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CANNIBALISM PERFORMANCE OF ASIAN REDTAIL CATFISH (*Hemibagrus nemurus*) FED RATION SUPPLEMENTED WITH DIFFERENT DOSES OF 17α-METHYLTESTOSTERONE

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ABSTRACT

One of the obstacles in Asian redtail catfish hatchery is the high cannibalism incidence. Cannibalism is associated with aggressive behavior caused by hormonal metabolism especially of testosterone. The purpose of this study was to evaluate the effect of testosterone administration on the cannibalism incidence in the Asian redtail catfish juveniles. The experiment was conducted by rearing fish with initial length of 4.09±0.19 cm in 16 of 20 L aquariums with a density of 2 fish L^{-1}. The experiment was designed with a completely randomized design with 4 treatments which were different level of 17α-methyltestosterone supplementation in feed, i.e., 0 mg kg^{-1} feed (A) as control, 7.5 mg kg^{-1} feed (B), 15 mg kg^{-1} feed (C), and 30 mg kg^{-1} feed (D). Each treatment has 4 replications. Fish fed experimental diet (40% protein) 4 times a day to satiation for 30 days. The parameters observed were type and index of cannibalism, aggressive behavior, survival rate, normal mortality, growth performance, hormones concentrations (estradiol, testosterone, and cortisol), and water quality. The results showed that cannibalism type II (the fish eaten completely or missing) and cannibalism index increased with the increasing doses of testosterone administration in the feed with the highest cannibalism incidence was 40.63%. The highest survival rate was found in treatment B (73.75±2.50%) and was not significantly different from the control treatment (69.38±2.39%). No differences in testosterone concentration and in the growth performance among the treatments. However, there was a trend of decrease in the estradiol concentration of Asian redtail catfish juveniles fed ration supplemented with the increasing doses of 17α-methyltestosterone. Based on the results obtained in this research, estradiol changed in the body's plasma, it appears that there was a role for plasma estradiol concentration in controlling cannibalism of Asian redtail catfish juveniles. It concluded that the testosterone administration affected the cannibalism incidence in the Asian redtail Catfish.

KEYWORDS: cannibalism; testosterone; estradiol; *Hemibagrus nemurus*

INTRODUCTION

Asian redtail catfish (*Hemibagrus nemurus*) is an economical fish in the Southeast Asian countries. However, in the breeding activity, this fish exhibits rather pronounced cannibalism behavior. The cause of death of Asian redtail catfish juveniles was often associated with cannibal behavior (Baras *et al.,* 2013; Rahmah *et al.,* 2014; Heltonika & Karsih, 2017; Kusmini *et al.,* 2019). Cannibal behavior of Asian redtail catfish increased with increasing stocking densities (Heltonika *et al*., 2021; 2022). Cannibalism is a predatory predation strategy which includes eating and killing an individual belonging to the same species (Naumowicz *et al*., 2017). In general, several factors influence cannibalism are divided into two categories, namely size and habit. Factors related to size are caused by population density, feed availability, protection, light intensity, water clarity, frequency of feeding, and alternative feed given (Fessehaye *et al*., 2006). Cannibalism is also closely related to aggressiveness behavior, where one of the hormones responsible for aggressive behavior is testosterone. Barki *et al*. (2006) when androgen glands of juvenile crayfish (*Cherax quadricarinatus*) was removed, these crayfish, do not fight with the other males when reach mature. There is an indirect relationship between

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the influence of testosterone and the aggressive behavior of crayfish (*Cherax quadricarinatus*). Several studies have described the role of testosterone in aggressiveness in fish, where high testosterone concentration in the body's plasma increases the aggressiveness of Juncos *Junco hyemalis* (Peterson *et al*., 2013), fighting fish *Betta splendens* (Kania *et al*., 2012), Mangrove rivulus *Kryptolebias marmoratus* (Chang *et al*., 2012), and Orange-fringed largemouth *Astatotilapia burtoni* (Alcazar *et al*., 2016).

Zairin *et al*. (2002) stated that increase in testosterone concentration in African catfish *Clarias batrachus* is related to the maturation of gonads and the process of vitellogenesis. Under these conditions, the testosterone content accumulates in the eggs and then passes to the larval developmental stage. The content of the innate testosterone hormone of the parents triggers aggressive behavior that can lead to cannibalism. This phenomena was also proven by (Rahmadiah *et al*., 2019) in *Clarias gariepinus* by regularly injecting catfish brood stock with 17α-methyltestosterone and this injection causes an increase in the content of 17α-methyltestosterone in brood stock and juveniles. The increased 17α-methyltestosterone concentration also have an effect on increasing the aggressiveness and cannibalism of catfish juveniles. One approach to delivery 17α-methyltestosterone to fish is through feed supplementation (Greisy & Gamal, 2012; Jensi *et al*., 2016; Sulaeman & Fotedar, 2017; Mangaro *et al*., 2018; Muniasamy *et al*., 2019).

Study on cannibalism in Asian redtail catfish still needs in depth observation to more understanding it mechanism. Our previous study showed the effect of stocking density on cannibalism behavior of Asian redtail catfish juveniles, where increased stocking density, increased cannibals incidence (Heltonika *et al*., 2021; 2022). Further more, evaluation of the behavior change caused by hormones including testosterone is necessarily to be conducted to understand whether it can increase cannibalistic behavior in juveniles of Asian redtail catfish. Therefore, this study aimed to evaluate the effects of 17á-methyltestosterone through supplementation in feed on the level of cannibalism in the juveniles of Asian redtail catfish.

MATERIALS AND METHODS

This research was conducted from March – April 2020, at Laboratory of Fish Breeding and Hatchery, Faculty of Fisheries and Marine Science, University of Riau. Analysis of several parameters observed was carried out at Laboratory of Breeding and Hatchery, Faculty of Fisheries and Marine Sciences, University of Riau and at Ornamental Fish Research Center, Depok, Ministry of Marine Affairs and Fisheries.

Experimental Design

This study was designed into a completely randomized design (Mangaro *et al*., 2018) consisting of four treatments, with four replicates in the form of. The treatments were different doses of 17α-methyltestosterone (17α-MT, specific for Aquaculture, Argent Laboratories Inc.) supplemented in commercial feed at 0 $mgkg^{-1}$ feed with 17α-MT (MT0), 7.5 $mgkg^{-1}$ feed with 17α-MT (MT1), 15 $mgkg^{-1}$ feed with 17α- MT (MT2), and 30 $mgkg^{-1}$ feed with 17α-MT (MT3).

Commercial feed use was specific for catfish, contained 40% protein content. Preparation of the test feed was carried out by spraying the hormone on feeds according to the predetermined dose of 17α-methyltestosterone supplementation, then stored in the freezer until use. Before the feeds stored in the freezer was used for fish, the feed was removed 30 minutes before it is given, to decreases the feeds temperature from inside the freezer

Research procedure

Redtail catfish juveniles with the initial length of 4.09±0.19 cm was used in this experiment. The juveniles were artificially bred s and reared until reaching the desired size for the experiment. Selected juveniles were stocked in aquarium (40x25x35 cm^3) with a volume of 20 L, with a density of 2 fish L^{-1}. During the experiment, fish fed 17α-MT supplemented feed 4 times a day to satiation for 30 days. Feces and uneaten feed were siphoned out everyday and exchanged water at 10-30%.

Observed Parameters

Parameters observed in this experiment were the type and index of cannibalism, aggressive behavior, normal mortality, survival rate, concentrations of steroid hormones including testosterone, estradiol, and cortisol. Growth performances were measured for total weight and length, then specific growth of weight and length, and water quality of temperature, pH, and dissolve oxygen. Observation of cannibalism characteristics was performed for number of dead fishes observed every six hours, number of cannibalisms based on types I and II, and the cannibalism index. Type I cannibalism is a dead fish condition identified with a damage on the tail, intact with stomach or head marks and the whole body eaten and left-body parts found. Type II cannibalism is condition of the fish eaten completely or missing (Król *et al*., 2014). The cannibalistic category was calculated according to Król & Zakêœ (2016), using equation: Cannibalism type I (%) =

$$\frac{\text{Number of dead woundded fish}}{\text{Number of intial fish}} \text{x100}$$

and Cannibalism type II (%) =

$$\frac{\text{Number of missing fish}}{\text{Number of initial fish}} \text{x100}$$

The cannibalistic rate measured according to Obirikorang *et al*. (2014) as a Cannibalism index (%) =

$$\frac{\text{Number of missing and dead woundded Fish}}{\text{Number of initial fish}} \text{X100}$$

Mortality is the number of normal death, not due to cannibalistic. calculated using the formula:

$$\text{Mortality (\%)} = \frac{\text{Number of normal dead fish}}{\text{Number of initial fish}} \text{x100}$$

Survival is the number of living fishes at the end of the study. Calculated as:

$$\text{Survival rate (\%)} = \frac{\text{Number of fish at the end study}}{\text{Number of initial fish}} \text{x100}$$

The aggressiveness level observed in every seven days for three minutes immediately after feeding, and 30 minutes afterward. The aggressiveness was quantified based on the ethogram in percentage according to Nieuwegiessen (2009) (Table 1).

Table 1. Aggressive behavior

No	Behavior	Definition
1	Agonistic	Chasing/biting fish chased/bitten by other fish
2	Flee/escape	Fish move to water surface for some time with gill covers closed
3	Rest	Passive movement below surface of entertainment medium
4	Stereotypic	Distinct swimming movement pattern below and continuously for 10 seconds
5	Swimming	Movement of body to find and move food

Growth performances were calculated for total length and weight. Weight gain was calculated using formula according to Siregar *et al*. (2021), Wm = Wt – Wo, where Wm is the total weight growth (g), Wt is the final weight (g) and Wo is initial weight (g). The Specific growth rate (SGR) was calculated by the formula SGR (%/day) = $\frac{\text{LnWt} - \text{LnWo}}{t} \text{x100}$, where Wt is final weight at time t (g), Wo is initial weight (g), and t is duration of rearing time (day). Total length growth was calculated using the formula of Siregar *et al*. (2021), Lm = Lt – Lo, where Lm is the total length (cm), Lt is final length (cm) and Lo is initial length (cm). The Specific length growth rate (SLR) was calculated using the formula SLR (%/day) = $\frac{\text{LnLt} - \text{LnLo}}{t} \text{x100}$, where Lt is final length (cm), Lo is initial length (cm), and t is duration of rearing time (day).

For measuring the steroid concentration, bodies of the fish were sampled at days 0, 15, and 30 of the rearing periods. Two grams of the collected fish were used to prepare 1 mL of supernatant. To reduce the impact of stress, before kill the experimental fish anesthetized using 0.35% phenoxy ethanol. The samples were washed with distilled water, then extracted by grinding the samples and homogenized in a phosphate buffered saline (PBS) solution containing 0.05% Tween-20 (pH 7.2) with a ratio of 1:4. The samples were then centrifuged at 5000 rpm for 10 minutes (Sukenda *et al*., 2017). The supernatant was produced by centrifugation and collected as the serum and then stored at -20 °C for determining the concentrations of testosterone, estrogen, and cortisol in the fish body's plasma. Steroid analysis was performed using the ELISA method. Kit used for the hormone analysis was for human including estradiol for females, testosterone kit for males, and cortisol kit for cortisol, (size 96 wells, from DRG diagnostics kit). The method of ELISA measurement followed the protocol from DRG International Inc. USA version 11.1 from 2010. Water quality of pH measured used pH-meter and dissolved oxygen (DO) measured used DO-meter was done once in every seven days, while the temperature measured used thermometer was done daily.

All data collection were analyzed by analysis of variance (ANOVA) through SPSS program version 22 with 95% confidence interval. If the analysis of variance showed significantly different results, Duncan's method was used to further tests.

RESULTS AND DISCUSSION

Type and index of cannibalism, normal death, and survival rate (SR) of fish are presented in Table 2. there was no difference among treatments for cannibalism type I based on the death injured due to predation, with the bodies of fish often damaged by feeding actions. Meanwhile, the incidences of cannibal-

Table 2. Cannibalism types I and II, index of cannibalism, normal death, and survival rate (%) of fish juveniles fed ration supplemented with different dose of 17α-MT

Treatment	Cannibalism Type I	Cannibalism Type II	Index of Cannibalism	Normal Death	SR
MT0	23.13±2.39[a]	5.63±3.15[a]	28.75±3.23[a]	1.88±1.25[a]	69.38±2.39[b]
MT1	21.88±1.25[a]	2.50±0.00[a]	24.38±1.25[a]	1.88±1.25[a]	73.75±2.50[b]
MT2	24.38±3.75[a]	16.25±3.23[b]	40.63±4.27[b]	1.88±1.25[a]	57.50±4.56[a]
MT3	23.13±3.15[a]	12.50±7.07[b]	35.63±5.15[b]	3.75±1.44[a]	60.63±4.27[ab]

Different superscripts in the same column indicate significant difference (P<0.05). Note: MT0 (Administration of 17α-MT at a dose of 0 mg kg^{-1} feed), MT1 (Administration of 17α-MT at a dose of 7.5 mg kg^{-1} feed), MT2 (Administration of 17α-MT at a dose of 15 mg kg^{-1} feed), and MT3 (Administration of 17α-MT at a dose of 30 mg kg^{-1} feed).

ism type II were significantly higher (P<0.05) in treatment MT2 and MT3 compared to control (MT0) and MT1 (P <0.05). Likewise, the cannibalistic index value at treatment MT2 and MT3 were significantly higher than treatment MT1 (P <0.05). Fish fed feed supplemented with 17α-MT at treatment MT2 has the lowest SR value (P<0.05), while normal mortality has no statistical difference (P>0.05). Furthermore, fish fed feed supplemented with 17á-methyltestosterone at treatment MT2 had the highest cannibal index and the lowest survival rate compared to the other treatments. Based on those data, the incidence of cannibals significantly (P<0.05) affected survivals.

Figure 1 shows the pattern of the mean incidence of type I cannibalism during days, there was an increase in the incidence until day 14, then decreased on days 21 and 30 in fish fed ration supplemented with 17α-MT at treatment MT0 and MT1. Fish fed

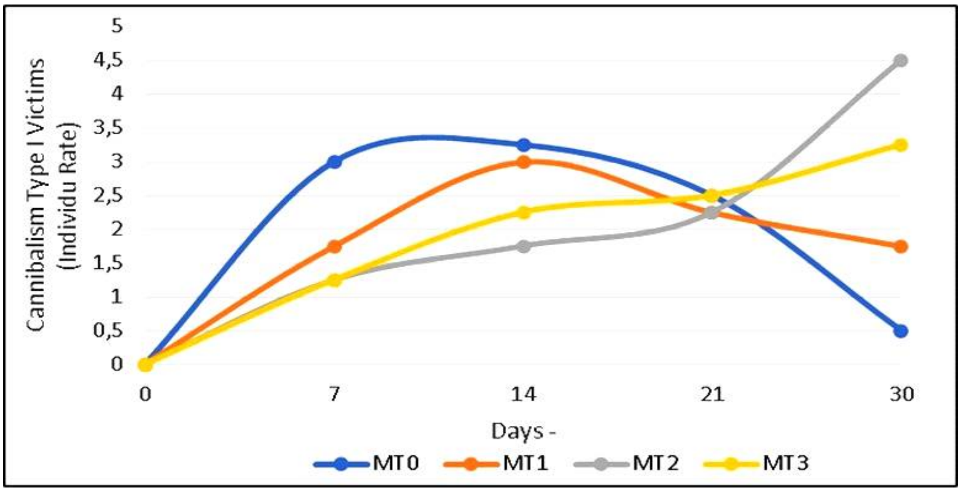

Figure 1. Tendency rate of cannibalism type I on Asian redtail catfish juveniles reared for 30 days.

supplemented with 17α-MT at treatment MT2 and MT3 had different trend that the incidence of type I cannibalism increased from day 7 until day 30, and with the highest incidence occurred in the C group in the last day of the treatment. The results found the present study were in line with the previous studies where the administration of the 17α-MT has the effect in increasing aggressiveness which further affected on the cannibalism in fish (Zairin *et al*., 2002; Kania *et al*., 2012; Chang *et al*., 2012; Peterson *et al*., 2013; Alcazar *et al*., 2016; Rahmadiah *et al*., 2019), need a certain number of doses testosterone (Batrinos, 2012; Dreher *et al.,* 2016).

Aggressive behavior of agonistic and running away were not statistically significant for any treatments and at the time of observation (Tables 3) (P >0.05). However, in resting behavior, there was a difference between treatments (P <0.05), an observation while feeding there was decreased then observation on 3 minute before and 30 minute after feeding suggested because increased in stereotypic and swimming behaviors. There were also differences in stereotypies and swimming of fish among treatments. The smallest resting behavior at all times of observation was recorded in treatment MT3, but the highest number of resting behavior was different at different times

Table 3. Aggressive behavior (%) of Asian redtail catfish juveniles observed 3 minutes before feeding, at the time of feeding, and 30 minutes after feeding with diets supplemented with different dose of 17α-MT

Treatment		Agonistic	Flee/Escape	Rest	Stereotypic	Swimming
3 minutes before feeding	MT0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	39.04 ± 5.30^{ab}	18.14 ± 2.54^{ab}	42.81 ± 4.18^{a}
	MT1	0.00 ± 0.00^{a}	0.83 ± 1.67^{a}	44.26 ± 4.57^{ab}	15.03 ± 4.71^{ab}	39.88 ± 2.72^{a}
	MT2	1.32 ± 2.63^{a}	0.66 ± 1.32^{a}	45.48 ± 4.62^{b}	11.71 ± 5.26^{a}	40.84 ± 3.18^{a}
	MT3	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	36.53 ± 5.21^{a}	21.32 ± 7.65^{b}	42.15 ± 8.49^{a}
while feeding	MT0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	8.79 ± 1.16^{ab}	38.90 ± 9.49^{b}	52.31 ± 10.26^{a}
	MT1	3.13 ± 6.25^{a}	1.04 ± 2.08^{a}	19.10 ± 10.46^{b}	29.50 ± 14.44^{ab}	47.23 ± 12.53^{a}
	MT2	1.79 ± 3.57^{a}	0.00 ± 0.00^{a}	12.42 ± 7.05^{ab}	25.92 ± 8.55^{ab}	59.87 ± 9.11^{a}
	MT3	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	5.95 ± 3.22^{a}	18.13 ± 5.98^{a}	75.93 ± 5.96^{b}
30 minutes after feeding	MT0	1.56 ± 3.13^{a}	0.00 ± 0.00^{a}	34.91 ± 7.06^{bc}	33.29 ± 8.41^{b}	30.24 ± 5.53^{a}
	MT1	0.00 ± 0.00^{a}	0.78 ± 1.56^{a}	40.79 ± 5.06^{c}	25.87 ± 9.19^{b}	32.56 ± 8.38^{ab}
	MT2	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	30.51 ± 5.64^{ab}	10.86 ± 6.91^{a}	58.63 ± 9.25^{c}
	MT3	0.64 ± 1.28^{a}	0.00 ± 0.00^{a}	24.92 ± 2.71^{a}	31.74 ± 8.95^{b}	42.70 ± 6.54^{bc}

Different superscripts in the same column for each group (3 minutes before feeding, while feeding and 30 minutes after feeding) indicate significant difference ($P < 0.05$). Note: MT0 (Administration of 17α-MT at a dose of 0 mg kg^{-1} feed), MT1 (Administration of 17α-MT at a dose of 7.5 mg kg^{-1} feed), MT2 (Administration of 17α-MT at a dose of 15 mg kg^{-1} feed), and MT3 (Administration of 17α-MT at a dose of 30 mg kg^{-1} feed).

of observation. Before feeding observation the highest value was found in treatment MT2, while for immediately and 30 minutes after feeding observation, it was found at treatment MT1. Swimming behavior, at 3 minutes before feeding had no differences among treatments. However, at the time of feeding, swimming behavior of fish fed with 17α-MT at treatment MT3 was significantly higher ($P < 0.05$) than the other treatments. In observations 30 minutes after feeding, it looks different among treatments where fish fed supplemented with 17α-MT at treatment MT0 had the lowest swimming movement and the highest value was observation in juveniles at treatment MT2.

It was difficult to observe the aggressive behavior for agonistic behavior and running away, because the Asian redtail catfish juveniles moved quickly and grouped at corner of aquaria. Therefore, the incidence of attack and attacked was quite difficult to recognize. Based on the observations, fish injured or even cut off the tails were frequently attacked 1 hour after feeding. Most likely this behavior was not due to lack of feed, but rather to the high aggressiveness. The highest swimming behavior at 30 minutes after feeding occurred in fish fed diet with 17α-MT at treatment MT2. This condition was different from African catfish where the more swimming movements, the lower the aggressive behavior (Mukai *et al*., 2013).

Table 4. Growth performance of fish fed diet supplemented with different doses 17α-MT

Treatment	Wm (g)	Lm(mm)	SGR (%)	SLR (%)
MT0	1.70 ± 0.11^{a}	29.12 ± 1.33^{a}	2.89 ± 0.13^{a}	15.67 ± 0.06^{a}
MT1	1.57 ± 0.16^{a}	28.67 ± 2.20^{a}	2.74 ± 0.20^{a}	15.64 ± 0.11^{a}
MT2	1.55 ± 0.41^{a}	29.10 ± 3.24^{a}	2.69 ± 0.47^{a}	15.66 ± 0.15^{a}
MT3	1.49 ± 0.40^{a}	27.83 ± 2.48^{a}	2.62 ± 0.49^{a}	15.60 ± 0.12^{a}

Different superscripts in the same column indicate significant difference ($P < 0.05$). Note: A (Administration of 17α-MT at a dose of 0 mg kg^{-1} feed), B (Administration of 17α-MT at a dose of 7.5 mg kg^{-1} feed), C (Administration of 17α-MT a dose of 15 mg kg^{-1} feed), and D (Administration of 17α-MT at a dose of 30 mg kg^{-1} feed).

There was no difference in growth performances among treatments ($P > 0.05$) both gain in total weight and length, and growth of specific weight and length (Table 4). However, if we look at the total weight growth, fish fed ration supplemented with 17α-MT at treatment MT0 had the highest value and fish fed ration supplemented with 17α-MT at treatment MT3 had the lowest specific growth rate. This condition is also the same for the growth of total length and growth of specific length (Table 4).

Concentration of the plasma testosterone did not differ significantly ($P > 0.05$) among group (Table 5). However, there was a trend of a decrease in the level of the plasma testosterone in juveniles of Asian redtail catfish, when compared with treatment without supplementation with 17α-MT (MT0) on the day 15 and day 30 of observation. Furthermore, concentration of plasma estradiol, was the lowest estradiol body's plasma values statistically ($P < 0.05$), namely in juveniles fed ration supplemented with 17α-MT at MT2 on day 15 and juveniles fed ration supplemented with 17α-MT at treatment MT3 on day 30. However, on day 15, the highest content of the plasma estradiol was found in juveniles fed ration supplemented with 17α-MT at treatment MT3 and decreased to be lowest on day 30. On the other hand, this phenomenon also occurs in the administration of estradiol-17α to post larvae of Asian redtail catfish, where at highest doses, longest time and most common immersion gave the highest incidence of cannibalism has an effect on the highest plasma testosterone concentration occurred on day 15, but has an lowest concentration on day 30 (Heltonika *et al*., 2023). The concentration of cortisol was not difference among

Table 5. Testosterone, estradiol, and cortisol concentrations of fish fed diet containing different doses of 17α-MT

	Testosterone (pg ml^{-1})		Estradiol (ng ml^{-1})		Cortisol (pg ml^{-1})	
Days-0	0.4913±0.03		0.7755±0.74		27.9795±4.89	
	Day-15	Day-30	Day-15	Day-30	Day-15	Day-30
MT0	0.72±0.35^{a}	0.54±0.06^{a}	1.03±0.93^{ab}	2.01±0.17^{b}	38.76±1.86^{b}	27.86±5.12^{a}
MT1	0.50±0.04^{a}	0.48±0.06^{a}	0.90±0.56^{ab}	1.66±0.40^{b}	25.38±6.98^{ab}	17.70±3.61^{a}
MT2	0.47±0.13^{a}	0.44±0.20^{a}	0.67±0.21^{a}	1.55±0.39^{b}	18.39±9.80^{a}	25.87±4.99^{a}
MT3	0.50±0.09^{a}	0.45±0.10^{a}	1.86±0.31^{b}	0.70±0.27^{a}	23.48±11.99^{ab}	22.99±10.39^{a}

Different superscripts in the same column indicate significant difference (P<0.05). Note: A (Administration of 17α-MT at a dose of 0 mg kg^{-1} feed), B (Administration of 17α-MT at a dose of 7.5 mg kg^{-1} feed), C (Administration of 17α-MT at a dose of 15 mg kg^{-1} feed) and D (Administration of 17α-MT at a dose of 30 mg kg^{-1} feed).

the treatments on day 30, but on day 15 there was a significant difference where control had the highest cortisol concentration, and the lowest concentration was found in the juveniles fed supplemented with 17α-MT at treatment MT2. Looking at the steroid biosynthesis pathway, the steroidogenic enzymes (aromatases) exclusively converted androgens to estrogens in the steroidogenic pathway (Nelson & Habibi, 2013). Therefore, it is very likely that the decrease in estradiol contents in the body's plasma occurs through the negative feedback signal of biosynthesis. The decline of the hormone concentration occurs through a negative feedback mechanism (Dinsdale & Ward, 2010). Several internal and external factors may affect the metabolic clearance pathways of hormones which further impact on reducing hormone concentrations (Thibaut & Porte, 2004).

There is an indication of reduction in the plasma estradiol at the treatment MT2 on day 15 and MT3 on day 30 and cortisol concentrations at MT2 on day 15, due the supplementation of 17α-MT. Which was in line with the cannibal index value. This indicated that the administration of 17α-MT tended t decrease the concentrations of estradiol and cortisol in body's plasma and further increasing the incidence of cannibalism. This research were different from the opinion that increasing plasma androgen will increase aggressiveness and decrease parental care in fish (Matsumoto *et al*., 2018). In the present condition, concentration of the androgen hormone (testosterone) in the experimental fish did not differ among treatments. However, concentration of the plasma estrogen (estradiol) was different which was in line with the increasing incidence of cannibalism in juveniles of Asian redtail catfish. Indicating that low content of estradiol in the body's plasma of juveniles showed the highest incidence of cannibalism.

Aggressive behavior is related to the action of androgen receptors (Cunningham *et al*. 2012; Yang *et al*., 2017). The increased incidence of cannibal in 17α-MT treatments was related to the androgen receptor. In mammals, the neurobiological mechanism underlying this aggressive behavior was due to stimulation by androgens through receptors in the amygdala and orbitofrontal cortex. The amygdala area functions to regulate emotion, aggressiveness, and dominance (Eisenegger *et al*., 2011; Nguyen *et al*., 2016; Geniole *et al*., 2019; Zhang, 2021). Furthermore, Zhang (2021) revealed that release of testosterone induced by aggression could lead to an increase in estrogen in the brain. This mechanism is through the neuroendocrine system (Munley *et al*., 2018).

Other pathway needs to be determined at in the future is the role of aromatase pathway on Asian redtail catfish, with the high concentration of estradiol detected on day 15 on highest testosterone, although statistically it was not significantly different. Conversion of testosterone to become estradiol in the body's plasma was regulated by the action of aromatase, which is responsible for the increased aggressiveness and leads to increased cannibalism. Cunningham *et al*. (2012) stated that an increased in aromatase activity in the conversion of testosterone to estradiol increases aggressiveness (cannibalism). Furthermore, Huffman *et al*. (2013) revealed when fadrozole (aromatase inhibitor) administered to inhibit the formation of the estradiol hormone from testosterone, the incidence of aggression (aggressiveness) was decrease in the in these fish indicating that aromatase was a regulator of aggressiveness in male *Astatotilapia burtoni*.

Water quality parameters are presented by Table 6 in which the range were in normal condition and supported the growth of the juveniles. The temperature ranged from 28 to 29 °C. The pH ranged from 6.0 to 6.5and dissolved ranged from 6.3 to 7.5 ppm.

Table 6. Water quality in rearing sed of Asian redtail catfish fed ration supplemented with different doses of 17α-MT.

Treatment	Temperature (°C)	pH	Dissolved oxygen (ppm)
MT0	28 – 29	6.0 – 6.5	6.3 – 7.4
MT1	28 – 29	6.0 – 6.5	6.5 – 7.3
MT2	28 – 29	6.0 – 6.5	6.4 – 7.5
MT3	28 – 29	6.0 – 6.5	6.5 – 7.5
(Yudha *et al*., 2018)	27 – 30	5.0 – 7.0	5.0 – 7.0

CONCLUSION

Administration of the hormone testosterone through feed increased cannibalism in Asian redtail catfish, with highest cannibalism index of 40.63 ± 4.27 (MT2) and 35.63 ± 5.15 (MT3). No significant effect of the treatments in the growth performances. Based on estradiol change in the body's plasma, it was suspected that plasma estradiol concentration might play a role in controlling cannibalism of Asian redtail catfish juveniles.

CRediT authorship contribution statement and declaration of competing interest

Benny Heltonika: Methodology, conceptualization, writing, analysis of data, review and editing. **Agus Oman Sudrajat:** Writing, review, editing and supervision. **Widanarni:** Writing, review, editing and supervision. **Agus Suprayudi:** Writing, review, editing and supervision, **Wasmen Manalu:** Writing, review, supervision and editing, **Yani Hadiroseyani:** Writing, review, supervision and editing, **Muhammad Zairin Junior**: Methodology, conceptualization and Supervision.

DECLARATION OF COMPETING INTEREST

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

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