

Cannibalism control of Asian seabass *Lates calcarifer* fry by melatonin hormone administration

Pengendalian kanibalisme benih ikan kakap putih *Lates calcarifer* melalui pemberian hormon melatonin

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ABSTRACT

Cannibalism is one of the causes low survival rate white snapper fry due to its aggressiveness. Melatonin is a hormone that can modulate aggressive interactions in fish. This study aims to evaluate effect of melatonin hormone on the level cannibalism sea bass. This study used completely randomized design (CRD) consisting of four treatments and three replications, namely control (without melatonin hormone administration), melatonin doses of 5, 10, and 15 µg/g fish. Hormone administration was done orally through feed supplementation. Sea bass fry used were 2.82 ± 0.41 cm average length and 0.53 ± 0.08 g average weight. Stocking density was 2 fish/L. Sea bass were reared for 30 days with flow through system and fed three times a day in restricted manner (Feeding rate 15%). The results showed the administration of melatonin hormone can reduce level cannibalism, increase melatonin hormone levels, increase estradiol 17β hormone, and have no effect on body glucose levels sea bass. Melatonin dose 10 µg/g fish can reduce level cannibalism by 40.67% ($P < 0.05$). The highest total cannibalism was found in the control treatment with a value of 58.67% ($P < 0.05$). The administration of melatonin hormone did not affect the growth body weight and length of sea bass ($P > 0.05$). Increasing dose melatonin beyond 10 µg/g fish tends to increase cannibalism. These results indicate the administration of melatonin hormone at a dose of 10 µg/g fish is the optimal dose to reduce cannibalism and increase fry survival, which is expected to increase the productivity of sea bass hatcheries.

Keywords: aggressiveness, Asian seabass, cannibalism, melatonin

ABSTRAK

Kanibalisme merupakan salah satu penyebab rendahnya tingkat kelangsungan hidup benih kakap putih akibat sifat agresivitasnya. Hormon melatonin merupakan hormon yang dapat memodulasi interaksi agresif pada ikan. Penelitian ini bertujuan untuk mengevaluasi pengaruh hormon melatonin terhadap tingkat kanibalisme ikan kakap putih. Penelitian ini menggunakan rancangan acak lengkap (RAL) yang terdiri dari empat perlakuan dan tiga kali ulangan, yaitu kontrol (tanpa pemberian hormon melatonin), melatonin dosis 5, 10, dan 15 µg/g ikan. Pemberian hormon dilakukan secara oral melalui suplementasi pakan. Benih kakap putih yang digunakan berukuran panjang rata-rata $2,82 \pm 0,41$ cm dan berat rata-rata $0,53 \pm 0,08$ g. Padat tebar yaitu 2 ekor/L. Benih kakap putih dipelihara selama 30 hari dengan sistem flow through dan pemberian pakan sebanyak tiga kali sehari secara restricted (Feeding rate 15%). Hasil penelitian menunjukkan bahwa pemberian hormon melatonin dapat menurunkan tingkat kanibalisme, meningkatkan kadar hormon melatonin, cenderung meningkatkan hormon estradiol 17β, dan tidak berpengaruh terhadap kadar glukosa tubuh benih kakap putih. Melatonin dosis 10 µg/g ikan dapat mengurangi tingkat kanibalisme sebesar 40,67% ($P < 0,05$). Total kanibalisme tertinggi terdapat pada perlakuan kontrol dengan nilai sebesar 58,67% ($P < 0,05$). Pemberian hormon melatonin tidak berpengaruh terhadap pertumbuhan bobot dan panjang tubuh benih kakap putih ($P > 0,05$). Peningkatan dosis melatonin melebihi 10 µg/g ikan cenderung meningkatkan kanibalisme. Hasil ini menunjukkan bahwa pemberian hormon melatonin dosis 10 µg/g ikan merupakan dosis optimal untuk mengurangi kanibalisme dan meningkatkan kelangsungan hidup benih, sehingga diharapkan dapat meningkatkan produktivitas pembenihan kakap putih.

Kata Kunci: agresivitas, kakap putih, kanibalisme, melatonin



INTRODUCTION

The Asian seabass (*Lates calcarifer*) is one of the most important commercial fish species, widely cultivated across Southeast Asia, Australia, the Middle East, and the Americas (Terence *et al.*, 2021). This species is known by several common names, such as *barramundi* in Australia and *Asian seabass* in Southeast Asia (Haque *et al.*, 2021). Its distribution is extensive, covering the Western Indo-Pacific region, including India, Pakistan, Myanmar, Sri Lanka, Malaysia, Indonesia, the Philippines, Papua New Guinea, the Persian Gulf, Northern Australia, and Southern China (Haque *et al.*, 2021). Indonesia is one of the major exporters of Asian seabass, with substantial market demand (DJPB, 2018). According to data from Statistics Indonesia (BPS, 2018), the total export value of Asian seabass in 2017 reached USD 37,136, representing an increase of 69.58% compared to 2016, which amounted to USD 21,897. Furthermore, the Ministry of Marine Affairs and Fisheries (DJPB, 2022) reported that national seabass production in 2022 reached 10,364 tons, an increase from 9,583 tons in 2017.

These data indicate a modest increase in Asian seabass production, averaging 7.27%. The market price of Asian seabass reaches approximately IDR 80,000 per kilogram (Yuniarti *et al.*, 2022). The species is widely cultured due to its favorable biological and economic characteristics (Astuti *et al.*, 2023), including relatively rapid growth (Linayati *et al.*, 2025), high nutritional value (Nurmasyitah *et al.*, 2018), and broad environmental tolerance (Rayes *et al.*, 2013). However, challenges persist in the hatchery phase of Asian seabass aquaculture. One of the major issues is the low survival rate of juvenile fish (Mainisa *et al.*, 2021). This problem arises primarily due to the species' strong natural cannibalistic behavior, which is difficult to control and constitutes a major cause of mortality among fish measuring 2–7 cm (Pham *et al.*, 2020), resulting in a survival rate of only about 28% (Juharni *et al.*, 2022).

Cannibalism is defined as the act of consuming all or part of individuals of the same species (Duk *et al.*, 2017). According to Mukai *et al.* (2015), cannibalism in fish is regulated by physiological, morphological, and behavioral factors. Several factors contribute to the occurrence of cannibalism in fish, including size variation (Xi *et al.*, 2016), feeding regime (Ribeiro & Qin, 2016), stocking density (Waluyo *et al.*, 2024), light

intensity (Heltonika & Karsih, 2017; Pati *et al.*, 2023), environmental conditions (Pereira *et al.*, 2017), elevated testosterone levels (Siregar *et al.*, 2021; Heltonika, 2023; Putri, 2019a), and genetic factors (Liu *et al.*, 2017). Based on predation patterns, cannibalism can be categorized into two types: Type I and Type II (Xi *et al.*, 2017).

Type I cannibalism involves predation starting from the tail and sometimes leaving the head intact, typically occurring among fish of similar size. Type II cannibalism involves consuming the entire body beginning from the head, generally occurring between individuals of different sizes. Several studies have been conducted to control cannibalism in Asian seabass, including approaches such as size grading and feeding management (Kumar *et al.*, 2016), photoperiod manipulation (Badruzzaman *et al.*, 2021), the use of shelters for hiding (Nazlia *et al.*, 2021), and varying stocking densities (Suresh *et al.*, 2018; Khan *et al.*, 2021). However, these efforts have not yet achieved optimal reduction in cannibalism rates, indicating the need for alternative strategies to mitigate this issue in Asian seabass culture. A thorough understanding of the underlying causative factors is essential to achieve effective control outcomes. One potential approach to mitigating cannibalism in fish is through hormonal manipulation, particularly via the administration of exogenous hormones. Endocrinology plays a critical role in regulating aggressive behavior, which is closely associated with the occurrence of cannibalism (Vallon *et al.*, 2016).

The hormone used in this study is melatonin. Melatonin is a lipophilic compound derived from the amino acid tryptophan (Maitra & Hasan, 2016). In fish, melatonin is synthesized primarily in the pineal organ (Kulczykowska *et al.*, 2018) and the retina (Takahashi & Ogiwara, 2021). This hormone plays a crucial role in regulating circadian rhythms, reproductive cycles, feed intake, and growth in fish (Falcón *et al.*, 2007). Additionally, melatonin is known to act as a sedative agent (Acharyya *et al.*, 2021), and therefore, it is expected to reduce aggressive behavior in Asian seabass (*Lates calcarifer*). Previous studies have demonstrated the influence of melatonin on fish aggression. Munro (1986) observed that administering melatonin at a dose of 10 µg/µL sample effectively reduced aggressive behavior in *Aequidens pulcher*. Similarly, Amaral *et al.* (2020) reported a decrease in aggressiveness in *Brycon amazonicus* treated with melatonin at a concentration of 1 µmol/L.

Cannibalism and aggressiveness are closely interrelated behavioral phenomena (Muntaziana *et al.*, 2017). However, the use of melatonin as a means of controlling cannibalism in Asian seabass has not yet been explored. Therefore, this study is expected to contribute new insights and references for the development of strategies to control cannibalism in *L. calcarifer*. This study aims to analyze and evaluate the effect of melatonin hormone administration on the cannibalism rate of Asian seabass (*Lates calcarifer*).

MATERIALS AND METHODS

Experimental design

This study employed a completely randomized design (CRD) consisting of four treatments including a control group (without melatonin administration) and three melatonin-supplemented groups at doses of 5, 10, and 15 µg/g body weight. Every treatment has three replications.

Preparation of experimental tanks and test fish

The experimental units consisted of circular tanks with a diameter of 30 cm and a water volume of 25 L. Each tank was thoroughly cleaned, disinfected using chlorine, rinsed with clean water, and equipped with a single aeration point. The seawater used was aged for three days, sterilized with 15 ppm calcium hypochlorite (Ca (OCl)₂), and adjusted to a salinity range of 29–32 ppt. The test organisms were Asian seabass (*Lates calcarifer*) juveniles with an average total length of 2.82 ± 0.41 cm and an average body weight of 0.53 ± 0.08 g, stocked at a density of 2 fish/L. A total of 600 fish were obtained from the Marine Aquaculture Center, Batam. Initial weight sampling was conducted to determine the appropriate melatonin dosage for each treatment group.

Preparation of hormone and experimental feed

The hormone used in this study was melatonin (C₁₃H₁₆N₂O₂) in powdered form with a white crystalline appearance. Melatonin treatments were administered orally via feed. The feed used was a commercial pellet diet (Otohime brand) containing 50% protein, 10% lipid, 3% fiber, 16% ash, 2.3% calcium, and 1.5% phosphorus.

Melatonin was incorporated into the feed using the coating method with 1% carboxymethyl cellulose (CMC) as a binder. The coating process consisted of the following steps. The melatonin

powder was weighed according to the designated dose and placed into a test tube. Distilled water was added to the tube, and the solution was homogenized using a vortex mixer until clear, followed by dilution with 100 mL of distilled water per kg of feed. The melatonin solution was evenly sprayed onto the feed pellets and allowed to stand for 20 minutes. The feed was then air-dried at room temperature for 12 hours. The dried feed was stored in a sealed container at –20°C until use. Control feed was prepared using the same procedure without the addition of melatonin.

Rearing procedure

Asian seabass juveniles were reared for 30 days under controlled conditions. Fish were fed three times daily (morning, midday, and afternoon) using a restricted feeding regime with a feeding rate (FR) of 15% body weight per day. Sampling of total length and individual body weight was conducted every 10 days. Water exchange was performed daily, replacing 50% of the total tank volume. Mortality was recorded daily, and dead fish were removed promptly without replacement.

Observation and collection of dead fish

Observations were conducted daily from day 1 of the rearing period. Monitoring included both non-cannibalistic and cannibalistic mortality. Each dead fish sample was examined for external injury marks to determine the cause of death. Fish exhibiting potential cannibalistic behavior were identified based on individuals measuring 2–3 times larger than the average size of their tank mates. Aggressive behavior observations were carried out daily, specifically three minutes before and after feeding, to assess changes in aggression levels associated with feeding activity.

Analysis of melatonin, estradiol-17β, and blood glucose levels

Sampling for hormone assays was performed on days 0, 15, and 30 of the rearing period. Each fish sample was weighed, then placed into a tube and homogenized until fully crushed. Phosphate-buffered saline (PBS, pH 7.4) was added to the homogenate at a ratio of 1:4 for hormone analysis and 1:2 for glucose analysis. The homogenized samples were then centrifuged at 5000 rpm for 10 minutes at 4°C. The resulting clear supernatant was carefully collected using a micropipette and transferred into clean tubes. The supernatant samples were subsequently stored at –20°C until further analysis (Kusuma, 2021).

Measured parameter

Total cannibalism

Cannibalism was observed and recorded daily, and the total number of cannibalistic events was summed at the end of the experiment. The total cannibalism rate was calculated using the formula proposed by Obirikorang *et al.* (2014) as follows:

$$\text{Cannibalism (\%)} = \frac{\text{number of fish lost}}{\text{total number of fish}} \times 100$$

Cannibalism potential

The potential for cannibalism was assessed on days 0, 15, and 30 of the rearing period. Fish were considered potential cannibals if their body weight was 2 to 3 times greater than the average body weight of the population at the end of the rearing period (Król & Zakęś, 2015). The cannibalism potential was calculated using the following formula.

$$\text{Cannibalism potential (\%)} = \frac{\text{number of fish identified as potential cannibal}}{\text{total number of fish at the end of experiment}} \times 100$$

Types of cannibalism

The types of cannibalism were observed daily throughout the rearing period. Based on the extent of body damage, cannibalism was categorized into two types (Król & Zakęś, 2015). Type I is partial cannibalism, the damage occurs on specific body parts such as the tail, abdomen, or head, or only a portion of the body is consumed. Meanwhile Type II is complete cannibalism, the entire body of the fish is consumed or devoured. The percentage of each cannibalism type was calculated using the following formula.

$$\text{Type I (\%)} = \frac{\text{number of injured dead fish}}{\text{total number of fish}} \times 100$$

$$\text{Type II (\%)} = \frac{\text{number of fish lost due to cannibalism}}{\text{total number of fish}} \times 100$$

Survival rate

The survival rate was calculated at the end of the experiment to determine the proportion of fish that survived during the rearing period. The calculation followed the method described by Nazar (2022), as shown below.

$$\text{Survival rate (\%)} = \frac{\text{total number of fish at the end of experiment}}{\text{total number fish st the beginning of experiment}} \times 100$$

Absolute length and weight growth

The absolute growth in length and weight was measured on days 0, 15, and 30 of the rearing periods. Calculations were performed following the formula by Effendie (1997) as follows.

$$\text{Absolute length} = L_t - L_0$$

$$\text{Absolute weight} = W_t - W_0$$

Note:

- L_t = Average total length of fish at the end of the rearing period (cm)
- L₀ = Average total length of fish at the beginning of the rearing period (cm)
- W_t = Average body weight of fish at the end of the rearing period (g)
- W₀ = Average body weight of fish at the beginning of the rearing period (g)

Specific growth rate in weight and length

The specific growth rate (SGR) for both body weight and total length was measured on days 0, 15, and 30 of the rearing period. The calculation followed the formula proposed by Huisman (1987) as follows.

$$\text{Specific growth rate in weight (\%/day)} = \frac{\text{Ln}W_t - \text{Ln}W_0}{t} \times 100$$

$$\text{Specific growth rate in length (\%/day)} = \frac{\text{Ln}L_t - \text{Ln}L_0}{t} \times 100$$

Note:

- W_t = Average body weight at the end of the observation period (g)
- W₀ = Average body weight at the beginning of the observation period (g)
- L_t = Average total length at the end of the observation period (cm)
- L₀ = Average total length at the beginning of the observation period (cm)
- t = Duration of rearing period (days)

Coefficient of length variation

The coefficient of length variation was calculated following the method described by Nazar (2022) using formula below.

$$\text{Coefficient of length variation (\%)} = \frac{\text{Standard deviation of fish length}}{\text{Mean total length of fish}} \times 100$$

Measurement of melatonin hormone levels

Melatonin hormone levels were measured using a Cortisol Kit (EIA 1887) following the enzyme-linked immunosorbent assay (ELISA) method. The concentration of melatonin hormone

was determined using the MPM 6/Gen 5 software for data processing and analysis.

Measurement of body glucose levels

Glucose concentration was measured using a spectrophotometer at a wavelength of 500 nm with the GluL 1000 Kit (PLIVA-Lachema; Czech Republic). The glucose concentration was then calculated using the following formula:

$$\text{Glucose concentration (mg/dL)} = \frac{A_s}{A_{st}} \times C_{st}$$

Note:

- A_s = Absorbance of the sample
 A_{st} = Absorbance of the standard solution
 C_{st} = Concentration of the standard solution (mg/dL)

RESULTS AND DISCUSSION

Result

According to the conducted study, the results of the cannibalism parameters in Asian seabass (*Lates calcarifer*), including total cannibalism, cannibalistic potential, types of cannibalism, non-cannibalistic mortality, and survival rate are presented in Table 1. The results indicated that total cannibalism, cannibalistic potential, and type

II cannibalism were highest in the control group (K) ($P < 0.05$). This finding suggests that melatonin hormone administration effectively reduced the occurrence of cannibalism as well as the potential for cannibalistic behavior in Asian seabass. The type I cannibalism values among treatments did not differ significantly ($P > 0.05$). The lowest survival rate was observed in the control group (K) ($P < 0.05$), indicating that melatonin hormone supplementation also contributed to improved survival of Asian seabass juveniles.

The growth performance parameters of Asian seabass (*Lates calcarifer*), including final weight, final length, absolute weight gain (AWG), absolute length gain (ALG), specific growth rate by weight (SGRw), specific growth rate by length (SGRI), and The coefficient of length variation are presented in Table 2. The results after 30 days of rearing showed no significant differences among treatments ($P > 0.05$). This indicates that the administration of hormonal treatments had no significant effect on the growth performance of Asian seabass. Based on observations during the experiment, three modes of predation were identified in Asian seabass (*Lates calcarifer*) as shown in the figures below. This predatory behavior was rarely observed and was typically found in dead fish specimens. It assumed that

Table 1. The parameters of cannibalism in Asian seabass (*Lates calcarifer*).

Parameters (%)	Treatments			
	K	M5	M10	M15
Total cannibalism	58.67 ± 9.87 ^b	22.00 ± 5.29 ^a	18.00 ± 2.00 ^a	31.33 ± 19.01 ^a
Cannibalistic potential	18.97 ± 14.91 ^b	2.08 ± 3.61 ^a	0.00 ± 0.00 ^a	2.38 ± 4.12 ^a
Type I cannibalism	11.33 ± 1.17 ^a	15.33 ± 9.02 ^a	13.33 ± 5.77 ^a	12.67 ± 9.87 ^a
Type II cannibalism	47.33 ± 11.02 ^b	6.67 ± 4.16 ^a	4.67 ± 4.16 ^a	18.67 ± 20.23 ^a
Survival rate	41.33 ± 9.87 ^a	78.00 ± 5.29 ^b	82.00 ± 2.00 ^b	68.67 ± 19.01 ^b

Table 2. The growth performance in Asian seabass (*Lates calcarifer*).

Parameters	Treatments			
	K	M5	M10	M15
Initial weight (g/fish)	0.52 ± 0.08 ^a	0.56 ± 0.09 ^a	0.61 ± 0.09 ^a	0.60 ± 0.03 ^a
Final weight (g/fish)	4.11 ± 0.21 ^a	4.23 ± 0.82 ^a	4.82 ± 0.80 ^a	4.52 ± 0.50 ^a
Final length (cm/fish)	6.64 ± 0.63 ^a	6.36 ± 0.43 ^a	6.61 ± 0.21 ^a	6.47 ± 0.25 ^a
AWG (g/fish)	3.59 ± 0.28 ^a	3.60 ± 0.74 ^a	4.21 ± 0.75 ^a	3.92 ± 0.48 ^a
ALG (cm/fish)	3.71 ± 0.71 ^a	3.40 ± 0.37 ^a	3.54 ± 0.21 ^a	3.38 ± 0.28 ^a
SGRw (%/day)	11.97 ± 0.95 ^a	12.01 ± 2.45 ^a	14.03 ± 2.51 ^a	13.07 ± 1.60 ^a
SGRI (%/day)	12.38 ± 2.36 ^a	11.34 ± 1.23 ^a	11.79 ± 0.70 ^a	11.27 ± 0.92 ^a
The coefficient of length (%)	28.77 ± 1.60 ^a	29.11 ± 0.91 ^a	28.58 ± 0.43 ^a	28.86 ± 0.51 ^a

the fish had already died prior to being bitten by others. The abdominal part was presumed to be targeted because it is the softest part of the body compared to other parts.

The predatory attack usually begins at the head part, particularly targeting the eyes. After being attacked, the victim fish becomes stressed and eventually dies. This behavior commonly occurs among fish of similar size. This predatory behavior represents an unsuccessful attempt by the predator fish to swallow its prey. It typically occurs between individuals of slightly different sizes, where the predator is unable to ingest the prey completely. In some cases, the predator successfully regurgitates the prey and survives, although the prey ultimately dies. In contrast, as shown in Figure 3, the predator fish fails to regurgitate its prey, leading to respiratory distress and eventual death.

Based on the results of the present study, the concentrations of estradiol-17 β , body glucose, and melatonin hormones in barramundi (*Lates calcarifer*) were determined. The estradiol-17 β concentration values are presented in Figure 4. Hormonal fluctuations were observed in treatments K, M10, and M15, while treatment M5 showed a continuous increase from day 0 to day 30. The estradiol-17 β hormone levels showed no significant differences among treatments ($P>0.05$), indicating that melatonin administration did not affect estradiol-17 β concentrations in juvenile barramundi. The glucose levels are presented in Figure 5. There are no significant differences among treatments ($P>0.05$), indicating that melatonin administration did not cause an increase in body glucose levels in juvenile barramundi (*Lates calcarifer*).

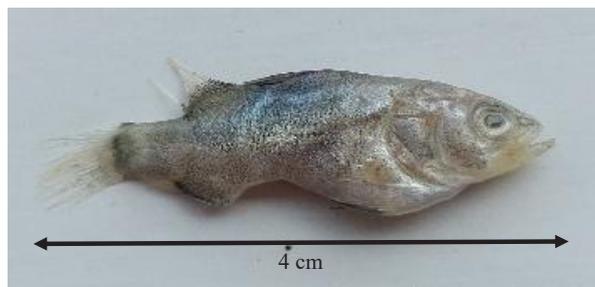


Figure 1. Cannibalism victim fish showing bite marks on the abdominal part (Type I cannibalism).



Figure 2. Cannibalism victim fish attacked in the eye region (Type II cannibalism).



Figure 3. Predation starting from the head part then extending to the entire body (Type III cannibalism).

The melatonin hormone levels of barramundi (*Lates calcarifer*) are presented in Figure 6. A significant increase in hormone concentration was observed in treatments M5, M10, and M15 ($P < 0.05$). This finding indicates that melatonin supplementation can enhance the melatonin hormone levels in barramundi juveniles.

Discussion

Cannibalism is one of the major factors contributing to high mortality rates in barramundi (*Lates calcarifer*), particularly among individuals measuring 2–7 cm in length (Pham *et al.*, 2020). Based on this size range, the initial size of fish used in the present study was 2–3 cm. After 30 days of rearing, a significant difference in total

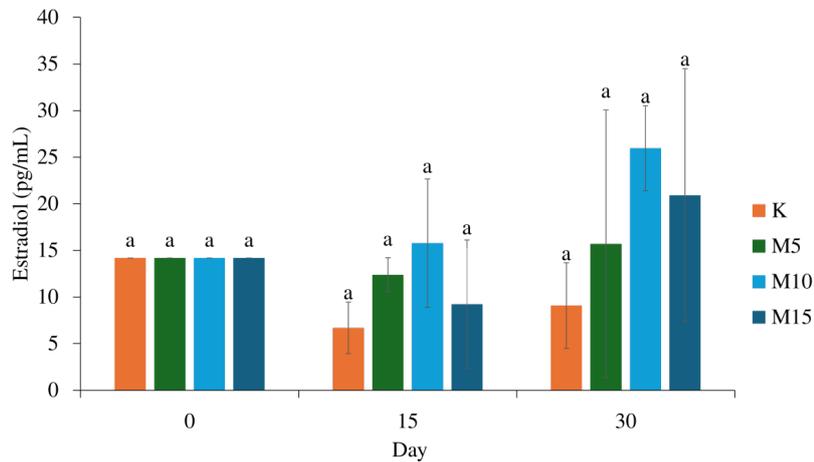


Figure 4. Estradiol-17 β hormone levels in barramundi (*Lates calcarifer*).

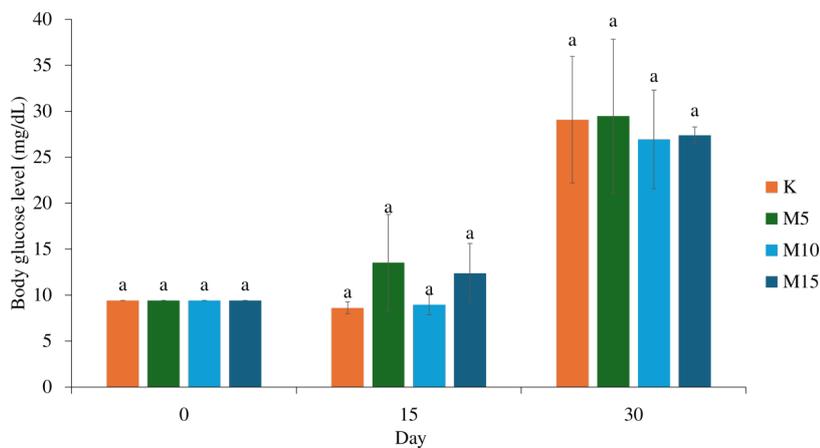


Figure 5. Fish glucose level in the body.

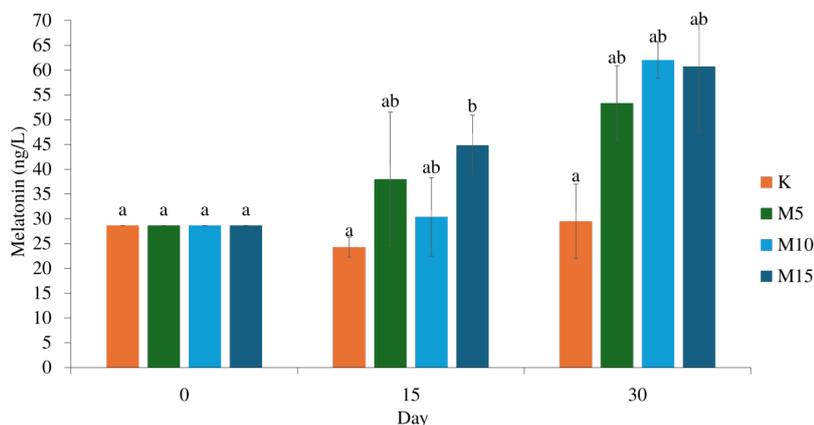


Figure 6. Melatonin hormone levels in barramundi (*Lates calcarifer*).

cannibalism was observed among treatments. The administration of melatonin hormone at the M10 dosage resulted in a 40.67% reduction in cannibalism compared to the control treatment ($P < 0.05$).

The total rate of cannibalism was inversely proportional to survival rate; as cannibalism increased, survival decreased, and vice versa. The M10 treatment improved the survival rate of barramundi by 40.67%. This finding indicates that melatonin supplementation at specific doses can effectively reduce cannibalistic behavior and enhance the survival of barramundi. The reduction in cannibalism is likely due to elevated melatonin levels in the body, which reduce aggressive behavior in the fish. This observation is consistent with the findings of Munro (1986) and Amaral *et al.* (2020).

Based on the measurement results of estradiol 17- β hormone levels, no significant differences were found among treatments ($P > 0.05$). However, the highest estradiol 17- β concentration was observed in the M10 treatment. Estrogen is a hormone directly involved in the brain's neuroendocrine system, influencing both reproductive behavior and aggressiveness in fish (Heltonika *et al.*, 2023). The elevated estradiol 17- β levels in the M10 group suggest lower aggressiveness compared to other treatments. This trend was consistent with melatonin hormone measurements. Significant differences in melatonin levels were observed among treatments on days 15 and 30 of the rearing period, with the highest concentration recorded in the M10 treatment on day 30. These results further support that melatonin administration (M10) can effectively reduce aggressive behavior in barramundi (*Lates calcarifer*).

Cannibalistic behavior leads to size variation among fish, resulting in the presence of larger individuals that are more likely to attack or prey upon smaller ones. This occurs because predatory fish tend to grow faster when they successfully consume conspecifics. The results of this study showed that the M10 treatment exhibited the lowest cannibalism potential. According to Pham *et al.* (2020), barramundi (*Lates calcarifer*) became cannibalistic when size differences reach approximately 50%, and when body size differences range between 22–28% (Ribeiro & Qin, 2013). In the present study, barramundi exhibited cannibalistic behavior when the size difference reached about 2 cm. Observations throughout the experiment revealed that fish

in the M10 treatment group displayed a more uniform size compared to other treatments. This uniformity likely contributed to the reduced cannibalism potential in the M10 group. Halawa (2023) also reported that size disparities among fish can increase the incidence of cannibalistic behavior.

Differences in body size can also determine the type of cannibalism exhibited (Nazar, 2022). Based on feeding behavior, cannibalism can be classified into two types: Type I and Type II (Xi *et al.*, 2017). Type I cannibalism occurs when the attack starts from the tail and sometimes leaves the head intact, typically observed among fish of similar sizes. Type II cannibalism involves complete consumption of the body, beginning from the head, and usually occurs among fish of different sizes. In the present study, Type II cannibalism predominated, with the highest incidence observed in the control group (47.33%). Meanwhile, in Type I cannibalism among treatments were no significant differences. These findings are consistent with those of Khan *et al.* (2021), who reported that Type II cannibalism is the dominant form observed in barramundi (*Lates calcarifer*).

Growth performance in barramundi (*Lates calcarifer*) showed no significant differences among treatments. Administration of melatonin hormone treatments did not affect growth performance. However, these findings also indicate that melatonin supplementation did not exert any negative effects on fish health, suggesting that the fish remained in normal physiological condition with no reduction in feeding activity. This is further supported by the glucose level data, which showed no significant differences among treatments after 30 days of rearing ($P > 0.05$).

Blood glucose levels are often used to assess the physiological response of fish to experimental treatments (Rahmadiyah, 2018). Glucose concentration can serve as an indicator of stress induced by environmental changes and generally increases when fish expose to stress (Putri, 2019b). In addition, glucose levels can also reflect energy utilization from metabolic processes that regulate neuronal and neurotransmitter activity, which influence fish behavior patterns (Howarth *et al.*, 2012).

Melatonin administration in barramundi juveniles can be applied as a strategy to reduce cannibalism arising from aggressive behavior, thereby improving hatchery productivity.

Melatonin supplementation may also be beneficial for other fish species exhibiting high aggressiveness; however, further research is needed to evaluate its effectiveness across different species.

CONCLUSION

The supplementation of melatonin hormone in the diet reduced cannibalism levels by 40.67% and did not affect the growth performance of barramundi (*Lates calcarifer*) juveniles, with the optimal dosage being 10 µg of melatonin per gram of fish.

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