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Research Article

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Effectively Of 17α-Methyltestosterone on Tropical Eel, *Anguilla bicolor* McClelland Masculinization in Different Salinity Culture

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ABSTRACT

Eel population in nature reaches critical number, so that culture strategy is urgently needed to fulfil the high demand of this fish. A shortcut to get functional male, which proven difficult to be founded from natural catching, is masculinization. This research aimed to induce masculinization of tropical eel, *Anguilla bicolor* McClelland supplemented with various doses of 17α-methyltestosterone during a month culture in freshwater (0 ppt) or brackish water (10 ppt). The eel was grouped and fed with supplementation of 17α-methyltestosterone at various doses, depending upon treatments, namely 0 mg Kg^{-1} diet (control), 40, 80 or 120 mg Kg^{-1} diet. Eels size were similar, at approximately 16,78 g ± 0,62 in weight and 25,38 cm ± 0,15 in length were either culture in freshwater or brackish during the experiment for eight weeks. Sex gonad, based on anatomical histological structures, Eye Index and Fin Index were measured after time culture treatment achieved, as well as body length, weight, eye diameter and the length of the pectoral fin were measured. Results showed that supplementation 17α-methyltestosterone 80 mg/Kg diet culture in brackish water has the highest number of male (90%). This study proven that, the hormone was effective for masculinization in eels, It useful for masculinization in eels. Results proved that the 17α-methyltestosterone highly significant ($P<0.01$) effect on the Eye index (3.63 – 5.14) and Fin Index (3.03 – 4.08) of eels. This study concluded, that 17α-methyltestosterone more effective in improving the number of males in brackish water than in freshwater culture.

Keywords: *Eel, masculinization, 17α-methyltestosterone, salinity*.

1. Introduction

Mature male eel is very important as mature female for artificial insemination as part of eel culture needed. Female broodstock in nature was easily founded, as compared to mature male in capture (Rachmawati and Susilo, 2011; Arai and Kadir, 2017). Information about natural eel maturation is lacking (Churcher et al., 2014). So, the male functional for broodstock by induction for eel culture purposes urgently needed.

The effectiveness use of male sex hormone, 17α-methyltestosterone has been reported for some purposes including to induce masculinization, as a mean of sex reversal, on Tilapia (Marjani et al, 2009;) or on zebra fish (Orn et al., 2003), as well as for sex inversion in grouper fish (Sarter et al, 2006) use of the hormone as inplant to induce permanent sex inversion of 1-year-old juvenile protogynous dusky grouper (*Epinephelus marginatus*) resulted in complete sex change after 12 weeks, with the gonad organized in lobules and cysts filled with germ cells at all stage of spermatogenesis.

Masculinization, in term of getting functional male, not as a mean of sex revearsal, applied 17α-methyltestosterone on tropical eel has been reported. Rovara *et al* (2005) applied 17α-methyltestosterone to eels of sizes 20–30 cm (yellow eel stage) with the doses of 0, 100 and 200 mg.Kg^{-1} diet for a month resulted in 80% population was male. To induce tropical eel maturation aplked the 17α-methyltestosterone in combination with PMSG, AD and MT of 100-150 gr sizes (yellow eel stage), resulted in 100% male and enhanced the gonad maturity also has been reported by Aryani et al. (2015). As we aware ell life cycle is complex, consist of stages : larvae leptocephalus, young eel at glass eel, young eel of elver, young eel of yellow ell and adult of silver eel (Van den Thillart & Dufour, 2009; Macri et al, 2014; Simon, 2015). This report assess effectiveness of the hormone applied on tropical eel of yellow eel (15 – 30 cm in length)

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to induce masculinization as a mean of maturation or functional male.

In European eel sexual maturity has been detected via metamorfosis processes including changes in skin colours, increase in body weight, and eye size culture in saltwater other than fresh water induce reproductive status better (Nowosad et al., 2014). Eel maturity status has been claimed for sometime can be seen from size of particular part including body length, particularly the Eye Iindex and Fin Index increased during gonad maturity (Nowosad et al., 2014). As well as induce masculinization of eels, it is important to stimulate gonad maturity by rearing in the seawater. Effect of 17α-methyltestosterone on maturation characters of european eels has long ago reported as made darker skin in colour and enlarged of eye diameter (Olivereau and Olivereau, 1985). Methyltestosterone, an inhibitor of aromatase (Kitano et al., 2000). A 17α-methyltestosterone is synthetic hormone to mimic natural testosterone functions, or male sex hormone, some function such as determination of secondary reproductive characters, induction of male maturation step processes, including function in male gametogenesis, etc.

The use of 17α-methyltestosterone in sex reversal of zebrafish effective use the dose of 26-1000 ngL^{-1} (Orn et al., 2003). The 17α-methyltestosterone effectively in large taxonomic fishes for sex reversal such as review by Pandian and Sheela (1995). The 17α-methyltestosterone also use to induced somatic growth in European eel give significant in liner growth of elver eel (Degani, 1986), as well as the hormone give linier increased in body composition of eel after dietary with the hormone treatment (Degani, 1985).

Histological male gonad of single male yellow wild giant mottled eel has been reported. (*Anguila marmorata*) having immature male germ cell (Matsubara et al., 2013), and the study proven that gonadal development correspond to their morphological changes. Early maturation stage of male gonad has also been reported in two freshwater eels as having seminiferous tubules with spermatogonia or in later stage with spermatocyte (Arai and Kadir, 2017). Freshwater eels of two species has similar maturation stage of male as characteristic by eye and fin (Arai and Kadir, 2017).

This research aimed to induce masculinization of tropical eel, *Anguilla bicolor* McClelland supplemented with various doses of 17α-methyltestosterone during eight weeks culture in freshwater (0 ppt) or brackish water (10 ppt), based on histological assessment data to determine the sex of eel results (histological structural sex determination of the gonad).

2. Materials and Methos

Animal Handling

The experimental animals, which homogenous eels of yellow eel stage, were obtained from PT LABAS, Bogor, then were acclimated for about a week on the fibre aquarium (220 x 120 x 40 cm^3) filled with 528 L of freshwater. During the acclimation eels were fed with commercial pellet (PT LABAS Indonesia, Protein: 56.56%; fat: 17.25%; fibers: 2.29%; Water: 4.85% and BETN: 5.70%) satiated *ad libitum*. After the acclimation, eels were transferred to the experiment fibre aquarium (40 x 60 x 60 cm3) with 20 fishes per fibre aquarium according to the treatment. During experimentation, eels were fed with commercial pellet (PT LABAS Indonesia) once a day at 5 pm as much as 3% of body weight and kept for 8 weeks (two months). The food were supplemented with various doses of Methyltestosterone 0, 40, 80, and 120 $mg.Kg^{-1}$ diet and culture in freshwater (0 ppt) or brackish water (10 ppt).

17α-Methyltestosterone Preparation for Diet

Method for hormone preparation for diet was modification from Hendry *et al*. (2003). The Methyltestosterone (Sigma, M-7252) powder 40 mg was dissolved in 10 mL of 70% Alcohol then were mixed thoroughly with one Kg commercial pellet with the help of water. Similar procedure for others doses, 80 mg hormone or 120 mg was dissolved in 10mL 70% alcohol then each were mixed thoroughly by adding water as needed. Food contain hormone then was air dried during the day under sunlight, as modification methods of Hendry et al.(2003). Control group food was saturated with Alcohol only.

Data Collection

After week 8^{th} of treatments was achieved, eels were anesthetized with clove oil 5 ppm for about 30 minutes (Rachmawati & Susilo, 2009). Then, each individual eel was weighed using technical scale, data recorded, it's length body was measured using a ruler, data recorded, eye diameter and pectoral fin length were measured using a calliper. Fish then were dissected through the abdomen from the anus to the pectoral for gonadal isolation. Gonad was weighed using an analytical scale

(Explorer OHAUS) to get the GSI (Gonadosomatic Index).

The horizontal (A) and vertical diameter (B) of the orbital eye were measured using a calliper, to get eye diameter. Eye Index is {(A + B) / 4} 2 x π / Body Length (mm) x 100 (Yokouchi et al., 2009). Measurement of pectoral fin length using a calliper by measuring the length of the fins (FL) ranging from base to tip. Fin index is FL (mm) / Body Length (mm) x 100 (Yokouchi et al., 2009).

Each isolated gonad, marked by it's treatments, then was fixed in NBF solution for Paraffin Method preparation gonad sample follow by Haematoxylin-Eosin. All labelled fixed gonad were sent to the Research laboratory of Faculty of Medicine UNSOED for further processes. The analysis of gonadal histology structure refers to Arai & Kadir (2017) to evaluate sex and gonad maturity status under microscope.

Experimental Design

This experimental study applied eight treatments with 20 replications as an individual unit. Eels were grouped and fed with supplementation of 17α-methyltestosterone died at various doses, depending upon treatments, namely 0 mg Kg^{-1} diet (control), 40, 80 or 120 mg Kg^{-1} diet. Eels size were approximately similar, averages size 16.78 g ± 0.62 in weight and 25.38 cm ± 0.15 in length were either culture in freshwater or brackish during the experiment for two months. The observed variables were survival rate, sex (based on histological structure of gonad), Eye Index and Fin Index.

The research was conducted at Experiment Station and Animal Physiology Laboratory of Biology Faculty UNSOED from April to July 2017. Data obtained from the study: Eye index and fin index were analysed by one-way ANOVA and the number of males were analysed descriptively (Steel & Torrie 1981).

3. Results and Discussion

The results showed the highest number of male (90%) in the eel from treatment of 17α-methyltestosterone 80 $mg.kg^{-1}$ diet in brackish water culture whereas in the same doses, which culture in freshwater the number of a male only 55,56% (Figure 1).

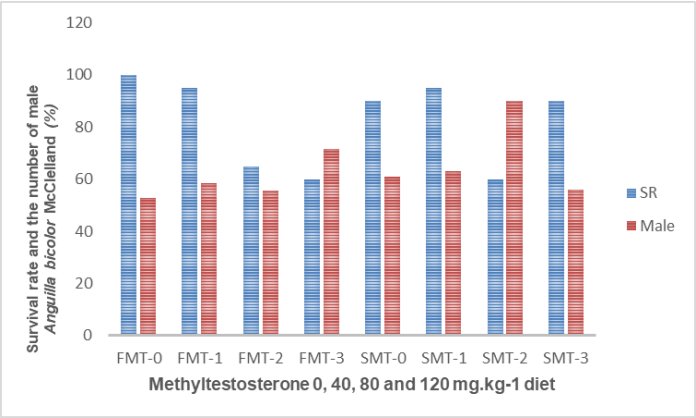

Figure 1. Survival rate (SR) and male number of the experimentation of *Anguilla bicolor* McClelland after 8 weeks supplemented with various doses of 17α-methyltestosterone in freshwater (0 ppt) or brackish water (10 ppt). FMT-0: Methyltestosterone 0 $mg.Kg^{-1}$ diet in freshwater culture (control); FMT-1: Methyltestosterone 40 $mg.Kg^{-1}$ diet in freshwater culture; FMT-2: Methyltestosterone 80 $mg.Kg^{-1}$ diet in freshwater culture; FMT-3: Methyltestosterone 120 $mg.Kg^{-1}$ diet in freshwater culture; SMT-0: Methyltestosterone 0 $mg.Kg^{-1}$ diet in brackish water culture; SMT-1: Methyltestosterone 40 $mg.Kg^{-1}$ diet in brackish water culture; SMT-2: Methyltestosterone 0 $mg.Kg^{-1}$ diet in brackish water culture; SMT-3: Methyltestosterone 120 $mg.Kg^{-1}$ diet in brackish water culture.

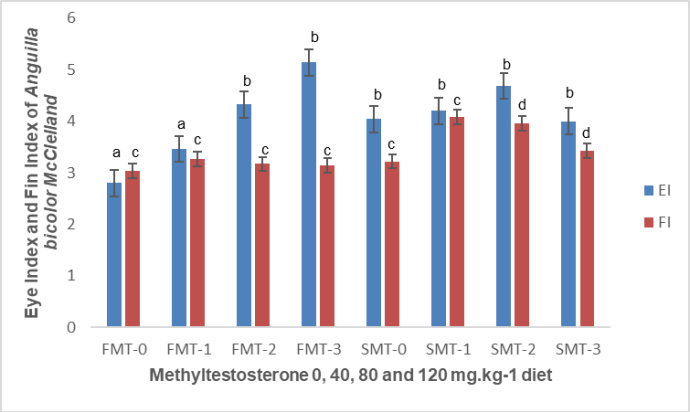

Figure 2. Eye Index (EI) and Fin Index (FI) of *Anguilla bicolor* McClelland supplemented with various doses of 17α-methyltestosterone in freshwater (0 ppt) or brackish water (10 ppt). FMT-0: Methyltestosterone 0 mg.Kg^{-1} diet in freshwater culture (control); FMT-1: Methyltestosterone 40 mg.Kg^{-1} diet in freshwater culture; FMT-2: Methyltestosterone 80 mg.Kg^{-1} diet in freshwater culture; FMT-3: Methyltestosterone 120 mg.Kg^{-1} diet in freshwater culture; SMT-0: Methyltestosterone 0 mg.Kg^{-1} diet in brackish water culture; SMT-1: Methyltestosterone 40 mg.Kg^{-1} diet in brackish water culture; SMT-2: Methyltestosterone 0 mg.Kg^{-1} diet in brackish water culture; SMT-3: Methyltestosterone 120 mg.Kg^{-1} diet in brackish water culture. Similar letter has no significant, different letter significant (P<0.05) or higly significant (P<0.01).

This study showed that 17α-methyltestosterone effectively induce male especially in marine or brackish water (Figure 1.). The 17α-methyltestosterone hormone, actually more effective for masculinization in freshwater culture than the marine or brackish culture one (Figure 1).

The 17α-Methyltestosterone improved eye index as well as the fin index significantly (Figure 2). Both data culture, freshwater and brackish water, significantly improve the eye and fin index (Figure 2.). This study also confirm earlier study that no real correlation between sex (male) and length (Table 1), except that eel of over ≥ 45 cm in length were females (Colombo et al., 1984; Colombo & Grandi, 1990; Colombo & Grandi, 1995; Tesch, 2003; Rachmawati & Susilo, 2012; Rachmawati et al., 2017; Arai & Kadir, 2017). In nature eel needs marine or brackish water to get maturation stage for reproduction, so we call eel as a catadromous species. This means that brackish water as an environmental factor for effective eel maturation factor. This study had been proven this natural phenomenon factor for eel maturation stage.

Table 1. Average (± SD) of Lenght and Weight of tropical eel *Anguilla bicolor* McClelland before and after 8 weeks culture in fershwater or brakish one.

Treatments	BW=0 (g)	BW=t (g)	BL=0 (cm)	BL=t (cm)
Control FMT0	22.95±3.5	16.99±5.9	25.06±1.0	25.53±1.3
FMT 40	24.9±5.0	14.79±3.3	20.45±1.1	25.02±1.2
FMT 80	21±3.0	15.85±2.6	24.73±1.5	25.23±1.4
FMT 120	22.95±3.6	14.5±2.3	25.29±1.2	25.47±1.1
Control SMT0	23.3±3.1	25.44±13.8	25.9±1.6	26.53±2.5
SMT 40	21.9±3.5	16.34±4.2	25.43±1.4	25.51±1.7
SMT 80	20.9±2.6	15.08±2.8	24.93±1.3	24.49±1.1
SMT 120	20.75±3.4	15.22±3.6	24.64±1.4	25.23±1.7

Results from LSD analysis of eye index and fin index data proven the effectiveness of the hormone. Results shown no hormone in fresh water culture (control) group was high significant different (P<0.01) to all other 7 (seven) treatments, meaning that even low concentration and in fresh water culture, the hormone actually as effective as in brackish water culture induce masculinization of eel gonads. The hormone act as an aromatase inhibitor gene expression, and consequently the resultant decrease in the amount of natural oestrogen (Kitano et al., 2000). This possible explanation has been proven by histological data of this study showing male gonad structure, not the female one. In other word, this study proven that the hormone effectively induced a male gonad structure. Interesting finding from this study that the hormone gives different response in freshwater as compared to higher salinity water results.

The concentration of 17α-Methyltestosterone 80 mg Kg^{-1} diet gave the highest number of males, so that the normal curve had been achieved from 4 doses applied. Contrary, male number from freshwater culture give linier curve result, the highest the hormone concentration give the highest number of male (Figure 1). This study confirms previous report on other eel that salinity culture better than freshwater one. In other word this finding support hypothesis of Nowosad et al. (2014) that saltwater gives better result than freshwater in inducing maturation reproduction of European eel. This tropical eel finding also give similar phenomenon that the brackish water culture is better than the fresh water culture.

4. Conclusion

This study concluded, that 17α-methyltestosterone more effective in improving the number of males in brackish water than in freshwater culture.

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Authors' contributions

FNR, RA,and YS design the study. FNR and YS analysed data and wrote manuscript.

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