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Evaluation of dietary administration of oxitriptan to control cannibalism in juvenile Asian seabass *Lates calcarifer*

Evaluasi pemberian oxitriptan melalui pakan terhadap pengendalian kanibalisme benih ikan kakap putih *Lates calcarifer*

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ABSTRACT

Cannibalism is a clear example of a predatory trait between similar species in a population that is triggered by aggressiveness. This study definitively shows that increasing serotonin in the body can suppress aggressiveness. 5-HTP (oxitriptan) is the name of the substance used in the study to achieve this, as it acts as a precursor for forming serotonin. The serotonin pathway is a hormonal pathway that reliably reduces aggressive behaviour. The study will evaluate the effect of oxitriptan on cannibalism of juveniles sea bass. The research method was an experimental, completely randomised design, with 5 treatments and 3 replications for each. It did not include l-tryptophan and oxitriptan (K), 5% l-tryptophan/g fish (T5), 5 µg oxitriptan/g fish (O5), 10 µg oxitriptan/g fish (O10), and 15 µg oxitriptan/g fish (O15). The juveniles seabass used were 30 days old, with an average length of 3.48 ± 0.15 cm and an average weight of 1.00 ± 0.1 g. The juveniles are maintained in a flow-through system for 30 days, with a feeding rate of 15%. The results showed that oxitriptan supplementation reduced the level of cannibalism ($P<0.05$). The 10 µg (O10) treatment was undoubtedly the most effective, reducing cannibalism by 20.67 percentage points and improving survival rate by the same amount ($P<0.05$). Oxitriptan is the solution to reduce cannibalism and increase the survival of juveniles seabass.

Keywords: cannibalism, oxitriptan, seabass juvenile, survival rate

ABSTRAK

Kanibalisme merupakan sifat memangsa antara spesies sejenis dalam suatu populasi yang dipicu oleh agresivitas. Studi ini secara definitif menunjukkan bahwa peningkatan serotonin dalam tubuh dapat menekan agresivitas. 5-HTP (oksitriptan) adalah asam amino yang bertindak sebagai prekursor untuk membentuk serotonin. Jalur serotonin adalah jalur hormonal yang secara efektif mampu memodulasi perilaku agresif. Penelitian ini bertujuan untuk mengevaluasi efek oksitriptan terhadap kanibalisme dan tingkat kelangsungan hidup benih ikan kakap putih. Metode penelitian yang dilakukan adalah eksperimental, rancangan acak lengkap, terdiri dari 5 perlakuan dan 3 kali ulangan untuk masing-masing perlakuan. Tanpa pemberian triptofan dan oksitriptan(K), 5% l-triptofan/g ikan (T5), 5 µg oxitriptan/g ikan (O5), 10 µg oksitriptan/g ikan (O10), dan 15 µg oksitriptan/g ikan (O15). Benih kakap putih yang digunakan berumur 30 hari, dengan panjang rata-rata 3,48 ± 0,15 cm dan berat rata-rata 1,00 ± 0,1 g. Benih kakap putih dipelihara dalam sistem *flow through* selama 30 hari, dengan *feeding rate* sebesar 15%. Hasil penelitian menunjukkan bahwa suplementasi oksitriptan menurunkan tingkat kanibalisme dan meningkatkan tingkat kelangsungan hidup benih ($P<0,05$). Perlakuan 10 µg (O10) merupakan yang paling efektif, menurunkan kanibalisme hingga 20,67 serta meningkatkan tingkat kelangsungan hidup dengan persentase yang sama ($P<0,05$). Oxitriptan dapat digunakan sebagai solusi dalam mengurangi kanibalisme dan meningkatkan kelangsungan hidup benih kakap putih.

Kata kunci: kanibalisme, oxitriptan, benih kakap putih, tingkat kelangsungan hidup

INTRODUCTION

Asian seabass (*Lates calcarifer*) is a catadromous and euryhaline fish species widely distributed throughout the tropical and subtropical Indo-Pacific region, including Australia, the Philippines, and coastal areas surrounding the Arabian Sea (Solanki *et al.,* 2023). This species is highly valued due to its superior nutritional and commercial attributes, including high protein and omega-3 fatty acid contents, high economic value, rapid growth rate, and remarkable tolerance to environmental fluctuations (Nhan *et al.,* 2022). Under optimal culture conditions, Asian seabass can gain a body weight of approximately 2,000 g within one year (Hassan *et al.,* 2021). The increasing market demand for Asian seabass has intensified the need for continuous seed production, thereby promoting the expansion of aquaculture practices (Fitrinawati & Sri, 2023). The hatchery production remains constrained by the high incidence of cannibalism during the larval and juvenile stages, resulting in reduced seed production. In some cases, cannibalism has been reported to account for losses of up to 90% of the potential seed production (Inoue *et al.,* 2016). Furthermore, cannibalism contributes to approximately 1–2% of the total mortality in Asian seabass juveniles, depending on feed availability (Liu *et al.,* 2017).

Cannibalism is a form of intraspecific predation characterized by the consumption of conspecific individuals within the same population (Duk *et al.,* 2017). Based on feeding behavior, cannibalism can be classified into two major types. Type I involves partial predation, in which the predator consumes the prey beginning from the caudal region toward the body, whereas Type II involves complete ingestion of the prey from the tail to the head (Grześkiewicz & Fey, 2020). The factors triggering cannibalistic behavior can be categorized into endogenous and exogenous factors. Endogenous factors include genetic relatedness (Liu *et al.,* 2017), size heterogeneity among individuals (Król & Zakęś, 2016), and neuromodulatory or neurotransmitter activity (Naumowicz *et al.,* 2017). In contrast, exogenous factors include stocking density, water temperature, water transparency, light intensity, feed availability, and feeding frequency (Vallon *et al.,* 2016).

Several strategies have been investigated to reduce cannibalism in Asian seabass juveniles. Dietary supplementation with tryptophan has been explored as a nutritional approach to suppress cannibalistic behavior (Kumar *et al.,* 2017), while size grading at a stocking density of 0.03 individuals L^{-1} has been employed to minimize aggressive interactions among individuals (Khan *et al.,* 2021). These approaches have not yet produced optimal outcomes, indicating the need for more effective interventions. Cannibalistic behavior is associated with aggression, which is regulated by hormonal activity through neuromodulatory neurotransmitter pathways (Naumowicz *et al.,* 2017). Among these pathways, serotonin has consistently been identified as one of the principal modulators of aggressive behavior across vertebrate taxa (Hulse, 2017).

Serotonin plays a critical role in regulating stress responses, social dominance, reproductive behavior, and aggression (Courtiol *et al.,* 2021). Recent findings demonstrated that dietary supplementation with serotonin at a dose of 0.5 mg/g has significantly reduced cannibalism in catfish (Halawa, 2023). Serotonin, also known as 5-hydroxytryptamine (5-HT), is an endogenous autacoid that exerts a wide range of physiological and pharmacological effects even at very low concentrations (Backström & Winberg, 2017). Serotonin is synthesized in neurons using the essential amino acid tryptophan as its metabolic precursor (Park *et al.,* 2021). Its biosynthesis begins with the hydroxylation of tryptophan into 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase, followed by the decarboxylation of 5-HTP by aromatic L-amino acid decarboxylase to produce serotonin (5-HT) (Höglund *et al.,* 2019).

Previous studies have attempted to enhance endogenous serotonin production through dietary tryptophan supplementation to control cannibalism; however, survival rates remained relatively low (Kumar *et al.,* 2017). Therefore, alternative approaches involving oxitriptan supplementation warrant further investigation. The 5-Hydroxytryptophan (5-HTP) is commonly referred to as oxitriptan, is an aromatic amino acid naturally synthesized in the body

from the essential amino acid tryptophan (Maffei, 2021). Animal studies have shown that approximately 70% of administered 5-HTP is converted into serotonin, whereas only about 5% of administered tryptophan undergoes the same conversion (Höglund *et al.,* 2019). Furthermore, 5-HTP crosses the blood-brain barrier (BBB) more efficiently than tryptophan, with approximately 1 mg of oxitriptan estimated to yield 0.1–0.2 mg of serotonin, depending on individual metabolic capacity (Maffei, 2021). Despite these advantages, no previous studies have evaluated the effectiveness of oxitriptan in reducing aggression and cannibalistic behavior in fish. Therefore, the present study was conducted to evaluate the role of oxitriptan and determine its effective dosage for controlling cannibalism and improving the survival rate of Asian seabass juveniles.

MATERIAL DAN METHOD

Study site and experimental period

The experiment was conducted at the Batam Marine Aquaculture Center (Balai Perikanan Budidaya Laut Batam), Batam City, Riau Islands, Indonesia, from June to July 2023.

Experimental design

A completely randomized design was used, consisting of five dietary treatments with three replicates each: (1) control without tryptophan or oxitriptan supplementation (K), (2) 5% tryptophan/g fish (T5), (3) 5 µg oxitriptan/g fish (O5), (4) 10 µg oxitriptan/g fish (O10), and (5) 15 µg oxitriptan/g fish (O15). The dietary supplementation level of 5% tryptophan was adopted from Kumar *et al.* (2017), who reported this concentration as the most effective dosage. Accordingly, the tryptophan treatment served as a positive control for comparison in the present study. The oxitriptan dosages were selected based on the study of De Pedro *et al.* (1998), in which these concentrations significantly affected fish behavior, as indicated by reduced feed intake for up to 8 h.

A total 1,300 juveniles were available for the study, of which 750 were allocated to the experimental units (mean total length: 3.48 ± 0.15 cm; mean body weight: 1.00 ± 0.10 g) obtained from the Batam Marine Aquaculture Center were used in the experiment. Fish were stocked in plastic containers containing 25 L of water, each equipped with water inlet and outlet systems and continuous aeration. The stocking density was maintained at 2 fish/L, which is categorized as a high stocking density according to Khan *et al.* (2021). The fish were reared in a flow-through culture system with a water flow rate of 1.5 L/min for 30 days without size grading. Fish were fed three times daily (08:00, 11:00, and 14:00) at a feeding rate of 15% of total biomass (BBPBL, 2021). Body weight and total length were measured on Days 0 and 15 to adjust the dosage of dietary supplements according to fish biomass. Fifteen fish were randomly sampled for each measurement.

A commercial diet containing 54% crude protein was used throughout the experiment. Oxitriptan was incorporated into the feed using the coating method described by Kumar *et al.* (2017). Briefly, oxitriptan was homogenized using a vortex mixer, diluted with distilled water at a ratio of 100 mL/kg feed, and sprayed onto feed pellets previously coated with 1% carboxymethyl cellulose (CMC), which served as a binding agent between the feed and oxitriptan. The coated feed was air-dried at room temperature for 12 h and subsequently stored at 4°C until use. The same coating procedure was applied to the dietary tryptophan treatment.

Serum preparation for hormone and glucose analyses

Three fish from each treatment were randomly collected on Days 0, 15, and 30 for hormone and glucose analyses. Whole fish samples were homogenized using a mortar and pestle, followed by homogenization in phosphate-buffered saline (PBS; pH 7.4). The sample-to-buffer ratio was 1:4 (w/v) for hormone analysis and 1:2 (w/v) for glucose analysis. The homogenates were centrifuged at 5,000 rpm for 10 min at 4°C following the method described by Sukenda *et al.* (2017). The resulting supernatants were collected and stored at −20°C until biochemical analyses were performed.

Measured parameters

The evaluated parameters included cannibalism, growth performance, survival rate, hormone concentrations, glucose concentration, and water quality. Cannibalism assessment included total cannibalism, Type I cannibalism, Type II cannibalism, and survival rate. Growth performance was evaluated based on absolute weight gain, absolute length gain, specific

daily weight growth rate, daily length growth rate, length coefficient of variation, and size heterogeneity. Serotonin concentration was determined using a Fish Serotonin ELISA Kit (BZ-08062520-EB), whereas estradiol-17β concentration was measured using an Estradiol-17β ELISA Kit (DRG EIA 2693). Hormone analyses were performed using the enzyme-linked immunosorbent assay (ELISA) method. Glucose concentration was determined spectrophotometrically at a wavelength of 635 nm. Water quality parameters were monitored throughout the experimental period as supporting environmental variables.

Total cannibalism

Total cannibalism was defined as the cumulative number of juvenile fish that died as a result of both Type I and Type II cannibalism during the 30-day experimental period. Total cannibalism was calculated according to the formula described by Khan *et al.* (2021) as follows:

$$\text{Cannibalism (\%)} = \frac{\text{Number of missing or injured fish}}{\text{Total initial of fish}} \times 100$$

Cannibalism type

Type I and Type II cannibalism were quantified according to the equations described by Król and Zakęś (2016) as follows:

$$\text{Type I (\%)} = \frac{\text{Number of missing or injured fish}}{\text{Total initial of fish}} \times 100$$

$$\text{Type II (\%)} = \frac{\text{Number of fish completely consumed}}{\text{Total initial of fish}} \times 100$$

Survival rate

Survival rate was determined at the end of the experimental period according to the equation described by Rahmadiah *et al.* (2019) as follows:

$$\text{Survival rate (\%)} = \frac{\text{Total fish at the end of the study}}{\text{Total fish at the initial of the study}} \times 100$$

Absolute length and weight growth

Absolute length growth and absolute weight growth were calculated according to the equations described by Khan *et al.* (2021) as follows:

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

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

Notes:

L_t = Mean total length of fish at the end of the experimental period (cm)

L_0 = Mean total length of fish at the beginning of the experimental period (cm)

W_t = Mean body weight of fish at the end of the experimental period (g)

W_0 = Mean body weight of fish at the beginning of the experimental period (g)

Specific weight and length growth rates

The specific weight growth rate (SWGR) and specific length growth rate (SLGR) were calculated at the end of the experimental period according to the equations described by Rachmawati *et al.* (2021) as follows:

$$\text{Specific growth rate in weight (\%/day)} = \frac{\text{LnWt} - \text{LnW0}}{t} \times 100$$

$$\text{Specific growth rate in length (\%/day)} = \frac{\text{LnLt} - \text{LnL0}}{t} \times 100$$

Notes:

W_t = Mean body weight of fish at time *t* (g)

W_0 = Mean body weight of fish at the beginning of the experimental period (g)

t = Duration of the experimental period (days)

L_t = Average length of fish at the time t (cm)

L_0 = Mean total length of fish at time t (cm)

t = Mean total length of fish at the beginning of the experimental period (cm)

Coefficient of variation in length

The coefficient of variation (CV) of fish length was determined at the beginning and the end of the experimental period according to the equation described by Kumar *et al.* (2017) as follows:

$$\text{Coefficient of variation of fish length (\%)} = \frac{\text{Standard deviation of total body length}}{\text{Mean total body length at the beginning/end of the experimental periodof fish}} \times 100$$

Water quality

The water quality parameters observed in this study included temperature, pH, dissolved oxygen

(DO), and total ammonia nitrogen. Water quality measurements were conducted before and after each water exchange using a thermometer for temperature, a pH meter for pH, and a dissolved oxygen (DO) meter for dissolved oxygen. Total ammonia nitrogen was measured according to standard laboratory procedures.

Data analysis

Data on cannibalism and growth performance were tabulated using Microsoft Excel 2021 and subsequently analyzed by one-way analysis of variance (ANOVA) using IBM SPSS Statistics at a 95% confidence level. When significant differences among treatments were detected ($P<0.05$), Duncan's multiple range test was performed as a post hoc analysis at a significance level of $\alpha = 0.05$. Data on serotonin hormone profile, estradiol-17β concentration, blood glucose level, and water quality were analyzed descriptively.

RESULT AND DISCUSSION

Result

Cannibalism

The percentages of cannibalism and survival rate of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan, with tryptophan serving as a positive control, are presented in Table 1. Type I cannibalism differed significantly among treatments ($P<0.05$). The control treatment (K) exhibited a significantly higher percentage of Type I cannibalism than all other treatments, with the highest value recorded in the control group (18.00%) and the lowest in the O15 treatment (4.67%). In contrast, Type II cannibalism showed a different response pattern. The T5 treatment exhibited a significantly higher percentage ($P<0.05$) than all other treatments, reaching 24.67%, whereas the lowest percentage was observed in the O15 treatment (5.33%). Overall, the incidence of Type II cannibalism was greater than that of Type I, indicating that juvenile Asian seabass exhibit a high degree of cannibalistic behavior during the rearing period.

Total cannibalism, calculated as the cumulative incidence of Type I and Type II cannibalism, was significantly lower ($P<0.05$) in the oxitriptan-treated groups (O5, O10, and O15) than in the control treatments (K and T5). The lowest total cannibalism was recorded in the O15 treatment (10.00%), whereas the highest was observed in the untreated control (K), reaching 36.00%. Dietary supplementation with oxitriptan significantly reduced total cannibalism by 26 percentage points compared with the control treatment ($P<0.05$). The temporal patterns of Type I and Type II cannibalism throughout the experimental period are presented in Figure 1. Overall, the pattern of cannibalistic behavior was similar across all treatments. Cannibalism-induced mortality began on Day 4, reached its peak between Days 7 and 14, and subsequently declined from Day 21 until the end of the experiment (Day 30).

Growth performance

The growth performance of Asian seabass (*Lates calcarifer*) juveniles is presented in Table 2. Increasing dietary oxitriptan supplementation significantly affected absolute weight gain, specific weight growth rate, final body weight, and fish biomass ($P<0.05$). In contrast, absolute length gain, specific length growth rate, and final body length did not differ significantly among treatments ($P>0.05$). While no significant differences were observed in length-related growth parameters between the control and oxitriptan-treated groups. The control treatment showed the highest absolute weight gain and specific weight growth rate, although significant differences were observed only between the control and O10-O15 treatments for absolute weight gain and between the control O15 treatment for specific weight growth rate ($P<0.05$).

The final total length, biomass, and coefficient of variation in length (CVL) of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan are presented in Table 3. Oxitriptan supplementation resulted in significantly greater final total length and biomass than those observed in the control and tryptophan treatments ($P<0.05$). However, the coefficient of variation in length did not differ significantly among treatments ($P>0.05$).

Hormone and glucose concentrations

The serotonin concentrations of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan are presented in Figure 2. On Day 15, serotonin concentrations increased in the T5, O5, O10, and O15 treatments, whereas a decrease was observed in the control group (K). By Day 30, serotonin concentrations increased in the K, O10, and O15 treatments but declined in the T5 and O5 treatments. Although serotonin levels in the control group increased between Days 15 and 30, they remained lower

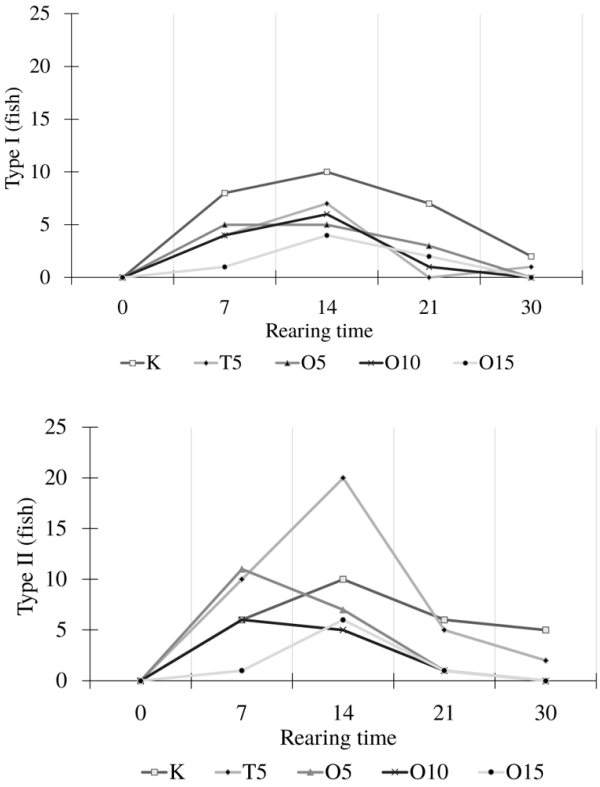

Figure 1. Temporal patterns of Type I and Type II cannibalism during the rearing period. K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

Table 1. Percentage of cannibalism, survival rate, and mortality of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan.

Treatments	Type I cannibalism	Type II cannibalism	Total cannibalism	**Survival rate**
K	18.00 ± 7.21^{b}	18.00 ± 12.49^{ab}	36.00 ± 5.29^{b}	64.00 ± 5.29^{a}
T5	8.00 ± 2.00^{a}	24.67 ± 10.26^{b}	32.67 ± 9.86^{b}	67.33 ± 9.86^{a}
O5	8.67 ± 3.05^{a}	12.67 ± 6.11^{ab}	21.33 ± 3.05^{a}	78.67 ± 3.05^{b}
O10	7.33 ± 1.15^{a}	8.00 ± 2.00^{a}	15.33 ± 1.15^{a}	84.67 ± 1.15^{b}
O15	4.67 ± 2.30^{a}	5.33 ± 4.61^{a}	10.00 ± 6.92^{a}	90.00 ± 6.92^{b}

Note: Different superscript letters within the same column indicate statistically significant differences among treatments ($P < 0.05$). K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

Table 2. Growth performance of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan.

Treatments	**PPM**	**PBM**	**LPSP**	**LPSB**
K	4.32 ± 0.21^{ab}	7.01 ± 1.03^{c}	2.64 ± 0.13^{ab}	6.91 ± 0.20^{b}
T5	4.22 ± 0.26^{a}	5.21 ± 0.62^{ab}	2.68 ± 0.07^{ab}	6.24 ± 0.31^{ab}
O5	4.79 ± 0.38^{b}	5.84 ± 0.50^{bc}	2.88 ± 0.11^{b}	6.30 ± 0.24^{ab}
O10	4.31 ± 0.15^{ab}	5.40 ± 0.36^{ab}	2.70 ± 0.21^{ab}	6.13 ± 0.71^{ab}
O15	3.82 ± 0.25^{a}	4.36 ± 0.73^{a}	2.47 ± 0.13^{a}	5.65 ± 0.67^{a}

Note: Different superscript letters within the same column indicate statistically significant differences among treatments ($P < 0.05$). PPM = absolute length gain; PBM = absolute weight gain; LPSB = specific weight growth rate; LPSP = specific length growth rate; K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

than those of the supplemented treatments throughout the experimental period. The highest serotonin concentration was recorded in the O15 treatment on Day 30 (172.49 ng/mL), whereas the lowest concentration was observed in the control treatment (160.25 ng/mL). These findings indicate that dietary supplementation with L-tryptophan or oxitriptan at appropriate doses can enhance serotonin concentrations in Asian seabass juveniles.

Estradiol-17β concentrations in Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan are presented in Figure 3. Overall, estradiol-17β concentrations increased from Day 15 to Day 30 in all treatments. However, the highest

Table 3. Coefficient of variation in length (CVL) and length heterogeneity of Asian seabass *(Lates calcarife*r) juveniles fed diets supplemented with different doses of oxitriptan.

Treatments	Total length (cm)	Final biomass (g)	Initial CV of length	Final CV of length
K	252.78 ± 24.43^{a}	257.96 ± 53.14^{ab}	11.12 ± 1.52^{a}	8.52 ± 0.36^{a}
T5	256.40 ± 36.15^{a}	205.98 ± 27.43^{a}	11.83 ± 1.49^{a}	10.98 ± 0.99^{a}
O5	352.12 ± 6.41^{b}	270.36 ± 14.56^{b}	10.15 ± 3.73^{a}	10.83 ± 1.78^{a}
O10	392.16 ± 7.05^{b}	272.51 ± 11.19^{b}	12.60 ± 2.60^{a}	10.54 ± 1.86^{a}
O15	328.77 ± 28.67^{b}	239.84 ± 28.46^{ab}	15.10 ± 3.02^{a}	11.46 ± 3.02^{a}

Note: Different superscript letters within the same column indicate statistically significant differences among treatments (P < 0.05). Initial CVL = initial coefficient of variation in length; Final CVL = final coefficient of variation in length; K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

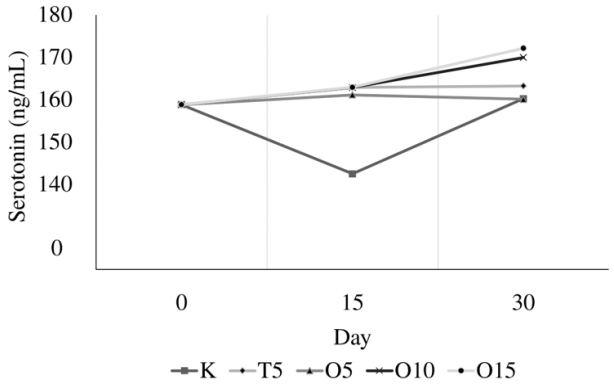

Figure 2. Serotonin concentrations in Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan. K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

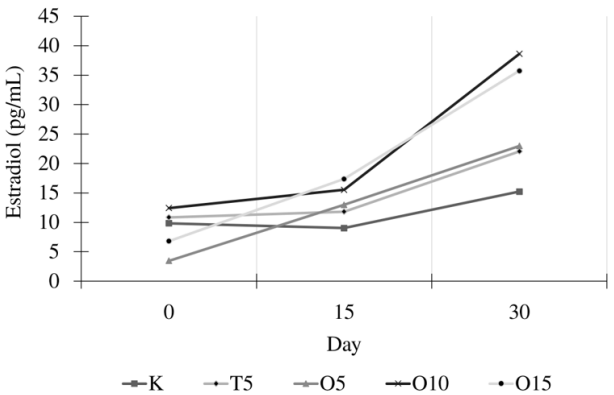

Figure 3. Estradiol-17β concentrations in Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan. K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

concentrations were observed in the O10 (10 µg oxitriptan/g fish) and O15 (15 µg oxitriptan/g fish) treatments, whereas the lowest concentration was recorded in the control group. These results indicate that dietary oxitriptan supplementation enhances estradiol-17β concentrations in Asian seabass juveniles.

Body glucose concentrations in Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan are presented in Figure 4. With the exception of the O15 treatment, in which glucose concentration decreased, body glucose concentrations increased in all treatments on Day 15 and subsequently showed a declining trend by Day 30. In contrast, body glucose concentrations increased on Day 30 in the O15 and T5 treatments; however, the glucose concentration in the O15 treatment remained lower than that observed in the T5 and O5 treatments. The water quality parameters recorded during the rearing of Asian seabass (*Lates calcarifer*) juveniles fed diets supplemented with different doses of oxitriptan are presented in Table 4. Throughout the experimental period, water quality remained relatively stable and showed no significant fluctuations. All measured parameters were within the acceptable ranges specified by the Indonesian National Standard (SNI, 2014) for Asian seabass seed production.

Discussion

The study demonstrated that oxitriptan (5-hydroxytryptophan; 5-HTP) supplementation effectively reduced cannibalism while improving the survival rate (SR) of Asian seabass (*Lates calcarifer*) juveniles. The optimal dose was 15 µg/g fish, which significantly decreased cannibalism by 26 percentage points and increased survival by 26 percentage points compared with the control treatment ($P<0.05$). In addition, oxitriptan supplementation increased circulating estradiol-17β concentrations. Otherwise, blood glucose levels exhibited variable responses, indicating that increased serotonin activity may either elevate or reduce glucose concentrations depending on physiological conditions.

Oxitriptan supplementation has been reported to increase serotonin concentrations in fish

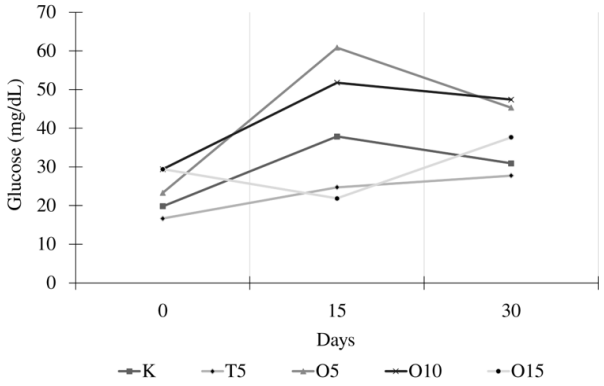

Figure 4. Body glucose concentrations in Asian seabass (Lates calcarifer) juveniles fed diets supplemented with different doses of oxitriptan. K = control (without tryptophan or oxitriptan supplementation); T5 = 5% L-tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

Table 4. Water quality parameters during the rearing of Asian seabass (Lates calcarifer) juveniles fed diets supplemented with different doses of oxitriptan.

Treatments	Temperature (ºC)	pH	DO (mg/L)	Salinity (ppt)
K	29.5-30.5	7.84-8.26	5.8-5.9	29
T5	29.5-30.9	7.72-8.16	5.8-6.2	29
O5	29.7-30.7	7.75-8.15	5.8-5.9	29
O10	29.7-30.7	7.73-8.09	5.6-5.9	29
O15	29.8-30.7	7.77-8.14	5.6-6.3	29
SNI 6145.4:2014	28 – 32	7,5 – 8,5	>4	28-33

Note: K = control (without tryptophan or oxitriptan supplementation); T5 = 5% tryptophan/g fish; O5 = 5 µg oxitriptan/g fish; O10 = 10 µg oxitriptan/g fish; and O15 = 15 µg oxitriptan/g fish.

(Maffei, 2021). Enhanced serotonergic activity in the brain suppresses aggressive behavior in vertebrates, thereby reducing the incidence of cannibalism (Sahu *et al.,* 2020). This mechanism likely explains the reduced cannibalism and the corresponding improvement in survival observed in the present study. Serotonin also plays an important role in emotional regulation and stress alleviation (Backström & Winberg, 2017). Serotonin exerts its effects primarily through 5-HT1A and 5-HT2A receptors, transmitting signals to target organs that promote relaxation and reduce stress responses (Höglund *et al.,* 2019).

The increase in estradiol-17β observed following oxitriptan supplementation may also have contributed to the reduction in aggressive behavior. Estradiol is known to exert dual effects on aggression in fish, acting either as an inhibitor or promoter depending on the receptor subtype involved (Indriastuti *et al.,* 2022). Estradiol binding to estrogen receptor α (ERα) enhances physiological responses associated with increased aggression, whereas binding to estrogen receptor β (ERβ) suppresses aggressive behavior (Cunningham *et al.,* 2012). Activation of ERβ stimulates tryptophan hydroxylase-2 (TPH2), the rate-limiting enzyme in serotonin synthesis (Heltonika, 2022). It produces antidepressant-like effects comparable to selective serotonin reuptake inhibitors (SSRIs) by enhancing serotonergic neurotransmission and reducing aggressive behavior (Hansen *et al.,* 2017). However, excessive estradiol concentrations may preferentially activate ERα, resulting in increased aggression.

Heltonika (2022) reported that an estradiol-17β concentration of 2.1 ng/mL increased cannibalism and reduced survival in Asian redtail catfish (*Hemibagrus nemurus*) larvae. The hormonal responses are influenced by brain differentiation, which should also be considered when interpreting the physiological effects of estradiol. Blood glucose concentration is recognized as a secondary physiological indicator of stress and environmental adaptation. On day 15, fish receiving 15 µg/g oxitriptan exhibited reduced blood glucose levels, whereas glucose concentrations increased in the other treatments. By day 30, however, glucose levels had increased across all treatments. These findings suggest that enhanced serotonergic activity may either decrease or increase blood glucose depending on the site and mechanism of serotonin action.

Zhang *et al.* (2022) demonstrated that serotonin improves glucose homeostasis by regulating glucose metabolism, thereby reducing blood glucose concentrations. Central serotonin primarily lowers glucose through appetite regulation and metabolic control, whereas peripheral serotonin, produced in the intestine, liver, and pancreas, may increase blood glucose through stimulation of gluconeogenesis and inhibition of insulin secretion (Tubío *et al.,* 2010). Serotonin also stimulates insulin release from pancreatic β-cells via 5-HT1A and 5-HT2B receptors, promoting glucose uptake by peripheral tissues. In the hypothalamus, serotonin activates pathways that enhance insulin sensitivity and suppress hepatic glucose production through 5-HT2C receptors (Almaça *et al.,* 2016). Otherwise, the activation of pancreatic 5-HT3 receptors inhibits insulin secretion, thereby increasing blood glucose concentrations (Kim *et al.,* 2015).

Population density may also influence glucose metabolism, as higher stocking densities have been associated with elevated glucose indices (Pellegrin *et al.,* 2023). In marine fish, physiological stress has consistently been linked to elevated blood glucose concentrations. For example, hybrid grouper exhibited glucose levels ranging from 61 to 114 mg/dL, exceeding the normal range of 28.8–57.5 mg/dL (Putri *et al.,* 2025). Type II cannibalism resulted in higher mortality than Type I cannibalism, accounting for 103 (57%) of the 173 fish that died from cannibalistic events. This type of cannibalism involved both successful predation, where the predator completely swallowed its prey, and unsuccessful predation, where the prey could not be completely ingested. Type II cannibalism generally occurs when prey length reaches approximately 50% of predator body length (Ribeiro & Qin, 2016).

In some cases, predation occurred between fish of similar size, preventing complete ingestion or digestion and ultimately resulting in mortality. Uneven size distribution among juveniles (Khan *et al.,* 2021) and limited food availability (Pham *et al.,* 2020) have also been identified as important factors promoting Type II cannibalism. Size uniformity was evaluated using the coefficient of variation (CV), classified as low (<10%), moderate (10–20%), or high (>20%) according to Maulida *et al.* (2022). The initial length CV in this study was categorized as moderate, whereas the final CV was classified as low, indicating improved size uniformity over the rearing period. This pattern

corresponded closely with cannibalism dynamics, which peaked between days 4 and 14. Variation in fish length at the end of the experiment was likely caused by competition for feed and space (Pham *et al.,* 2020). In addition, the transition to a larger pellet size may have temporarily affected growth because juveniles required time to adapt to the new feed size.

The greatest increase in body length was observed in the O10 treatment (10 µg g^{-1}), suggesting that serotonin may promote somatic growth by stimulating growth hormone (GH) secretion. Similar findings have been reported by Mainisa *et al.* (2024) and Khan *et al.* (2023), who demonstrated that dietary tryptophan supplementation enhanced length growth in Asian seabass juveniles. According to Bromek *et al.* (2019), serotonin stimulates GH secretion through activation of 5-HT2 receptors. Serotonin regulates GH release by acting on hypothalamic receptors, where activation of 5-HT1A and 5-HT1B receptors stimulates growth hormone-releasing hormone (GHRH) secretion, which subsequently induces GH release from the pituitary gland. In contrast, activation of 5-HT2C receptors increases somatostatin secretion and suppresses GH release, whereas antagonism of this receptor produces the opposite effect (Bromek & Daniel, 2021).

These opposing regulatory pathways indicate that serotonin-mediated GH secretion depends on the receptor subtype activated. Consequently, higher oxitriptan doses, particularly in the O10 and O15 treatments, may have promoted somatostatin release, thereby limiting growth. The increased stocking density in juvenile Asian seabass intensifies intraspecific competition, resource limitation, and social stress, all of which negatively affect growth performance (Refaey *et al.,* 2018; Roberts *et al.,* 2026). Similar density-dependent growth reductions have also been reported in European seabass (*Dicentrarchus labrax*) and blunt snout bream (*Megalobrama amblycephala*) (Qi *et al.,* 2016; El-Sayed *et al.,* 2021).

Body weight growth showed a different response from length growth. Increasing oxitriptan dosage resulted in lower weight gain and specific growth rate, while the control treatment exhibited significantly greater weight growth than the supplemented groups ($P<0.05$). One possible explanation is the difference in stocking density among treatments. Higher stocking densities generally reduce weight gain because of increased competition for resources (Li *et al.,* 2021). Similar density-related reductions in growth have been reported in largemouth bass (*Micropterus salmoides*) and blackchin tilapia (*Sarotherodon melanotheron*) (Ni *et al.,* 2021; Tine *et al.,* 2024).

In the present study, oxitriptan-treated groups exhibited higher survival, resulting in greater stocking densities throughout the experimental period. In contrast, the control group experienced greater cannibalism-induced mortality, leading to lower fish density and consequently greater feed availability per individual. Environmental conditions may also contribute to differences in weight gain (Matthias *et al.,* 2018). Moreover, this parameter was used to evaluate feeding activity. Throughout the 30-day experiment, oxitriptan supplementation at 5, 10, and 15 µg/g did not appear to affect appetite. Fish actively consumed the pellets during feeding, and no uneaten feed was observed at the bottom of the culture tanks.

Water quality remained within the recommended range for rearing Asian seabass juveniles throughout the study and therefore did not adversely affect the experimental treatments. Water temperature ranged from 28 to 32°C, dissolved oxygen remained above 4 mg/L, pH ranged from 7.5 to 8.5, and salinity ranged from 28 to 33 ppt, all of which complied with the Indonesian National Standard (SNI, 2014) for Asian seabass juvenile culture. Therefore, non-cannibalistic mortality observed in this study was unlikely to have been caused by differences in water quality among treatments.

CONCLUSION

Based on the findings of this study, dietary supplementation with oxitriptan at a dose of 10 µg/g body weight of Asian seabass (*Lates calcarifer*) juveniles effectively reduced cannibalism by 20.67 percentage points and increased survival rate by 20.67 percentage points compared with the control treatment. These findings provide valuable insights for improving Asian seabass hatchery practices by demonstrating that oxitriptan supplementation is a promising strategy for mitigating cannibalism and enhancing the survival performance of juvenile fish.

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