The effect of taurine in artificial feed on protein digestibility, feed utilization efficiency, and growth of Java barb (*Barbonymus gonionotus*) seeds

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Abstract. Substituting fishmeal with plant-based protein in fish feed offers economic benefits. Fishmeal is abundant in taurine, which is lacking in plant protein sources. Taurine supplementation is known to enhance fish growth. This study aimed to evaluate how taurine in fish feed affects protein digestibility, feed efficiency, and the growth of Java barb (*Barbonymus gonionotus*) seeds. Java barb seeds weighing 2.57±0.15 g were reared in 1 m^3 fiberglass tanks with 15 fish per tank. They were fed the experimental diet three times daily until satiation. The research comprised two phases: a digestibility test and a growth test. Taurine was added at 0, 0.5, 1, and 1.5% doses to the isoprotein and isoenergy experimental diets, each experimental diet was repeated three times. The digestibility test spanned 20 days, involving feces collection on the seventh day after feeding. The growth test ran for 53 days, with a 30% water exchange before each feeding. Results demonstrated that adding 15 g taurine/kg of feed improved total digestibility (77.52±0.23%), energy digestibility (68.24±0.16%), protein digestibility (94.18±0.15%), and enzyme activity (6.72±0.04 U g^{-1} protein for protease and 0.89±0.03 U g^{-1} protein for lipase) of Java barb seeds. Therefore, it can be concluded that adding taurine at a dose of 15 g kg^{-1} feed is the best dose for Java barb seeds.
Key Words: amylase, enzyme, energy, fish, protease.

Introduction. Java barb (*Barbonymus gonionotus*) is a native freshwater fish species in Indonesia. The success of Java barb cultivation depends on the availability of high-quality fish feed. Quality fish feed not only contains the right amount of protein for the need of the fish but also has a complete profile of essential amino acids to promote growth (NRC 2011). Fishmeal is used as an animal protein source in fish feed formulations, but its cost has increased due to rising demand (Sampath et al 2020). As a result, feed costs account for up to 60% of the total production costs of fish farming (Yang et al 2013). Because there is a limited supply of fishmeal, overfishing has increased without regard for the sustainability of the natural ecosystem (Pelletier et al 2018).

To overcome the world's fishmeal deficiency and the development of the feed industry in sustainable aquaculture activities, most feeds use plant proteins that are widely available and economical (Fiorella et al 2021). However, the high use of plant protein in feed can lead to reduced feed consumption, feed utilisation efficiency, and slow fish growth (Gunathilaka et al 2019). This is because plant protein sources lack the amino acid taurine (Cheng et al 2018). Taurine is a conditionally essential amino acid for fish, which is abundant in fishmeal, whereas most sources of plant material contain almost no taurine (Lin et al 2020). Lack of taurine in feed can result in decreased protein digestibility, feed utilisation efficiency, and fish growth (Li et al 2022). Efforts to overcome the problems mentioned above were oriented to make feed with the addition of taurine. Supplementation of taurine in feed can improve feed utilisation efficiency and fish growth performance (Zhang et al 2019; Sampath et al 2020).

Taurine in feed has been reported to have several physiological functions for fish including improving growth (Li et al 2022), feed efficiency (Zhang et al 2019), protein digestibility and protein retention (Wei et al 2019), and fish immune response (Yan et al 2019).

Several studies of taurine supplementation in feed to enhance efficiency of feed utilisation and growth have been conducted on several fish species, including: *Pangasianodon hypophthalmus* (Peter et al 2021), *Scophthalmus maximus* (Zhang et al 2019), *Takifugu rubripes* (Wei et al 2019), *Oncorhynchus mykiss* (Huang et al 2021), *Mylopharyngodon piceus* (Zhang et al 2019), *Cyprinus carpio* (Abdel-Tawwab & Monier 2018), *Dicentrarchus labrax* (Martins et al 2018). At this time, there is no information available regarding taurine supplementation in Java barb feed. The aim of the study is to examine the effect of taurine supplementation in feed on on protein digestibility, feed utilization efficiency, and growth of Java barb. The urgency of this research is to develop a nutritional method of taurine supplementation in feed to improve the growth of Java barb to accelerate production.

Material and Method

Research period and location. This research was conducted from March to June 2024 at the Freshwater Fish Breeding and Cultivation Center in Muntilan, Magelang, Central Java.

Research design. This research consists of two phases: the digestibility and growth tests. The study employed a completely randomized design comprising four treatments with three replications. Treatments were determined based on different taurine doses, namely 0, 5, 10, and 15 g kg^{-1} of feed.

Test feed preparation. The test feed formulation used taurine at different doses as treatment, and tapioca was used as a supplementary ingredient to make up 100% of the feed formula (Table 1).

Table 1

Test feed ingredients and formula (g kg^{-1} of feed)

Raw ingredient (% dry weight)	*Addition of taurine in the feed (g kg^{-1} of feed)*			
	0	*5*	*10*	*15*
Fish flour	280.00	280.00	280.00	280.00
Soybean meal	250.00	250.00	250.00	250.00
Cornstarch	100.00	100.00	100.00	100.00
Wheat bran	220.00	215.00	210.00	200.00
Fish oil	20.00	20.00	20.00	20.00
Corn oil	20.00	20.00	20.00	20.00
Tapioca	80.00	80.00	80.00	80.00
Vitamin & minerals[1)]	15.00	15.00	15.00	15.00
Choline chloride	5.00	5.00	5.00	5.00
Taurine	0.00	5.00	10.00	15.00
Attractant	5.00	5.00	5.00	5.00
Cr_2O_3	5.00	5.00	5.00	5.00
Total	1000	1000	1000	1000

Note: [1)]Vitamin and Mineral mix kg^{-1}: magnesium (Mg) 1.900 mg, calcium (Ca) 219 mg, potassium (K) 150 mg, sodium (Na) 117 mg, selenium (se) 150 mg, vit. A 36,000 I.U., vit. B1 52 mg, vit. B2 97 mg, vit. B6 46 mg, vit. B12 60 mg, 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, iodine (KI) 1.8 mg, cobalt (Co) 450 mg, pantothenic acid 93 mg, niacin 130 mg, folic acid 10 mg, inositol 225 mg, biotin 450 mg, vit. E 187 mg, vit. K3 19 mg.

Before the feed was prepared, the raw materials used in the feed formulation were first subjected to proximate analysis to determine the nutrients contained in them. Subsequently, the prepared raw materials were weighed according to the predetermined feed formulation. The prepared raw materials were thoroughly mixed and then shaped using an extruder pelletizing machine (H 2700, China) with a diameter of 2 mm. The pellets were then dried in an oven at 30°C for 24 hours. The test feed, now in pellet form, was subjected to proximate analysis to ensure its content matched the predetermined values (Table 2). The test feed was formulated to have isoprotein (30.28±0.30%) and isoenergy (360.07±0.21 kcal g^{-1} of feed). The labeled test feed was stored in airtight plastic containers.

Table 2
Proximate (% dry weight), gross energy (kcal g^{-1}), and the ratio of the protein energy in the test feed with different doses of added taurine

Parameters	*Addition of taurine in the feed (g kg^{-1} of feed)*			
	0	*5*	*10*	*15*
Crude protein*)	30.23	30.01	30.70	30.18
Crude fat*)	6.64	6.55	6.62	6.64
Ash*)	8.59	8.59	8.50	8.62
Crude fiber*)	5.84	5.32	5.53	5.41
NFEM*)	45.70	45.53	45.65	46.15
GE1)	358.84	359.05	362.68	359.72
C/P ratio2)	11.83	11.96	11.81	11.92

Note: *) NFEM = nitrogen-free extract materials. *) Proximate analysis results from the Laboratory of Animal Feed, Faculty of Animal Husbandry and Agriculture, Universitas Diponegoro, 2024. 1) GE = gross energy: 1 g protein = 5.6 kkal, 1 g NFEM = 4.1 kkal, 1 g fat = 9.4 kkal (NRC 2011); 2) C/P: energy-protein ratio.

Containers and test fish preparation. The containers used for the research were 16 units of 1 x 1 x 1 m^3 fiberglass tanks. These fiberglass tanks were sanitized by washing, disinfecting, rinsing, and drying. Each fiberglass tank was equipped with a recirculation system to maintain the water quality within an optimal range. In this research, the maintenance medium used was water sourced from a reservoir that had been settled and stored beforehand. The test fish consisted of 300 Java barb seeds from the Freshwater Fish Breeding and Cultivation Center in Muntilan, Central Java. The test fish were acclimated to the maintenance medium and artificial feed for seven days to allow the test animals to adapt to the new diet and environment. The fish were fed commercial feed three times daily during the adaptation period until they were completely satiation. Before being introduced into the aquarium, the Java barb seeds were selected based on uniform size, absence of deformities, completeness of body organs, physical health, and the absence of potential diseases (Rachmawati et al 2023).

Feed digestibility test. After being acclimatized, the Java barb seeds had an initial weight of 2.57±0.15 g and were stocked at a density of one fish per liter in fiberglass tanks containing 30 liters of water. The random distribution of the Java barb seeds was performed. The Java barb seeds fish were given the test diet until full at 7:00, 12:00, and 17:00 of Western Indonesian Time (WIT) for 20 days. The collection of fish feces began on the seventh day after the fish were given the test feed. Cr_2O_3 in the feed was used as a digestibility indicator. Fish feces were collected 1 hour after feeding using a siphoning method with a hose. The collected feces were then placed in labeled film bottles and stored in a refrigerator to maintain their freshness for subsequent proximate and Cr_2O_3 content analysis.

Growth test. Following acclimatization, the Java barb seeds with initial weight of 2.57±0.15 g were randomly dispersed into fiberglass aquariums with a stocking density of 15 fish per tank. During the course of 56 days, the Java barb seeds were given the test meal until they were satiation at 07:00, 12:00, and 17:00 WIT. Siphoning and water replacement, amounting to 30% of the rearing media volume, were carried out in the morning before feeding. Measurements of temperature were collected each morning and evening, while measurements of dissolved oxygen, pH, and total ammonia nitrogen (TAN) were obtained twice during the maintenance period, once on day 0 and on day 30. In the case of any mortality, the number and weight of the fish were recorded. The daily feed consumption during the maintenance period was calculated by weighing the feed before and after it was given.

Research variables. Proximate analysis was conducted on raw materials, test feed, feces, initial test fish bodies, and final test fish bodies of Java barb seeds. Proximate analysis included moisture content, protein, fat, crude fiber, ash, and TAN analysis (AOAC 2005). Analysis of Cr_2O_3 content in the feed and feces was performed based on Takeuchi (1988). The study of digestive enzyme activities included protease following Bergmeyer (2012) method, amylase according to Worthington's (1993) method, and lipase following Borlongan's (1990) method. The concentration of soluble protein was measured using the Bradford (1976) method. Protein and fat retention values were calculated based on the formula proposed by Guo et al (2012). The relative growth rate (RGR) of the fish was calculated using the equation provided by Yu et al (2016). Feed utilization efficiency (FUE) was calculated using the equation from Pohlenz et al (2012). Survival rate was calculated using the formula from Han et al (2014). Each parameter was calculated based on the following procedures:

$$TD = 100 \times (1 - b/b')$$
$$PD/ED = 100 \times [1 - (a'/a \times b/b')]$$

where: TD = total digestibility (%);
PD = protein digestibility (%);
ED = energy digestibility;
a = amount of protein/carbohydrate in the test feed (% of dry weight);
a' = amount of protein/carbohydrate in the feces (% of dry weight);
b = amount of Cr_2O_3 indicator in the test feed (% of dry weight);
b' = amount of Cr_2O_3 in the feces (% of dry weight).

$$PR = (Pt - Po)/Pp \times 100$$

where: PR = protein retention (%);
Pt = amount of protein in the fish at the end of period (g);
Po = amount of protein in the fish at the beginning of period (g);
Pp = amount of protein in the feed consumed by the fish (g).

$$FR = (Lt - Lo)/LI \times 100$$

where: FR = fat retention (%);
Lt = initial amount of fat in the fish (g);
Lo = final amount of fat in the fish (g);
LI = amount of fat in the feed consumed by the fish (g).

$$RGR = ((LnWt - LnWo))/t \times 100$$

where: RGR = relative growth rate (% day^{-1});
Wt = final fish weight (g);
Wo = initial fish weight (g);
t = rearing period.

$$FUE = ([(Wt + Wd) - Wo])/F \times 100$$

where: FUE = feed utilization efficiency (%);
Wt = average of the final fish weight (g);
Wo = initial fish weight (g);
Wd = weight of dead fish during the period (g);
F = amount of feed along the period (g).

The calculation of feed consumption was done by weighing the amount of feed consumed by the fish each day during the rearing period.

$$SR = Nt/No \times 100$$

where: SR = survival rate (%);
Nt = final amount of fish;
No = initial amount of fish.

Statistical analysis. The research variables were analyzed using analysis of variance (ANOVA), and significant value was continuing with post hoc Duncan multiple range test ($p < 0.05$). Statistical testing was performed using SPSS software version 22.

Results. Adding taurine to the Java barb seeds feed can improve feed digestibility (Table 3). The highest values for total digestibility, energy digestibility, and protein digestibility were found in the treatment with a 15 g kg^{-1} of feed dose, and these values were significantly different ($p < 0.05$) from the other treatments.

Table 3

Digestibility of the Java barb seeds feeds provided with the test feed for 20 days

Parameters (%)	*Addition of taurine in the feed (g kg^{-1} of feed)*			
	0	*5*	*10*	*15*
Total digestibility	60.36 ± 0.27^a	67.38 ± 0.22^b	72.19 ± 0.24^c	77.52 ± 0.23^d
Energy digestibility	52.17 ± 0.18^a	55.26 ± 0.23^b	62.47 ± 0.25^c	68.24 ± 0.16^d
Protein digestibility	73.42 ± 0.14^a	80.42 ± 0.18^b	84.52 ± 0.15^c	94.18 ± 0.15^d

Note: The upper letters behind the standard deviation values that differ in each row signify statistically significant differences ($p < 0.05$).

The highest values for protease and lipase enzyme activities were found in the treatment with dose taurine 15 g kg^{-1} of feed, and these values were significantly different ($p < 0.05$) from the other treatments (Table 4). However, the amylase enzyme activity showed no significant difference ($p > 0.05$) among the treatments.

Table 4

Digestive enzyme activities of Java barb seeds fed with the test feed for 56 days

Parameter (U g^{-1} protein)	*Addition of taurine in the feed (g kg^{-1} of feed)*			
	0	*5*	*10*	*15*
Protease	2.68 ± 0.04^a	3.54 ± 0.08^b	5.26 ± 0.03^c	6.72 ± 0.04^d
Amylase	0.48 ± 0.05^a	0.49 ± 0.03^a	0.50 ± 0.02^a	0.50 ± 0.02^a
Lipase	0.40 ± 0.03^a	0.50 ± 0.02^b	0.60 ± 0.01^c	0.89 ± 0.03^d

Note: The upper letters behind the standard deviation values that differ in each row signify statistically significant differences ($p < 0.05$).

Table 5 shows that the values for final fish weight (Wt), feed consumption (FC), protein retention (PR), fat retention (FR), feed efficiency (FUE), nd relative growth rate (RGR) were significantly different ($p < 0.05$) among the treatments. However, there was no significant difference ($p > 0.05$) in the initial fish weight (Wo) and survival rate of the Java barb seeds.

Table 5

Initial fish weight (Wo), final fish weight (Wt), feed consumption (FC), protein retention (PR), fat retention (FR), feed efficiency (FE), relative growth rate (RGR), and survival rate (SR) of Java barb seeds fed with the test feed for 53 days

Variables	*Addition of taurine in the feed (g kg^{-1} of feed)*			
	0	*5*	*10*	*15*
Wo (g^{-1})	2.57 ± 0.15^a	2.56 ± 0.16^a	2.58 ± 0.14^a	2.57 ± 0.14^a
Wt (g^{-1})	10.16 ± 0.22^a	22.44 ± 1.32^b	25.45 ± 1.14^c	29.28 ± 1.26^d
FC (g^{-1})	19.48 ± 1.30^a	24.73 ± 1.44^b	28.16 ± 1.19^c	32.83 ± 1.25^d
PR (%)	25.30 ± 1.52^a	32.82 ± 1.62^b	43.28 ± 1.38^c	49.29 ± 1.62^d
FR (%)	47.63 ± 1.17^a	59.86 ± 1.75^b	79.68 ± 1.42^c	88.62 ± 1.47^d
FUE (%)	56.42 ± 1.24^a	65.38 ± 1.40^b	76.49 ± 1.26^c	89.35 ± 1.18^d
RGR (% day^{-1})	2.43 ± 0.02^a	3.09 ± 0.15^b	3.85 ± 0.08^c	4.86 ± 0.03^d
SR	90.00 ± 2.00^a	90.00 ± 2.00^a	92.00 ± 2.00^a	92.00 ± 2.00^a

Note: The upper letters behind the standard deviation values that differ in each row signify statistically significant differences ($p < 0.05$).

During the research study, water quality parameters remained within suitable ranges for Java barb seeds aquaculture, including a temperature range of 28-31°C, dissolved oxygen levels of 5.2-6.6 mg L^{-1}, pH levels between 6.77 and 7.30, and total ammonia nitrogen ranging from 0.03 to 0.39 mg L^{-1}. These parameters are essential for maintaining a healthy environment for fish rearing.

Discussion. The Java barb seeds fed with the treatment feed without adding taurine exhibited lower total digestibility, energy digestibility, and protein digestibility than the treatment with taurine supplementation (Table 3). The results of this study are consistent with research reported by Peter et al (2021) on *P. hypophthalmus*, Zhang et al (2019) on *S. maximus* and Wei et al (2019) on *T. rubripes*. The results of this study demonstrate that total digestibility, energy, and protein increase with the addition of taurine to the feed. This demonstrates the significance of taurine in fish nutrition use. As a result, the addition of taurine to feeds in which some or all of the fishmeal has been substituted with plant-based protein sources is required (Tan et al 2018).

The digestibility of feed is determined by digestive enzyme activity and enzyme activity levels in a fish's digestive tract (Liao et al 2015). Protease enzyme activity significantly differs ($p < 0.05$) among the treatments (Table 4), while amylase enzyme activity did not significantly different ($p > 0.05$). The increasing taurine dose in the feed for each treatment increases protease enzyme activity. This is because taurine is an amino acid derivative of protein (Huang et al 2021), and protease enzymes play a role in protein digestion (NRC 2011). Lipase enzyme activity also increases with the increasing taurine dose in the feed. Similar research results in *D. labrax* (Martins et al 2018), *Takifugu obscurus* (Cheng et al 2018) and *Sparus aurata* (Gaon et al 2021).

Adding taurine to the feed leads to an increase in the fish's appetite, as indicated by the increased feed consumption (Table 5). This increased appetite in fish is attributed to the role of taurine as a signaling molecule that regulates appetite (Coutinho et al 2016). Additionally, feed consumption is influenced by feed palatability. Palatability is closely related to attractability, which affects the fish's search, intake, and consumption of feed related to nutrient content, especially certain free amino acids like glycine, glutamate, and taurine (Sampath et al 2020). The number of nutrients that a fish is able to absorb is proportional to the amount of feed that it takes in. Values of digestibility reflect the content of nutrients that can be absorbed and used for growth, as well as the metabolic waste products (Peter et al 2021). The highest total digestibility value was found in the 15 g kg^{-1} of feed treatment, at 77.52±0.23% (Table 3), meaning that the Java barb seeds can digest 77.52±0.23% of the nutrients in the feed provided. Meanwhile, the energy digestibility value indicates the amount of carbohydrates, fats, and proteins in the feed that the fish can digest. The 15 g kg^{-1} of feed treatment also produced the highest protein digestibility value, 94.18±0.15%. Higher protein digestibility means that the fish can utilize more protein for growth.

The quantity of feed consumed, digested, absorbed, and utilized by the fish will increase retention values. The protein retention and fat retention values increase with the increasing taurine dose in the feed (Table 5). This research demonstrates that the addition of taurine to the feed affects the fish's body protein, resulting in increased values for protein retention and fat retention. Numerous factors, such as the protein content of the meal, the balance of amino acids, and the feed's energy-to-protein ratio affect protein retention levels (Pohlenz et al 2012). Additionally, the increase in body fat content in the fish affects fat retention, where fat retention of the Java barb in the 15 g kg^{-1} of feed treatment has the highest value, exceeding 80%. This means that the amount of fat converted into the fish's body exceeds the fat intake from the feed. This occurs because excess glucose is converted into fat stores through lipogenesis and stored in the fish's body tissues. Abdel-Tawwab & Monier (2018) revealed that fatty acids can be synthesized from glucose derived from carbohydrates if there is an excess of glucose intake.

Retention values will affect the fish's weight, related to growth rate and feed utilization efficiency. This research demonstrates that adding taurine to the feed can increase the growth rate of Java barb seeds as the taurine dose in the feed increases.

According to Green et al (2002), abundant free amino acids available for fish consumption allow essential amino acids in the body to form tissues so they are not metabolized into energy in the body. Taurine serves as an energy source, and inside the body, taurine is converted into glutamate, which is then converted into alpha-ketoglutarate, used in the Krebs cycle to produce ATP (Campbell & Anderson 1991). The feed utilization efficiency value compares growth and feed consumption in percentage units. The treatment with a concentration of 15 g kg^{-1} of feed had a feed utilization efficiency value that was considerably highest between in the other treatments (Table 5). The increase in feed utilization efficiency is related to the amount of feed consumed and feed nutrients stored in the fish's body. The higher the feed nutrients stored in the fish, the higher the RGR and feed utilization efficiency value. This research shows that adding taurine to the feed does not affect the survival of Java barb seeds. The survival of Java barb seeds in all taurine addition treatments (0, 5, 10 and 15 g kg^{-1} of feed) did not significantly differ ($p > 0.05$) between the treatments. Similar results were reported by Tan et al (2018), Peter et al (2021), and Martins et al (2019). The mortality of Java barb seeds during the research may be due to the fish experiencing stress during sampling during the study.

Conclusions. Adding taurine at a dose of 15 g kg^{-1} of feed seeds can increase total digestibility, energy digestibility, protein digestibility, protease and lipase enzyme activities, feed utilization efficiency, relative growth rate, and protein retention of Java barb. Therefore, it can be concluded that adding taurine at a dose of 15 g kg^{-1} feed is the best dose for Java barb seeds.

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