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Cannibalism, Growth Performance, and Glucose Levels of Snakehead Larvae (Channa striata) Administered the 17β-Oestradiol and Varying Temperatures

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Abstract

Cannibalism remains a significant constraint in snakehead fish farming, leading to seed mortality rates of over 55%. Key factors contributing to this issue include non-uniform fish size, delayed feeding, high testosterone levels, and inappropriate rearing temperatures. A randomized complete factorial design (RAL-F) with two doses of 17β-Oestradiol (30 and 50 mg/L) and three temperature treatments (28–29, 32–33, and 36–37°C) was used to investigate their effectiveness in mitigating cannibalism. Each treatment was replicated twice. Fourteen-day-old fish larvae were subjected to these treatments for 45 days. All performance and cannibalism parameters were collected throughout the 45-day trial, with data analyzed using ANOVA and Duncan's post-hoc test. The results showed that 17β-Oestradiol had a significant effect on reducing total cannibalism, specifically type-II cannibalism, and on increasing the percentage of females. Conversely, different temperature treatments significantly influenced mortality from other factors and affected the glucose levels of the larvae on day 45. In conclusion, administering 17β-Oestradiol at a dose of 30 mg/kg of feed was effective in significantly reducing total and type-II cannibalism, while temperature had no significant effect.

Keywords: Mortality; Hormone Therapy; Aquaculture; Survival rate; Fish Farming

1. Introduction

The production of Channa striata, commonly known as the snakehead fish, has seen a remarkable increase of 238.78% between 2015 and 2019, averaging approximately 59.7% annually (KKP, 2020). This species is highly valued for its product diversification, attributed to its unique flavor and high nutritional content. The fish's nutritional composition includes 16.76% protein, 1.37% fat, and 1.28% carbohydrates. Notably, the fish is rich in albumin, with concentrations of approximately 1.25% to 1.96% of its meat's weight (Hidayati et al., 2023), which has been shown to promote the healing of various wounds, including burn wounds (Nofriyanti et al., 2020), postoperative wounds (Ab Wahab et al., 2015), and diabetic ulcers (Udayanti & Noviyani, 2022).

Given its beneficial properties, there is a pressing need for snakehead culture to meet market demand and prevent its extinction in the wild. However, the aquaculture of this species faces significant challenges, particularly cannibalism. This behavior is a major cause of high mortality rates in seed production, with reported losses reaching 90% in barramundi (Khan et al., 2021), 30-40% in catfish (Cahyanurani et al., 2023), over 50% also in catfish (Putri, 2020), and more than 55% in snakehead fed with fresh fish like nilem (Osteochilus sp) and earthworms (Lumbricina sp) (Safir et al., 2022). Cannibalism can be influenced by various factors, including size heterogeneity among fish (Tasyah et al., 2020), feeding delays (Feranita et al., 2019), elevated testosterone levels (Putri et al., 2020), and environmental temperature (Kasihmuddin et al., 2024).

2. Theory

Several approaches can be used to control cannibalism, such as administering 17β-Oestradiol to lower elevated testosterone levels and managing rearing temperatures (Figure 1). Research by Utama (2022) demonstrated a reduction in cannibalism among snakehead fry treated with estrogen. The lowest incidence of cannibalism was observed in larvae fed a diet containing 30 mg/kg of the hormone, in contrast to treatments with 0 and 60 mg/kg. Similarly, a study on catfish (Putri et al, 2020) found that an effective hormone dosage of 50 mg/kg of feed at 28°C resulted in minimal injuries to the head, body, and tail.

In a separate study by Sari (2022), cannibalism was effectively controlled through a combination of temperature and density treatments. The optimal conditions were a temperature range of 31-32°C and a stocking density of 2 individuals per liter. Conversely,

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lower temperatures of 24°C and 28°C were insufficient to suppress cannibalism in snakehead larvae.

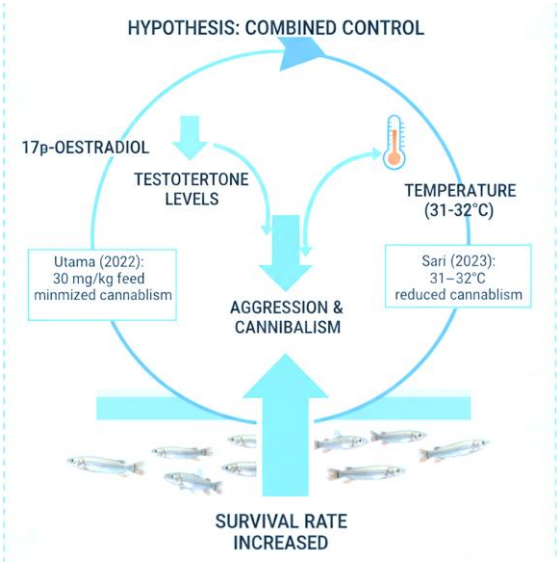

Figure 1. Mechanism of Hormonal and Temperature Control in Improving Fish Survival

These findings suggest the need to explore higher temperature treatments and hormone dosages greater than 30 mg/kg of feed but below 60 mg/kg to determine the most effective strategy for reducing cannibalism from the larval to fry stages of snakehead fish.

This study will investigate the effects of 17β-Oestradiol and temperature on cannibalism in snakehead larvae. It will use temperatures of 28°C, 32°C, and 36°C, along with hormone dosages of 30 mg/kg and 50 mg/kg of feed. The objective was to evaluate the most effective strategy for mitigating cannibalistic behaviour, its effect on gonadal development, and improving survival rates in aquaculture.

3. Method

3.1. Materials

This study utilized the following materials: twelve aquariums, each measuring 50 x 40 x 30 cm³; one-week-old snakehead larvae with an average weight of 0.034±0.017 grams and an average length of 1.58±0.15 cm. Additionally, the equipment included a heater, a filtration system, and 17β-Oestradiol (Argent, produced by Argent Chemical Laboratories, USA). The diet consisted of Moina sp., Artemia sp., and commercial pellet feed (Feng Li, size FL 1).

3.2. Experimental Design

The research employed a factorial complete randomized design (CRD) to investigate the effects of two factors: temperature and 17β-Oestradiol dosage. The temperature treatments were T1 (28-29°C), T2 (32-33°C), and T3 (36-37°C). The 17β-Oestradiol dosage treatments were D1 (30 mg/kg of feed) and D2 (50 mg/kg of feed). This design resulted in six treatment combinations, each replicated twice. The study's methodology involved mixing hormones with feed and regulating the water temperature using a heater.

3.3. Test Feed Preparation

Hormone administration was conducted by spraying the hormone onto the feed at the specified dosages. The hormone is a fine white powder with a purity of 99%. The hormone was weighed according to the designated dosages (30 and 50 mg/kg of feed), placed into a tube with 300 mL/kg of 95% ethanol, and homogenized using a vortex mixer. This hormone solution was then evenly sprayed onto the commercial feed and allowed air to dry until the ethanol odor dissipated. The prepared feed was stored in a sealed container (e.g., a jar or zip-lock bag) in a refrigerator. Before each feeding, the feed was removed from the refrigerator and allowed to acclimate to room temperature to prevent it from being too cold for the fish.

3.4. Fish Rearing

The larvae were reared and treated for a total of 45 days. Feeding occurred five times daily at 07:00, 10:00, 13:00, 16:00, and 19:00 WIB. The feeding method was ad libitum. During the first week (larval age of 7-14 days), live feeds, specifically Moina sp. and Artemia sp. were provided. This live feed was either untreated or enriched with 17β-Oestradiol. To enrich Artemia sp., the hormone was first dissolved in 1 mL of 95% ethanol at the specified dosage and vortexed for homogeneity. This hormone solution was then mixed with 950 mL of water and aerated for 6 hours. After aeration, 3 grams of newly hatched Artemia were harvested, thoroughly rinsed with freshwater, and then combined with 100 mL of freshwater before being divided into two equal portions. Each portion received 50 mL of the hormone solution and was aerated for an additional 2 hours.

3.5. Research Parameters

The performance parameters observed in this study included absolute weight gain, absolute length, and specific growth rate. The instances of Type-I cannibalism and mortality from other causes were monitored daily, with observations conducted every six hours at 06:00, 12:00, 18:00, and 24:00 WIB. Data was compiled every 15 days. Total cannibalism, Type-II cannibalism, potential cannibalism, and survival rates were assessed at the conclusion of the rearing period. Glucose levels in the fish were tested three times on days 15, 30, and 45 of the rearing period using three fish per replicate. Blood glucose was measured from individual fish (n=3 per replicate per

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sampling day), and each fish was analyzed separately to preserve biological variation among individuals.

The parameters of this study were calculated using the following equations:

- Weight gain (g) = final weight (g) – initial weight (g)
- Length gain (cm) = final length (cm) – initial length (cm)
- Specific growth rate (% day-1) = [(ln final weight – ln initial weight)/number of rearing days]x100
- Blood glucose level (mg/dL) = [sample absorbance/standard absorbance] x standard glucose concentration
- Type-I cannibalism (%) = [number of fish that died injured/number of fish at the start of the study] x100
- Type-II cannibalism (%) = 100-[Type-I cannibalism+death due to other factors+survival rate]
- Death due to other factors (%) = [number of whole dead fish-number of leftover fish]x100%
- Survival rate (%) = [Number of fish survived at the end of experiment / Initial number of fish stoked] x 100
- Female Percentage (%) = [number of female gonad/total fish] x 100

3.6. Data Analysis

Data were tabulated using Microsoft Office Excel 2019 and subsequently analyzed through analysis of variance (ANOVA) using SPSS 26, with a confidence interval of 95%. If the ANOVA results indicated significant differences, post-hoc testing was conducted using the Duncan test.

4. Results and Discussion

4.1. Effects of 17β-Oestradiol

The administration of 17β-oestradiol at different doses affected cannibalism and sex ratio but did not significantly influence survival rate or growth performance. The 50 mg/kg dose resulted in a higher percentage of females and higher overall cannibalism, particularly Type-II cannibalism, compared with the 30 mg/kg treatment (Table 1). In contrast, survival rate, length gain, weight gain, and specific growth rate did not differ significantly between treatments ($P > 0.05$).

4.2. Effects of Rearing Temperature

Rearing temperature affected sex ratio but did not significantly influence survival rate or growth performance of juvenile C. striata. The percentage of females was highest at 28–29°C and decreased significantly with increasing temperature, as indicated by different superscript letters among temperature treatments (Table 2).

Table 1. Growth performance, cannibalism, survival rate, and female percentage of C. striata juveniles in a 17β-Oestradiol factor (mg/kg of feed) experiment. Different superscript letters on the same factor show a significantly different effect ($P<0.05$)

Parameters	Doses of 17ß-Oestradiol (mg/kg of feed)	
	30	50
Length gain (mm)	35.65 ± 6.66[a]	37.34 ± 4.04[a]
Weight gain (g)	1.22 ± 0.35[a]	1.33 ± 0.39[a]
Specific growth rate (%/day)	7.91 ± 0.66[a]	7.91 ± 0.66[a]
Cannibalism (%)	62.56 ± 14,82[a]	82.33 ± 1,83[b]
Cannibalism type-I (%)	0.85 ± 0,47[a]	1.52 ± 1,10[a]
Cannibalism type-II (%)	61.70 ± 15,29[a]	80.81 ± 2,41[b]
Cannibalism due to other factors (%)	8.04 ± 7,07[a]	3.85 ± 2,30[a]
Survival rate (%)	25.63 ± 10.27[a]	13.81 ± 1.31[a]
Female (%)	35.42 ± 1.21[a]	49.43 ± 1.52[b]

In contrast, survival rate did not differ significantly among temperature groups ($P > 0.05$), despite a numerical decline at higher temperatures. Similarly, length gain, weight gain, and specific growth rate showed no significant differences across the tested temperature ranges (Table 2).

Table 2. Growth performance, cannibalism, survival rate, and female percentage of C. striata juveniles in rearing temperature factor. Different superscript letters on the same factor show a significantly different effect ($P<0.05$)

Parameters	Temperature (℃)		
	28-29	32-33	36-37
Length gain (mm)	36.05 ± 5.35[a]	35.98 ± 3.72[a]	37.44 ± 6.98[a]
Weight gain (g)	1.14 ± 0.34[a]	1.30 ± 0.20[a]	1.38± 0.57[a]
Specific growth rate (%/day)	8.52 ± 0.82[a]	7.48 ± 0.98[a]	8.11 ± 1.27[a]
Cannibalism (%)	68.44 ± 13,67[a]	74.56 ± 5,81[a]	74.33 ± 5,50a
Cannibalism type-I (%)	0,61 ± 0,86[a]	1,44 ± 0,79[a]	1,50 ± 0,71[a]
Cannibalism type-II (%)	67.83 ± 14,53[a]	73.11 ± 5,81[a]	72.83 ± 6,21[a]
Cannibalism due to other factors (%)	0.94 ± 0,71[b]	6.11 ± 7,07[ab]	16.44 ± 6,29[a]
Survival rate (%)	30.61 ± 13.91[a]	19.33 ± 2.51[a]	9.22 ± 0.94[a]
Female (%)	49.51± 1.56[a]	41.69 ± 0.84[b]	36.09± 0.78[c]

The glucose levels of juvenile C. striata were significantly affected by both the 17β-oestradiol dose and the rearing temperature, especially by the end of the 45-day maintenance period. Figure 1 shows that by

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day 45, the lower dose of 30 mg/kg resulted in a dramatically higher glucose level (103.38 mg/dL) compared to the 50 mg/kg dose (32.19 mg/dL). Concurrently, Figure 2 reveals a clear correlation between temperature and glucose levels, where the highest temperature group (36-37°C) showed a significantly elevated glucose level (87.96 mg/dL) on day 45 compared to the lower temperature groups. Together, these findings highlight that both hormonal dosage and environmental temperature are critical factors influencing the fish's metabolic state, with higher glucose levels potentially indicating metabolic stress induced by either a specific hormonal dose or a challenging thermal environment.

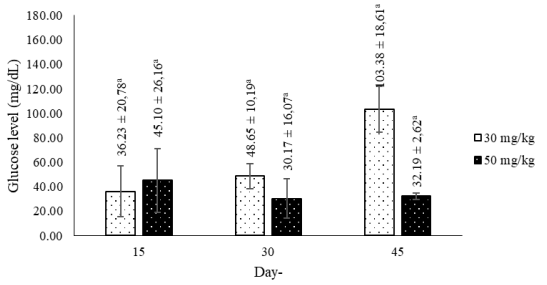

Figure 1. Glucose level of C. striata juvenile in factor of different doses of 17β-Oestradiol (mg/kg of feed) with 45 days of maintenance. Different superscript letters on the same factor show significantly different effects (P<0.05).

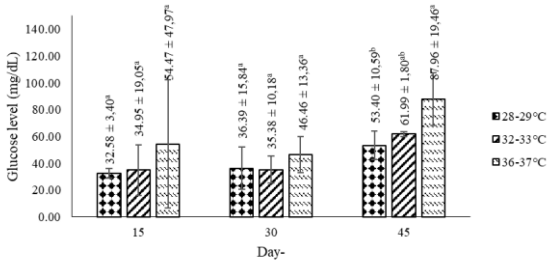

Figure 2. Glucose level of C. striata juvenile in factor of different rearing temperature with 45 days of maintenance. Different superscript letters on the same factor show significantly different effects (P<0.05).

4.3. Discussion

The administration of 17β-Oestradiol at varying doses exerts significantly different effects on cannibalism parameters and type-II cannibalism (Table 1), with the higher dosage leading to a corresponding rise in aggressive, cannibalistic behavior. The increase in cannibalism at the higher 17β-oestradiol (E2) dose may reflect a non-linear, context-dependent effect of estrogenic signaling on agonistic behavior rather than a simple suppression of testosterone results in a lower level of the aggression pathway. In teleosts, estrogens can modulate aggression through multiple routes, including central estrogen receptor signaling, aromatase-linked neurosteroid dynamics, and downstream neuromodulators involved in social dominance and competitive interactions (Stennette & Godwin, 2024). Moreover, exposure to estrogenic compounds can alter aggressive responses via stress-axis and monoaminergic pathways (e.g., serotonin/dopamine), producing either suppression or escalation of aggression depending on dose, duration, and social context (Zhang et al., 2025). Therefore, the higher cannibalism observed at 50 mg/kg (Table 1) may indicate that the dose exceeded the optimal endocrine window for behavioral stabilization, potentially triggering compensatory neuroendocrine responses or altered social dynamics that increase attack frequency and type-II cannibalism.

Importantly, the observed cannibalism patterns may arise from a combination of direct endocrine effects and indirect effects mediated by the social environment. Because mortality differed across treatments, the surviving fish experienced different effective stocking densities over time, which can modify encounter rates and competitive pressure and thereby influence cannibalism risk.

In contrast to the mortality resulting from cannibalism, the mortality from other factors was similar across the tested estradiol dosages (Table 1), indicating that the hormone itself does not exhibit direct toxic effects on the fish. This aligns with the findings of Indriastuti et al., 2022 in catfish. Similarly, glucose levels, as shown in Figure 1, did not consistently show a dose-dependent effect, supporting the notion that estradiol does not exert a significant influence on glucose levels (Woo et al., 1993). Glucose responses should be interpreted within the framework of fish stress physiology, where primary stress signaling activates secondary metabolic adjustments, including mobilization of glucose to meet elevated energetic demands (Canosa & Bertucci, 2023; Lemos et al., 2023). In this study, glucose differed by dose and temperature toward day 45, suggesting that endocrine treatment and thermal conditions altered metabolic state. Notably, the higher glucose observed in the 30 mg/kg group at day 45 may reflect density- or interaction-mediated stress (i.e., more survivors leading to higher effective density) rather than a purely direct hormonal effect, consistent with the need to separate endocrine action from social-environmental drivers.

Unlike the hormonal factor, the cannibalism resulting from the tested temperature treatments exerted a similar influence (Table 2), aligning with Sari (2023) who also found that varying temperatures had a similar effect on snakehead larvae cannibalism. However, temperature treatments had distinct effects on mortality rates attributable to other factors. Temperatures ranging from 36-37°C resulted in the highest mortality due to other factors, as supported by Ridwantara et al. (2019) who noted that both excessively low and high temperatures can increase mortality.

Furthermore, the temperature treatment exhibited a distinct influence on glucose levels on day 45 (Figure 2). Higher temperatures corresponded with elevated glucose concentrations, a finding consistent with Widiastuti & Widodo (2022). Glucose serves as an early stress indicator (Templeman et al., 2014), and the elevated temperatures likely enhance aggression, exacerbating stress conditions. This leads to an increase in glucocorticoids, which correlates with the rise in glucose levels to meet the high energy demands associated with stress (Canosa & Bertucci, 2023).

Absolute length growth, absolute weight growth, and specific growth rate did not show significant differences in either the hormonal (Table 1) or temperature treatments (Table 2). This is consistent with Reis & Almeida (2019), who found that varying doses of 17β-Oestradiol did not significantly impact fish length and weight. Similarly, Nurvadilla, 2022; Tu et al., 2022, concluded that different temperatures do not significantly affect the growth of snakehead larvae. While food intake and energy consumption may increase with rising temperatures (Henken et al., 1986; Volkoff & Rønnestad , 2020; Langi et al., 2024), these factors did not translate into statistically significant differences in overall growth performance in this study.

5. Conclusion

This research highlights a critical trade-off: while a higher dose of 17β-Oestradiol and a higher rearing temperature can influence female differentiation, they do so at the significant cost of increased cannibalism and reduced survival. The lack of a substantial effect on growth parameters suggests that the main impacts of these treatments are behavioral and physiological. Therefore, for optimal aquaculture outcomes, a lower temperature (28-29°C) combined with a moderate hormonal dose appears to be a more viable strategy.

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