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Masculinization of *Nile tilapia* (*Oreochromis niloticus*) using 17α-methyl testosteron

Yuni Widyawati[1,2*], Muhammad Fakhri[1,2], Muhammad Dailami[1], Nasrullah Bai Arifin[1,2]

[1]Study Program of Aquaculture, Faculty of Fisheries and Marine Science, Brawijaya University, Malang 65145, Indonesia,
[2]Aquatic Biofloc Research Group, Faculty of Fisheries and Marine Science, Brawijaya University, Malang 65145, Indonesia
*Corresponding author: yunirasyad_bp@ub.ac.id

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ABSTRACT

The biological nature of tilapia which causes uncontrolled mating and has an impact on reducing the quality of the fish has become one of the problems in tilapia aquaculture production. 5 times faster than female tilapia. The production of male monosexual tilapia can be obtained in several ways, including the application of low doses of hormones. The purpose of this study was to determine the effectiveness of using low doses of 17α Methyl Testosterone (MT) in immersing tilapia larvae and to obtain superior quality male tilapia results. This study used tilapia larvae that were 1 week old and were stocked in a tank measuring 30x60cm with a dose of MT 1 mg / l, 3 mg / l and 5 mg / l with 3 replications and 1 control. stocking density of 250 birds per cage. Soaking is carried out for 8 hours. The results of immersion of tilapia larvae using MT at a dose of 3 mg / l until the 60th day showed the highest results with the proportion of 72% compared to the untreated dose, 1 mg / l and 5 mg / l. It can be ignored that the use of low doses of 17α Methyl testosterone can affect sex changes in tilapia larvae. The results showed that the dose of 5 mg / l was the best dose with the proportion of male genitalia at 97%.

INTRODUCTION

Tilapia production in 2017 reached 1.5 million tons or an increase of 3.6 percent from 2016 which reached 1.14 million tons. In 2017, tilapia exports reached 9,179 million tons with a value reaching 57.43 million United States dollars. Although the technology for tilapia aquaculture is very well mastered, but until now the increase in production is still experiencing obstacles. The obstacles that help in tilapia aquaculture activities are mainly the biological

characteristics that are genetically mature. This tilapia also has sex dimorphism, where the growth of male tilapia is played by female fish faster, so that male cultivation is more profitable two times over (Srisarkutiew, et al, 2019). Furthermore, excess is causing wars in competition for food and space so that nearly 50% of tilapia cannot reach consumption sizes at harvest (Herrera *et al*., 2011). This is one of the requests for fulfilling the export demand for tilapia at sizes above 600 grams uniformly in large quantities.

The biological nature of tilapia which causes uncontrolled mating and has an impact on reducing the quality of the fish is one of the problems in tilapia aquaculture production so far. Various efforts have been made, among others, by cultivating male monosec tilapia, which naturally has been known to grow 1.3 - 1.5 times faster than female tilapia. The production of male monosexual tilapia can be obtained in several ways, including; hormone application, hybridization between strains, chromosome changes artificially to produce tilapia which has YY sex chromosome (Supermale) (Dagne A et all, 2013)

Cultivation of male monosex (single sex) tilapia is carried out with the aim; (1) obtained faster growth (2) controlled wild spawning, and (3) obtained a more attractive appearance (Muslim et al, 2011). One of the techniques to get male monosexual tilapia seed is through the sex transfer technique by administering male hormone (testosterone). The hormone commonly used is synthetic hormone 17α-methyltestosterone (MT). Minister of Marine Affairs and Fisheries Decree No: KEP.52 / MEN / 2014 the use of MT has been banned in Indonesia, due to the potential dangers it causes. but in the International Standards for Responsible Tilapia Aquaculture, the use of methyl and ethyltestosterone can still be used at low doses and can still be tolerated. In previous studies it was reported that the hormone methyl testosterone was quickly metabolized and excreted.

The MT concentration in tilapia plasma decreased at 22 hours after cessation of feed (Richard, et all, 2019). The MT concentration in the fish body after 24 hours decreased to 2.5–3.0% (Curtiz et al, 1991) and after 100 hours it decreased to 1% (Johnstone et al, 2013). Monosec activity using this hormone is expected to help shorten the progeny test process so that the time for male tilapia production will be more efficient. The purpose of this study was to determine the effectiveness of using low-dose MT in immersing tilapia larvae and obtaining superior quality male tilapia results.

MATERIAL AND METHODS

Spawing. Tilapia broodstock was obtained from one of the farmer groups in Lamongan Regency, East Java. The selected broodstock is a parent with a good performance and superior characteristics. Spawning is done naturally with the stocking density of the spawning pond is 1 fish / m². The spawning process is carried out with a male and female ratio of 1: 3. The process of incubating the eggs usually takes about one week before the eggs hatch into larvae. After 5 days of age, the tilapia larvae are taken and transferred to a special place for adaptation before being given treatment.

Soaking 17α Methyl testosteron. 17α Methil Testosterone (MT) immersion was given to tilapia larvae that were 1 week old, spread at a size of 30x60cm with a dose of MT 1 mg / l, 3 mg / l and 5 mg / l with 3 repetitions and 1 control. stocking density of 250 birds per cage. Soaking is carried out for 8 hours

Observation of Gender. Observation of sex to see differences in organs from each sex sample of tilapia was carried out on the first sampling on the 30th day and on the 30th day.

The observations observed were primary sex characteristics, male and female tilapia can be distinguished based on the number of holes around the anus. In male tilapia there are two holes, namely the anus and urogenital holes, while in female tilapia there are three holes, namely the anus, ureteric holes, and genital holes (Darwasito et al, 2015).

RESULTS AND DISCUSSION

The results of the observations observed were primary sex characteristics, male and female tilapia can be distinguished based on the number of holes around the anus. In male tilapia, there are two holes, namely the anus and urogenital holes, while in female tilapia there are three holes, namely the anus, ureter and genital holes (Zairin et all, 2003). The results of the differences in the characteristics of male and female fish are presented in Figure 1 (A and B). 50 tilapia fish in each aquarium shows that at a dose of 5 mg / l shows the highest percentage for male sex compared to other doses. The results showed that the testosterone levels in all treated fish at the beginning of the observation were very high compared to the control treatment without giving methyl testosteron

Figure 1. Observation of differences in sex characteristics in tilapia

The percentage calculation from the results of tilapia sampling with the male sex of each treatment can be seen in Table 1.

treatment	Number of fish		Gender day of 60		Male (%)
	early	end	male	female	
Kontrol	250	220	110	110	50
1 mg/l	250	238	195	43	82
3 mg/l	250	210	197	13	94
5 mg/l	250	224	218	6	97
Kontrol	250	220	110	110	50

The research was conducted using tilapia larvae aged 7 days after hatching with a total length of 9-11 mm. Fish of this size and length have not yet experienced a sex differential (Torrans et all, 2018). When fish larvae are 9 mm in size is a good time to initiate sex differential manipulation with a treatment time of six weeks. Although the success of sex change is also influenced by several other factors such as the dose and type of hormone used, the method of hormone administration, the length of treatment time, and the type of fish used in the study (Hines et al, 1995). Observations were made on the 60th day of maintenance in addition to the percentage of sex, histology was also carried out on tilapia.

Observations carried out on the 60th day after immersion were histological observations carried out by observing male and berina gonads in tilapia. One of the methods used to quickly identify gonads in fish seeds is staining using acetocarmine. The method in this study refers to (Soelistyowati et al, 2007) and (Hines et al, 1995). Acetokarmine is easily absorbed by the gonadal tissue. Its differentiation absorption by several gonad structures makes it possible to provide contrasting and distinct colors between the developing gonad and the surrounding connective tissue (Guerro et al).

The way gonad identification works in this study is by taking the gonads from the fish samples then mashed and placed on a glass object. then dripped with acetocarmine solution and used as a smear preparation. The observation of the preparations was carried out using a microscope to reveal the ovary and testicular tissue. Tilapia seeds given acetocarmine will be identified positively as ovaries if the tissue shows a round oocyte, with a slightly pale nucleus and surrounded by darker cytoplasm (Hines et al, 1995). as shown in Figure B. Observations were made on the 60th day of maintenance other than the percentage of sex was also performed histology on tilapia. The results of histological observations on tilapia are presented. The results of histological observations on tilapia are presented in Figure 2

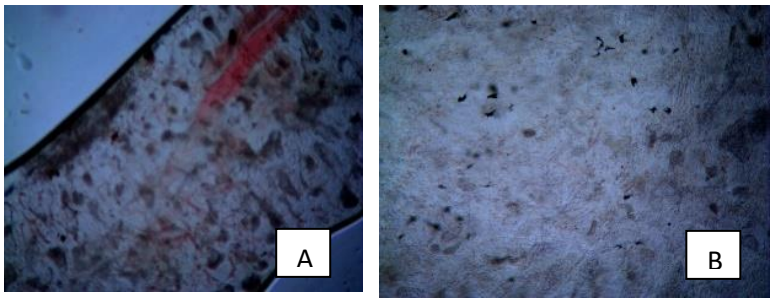

Figure 2. Gonad visualization of male (A) and female (B) fish on day 30

The ovule cell consists of small dots, while the ovule cell appears to be in the form of a large sphere and the nucleus is in the middle with a paler color surrounded by a clearer colored cytoplasm [9]. One month old tilapia seeds were observed to have a weight range between 1.3 - 3.2 grams and a total body length of about 3- 5 cm and have complete fins. The results of the characterization of sexual dimorphism of tilapia seeds showed that the shape and length of the dorsal fin tips between male and female seeds were not clearly visible, as there were differences that could be recognized easily and quickly in adult tilapia.

Likewise, the urogenetalia hole is still difficult to recognize because its size is still very small. The results of anatomical observations of the body of one month old fish seed showed that topographically, the location of the male and female gonads was under the swim bladder, but they were still difficult to find because the gonads were threadlike, thin and white in color. The results of histological observations of the gonads with acetocarmine staining showed that the female fish seeds which had a total body length of 3.5 cm had seen a picture of relatively large sized spheres identified as eggs, while the histological features of the male seeds were one month old with the total length. body 4 cm in the form of small dots and identified as testes

CONCLUSION

The use of low doses of 17α Methyl testosterone can affect sex changes in tilapia larvae. The results showed that the dose of 5 mg/l was the best dose with a male sex percentage of 97%.

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