Aggressive and cannibalistic behavior of African catfish larvae: effect of different doses of methyltestosteron injecting to female broodstock and larval stocking densities

Tingkat agresivitas dan kanibalisme larva ikan lele Afrika *Clarias gariepinus*: pengaruh dosis hormon MT disuntikkan ke induk betina dan padat tebar larva berbeda

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ABSTRACT

This study aimed to evaluate the effect of 17α-methyltestosterone hormone (MT) injecting to female broodstocks and stocking densities on the aggressive and cannibalistic behavior of African catfish larvae. Two-day-old post-hatching larvae were used in this experiment. Larval rearing was started at three-day-old post-hatching (body weight 0.004 ± 0.003 g and total length 0.2 ± 0.05 cm) in a 40 cm×30 cm×25 cm rearing aquaria. This study used a completed randomized factorial design which consisted of two factors. The first factor were the larvae from females broodstocks without MT injection (0 µg/g body weight) (A), injected with MT (1 µg/g body weight) (B), and injected with MT (2 µg/g body weight) (C), while the second factor were larval stocking densities of $3.000/m^2$ (V1) and $6.000/m^2$ (V2), with three replications. The results showed that the larvae from female broodstocks injected with MT 2 µg/g body weight (C) and stocking densities $6.000/m^2$ (V2) increased the aggressiveness of swim and decreased cannibalism. The highest cannibalistic behavior occurred from 00.00–06.00.

Keyword: aggressiveness, cannibalism, *Clarias gariepinus*, methyltestosterone

ABSTRAK

Penelitian ini bertujuan untuk mengevaluasi pengaruh hormon 17α-metiltestosteron (MT) yang diinjeksikan pada induk betina dan padat tebar terhadap tingkat agresivitas dan kanibalisme larva ikan lele. Ikan uji yang digunakan adalah larva berumur dua hari setelah penetasan. Pemeliharaan larva dimulai saat larva berumur tiga hari setelah penetasan (bobot tubuh $0,004 \pm 0,003$ g dan panjang total 0.2 ± 0.05 cm) yang dipelihara di dalam akuarium berukuran 40 cm×30 cm×25 cm. Penelitian ini menggunakan rancangan acak lengkap faktorial yang terdiri atas dua faktor. Faktor yang pertama adalah larva dari induk tanpa diinjeksi dengan hormon MT (0 µg/g bobot tubuh) (A), diinjeksi dengan hormon MT (1 µg/g bobot tubuh) (B), dan diinjeksi dengan hormon MT (2 µg/g bobot tubuh) (C), sedangkan faktor yang kedua adalah padat tebar 3.000 ekor/m² (V1) dan 6.000 ekor/m² (V2), dengan tiga kali ulangan. Hasil penelitian menunjukkan bahwa larva ikan lele dari induk yang diinjeksi hormon MT dosis 2 µg/g bobot tubuh dan dipelihara pada padat tebar tinggi 6.000 ekor/m² (V2) dapat meningkatkan agresivitas berenang dan menurunkan kanibalisme. Tingkat kanibalisme tertinggi terjadi pada pukul 24.00–06.00 WIB.

Kata kunci: agresivitas, kanibalisme, *Clarias gariepinus*, metiltestosteron

INTRODUCTION

The total production of catfish in 2017 reached 1.8 million tons or 131.7% of the previous year (MAF, 2018). However, the increased in catfish production has not been supported by seed sustainability. High cannibalism at larval and juvenile stage tends to increase the mortality. A high level of mortality straightly reduces the harvest and total income of the catfish farmer.

Cannibalism is a predation habit to entirely or partially consume an individual of the same population. According to the development stage, cannibalism is divided into pre-hatching cannibalism and post-hatching cannibalism (larvae, seed, and mature). Cannibalism in the pre-hatching phase occurs on the unhatched egg and it will be eaten by the broodstock. Genetically, cannibalism is sorted into broodstock-offspring, offspring-offspring, and unrelated individual cannibalism. The broodstock-offspring cannibalism often happens to parental-care species, while the offspring-offspring happens frequently to identical individuals. There is also cannibalism amongst two unrelated individuals, such as the fluid and aroma of rainbow trout egg will attract goby fish. Based on maturity, cannibalism is sorted into an identical maturity level and different maturity levels. The cannibalism in the identical age/maturity usually happens in the same species, yet different sex, for example, Vundu catfish. The cannibalism in the different age/maturity levels often occurs amongst bigger individuals versus the smaller one. According to the size difference, cannibalism is categorized as type I and type II. Type I refers to partially bite and attack several part of prey, while type II assigns to the term of swallow the intact body of the prey (Hecht & Appelbaum, 1988; Smith & Reay, 1991; Yavno & Corkum, 2011; Bystro *et al*., 2012; Krol *et al*., 2014; Naumowicz *et al*., 2017). In a catfish rearing, cannibalism often occurs amongst identical age.

Several factors affected cannibalism, i.e. stock density, feed supply, temperature, grading, balanced nutrition, light, water brightness, and size and shape of the rearing media (Pereira *et al*., 2017; Muslimin *et al*., 2011). Aderolu *et al.* (2010) explained that feeding frequency four times a day caused a decline in the fish's aggressivity compared to three times a day of feeding. Eyo & Ekanem (2011) added that the catfish feeding three times a day resulted in a lower survival rate (82.5 ± 7.5%) compared to four times a day of feeding (92.5 ± 2.5%). Thus,

besides feed consumption, another possible factor of fish cannibalism is required to be investigated.

Kania *et al.* (2012), Yang *et al.* (2015), and Vallon *et al.* (2016) described that endocrinology had an essential role in triggering aggressivity which led to cannibalism. The attack in cannibalism amongst the group is related to hormonal activity through different modulators, one of them is testosterone. Testosterone affects aggressive activity to defend its place. In *Clarias batrachus,* there is a high content of maternal testosterone in a mature broodstock. Zairin *et al.* (1992) explained that the testosterone content in *Clarias batrachus* rose in the 8th month along with the vitellogenesis and gonad maturation. Mehta and Beer (2010), Huntingfold *et al.* (2012), Nakamura (2013), and Kang *et al.* (2015) also explained that testosterone is not only stored in the egg embryo, but also the egg yolk. Testosterone will keep increasing along with the larvae growth. However, the specific level of testosterone which directly affects aggressivity and cannibalism in catfish. Therefore, a further study to evaluate testosterone hormone impact on aggressivity and cannibalism through broodstock and stock density is required.

MATERIALS AND METHODS

Experimental design

This study applied a complete randomized design using two factors. The first factor is the larvae from a broodstock without 17α-methyltestosterone hormone (MT) injection (A), injection with MT 1 μg/g of body weight (B), and 2 μg/g of body weight (C). The second factor was larval stocking density of 3000 individuals/m² (V_1) and 6000 individual/m² (V_2).

Experimental fish procurement

The experimental larvae were obtained from broodstocks that have been injected by MT using different dosages. The two-day-old larvae were moved to the aquaria (size 40 cm×30 cm×25 cm) with 15 cm of water level. The larvae were acclimatized for 24 hours. The rearing period was started on the 3rd day after hatching. Each aquarium was equipped with an aeration system and water heater to maintain a temperature of approximately 27−30°C.

Larval rearing

During the rearing period, the larvae were fed using commercial feed (No. 0 Feng-Li) at apparent satiation. Feeding was done three times

at 7.00, 13.00, and 19.00. Water exchange was conducted 10–30% of the total volume. The larvae were reared for 30 days or until 33 days after hatching. To observe the growth, the larvae weight and total body length were measured at the beginning and the end of the study. The observation of aggressivity was performed once a week, about three minutes before feeding. To presume cannibalism level, the mortal population was observed every six hours, then identified according to the cannibalism type (type I and II) and also the cannibalism index. The current population was calculated every week.

Body fluid preparation

The body fluid of the larvae was collected on day 0, day 10, day 20, and day 30. The larvae samples were 4 g or about 10,000 larvae (5,000 larvae/2 g resulted in 1 mL of supernatant). The sample was rinsed using distilled water, then extracted by grinding the sample. Moreover, it was mix thoroughly in phosphate-buffered saline (PBS) which contains 0.05% Tween-20 (pH 7.2) with ratio 1 (larvae): 4 (PBS). The sample was centrifuged in 5,000 rpm for 5–10 minutes (Kurniaji *et al.*, 2018). The supernatant was considered as serum. Furthermore, the serum was stored at -20°C to analyze the testosterone content.

Experimental parameters

Aggressivity level

Aggressivity level measurement is referred to as the following formula (Nieuwegiessen *et al.*, 2009):

Cannibalism index

$$\text{Aggressivity level (\%)} = \frac{\sum \text{Observed fish (individual)}}{\sum \text{fish sample (individual)}}$$

Index of cannibalism is determined using this following formula (Appelbaum & Arockiaraj, 2010):

The lost population term is referred to the weekly population minus the mortal fish. The

$$\text{Aggressivity index (\%)} = \frac{\sum \text{lost population} - \sum \text{mortal population}}{\sum \text{initial population}}$$

mortal fish is particularly caused by cannibalism between individuals.

Testosterone level

This particular parameter was analyzed using the ELISA testosterone kit (DRG Diagnostic EIA 1559). Moreover, the sample was analyzed using a spectrophotometer in 450 nm of wavelength (Kurniaji *et al.*, 2018).

Mortality

Mortality is a parameter to calculate the population which normally mortal, not because of cannibalism. This parameter is calculated based on the following formula (Appelbaum & Arockiaraj, 2010):

$$\text{Mortality (\%)} = \frac{\text{Mortal pupulation}}{\text{Initial population}} \times 100$$

Survival rate

The survival rate is the percentage of a population that lives in a certain amount of time. It is determined according to the following formula (Appelbaum & Arockiaraj, 2010):

$$\text{SR (\%)} = \frac{\text{Final pupulation}}{\text{Initial population}} \times 100$$

Total length growth

The total length growth was measured to identify the length extension during the rearing period using this following formula (Appelbaum & Arockiaraj, 2010):

$$\text{Lm} = TL_1 - TL_0$$

Note:
Lm: Total length growth (cm)
TL_1: Final total length (cm)
TL_0: Initial total length (cm)

Total weight growth

Total weight was measured to determine the weight gain of the fish during the rearing period. This parameter was calculated using the following formula by Appelbaum and Arockiaraj (2010).

$$\text{Wm} = WL_1 - WL_0$$

Note :
Wm: Total weight gain (g)
WL_1: Total final weight (g)
WL_0: Total initial weight (g)

Specific length rate

Specific length rate is measured according to the following formula (Appelbaum & Arockiaraj, 2010):

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

Note :
SLR : Specific length rate (%/day)
Lt   : Average length at a certain time (t) (cm)
Lo   : Initial average length (cm)
t   : Rearing period (days)

Specific growth rate

The specific growth rate is determined using the formula by Appelbaum and Arockiaraj (2010).

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

Note :
SGR : Specific growth rate (%/day)
Wt   : Average weight at a certain time (t) (g)
Wo   : Initial average growth (g)
t   : Rearing period (days)

Water quality

The measured water quality parameters during the study consisted of pH and temperature, which is daily measured. The dissolved oxygen, ammonia, nitrite, and nitrate were observed at the beginning of the study, in the middle, and at the end of the study.

Data analysis

The quantitative data were aggressivity level, cannibalism, and production performance of the larvae. Data were tabulated using Microsoft Excel 2010 and analyzed using analysis of variance using SPSS 16.0 at 95% confidence level. A significant result was processed through Duncan's posthoc test. The qualitative analysis was conducted on the testosterone data and water quality parameters and presented in a table and graphic.

RESULTS

Aggressivity level

According to the statistical analysis in Table 1, the aggressivity level before feeding is significantly different (P<0.05). In contrast, stereotypic behavior does not differ significantly (P>0.05). The MT treatment injected through the broodstock presented interaction with swimming behavior and agonistic. These two behaviors were related to inversely. The MT hormone injection in a dosage of 2 μg/g[1] boosted the swimming behavior, however, the agonistic decreased. Moreover, the stocking density also had an inverse relation with stereotypic and rest behavior. The 6000 ind/m² (V₂) of stocking density presented a higher swimming behavior compared to the 3000 ind/m² of stocking density (V₁). On the contrary, the V₂ treatment experienced a lower stereotypic and rest behavior compared to the V₁.

According to the observation, the aggressivity of catfish larvae just a moment before feeding and after feeding were nearly similar. Table 2 described that the swimming behavior while feeding differed significantly (P<0.05), while the stereotypic, flee, rest, and agonistic behavior did not significantly differ (P>0.05). The highest level of swimming behavior was presented in the CV2 treatment, whereas the lowest was the AV1

Table 1. Aggressivity level of catfish observed before feeding for 30 days of rearing.

Treatment	Ethogram of aggressivity (%)				
	Swimming	Stereotypic	Flee	Rest	Agonistic
AV₁	47.1 ± 0.87[b d h]	3.3 ± 1.47[a c h]	3.1 ± 0.10[a c h]	3.5 ± 2.34[a c h]	11.0 ± 1.20[a c h]
AV₂	69.1 ± 0.98[b c h]	1.8 ± 0.79[a d h]	3.3 ± 0.22[a c h]	18.2 ± 2.78[a d h]	7.5 ± 3.33[a c h]
BV₁	50.0 ± 1.07[b d h]	5.2 ± 1.80[a c h]	4.8 ± 1.64[a c h]	32.4 ± 3.13[a c h i]	7.6 ± 0.65[a b c h]
BV₂	65.7 ± 3.13[b c h]	1.5 ± 0.37[a d h]	4.9 ± 1.76[a c h]	21.5 ± 0.79[a d h i]	6.5± 2.35[a b c h]
CV₁	68.4 ± 3.71[a d h]	1.5 ± 0.62[a c h]	5.7 ± 2.23[a c h]	21.2 ± 5.10[a c h i]	3.3 ± 2.15[b c h]
CV₂	72.8 ± 1.72[a c h]	0.8 ± 0.35[a d h]	6.3 ± 2.38[a c h]	18.1 ± 2.63[a d h]	2.1 ± 0.95[b c h]

Note: AV₁: larvae from broodstock A and 3000 ind/m² of stocking density; AV₂: larvae from broodstock A and 6000 ind/m² of stocking density; BV₁: larvae from broodstock B and 3000 ind/m² of stocking density; BV₂: larvae from broodstock B and 6000 ind/m² of stocking density; CV₁: larvae from broodstock C and 3000 ind/m² of stocking density; CV₂: larvae from broodstock C and 6000 ind/m² of stocking density. Different superscript in the same column indicates a significant difference (Tukey test; P<0.05); The [ab] letter states the correlation of the treatment and the aggressivity; The [cd] letter states the relation of the hormone and the aggressivity; The [hi] letter states the correlation of the stocking density and the aggressivity.

Table 2. Aggressivity level of catfish observed while feeding for 30 days of rearing.

Treatment	Ethogram of aggressivity (%)				
	Swimming	Stereotypic	Flee	Rest	Agonistic
AV1	65.8 ± 2.50[bdIj]	0.8 ± 0.10[ach]	2.5 ± 1.46[ach]	29.8 ± 2.33[ach]	1.2 ± 0.46[ach]
AV2	78.8 ± 1.79[achi]	0.4 ± 0.10[ach]	0.4 ± 0.31[ach]	16.1 ± 3.04[adh]	4.2 ± 0.60[ach]
BV1	69.7 ± 2.05[bdhIj]	1.6 ± 0.36[ach]	0.2 ± 0.10[ach]	26.0 ± 5.31[ach]	2.5 ± 1.14[ach]
BV2	78.2 ± 2.00[achij]	0.6 ± 0.17[ach]	0.2 ± 0.10[ach]	20.3 ± 2.66[adh]	0.7 ± 0.64[ach]
CV1	67.3 ± 2.75[adIj]	1.3 ± 1.01[ach]	1.8 ± 1.36[ach]	29.0 ± 4.56[ach]	0.7 ± 0.26[ach]
CV2	82.0 ± 4.29[ach]	0.7 ± 0.10[ach]	0.2 ± 0.17[ach]	15.0 ± 6.85[adh]	2.0 ± 1.32[ach]

Note: AV1: larvae from broodstock A and 3000 ind/m² of stocking density; AV2: larvae from broodstock A and 6000 ind/m² of stocking density; BV1: larvae from broodstock B and 3000 ind/m² of stocking density; BV2: larvae from broodstock B and 6000 ind/m² of stocking density; CV1: larvae from broodstock C and 3000 ind/m² of stocking density; CV2: larvae from broodstock C and 6000 ind/m² of stocking density. Different superscript in the same column indicates a significant difference (Tukey test; P<0.05); The $^{a\,b}$ letter states the correlation of the treatment and the aggressivity; The ^{cd} letter states the correlation of the hormone and the aggressivity; The ^{hi} letter states the correlation of the stocking density and the aggressivity.

Table 3. The survival rate, mortality, and cannibalism level of catfish

Treatment	Survival rate (%)	Mortality (%)	Cannibalism type (%)		Cannibalism index (%)
			Type I	Type II	
AV1	51.9 ± 2.78[bdij]	11.0 ± 2.74[abchi]	26.1 ± 3.27[abchi]	11.0 ± 2.74[ach]	37.1 ± 1.31[abchi]
AV2	60.0 ± 5.80[bchi]	11.2 ± 1.55[abchi]	21.2 ± 2.97[abchi]	7.8 ± 2.53[adh]	29.0 ± 4.81[abdhi]
BV1	65.3 ± 6.96[adh]	7.5 ± 0.96[bcj]	19.7 ± 5.30[bci]	7.5 ± 0.96[ach]	27.2 ± 6.11[bci]
BV2	64.4 ± 3.86[achi]	9.2 ± 1.05[bchi]	20.4 ± 1.76[bci]	6.1 ± 1.68[adh]	26.4 ± 3.43[bdi]
CV1	45.7 ± 4.32[bdij]	13.2 ± 2.94[ach]	31.8 ± 6.47[ach]	9.4 ± 0.96[ach]	41.2 ± 6.90[ach]
CV2	53.7 ± 2.15[bchIj]	13.8 ± 1.74[ach]	25.1 ± 2.10[achi]	7.3 ± 1.53[adhIj]	32.5 ± 2.63[adh] i

Note: AV1: larvae from broodstock A and 3000 ind/m² of stocking density; AV2: larvae from broodstock A and 6000 ind/m² of stocking density; BV1: larvae from broodstock B and 3000 ind/m² of stocking density; BV2: larvae from broodstock B and 6000 ind/m² of stocking density; CV1: larvae from broodstock C and 3000 ind/m² of stocking density; CV2: larvae from broodstock C and 6000 ind/m² of stocking density. Different superscript in the same column indicates a significant difference (Tukey test; P<0.05); The ^{ab} letter states the correlation of the treatment and the aggressivity; The ^{cd} letter states the correlation of the hormone and the aggressivity; The ^{hi} letter states the correlation of the stocking density and the aggressivity.
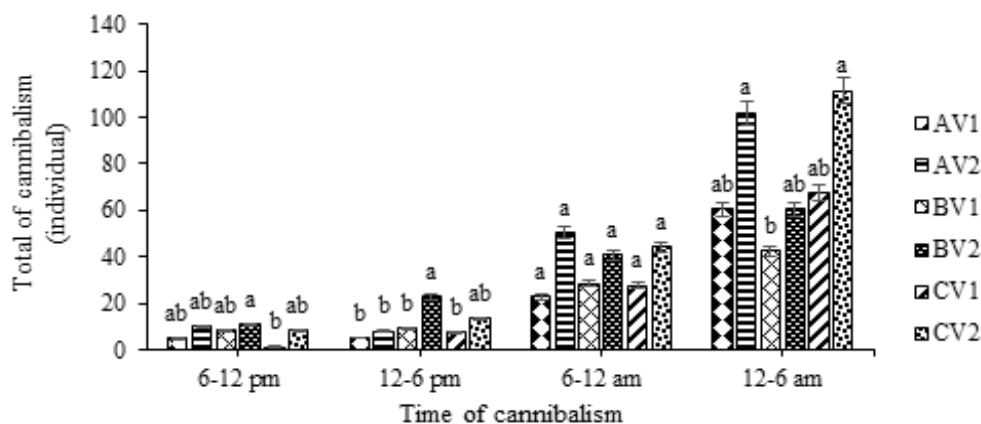

Figure 1. Daily cannibalism dynamic of catfish for 30 days of rearing. AV1: larvae from broodstock A and 3000 ind/m² of stocking density; AV2: larvae from broodstock A and 6000 ind/m² of stocking density; BV1: larvae from broodstock B and 3000 ind/m² of stocking density; BV2: larvae from broodstock B and 6000 ind/m² of stocking density; CV1: larvae from broodstock C and 3000 ind/m² of stocking density; CV2: larvae from broodstock C and 6000 ind/m² of stocking density. Different superscript in the same column indicates a significant difference (Duncan test; P<0.05)

treatment. The stocking density interacted with the swimming and rest behavior. The swimming behavior in the V2 treatment was higher than the V1. On the other hand, the V2 treatment had a lower level of stereotypic and rest behavior than V1.

Cannibalism

Table 3 showed that the survival rate, mortality, cannibalism type 1, and cannibalism index were significantly different amongst treatment, while the cannibalism type II did not differ significantly (P>0.05).

The highest survival rate was presented by BV1 treatment and the lowest was CV1. The mortality and cannibalism type I showed was related to each other inversely. The mortality rate and cannibalism type I were low in the BV1 treatment, whereas in the CV1 treatment the mortality rate and cannibalism type I was high. For the cannibalism index, the CV1 treatment showed a high value, while the BV2 was low. The MT hormone injected into the broodstock interacted with the swimming behavior, mortality, cannibalism type I, and cannibalism index. The higher dosage caused a lower level of survival rate, at the same time increased the mortality rate, cannibalism type I, and cannibalism index. The stocking density treatment had interactions with survival rate, cannibalism type II, and cannibalism index. The 6000 ind/m^2 (V2) resulted in a higher survival rate compared to the 3000 ind/m^2 (V1). On the contrary, the stocking density of 6000 ind/m^2 had a lower cannibalism type II and a cannibalism index than the 3000 ind/m^2 stocking density.

The exact time of cannibalism in a day was not identical. It depended on the natural behavior of catfish which actively moves at night. The amount of eaten larvae in 24 hours for 30 days is explained in Figure 1.

Testosterone hormone of the larvae

Figure 2 showed that the testosterone hormone content amongst treatments relatively varied. The highest content was BV2, AV1 (day 10), BV1 (day 20), and CV2 (day 30). It can be concluded that the injection of the MT hormone did not affect the testosterone of the larvae.

Production performance of larvae

The injection of MT hormone aimed to maintain the stabilization of testosterone hormone which presumably affects larvae growth. On the contrary, the result showed that both hormone injection and without injection did not affect the production performance of the larvae (P>0.05).

Water quality

The result of the water quality measurment is presented in Table 4.

Discussion

Cannibalism is started with the role of testosterone, however, the source hasn't known yet. A matured broodstock of *Clarias batrachus* has a high level of maternal testosterone. Zairin *et al.* (1992) explained that the testosterone content in *C. batrachus* increased in the 8th month along with the vitellogenesis process and gonad maturation. Mehta & Beer (2009), Huntingfold *et al.* (2012), and Nakamura (2013) also stated that testosterone is not only stored in the embryo of the egg but also the egg yolk and it keeps increasing along with the larvae development.

The predation is usually started with aggressivity amongst individuals. Kania *et al.* (2012), Yang *et al.* (2015), and Vallon *et al.* (2016) explained that endocrinology holds an essential role in triggering aggressivity and cannibalism. Attack amongst the different group and it is related to the hormonal mechanism through different modulators, one of them is testosterone. Testosterone influences aggressive behavior in

Table 4. The range of water quality of catfish larvae

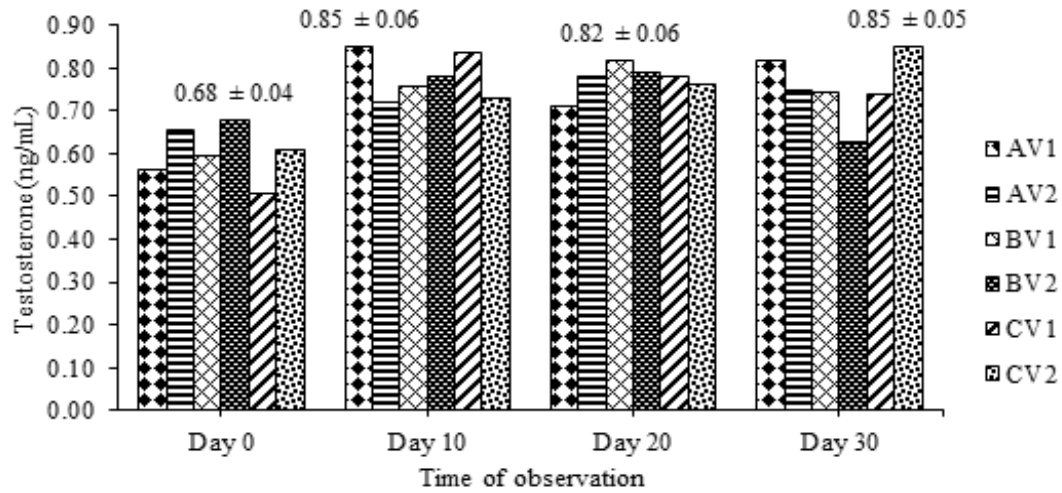| Parameter	Results	References
Temperature (°C)	25.0−26.3	25.0−30.0 (INS, 2014)
pH	6.8−8.0	6.5−8.5 (INS, 2014)
		Minimal 3 (INS, 2014)
		Maximal 0.01 (INS, 2014)
Dissolved oxygen (DO) (mg/L)	3.5−7.0	<0.006 (EGR, 2001)
Total ammonia nitrogen (TAN) (mg/L)	0.003−0.019	20 (EGR, 2001)
Nitrite (mg/L)	0.029−4.947	
Nitrate (mg/L)	2.617−4.874	

Figure 2. Testosterone content in catfish larvae during 30-day of rearing. AV1: larvae from broodstock A and 3000 ind/m² of stocking density; AV2: larvae from broodstock A and 6000 ind/m² of stocking density; BV1: larvae from broodstock B and 3000 ind/m² of stocking density; BV2: larvae from broodstock B and 6000 ind/m² of stocking density; CV1: larvae from broodstock C and 3000 ind/m² of stocking density; CV2: larvae from broodstock C and 6000 ind/m² of stocking density.
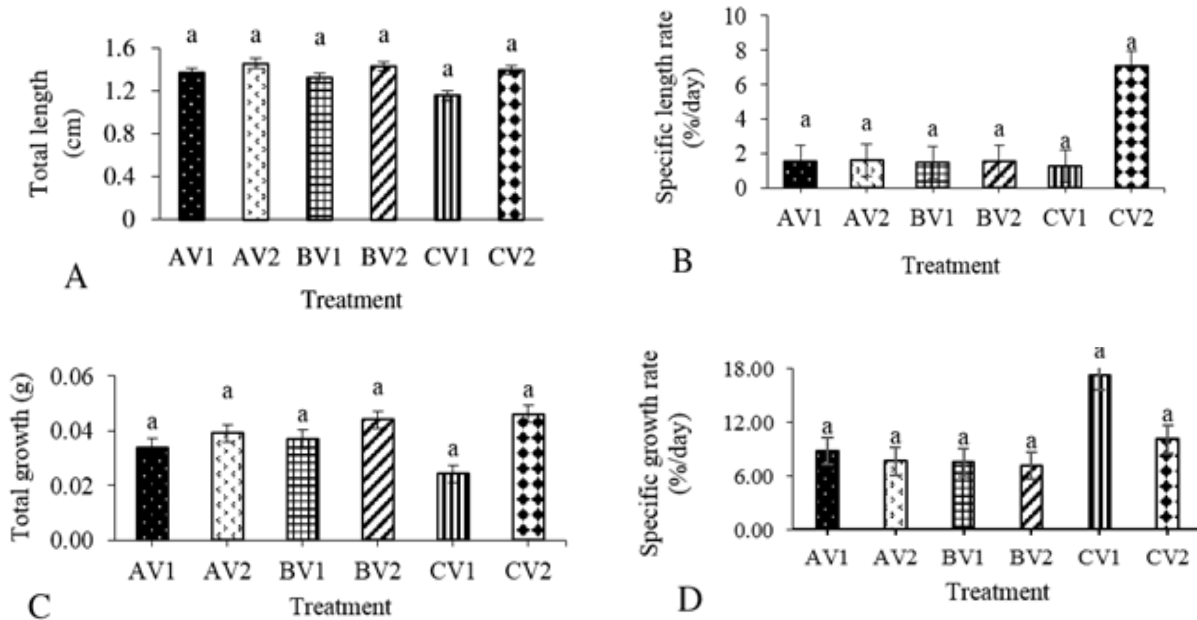

Figure 3. Body length (A and B) and weight (C and D) growth on catfish larvae during the 30-day of rearing. AV1: larvae from broodstock A and 3000 ind/m² of stocking density; AV2: larvae from broodstock A and 6000 ind/m² of stocking density; BV1: larvae from broodstock B and 3000 ind/m² of stocking density; BV2: larvae from broodstock B and 6000 ind/m² of stocking density; CV1: larvae from broodstock C and 3000 ind/m² of stocking density; CV2: larvae from broodstock C and 6000 ind/m² of stocking density. Different superscript in the same column indicates a significant difference (Duncan test; $P<0.05$)

defending a certain area. The aggressivity level of catfish larvae showed a similar result before and after feeding. The result demonstrated that stocking density was crucial in the overall movement, i.e. swimming (CV1), rest (BV1), and agonistic (AV2). The 6000 ind/m² of stocking density (AV2, BV2, and CV2) showed a higher level of movement (swimming, rest, and agonistic),

compared to the 3000 ind/m² of stocking density (AV1, BV1, and CV1). The result was supported by Nieuwegiessen *et al.* (2009) dan Manley *et al.* (2015) who explained that stocking density is closely related to swimming and agonistic behavior. The swimming behavior consists of finding something to eat and breathing, while agonistic behavior causes individual competition, such as an attack, chase, and catch the prey.

The stocking density affects the cannibalism level of catfish. The 6000 ind/m² of stocking density (AV$_2$, BV$_2$, and CV$_2$) experienced a lower cannibalism index than the 3000 ind/m² of stocking density (AV$_1$, BV$_1$, and CV$_1$). According to analysis the correlation between the aggressivity level (swimming behavior) and cannibalism (type II and mortality) during the treatment, it can be identified that the larvae with low aggressivity (swimming behavior) tended to have higher cannibalism and vice versa. The result was not in line with Baras *et al*. (2010), Kania *et al*. (2012), Yang *et al*. (2015), and Vallon *et al*. (2016) who stated that a high level of aggressivity and movement will be followed by a high level of cannibalism caused by stocking density which affects swimming behavior and agonistic. A high stocking density caused low space to move around so that predation would happen without attacking. When space is enough, the attack, chase, and predation will freely happen.

The predation behavior was evaluated using the cannibalism type method. Cannibalism is sorted into two types, i.e. type I and type II. Cannibalism type I refers to partial predation. The larvae tend to bite and attack their prey. Type I often occurs when the size variance is high and the egg yolk is depleted. Cannibalism type II occurs when a fish completely swallows its prey as a whole. This type of cannibalism is frequently used as a term to describe a tendency of predation related to size variance (Hecht & Appelbaum, 1988; Krol *et al*., 2014; Ribeiro, 2015). During the rearing period, the catfish larvae showed generally cannibalism type I (31.80 ± 6.47%), while cannibalism type II was a lot lower (9.40 ± 0.96%).

Aggressivity and cannibalism straightly influence the physiology mechanism. The blood profile is commonly analyzed to evaluate physiology responses caused by adaptation towards environmental changes. Huntingfold *et al.* (2012), Nardocci *et al*. (2014), and Yarahmadi *et al.* (2015) added that generally there are three types of physiology responses, i.e. primary, secondary, and tertiary response. According to Nieuwegiessen *et al.* (2009), catfish response stress through the increase of blood glucose. Cowey *et al.* (1977), Wingfield *et al.* (2008), Mousavi & Yousefian (2012), and Junior *et al.* (2016) also explained that the testosterone contributed in high energy demand and lipid decrease. It caused an increase in cortisol and glucose. Catecholamine affects glucose production through glycogenolysis and cortisol holds an essential role in gluconeogenesis activation. Gluconeogenesis will affect the health status of fish in a long period and change the pH level of lipid deposition.

In larvae observation, the testosterone content is in line with blood glucose content. The blood glucose level was fluctuating, then stable, decrease in day 20, and increase at the end of the study. The decrease of blood glucose content was believed that in a certain time, the tested fish utilized the glucose as an energy source to adapt towards a certain stress response (Diatin *et al.*, 2014). A different pattern was also found that a high level of blood glucose content triggered cannibalism, even though it was not followed by aggressive behavior. The result of blood glucose content was in the normal range of 53.0–73.1 mg/dL. It is supported by Hastuti and Subandiyono (2015) who stated that a normal range for blood glucose in catfish is 70−100 mg/dL.

Srivastava *et al.* (2012) performed a blood glucose analysis towards the other stressor, such as photoperiod. A population of *C. batrachus* was exposed for 24 hours under direct sunlight and did not show any sign of blood glucose escalation. It is closely related to the circadian rhythm of catfish. Mukai *et al.* (2013), Sierra-Flores *et al.* (2016), and Barmann *et al.* (2017) added that swimming and attack behavior is influenced by light intensity. Regarding the natural behavior of catfish which more likely to active at night, the exact time of cannibalism was observed. The cannibalism was already started at 18.00–24.00 and cannibalism happened frequently at 24.00–06.00. The result was supported by several studies by Ghosh and Pati (2004) and Ramteke *et al.* (2009). The phototaxis peak happened at night or predawn with swimming behavior, seeking prey, and predation. Naumowicz *et al.* (2017) added that blue light with 0.017–0.021 lmol/m²/s of wavelength can reduce cannibalism.

The daily weight in the 6000 ind/m² treatment decreased (AV2, BV2, and CV2). Stocking density and feeding activity are some factors that affect the growth parameter (Bystro *et al.,* 2012; Yatuha *et al*., 2012; Jiwyam & Nithikulworawong, 2014; Umanah & Dapa, 2017). The stocking density influences energy utilization as well (Jensen *et al.,* 2013, Nwipie *et al.,* 2018). It will affect the growth, survival rate, and aggressivity. The low level of growth parameter and cannibalism rate in this study were presumably caused by improper commercial feeding at the beginning of the study. Akbary *et al.* (2010) stated that the decrease of aggressivity and cannibalism happened when the

live feed was added to the rearing media of African catfish and Vundu *Heterobranchus longifilis*. The feeding time interval was too excessive and it led to cannibalism (Conceição *et al*., 2010). In this study, the feeding frequency was three times a day with 6 hours of interval. It resulted cannibalism index 27.2–41.2 %.

The water parameter changes, such as temperature, DO, pH, ammonia, and nitrogen affect the behavior amongst predators and prey. According to the water quality parameter, the temperature, DO, and pH was in the tolerable range. On the other hand, TAN and nitrite exceeded the tolerable range of catfish. Zakęś (2012) and Wu *et al.* (2015) mentioned that a warm temperature (22–24°C) is the potential to boost feed consumption so that cannibalism happened. Besides, Solomon and Udoji (2011) and Kawamura *et al*. (2017) explained that DO, pH, ammonia, nitrogen, and salinity are considered to affect the eating habit, stress, and survival rate. Environment temperature is closely related to fish metabolism, whereas DO and pH are strongly associated with several toxic gases, such as ammonia and nitrate, which threaten the survival rate. Kelabora (2010) and Yulan *et al.* (2013) explained that a high temperature tends to reduce the dissolved oxygen and affects the fish's appetite. A high temperature can increase salinity as well. An increase in water temperature causes the elevation of viscosity, chemical reaction, evaporation, volatilization, and decrease gas dissolved in the water.

CONCLUSION

The tested larvae from the broodstock injected by MT hormone in a dosage of 2 μg/g (C) and reared in 6000 ind/m² of stocking density experienced higher aggressivity (swimming behavior) with a low level of cannibalism.

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