The effect of papaya (*Carica papaya*) leaf extract addition in feed on growth performance, feed efficiency, and body nutrient composition of Java barb (*Barbonymus gonionotus*) at the grow-out stage

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Abstract. This study aimed to examine the effect of papaya (*Carica papaya*) leaf extract in feed on the growth performance, feed efficiency, and body nutrient composition of Java barb (*Barbonymus gonionotus*) at the grow-out stage. The study used a completely randomized design with four treatments and three replicates. The test fish were Java barb at the grow-out stage with an average weight of 50.46±0.35 g fish^{-1}, obtained from the Fish Hatchery Center (BBI) Sawangan, Magelang, Central Java, Indonesia. The test feeds were artificial feeds supplemented with different doses of papaya leaf extract: A (0 g kg^{-1} feed), B (10 g kg^{-1} feed), C (20 g kg^{-1} feed), and D (30 g kg^{-1} feed). The research parameters included total feed intake (TFI), apparent digestibility coefficient for protein (ADCp), feed utilization efficiency (EFU), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), survival rate (SR), and body nutrient composition of Java barb. The results showed that the addition of papaya leaf extract to the feed had a significant effect ($p < 0.05$) on TFI, ADCp, EFU, FCR, PER, and RGR, but had no significant effect ($p > 0.05$) on the SR and body nutrient composition of Java barb. The addition of papaya leaf extract at a dose of 30 g kg^{-1} feed yielded the best results for improving the growth performance and feed efficiency of Java barb at the grow-out stage.
Keywords: antinutritional, aquaculture, enzyme, digestibility, papain.

Introduction. The Java barb (*Barbonymus gonionotus* (Bleeker, 1849)) is a freshwater fish species with high economic value and aquaculture potential. It has the advantage of not requiring land with specific criteria and the capability for year-round cultivation (Rachmawati et al., 2025). The success of fish farming depends greatly on the availability of sustainable and high-quality formulated feeds. Feed is the largest cost factor and is crucial for the success of aquaculture (NRC, 2011). Fishmeal remains an important ingredient in fish feed and is widely used in most aquaculture feeds. Along with the expansion of aquaculture, the demand for fishmeal has increased; however, as its supply from wild fisheries has not grown, this can lead to higher prices and increased pressure on marine ecosystems (Hassaan et al., 2021). Therefore, there is a clear gap between the shortfall in fishmeal production and the ever-growing needs of the aquaculture industry. In response to these challenges, there is growing interest in incorporating high-quality feed ingredients that can be efficiently utilized in fish diets to enhance growth performance and replace fishmeal, especially in developing countries that seek cost-effective aquaculture production (Hassaan et al., 2018). Recent studies have investigated the replacement of fish meal in fish feed with various plant-based protein sources; however, feeding fish diets high in plant protein, such as soybean meal, can negatively affect fish growth because these ingredients contain anti-nutritional compounds, such as trypsin inhibitors, phytic acid, and saponins, which interfere with feed intake and nutrient absorption (Hassaan et al., 2020). One solution to address this issue is the addition of exogenous enzymes to fish feed.

Exogenous enzymes in fish feed play a role in deactivating antinutritional compounds and releasing beneficial nutrients, thereby enabling a higher inclusion of plant-based proteins in the feed without causing negative effects (Fan et al., 2025). Papain is an exogenous enzyme derived from the papaya plant (*Carica papaya*). Papain is produced in various parts of the papaya plant. However, papaya leaves are an attractive source of papain because they have not been optimally utilized, making them readily available and potentially reducing production costs while adding value to local resources. Papaya leaves, especially young ones, are known to contain papain in high concentrations, with a proteolytic activity of 0.004±0.001 U, compared to 0.001±0.000 U in papaya peel (Khatun et al., 2023).

Papain is an exogenous proteolytic enzyme capable of hydrolyzing complex proteins, including plant proteins, into peptides and simple amino acids, thereby facilitating their absorption in the digestive tract of fish (Hamid et al., 2022). Papain offers advantages over other proteolytic enzymes, such as broad proteolytic activity within the pH range of 5-8. This range matches the conditions of the fish digestive tract, remains stable at the temperatures encountered during feed production, and its raw material is readily available owing to the abundance of papaya plants in Indonesia (Rachmawati et al., 2020). Thus, the use of papaya leaf extract not only increases the efficiency of protein utilization but also optimizes the use of plant-based ingredients as substitutes for fish meals. In addition to its impact on growth and feed efficiency, the improvement in protein digestibility resulting from papaya leaf extract supplementation has the potential to enhance fish overall health. Studies on the addition of various parts of papaya to fish feed have been conducted by several researchers on different species, as reported by Sundararajan et al. (2024) in *Labeo rohita*, Hamid et al. (2022) in *Oreochromis mossambicus* × *O. niloticus* hybrid, Afriani & Oktari (2024) in *Chanos chanos*, Oparaku et al. (2024) in *Clarias gariepinus*, Giri et al. (2026) in *Cyprinus carpio*, and Silaen et al. (2023) in *Cyprinus rubrofuscus*. This study aimed to examine the effect of papaya leaf extract in feed on the growth performance, feed efficiency, and nutrient composition of Java barb at the grow-out stage.

Material and Method

Fish research design and test fish preparation. This study was conducted from February to April 2026 at the Fish Hatchery Center (BBI) Sawangan, Magelang, Central Java, Indonesia. The study used an experimental method, a Completely Randomized Design (CRD), with four treatments and three replicates per treatment. The test fish were Java barb at the grow-out stage, with an average body weight of 50.46±0.35 g fish^{-1}, totaling 600 fish, obtained from the Fish Hatchery Center (BBI) Sawangan, Magelang, Central Java, Indonesia. The preparation of the test fish began with an adaptation process of the Java barb to the environment and feed for one week, during which the feed provided was an artificial feed that did not contain papaya leaf extract. Feed was provided ad satiation in the morning, afternoon, and evening daily. Before being used as test fish, Java barb were selected based on uniform size and weight, active swimming, health, and freedom from deformities (Rachmawati et al., 2023). The test fish were then fasted for one day before the experiment to clear any remaining feed from their digestive tracts.

Preparation of papaya leaf extract. Crude papaya leaf extract was prepared as described by Babalola et al. (2023). Young papaya leaves (50.0 g) were placed in 100 mL of cold 0.1 N phosphate buffer (pH 5.5) and divided into five containers for extraction. The samples were homogenized using a Waring blender (Thomas Scientific 3390D40). The homogenate was collected and filtered, and the residue was discarded afterward. A total of 500 mL of the resulting filtrate was centrifuged at 3,000 rpm for 10 min at 4°C using a refrigerated centrifuge (Lacter-21). The supernatant was discarded, and the obtained pellet was rinsed with 1 mL of cold 0.1 M phosphate buffer (pH 5.5). The crude extract was stored at 4°C for 10 min. The papain enzyme activity in the papaya leaf extract was 187.24 U mL^{-1} (Laboratory of Life Sciences, Engineering, and Technology, Airlangga University, Airlangga, 2026).

Preparation of the test feed. The test feed consisted of a formulated isonitrogenous feed (30%) and isocaloric (252.06 kcal g^{-1}) following Rachmawati et al. (2025), supplemented with Cr_2O_3 as a protein digestibility marker (NRC, 2011) and papaya leaf extract at doses according to the respective treatments, namely A (0 g kg^{-1} feed), B (10 g kg^{-1} feed), C (20 g kg^{-1} feed), and D (30 g kg^{-1} feed), modifying the methods described by Hamid et al. (2022). The feed formulation was composed based on the proximate analysis results of raw materials using Microsoft Excel. The test feed included fish meal as the animal protein source and soybean meal as the plant protein source. Corn flour and bran served as carbohydrate sources, while tapioca flour functioned as a binder. Fish oil was used as the source of both animal- and plant-based fats, and a vitamin-mineral mix supplement was added to meet the fish vitamin and mineral requirements. The preparation of the test feed began with raw material preparation. All raw materials were ground and sieved. The finely ground raw materials were weighed using a digital scale to ensure that their composition matched the predetermined formula (Table 1). The next stage was the gradual mixing of raw materials in a basin, starting from the lowest to the highest percentage. Once evenly mixed, water was added to the mixture at 200 mL kg^{-1} of feed ingredient and poured gradually while stirring with a mixer until a homogeneous consistency was achieved. The prepared dough is then placed into an extruder machine (LX 75, China) to produce artificial feed in pellet form with a diameter of 2 mm, molded into floating pellets, and followed by a drying process at room temperature (approximately 26°C). The feed was then packed in appropriate containers, labeled, and stored at –4°C until further use.

Table 1

The test feed formulations (1000 g) and proximate analysis results

Ingredient (g 1000 g^{-1})	*Treatment*			
	A	*B*	*C*	*D*
Fish meal	280	280	280	280
Soybean meal	270	270	270	270
Corn meal	179.5	169.5	159.5	149.5
Bran meal	180	180	180	180
Tapioca flour	60	60	60	60
Fish oil	10	10	10	10
Corn oil	10	10	10	10
Mix of vitamin-mineral [1)]	10	10	10	10
Cr_2O_3	0.5	0.5	0.5	0.5
Papaya leaf extract	0	10	20	30
Total	1000	1000	1000	1000
	Proximate analysis			
Protein (%)*	30.08	30.02	30.09	30.08
Fat (%)*	8.23	8.36	8.32	8.24
Ash (%)*	27.93	27.89	27.90	27.92
Fiber (%)*	4.25	4.30	4.28	4.27
Moisture (%)*	7.18	7.17	7.20	7.16
BETN (%)*	22.33	22.40	22.38	22.34
Energy (kkal g^{-1})[2)]	256.26	256.30	256.26	256.28
Energy/Protein Ratio[3)]	8.52	8.54	8.52	8.52

Note: [1)] Vitamin and Mineral mix kg^{-1}: vitamin D3 (8,00,000 IU); vitamin E (40,000 IU); vitamin K3 (1600 mg); folic acid (800 mg); biotin (100 mcg); vitamin A (4,000,000 IU); vitamin B1 (4000 mg); vitamin B2 (3000 mg); vitamin B6 (3800 mg); vitamin B12 (3 mg); coated vitamin C (60,000 mg); inositol (10,000 mg); nicotinic acid (18,000 mg); pantothenic acid (8000 mg); choline chloride (120,000 mg); lysine (10,000 mg); manganese (6000 mg); zinc (20,000 mg), iron (8000 mg); iodine (400 mg); selenium (40 mg); cobalt (150 mg); copper (800 mg); [2)] Calculated based on Digestible Energy values according to NRC, (2011): 3.5 kcal g^{-1} for protein, 8.1 kcal g^{-1} for fat, and 2.5 kcal g^{-1} for carbohydrate; [3)] According to NRC, (2011), the E/P ratio for optimal fish growth ranges from 8 to 12 kcal g^{-1}. *Proximate analysis results from the Animal Feed Science Laboratory, Faculty of Animal and Agricultural Sciences, Diponegoro University (2026).

Preparation of research containers. The containers used in this study were happas measuring 1 × 1 × 0.6 m^3, totaling 12 units consisting of four treatments with three replicates each. Before use, the happas were thoroughly inspected to ensure that there was no damage or holes that could allow fish to escape or predators to enter the traps. Preparation was carried out by washing the happas with clean water and soaking them in a potassium permanganate ($KMnO_4$) solution at 30 mg L^{-1} for 24 hours (Afriani & Oktari, 2024) to remove debris and kill attached pathogens, then rinsed with clean water and dried under sunlight until completely dry as a natural sterilization step to prevent contamination. The happas were installed in earthen ponds using bamboo supports at each corner, tied with ropes to prevent them from coming loose, and weighted with stones inside to ensure that the happas sank to the bottom of the pond. Laminated treatment labels were randomly attached to each rearing container to facilitate the identification of each experimental unit according to its treatment group. The happas were subsequently left for one day to allow the water quality in the earthen ponds to return to normal before use in the experiment.

Proximate analysis of test feeds and test fish. Proximate analyses of the test feed and fish were performed according to the AOAC, (2005). Crude protein was calculated by measuring the total nitrogen (using the Kjeldahl method) and multiplying it by a conversion factor. This is the primary determinant of livestock growth in the region. Crude fat was extracted using a non-polar solvent via the Soxhlet method. Crude fiber is used to measure complex carbohydrates (cellulose, hemicellulose, and lignin) that are difficult to digest and to determine the digestibility value of the feed. The ash content indicates the total minerals (inorganic), such as calcium and phosphorus, remaining after the material is burned at high temperatures (approximately 600°C). Moisture content measures the weight of water that evaporates from the feed. A high moisture content promotes mold growth. Nitrogen-Free Extract (NFE) is an easily digestible carbohydrate fraction (sugars and starch) calculated by subtracting the percentages of water, ash, protein, fat, and fiber from the total weight of the material.

Protein digestibility analysis*.* Protein digestibility was measured by collecting the feces of the test fish in the morning, afternoon, and evening throughout the study. The collected test fish feces were then filtered using plankton cloth nets mesh size 150-200 micron, and the filtered test fish feces were stored in a refrigerator at 4°C. In the next step, the test fish feces were dried in an oven (Gravity Oven) at 6°C for 24 h and analyzed. The protein and Cr_2O_3 contents in the fish feces were analyzed using an SSA spectrophotometer at 350 nm (Pérez-Jiménez et al., 2009).

Implementation of the research*.*The implementation phase of the research began by weighing and measuring the total length of the test fish using a digital scale and a millimeter block as baseline data before stocking the fish. The test fish were then stocked into individual hapas measuring 1 × 1 × 0.6 m³ at a stocking density of 30 fish L^{-1} (Rachmawati et al., 2025). The feed used was a formulated feed containing 30% protein with the addition of different doses of papaya leaf extract for each treatment. Feeding was carried out at satiation three times per day, namely at 08.00, 12.00, and 16.00 WIB. The test fish rearing period lasted 49 days. Throughout this period, growth sampling was conducted every seven days by randomly collecting weight and length data of the fish to determine the growth and survival rate of the test fish. The observed water quality parameters included ammonia content, determined using an ammonia meter (Hanna Instrument HI715, Indonesia), temperature (25-30°C), dissolved oxygen (DO) (≥ 3 mg L^{-1}), and pH (6.5-8.6), using a Water Quality Checker (AZ8603, China) according to the method of Boyd & Tucker (2012).

Observed parameters. The parameters observed during the study included weight gain (WG) according to NRC (2011), total feed intake (TFI) according to Pereira et al. (2007), apparent digestibility coefficient for protein (ADCp) according to Pérez-Jiménez et al (2009), feed utilization efficiency (FUE), feed conversion ratio (FCR), protein efficiency ratio (PER), relative growth rate (RGR), survival rate (SR) according to NRC, (2011), and proximate analysis to determine the nutritional composition of the test feed and test fish using the AOAC (2005) method. The formulas for these calculations are as follows :

$$WG\ (g) = Final\ weight\ (g) - Initial\ weight\ (g)$$

$$TFI\ (g) = Amount\ of\ feed\ on\ day\ 1 + Amount\ of\ feed\ on\ day\ 2 + \ldots + Amount\ of\ feed\ on\ day\ n$$

$$ADCp\ (\%) = 100 - \{100 \times Cr_2O_3\ in\ the\ feed\ /\%\ Cr_2O_3\ in\ the\ feces \times \%\ protein\ in\ the\ feces/\ \%\ protein\ in\ the\ feed\}$$

$$FUE\ (\%) = Final\ weight - Initial\ weight/the\ weight\ of\ feed\ consumed \times 100$$

$$FCR = Feed\ intake\ (g)/body\ weight\ gain\ (g)$$

$$PER = 100 \times (Final\ weight - Initial\ weight)/The\ amount\ of\ diet\ consumed \times Protein\ content\ of\ diet$$

$$RGR\ (\%) = 100 \times (final\ weight - initial\ weight)/(times\ of\ experiment \times initial\ weight)$$

$$SR\ (\%) = 100\ (final\ count/initial\ count)$$

Statistical analysis. Data on growth performance variables, feed efficiency, and body composition of tawes fish were first tested to determine their homogeneity, additivity, and normality before ANOVA was conducted. Analysis of variance (ANOVA) was used to analyze the effect of extract supplementation in feed on the observed variables. Duncan's multiple range test was used to identify specific differences between mean values (Steel et al., 1997).

Results. The WG, TFI, FCR, PER, FUE, ADCp, RGR, and SR of the Java barb during the study are shown in Table 2.

Table 2

Mean values of weigh gain (WG) total feed intake (TFI), feed conversion ratio (FCR), protein efficiency ratio (PER), feed utilization efficiency (FUE), apparent digestibility coefficient of protein (ADCp), relative growth rate (RGR), and survival rate (SR) of Java barb during the study

Parameters	*Treatment*			
	A	*B*	*C*	*D*
Initial weight (g)	50.46 ± 0.35^a	50.50 ± 0.22^a	50.44 ± 0.28^a	50.42 ± 0.32^a
Final weight (g)	126.15 ± 0.16^d	146.45 ± 0.25^c	161.40 ± 0.18^b	188.07 ± 0.20^a
Weight gain (g)	75.69 ± 0.26^d	95.95 ± 0.24^c	110.96 ± 0.23^b	137.65 ± 0.26^a
TFI (g)	366.45 ± 1.28^a	348.49 ± 1.32^b	323.45 ± 1.35^c	308.00 ± 1.20^d
FUE (%)	55.27 ± 0.32^d	69.58 ± 0.31^c	75.64 ± 0.23^b	80.24 ± 0.23^a
FCR	1.82 ± 0.12^d	1.74 ± 0.12^c	1.52 ± 0.11^b	1.26 ± 0.10^a
PER	1.64 ± 0.12^d	2.72 ± 0.10^c	3.05 ± 0.16^b	3.29 ± 0.18^a
ADCp (%)	52.37 ± 0.16^d	68.74 ± 0.27^c	76.53 ± 0.20^b	88.26 ± 0.26^a
RGR (% day^{-1})	2.50 ± 0.23^d	2.90 ± 0.21^c	3.19 ± 0.17^b	3.73 ± 0.23^a
SR (%)	100^a	100^a	100^a	100^a

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

Table 2 shows that the addition of papaya leaf extract to the feed significantly affected WG, TFI, PER, EFU, ADCp, RGR, and FCR but did not significantly affect the SR of the fish. The Java barb fed diets supplemented with papaya leaf extract exhibited higher PER, EFU, ADCp, and RGR values and lower FCR and TFI values than those without supplementation. The addition of 30 g kg^{-1} papaya leaf extract to the feed was the optimal dose for Java barb, as it resulted in the highest WG, PER, FUE, ADCp, and RGR values and the lowest FCR and TFI values compared to the other treatment.

The results of the proximate analysis to determine the nutritional composition of Java barb fish meat are shown in Table 3. The addition of papaya leaf extract to the feed did not significantly affect ($p > 0.05$) the moisture, protein, lipid, carbohydrate, and ash content of the fish.

Table 3

Nutritional composition of Java barb bodies after 49 days of feeding with test diets

Nutritional composition (%)	*Treatment*			
	A	*B*	*C*	*D*
Moisture	70.07±0.25[a]	70.08±0.22[a]	70.10±0.22[a]	70.08±0.28[a]
Protein	18.52±0.31[a]	18.54±0.34[a]	18.53±0.31[a]	18.52±0.32[a]
Lipid	13.48±0.23[a]	13.42±0.22[a]	13.46±0.20[a]	13.48±0.21[a]
Carbohydrate	0.58±0.24[a]	0.57±0.22[a]	0.58±0.23[a]	0.57±0.24[a]
Ash	2.56±0.30[a]	2.55±0.33[a]	2.56±0.32[a]	2.56±0.31[a]

Note: Mean values with different superscripts in the same row indicate significant differences ($p < 0.05$).

Discussion. The average WG results showed that the highest value was obtained in treatment D (137.65±0.26 g), followed by treatment C (110.96±0.23 g), treatment B (95.95±0.24 g), and the lowest WG value was obtained in treatment A (75.69±0.26 g). This indicates that the increase in fish WG is likely due to the main factor of providing high-quality feed and the correct dose of papaya leaf extract in the feed, as well as being supported by suitable water quality for the maintenance of Java barb. According to NRC (2011), the increase in WG is caused by external factors (environment and feed) and internal factors (genetics, health, and age of the fish).

The average TFI results showed that the highest value was obtained in treatment A at 366.45±1.28 g, followed by treatment B at 348.49±1.32 g, treatment C at 323.45±1.35 g, and the lowest TFI value was obtained in treatment D at 308.00±1.20 g. This indicates that the obtained TFI values are related to the palatability of the test feed. Palatability refers to the degree to which fish prefer the provided feed within a certain time frame and is influenced by the organoleptic characteristics of the feed, such as texture, color, and aroma. Observations during the study showed that the feed in treatment A without papaya leaf extract had better palatability than treatments B, C, and D, as reflected in the higher TFI value. The high palatability of feed A is thought to be due to the absence of secondary compounds originating from papaya leaf extract, resulting in a taste, texture, and aroma that are more familiar and readily accepted by fish. Conversely, the higher the dose of papaya leaf extract added to the feed in treatments B, C, and D, the greater the concentration of secondary compounds, such as alkaloids, tannins, and saponins, which are naturally present in the papaya leaves. These compounds are known to contribute to the distinctive bitter taste of the feed; therefore, at higher doses, fish tend to reduce their feed intake. This is supported by Hamid et al. (2022), who stated that papaya leaf extract contains polyphenols, saponins, flavonoids, and tannins that can affect palatability and nutrient absorption at high concentrations. These compounds generally have bitter and astringent taste characteristics that may influence fish feed preference.

Although the TFI value in treatment A was higher than that in the other treatments, this increase in feed intake was not accompanied by an improvement in FUE. This is possibly due to the absence of papain-containing papaya leaf extract in the feed for treatment A; therefore, the protein digestion process relied entirely on endogenous enzymes produced by the fish's digestive system. Without the support of exogenous papain, the ability of the fish digestive tract to break down complex proteins into forms that can be absorbed by the body becomes limited, thereby requiring the fish to consume more feed to meet its daily nutritional needs. This is consistent with the statement by Thaiso et al. (2024) that exogenous enzymes in feed can improve growth performance by enhancing feed utilization and nutrient digestibility, increasing digestive enzyme activity, and improving intestinal histological structure and health. The addition of papaya leaf extract to the feed significantly increased the final body weight of the fish compared to no addition, indicating that enzyme-supplemented feed was utilized more efficiently, thereby reducing the total feed consumption required to achieve equivalent growth (Wiszniewski et al., 2022).

The Java barb fed on diets supplemented with papaya leaf extract (B, C, and D) had higher FUE values than those fed diets without supplementation (A). This is likely due to the supplementation of papaya leaf extract, which contains papain that breaks down complex proteins into simple proteins, resulting in better nutrient absorption and increased feed utilization efficiency. Papain plays a role in hydrolyzes complex proteins, including plant proteins, into peptides and simple amino acids, making them more easily absorbed by the fish digestive tract, thereby increasing FUE (Hamid et al., 2022). The highest FUE value was obtained in treatment D at 80.24±0.23%, indicating that the addition of papaya leaf extract at a dose of 30 g kg^{-1} feed is an appropriate dosage for the papain contained in the papaya leaf extract to break down complex proteins into simple proteins, thereby increasing nutrient absorption and resulting in a higher FUE value. According to Fakoya et al. (2019), a high FUE value indicates that protein use for metabolism is relatively low; therefore, more protein can be utilized for fish growth. Yadav et al. (2024) stated that high FUE also reflects good feed quality, as the nutrient content and proportion of each nutrient component are the main factors determining its optimal utilization. Furthermore, Rachmawati & Prihanto (2019) noted that high FUE is demonstrated by better feed utilization to achieve better fish growth. Afriani & Oktari (2024) stated that high FUE is shown by using most of the feed for growth and only a small portion for energy. The lowest FUE value was obtained for treatment A without the addition of the papaya leaf extract. This is presumed to be due to the absence of papain in the feed; therefore, the hydrolysis of complex proteins relies solely on endogenous enzymes and cannot proceed optimally. Zheng et al. (2020) reported that papain in papaya leaves has the ability to improve protein utilization, thus increasing efficiency in enhancing growth, survival, and feed conversion. The results of this study indicate that the FUE value in each treatment was categorized as quite good because it was > 50%. Singh et al. (2011) stated that FUE is considered good if the value exceeds 50% and can even reach up to 100%.

Analysis of variance showed that the papaya leaf extract in the feed had a significant effect ($p < 0.05$) on the FCR of Java barb. This study demonstrated that the test diets supplemented with papaya leaf extract in treatments B, C, and D had lower FCR values than those without supplementation (A). Elvy et al. (2022) stated that a lower FCR indicates that less feed is required to produce 1 kg of fish meat, and a decreasing FCR value indicates increasing feed efficiency, where the amount of feed required to achieve a certain weight gain is reduced. This is supported by the study results showing that the high FUE value in treatment D (80.24±0.23%) resulted in the lowest FCR value of 1.26±0.10. Shanthanna et al. (2024) stated that the high ability of fish to utilize feed for growth can increase body weight, thereby lowering the FCR. This indicates that the nutrients in the feed can be digested and optimally utilized by fish to support growth. Shanthanna et al. (2024) found that the addition of papaya leaf extract to artificial feed for *Labeo rohita* significantly improved feed conversion efficiency. Research conducted by Hamid et al. (2022) evaluated the effect of adding papaya leaf extract to feed for red hybrid tilapia (*Oreochromis mossambicus* × *O. niloticus*), resulting in a lower FCR than that of the feed not supplemented with the papaya leaf extract enzyme. The papain enzyme in papaya leaves can hydrolyze complex proteins into simpler peptides and amino acids, making them more easily absorbed by the intestines (Zheng et al., 2020). This enzymatic activity can reduce the workload of the digestive system, thereby increasing the efficiency of essential amino acid and nutrient absorption. This ultimately supports improved growth and feed utilization efficiency in fish (Masooma et al., 2024).

The results of this study showed that the PER value of the test feed with papaya leaf extract addition (B, C, and D) was higher than that of the test feed without supplementation. The highest PER value was obtained in treatment D, at 3.29±0.18. This is likely because this dosage provides an appropriate amount of papain to hydrolyze protein macromolecules into amino acid compounds, thus enabling protein synthesis (Siddik et al., 2021). The higher the PER, the better the feed quality; therefore, feed efficiency increases. The rate of protein absorption is higher when the feed is enriched with papaya leaf extract containing papain, which can increase protein digestibility and, thereby, directly affect growth (Giri et al., 2026). Furthermore, Kirimi et al. (2022) stated that the suitability of amino acid composition enables the absorption and utilization of nutrients to proceed more optimally, thereby stimulating metabolism, which results in increased FUE and fish body weight gain. Papain is a proteolytic enzyme that functions in protein digestion in the body. In the fish digestive system, protein from feed cannot be absorbed directly; it must first be broken down by protease enzymes into simpler compounds, such as amino acids and peptides, so that they can be absorbed by the body. Protein breakdown occurs in the stomach and intestines, whereas the absorption of digestion products occurs in the intestines (Gopalraaj et al., 2022). The low PER value in treatment A is likely due to the absence of papain content from papaya leaf extract, which plays a role in accelerating the protein hydrolysis process in the feed, resulting in less protein being broken down into amino acids and consequently a lower amount of amino acids that can be absorbed by the body (Rachmawati & Prihanto, 2019). The addition of papaya leaf extract to fish feed can play an important role in improving overall growth and feed efficiency (Hamid et al., 2022). According to Ullah-khan et al. (2019), PER is a measurement used to indicate how well the protein in the feed can provide essential amino acids to the fish.

The ADCp value increased with increasing doses of papaya leaf extract in the test diet. This indicates that the addition of fish feed with papaya leaf extract containing papain significantly enhanced the protein digestibility. Papain, a protease enzyme extracted from papaya latex, acts as a biocatalyst that hydrolyzes complex feed protein molecules into simpler peptides and amino acids, facilitating faster nutrient absorption by fish (Singh et al., 2011). In addition, papain helps break peptide bonds, especially in plant-based proteins, thereby minimizing feed residue wastage (Patil & Singh., 2014). The highest ADCp value was found in treatment D, reaching 88.26±0.26%. This is likely because this dose of papaya leaf extract containing papain is adequate for breaking down complex proteins into simpler proteins, resulting in higher protein digestibility than that in test feeds A, B, and C. Papain increases protein digestibility, which supports accelerated metabolism and nutrient absorption, ultimately leading to improved growth (Yadav et al., 2024). Furthermore, Mo et al. (2016) stated that the addition of papain to feed increases protein digestibility. Papain can break peptide bonds in complex protein molecules into simpler forms, such as peptides and amino acids, resulting in increased protein digestibility, allowing for easier absorption of protein is more easily absorbed by fish (Rachmawati et al., 2020).

The highest RGR value in Java barb was obtained in treatment D, amounting to 3.73±0.23% day^{-1}. The results of this study showed that RGR tended to increase in line with high FUE, low FCR, and high PER and ADCp values. This indicates that the feed provided could be utilized optimally by Java barb, resulting in minimal nutrient losses. In addition, the greater the nutrient content of the feed that can be absorbed and stored in the fish body, the higher the RGR (Besson et al., 2020). This is related to the fish’s ability to digest and utilize nutrients for growth. Thus, the more nutrients are successfully absorbed from the feed, the faster, more efficient, and more optimal the fish growth will be. This is in line with the research by Rachmawati & Prihanto (2019), who stated that the presence of papain enzyme in feed can hydrolyze protein into amino acids, thereby making protein easier to digest and improving fish growth. Sheng et al. (2022) stated that papain can hydrolyze polypeptides into short-chain peptides. The availability of short-chain peptides is an important factor in improving protein digestibility, nutrient absorption and growth.

The results showed that feed supplementation with papaya leaf extract had no significant effect ($p > 0.05$) on the survival rate of the Java barb. This is likely because the papain in the papaya leaf extract in the feed was non-toxic. Wiszniewski et al. (2022) reported that papain in fish feed has been proven to be safe for both fish and the aquatic environment. Furthermore, Yadav et al. (2024) stated that papain is safe and does not cause toxicity that threatens the survival of cultured fish. Similar findings have been reported by Rachmawati & Prihanto (2019), Zheng et al. (2020) and Silaen et al. (2023).

The results of the proximate analysis of the nutritional composition of the Java barb bodies (Table 3) showed that the addition of papaya leaf extract to the feed did not affect ($p > 0.05$) the nutritional composition (moisture, protein, fat, carbohydrate, and ash) of the fish. The nutritional composition of fish is a result of internal metabolic processes, not from the enzymes in the feed acting directly within fish tissues (Mohammady et al., 2025). Similar results were reported by Hamid et al. (2022), who stated that the moisture, protein, and lipid contents of red hybrid tilapia *(Oreochromis mossambicus* × *Oreochromis niloticus*) were not affected by the addition of papaya leaf extract to their feed. Shanthanna et al. (2024) noted that the addition of papaya leaf extract to feed did not affect the nutritional composition of *Labeo rohita*. Furthermore, Oparaku et al. (2024) reported that the nutritional composition of *Clarias gariepinus* was not affected by the addition of papaya leaf extract to the feed.

Conclusions. Papaya leaf extract in feed had a significant effect on growth performance and feed efficiency but did not significantly affect the survival rate and body nutrient composition of Java barb at the Grow-Out Stage. Papaya leaf extract in feed at 30 g/kg improved the growth performance and feed efficiency of Java barb at the Grow-Out Stage. Therefore, papaya leaf extract in feed at 30 g/kg feed is recommended as a feed additive for the cultivation of Java barb during the Grow-Out Stage.

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

Data Availability. The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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