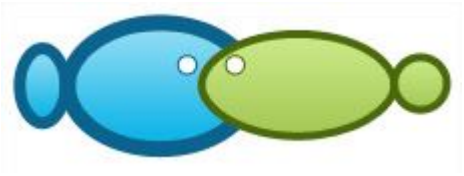

Aggressiveness and growth of male Siamese fighting fish treated with different dose and duration of 17α-methyltestosterone immersion

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Abstract. Siamese fighting fish (*Betta splendens*) is famous as ornamental and "fighting fish" due to its aggressiveness. This study aimed to determine the dose and duration of 17α-methyltestosterone (MT) immersion to increase the aggressiveness of Siamese fighting fish. Three different doses of MT were tested, namely 0 mg L^{-1} (control), 0.2 mg L^{-1} and 0.8 mg L^{-1}, and immersion was performed for 12 and 24 hours. As replication, eight fish were used in each treatment. Fish were reared for 8 weeks in plastic cups filled with 250 mL water and fed with blood worms three times daily at apparent satiation. MT was given three times at interval of two weeks, and agonistic behavioral test was performed seven times with one week interval. After immersion, fish were challenged to fight with non MT-immersed fish by placing them close to each other. The results revealed that MT immersion increased aggressiveness shown by increasing number of gill flaring, number of attack and duration ($p < 0.05$). Immersion duration gave insignificantly effect on aggressiveness ($p > 0.05$). Highest number of gill flaring (103 times), number of attacks (95 times) and attacks duration (91 min) was obtained in 0.2 mg L^{-1} immersion dose at first agonistic test. Higher growth was obtained in 0.8 mg L^{-1} MT treated fish, while 0.2 mg L^{-1} treatment was similar with control. However, survival of 0.8 mg L^{-1} treatment was lower than 0.2 mg L^{-1} and control treatments. In conclusion, MT immersion at dose of 0.2 mg L^{-1} for 12 hours was effective to increase male Siamese fighting fish aggressiveness.

Key Words: *Betta splendens*, immersion, 17α-methyltestosterone, aggressiveness.

Introduction. Siamese fighting fish, *Betta splendens* Regan, 1910 is among popular ornamental fishes in South East Asian countries, such as in Thailand, Malaysia, Vietnam, and Indonesia. Not only as ornamental fish, Siamese fighting fish is also known as fighting fish for its aggressiveness to its own kind (Tilberg 2010). Aggressiveness and endurance can be attractive features of Siamese fighting fish. These two points can be observed through agonistic behavior when Siamese fighting fish sighted enemy that it could either act more aggressively, or instead being intimated (Irawan & Afiati 2006). Aggressiveness in Siamese fighting fish is usually increased by regular spar to habituate agonistic interaction between fishes. Agonistic interaction and competition between males are associated with endocrine response, especially those that influence the rise of androgen hormone (Oliveira et al 2002). One of natural androgen hormone within animal body is testosterone, while synthetic testosterone hormone commonly used is 17α-methyltestosterone (MT). To date MT hormone in aquaculture field is used for sex reversal, which is a technique to reverse sex artificially before sex differentiation period. Administration of MT hormone in emulsion form can increase testosterone concentration in blood plasma of Asian redtail catfish, *Hemibagrus nemurus* (Valenciennes, 1840) because of the increasing activity of FSH hormone as a positive feedback from hormone administration (Supriyadi 2005; Suriansyah 2010). In addition, androgen hormone also can influence aggressive behavior in territorial context (Ros et al 2004). Aggressive behavior in territorial context is one of Siamese fighting fish characters that hobbyist are attracted to. Various response of MT hormone administration is closely related to given doses. The doses of MT are given based on species, age, sex, reproduction status, environmental condition, and also method of administration. There has been no

information about MT hormone administration doses in its usage of increasing aggressiveness in fish. This research aimed to study MT hormone administration with different dose and duration of administration in its use of increasing male Siamese fighting fish aggressiveness.

Material and Method. This study used factorials design with two factors, i.e. (1) 17α-methyl testosterone (MT), and (2) duration of hormone immersion. Dose used were 0.0, 0.2, and 0.8 mg L^{-1}, while the duration of hormone immersion were 12 hours and 24 hours. Agonistic behavior tests were done 7 times with one week intervals (Test 0, I, II, III, IV, V, and VI). During experimental period of March to May 2015, MT were given three times with two weeks interval (at Test I, III, and V).

Experimental fish and maintenance. Siamese fighting fish of halfmoon strain aged 5 months (average body weight: 1.193±0.092 gram, average body length: 3.03±0.15 cm) was obtained from a breeder in West Java, Indonesia. Total fish used were 96 fishes with 48 fishes as treatment fish, and 48 fishes as the opponent of treatment fish during aggressiveness test. Determination of treatment fish and treatment fish opponent was based on body weight and length of treatment fish.

Fishes were kept for 8 weeks in plastic glasses filled with 250 mL water. Six plastic glasses were placed in 30 × 20 × 15 cm^3 aquarium filled with 1,500 mL water. Treatment container for treatment fish was 20 × 15 × 15 cm^3 aquarium filled with 1,000 mL water. Meanwhile, untreated fish were kept in plastic glass filled with 250 mL water. Fishes were fed on blood worms, *Tubifex* sp. at satiation, 3 times a day (08.00, 14.00, and 18.00). At amount of 10% water volume was replaced every day to dispose of filth in maintenance container, and 100% water replacement was done once a week.

MT hormone immersion. Hormone used in this research was synthetic 17α-methyltestosterone (MT) hormone (Sigma Chemical, USA). Hormone was weighted according to treatment doses, diluted into 100 µL 70% ethanol solution and then diluted into 1,000 mL water and aerated. Immersion was done for 2 hours.

Aggressiveness test simulation. Test simulation was conducted inside treatment container to observe aggressiveness behavior. After fish was immersed in MT hormone solution, untreated fish (treatment fish's opponent) inside plastic glasses were put inside treatment container, so that treatment fishes and their opponents will fight each other while being separated.

Test parameters related to aggressiveness observed were the number of gill flaring, number of attacks, the duration of attacks, and the size of bubble nest. The number of gill flaring is one of secondary characteristics of fighting fish when threatened or sighting opponents, so it can be used as aggressiveness markers. In this research the number of gill flaring in treatment fish was observed for 120 minutes.

The number of attacks is also one of the secondary characteristics of fighting fish when threatened or sighting opponent. Attack parameter is a condition when treatment fish attacked his opponent. The observation was done for 120 minutes. Attack duration is the duration of fighting fish showing attacking opponent aggressive behavior. Furthermore, the size of bubble nest is qualitatively observed by measuring the width of bubble nest formed based on documented image taken by digital camera (Canon powershoot A3400 IS HD). The documentation images were analyzed by software ImageJ.

Growth performance. Test parameter concerning growth performance observed was total body weight and length gains, survival, and feed consumption. Weight gain is the increased of body weight during fish maintenance period, and was calculated using the formula: PB = Bt - Bo. Bt is the weight of the fish at the end of maintenance (gram $fish^{-1}$), and Bo: the initial body weight of fish (gram $fish^{-1}$). Total length gain was the difference of total body length at the end of maintenance and initial body length. Survival is the percentage of the ratio of the number of fish alive at the end of maintenance with the initial number of living fish.

Feed consumption of treatment fish was calculated by weighting the amount of feed eaten throughout the research. Fresh blood worms were weighted everyday by using digital balance (type DJ1002C with 0.01 mg readability).

Statistical analysis. Data was processed by using Microsoft Excel 2007 then analyzed by using multivariate analysis of variance (MANOVA) method and Duncan test at p = 0.05 with the help of SPSS 16.0 software.

Result and Discussion. The result of fish gill flaring observations is shown on Figure 1. MT administration based on dose influenced the number of gill flaring in V test ($p < 0.05$), while immersion duration factor and the interaction between the two factors towards the number of gill flaring in each treatment were not statistically different ($p > 0.05$). Administration of MT in 0.2 mg L^{-1} dose in V test caused the number of gill flaring to be higher than 0.8 mg L^{-1} dose and control ($p < 0.05$). The highest number of gill flaring (103 times) was obtained from 0.2 mg L^{-1} MT dose administration with 24 hour of immersion time on test I.

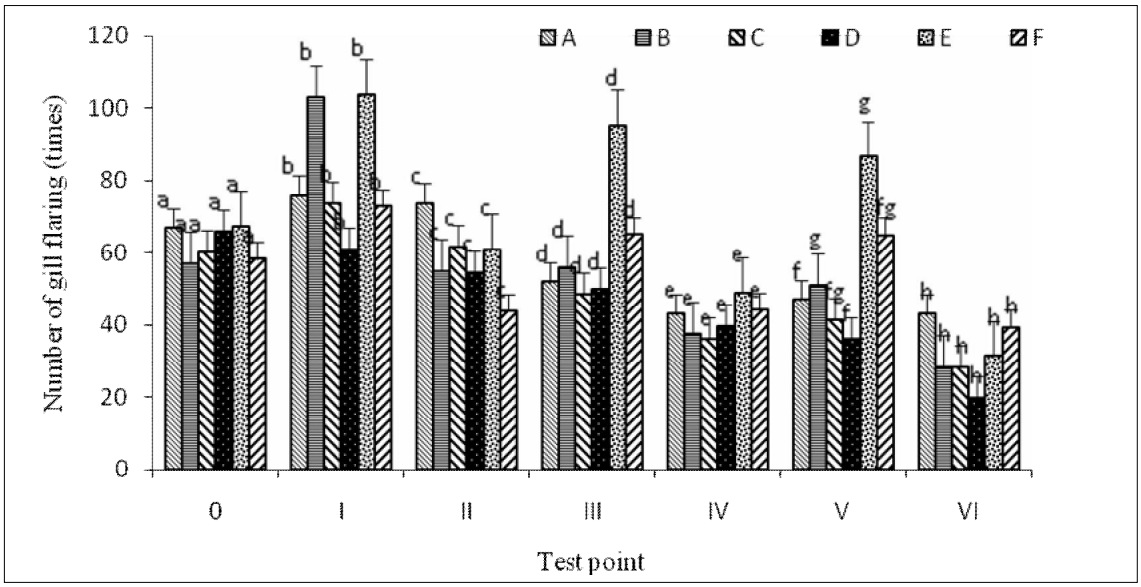

Figure 1. The number of gill flaring of Siamese fighting fish that has been immersed into water containing 17α-methyltestosterone (MT) hormone (caption: 0 = initial test, I = MT immersion Test I, II = test 2, III = 17α-MT immersion Test 3, IV =Test 4, V = MT immersion Test 5, VI = Test 6, A = 0 mg L^{-1} MT 12 hours of immersion, B = 0.2 mg L^{-1} MT 12 hours of immersion, C = 0.8 mg L^{-1} MT 12 hours of immersion, D = 0 mg L^{-1} 24 hours of immersion, E = 0.2 mg L^{-1} MT 24 hours of immersion, F = 0.8 mg L^{-1} MT 24 hours of immersion). Different letters in every test point indicated significantly different result ($p < 0.05$).

The number of attacks from treatment fish towards his opponent is shown on Figure 2. Dose factor of MT administration has significant influence towards the number of attacks in Test I and V ($p < 0.05$) while the duration of immersion and the interaction between the two factors against the number of attack were not significantly different in every test ($p > 0.05$). The number of attack observed in 0.2 mg L^{-1} dose MT immersion in test I and V were higher than 0.8 mg L^{-1} dose treatment and control ($p < 0.05$). Based on Figure 2, the highest number of attacks (95 times) was obtained from 0.2 mg L^{-1} dose MT immersion with 12 hours of immersion in Test I, and 0.2 mg L^{-1} dose MT immersion with 24 hours immersion (91 times).

Attack duration is the endurance of MT hormone treated fish on the last time he attacked his opponent within 120 minutes time period. Observation of fish endurance is presented on Figure 3. Research result showed that dose factor of MT administration had significant influence to attacking endurance response on Test I, III, and V ($p < 0.05$), while immersion duration and interaction between two factors towards endurance response on every test has no significant difference ($p > 0.05$). Attack duration of fish

treated by 0.2 mg L^{-1} dose Mt on Test I, III, and V is higher than 0.8 mg L^{-1} dose treated fish and control ($p < 0.05$). Based on Figure 3, the longest endurance was obtained from fish treated by 0.2 mg L^{-1} dose of MT with 12 hours of immersion in Test I (91 minutes), and 0.2 mg L^{-1} dose with 24 hours of immersion in Test I (84 minutes).

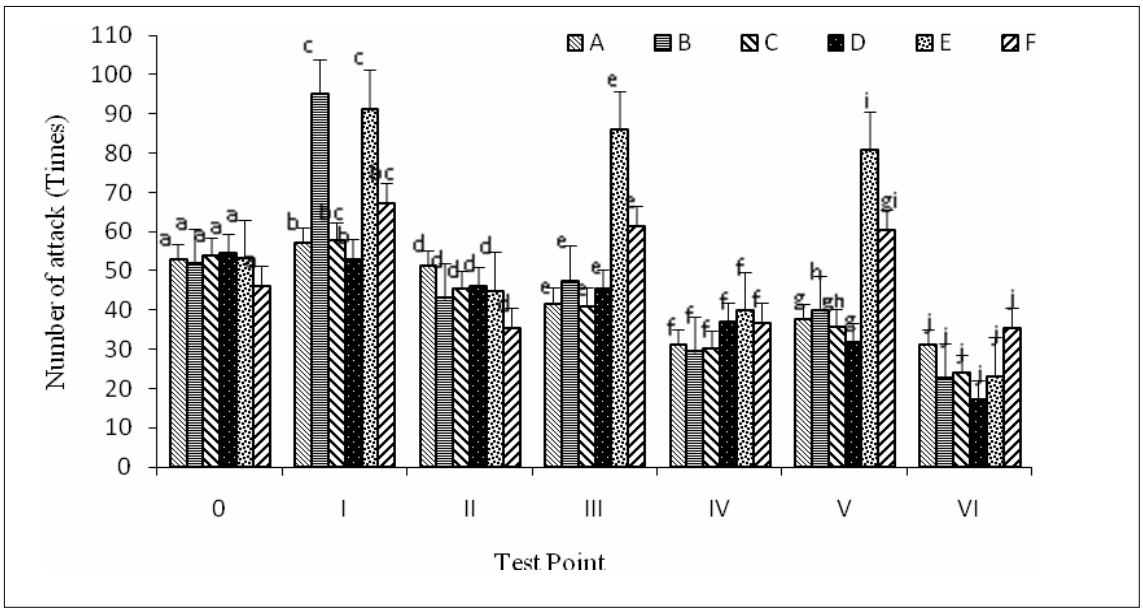

Figure 2. The number of attack by male Siamese fighting fish after immersed in water containing 17α-methyl testosterone (MT) hormone against untreated opponent fish (caption: 0 = initial test, I = MT immersion Test I, II = test 2, III = 17α-MT immersion Test 3, IV =Test 4, V = MT immersion Test 5, VI = Test 6, A = 0 mg L^{-1} MT 12 hours of immersion, B = 0.2 mg L^{-1} MT 12 hours of immersion, C = 0.8 mg L^{-1} MT 12 hours of immersion, D = 0 mg L^{-1} 24 hours of immersion, E = 0.2 mg L^{-1} MT 24 hours of immersion, F = 0.8 mg L^{-1} MT 24 hours of immersion). Different letters in every test point indicated significantly different result ($p < 0.05$).

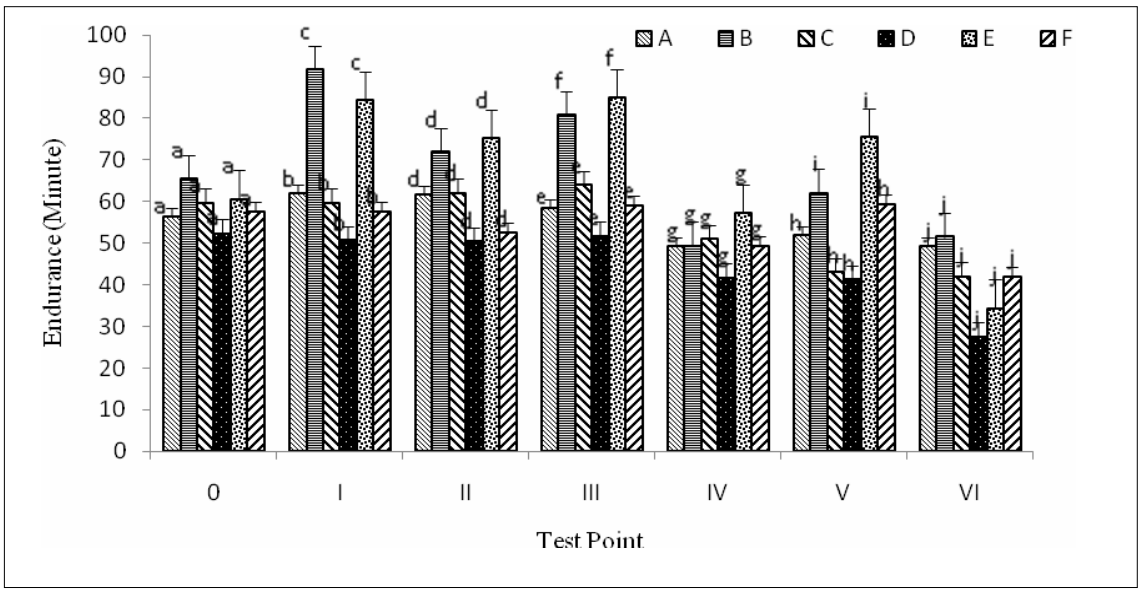

Figure 3. Endurance of male Siamese fighting fish immersed in water containing 17α-methyl testosterone (MT) hormone against untreated opponent fish (caption: 0 = initial test, I = MT immersion Test I, II = test 2, III = 17α-MT immersion Test 3, IV =Test 4, V = MT immersion Test 5, VI = Test 6, A = 0 mg L^{-1} MT 12 hours of immersion, B = 0.2 mg L^{-1} MT 12 hours of immersion, C = 0.8 mg L^{-1} MT 12 hours of immersion, D = 0 mg L^{-1} 24 hours of immersion, E = 0.2 mg L^{-1} MT 24 hours of immersion, F = 0.8 mg L^{-1} MT 24 hours of immersion). Different letters in every test point indicated significantly different result ($p < 0.05$).

Bubble nest size is presented on Figure 4. Result showed that MT hormone administration has no significant difference towards the size of bubble nest made by male Siamese fighting fish ($p > 0.05$).

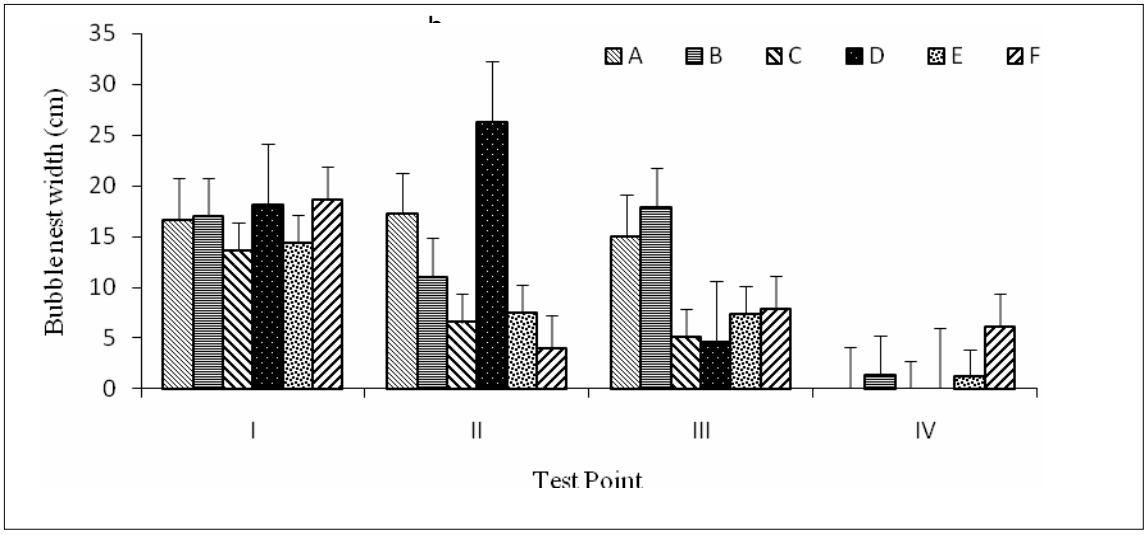

Figure 4. The presence of bubble nest made by male Siamese fighting fish immersed in water containing 17α-methyltestosterone (MT) hormone against untreated opponent fish (caption: 0 = initial test, I = MT immersion Test I, II = test 2, III = 17α-MT immersion Test 3, IV =Test 4, V = MT immersion Test 5, VI = Test 6, A = 0 mg L^{-1} MT 12 hours of immersion, B = 0.2 mg L^{-1} MT 12 hours of immersion, C = 0.8 mg L^{-1} MT 12hours of immersion, D = 0 mg L^{-1} 24 hours of immersion, E = 0.2 mg L^{-1} MT 24 hours of immersion, F = 0.8 mg L^{-1} MT 24 hours of immersion). Different letters in every test point indicated significantly different result ($p < 0.05$).

MT hormone is testosterone which biochemical structure has been modified by the addition of methyl compound (CH_3) in its 17th carbon chain that can extend its period of activity, prevent oxidation, and not quickly inactivated upon entering the body by digestive enzyme which caused it to have longer duration (Supriyadi 2005; Arisandi 2007). Generally MT hormone in fishery is used for masculinization (Carman et al 2008), among them is application on guppy parent fish with 24 hours immersion of 2 mg L^{-1} hormone to produce 100% male phenotype offsprings (Zairin et al 2002). Research of MT hormone administration on male Siamese fighting fish in order to increase aggressiveness is the first to have been done in Indonesia. Increase of aggressiveness has been reported before by Dunlap et al (2002) in brown ghost knifefish male, *Apteronotus leptorhynchus* (Ellis, 1912) given cortisol hormone. In this research, aggressiveness is observed by several parameters which are gill flaring, treatment fish attacks, and attack endurance of treatment fish. Based on the result of this research, administration of 0.2 mg L^{-1} MT hormone can increase male Siamese fighting fish aggressiveness. However, with the same dose, the duration of immersion does not affect level of aggressiveness. This is in line with Ros et al (2004) that reported androgen hormone can influence aggressive behavior in territorial context. Agonistic interaction and competition between males are associated with endocrine response which especially related to androgen hormone increasing triggers (Oliveira et al 2002). In addition, Siamese fighting fish level of aggressiveness is also associated with inherited trait from its parents, however until now the influence of genetic factor is not known in details (Karino & Someya 2007). This may also affect the aggressiveness level in this research, since the fishes used in this research did not come from the same parents. Aggressiveness during fighting period can also be influenced by social environment (Karino & Someya 2007). Social environment mentioned here is the presence of opponent.

MT hormone administration on male Siamese fighting fish does not influence the size of bubblenest. Presence of bubble nest possibly influenced by the presence of opposite of sex, environment, and territorial behavior (Dzieweczynski et al 2006). Administration of MT hormone was presumed to increase aggressiveness in territorial

context. The more intensive a Siamese fighting fish in protecting its territory, the less of possibility of it making bubble nest. Fish given excessive MT may undergo stress. Schreck (2010) stated that fish stress level is related to hormonal system and has negative correlation to its reproductive process. Fish environmental factor also influences fish reproductions with its interaction with stress response from endocrine hormone.

Growth performance. Daily weight growth (WG), length growth (LG), feed consumption amount (FCA), and survival (SR) are shown on Table 1. WG of Siamese fighting fish in 0.2 mg L^{-1} dose MT treatment was significantly different with 0.8 mg L^{-1} dose, however both were not significantly different with control. Highest WG was obtained from 0.8 mg L^{-1} dose with 24-hour immersion (0.2375±0.48 g). Growth has positive correlation with FCA. Highest FCA was obtained from 0.8 mg L^{-1} dose MT hormone with 24-hour immersion (43.0±0.160 g).

Table 1

Daily weight growth (WG), length growth (LG), feed consumption amount (FCA), and survival (SR) of male Siamese fighting fish immersed in water containing 17α-methyltestosterone (MT) hormone

Treatment	*WG (gram)*	*LG (cm)*	*FCA (gram)*	*SR (%)*
A	0.2025±0.037^{ab}	0.000±0.000^{a}	22.9±0.999	100.0
B	0.2363±0.047^{a}	0.050±0.053^{ab}	30.5±0.104	100.0
C	0.0975±0.150^{b}	0.050±0.100^{b}	37.1±0.137	50.0
D	0.2238±0.040^{ab}	0.013±0.035^{a}	26.3±0.159	100.0
E	0.2313±0.033^{a}	0.038±0.052^{ab}	33.9±0.087	100.0
F	0.2375±0.048^{b}	0.050±0.053^{b}	43.0±0.160	62.5
		Two-way ANOVA		
17α-MT (P) dose		$p < 0.05$	$p < 0.05$	
Immersion duration (R)		$p < 0.05$	$p > 0.05$	
Interaction (P*R)		$p < 0.05$	$p > 0.05$	

Legend: A = 0 mg L^{-1} MT 12 hours of immersion, B = 0.2 mg L^{-1} MT 12 hours of immersion, C = 0.8 mg L^{-1} MT 12 hours of immersion, D = 0 mg L^{-1} 24 hours of immersion, E = 0.2 mg L^{-1} MT 24 hours of immersion, F = 0.8 mg L^{-1} MT 24 hours of immersion). Different letter in every test point indicated significantly different result ($p < 0.05$).

Based on the result, it is known that MT hormone administration can increase eating response and the growth of Siamese fighting fish. The increase of fish response upon administration of MT hormone towards feed is presumed to be caused by the fish's needs for energy in order to adapt with its environment. This energy usage is related to metabolism and stomach emptying rate. The increasing fish response towards feed has positive correlation with stomach emptying rate of treatment fish. In addition, testosterone hormone is also included in anabolic hormone criteria, where testosterone hormone can stimulate growth. It is supported by Carman et al (2008) who stated that the rise of MT hormone level may influence growth hormone activity which resulted in higher rate of growth.

However, MT hormone administration has negative correlation with fish life viability. The higher the given dose, the lower the life viability of fish. In this research the lowest life viability value was obtained from 0.8 mg L^{-1} dose of MT hormone for 12 hours (50%), and 0.8 mg L^{-1} dose of MT hormone for 24 hours (62.5%), while in control and 0.2 mg L^{-1} dose of MT hormone administration fish survival value was 100%.

Excessive MT hormone administration is presumed to heighten stress in fish that it can cause death. The rise of hormone level in blood can change secondary physiological responses, including to its organ system (Schreck 2010). Also, synthetic material entering the body can give biphasic effect to antibody system, which at first it may induce antibody production, but may further has immune reaction inhibiting effect. Long term usage of synthetic compound whether in low dose or high dose can destroy immune cell ability to multiply (Connell & Miller 1984).

Conclusions. MT hormone administration via immersion of 0.2 mg L^{-1} dose for 24 hours can increase male Siamese fighting fish's aggressiveness, but has no effect to its production performance. Male Siamese fighting fish that has been immersed in 0.2 mg L^{-1} dose of MT hormone is suggested to be bred in order to see its reproductive performance and its aggressive trait inheritance to its offspring.

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