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Research Article

Immersion Effect of Estradiol-17β on Cannibalism of Asian Redtail Catfish (*Hemibagrus nemurus*) Post Larvae

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Abstract

The cannibalistic behavior of Asian redtail catfish greatly hampers the supply of these fish seeds, invoking the need to be controlled. Estradiol is one of the hormones that has been known to reduce cannibalistic behavior on fish. This study aimed to evaluate the effect of estradiol-17β immersion on the incidence of cannibalism in the rearing of post larvae of Asian redtail catfish. Post larvae was treated with a combination of doses of estradiol hormone 0, 1, and 2 ppm and immersion time of two and four hours with immersion intervals of three and six days with a completely randomized design. Each treatment had a fish density of 10 fish L^{-1}, with mean size individual length of 6.73 ± 0.73 mm (three days of age after hatched), with four replications. Fish were fed with tubifex up until satiation point and reared for 30 days. The results showed that the administration of the estradiol-17β through immersion with a dose of 1 ppm for two hours and interval of six days was able to reduce the level of cannibalism and increase the survivability. A further increase in dose, immersion time, and interval had the opposite effect. Estradiol-17β immersion with certain dose, duration, and interval affected the rate of cannibalism, growth rate, and survival. Estradiol-17β immersion at a dose of 1 ppm for two hours and six days interval was the best to reduce the level of cannibalism and normal mortality in post larvae of Asian re

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1. Introduction

Asian redtail catfish (*Hemibagrus nemurus*) has an economic value and has become an icon, especially for the Malay community. Cultivation of this fish has also been carried out since 2006 in Riau, Indonesia. However, low survival rate in cultivation activities is the main obstacle. The survival rates of Asian redtail catfish seeds are only 20-28% in the study by Aysah (2014) and 44-48% in the study by Heltonika and Karsih (2017), this low survival rate was dominantly caused by cannibalism (Rahmah *et al.*, 2012; Heltonika *et al.*, 2021; 2022). The level of cannibalism is closely related to the aggressive behavior of the fish. Fish with aggressive behavior are more active, aggressive, and prey on intracohort. The aggressive behavior of cannibalism is closely related to the role of hormones, one of which is testosterone. In general, there are two hormonal approaches to reduce cannibalism in fish, namely through the steroid hormone pathway in the form of estradiol (Filby *et al.*, 2012; Brown *et al.*, 2014; Siregar *et al.*, 2021) and amino acids in the form of tryptophan (Muslimin *et al.*, 2011; Król and Zieliński, 2015).

Several studies regarding the role of estradiol in reducing fish cannibalism have been described by Siregar *et al.* (2021) where enrichment of feed with the estradiol-17β could reduce the level of cannibalism in African catfish (*Clarias gariepinus*). Furthermore, there was also a decrease in aggressiveness with the administration of estrone and estradiol in *Pimephales promelas* (Dammann *et al.*, 2011), environmental estrogen (EE2) which reduced the aggressiveness of zebrafish (Filby *et al.*, 2012), 17α-ethinyl estradiol in male zebrafish *Danio rerio* (Colman *et al.*, 2009), and the phytoestrogens-sitosterol and genistein in *Betta splendens* (Brown *et al.*, 2014).

The immersion technique is the most effective method approach for the estradiol hormone treatment in larvae and juveniles of fish (Hoga *et al.*, 2018). The success of the immersion treatment using the estradiol in fish is strongly influenced by the use of the right dose (Duk *et al.*, 2017) and the length of immersion time (Rosmadiar *et al.*, 2012; Firmansyah *et al.*, 2016). The doses of estrogen immersion and the optimal length of immersion time are critical because they determine fish larvae survival (Firmansyah *et al.*, 2016; Uğuz, 2017; Koyakomanda *et al.*, 2019; Ikhwanuddin *et al.*, 2019).

In this study, the use of estradiol-17β in post larvae (three days after hatching) used different doses, immersion times, and immersion intervals. This was done to evaluate the best combination to reduce aggressiveness in Asian redtail catfish post larvae. The aim of this study is to evaluate the effect of the estradiol-17β on the level of cannibalism of Asian redtail catfish by reducing aggressiveness through the approach of estradiol-17β. This study also analyzes the level of post larvae cannibalism in Asian redtail catfish treated with estradiol-17β immersion.

2. Materials and Methods

2.1 Research Time and Place

This study was carried out in April – May 2020. Spawning and larval rearing were carried out at the Fish Breeding and Hatchery Laboratory of Faculty of Fisheries and Marine Science Riau University, Indonesia. Analysis of several test parameters were carried out at the Breeding and Hatchery Laboratory of the Faculty of Fisheries and Marine Science, Riau University, and Depok Ornamental Fish Research Institute, Ministry of Marine Affairs and Fisheries, Indonesia.

2.2 Experimental Design

This was a completely randomized design experiment with nine treatments consisting of a combination of dose, immersion time, and interval (repeat), with each treatment having four replications. The treatments of this study were: A (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 0 ppm as control), B (immersion of post larvae of Asian redtail catfish with estradiol-17β (Argent Laboratories Inc, Philippines) at a dose of 1 ppm, for two hours, and conducted once every three days), C (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 2 ppm, for two hours, and conducted once every three days), D (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 1 ppm, for two hours, and conducted once every six days), E (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 2 ppm, for two hours, and conducted once every six days), F (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 1 ppm, for four hours, and conducted once every three days), G (immersion of post larvae of Asian redtail catfish with stradiol-17β at a dose of 2 ppm, for four hours, and conducted once every three days), H (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 1 ppm, for four hours, and conducted once in six days), and I (immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 2 ppm, for four hours, and conducted once in six days) (Table 1).

2.3 Research Procedure

2.3.1 Seed and feed preparation

The post larvae of Asian red tail catfish were obtained from the artificial spawning of brood stock by inducing sGnRH and anti-dopamine (Ovaprim; Syndel Laboratories Ltd, Canada), at a dose of 0.25 mL kg^{-1} for male and 0.5 mL kg^{-1} for female brood stock dissolved in 0.9% NaCl in a ratio of 1:2. The feed given during rearing was silkworm (Tubificidae) and it was cleaned before being fed to the experimental fish.

2.3.2 Seed maintenance

The seeds of Asian red tail catfish with the length of 6.73 ± 0.73 mm were reared in an aquarium with the size of 40x25x35 cm^3 with a water volume of 20 L and a stocking density of 10 fishes L^{-1}. The temperature of the rearing water was conditioned at 28±1°C. The experimental fish were reared for 30 days, and silkworm (Tubificidae) feeding was done ad libitum at all times. During rearing, siphoning of feces and feed residues as well as water changes of 10-30% every day was carried out once a day in the morning.

2.3.3 Observation parameters

The cannibalism observations included the number of dead fish observed every six hours, the number of cannibalisms classified as types I and II, and the cannibalism index. Type I is a fish condition where damage occurs in the tail, stomach, head, and part of the body being eaten. Type II is the condition of the fish being eaten whole (Król *et al*., 2014a). Cannibalism index was calculated by this formula:

Cannibalism index (%) =

$$\frac{\text{Numbers of fish that dead due to cannibalism (fish)}}{\text{Numbers of fish initial observation (fish)}} \times 100$$

The number of fish that died naturally and not as a result of cannibalism during the 30-day rearing period is known as normal mortality. The formula for calculating normal mortality was:

Normal mortality (%) =

$$\frac{\text{Numbers of normal mortality (fish)}}{\text{Numbers of fish initial observation (fish)}} \times 100$$

The number of opportunities for fish to survive after 30 days of rearing is referred to as survival. The formula used to calculate survival was:

Survival (%) =

$$\frac{\text{Numbers of fish that lived at the end of the observation (fish)}}{\text{Numbers of fish initial observation (fish)}} \times 100]$$.

Hormone analyses were conducted to determine the concentration of testosterone, estradiol, and cortisol in the body fluids of Asian redtail catfish. Seed body fluids were collected by collecting larvae on days 0, 15, and 30 of the rearing periods. The collected seeds were then washed with distilled water, then extracted by grinding the sample and homogenized in a phosphate buffered saline (PBS) solution containing 0.05% Tween-20 (pH 7.2) with a ratio of 1:4. The samples were centrifuged at 5000 rpm for 5-10 minutes. The supernatant formed from the centrifugation process as serum was then stored at -20 °C for testing hormone concentrations (Sukenda *et al*., 2017). Steroid analysis was performed using the ELISA method (human estradiol kit for females, human testosterone kit for males, and human cortisol kit for cortisol, with the size of 96 wells, DRG diagnostics). The use of these kits on fish is quite common. The ELISA measurement method uses a protocol from DRG International Inc. USA version 11.1 in 2010.

Table 1. Immersion pattern of Asian redtail catfish post larvae with estradiol-17b

Treatments / Days -	1	4	7	10	13	16	19	22	25	28
A (0 ppm, 0 hours, frequency 0)										
B (1 ppm, 2 hours, frequency 3)	B	B	B	B	B	B	B	B	B	B
C (2 ppm, 2 hours, frequency 3)	C	C	C	C	C	C	C	C	C	C
D (1 ppm, 2 hours, frequency 6)	D		D		D		D		D	
E (2 ppm, 2 hours, frequency 6)	E		E		E		E		E	
F (1 ppm, 4 hours, frequency 3)	F	F	F	F	F	F	F	F	F	F
G (2 ppm, 4 hours, frequency 3)	G	G	G	G	G	G	G	G	G	G
H (1 ppm, 4 hours, frequency 6)	H		H		H		H		H	
I (2 ppm, 4 hours, frequency 6)	I		I		I		I		I	

The test parameters for seed production performance consisted of growth in total weight, total length, specific weight growth rate, and specific length growth rate based on data at the beginning and at the end of the study. Total weight growth was calculated to determine the total weight gain of fish during rearing through this formula:

$$Wm = Wt - Wo$$

Where:
Wm = absolute weight growth (g)
Wt = average weight at the end of the study (g)
Wo = average weight at the start of the study (g)

The total length growth was calculated to determine the increase in fish length during rearing through this formula:

$$Lm = Lt - Lo$$

Where:
Lm = absolute length growth (cm)
Lt = final average length of the study (cm)
Lo = initial average length study (cm)

The Specific weight growth rate was calculated through this formula:

$$SGR\ (\%/day) = \frac{\ln Wt - \ln Wo}{t} \times 100$$

Where:
SGR = specific weight growth rate (%/day)
Wt = average fish weight over time t (g)
Wo = mean weight of fish at the beginning of rearing (g)
t = length of experiment (days).

The specific length growth rate was calculated through this formula:

$$SLR\ (\%/day) = \frac{\ln Lt - \ln Lo}{t} \times 100$$

Where:
SLR = specific length growth rate (%/day)
Lt = average length of fish at time t (cm)
Lo = average length of fish at the beginning of rearing (cm)
t = length of experiment (days)

Water quality in the form of dissolved oxygen (DO) and pH was measured once every seven days, while temperature was measured every day on each rearing medium. Measurement of dissolved oxygen was done by using a dissolved Oxygen meter (Lutron 5510), pH used pH meter (Krisbow, and confirmed with Merck Universal pH paper), and temperature was observed by reading a thermometer in each rearing medium.

2.4 Data Analysis

The data obtained were tabulated using the Microsoft Office Excel 2010 program, analyzed through two ways analysis of variance (ANOVA) using the SPSS program with a 95% confidence interval. If the analysis of variance showed significantly different results, then Duncan's further test was carried out.

3. Results and Discussion

3.1 Results

The results of the immersion treatment of post larvae of Asian redtail catfish with estradiol-17β showed that the incidence of death of type I cannibals did not differ between treatments, but the incidence of death of cannibals of type II varied, with the highest value of incidence occurred in treatment G and the lowest in treatment D, and this value was in line with the cannibal index ($p<0.05$) (Table 2). Therefore, there was an effect of estradiol-17β on the incidence of cannibals in Asian redtail catfish. Based on the normal mortality value, the estradiol-17β affected the ability of Asian redtail catfish to maintain high survival in high stocking densities, and this was seen from the largest normal mortality in treatment A (control) ($p>0.05$). Meanwhile, in treatment G (immersion with estradiol at a dose of 2 ppm, for four hours duration, and once every three days) had the lowest normal mortality rate. Based on the survival value, the lowest value occurred in treatment A (control), where the normal mortality rate greatly affected the low survival rate, and the highest value was found in treatment D (immersion with estradiol-17β at a dose of 1 ppm, for two hours, and was conducted every six days).

Based on analysis of variance, there was no significant effect between dose concentration, exposure duration and exposure frequencies on the incidence of type I cannibals ($p>0.05$). However, in the incidence of type II cannibals, based on analysis of variance, dose and frequency had no significant effect on the incidence of type II cannibals ($p>0.05$), but the duration of immersion had a significant effect on the incidence of type II cannibals ($p<0.05$). The same thing goes for cannibal index, where only the duration of immersion had a significant effect on the cannibal index ($p>0.05$). Furthermore, for normal mortality and survival rate, based on analysis of variance, it was shown that dose, duration of immersion and frequency of immersion had a significant effect ($p<0.05$), where estradiol-17β treatment was able to reduce normal mortality and increase survival rate.

Table 2. Types of cannibals I and II, cannibal index, normal mortality, and survival (%) with estradiol-17β immersion treatment

Treatment	Cannibal type I	Cannibal Type II	Cannibal Index	Normal mortality	Survival rate (SR)
A	0.00±0.00^{a}	21.00±8.73^{ab}	21.00±8.73^{ab}	40.50±3.54^{d}	38.50±6.28^{a}
B	0.88±0.85^{a}	19.13±4.87^{ab}	20.00±5.52^{ab}	18.38±15.41^{ab}	61.63±15.65^{bc}
C	1.63±2.29^{a}	18.63±7.22^{ab}	20.25±8.91^{ab}	13.63±12.60^{ab}	66.13±16.57^{cd}
D	1.13±2.25^{a}	10.38±9.37^{a}	11.50±8.32^{a}	11.13±11.14^{ab}	77.38±17.37^{d}
E	0.00±0.00^{a}	23.25±3.84^{ab}	23.25±3.84^{ab}	12.38±10.02^{ab}	64.38±8.56^{bc}
F	0.25±0.29^{a}	25.63±14.22^{ab}	25.88±14.08^{ab}	9.00±9.19^{ab}	65.13±17.39^{cd}
G	0.00±0.00^{a}	46.50±12.25^{c}	46.50±12.25^{c}	3.25±1.89^{a}	50.25±13.07^{ab}
H	0.13±0.25^{a}	27.63±8.04^{b}	27.75±7.86^{b}	28.88±14.87^{cd}	43.38±9.59^{ab}
I	0.00±0.00^{a}	19.38±10.99^{ab}	19.38±10.99^{ab}	21.13±5.44^{bc}	59.50±8.46^{bc}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$)

Table 3. Concentrations of estradiol, testosterone, and cortisol in the post larvae of Asian redtail catfish immersed with estradiol-17β

	Estradiol (ng ml^{-1})		Testosterone (pg ml^{-1})		Cortisol (pg ml^{-1})	
Day-0	**2.1±0.00**		**1.44±0.49**		**23.89±6.32**	
Treatment	**Days-15**	**Days-30**	**Days-15**	**Days-30**	**Days -15**	**Days -30**
A	2.1±0.00^{a}	2.1±0.00^{a}	0.90±0.12^{a}	0.56±0.09^{b}	22.89±6.94^{b}	23.88±4.56^{b}
B	2.1±0.00^{a}	2.1±0.00^{a}	0.99±0.04^{a}	0.48±0.12^{ab}	11.17±3.70^{a}	22.70±5.73^{ab}
C	2.1±0.00^{a}	2.1±0.00^{a}	0.70±0.29^{a}	0.53±0.14^{ab}	27.25±3.50^{b}	18.33±7.14^{ab}
D	2.1±0.00^{a}	2.1±0.00^{a}	0.89±0.11^{a}	0.53±0.18^{ab}	25.84±5.33^{b}	23.43±11.71^{b}
E	2.1±0.00^{a}	2.1±0.00^{a}	0.79±0.22^{a}	0.45±0.10^{ab}	19.54±6.50^{ab}	23.31±4.81^{b}
F	2.1±0.00^{a}	2.1±0.00^{a}	0.70±0.19^{a}	0.50±0.06^{ab}	20.34±6.47^{ab}	13.81±3.51^{ab}
G	2.1±0.00^{a}	2.1±0.00^{a}	1.48±0.55^{b}	0.33±0.02^{a}	28.35±1.04^{b}	8.23±10.12^{a}
H	2.1±0.00^{a}	2.1±0.00^{a}	0.78±0.09^{a}	0.51±0.06^{ab}	26.09±9.64^{b}	14.34±9.56^{ab}
I	2.1±0.00^{a}	2.1±0.00^{a}	0.95±0.20^{a}	0.50±0.09^{ab}	17.28±3.40^{ab}	21.11±7.72^{ab}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$).

Table 4. Performance of seed production of Asian redtail catfish by immersion with the estradiol-17β at different doses

Treatment	Absolute weight growth (g)	Absolute length growth (mm)	The Specific weight growth rate (%/day)	The Specific length growth rate (%/day)	Biomass weight growth (g)
A	0.18±0.03^{d}	34.56±1.82^{c}	14.50±0.51^{c}	14.06±0.19^{c}	13.92±2.84^{ab}
B	0.12±0.03^{ab}	28.74±3.29^{ab}	12.76±1.20^{ab}	13.39±0.42^{ab}	14.67±7.20^{ab}
C	0.09±0.01^{a}	26.60±0.52^{a}	11.80±0.50^{a}	13.13±0.07^{a}	10.99±1.95^{ab}
D	0.16±0.02^{cd}	34.01±0.83^{c}	13.99±0.36^{bc}	14.01±0.09^{c}	24.29±6.39^{c}
E	0.14±0.02^{bc}	32.83±0.95^{bc}	13.60±0.43^{bc}	13.88±0.10^{bc}	18.05±3.02^{bc}
F	0.12±0.02^{ab}	29.45±1.92^{ab}	12.92±0.68^{ab}	13.49±0.23^{abc}	15.44±5.41^{ab}
G	0.10±0.03^{a}	25.95±5.69^{a}	11.83±1.72^{a}	12.96±0.90^{a}	9.76±5.29^{a}
H	0.15±0.02^{bc}	31.60±2.51^{bc}	13.76±0.53^{bc}	13.74±0.28^{bc}	12.80±3.50^{ab}
I	0.15±0.01^{bc}	32.31±1.04^{bc}	13.83±0.25^{bc}	13.82±0.11^{bc}	17.89±3.77^{bc}

Note: Different superscripts in the same column indicate a significant difference ($p<0.05$).

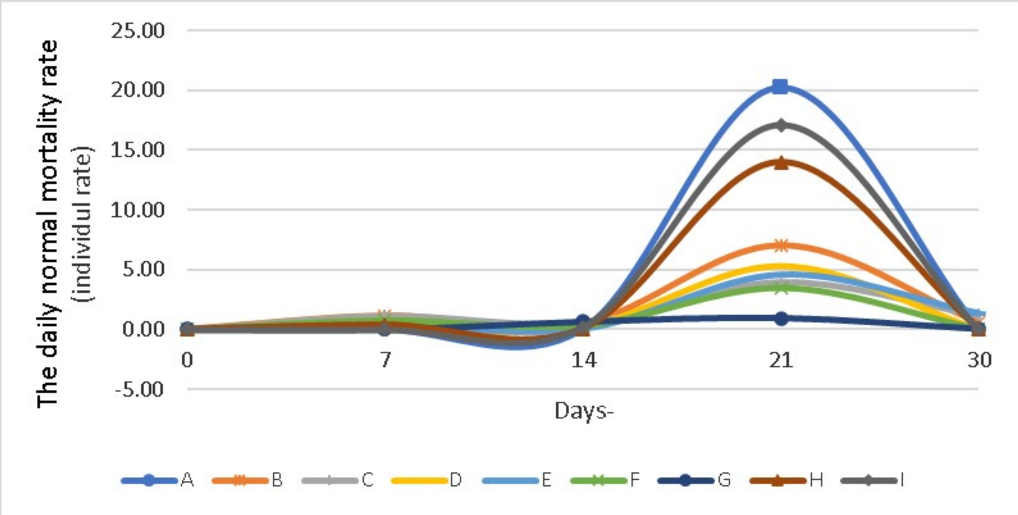

Figure 1. The daily normal mortality rate of Asian redtail catfish post larvae immersed with estradiol-17β treatment.

The highest average individual mortality in normal mortality was found on day 15 to day 21. The highest normal mortality incidence was found in treatment A, but in treatment G, normal mortality was relatively constant from day one to day 30 (Figure 1). Furthermore, on day 22 to day 30, all treatments experienced a decrease in normal mortality. Based on this incident, it is assumed that the administration of estradiol-17β has an effect on the viability at high stocking densities.

Based on the results of measurements of body hormone concentrations (Table 3), there was no difference in estradiol concentrations between treatments for both 15th and 30th days. These similar results were related to the estradiol concentrations that were above the threshold of the instrument's ability to read estradiol concentration. Furthermore, the highest body testosterone concentration on day 15 was found in treatment G, which was significantly different from the other treatments ($p<0.05$). However, on day 30, it had the lowest testosterone concentration compared to the other treatments. Meanwhile, the highest testosterone on day 30 was found in treatment A, which was significantly different from treatment G. The high testosterone concentration on day 15 of treatment G was closely related to the high incidence of cannibals in treatment G. The lowest cortisol concentration on day 15 was found in treatment B.

Based on analysis of variance, there was no significant effect between dose concentration, exposure duration, and exposure frequencies on body estradiol concentration both on day 15 and day 30 ($p>0.05$). The same thing goes for testosterone and cortisol concentration in the body, based on analysis of variance, dose concentration, exposure duration, and exposure frequencies did not significantly affect the testosterone and cortisol content of the body on day 15 and day 30 ($p>0.05$).

From the results of measuring the performance of Asian redtail catfish seed production, the best weight and total length growth as well as the specific weight growth rate and specific length growth rate were found in treatment A ($p<0.05$), and the lowest was found in treatments G and C (Table 4). However, when comparing between treatment A and D, there was no difference in length growth rate and weight increase rate. Based on the increase of biomass, treatment D had the best value and was significantly different from the other treatments, and treatment G had the lowest growth value of biomass weight ($p<0.05$).

Table 5. Water quality in rearing sed of Asian redtail catfish by immersion with the estradiol-17β at different doses

Treatment	Temp (°C)	pH	Dissolve Oxygen (ppm)
A	27 - 29	6 - 6.5	6.3 - 7.2
B	27 - 29	6 - 6.5	6.4 - 7.5
C	27 - 29	6 - 6.5	6.3 - 7.5
D	27 - 29	6 - 6.5	6.5 - 7.3
E	27 - 29	6 - 6.5	6.4 - 7.4
F	27 - 29	6 - 6.5	6.5 - 7.2
G	27 - 29	6 - 6.5	6.5 - 7.5
H	27 - 29	6 - 6.5	6.2 - 7.5
I	27 - 29	6 - 6.5	6.3 - 7.4

Based on analysis of variance, there was a significant effect between dose concentration, exposure duration, and exposure frequencies on absolute weight growth, absolute length, specific weight growth and specific length growth (p>0.05), where estradiol-17β administration had an impact on decreasing absolute weight growth, length absolute growth, specific weight growth and specific length growth. However, on the growth of biomass, the dose concentration and exposure duration did not produce significant difference (p>0.05), but the exposure frequencies had an effect on biomass growth (p<0.05).

The water quality of the rearing medium during the study was still within the tolerance limits of Asian redtail catfish, where the range of temperature was 27-29°C, pH range of 6-6.5, and DO range of 6.2-7.5 ppm (Table 5). According to Yudha *et al.* (2018), the range of water quality for Asian redtail catfish culture is a temperature of 27-30°C, pH of 5.0-7.0, and dissolved oxygen of 5.0-7.0 ppm.

3.2 Discussion

Administration of exogenous estradiol-17β hormone through immersion has an impact on the incidence of cannibalism in the post larvae of Asian redtail catfish. Immersion of post larval with estradiol-17β with the lowest immersion dose, the shortest immersion time, and the lowest frequency (D) gave the lowest value of cannibal index incidence and gave the highest survival rate. Several studies have shown that estradiol administration has an effect in reducing cannibalism in fish (Dammann *et al*., 2011; Filby *et al*., 2012; Brown *et al*., 2014). Based on this study, there is an optimum point that must be considered in the treatment of estradiol-17β, and when it is exceeded, it has the opposite effect, namely increasing the incidence of cannibalism in post larvae of Asian redtail catfish. Based on the data obtained, the highest incidence of cannibalism occurred in treatment G (immersion with estradiol-17β at a dose of 2 ppm, for four hours, conducted once every three days), which corresponded to the highest testosterone and cortisol concentration in the body on day 15, despite having a higher cortisol value with the lowest testosterone at the end of the study. The possibility of this incident was the impact of positive feedback from the administration of the estradiol exogenously. O'Connell and Hofmann (2012) stated that giving estradiol to male *A. Burtoni* fish increased the incidence of aggressiveness. However, in general, several studies have reported that exogenous administration of estradiol-17 causes a significant decrease in testosterone concentration accompanied by an increase in estradiol concentration in fish (Yuan *et al*., 2012; Khara *et al*., 2013). Another impact of increased testosterone was an increase in aggressiveness in fish which was closely related to the incidence of cannibalism in fish (Maruska and Fernald, 2010; Black *et al*., 2011; Kania *et al*., 2012; Chang *et al*., 2012; Peterson *et al*., 2013; Alcazar *et al*., 2016; Rahmadiah *et al*., 2019). Alcazar *et al.* (2016) stated that there was a relationship between steroid hormones and aggression in fish.

These results indicate that the success of the estradiol immersion treatment in fish is strongly influenced by the use of the correct dose (Duk *et al*., 2017) and duration of immersion (Rosmadiar *et al*., 2012). Optimal conditions were crucial, because they determine the survival of fish larvae (Firmansyah *et al*., 2016; Uğuz, 2017; Koyakomanda *et al*., 2019; Ikhwanuddin *et al*., 2019). In addition, when viewed from the cortisol concentration, the fish given estradiol-17β had relatively lower cortisol concentration indicating that it has an effect on the healthier condition of the fish.

However, based on this study, there is an optimum treatment that must be considered to provide a treatment with the hormone of estradiol-17β, where an excess of the optimum point will cause the opposite effect, namely an increase of cannibalism in Asian red-tailed catfish. This is most likely due to the positive feedback loop caused by the administration of exogenous estradiol. O'connell and Hofmann (2012) stated that giving estradiol to male *A. burtoni* fish increases the incidence of aggressiveness, while Huffman *et al.* (2013) revealed that aromatase is a regulator of aggressiveness in male *A. burtoni* fish. Based on this study, giving fadrozole (an aromatase inhibitor) to these fish to inhibit the formation of the hormone estradiol from testosterone reduced the incidence of attack (aggressiveness). However, testosterone levels increased while estradiol levels decreased. Furthermore, the low testosterone concentration at the end of the study in the estradiol-17β treatment was linked to decreased testosterone synthesis in the fish's body. This decrease occurred as a result of a negative feedback mechanism (Dinsdale and Ward, 2010). Several types of fish are also known to experience a decrease in testosterone concentration after estradiol-17β administration (Yuan *et al*., 2012; Khara *et al*., 2013; Siregar *et al*., 2021). Increased levels of estrogen cause suppression of testosterone levels in the body (Kim and Park, 2012).

Thus, in this study's optimal treatment (D), which was evaluated based on the body's testosterone concentration and was not significantly different from the control treatment, the possibility of giving estradiol-17β hormone was sufficient to only provide an estrogenic effect without changing the balance of estradiol and testosterone. There was no difference in the concentration of the body's testosterone with the control treatment. Therefore, there is no increase in aromatase activity, which is responsible for the conversion of testosterone to estradiol, whereas increased aromatase activity causes an increase in aggressiveness, which leads to an increase in cannibalism. Another possibility to consider is the increased incidence of cannibalism in the administration of estradiol at a high dose which influences the brain's differentiation response. Hormones determine morphological differences in brain nuclei, and morphological differences are the basis of hormones that influence sexual and aggressive behavior (Pfannkuche *et al*., 2009). This incident is also closely related to the response of estradiol receptors in the brain. Cunningham *et al*. (2012) stated that an increase of an aromatase activity in the conversion of testosterone to estradiol can also increase aggressiveness. Furthermore, Cunningham *et al*. (2012) revealed that increased activation of the estradiol receptor α was associated with an increase in aggressive behavior. Meanwhile, the activation of the estradiol receptor β was associated with a decrease in aggressive behavior. According to this viewpoint, it is possible that at the optimal dose in this study, the dominant hormone estradiol-17β only binds to the estrogen receptor β, resulting in weaker aggressive behavior and a decrease in cannibalism. Meanwhile, in treatment with values above the optimal point, estradiol-17β not only binds to the estrogen receptor β, but also to the estrogen receptor α. The more estradiol-17β binds to the receptor α, the more aggressive it becomes, which increases the incidence of cannibalism in Asian redtail catfish post larvae. These behavioral changes are most likely due to the involvement of nervous system pathways.

Based on normal post larvae mortality, there is a possibility that administering the hormone estradiol-17β provides fitness to Asian redtail catfish post larvae. Although, cortisol levels in the bodies of Asian redtail catfish post larvae did not indicate this condition. Therefore, the condition improved the survival of post larvae in high stocking densities (10 L-1 individuals). Based on this study, it was stated that the optimal stocking density for rearing Asian redtail catfish post larvae was 5 L-1 tails (Nasution *et al*., 2021). Furthermore, Faizati *et al*. (2021) revealed that stocking density affects the survival of Asian redtail catfish seeds, and one of the death causes is cannibalism. The results of this study are in line with Siregar *et al*. (2021), where the administration of estradiol-17β gave catfish seed viability at high stocking densities by suppressing normal mortality, providing fitness for fish with low cortisol. However, this condition was different from Król *et al*. (2014b) which stated that the administration of estradiol had a negative impact on fish survival. Production performance data revealed that the higher the dose of estradiol, the longer the immersion time, and the more frequent the immersion, the more stress the estradiol-17β could cause in the post larvae stage, with the best final growth found in the control post larvae of redtail catfish with no treatment of estradiol (A). It was also revealed that the administration of estrogen had a negative impact on growth and even suppressed the growth of the fish (Lerner *et al*., 2007; Shved *et al*., 2007; Yang *et al*., 2008; Hoseini and Tarkhani, 2013; Król *et al*., 2014b; Tarkhani *et al*., 2015).

Based on this study, immersion of post larvae of Asian redtail catfish with estradiol-17β at a dose of 1 ppm, for two hours, and immersed once every six days (treatment D) gave the best response with the increased survival and the reduced incidence of cannibalism. On the other hand, soaking the post larvae of Asian redtail catfish with estradiol-17β at a dose of 2 ppm, for four hours, and immersed once every three days (treatment G) gave an increase in the incidence of cannibalism in the post larvae of Asian redtail catfish. This condition is in line with the results of several studies that the success of immersion treatment using the estradiol in fish is strongly influenced by the use of the correct dose (Duk *et al*., 2017) and the duration of immersion time (Rosmadiar *et al*., 2012; Firmansyah *et al*., 2016). The doses and the optimal duration of immersion are very critical, because they determine the survival of fish larvae (Firmansyah *et al*., 2016; Uğuz, 2017; Koyakomanda *et al*., 2019; Ikhwanuddin *et al*., 2019).

4. Conclusion

The dose, duration of immersion, and time interval of immersion of the estradiol-17β affect the rate of cannibalism and increase the seed survival of Asian redtail catfish. Administration of the estradiol-17β at a dose of 1 ppm for 2 hours, once every 6 days for 1 month, reduced the incidence of cannibalism and normal mortality of Asian redtail catfish seeds.

Based on this study, administration of the hormone estradiol-17β through immersion could be a solution to reduce the rate of cannibalism and normal mortality in high density of post larvae of Asian redtail catfish. Further studies are needed to find the optimal dose below 1 ppm, soaking time below 2 hours, and soaking interval above 6 days to get a higher SR (above 77.38%).

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Authors' contributions

The contribution of each author is as follow, Benny Heltonika; conducted the methodology, conceptualized, wrote, analyzed the data, reviewed, and edited the manuscript. Agus Oman Sudrajat, Widanarni, Agus Suprayudi, Wasmen Manalu, and Yani Hadiroseyani; wrote, reviewed, edited the manuscript, and supervised the research. Muhammad Zairin Junior; conducted the methodology, conceptualized, and supervised the research. All authors discussed the results and contributed to the final manuscript.

Conflict of interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the completion of this paper.

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