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## EFFECT OF A SUPPLEMENTED DIET WITH MELATONIN ON PERFORMANCE OF JAVAEN BARB *Systemus orphoides* (Valenciennes, 1842) JUVENILE

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### ABSTRACT

Javaen barb is a native fish in Indonesian inland water with economic value and the potential to be developed as cultured fish resources and ornamental commodities. In the development of aquaculture, there are still problems, such as the low adaptability of the larvae, so that their survival is also low. This study was conducted to evaluate the effect of melatonin supplementation on the productivity of Javaen barb fish juveniles in culture containers. This study was conducted experimentally using a completely randomized design consisting of four treatments with different doses of melatonin supplementation, i.e., A) control (without melatonin supplementation), B) 0.2 mg/100 g of feed, C) 0.4 mg/100 g feed, and D) 0.6 mg/100 g feed, each treatment was repeated three times. Javaen barb juveniles were kept in an aquarium measuring 30 × 36 × 60 cm with a water level of 40 cm, consisting of 12 units. Each aquarium was stocked with 150 individuals and given an aeration system with the same air pressure intensity. Feeding was carried out *ad-libitum* with a frequency of three times daily for 180 days of rearing. The results showed that melatonin supplementation of as much as 0.6 mg/100 g of feed was able to increase the growth of Javaen barb fish juvenile with the highest survival rate (81.33 ± 0.54 %) and feed conversion ratio (2.61 ± 0.14). Melatonin supplementation of 0.6 mg/100 g of feed had higher total leukocyte (3.41 ± 0.73 × 10<sup>4</sup> cells/mm<sup>3</sup>) and hemoglobin (5.07 ± 0.12 g%) values and provided the best production performance in Javaen barb juveniles.

**KEYWORDS:** FCR; growth; haematology; productivity; survival rate

### INTRODUCTION

Javaen barb is a native fish in Indonesian inland water with economic value and the potential to be developed as cultured fish resources and ornamental commodities (Dewi *et al.*, 2021). In Southeast Asia, this fish was found in the waters of Myanmar, Thai-

land, Mekong, and the Malay Peninsula (Fishbase, 2023; Iswantari *et al.*, 2021; Ut *et al.*, 2020; Cahyanti *et al.*, 2020; Panprommin *et al.*, 2019). In aquaculture development, the problems that often occur are not only in their reproductive performance but also in the adaptation process and low survival. Observational data showed that the survival rate of Javaen barb fish juveniles was only around 40-60% (Dewi *et al.*, 2020). In addition, when rearing in aquaculture containers, Javaen barb fish are susceptible to disease, especially *Aeromonas hydrophila*. It can cause a mortality rate of up to 80% (Dewi *et al.*, 2020). Therefore, efforts are needed to overcome these

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problems to support the development of Javaen barb fish farming.

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone that originates from the pineal gland and is categorized into compounds that have various therapeutic and pharmacological effects. Melatonin has also been detected in fish in several tissues, such as the retina, intestine, liver, and ovary (Hasan *et al.*, 2016). Melatonin and the pineal organ are heavily involved in controlling various processes and functions in fish's biochemical, physiological, and behavior, both daily and annual rhythms (Nisembaum *et al.*, 2021). The daily rhythm includes migration and behavioral activity (Ben-Moshe *et al.*, 2016), reproduction, and spawning (Falcon & Zohar, 2018), and influences the immune system (Ngasainao & Lukram, 2016), while the annual rhythm includes development and growth (Kouhi-Dehkordi & Bani, 2017). In addition, melatonin also contributes to lipid and glucose metabolism, neuroendocrine hormonal rhythms, cell division, and regeneration (Maitra *et al.*, 2013). Melatonin is an antioxidant in reducing intracellular stress (Maitra & Hasan, 2016).

Several studies related to the application of melatonin in fish have been carried out. Melatonin application can be made through coating on feed or direct induction into the fish's body. Several studies on the application of melatonin through a feed that have been carried out include Nile tilapia at doses of 0.3 and 3 mg/Kg of feed (Kim *et al.*, 2017), Nile tilapia at doses of (50 and 200 mg/Kg of feed (Veisi *et al.*, 2021), masu salmon at a dose of 1g/2 Kg of feed (Amano *et al.*, 2004), *Clarias macrocephalus* at doses of 0.05 and 0.25 mg/Kg of feed (Aripin *et al.*, 2014), sea bream *Sparus aurata* at a dose of 40 mg/Kg feed (Amri *et al.*, 2020), while research on melatonin application through induction has been carried out on sea bass using a dose of 3.81 mg/g body weight (Alvarado *et al.*, 2015). In general, the study's results reported that melatonin played a more dominant role in reproductive performance and physiology in fish. Melatonin supplementation in Javaen barb fish is the first research conducted. This study evaluated the effect of melatonin supplementation in feed on the performance of Javaen barb juveniles. This research is also expected to provide data and information related to the fitness of Javaen barb juveniles so that it is useful in meeting the availability of Javaen barb juveniles throughout the year.

## MATERIAL AND METHODS

The research was conducted in May-November 2022. This study was conducted at the Research Station for Freshwater Fisheries Germplasm, Cijeruk,

West Java, which belongs to the Research Institute for Freshwater Aquaculture and Fisheries Extension (BRPBATPP), Ministry of Marine Affairs and Fisheries, Republic of Indonesia.

### Experimental fish

The fish used in this study were Javaen barb fish (2 months old) measuring  $1.5 \pm 0.0$  cm in standard length and  $0.054 \pm 0.001$  g in body weight, a total of 1,800 individuals. Juveniles result from semi-artificial spawning of Javaen barb fish using 5 females and 10 males broodstock.

### Melatonin supplementation

This study was conducted experimentally using a completely randomized design consisting of four treatments, and each repeated three times. The feed used in this study was commercial feed containing 30% protein in crumble form. Then it was coated with melatonin according to the treatment dose, namely: A) control (without melatonin supplementation), B) 0.2 mg/100 g feed, C) 0.4 mg/100 g feed, and D) 0.6 mg/100 g feed. Mixing melatonin in the feed uses a binder of 2 egg yolks per kg of feed (modification of research by Budi *et al.*, 2016). The feed and melatonin are stirred until evenly distributed and then sprayed with water using a *sprayer*. The feed is air-dried at room temperature and protected from direct sunlight for 6-8 hours. Dry feed is stored in jars and placed at room temperature (24-29°C).

### Rearing of fish

Javaen barb juveniles were reared using 12 aquariums measuring 30 cm × 36 cm × 60 cm. Each aquarium was filled with a water level of 40 cm, and 150 individuals were stocked. The aquarium has an aeration system with the same air pressure intensity. During 180 days of rearing, the Javaen barb was fed using *ad-libitum*. Feeding was done with a frequency of three times a day (08:00 AM, 01:00 PM, and 05:00 PM). Water quality in the aquarium is maintained by siphoning and changing the water by 30-50% every three days.

### Growth and survival rate

The growth and survival of Javaen barb fish juveniles were observed every month of rearing. The growth in length and weight is the difference between the final length or weight and the initial length or weight of rearing. Growth was observed by measuring the length and weight of individuals as much as 25% of the total population. Survival is the percentage of juveniles that live at the end of the observation compared to the number of juveniles at the be-

ginning of rearing. Growth and survival were calculated based on the formula used in the study by Kusmini *et al.* (2020) on Asian redbtail catfish.

### Feed Conversion Ratio

The amount of feed given is then used to calculate the feed conversion ratio or FCR. FCR was calculated using the formula (Mohanta *et al.*, 2013):

$$FCR = \frac{F}{(Bt + Bd) - Bo}$$

Where:

- Bt = Fish biomass at the end of the rearing (g)
- Bo = Fish biomass at the initial of the rearing (g)
- Bd = Dead fish biomass during rearing (g)
- F = Total feed intake (g)

### Measurement of blood hematology and cortisol hormone

Blood samples were collected at the end of the observation. Before blood collection, the fish were fasted for 24 hours and then anesthetized with 0.3 mL/L arowana stabilizer solution. Blood samples were obtained from three Javaen barb fish in each treatment which was taken randomly for each parameter, namely hematology, and cortisol. For hematological measurements (leukocytes, erythrocytes, and hemoglobin), blood was taken from the caudal artery of Javaen barb fish as much as 1 mL using a 3 mL syringe given heparin sodium. The differential measurement of leukocytes and erythrocytes refers to the method of Blaxhall & Daisley (1973), while the hemoglobin calculation is based on the method of Wedemeyer & Yasutake (1977). For the cortisol parameter, 0.5 mL

of blood was taken from the caudal artery using a 1 mL syringe given heparin sodium. Blood samples were centrifuged at 3000 rpm for 15 minutes and stored at -20°C before further analysis. The ELISA method measured Cortisol in blood plasma using a kit (DRG EIA 1887).

### Data analysis

Parameter data for evaluating the productivity of Javaen barb juveniles (length, weight, FCR, survival rate, hematology, and cortisol) were analyzed quantitatively. The data were tabulated (mean±SD) in tabular form and analyzed statistically (IBM SPSS Statistics 23), and a one-way ANOVA test was performed at a 95% confidence interval. Differences between treatments were evaluated using Duncan's advanced test.

## RESULTS AND DISCUSSION

### Growth performance of Javaen barb fish

Growth values in length, weight, specific growth rate (SGR), and FCR in Javaen barb juveniles supplemented with melatonin using different doses are presented in Table 1. The results of the 180-day (6-month) rearing of Javaen barb juveniles supplemented with melatonin 0.6 mg/100 g of feed gave the best performance with growth values in length (3.9 ± 0.1 cm), weight (3.89 ± 0.20 g), length SGR (0.70 ± 0.02 %/day), weight SGR (2.39 ± 0.05%/day), and FCR (2.61±0.14), where the resulting values statistically show a significant difference (P<0.05) compared to the control treatment with values for growth in length (3.2±0.2 cm), weight (2.64±0.34 g), length SGR (0.63±0.03 %/day), weight SGR (2.18±0.07 %/day), and FCR (3.95±0.53).

Table 1. Growth in length, weight, SGR, and FCR of Javaen barb fed diet supplemented with melatonin for 180 days of rearing.

Growth parameters	Melatonin supplementation treatments			
	A (Without supplementation)	B (0.2 mg/100 g feed)	C (0.4 mg/100 g feed)	D (0.6 mg/100 g feed)
Initial length (cm)	1.5±0.0	1.5±0.0	1.5±0.0	1.5±0.0
Initial body weight (g)	0.053±0.002	0.053±0.002	0.055±0.002	0.053±0.002
Final length (cm)	4.8±0.2	4.8±0.2	4.9±0.4	5.5±0.1
Final body weight (g)	2.70±0.34	2.97±0.29	3.24±0.81	3.94±0.20
Absolute length gain (cm)	3.2±0.2 <sup>a</sup>	3.3±0.2 <sup>a</sup>	3.4±0.4 <sup>ab</sup>	3.9±0.1 <sup>b</sup>
Absolute weight gain (g)	2.64±0.34 <sup>a</sup>	2.92±0.29 <sup>ab</sup>	3.19±0.81 <sup>ab</sup>	3.89±0.20 <sup>b</sup>
Specific growth rate of length (%/day)	0.63±0.03 <sup>a</sup>	0.64±0.02 <sup>ab</sup>	0.65±0.04 <sup>ab</sup>	0.70±0.02 <sup>b</sup>
Specific growth rate of weight (%/day)	2.18±0.07 <sup>a</sup>	2.23±0.08 <sup>a</sup>	2.25±0.13 <sup>ab</sup>	2.39±0.05 <sup>b</sup>
FCR	3.95±0.53 <sup>a</sup>	3.59±0.35 <sup>ab</sup>	3.45±0.70 <sup>ab</sup>	2.61±0.14 <sup>b</sup>

Remarks: Numbers followed by the same superscript letter in the same line indicates no significantly difference (P>0.05).

Melatonin supplementation at a dose of 0.6 mg/100 g of feed showed the best performance on growth (length and weight) and was able to utilize feed optimally compared to the control treatment ( $P < 0.05$ ). From the growth value of the fish obtained in the melatonin supplementation treatment at a dose of 0.6 mg/100 g of feed, the length was 22.33% larger, and the weight was 47.13% larger, with a decrease in FCR value of 33.90% compared to the control treatment. Melatonin plays a central role in the adaptive behaviour of fish to the environment, including fish growth (Lima-Cabello *et al.*, 2014; Piccinetti *et al.*, 2010). Melatonin can increase metabolic and physiological functions, thereby stimulating growth. The results of this study align with the research conducted by Porter *et al.* (1998), who reported that melatonin administration had an increasing effect on the growth of salmon.

In this study, the treatment of melatonin supplementation at doses of 0.2 and 0.4 mg/100 g of feed did not show different results from the control treatment ( $P > 0.05$ ). It is suspected that the dose of melatonin given was still relatively small and had no maximum effect on the performance of Javaen barb

juveniles. Melatonin doses and fish species are thought to greatly influence the resulting growth performance. Research by Kim *et al.* (2018) reported that giving melatonin supplementation at  $< 0.4$  mg/100 g of feed in Nile tilapia broodstock did not perform optimally.

### Survival and blood profile of Javaen barb juvenile

The survival rate of Javaen barb fish juveniles after being reared for 6 months is presented in Figure 1, while the blood profile (leukocytes, erythrocytes, and hemoglobin) of Javaen barb juveniles supplemented with melatonin for 6 months of rearing is presented in Table 2. The results showed that the survival rate of Javaen barb juveniles fed a melatonin-added diet was the same ( $P > 0.05$ ) and higher than that of the control not given melatonin ( $P < 0.05$ ). The blood profile showed that the treatment with melatonin supplementation of 0.4 and 0.6 mg/100 g of feed had higher total leukocyte and hemoglobin values than the control treatment ( $P < 0.05$ ). Meanwhile, all treatments total erythrocyte value was the same ( $P > 0.05$ ).

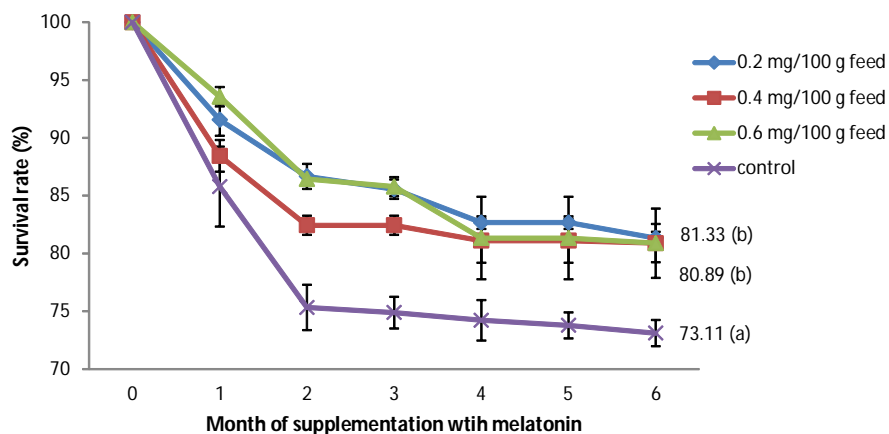


Figure 1. The survival rate of Javaen barb juveniles fed diet supplemented with melatonin for 180 days of rearing.

Table 2. The blood profile of Javaen barb juveniles fed diet supplemented with different melatonin doses for 180 days of rearing

Blood profile	Melatonin supplementation treatments			
	A (Without supplementation)	B (0.2 mg/100 g feed)	C (0.4 mg/100 g feed)	D (0.6 mg/100 g feed)
Total erythrocytes ( $\times 10^6$ cells/mm <sup>3</sup> )	1.85 ± 0.37 <sup>a</sup>	1.87 ± 0.39 <sup>a</sup>	2.32 ± 0.40 <sup>a</sup>	2.10 ± 0.07 <sup>a</sup>
Total leukocytes ( $\times 10^4$ cells/mm <sup>3</sup> )	2.25 ± 0.09 <sup>a</sup>	2.64 ± 0.41 <sup>ab</sup>	3.21 ± 0.80 <sup>b</sup>	3.41 ± 0.73 <sup>b</sup>
Hemoglobin (g%)	4.77 ± 0.21 <sup>a</sup>	4.77 ± 0.42 <sup>ab</sup>	5.10 ± 0.22 <sup>b</sup>	5.07 ± 0.12 <sup>b</sup>

Remarks: Numbers followed by the same superscript letter in the same line indicates no significantly difference ( $P > 0.05$ ).

Melatonin supplementation at a dose of 0.2-0.6 mg/100 g of feed showed survival rates that were not significantly different, with a range of 80.89-81.33%. Melatonin supplementation can reduce the mortality rate in Javaen barb juveniles. This is presumably because melatonin controls homeostasis, the body's immune system and suppresses stress levels in fish, thereby reducing the mortality rate (Gharaei *et al.*, 2020; Ngasainao & Lukram, 2016; Hasan *et al.*, 2014). This condition is also supported by observing blood profiles in Javaen barb juveniles (Table 2), where blood hematological concentrations showed that treatment with melatonin supplementation of 0.4 and 0.6 mg/100 g of feed had higher total leukocyte and hemoglobin values. Leukocyte and hemoglobin concentrations in fish can be used to identify stress levels due to environmental conditions. An increase in leukocyte values indicates that melatonin can increase non-specific immunity in Javaen barb juveniles, while an increase in hemoglobin indicates a physiological response strategic aimed at increasing the absorption of dissolved oxygen in the blood (Gharaei *et al.*, 2020; Eslamloo *et al.*, 2014).

In the immune system, melatonin has been observed to affect the development of the thymus and spleen glands which are the main organs of the immune system (Ngasainao & Lukram, 2016; Carrillo-Vico *et al.*, 2005). In vitro and in vivo, melatonin regulates innate and adaptive immune responses. Research by Kepka *et al.* (2015) reported that melatonin stimulation can directly response to leukocytes in common carp *Cyprinus carpio*. Administration of melatonin in vitro and in vivo (2 mg/mL melatonin @ 1 mL/20 g body weight) in common carp *Cyprinus carpio* (60-70 g) was able to induce a decrease in respira-

tory burst on inflammatory leukocytes and a reduction in the number of neutrophils (in vivo) during zymosan-induced peritonitis. The results of this study indicate that in common carp, melatonin performs important pleiotropic and extra-pineal functions in maintaining the pro- and anti-inflammatory balance during infection.

### Blood cortisol hormone profile

The cortisol hormone profile in the blood plasma of Javaen barb supplemented with melatonin for 180 days of rearing is presented in Figure 2. The measurement results showed no significant difference ( $P > 0.05$ ) in all treatments. Cortisol hormone concentration values ranged from 1.46 to 1.56 ng/mL.

Melatonin supplementation also did not cause the cortisol concentration in the blood plasma of Javaen barb juveniles to increase compared to the control treatment ( $P > 0.05$ ) (Figure 2), meaning that the condition of the fish during the rearing process was still under control. In fish, cortisol is released as a primary response to an environmental stress factor, overcrowding, handling, and inappropriate ingested feed. An increase in cortisol can prompt a rise in energy-demanding processes, which adversely impact growth, reproductive performance, neurogenesis, and the immune system (Gharaei *et al.*, 2020; Conde-Sieira *et al.*, 2018; Näslund *et al.*, 2013). The role of melatonin in the development of Javaen barb fish farming has been confirmed to increase the adaptive properties of fish, but in terms of fish growth parameters, further studies are still needed. The number of doses and the type of species will affect the level of effectiveness of melatonin on growth performance.

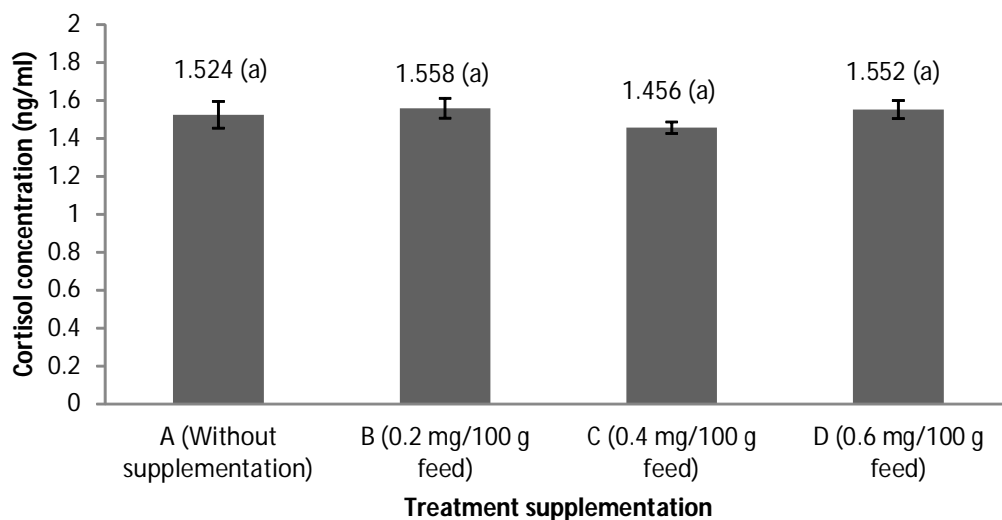


Figure 2. Cortisol hormone profile in the blood plasma of Javaen barb fish fed diet supplemented with melatonin for 180 days of rearing.

## CONCLUSIONS

Melatonin supplementation at a dose of 0.6 mg/100 g of feed provided the best production performance in Javaen barb fish juveniles with the highest survival value.

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