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The use of different 17β-estradiol hormone doses and water temperatures to control cannibalism in catfish *Clarias gariepinus* seed

Penggunaan dosis hormon estradiol-17β dan suhu pemeliharaan berbeda untuk pengendalian kanibalisme pada benih ikan lele *Clarias gariepinus*

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ABSTRACT

Cannibalism is a major problem in the intensive catfish hatchery that caused high mortality. This phenomenon is allegedly due to the high level of testosterone hormones in the early larvae and seed stages. Testosterone is a maternal steroid hormone that is transferred directly by the parent to the egg. Catfish broodstock has high testosterone levels during the gonad maturation phase and it enters the eggs during the process of vitellogenesis. A high level of testosterone is considered to cause catfish seeds to behave aggressively and subsequently encourage cannibalism. This testosterone level may be reduced by estrogen through a negative feedback mechanism. This experiment aimed to evaluate the use of several 17β-estradiol doses at different water temperatures to control cannibalism in catfish seeds. This experiment used two factors, i.e. 17β-estradiol doses (0, 20, and 50 mg/kg) coated in the diet and water temperatures (28 and 31°C). The results showed that 17β-estradiol levels in catfish seeds increased with increasing experimental length. The use of 17β-estradiol at low water temperature (28°C) was better in decreasing mortality, while the dose of 50 mg/kg17β-estradiol which applied at 28°C was the best combination in controlling cannibalism on catfish seeds.

Keywords: 17β-estradiol, cannibalism, *Clarias gariepinus*, seed.

ABSTRAK

Kanibalisme merupakan salah satu masalah utama dalam pembenihan ikan lele intensif karena menyebabkan kematian yang tinggi. Fenomena ini diduga karena kadar hormon testosteron yang tinggi pada tahap larva dan benih. Testosteron merupakan hormon steroid maternal yang ditransfer secara langsung oleh induk ke telur. Induk ikan lele memiliki kadar testosteron yang tinggi pada fase pematangan gonad dan masuk ke dalam telur selama proses vitelogenesis. Tingginya kadar testosteron diduga menyebabkan benih berperilaku agresif dan akan mendorong kanibalisme. Kadar testosteron dapat ditekan dengan meningkatkan kadar hormon estrogen melalui mekanisme *feedback* negatif. Penelitian ini bertujuan mengevaluasi penggunaan dosis estradiol-17β dan suhu pemeliharaan yang berbeda untuk mengendalikan kanibalisme pada benih ikan lele. Penelitian ini menggunakan dua faktor yaitu dosis estradiol-17β yang berbeda (0, 20, dan 50 mg/kg) yang diberikan melalui pakan, dan suhu pemeliharaan yang berbeda (28 dan 31°C). Hasil penelitian menunjukkan bahwa konsentrasi estradiol-17β pada benih ikan lele meningkat seiring dengan lamanya pemeliharaan. Penggunaan estradiol-17β pada suhu 28°C lebih baik dalam mengurangi mortalitas, sementara dosis estradiol-17β 50 mg/kg pada suhu pemeliharaan 28°C adalah kombinasi terbaik dalam mengendalikan kanibalisme pada benih ikan lele.

Kata kunci: benih, *Clarias gariepinus*, estradiol-17β, kanibalisme.

INTRODUCTION

Catfish *Clarias gariepinus* is one of the prospective freshwater commodities to be cultivated. The increase in catfish hatchery production has been carried out widely, but seed producers are still experiencing a loss of high mortality during the early seed stages. Deaths from cannibalism account for 70–83% of the total deaths accumulated during 46–50 rearing days in catfish at the larval and seed phases (Appelbaum & Van Damme, 1988). Furthermore, cannibalism is believed to be one of the causes of more than half of the total mortality in pikeperch seeds (Król & Zake, 2016). Several attempts have been made to control cannibalism in fish, such as manipulating the level of satiety through increasing the amount of feed and frequency of feeding, but it did not eliminate cannibalism in catfish seeds. Also, the size sorting conducted regularly is considered less effective in mass catfish farming activities and has not optimally reduced the level of cannibalism in catfish seed (Marimuthu *et al.,* 2011; Eyo & Ekanem, 2011; Biu *et al.,* 2015). Cannibalism triggers in intensive seeding systems are thought caused by the intraspecific aggressive behavior of catfish feeds (Onwuteaka & Prince, 2015).

Aggressive behavior that causes cannibalism in catfish is an effect of neuromodulators (Kania *et al.,* 2012) which is mediated by high levels of testosterone (Forsatkar *et al.,* 2013). Testosterone is a maternal steroid hormone that is transferred directly by the parent to the egg (Paitz *et al.,* 2015). Catfish broodstock is reported to have high testosterone levels in the gonad maturation phase (Zairin *et al.,* 1992) and enter the eggs during the process of vitellogenesis. Detected levels of the hormone testosterone begin to increase and stabilize on the 30th day or at the size of 15 mm (Rahmadiah, 2018). High levels of testosterone thought to cause seeds to behave aggressively and subsequently encourage cannibalism. A decrease in testosterone levels can be done by increasing estrogen levels, which causes a negative feedback mechanism (Dinsdale & Ward, 2010). Some studies using the estradiol hormone have been carried out, such as environmental estrogen (EE2) and 17α-ethinylestradiol in male zebrafish estrone and 17α-estradiol in *Pimephales promelas* (Filby *et al*., 2012; Colman *et al.*, 2009; Dammann *et al*., 2011). Besides, the use of 17β-estradiol has been shown to reduce cannibalism levels in African catfish seeds (Siregar, 2017), but the dosage used still needs to be evaluated. Furthermore, hormone levels are believed to be associated with environmental temperature, especially in fish that are poikilothermic. Therefore, further research is needed to evaluate the effect of the 17β-estradiol hormone combined with temperature on aggressive behavior and cannibalism in catfish seed stages. This experiment aimed to evaluate the use of several 17β-estradiol doses at different water temperatures to control cannibalism in catfish seeds.

MATERIALS AND METHODS

The experimental design

The method used for this experiment was a factorial completely randomized design consisted of two factors, namely 17β-estradiol doses (0, 20, and 50 mg/kg) coated to the diet and water temperatures (28 and 31°C). Each treatment had three replications.

Research procedures

Experimental time and location

The experiment was conducted from January–March 2019 in the Laboratory of Reproduction and Genetics of Aquatic Organism, Department of Aquaculture, IPB University, and Laboratory of Research Institute for Ornamental Fish Culture, Depok, West Java.

Aquarium preparation

The containers used were 30×20×20 cm aquaria and the water volume used in each aquarium was 8 L. Each aquarium was equipped

Table 1. Research experimental design

Treatment	The combination of 17β-estradiol treatment and temperature
E0028	17β-estradiol 0 mg/kg and water temperature at 28°C
E2028	17β-estradiol 20 mg/kg and water temperature at 28°C
E5028	17β-estradiol 50 mg/kg and water temperature at 28°C
E0031	17β-estradiol 0 mg/kg and water temperature at 31°C
E2031	17β-estradiol 20 mg/kg and water temperature at 31°C
E5031	17β-estradiol 50 mg/kg and water temperature at 31°C

with aerations and regulated heaters according to the temperature of the treatment. The aquaria were placed in a room with a temperature of 25–26°C.

Preparation of experimental feed

The hormone used in this study was estradiol-17β produced by Argent Chemical Laboratories, USA. The hormone was dissolved in 300 ml of alcoholic solution 96% and sprayed onto commercial feed using a coating method following Siregar (2017). Then, the feed was left to dry at room temperature.

Fish rearing

Each aquarium was stocked with catfish seeds at a density of 15 fishes per liter. The seeds used were 2.55 ± 0.14 cm in size obtained from catfish seed producers in Bogor, West Java. Rearing of hormones-fed seed was carried out for 30 days. During the experiment, the feed was given as much as 5% of the total weight of fish biomass. The feeding frequency was ~~of~~ three times a day in the morning (7:00 a.m.), noon (1:00 a.m.), and evening (7:00 a.m.).

Observation of aggressive behavior and cannibalism

Seed behavior and type of cannibalism were observed starting from the first day of the treatment. Behavior observations were carried out for 3 minutes before and during feeding moment, using a video action camera 4K ultra HD every seven days, while cannibalism observation was carried out every six hours. Dead fish was classified according to the characteristics based on the type of cannibalism, which consisted of cannibalism types I and II. Type I is the condition of the seed with tail damage, intact body but had bite marks on the stomach, head, or body partially eaten. Type II is the condition of the whole seed eaten from head to tail (Xi *et al*., 2017).

Parameter measurement

17β-estradiol and testosterone levels

Measurement of 17β-estradiol and testosterone levels were carried out on days 0, 15, and 30 during the rearing period. Three fish samples were taken from each treatment. The samples taken are body fluids (body serum). The samples were anesthetized using clove oil at a dose of 0.01 ml/L. The samples were crushed and added with PBS (phosphate buffer saline) with a ratio of 1:4 then centrifuged at 5000 rpm for 15 minutes. The supernatant was taken and stored in a freezer at -20°C until analyzed using the Vidas ELISA kit for 17β-estradiol (EIA–2693) and testosterone (EIA–1559). The data were analyzed using the Gen 5 (BioTek® Instruments, Inc.) program.

Seed behavior

Behavior observation was carried out for three minutes before and during feeding using a video camera every seven days. Behavior levels were classified according to Table 2 and calculated based on Nieuwegiessen (2009).

$$\text{Behavior (\%)} = \frac{\text{Observated seed (individual)}}{\text{Number of observed seed (individual)}} \times 100$$

Cannibalism

Cannibalism calculation is based on the total number of missing and consumed. The type of cannibalism was measured based on the formula of Król & Zakes (2016). The cannibal potential of seeds was calculated based on the size of the seeds that two to three times larger than the average size of the body at the end of the experiment (Król & Zakes, 2016).

$$\text{Cannibalism (\%)} = \frac{\text{Number of seed missing or consumed (individual)}}{\text{Initial number of seed (individual)}} \times 100$$

Table 2. Ethogram of catfish behavioral observations.

Elements of behavior	Definition
Agonistic behavior	Chasing or biting a fish, or being chased upon or bitten by another fish.
Escape	The fish moves to the water surface and it is head emerges from the water surface for a few hours with the covered gill.
Resting	Moving passively through the water or lying still at the bottom of the media.
Stereotypic behavior	Continuous and compulsive swimming under a fixed, repetitive pattern for at least 10 seconds.
Swimming	A displacement of the body, moving, and eating.

Source: Nieuwegiessen, 2009.

$$\text{Type 1 Cannibalism (\%)} = \frac{\text{Number of seed missing (individual)}}{\text{Initial number of seed (individual)}} \times 100$$

$$\text{Type 2 Cannibalism (\%)} = \frac{\text{Number of seed consumed (individual)}}{\text{Initial number of seed (individual)}} \times 100$$

$$\text{Cannibal potential (\%)} = \frac{\text{Number of seed cannibal potential (individual)}}{\text{Final number of seed (individual)}} \times 100$$

Mortality

The percentage of deaths is the number of the individual which normally died, not caused by cannibalism for 30 days of treatment. Normal death is death caused by the inability of seed to adapt to the environment, without bite marks or injuries (Król & Zakes, 2016). The mortality was calculated every day.

$$\text{Mortality (\%)} = \frac{\text{Number of dead seed (individual)}}{\text{Initial number of seed (individual)}} \times 100$$

Survival rates

Survival is the number of fish life opportunities during the 30-day maintenance period. Survival is calculated according to the formula as follows:

$$\text{Survival rate (\%)} = \frac{\text{Final number of seed (individual)}}{\text{Initial number of seed (individual)}} \times 100$$

Growth performance

Calculation of growth in length and absolute weight based on 30 days of treatment. The growth performance of catfish seeds was measured as fish absolute growth of body length (cm), fish absolute growth of body weight (g), fish absolute growth of body length specific length rate (SLR) (%/day), and specific growth rate (SGR) (%/day). Observation of body length and weight was done at the beginning and end of the study using a 0.1 cm scale ruler and a digital scale 0.001 g.

Data analysis

Data were tabulated using Microsoft Office Excel 2010 and analyzed using analysis of variance (ANOVA) through the Minitab 18.0 program with a confidence interval of 95%. If the variance analysis showed significantly different results, then further Tukey's test was carried out.

RESULTS AND DISCUSSION

Results

17β-estradiol and testosterone levels

The results of hormone measurements using the ELISA method and variance analysis are presented in Table 3. Based on Table 3, 17β-estradiol levels in catfish seeds increased with increasing experimental time. On the 15th day, the 17β-estradiol levels of the seeds given 17β-estradiol tended to increase higher than in the 0-day of observation (before being given 17β-estradiol). Furthermore, the seeds without the administration of 17β-estradiol at 28°C showed a higher value of 17β-estradiol levels than the seeds at 31°C.

Table 3. 17β-estradiol and testosterone levels (ng/mL) of catfish seeds at the beginning, middle, and end of the experiment.

Treatment	Measurement					
	Day 0		Day 15		Day 30	
	17β-estradiol	Testosterone	17β-estradiol	Testosterone	17β-estradiol	Testosterone
E0028	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	0.86 ± 0.03^{c}	0.61 ± 0.00^{a}	0.99 ± 0.07^{b}	0.62 ± 0.03^{a}
E2028	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	1.31 ± 0.07^{ab}	0.41 ± 0.05^{b}	1.87 ± 0.32^{a}	0.24 ± 0.02^{d}
E5028	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	1.46 ± 0.13^{a}	0.36 ± 0.01^{b}	2.10 ± 0.00^{a}	0.22 ± 0.02^{d}
E0031	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	0.46 ± 0.15^{d}	0.60 ± 0.02^{a}	0.72 ± 0.02^{b}	0.63 ± 0.15^{a}
E2031	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	1.10 ± 0.11^{bc}	0.55 ± 0.02^{a}	1.77 ± 0.29^{a}	0.46 ± 0.00^{b}
E5031	0.42 ± 0.00^{a}	0.62 ± 0.00^{a}	1.38 ± 0.11^{ab}	0.55 ± 0.04^{a}	2.06 ± 0.07^{a}	0.37 ± 0.05^{c}

Note: Different superscript letters in the same column show the effect of treatment that is significantly different (P<0.05). The values listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

The results also showed an interaction between the administration of 17β-estradiol at different temperatures to decrease testosterone levels in catfish seeds ($P<0.05$). On the 15th day, administration of 17β-estradiol at 28°C decreased testosterone significantly compared to the testosterone levels before 17β-estradiol administration. This condition continued until the 30th day, administration of 17β-estradiol at 28°C tends to cause testosterone levels to decrease to 63%, while at 31°C it only decreased by 34%. In addition, the results of hormone measurements showed that the seeds without the administration of 17β-estradiol contained testosterone level, which tends to be stable during the experiment.

Seed behavior

Results based on seed behavior observations before feeding for 30 days were obtained as shown in Table 4. The statistical analysis results showed an interaction between the administration of 17β-estradiol at different temperatures on the behavior of catfish seeds before feeding included agonistic, escape, resting, and swimming behavior ($P<0.05$). The seeds treated by 17β-estradiol were known to have lower agonistic behavior compared to treatment without 17β-estradiol administration. The lowest agonistic value was found in the dose of 50 mg/kg 17β-estradiol i.e. 3.47%. A similar behavior pattern also occurred in the behavior at feeding moment, which is presented in Table 5.

Based on Table 5, the administration of 17β-estradiol with different temperatures has interactions with catfish seed behavior during feeding, which included agonistic, resting, stereotypic, and swimming ($P<0.05$). Seeds without administration of 17β-estradiol at 31°C were known to have higher agonistic, escape, and stereotypic behavior than those seeds with the administration of 17β-estradiol at 28°C.

Cannibalism

Observations on survival, mortality, cannibalism, cannibalism type, and cannibal potential for 30 days of the experiment are presented in Table 6. The results showed an interaction between the combination of administration of 17β-estradiol with rearing temperature on survival, mortality, cannibalism level, type-I cannibalism, and cannibal potential

Table 4. Observation results of catfish seed behavior were observed before feeding for 30 days of the experiment.

Treatment	Elements of behavior (%)				
	Agonistic	Escape	Resting	Stereotypic	Swimming
E0028	6.44 ± 0.52^{a}	3.81 ± 0.16^{b}	11.4 ± 0.52^{c}	1.73 ± 0.44^{a}	76.6 ± 0.64^{a}
E2028	4.95 ± 0.02^{b}	3.68 ± 0.36^{bc}	16.6 ± 0.74^{b}	1.85 ± 0.24^{a}	73.0 ± 0.87^{bc}
E5028	3.47 ± 0.06^{c}	2.98 ± 0.20^{cd}	20.6 ± 0.53^{a}	2.04 ± 0.18^{a}	70.9 ± 0.66^{c}
E0031	7.16 ± 0.18^{a}	4.73 ± 0.49^{a}	11.1 ± 0.87^{c}	1.89 ± 0.34^{a}	75.1 ± 1.02^{ab}
E2031	4.55 ± 0.28^{b}	2.94 ± 0.20^{cd}	15.1 ± 1.40^{b}	2.04 ± 0.19^{a}	75.4 ± 1.29^{ab}
E5031	4.22 ± 0.19^{b}	2.85 ± 0.02^{d}	14.8 ± 1.31^{b}	1.77 ± 0.33^{a}	76.4 ± 1.36^{a}

Note: Different superscript letters in the same column show the effect of treatment that is significantly different ($P<0.05$). The values listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

Table 5. Observation results of catfish seed behavior observed feeding moment for 30 days of the experiment.

Treatment	Elements of behavior (%)				
	Agonistic	Escape	Resting	Stereotypic	Swimming
E0028	3.27 ± 0.24^{b}	2.29 ± 0.20^{b}	6.03 ± 0.40^{c}	1.17 ± 0.22^{b}	87.3 ± 0.70^{a}
E2028	2.24 ± 0.32^{c}	1.66 ± 0.37^{bc}	7.91 ± 0.96^{b}	0.74 ± 0.15^{b}	87.5 ± 0.81^{a}
E5028	1.88 ± 0.28^{c}	1.42 ± 0.23^{c}	11.0 ± 0.48^{a}	0.77 ± 0.40^{b}	84.9 ± 0.43^{bc}
E0031	4.92 ± 0.40^{a}	3.67 ± 0.52^{a}	6.31 ± 0.46^{c}	2.26 ± 0.30^{a}	82.8 ± 1.57^{c}
E2031	3.57 ± 0.14^{b}	2.51 ± 0.29^{b}	6.41 ± 0.56^{bc}	1.35 ± 0.16^{b}	86.2 ± 0.39^{ab}
E5031	2.33 ± 0.18^{c}	1.78 ± 0.16^{bc}	7.05 ± 0.26^{bc}	0.99 ± 0.28^{b}	87.8 ± 0.13^{a}

Note: Different superscript letters in the same column show the effect of treatment that is significantly different ($P<0.05$). The values listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

(P<0.05). Furthermore, administration of 50 mg/kg 17β-estradiol at 28°C showed the highest percentage of survival (76.7%), while treatment without administration of 17β-estradiol had a lower percentage of seed survival. This is due to differences in the level of cannibalism during the experiment. Administration of 50 mg/kg 17β-estradiol at 28°C showed a percentage of cannibalism of 15.8% and was able to reduce cannibalism levels by 45% compared to other treatments at the same temperature. Based on observations, treatment without administration of 17β-estradiol at 31°C increased the occurrence of higher cannibalism by 47.5% compared to treatment without the administration of 17β-estradiol at 28°C. In addition, the combination of treatment without administration of 17β-estradiol at 31°C also caused high cannibal potential during the experiment (P<0.05).

Furthermore, the timing of cannibalism showed varying values in each treatment. The highest peak of cannibalism took place from 12.00 p.m.–06.00 a.m. This is related to the habits and behavior of catfish seeds, which tend to be nocturnal. Time for cannibalism and the number of seeds type-I cannibalism observed per 24 hours for 30 days are presented in Figure 1.

Growth performance

The results of the growth performances are shown in Table 7. Results of seed growth measurements for 30 days of the experiment

Table 6. Observation of catfish seeds survival, mortality, cannibalism, and cannibal potential for 30 days of the experiment.

Treatment	Survival rate (%)	Mortality (%)	Cannibalism (%)	Cannibalism type		Cannibal potential (%)
				Type-I (%)	Type-II (%)	
E0028	65.3 ± 0.48^{c}	6.11 ± 0.96^{b}	28.6 ± 0.48^{b}	22.8 ± 0.48^{b}	5.83 ± 0.00^{ab}	8.09 ± 0.77^{b}
E2028	69.4 ± 0.48^{b}	6.39 ± 0.48^{b}	24.2 ± 0.83^{c}	18.6 ± 0.48^{c}	5.56 ± 1.27^{ab}	7.20 ± 1.15^{b}
E5028	76.7 ± 1.67^{a}	7.50 ± 1.67^{ab}	15.8 ± 0.00^{d}	10.8 ± 0.83^{d}	5.00 ± 0.83^{b}	6.17 ± 0.74^{b}
E0031	43.1 ± 0.96^{d}	9.44 ± 0.48^{a}	47.5 ± 0.83^{a}	39.4 ± 0.48^{a}	8.06 ± 0.48^{a}	11.6 ± 1.71^{a}
E2031	66.9 ± 0.48^{c}	6.11 ± 0.48^{b}	26.9 ± 0.96^{b}	21.4 ± 1.27^{b}	5.56 ± 2.10^{ab}	6.64 ± 0.67^{b}
E5031	69.7 ± 0.48^{b}	7.22 ± 0.96^{ab}	23.1 ± 0.48^{c}	17.8 ± 1.27^{c}	5.28 ± 0.96^{ab}	6.37 ± 0.67^{b}

Note: Different superscript letters in the same column show the effect of treatment that is significantly different (P<0.05). The values listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

Table 7. Observation results of catfish seed growth performance for 30 days of the experiment.

Measurement	Treatment					
	E0028	E2028	E5028	E0031	E2031	E5031
PA	2.55 ± 0.01	2.55 ± 0.01	2.55 ± 0.01	2.55 ± 0.01	2.55 ± 0.01	2.55 ± 0.01
BA	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01	0.16 ± 0.01
PF	3.96 ± 0.01	4.04 ± 0.02	3.98 ± 0.01	3.97 ± 0.01	4.24 ± 0.01	4.02 ± 0.01
BF	0.57 ± 0.01	0.58 ± 0.01	0.60 ± 0.01	0.58 ± 0.01	0.70 ± 0.01	0.58 ± 0.01
PPM (cm)	1.41 ± 0.01^{c}	1.48 ± 0.02^{b}	1.42 ± 0.01^{c}	1.42 ± 0.01^{c}	1.69 ± 0.01^{a}	1.47 ± 0.01^{b}
PBM (g)	0.41 ± 0.01^{b}	0.42 ± 0.01^{b}	0.44 ± 0.01^{b}	0.41 ± 0.01^{b}	0.54 ± 0.01^{a}	0.42 ± 0.01^{b}
SLR (%/day)	1.47 ± 0.00^{c}	1.53 ± 0.01^{b}	1.48 ± 0.00^{c}	1.47 ± 0.01^{c}	1.69 ± 0.00^{a}	1.52 ± 0.00^{b}
SGR (%/day)	4.22 ± 0.07^{b}	4.25 ± 0.06^{b}	4.37 ± 0.05^{b}	4.23 ± 0.06^{b}	4.89 ± 0.05^{a}	4.25 ± 0.08^{b}

Note: PA: the initial body length (cm); BA: the initial body weight (g); PF: the final body length (cm); BF: the final body weight (g); PPM: absolute length growth; PBM: absolute weight growth; SLR: specific length rate; SGR: specific growth rate. Different superscript letters in the same column showed the effect of treatment that was significantly different. (P<0.05). The values listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

showed an interaction between the dose of 17β-estradiol and the temperature on absolute length growth, absolute weight growth, specific length rate, and specific growth rate ($P<0.05$). The administration of 20 mg/kg 17β-estradiol at 31°C showed the highest value in growth performance than the other treatment.

Discussion

Based on hormone measurements, it is known that the seeds treated during the experiment showed that 17β-estradiol levels increased with increasing experimental time while the testosterone levels decreased with the administration of 17β-estradiol compared to seeds without administration of 17β-estradiol. On the 30th day, the combination of 17β-estradiol with the temperature had higher 17β-estradiol levels than the seeds without the administration of 17β-estradiol. Administration of 17β-estradiol is thought to increase the estrogen level in the catfish seed. A similar condition was reported by Yuan *et al.,* (2012) which stated that there was an increase of 17β-estradiol level after administering a dose of 20 mg/kg 17β-estradiol in Asian swamp eel. Some types of fish are also known to have decreased in testosterone levels after administration of 17β-estradiol as in *A. stellatus* females, *Monopterus albus*, and *Clarias gariepinus* (Khara *et al.,* 2013; Yuan *et al.,* 2012; Siregar *et al.,* 2017).

The increase in 17β-estradiol levels in this experiment is also known to reduce testosterone levels. This testosterone levels may be reduced by estrogen hormones through a negative feedback mechanism (Dinsdale & Ward, 2010). This is also following the results of the experiment by Khara *et al*., (2013) who reported that the administration doses of 25 and 50 mg/kg 17β-estradiol had a significant effect on the decrease in testosterone levels accompanied by an increase in the concentration of 17β-estradiol in stellate sturgeon.

The decrease in testosterone levels in catfish seeds is in line with the decrease in the incidence of cannibalism during the experiment. In the present experiment, a combination of 50 mg/kg 17β-estradiol at a temperature of 28°C was proven to be able to effectively control cannibalism behavior in catfish seeds with a percentage of 15.8% cannibalism, while the dose of 0 mg/kg 17β-estradiol at a temperature of 31°C showed the least effective in controlling cannibalism, with the highest percentage (47.5%). The level of cannibalism decreased due to the administration of 17β-estradiol showed that this hormone was being able to reduce the aggressive behavior of catfish seeds. The results showed that the administration dose of 50 mg/kg 17β-estradiol at 28°C had the lowest agonistic behavior before feeding time compared to other treatments. Aggressive behavior is known to be the basis of cannibal behavior (Vallon *et al.*, 2016). This condition is also reported by Siregar (2017) that cannibalism is associated with aggressive behavior which could be reduced by using 17β-estradiol. In addition, several reports regarding the effect of 17β-estradiol have shown a decrease in

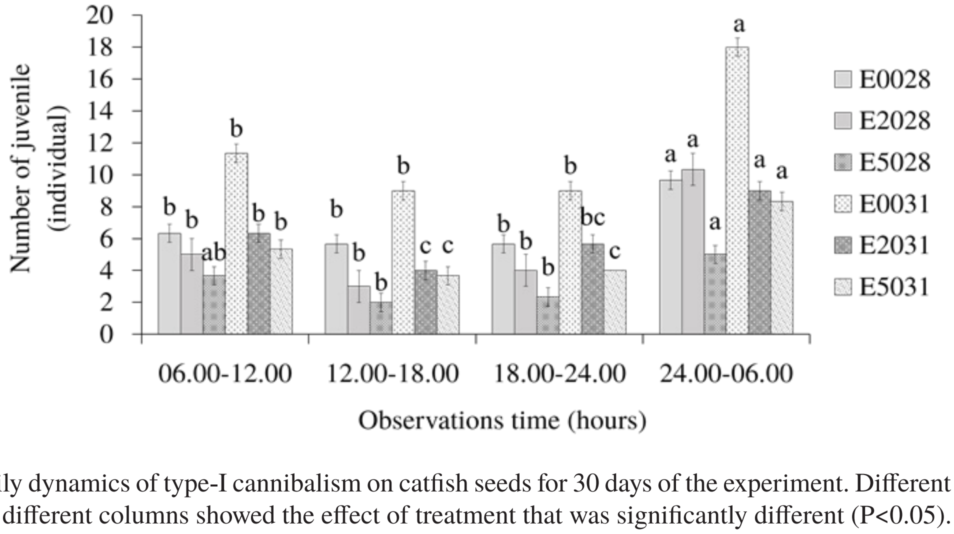

Figure 1. Daily dynamics of type-I cannibalism on catfish seeds for 30 days of the experiment. Different superscript letters in the different columns showed the effect of treatment that was significantly different ($P<0.05$). The values are listed as mean ± SD. E0028: 17β-estradiol 0 mg/kg and water temperature at 28°C, E2028: 17β-estradiol 20 mg/kg and water temperature at 28°C, E5028: 17β-estradiol 50 mg/kg and water temperature at 28°C, E0031: 17β-estradiol 0 mg/kg and water temperature at 31°C, E2031: 17β-estradiol 20 mg/kg and water temperature at 31°C, E5031: 17β-estradiol 50 mg/kg and water temperature at 31°C.

aggression and aggressiveness such as in male zebrafish (Filby *et al.*, 2012) and *Pimephales promelas* (Dammann *et al.*, 2011). Furthermore, high cannibalism at 31°C compared to 28°C is thought to be due to the rate of seed metabolism. The same thing happened in Baras *et al.* (2010), who reported that catfish seeds that were reared at high temperatures run into cannibalism.

The highest time for cannibalism tends to occur at 12.00 p.m.–06.00 a.m. The high incidence of cannibalism at that time was related to the nocturnal nature of catfish seedlings (Ramteke *et al.,* 2009). African catfish larvae and seeds are known to increase their feeding behavior at night (Appelbaum & Kamler, 2000). There are indications that feeding three times a day at 6-hour intervals causes foraging activities with high hunger motivation at 24.00–06.00. According to Conceicao *et al.* (2010), the interval of a feeding schedule that is too far can trigger cannibalism. Besides, Ribeiro & Qin (2016) and Baras *et al.* (2013) also added that all forms of feeding restrictions not only encourage foraging activities with hunger motivation but can also lead to food competition, variability in food intake, and the formation of a dominant hierarchy. In the growth parameters, the administration of 20 mg/kg 17β-estradiol at 31°C has a higher growth in length and weight than other treatments, while administration of 50 mg/kg 17β-estradiol has a low growth value. There are indications that high doses of hormones have a negative effect on growth. Research of Tarkhani *et al.* (2015) in goldfish *Carassius auratus* showed that high doses of 17β-estradiol might be toxic and reduced the growth parameters three months of the administration. To achieve the highest result, in which the maximum dose of hormones did not lead to adverse results (reduction in length and weight growth), the hormone must be used in the shortest possible time.

In the present study, there was no checking on the sex ratio in seeds due to the administration of the 17β-estradiol so that the sex ratio in seeds was unknown. However, according to Torrans *et al.* (1988), seeds aged 7 days after hatching or total length ranged from 9–13 mm, morphologically have not undergone sex differentiation, so in this study, it is expected that 17β-estradiol does not affect the sex ratio of catfish seeds.

This research is still in the early stages to prove the relationship between estradiol, testosterone, and cannibalism, so further research is still needed regarding their use directly in the field, especially costs. In addition to using natural estradiol (17β-estradiol) to enrich the body's estrogen, some estrogenic materials from plants can also increase the body's estrogen content such as phytoestrogens. According to Jargin (2014), phytoestrogens are natural decomposition products that are similar to. Flavonoid compounds are estrogenic because they can stimulate estrogen formation and reduce the secretion of testosterone hormone (Ashfahani *et al.,* 2010). Turmeric and soy are known to have phytoestrogenic potential which can increase estrogen in the body (Saraswati, 2015; Mishra *et al.,* 2011). Furthermore, according to Hoga *et al.* (2018), natural estradiol residues will easily disappear from fish meat in less than a month after administration, because they are quickly metabolized and excreted by the body.

CONCLUSION

Administration of 17β-estradiol at water temperature 28°C can reduce testosterone levels and cannibalism in catfish seeds more effectively than at higher temperatures (31°C). The dose of 50 mg/kg 17β-estradiol, which was applied at water temperature 28°C, was the best combination in controlling cannibalism in catfish seeds.

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