

## **Controlling the cannibalism of African catfish juvenile by 17 $\beta$ -estradiol hormone administration and the stocking density determination**

### **Pengendalian kanibalisme benih ikan lele Afrika *Clarias gariepinus* menggunakan hormon 17 $\beta$ -estradiol dan pengaturan padat tebar**

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#### **ABSTRACT**

An effort to increase the production of juvenile catfish is limited by the high mortality rate, especially caused by cannibalism. The hormonal treatment has been conducted as an effort to control cannibalism. This study used completely randomized factorial design, consisted of six treatments and three replications. There were two factors examined in this study, the first factor was different doses of 17 $\beta$ -estradiol hormone (0, 30, and 60 mg estradiol-17 $\beta$ /kg) incorporated in the feed, and the second factor was stocking density (150 and 300 fish/m<sup>2</sup>). Juvenile catfish with the size of 4.0  $\pm$  0.1 cm were reared for 30 days in the 84 L aquarium. The results showed that hormone treatment could reduce cannibalism rate, type-I and II cannibalism compared to control (P<0.05). The lowest of mortality was obtained in the treatment B (17 $\beta$ -estradiol administration of 30 mg/kg, at stocking density of 150 fish/m<sup>2</sup>; P<0.05). The lowest cortisol level was found in treatment F, and the highest estradiol level was found in treatment F (17 $\beta$  estradiol administration of 60 mg/kg, at stocking density of 300 fish/m<sup>2</sup>; P<0.05). The lowest of blood glucose level was found in treatment B (P<0.05). The highest specific weight growth was found in the stocking density of 150 fish/m<sup>2</sup> (P<0.05). The results of this study indicated that administration of 17 $\beta$  estradiol in feed could reduce the level of cannibalism in African catfish juvenile.

Keywords: cannibalism, Juvenile, catfish, estradiol-17 $\beta$ , cortisol

#### **ABSTRAK**

Upaya untuk meningkatkan produksi benih ikan lele dibatasi oleh tingginya angka kematian, terutama yang disebabkan oleh kanibalisme. Pendekatan hormonal telah dilakukan sebagai upaya pengendalian kanibalisme. Penelitian ini menggunakan rancangan acak lengkap faktorial, terdiri dari enam perlakuan dan tiga ulangan. Ada dua faktor yang diteliti dalam penelitian ini, faktor pertama adalah dosis hormon 17 $\beta$ -estradiol yang berbeda (0, 30, dan 60 mg/kg) yang diberikan melalui pakan, dan faktor kedua adalah padat tebar (150 dan 300 ekor/m<sup>2</sup>). Benih ikan lele berukuran 4.0  $\pm$  0.1 cm dipelihara selama 30 hari di akuarium (84 L). Hasil penelitian menunjukkan bahwa perlakuan hormon dapat menurunkan tingkat kanibalisme tipe-I dan II dibandingkan kontrol (P<0.05). Mortalitas terendah diperoleh pada perlakuan B (pemberian 17 $\beta$ -estradiol 30 mg/kg, pada padat tebar 150 ekor/m<sup>2</sup>; P<0.05). Kadar kortisol terendah ditemukan pada perlakuan F, dan kadar estradiol tertinggi ditemukan pada perlakuan F (pemberian 17 $\beta$ -estradiol 60 mg/kg, pada padat tebar 300 ekor/m<sup>2</sup>; P<0.05). Kadar glukosa darah terendah ditemukan pada perlakuan B (P<0.05). Pertumbuhan bobot spesifik tertinggi ditemukan pada padat tebar 150 ekor/m<sup>2</sup> (P<0.05). Hasil penelitian ini menunjukkan bahwa pemberian hormon 17 $\beta$ -estradiol dengan dosis 30 mg/kg pakan, dan padat tebar 150 ekor/m<sup>2</sup> dapat menurunkan tingkat kanibalisme pada benih ikan lele di Afrika.

Kata kunci: kanibalisme, juvenil, lele, 17 $\beta$ -estradiol, kortisol

## INTRODUCTION

African catfish *Clarias gariepinus* is one of the most popular freshwater species in Indonesia. Domestic production of African catfish showed a promising level with an increase of 26.43% per year (KKP, 2014). However, it cannot be said that African catfish culture was free of challenge. Cannibalism is one of the major challenges faced by the farmers because it impacted the survival. Król and Zakes (2016) supported the later statement that cannibalism contributes more than 50% of mortality during the rearing period. It is believed that intraspecific aggressive behavior is found in most catfish cannibalism cases (Onwuteaka & Prince, 2015). Particularly, it distracted most farmers because of its impact on production and undoubtedly profit. Cannibalism is often stated as killing and consuming amongst similar species, partially or entirely of the prey (Liu *et al.*, 2017). Cannibalism frequently happens on catfish started from larvae stadium (Mukai *et al.*, 2013). According to Obirikorang *et al.* (2014), cannibalism on catfish commonly occurs on fingerling amongst 700-1900 hours or 29-79 day old. At this particular time, cannibalism contributes 42.5% of mortality. Size sorting is required to be conducted regularly and low stocking density will help to reduce cannibalism. Nevertheless, the previously mentioned methods are high labor and need a wider area. It is not cost-effective considering that catfish can be cultured in high stocking density.

Catfish broodstock has a high testosterone content in the gonad maturation phase and right before ovulation (Zairin *et al.* 1992). A maternal steroid hormone, testosterone, for example, can be directly transferred by the broodstock to the egg and it will be detected until it hatched (Paitz *et al.*, 2015). High testosterone content particularly affected aggressive behavior. Kim and Park (2012) mentioned that the estrogen increase causes feedback mechanism that will decrease testosterone content. Several

former studies found that estrogenic compound and their waste (estradiol-17 $\beta$ , E2) and their analogue (17 $\alpha$ -ethinylestradiol, EE2) located in an aquatic environment have negative effects and influence aquatic reproduction organism. The 17 $\alpha$ -ethinylestradiol (Saaristo *et al.*, 2010) and 17 $\beta$ -estradiol (Clotfelter & Rodriguez, 2006) degrade the aggression behavior in male broodstock. Estrogen has other functions, i.e. antidepressant and antistress (Calmarza-Font *et al.*, 2012). Andrade *et al.* (2005) stated that estrogen deficiency is often related to stress, anxiety, and depression. Afterward, Prasad *et al.* (2015) presented that serotonin is essential in inhibiting aggressive behavior by boosting brain serotonergic. It has been proved by Kumar *et al.* (2017) that serotonin can reduce cannibalism on snapper. A similar result is assumed to be applied on African catfish by using 17 $\beta$ -estradiol. In brief, this study aimed to analyze and determine the 17 $\beta$ -estradiol dosage and stocking density to reduce cannibalism behavior on African catfish.

## MATERIALS AND METHODS

### Experimental fish

The tested fish was Sangkuriang catfish fingerling with average length  $4.0 \pm 0.1$  cm from the Center of Freshwater Aquaculture, Sukabumi. It was stocked according to the stocking density treatment (150 individuals/m<sup>2</sup> and 300 individuals/m<sup>2</sup>).

### Experimental design

This study used the factorial randomized complete design. There were two experimental factors, i.e., 17 $\beta$ -estradiol dosage (0 mg/kg, 30 mg/kg, and 60 mg/kg) and stocking density (150 individuals/m<sup>2</sup> and 300 individuals/m<sup>2</sup>) (Table 1). Each treatment was repeated three times. The 17 $\beta$ -estradiol hormone was delivered orally using the commercial feed with 39-41% of the protein content for 30 days. Feeding was done three times a day as much as 10% of the total biomass.

Table 1. Treatment combination of 17 $\beta$ -estradiol dosage and stocking density.

Treatment	Treatment combination of 17 $\beta$ -estradiol dosage and stocking density
A	0 mg estradiol-17 $\beta$ / kg of feed and 150 individuals/m <sup>2</sup>
B	30 mg estradiol-17 $\beta$ / of feed and 150 individuals/m <sup>2</sup>
C	60 mg estradiol-17 $\beta$ / kg of feed and 150 individuals/m <sup>2</sup>
D	0 mg estradiol-17 $\beta$ / kg of feed and 300 individuals/m <sup>2</sup>
E	0 mg estradiol-17 $\beta$ / kg of feed and 300 individuals/m <sup>2</sup>
F	0 mg estradiol-17 $\beta$ / kg of feed and 300 individuals/m <sup>2</sup>

### Experimental feed preparation

Each hormone treatment was weighed according to the treatment. After that, the feed was sprayed using alcohol 96% (300 mL of alcohol for 1 kg feed). Furthermore, it was sprayed to the feed. The feed was dried at room temperature for 12 hours to release the alcohol smell. Thereafter, it was stored at -20°C freezer.

### Fingerling sample preparation

This analysis was particularly aimed to measure the hormone and glucose content of the tested fingerling. Ten fingerlings were used for each treatment. The fingerling sample was fainted using a stabilizer with 1 mL/L dosage for 5 minutes. The sample was crushed and thoroughly mixed in PBS solution (phosphate buffer saline). The ratio was 1:4 and 1:2 for hormone and glucose measurement, respectively. Afterward, the samples were centrifuged at 5000 rpm and 4°C for 10 minutes. Supernatant then was separated into another microtube and stored at -20°C.

### Experimental parameters

The cannibalism was calculated using the formula by Obirikorang *et al.* (2014). The mortal fingerling with wounds was categorized as cannibalism type I, while the swallowed fingerling was classified as cannibalism type II. The mortal fingerling without wounds grouped in the mortality caused by other factors and the fingerlings with 2-3 times bigger than the others were categorized as a potential cannibal. Masing-masing parameter tersebut dihitung berdasarkan rumus yang dikemukakan oleh Król dan Zakes (2016). Production performance was calculated using a formula by Effendie (1997). Hormone analysis at the end of the study was conducted using ELISA (*enzyme-linked immunosorbent assay*) method. Estradiol, cortisol, and blood glucose were analyzed using Kit Estradiol-17β (DRG EIA 2693), Kit Cortisol (EIA 1887), and Kit GLUCOSE, respectively. Water quality analysis was managed at the beginning, in the middle, and at the end of the study. Water temperature was measured using a thermometer, pH using a pH meter. Dissolved oxygen and total ammonia nitrogen were measured using a DO meter and spectrophotometer, respectively.

#### Cannibalism

The cannibalism rate was measured using the formula according to Obirikorang *et al.* (2014).

$$\text{Cannibalism (\%)} = \frac{\text{Number of missing \& consumed fingerling}}{\text{Initial number of fish}} \times 100$$

#### Cannibalism category

Deceased and wounded fish were categorized into cannibalism type I. Meanwhile, completely lost or swallowed fish was categorized to cannibalism type II (Król & Zakes, 2016).

$$\text{Cannibalism type I} = \frac{\text{Number of dead wounded fingerling}}{\text{Number of initial fingerling}} \times 100$$

$$\text{Cannibalism type II (\%)} = \frac{\text{Number of missing / consumed fingerling}}{\text{Initial number of fish}} \times 100$$

#### Mortality caused by other factors

Mortal fish without bite wounds was categorized to mortality by other factors (Król & Zakes, 2016).

$$\text{Mortality by other factors} = \frac{\text{Number of dead intact fingerling}}{\text{Number of initial fingerling}} \times 100$$

#### Potential of cannibal

The bigger fish, two or three times bigger, from the average weight was categorized as a potential cannibal (Król & Zakes, 2016).

$$\text{Potential of cannibal (\%)} = \frac{\text{Number of potentially cannibal fingerling}}{\text{Number of final fingerling}} \times 100$$

#### Mortality

Total mortality was calculated at the end of the study.

$$\text{MR (\%)} = \frac{\text{Number of mortal fish}}{\text{Initial number of fish}} \times 100$$

#### Absolute growth

Absolute growth was calculated based on final length and weight minus the initial length and weight.

$$\text{PM} = \text{Pt} - \text{Po}$$

Note:

PM : Absolute growth (cm)

Pt : Final length (cm)

Po : Initial length (cm)

#### Specific growth rate

Specific growth rate was calculated at the end of the study.

$$\text{SGR (\%/day)} = \frac{\text{Ln Wt} - \text{Ln Wo}}{\text{T}} \times 100$$

Note:

SGR : Specific growth rate (%/day)

WT : Final average weight (g)

W0 : Initial average weight (g)

T : Rearing period (day)

#### *Coefficient of length variance*

Both length and weight variance were calculated at the initial and the end of the study.

$$CV (\%) = \frac{\text{Standard deviation of body length}}{\text{Average body length}} \times 100$$

#### *Feed conversion ratio*

Feed conversion ratio is defined as a certain amount of feed required to produce 1 kg of biomass. It was calculated using the following formula:

$$FCR = \frac{F}{[(Wt + Wm) - W0]}$$

Note :

FCR : Feed conversion ratio

P : Feed amount (g)

Wt : Final biomass (g)

Wm : Mortal fish biomass (g)

W0 : Initial biomass (g)

#### **Data analysis**

Data were tabulated using Microsoft Excel 2010. Cannibalism, production performance, hormone content, and blood glucose were analyzed using two-way ANOVA at  $P=0.05$  of significant level assisted by XLSTAT software

version 2014. A post-hoc test was also conducted at 95% of confidence level. Particularly, water quality parameters were explained descriptively.

## **RESULTS AND DISCUSSION**

### **Results**

#### *Mortality and cannibalism rate*

The result showed that there were interactions amongst the hormonal treatment and stocking density towards the mortality, cannibalism type, and mortality by other factors (Table 2;  $P<0.05$ ). Moreover, the lowest mortality was discovered in treatment B. It had 2.94 times of mortality and 3.84 times of cannibalism lower than the non  $17\beta$ -estradiol hormonal treatment (Table 2;  $P<0.05$ ). The application of  $17\beta$ -estradiol also reduced the cannibalism, both type I and type II (Table 2). The discovered cannibalism type I at the  $17\beta$ -estradiol hormonal treatment (B, F, C, and E) was lower than without  $17\beta$ -estradiol hormonal treatment (A and D). Additionally, the lowest mortality by other factors was also disclosed at treatment B (Table 2;  $P<0.05$ ). The potential of cannibal declined along with the  $17\beta$ -estradiol hormonal treatment (Table 2).

According to Figure 1A, mortality by other factors occurred from day 1 to day 14. However, the highest mortality of all treatments was found on day 7. Treatment E and F had the highest mortality, respectively. Moreover, mortality caused by cannibalism type I showed various results, the mortality started on day 1 to day 14 and the highest mortality was found on day 7

Table 2. Mortality and cannibalism of African catfish fingerling *Clarias gariepinus* treated with  $17\beta$ -estradiol hormonal feed and various stocking densities after 30 days of rearing.

Treatment	Mortality (%)	Cannibalism (%)	Cannibalism type		Mortality by other factors (%)	Potential of cannibalism (%)
			Type-I (%)	Type-II (%)		
A	59.03 ± 4.34 <sup>a</sup>	50.69 ± 6.36 <sup>a</sup>	19.44 ± 6.36 <sup>b</sup>	31.25 ± 8.33 <sup>a</sup>	8.33 ± 2.08 <sup>d</sup>	8.12 ± 1.88 <sup>a</sup>
B	20.14 ± 4.34 <sup>d</sup>	13.19 ± 2.41 <sup>b</sup>	6.25 ± 3.61 <sup>c</sup>	6.94 ± 3.18 <sup>c</sup>	6.94 ± 4.34 <sup>d</sup>	3.41 ± 1.39 <sup>b</sup>
C	40.28 ± 3.1 <sup>8b</sup>	19.44 ± 3.18 <sup>b</sup>	11.81 ± 1.20 <sup>c</sup>	7.64 ± 2.41 <sup>bc</sup>	20.83 ± 4.17 <sup>b</sup>	5.44 ± 1.86 <sup>ab</sup>
D	63.19 ± 3.94 <sup>a</sup>	50.35 ± 1.59 <sup>a</sup>	35.42 ± 2.08 <sup>a</sup>	14.93 ± 1.59 <sup>b</sup>	12.85 ± 2.62 <sup>cd</sup>	4.67 ± 2.90 <sup>ab</sup>
E	46.53 ± 4.34 <sup>b</sup>	16.67 ± 3.13 <sup>b</sup>	11.81 ± 4.92 <sup>c</sup>	4.86 ± 2.17 <sup>c</sup>	29.86 ± 1.59 <sup>a</sup>	3.85 ± 1.54 <sup>b</sup>
F	31.60 ± 1.59 <sup>c</sup>	15.63 ± 1.80 <sup>b</sup>	7.99 ± 1.20 <sup>c</sup>	7.64 ± 3.01 <sup>bc</sup>	15.97 ± 3.35 <sup>bc</sup>	3.73 ± 2.20 <sup>b</sup>

Different superscript in the same column indicates significant difference ( $P<0.05$ ). Note: A (0 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); B (30 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); C (60 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); D (0 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); E (30 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); F (60 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density).

(Figure 1B). Meanwhile, treatment D showed consistent mortality until day 21.

#### Hormone content and blood glucose

The result indicated interactions amongst  $17\beta$  estradiol hormonal treatment and stocking density towards estradiol, cortisol, and blood glucose of catfish fingerling (Table 3;  $P < 0.05$ ). Treatment F presented the highest estradiol content, but the lowest cortisol (Table 3;  $P < 0.05$ ). Meantime, blood glucose was reported declining along with the  $17\beta$ -estradiol hormonal treatment (Table 3;  $P < 0.05$ ).

#### Production performance

Stocking density had no significant difference towards length specific growth rate (SGR length), size variation, and feed conversion. In contrast, it decreased SGR weight (Table 4;  $P < 0.05$ ). Furthermore, the  $17\beta$ -estradiol hormonal treatment did not affect growth, size variance, and feed conversion (Table 4;  $P > 0.05$ ).

#### Water quality

Water quality results are shown in Table 5. The results indicated in the tolerable range to support the growth and survival of African catfish fingerling (Table 5).

#### Discussion

According to Naumowicz *et al.* (2017) cannibalism is aggressive behavior, caused by stress, variance, and environmental factors occurred in most live phases. Fundamentally, cannibalism is indicated by mortality caused by hurting, preying, and or killing. Different stocking density did not increase mortality and cannibalism (Table 2). However, the  $17\beta$ -estradiol hormonal treatment could diminish mortality and it was in line with the cannibalism decrease, both type I and II (Table 2). The decrease in mortality and cannibalism was presumably caused by the low level of aggressivity. Supported by Colman *et al.* (2009), who reported that the  $17\beta$ -estradiol hormonal treatment

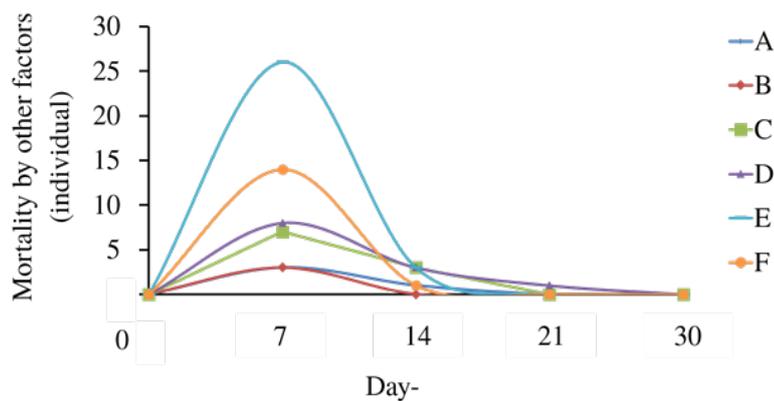


Figure 1A

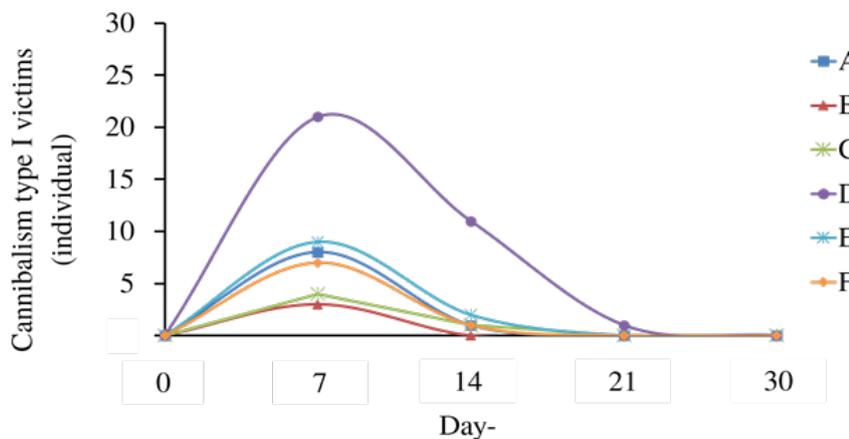


Figure 1B

Figure 1. (A) Daily mortality tendency caused by other factors on African catfish fingerling reared for 30 days. (B) Daily mortality tendency caused by cannibalism type I on African catfish fingerling reared for 30 days.

potentially reduced aggressive behavior on male broodstock of zebra danio *Danio rerio*, fathead minnows *Pimephales promelas* (Salierno *et al.*, 2009), and *Betta splendens* (Clotfelter & Rodriguez, 2006). Conversely, testosterone and aromatase inhibitors conceivably boost aggressivity (Forsatkar *et al.*, 2013). A similar result was also reported by Zairin *et al.* (2016) who discovered an aggressively boost caused by 17 $\alpha$ -methyltestosterone.

Rajakumar *et al.* (2012) reported in their study that testosterone and 11-ketotestosterone were detected in a 6 cm catfish fingerling. In this study, we used 4 cm in length catfish fingerlings so that we assumed that all of the experimental fingerlings already contented both two hormones mentioned before. Androgen hormone natural existence can be inhibited by increasing estrogen hormone through the feedback mechanism (Dinsdale & Ward, 2010). They also stated that 17 $\beta$ -estradiol could elevate the estradiol content in the catfish fingerling (Table 3). The feedback mechanism and estradiol would eliminate aggressivity, decrease mortality, and cannibalism in African catfish fingerling (Table 2).

According to Solomon and Udoji (2011), cannibalism type II is related to the mouth size along with the growth of catfish. It is also dependent on the size variation and the potential cannibal individuals. The potential cannibal individual undoubtedly will increase the cannibalism probability. In this study, the size variance (CV) at the end of the study did not differ significantly amongst treatments (Table 4). It was presumably caused by the mortality of the smaller individuals prey by the larger

and more aggressive individuals. Hseu and Huang (2014) stated that sorting can be done to prevent cannibalism on orange-spotted grouper larvae *Ephinephelus coioides*. The supported previous statement, Ribeiro and Qin (2013) also declared that sorting was the simplest and most practical solution in preventing cannibalism on directly ontogeny species, e.g. barramundi *Lates calcarifer* has the potential to be a cannibal when their size was 50% bigger than the overall population. Szczepkowski *et al.* (2011) added that sorting contributed a beneficial effect in boosting survival through inhibiting cannibal behavior on pikeperch. Biu *et al.* (2015) confirmed the latter theory on African catfish *Clarias gariepinus*.

Furthermore, the high potential cannibal in this study was found in treatment A (0 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density; P<0.05). Without 17 $\beta$ -estradiol hormonal treatment decreased cannibalism type II. In contrast, it increased cannibalism type I (Table 2). The decrease of cannibalism type II was impacted by the stocking density so that each individual has no space and opportunity to prey its kind. They tended to keep moving, forming groups, and unquestionably lessened the aggressivity (Nieuwegiessen *et al.*, 2008).

The lowest mortality was noticed in treatment B (30 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density) (Table 2). However, there was no significant difference in cannibalism between the hormonal treatment and both high and low stocking density. As mentioned in Table 2, the highest mortality caused by other factors was observed in treatment E. Thereby, lower mortality at treatment B was presumably

Table 3. The 17 $\beta$ -estradiol, cortisol, and blood glucose content of African catfish fingerling *Clarias gariepinus* at the end of the study.

Treatment	Parameter		
	17 $\beta$ -Estradiol (ng/mL)	Cortisol (ng/mL)	Blood glucose (mg/dL)
A	0.70 $\pm$ 0.08 <sup>b</sup>	15.12 $\pm$ 1.36 <sup>ab</sup>	187.22 $\pm$ 7.69 <sup>a</sup>
B	0.84 $\pm$ 0.08 <sup>b</sup>	14.06 $\pm$ 0.67 <sup>b</sup>	131.90 $\pm$ 4.54 <sup>d</sup>
C	0.75 $\pm$ 0.08 <sup>b</sup>	16.51 $\pm$ 0.50 <sup>a</sup>	159.90 $\pm$ 4.06 <sup>b</sup>
D	0.82 $\pm$ 0.01 <sup>b</sup>	16.67 $\pm$ 0.52 <sup>a</sup>	157.21 $\pm$ 7.94 <sup>b</sup>
E	0.85 $\pm$ 0.09 <sup>b</sup>	10.45 $\pm$ 0.76 <sup>c</sup>	144.43 $\pm$ 3.01 <sup>c</sup>
F	1.35 $\pm$ 0.12 <sup>a</sup>	8.72 $\pm$ 1.06 <sup>d</sup>	145.10 $\pm$ 5.42 <sup>c</sup>

Different superscript in the same column indicates significant difference (P<0.05). Note: A (0 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); B (30 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); C (60 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); D (0 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); E (30 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); F (60 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density).

caused by other factors. Table 2 also presented that mortality caused by other factors elevated along with stocking density.

Li *et al.* (2010) described that the increase of blood glucose was the secondary effect of stress, which is mediated by the corticosteroid and catecholamine release. Manuel *et al.* (2014) stated that the plasma cortisol content in catfish fingerling was below 10 ng/mL and increased when exposed by handling, sorting, and transport up to 50 ng/mL. The normal range of blood glucose content is 40-90 mg/dL and will reach two to three times higher than normal when exposed to stress (Patriche, 2009). Hastuti and Subandiyono (2015) stated a slightly different range of normal blood glucose, i.e. 70–100 mg/dL. Table 3 presented that the experimental catfish fingerling experienced stress during the study, which was described by the cortisol and blood glucose content. The 17 $\beta$ -estradiol hormonal treatment can presumably inhibit stress proved by the decline of cortisol and blood glucose content along with the 17 $\beta$ -estradiol hormone dosage (Table 3). Estradiol is known as an antidepressant

hormone, functions as an anxiety inhibitor of several animal species (Walf & Frye, 2009). The definition of estrogen is frequently related to stress, anxiety, and depression because of its ability to modulate neurotransmitter changes, included boosting serotonin and noradrenaline content. It also regulates the serotonin receptor and controls serotonergic neuron activity (Andrade *et al.*, 2005). Gore (2008) added that estrogen was involved in various aspects of the brain neuroendocrine system which affects behavior. The serotonergic activity rise in the fish brain decreases the aggressivity and cannibalism on pikeperch larvae *Sander lucioperca* (Król & Zakes, 2016).

According to SNI 01-6484.2–2000, the rearing of catfish fingerling (3–5 cm) in a plastic/concrete tank which applies 25 fish/m<sup>2</sup> of stocking density, shows an excellent survival, precisely >80%. Statistically, the 300 fish/m<sup>2</sup> treatment presented higher mortality than the 150 fish/m<sup>2</sup> treatment (P<0.05). The water quality in this study was considered as similar to each other and proper for fish culture (Table 5). Thereby, the difference

Table 4. Production performance from treatment combination of 17 $\beta$ -estradiol dosage and various stocking densities on African catfish fingerling *Clarias gariepinus* reared for 30 days.

Parameter	Treatment					
	A	B	C	D	E	F
Initial length (cm)	4.06 ± 0.04 <sup>a</sup>	4.06 ± 0.03 <sup>a</sup>	4.02 ± 0.02 <sup>a</sup>	4.07 ± 0.03 <sup>a</sup>	4.08 ± 0.01 <sup>a</sup>	4.07 ± 0.02 <sup>a</sup>
Final length (cm)	7.38 ± 0.11 <sup>a</sup>	7.19 ± 0.22 <sup>a</sup>	7.03 ± 0.58 <sup>a</sup>	6.79 ± 0.44 <sup>a</sup>	6.87 ± 0.11 <sup>a</sup>	7.21 ± 0.32 <sup>a</sup>
Initial weight (g)	0.53 ± 0.03 <sup>a</sup>	0.52 ± 0.04 <sup>a</sup>	0.49 ± 0.04 <sup>a</sup>	0.54 ± 0.02 <sup>a</sup>	0.55 ± 0.02 <sup>a</sup>	0.59 ± 0.03 <sup>a</sup>
Final weight (g)	2.84 ± 0.08 <sup>a</sup>	2.57 ± 0.21 <sup>a</sup>	2.71 ± 0.48 <sup>a</sup>	2.26 ± 0.33 <sup>a</sup>	2.43 ± 0.36 <sup>a</sup>	2.64 ± 0.40 <sup>a</sup>
Length SGR (%/day)	1.96 ± 0.06 <sup>a</sup>	1.91 ± 0.07 <sup>a</sup>	1.85 ± 0.27 <sup>a</sup>	1.70 ± 0.21 <sup>a</sup>	1.74 ± 0.07 <sup>a</sup>	1.91 ± 0.15 <sup>a</sup>
Weight SGR (%/day)	5.60 ± 0.21 <sup>a</sup>	5.35 ± 0.05 <sup>a</sup>	5.64 ± 0.67 <sup>a</sup>	4.75 ± 0.50 <sup>b</sup>	4.94 ± 0.47 <sup>b</sup>	4.98 ± 0.38 <sup>b</sup>
ALG (cm)	3.29 ± 0.12 <sup>a</sup>	3.13 ± 0.18 <sup>a</sup>	3.00 ± 0.57 <sup>a</sup>	2.7 ± 0.43 <sup>a</sup>	2.79 ± 0.12 <sup>a</sup>	3.15 ± 0.35 <sup>a</sup>
AWG (g)	2.31 ± 0.12 <sup>a</sup>	2.05 ± 0.17 <sup>a</sup>	2.22 ± 0.57 <sup>a</sup>	1.70 ± 0.43 <sup>a</sup>	1.88 ± 0.35 <sup>a</sup>	2.05 ± 0.38 <sup>a</sup>
Length CV (%)	12.39 ± 1.82 <sup>a</sup>	14.25 ± 0.33 <sup>a</sup>	17.27 ± 1.85 <sup>a</sup>	13.95 ± 5.09 <sup>a</sup>	13.12 ± 3.54 <sup>a</sup>	11.59 ± 0.14 <sup>a</sup>
Weight CV (%)	37.69 ± 5.79 <sup>a</sup>	41.48 ± 1.21 <sup>a</sup>	55.50 ± 9.70 <sup>a</sup>	41.57 ± 16.26 <sup>a</sup>	40.65 ± 12.26 <sup>a</sup>	34.17 ± 1.32 <sup>a</sup>
FCR	1.43 ± 0.61 <sup>a</sup>	1.16 ± 0.01 <sup>a</sup>	1.13 ± 0.19 <sup>a</sup>	1.28 ± 0.09 <sup>a</sup>	1.24 ± 0.12 <sup>a</sup>	1.14 ± 0.19 <sup>a</sup>

Different superscript in the same column indicates significant difference (P<0.05). Note: A (0 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); C (60 mg/kg of hormone dosage and 150 individuals/m<sup>2</sup> of stocking density); D (0 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); E (30 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density); F (60 mg/kg of hormone dosage and 300 individuals/m<sup>2</sup> of stocking density). ALG (absolute length growth), AWG (absolute weight growth), SGR (specific growth rate), CV (coefficient of variance), FCR (feed conversion ratio).

amongst mortality results (Table 2) was not caused by environmental factors. The high mortality caused by other factors in this study might be caused by the inability to quickly adapt in a new environment. It was supported by the high mortality on day 1 to day 7 in all treatments.

The hormonal treatment and stocking density did not affect the growth performance, included SGR length, but the increased stocking density diminished the SGR weight (Table 4). It was considerably caused by the low energy consumption in the wider area compared to the narrower area, where they tended to consume more energy (Slavič *et al.*, 2014). In a high stocking density, each individual tends to move actively and less rest so it requires more energy (Boerreigter *et al.*, 2015). It happened because, in high stocking density, they lack the space to move around and less chance on food as well. Nevertheless, the production performance on fingerling rearing is determined by the length and survival of the fingerling.

### CONCLUSION

The  $17\beta$ -estradiol hormone treatments could decrease the mortality and cannibalism on African catfish fingerling in high-density culture. The best treatment was reasonably the 30 mg/kg of  $17\beta$ -estradiol and 150 individuals/m<sup>2</sup>.

### REFERENCES

- Andrade TG, Nakamuta JS, Avanzi V. 2005. Anxiolytic effect of estradiol in the median raphe nucleus mediated by 5-HT<sub>1A</sub> receptors. *Behavioural Brain Research* 163: 18–25.
- Biu AA, Gwana AM, Bukar-Kolo YM. 2015. Effects of sorting on cannibalism in Catfish *Clarias gariepinus* rose in concrete tanks in maiduguri, North-Eastern Nigeria. *Animal and Veterinary Sciences* 3: 67–73.
- Calmarza-Font I, Lagunans N, Luis M, Garcia-Segura. 2012. Antidepressive and anxiolytic activity of selective estrogen receptor modulators in ovariectomized mice submitted to chronic unpredictable stress. *Behavioural Brain Research* 227: 287–290.
- Clotfelter ED, Rodriguez AC. 2006. Behavioral changes in fish exposed to phytoestrogens. *Environmental Pollution* 144: 833–839.
- Colman JR, Baldwin D, Jhonson L, Scholz NL. 2008. Effects of the synthetic estrogen,  $17\alpha$ -ethynylestradiol, on aggression and courtship behaviour in male zebrafish *Danio rerio*. *Aquatic Toxicology* 91: 346–354.
- Dinsdale EC, Ward WE. 2010. Early exposure to soy isoflavones and effects on reproductive health: a review of human and animal studies. *Nutrients* 11: 56–87.
- Effendie MI. 1997. *Biologi Perikanan*. Yogyakarta (ID) : Yayasan Pustaka Nusatama.
- Forsatkar MN, Abedi M, Nematollahi MA, Rahbari E. 2013. Effect of testosterone and fluoxetine on aggressive behaviors of fighting fish *Betta splendens*. *International Journal of Aquatic Biology* 1: 289–293
- Gore AC. 2008. Developmental programming and endocrine disruptor effects on reproductive neuroendocrine systems. *Frontiers in Neuroendocrinology* 29: 358–374.
- Hastuti S, Subandiyono. 2014. Production performance of African catfish *Clarias gariepinus* Burch were rearing with biofloc

Table 5. Water quality parameters (temperature, pH, DO, and TAN) of African catfish fingerling *Clarias gariepinus* reared for 30 days in different  $17\beta$ -estradiol dosage and stocking density.

Treatment	Water quality parameters			
	Temperature (°C)	pH	DO (mg/L)	TAN (mg/L)
Water source	25.1–25.4	6.9–7.0	6.0–7.2	0.095
A	25.1–25.5	6.9–7.2	6.0–7.9	0.67–1.00
B	25.1–25.6	6.9–7.0	6.2–7.4	0.75–0.84
C	25.1–25.3	7.0–7.1	7.0–7.8	0.72–0.84
D	25.1–25.3	7.1–7.4	7.1–7.8	0.71–1.03
E	25.1–25.2	6.9–7.2	6.2–7.4	0.71–1.03
F	25.3–25.4	6.9–7.2	6.0–7.2	0.89–1.10
References	25–30 (SNI 01-6483.4-2000)	6.5–8.0 (SNI 01 2000)	6483.4- >5 (SNI 01-6483.4-2000)	<4 (Stone & Thomforde, 2004)

- technology. *Jurnal Saintek Perikanan* 10: 37–42.
- Hseu JR, Huang WB. 2014. Application of logistic regression analysis to predict cannibalism in orange-spotted grouper *Epinephelus coioides* H. fry. *Aquaculture Research* 45: 868–873.
- Kim SH, Park MJ. 2012. Effects of phytoestrogen on sexual development. *Korean Journal of Pediatric* 55: 265–271.
- [KKP] Kementerian Kelautan dan Perikanan. 2014. Laporan kinerja (LKj) Direktorat Produksi tahun 2014. Direktorat Jenderal Perikanan Budidaya. Jakarta.
- Król J, Zake Z. 2016. Effect of dietary L-tryptophan on cannibalism, survival and growth in pikeperch *Sander lucioperca* (L.) post-larvae. *Aquaculture International* 24: 441–451.
- Kumar P, Kailasam M, Sethi SN, Sukumaran K, Biswas G, Subburaj R, Thiagarajan G, Ghosal TK, Vijayan KK. 2017. Effect of dietary l-tryptophan on cannibalism, growth and survival of Asian seabass *Lates calcarifer* (Bloch, 1790) fry. *Indian Journal Fisheries* 64: 28–32.
- Li P, Ray B, Gatlin DM, Sink T, Chen R, Lochmann R. 2009. Effect of handling and transport on cortisol response and nutrient mobilization of golden shiner, *notemigonus crysoleucas*. *Journal of the World Aquaculture Society* 40: 803–809
- Liu X, Xia J, Pang H, Yue G. 2017. Who eats whom, when and why? Juvenile cannibalism in fish Asian seabass. *Aquaculture and Fisheries* 2: 1–9.
- Manuel R, Boerrigter J, Roques J, Heul Jvd, Bos Rvd, Flik G, Vis Hvd. 2014. Stress in African catfish *Clarias gariepinus* following overland transportation. *Fish Physiological Biochemistry* 40: 33–44.
- Mukai Y, Sanudin N, Firdaus RF, Saad S. 2013. Reduced cannibalistic behaviour of African catfishes *Clarias gariepinus* larvae under dark and dim conditions. *Zoological Science* 30: 421–424.
- Naumowicz K, Padjak J, Terech- Majewska E, Szarek J. 2017. Intra-cohort cannibalism and methods for its mitigation in cultured freshwater fish. *Reviews in Fish Biology and Fisheries* 27: 193–208
- Nieuwegiessen PG, Boerlage AS, Verreth JAJ, Schrama JW. 2008. Assessing the effects of a chronic stressor, stocking density, on welfare indicators of juvenile African catfish *Clarias gariepinus* Burchell. *Applied Animal Behavior Science* 115: 233–243.
- Obirikorang K, Madkar HA, Boeateng A. 2014. A study of intra-cohort cannibalism in juvenile of African catfishes *Clarias gariepinus* under controlled conditions. *International Journal of Science and Technology* 3: 23–26.
- Onwuteaka J, Prince CO. 2015. Cannibalism of juvenile catfish *Clarias gariepinus* Burchell. *Journal of Environmental Science Toxicology and Food Technology* 9: 46–49.
- Paitz RT, Mommer BC, Suhrl E, Bell AM. 2015. Changes in the concentrations of four maternal steroids during embryonic development in the threespined stickleback *Gasterosteus aculeatus*. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* 323: 422–429.
- Patriche T. 2009. The importance of glucose determination in the blood of the cyprinids. *Zootehnie Biotehnologii* 42: 102–106.
- Prasad P, Ogawa S, Parhar IS. 2015. Role of serotonin in fish reproduction. *Frontiers in Neuroscience* 9: 195.
- Rajakumar A, Singh R, Chakrabarty S, Muruganathkumar R, Laldinsangi C, Prathibha Y, Sudhakumari CC, Dutta-Gupta A, Senthilkumaran B. 2012. Endosulfan and flutamide impair testicular development in the juvenile Asian catfish *Clarias batrachus*. *Aquatic Toxicology* 111: 123–132.
- Ribeiro FF, Qin JG. 2013. Modelling size-dependent cannibalism in barramundi *Lates calcarifer*: cannibalistic polyphenism and its implication to aquaculture. *PLoS ONE* 8: 1–8
- Saaristo M, Craft JA, Lehtonen KK, Lindstrom K. 2010. Exposure to 17 $\alpha$ - ethynylestradiol impairs courtship and aggressive behaviour of male sand gobies *Pomasthoscistus minutus*. *Chemosphere* 79: 541–546.
- Szczepkowski M, Zakeś Z, Szczepkowska B, Piotrowska I. 2011. Effect of size sorting on the survival, growth and cannibalism in pikeperch *Sander lucioperca* L. larvae during intensive culture in RAS. *Czech Journal of Animal Science* 56: 483–489

- Salierno JD, Kane AS. 2009.  $17\alpha$ -Ethinylestradiol alters reproductive behaviors, circulating hormones, and sexual morphology in male fathead minnows *Pimephales promelas*. *Environmental Toxicology* 28: 953–961.
- [SNI] Standar Nasional Indonesia 01-6484-2000. Benih ikan lele dumbo *Clarias gariepinus* kelas benih sebar. Direktorat Jenderal Perikanan Budidaya. Jakarta.
- Solomon RJ, Udoji FC. 2011. Cannibalism among cultured African catfishes *Heterobranchus longifilis* and *Clarias gariepinus*. *Natural and Science* 9: 1–13.
- Stone NM, Thomforde HK. 2004. Understanding your fish pond water analysis report. Arkansas (US): University of Arkansas Cooperative Extension Service Printing.
- Walf AA, Frye CA. 2009. Effects of two estradiol regimens on anxiety and depressive behaviors and trophic effects in peripheral tissues in a rodent model. *Gender Medicine* 6: 300–311.
- Zairin MJr, Fusukawaka K, Aida K. 1992. Changes in ovarian maturity in tropical walking catfish *Clarias batrachus*, reared under 23–25°C. *Nippon Suisan Gakkaishi* 58: 2033–2037.
- Zairin MJr, Rahmawati NR, Maulana F, Alimuddin. 2016. Aggressiveness and growth of male Siamese fighting fish treated with different dose and duration of  $17\alpha$ -methyltestosterone immersion. *AACL Bioflux* 9: 1364–1371.