = \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{weight of feed consumed (g)}} \times 100$$

$$\text{FCR} = \frac{\text{Feed intake (g)}}{\text{body weight gain (g)}}$$

$$\text{PER} = 100 \times \frac{\text{final weight (g)} - \text{initial weight (g)}}{\text{amount of diet consumed (g)} \times \text{diet protein content (\%)}}$$

$$\text{RGR} (\% \text{ day}^{-1}) = 100 \times \frac{\text{final weight (g)} - \text{initial weight (g)}}{(\text{times of experiments (day)} \times \text{initial weight (g)})}$$

$$\text{SR} (\%) = 100 \times \frac{\text{final fish count}}{\text{initial fish count}}$$

Statistical analysis. Data on ADC_p, FUE, FCR, PER, RGR, and SR were analyzed by one-way ANOVA. If the ANOVA results showed a significant effect ($p < 0.05$) or a highly significant effect ($p < 0.01$), Duncan's multiple range test was conducted to determine the differences in mean values among treatments (Steel et al 1997). All data were analyzed using SAS (V9.3. SAS Institute, Cary, NC, USA).

Results. The results of observations on growth and feed efficiency, which included ADC_p, FUE, PER, FCR, RGR, and SR of the Java barb juveniles during the study are shown in Table 2.

The results of the research in Table 2 show that the Java barb juveniles fed test diets supplemented with phytase enzyme had higher ADC_p, FUE, PER, and RGR values, and lower FCR compared to fish fed diets without phytase enzyme supplementation. The juvenile Java barbs fed the test diet E had higher ADC_p, FUE, PER, and RGR values, and lower FCR compared to the other test diets.

The effect of phytase enzyme supplementation in the feed on the chemical composition of the Java barb juveniles during the study is shown in Table 3, which shows that the Java barb juveniles fed with phytase enzyme supplementation had higher crude protein and ash content than those without phytase enzyme supplementation. Furthermore, there were no significant differences in crude fat and dry matter between the different test feed treatments during the of Java barb juveniles.

Table 2

Average initial fish weight (IW), final fish weight (FW), weight gain (WG), 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 Java barb juveniles during the study

Parameters	Test feed					
	A	B	C	D	E	F
IW (g)	125.42±0.26	125.63±0.24	126.34±0.24	126.42±0.26	125.46±0.28	126.32±0.26
FW (g)	152.30±0.20	168.20±0.22	174.58±0.20	190.10±0.22	200.32±0.22	182.18±0.20
WG (g)	26.88±0.23 ^f	42.57±0.23 ^e	48.24±0.22 ^d	63.68±0.24 ^b	74.86±0.25 ^a	55.86±0.23 ^c
ADCp (%)	52.32±0.21 ^f	65.18±0.14 ^e	69.46±0.12 ^d	74.27±0.22 ^b	82.74±0.22 ^a	72.25±0.25 ^c
FUE (%)	53.38±0.12 ^f	66.40±0.10 ^e	70.58±0.13 ^a	75.17±0.12 ^b	80.14±0.12 ^a	72.25±0.15 ^c
PER	1.36±0.10 ^f	1.64±0.05 ^e	1.73±0.09 ^d	2.27±0.10 ^b	2.52±0.04 ^a	2.05±0.10 ^c
FCR	1.76±0.20 ^f	1.48±0.26 ^b	1.33±0.02 ^a	1.27±0.04 ^b	1.17±0.03 ^a	1.25±0.03 ^c
RGR (% day ⁻¹)	2.52±0.12 ^f	2.86±0.17 ^e	3.14±0.13 ^d	3.82±0.12 ^b	4.36±0.10 ^a	3.48±0.12 ^c
SR (%)	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a	100±0.0 ^a

Note: Means in the same column with different superscripts are significantly different ($p < 0.05$).

Table 3

Chemical composition of the Java barb juveniles with varying doses of phytase enzyme supplementation during the study

Parameters	Test feed					
	A	B	C	D	E	F
Dry matter	26.98±0.34 ^b	24.12±0.32 ^a	24.22±0.30 ^a	24.20±0.25 ^a	24.32±0.22 ^a	24.30±0.26 ^a
Protein	55.14±0.17 ^b	57.42±0.13 ^a	57.53±0.16 ^a	57.48±0.18 ^a	57.53±0.12 ^a	57.62±0.16 ^a
Lipid	25.36±0.14 ^a	25.49±0.12 ^a	25.45±0.10 ^a	25.47±0.13 ^a	25.68±0.18 ^a	25.59±0.16 ^a
Ash	19.14±0.20 ^b	20.65±0.32 ^a	20.58±0.34 ^a	20.68±0.42 ^a	20.59±0.28 ^a	20.62±0.23 ^a

Note: Means in the same column with different superscripts are significantly different ($p < 0.05$).

Discussion. Phytic acids in feed cannot selectively bind with amino acids and reduce amino acid availability (Kumar et al 2012). In addition, phytic acid has been shown to inhibit the activity of enzymes, including trypsin and pepsin (Morales et al 2011). Supplementation of feed with phytase enzyme can increase the availability of protein and amino acids by breaking down the phytin-protein complex and neutralizing the negative effects of phytate on protein and other nutrients in the feed (Hussain et al 2017). This is because the phytic acid present in the feed has already been hydrolyzed by the phytase enzyme, thereby releasing the protein from the phytate complex and increasing feed protein digestibility. Xu et al (2022) stated that the addition of phytase enzyme can enhance the conversion of trypsinogen to trypsin enzyme, which breaks proteins into amino acids, thus increasing digestive enzyme activity and improving protein digestibility. The addition of phytase enzyme to feed can enhance protein digestibility, feed efficiency (Hussain et al 2020; Rachmawati et al 2023a) as well and fish growth (Adeshina et al 2023; Rachmawati et al 2024).

The results presented in Table 2 show that supplementation with phytase enzyme at a dose of 1000 FTU/kg feed yielded the highest protein digestibility value in the Java barb juveniles. This is presumed to be because the phytase enzyme in the feed at a dose of 1000 FTU/kg feed is the optimal dosage for hydrolyzing phytic acid in the feed, thereby increasing protein digestibility. The addition of phytase enzyme to feed can hydrolyze the phytate-protein complex compounds into amino acids that are easily digested for fish growth (Von Danwitz et al 2016). Norag et al (2018) reported that phytase supplementation assists in the hydrolysis of phytates, resulting in improved nutrient digestion in fish. Furthermore, Wang et al (2009) reported that breaking down phytate-protein complex bonds can increase trypsinogen and trypsin enzyme activities, which break down proteins into amino acids. Increased protein digestibility means greater absorption of amino acids, thus improving the overall protein digestion. Similar studies have been reported by Shahzad et al (2021) on *C. carpio*, Rachmawati et al (2024) on *O. niloticus*, Hussain et al (2017) on *Labeo rohita*, and Rachmawati et al (2023a) on *C. gariepinus*.

The Java barb juveniles fed with feed supplemented with phytase enzyme at a dose of 1000 FTU/kg (test feed E) had the highest FUE of $80.14 \pm 0.12\%$, and the lowest FUE was found in treatment A (0 FTU/kg feed) at 53.38 ± 0.12 . The highest FUE value in treatment E is suspected to be because the phytase enzyme dosage of 1000 FTU/kg feed is the optimal dose for hydrolyzing phytic acid, thereby allowing the protein to be efficiently utilized and digested by the fish, which increases the FUE. A high FUE value indicates that the fish can efficiently absorb protein to support its growth, rather than using it for metabolism, osmoregulation, and reproduction (Salem et al 2022). Furthermore, Kaiza et al (2023) stated that the addition of phytase enzyme could reduce the phytic acid content in feed ingredients, thus increasing the value of FUE. The FUE value is directly proportional to the fish growth rate (RGR) but inversely proportional to the FCR value. This can be observed in the FCR and RGR values (Table 2), which showed that treatment E had the lowest FCR value, while the RGR value was the highest compared to the other treatments. This is supported by Rachmawati et al (2023a), who reported that phytase enzyme supplementation in feed can result in the highest FUE and RGR, and lowest FCR values compared to other experimental feeds. Similar results were reported by Rachmawati et al (2024) for *O. niloticus*, Salem et al (2022) for *S. aurata*, and Rachmawati et al (2023b) for *C. carpio*.

The results of the study in Table 2 show that the Java barb juveniles fed with test feed E produced a higher PER value than other treatments. This is presumed to be because the phytase enzyme dosage used was appropriate and thus able to optimally hydrolyze phytic acid, resulting in increased protein digestibility, which in turn improved the PER of the Java barb juveniles. Nie et al (2017) stated that the addition of phytase enzyme in feed can increase the availability of protein, thereby improving the fish PER. Norag et al (2018) reported that phytase enzyme in feed can break down and separate the bonds between phytic acid and protein into their constituent amino acids, so that the protein is easily absorbed, increasing the protein efficiency ratio of the fish body. Furthermore, Rachmawati et al (2024) suggested that supplementation of feed with

phytase enzyme can hydrolyze complex phytate-protein compounds into amino acids that are easily digested for fish growth, thus, PER values increase. Similar results were reported by Rachmawati et al (2023a, b, 2024) and Xu et al (2022).

The highest RGR was obtained in the Java barb juveniles fed with feed supplemented with phytase enzyme at a dose of 1000 FTU/kg feed (test feed E) at $4.36 \pm 0.10\%$ day⁻¹, followed by a dose of 750 FTU/kg feed (test feed D) at $3.82 \pm 0.12\%$ day⁻¹, a dose of 1250 FTU/kg feed (test feed F) at $3.48 \pm 0.12\%$ day⁻¹, a dose of 500 FTU/kg feed (test feed C) at $3.14 \pm 0.13\%$ day⁻¹, a dose of 250 FTU/kg feed (test feed B) at $2.86 \pm 0.17\%$ day⁻¹, and a dose of 0 FTU/kg feed (test feed A) at $2.52 \pm 0.12\%$ day⁻¹. The highest RGR in the Java barb juveniles fed with feed supplemented with phytase enzyme at a dose of 1000 FTU/kg feed is thought to be due to this dose being the most optimal for helping the Java barb juveniles increase FUE, PER, and reduced FCR, thus improving their growth rate. The addition of phytase enzyme to feed can hydrolyze complex phytate-protein compounds into amino acids that are easily digested, thereby increasing FEU, fish growth and decreasing FCR (Nie et al 2017; Salem et al 2022; Shahzad et al 2022; Rachmawati et al 2024).

The results of the analysis of variance (Table 2) showed that supplementation of phytase enzyme in feed with different doses did not have a significant effect ($p > 0.05$) on the SR of the Java barb juveniles. This is thought to be due to the absence of a direct effect of phytase enzyme supplementation on the SR in the Java barb juveniles during the study. Yakuputiyage (2011) reported that feed did not affect the SR, because the SR was influenced by the initial treatment of the fish and water quality. Hepher (1988) stated that the SR of fish can be influenced by internal and external factors. Internal factors concern the fish health, age, genetics, and adaptability to the environment, while external factors concern the environmental conditions, such as cultivation management, environmental factors, competition and predation, and water quality. Aquaculture environment is one of the most important factors to consider because it can affect the SR of fish (Salem et al 2022).

Table 3 shows that phytase enzyme supplementation in the feed affects body protein content and ash levels, but does not affect fat content and dry matter in the Java barb juveniles. The increase in body protein content of the Java barb juveniles in this study was supported by an increase in PER in these fish (Table 2). In addition, the Java barb juveniles fed with feed supplemented with phytase enzyme at a dose of 1000 FTU/kg feed (test feed E) also had the highest protein digestibility, followed by the highest FUE compared to the other test feeds. Biswas et al (2019) stated that supplementation of phytase enzyme in feed increases protein digestibility, which is directly proportional to the increase in the PER and FUE in fish. Similar results were reported by Hussain et al (2017) in *L. rohita*, Bulbul et al (2015) in *M. japonicus*, and Rachmawati et al (2023a) in *C. gariepinus*. The results of this study also show that phytase enzyme supplementation in feed can increase the ash content in the body of the Java barb juveniles. This is consistent with the statement of Bulbul et al (2015) that adding phytase enzyme to feed can increase the ash concentration in the body of *M. japonicus*. Furthermore, Liebert & Portz (2005) also reported that ash content in the scales and backbone of *O. niloticus* increased significantly when fed a diet containing the phytase enzyme.

Conclusions. The results of this study indicated that supplementation of phytase enzyme in the feed had a significant effect ($p < 0.05$) on protein digestibility, feed utilization efficiency, growth, and body chemical composition of Java barb (*B. gonionotus*) juveniles. A phytase enzyme dosage of 1000 FTU/kg feed is the optimal dosage for supplementing phytase enzyme in the feed of the Java barb juveniles, resulting in the highest values for ADCp ($82.74 \pm 0.22\%$), FUE ($80.14 \pm 0.12\%$), PER (2.52 ± 0.04), and RGR ($4.36 \pm 0.10\%$ /day). Supplementation of phytase enzyme in the feed affects body protein content and ash levels, but does not affect the fat content or dry matter of the Java barb juveniles.

Acknowledgements. The authors would like to express their deepest gratitude to the Dean of the Faculty of Fisheries and Marine Sciences, Diponegoro University, for the research funding support provided through the Research Grant Research Activity Implementation Task Letter funded by the Additional State Revenue and Expenditure Budget (APBN) of Diponegoro University for the 2025 Fiscal Year Number: 02/UN7.F10/PP/III/2025.

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

References

- Adeshina I., Akpoilih B. U., Tiamiyu L. O., Badmos A. A., Emikpe B. O., Abdel-Tawwab M., 2023 Effect of feed supplementation with microbial phytase on growth, bone mineral, antioxidant status, innate immunity and resistance to disease in African catfish fed high soybean meal-based feed. *Journal of Animal Physiology and Animal Nutrition* 107(2):733-745.
- Biswas A., Araki H., Sakata T., Nakamori T., Takii K., 2019 Optimum fish meal replacement by soy protein concentrate from soymilk and phytase supplementation in diet of red sea bream, *Pagrus major*. *Aquaculture* 506:51-59.
- Boyd C. E., 2003 Guidelines for aquaculture effluent management at the farm level. *Aquaculture* 226(1-4):101-112.
- Bulbul M., Kader M. A., Ambak M. A., Hossain M. S., Ishikawa M., Koshio S., 2015 Effects of crystalline amino acids, phytase and fish soluble supplements in improving nutritive values of high plant protein based diets for kuruma shrimp, *Marsupenaeus japonicus*. *Aquaculture* 438:98-104.
- Cao L., Wang W., Yang C., Yang Y., Diana J., Yakupitiyage A., Luo Z., Li D., 2007 Application of microbial phytase in fish feed. *Enzyme and Microbial Technology* 40(4):497-507.
- Hepher B., 1988 Nutrition of pond fishes. Cambridge University, Cambridge, 388 pp.
- Hussain S. M., Afzal M., Nasir S., Javid A., Azmat H., Makhdoom S. M., Shah S. Z. H., Hussain M., Mustafa I., Iqbal M., 2017 Role of phytase supplementation in improving nutrient digestibility and growth performance for *Labeo rohita* fingerlings fed on canola meal-based diet. *Journal of Applied Animal Research* 45(1):15-21.
- Hussain S. M., Ahmad N., Shahzad M. M., Javid A., Aslam N., Hussain M., Arsalan M. Z. H., Riaz D., 2020 Efficacy of phytase enzyme and citric acid on growth performance, nutrients and mineral digestibility of *Cirrhinus mrigala* fingerlings fed guar meal-based diet. *Iranian Journal of Fisheries Sciences* 19(3):1573-1588.
- Kaiza V. E., Yildiz M., Eldem V., Golzaradabi S., Ofori-Mensah S., 2023 The effects of dietary microbial 6-phytase on growth parameters, intestinal morphometric properties and selected intestinal genes expression in rainbow trout (*Oncorhynchus mykiss*, Walbaum 1876). *Journal of Animal Physiology and Animal Nutrition* 107(6): 1517-1529.
- Kemigabo C., Abdel-Tawwab M., Lazaro J. W., Sikawa D., Masembe C., Kang'Ombe J., 2018 Combined effect of dietary protein and phytase levels on growth performance, feed utilization, and nutrients digestibility of African catfish, *Clarias gariepinus* (B.), reared in earthen ponds. *Journal of Applied Aquaculture* 30(3):211-226.
- Kumar V., Sinha A. K., Makkar H. P. S., De Boeck G., Becker K., 2012 Phytate and phytase in fish nutrition. *Journal of Animal Physiology and Animal Nutrition* 96(3): 335-364.
- Libert F., Portz L., 2005 Nutrient utilization of Nile tilapia *Oreochromis niloticus* fed plant based low phosphorus diets supplemented with graded levels of different sources of microbial phytase. *Aquaculture* 248(1-4):111-119.
- Morales G. A., Moyano F. J., Marquez L., 2011 *In vitro* assessment of the effects of phytate and phytase on nitrogen and phosphorus bioaccessibility within fish digestive tract. *Animal Feed Science and Technology* 170:209-221.
- National Research Council (NRC), 2011 Nutrient requirements of fish and shrimp. The National Academies Press, Washington DC., 392 pp.

- Nie X. Z., Chen S., Zhang X. X., Dai B. Y., Qian L. C., 2017 Effects of neutral phytase on growth performance and phosphorus utilization in crucian carp (*Carassius auratus*). *Journal of Zhejiang University-SCIENCE B* 18(10):886-896.
- Norag M. A. A., El-Shenawy A. M., Fadl S. E., Abdo W. S., Gad D. M., Rashed M. A., Prince A. M., 2018 Effect of phytase enzyme on growth performance, serum biochemical alteration, immune response and gene expression in Nile tilapia. *Fish and Shellfish Immunology* 80:97-108.
- Papatryphon E., Howell R. A., Soares Jr. J. H., 1999 Growth and mineral absorption by striped bass *Morone saxatilis* fed a plant feedstuff based diet supplemented with phytase. *Journal of the World Aquaculture Society* 30(2):161-173.
- Pérez-Jiménez A., Cardenete G., Morales A. E., García-Alcázar A., Abellán E., Hidalgo M. C., 2009 Digestive enzymatic profile of *Dentex dentex* and response to different dietary formulations. *Comparative Biochemistry and Physiology. Part A: Molecular and Integrative Physiology* 154(1):157-164.
- Rachmawati D., Riyadi P. H., Samidjan I., Elfitasari T., Chilmawati D., Windarto S., Amalia R., Nurhayati D., Yuniarti T., Yunanto, 2023a Phytase enzyme improves growth performance and body chemical composition of Sangkuriang catfish (*Clarias gariepinus* var. Sangkuriang) juvenile. *Pertanika Journals: Tropical Agricultural Science* 46(2):721-734.
- Rachmawati D., Riyadi P. H., Samidjan I., Elfitasari T., Chilmawati D., Windarto S., Amalia R., Nurhayati D., Yuniarti T., Yunanto, 2023b Phytase enzyme ameliorates growth performance, mineral digestibility, amino acid digestibility and body chemical composition of the common carp (*Cyprinus carpio* L.) at rearing stage. *Egyptian Journal of Aquatic Biology and Fisheries* 27(4):1-14.
- Rachmawati D., Elfitasari T., Chilmawati D., Yuniarti T., 2024 The effect of phytase enzyme in feed on growth performance, protein digestibility, feed utilization efficiency, mineral content, and nutrient composition in Nile tilapia (*Oreochromis niloticus*) fingerlings. *AAFL Bioflux* 17(5):2096-2108.
- Rachmawati D., Samidjan I., Elfitasari T., 2025 Impact of methionine in the diet on digestive enzyme activity, protein digestibility, feed utilization efficiency, growth, and body nutrient composition in the seed stage of catfish (*Pangasianodon hypophthalmus*). *AAFL Bioflux* 18(2):971-981.
- Salem M. E. S., Sallam A. E., El-Feky M. M. M., Abdel-Ghani H. M., 2022 Effect of phytase supplementation on growth performance, body mineral composition, and effluent phosphorus content of the seabream (*Sparus aurata*). *Egyptian Journal of Aquatic Biology and Fisheries* 26(3):149-166.
- Shahzad M. M., Bashir S., Hussain S. M., Javid A., Hussain M., Ahmed N., Khan M. K. A., Furqan M., Liaquat T., Rafique T., Khalid F., 2021 Effectiveness of phytase pre-treatment on growth performance, nutrient digestibility and mineral status of common carp (*Cyprinus carpio*) juveniles fed Moringa by-product based diet. *Saudi Journal of Biological Sciences* 28(3):1944-1953.
- Shahzad M. M., Liaquat I., Hussain S. M., Hussain M., Hussain Z., Chaudhary A., Abdullah S., Shah S. Z. H., Ahmad N., Khan M. K. A., Bashir S., Rafique M. T., 2022 Effects of dietary phytase (PHY) levels on nutrient digestibility, mineral absorption and growth performance of *Oreochromis niloticus* fingerlings fed Moringa based diets. *Pakistan Journal of Agricultural Sciences* 59(2):269-278.
- Shapawi R., Ebi I., Yong A., 2013 Soybean meal as a source of protein in formulated diets for tiger grouper, *Epinephelus fuscoguttatus* juvenile. Part I: Effects on growth, survival, feed utilization and body compositions. *Agricultural Sciences* 4(7): 317-323.
- Steel R. G. D., Torrie J. H., Dickey D. A., 1997 Principles and procedures of statistics: a biometrical approach. 3rd edition. McGraw Hill, New York, 666 pp.
- Von Danwitz A., van Bussel C. G. J., Klatt S. F., Schulz C., 2016 Dietary phytase supplementation in rapeseed protein based diets influences growth performance, digestibility and nutrient utilization in turbot (*Psetta maxima*). *Aquaculture* 450: 405-411.

- Wang F., Yang Y. H., Han Z. Z., Dong H. W., Yang C. H., Zou Z. Y., 2009 Effects of phytase pretreatment of soybean meal and phytase-sprayed in diets on growth, apparent digestibility coefficient and nutrient excretion of rainbow trout (*Oncorhynchus mykiss* Walbaum). *Aquaculture International* 17:143-157.
- Xu S. D., Zheng X., Dong X. J., Ai Q. H., Mai K. S., 2022 Beneficial effects of phytase and/or protease on growth performance, digestive ability, immune response and muscle amino acid profile in low phosphorus and/or low fish meal gibel carp (*Carassius auratus gibelio*) diets. *Aquaculture* 555:738157.
- Yakuputiyage A., 2013 On-farm feeding and feed management strategies in tropical aquaculture. In: On-farm feeding and feed management in aquaculture. Hasan M. R., New M. B. (eds), *FAO Fisheries and Aquaculture Technical Paper No. 583*, FAO, Rome, pp. 361-376.

Received: 13 July 2025. Accepted: 10 August 2025. Published online: 23 September 2025.

Authors:

Diana Rachmawati, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Diponegoro, 50275 Semarang, Central Java, Indonesia, e-mail: dianarachmawati1964@gmail.com
Sarjito, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Diponegoro, 50275 Semarang, Central Java, Indonesia, e-mail: sarjito_msdp@yahoo.com
Rosa Amalia, Department of Aquaculture, Faculty of Fisheries and Marine Sciences, University of Diponegoro, 50275 Semarang, Central Java, Indonesia, e-mail: rosa.amalia7@gmail.com

This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

How to cite this article:

Rachmawati D., Sarjito, Amalia R., 2025 The effect of phytase enzyme on protein digestibility, feed utilization efficiency, growth, and body chemical composition of Java barb (*Barbonymus gonionotus*) juvenile stage. *AACL Bioflux* 18(5):2123-2132.