FORMATION OF MALE SEX TILAPIA (*Oreochromis niloticus*) LARVAE BY IMMERSION METHOD IN NATURAL METHYLTESTOSTERONE HORMONE

ABSTRACT

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Aims: In cultivating Nile tilapia (*Oreochromis niloticus*), the growth of male tilapia is faster than female tilapia. Thus, to obtain productive tilapia, an effort can be made to change the sex of the tilapia larvae into male monosex tilapia. This study aimed to determine the effect of the duration of administration of the natural Methyltestosterone (MT) hormone on the formation of the male sex of tilapia using the immersion method.
Study design: The experimental design was completely randomized design (CRD) with 5 treatments and 3 replications
Place and Duration of Study: The study was conducted at the Northern Region Marine and Fisheries Service Branch, Wanayasa Freshwater Fish Seed Development Center, Purwakarta Regency, West Java Province.
Methodology: The experimental design used a completely randomized design (CRD) with 5 treatments and 3 replications. Treatment A: Control (-) without hormones, B: positive control (+) 17 α - MT at a dose of 100 mg/L for 5 h, C: natural MT at a dose of 350 mg/L for 5 h, D: natural MT at a dose of 350 mg/L for 10 h, E: natural MT at a dose of 350 mg/L for 15 h.
Results: Tilapia larvae used were aged 6 days after hatching and reared for 40 days, then the male and SR sex were observed. The results of this study showed that the duration of immersion of tilapia larvae using natural MT significantly affected the male tilapia which resulted in the highest male percentage at 15 h of immersion of 80.2%, then followed by 10-h immersion of 75.6% with the same SR value of about 90%.
Conclusion: Administration of natural MT using cow testis meal by immersion method at different durations significantly affects male sexual development in 6-day-old tilapia larvae. Based on the obtained data analyses, the percentage of male tilapia in the 40-day experimental study shows the most effective duration in treatment E (15 h) with a measure of 350 mg/l, which is 80.2%.

Keywords: *tilapia fish larvae, percentage of males, natural methyltestosterone, monosex Culture, bovine testicle,*

1. INTRODUCTION

One fishery product that is frequently imported by the US market is tilapia. After shrimp, salmon, and tuna, tilapia is the fourth most popular fish consumed in the United States (Pearson, 2023). Tilapia is ranked as one of the top ten major products of aquaculture activities and one of the freshwater fish aquaculture with significant economic values

(Department of Marine Affairs and Fisheries of the Republic of Indonesia, 2008). The demand for tilapia on the global market is 200,000 tons annually (Wijaya, 2011). There is a growing demand for tilapia because it remains one of the most reasonably priced sources of animal protein for people of all social classes. Numerous studies are still being conducted in Indonesia, in an attempt to produce productive tilapia (Hertanto, 2013). The formation of male tilapia larvae stimulated with the hormone methyl testosterone in pellet flour is one of these initiatives (Sarker et al., 2022).

The high reproductive potential of the fish makes it difficult to regulate and frequently results in inbreeding, which is a prevalent issue in tilapia aquaculture (Mangaro et al., 2018). This causes fish to grow slowly, taking a long time to reach the size needed for consumption, and sometimes even experiencing stalled growth (Tatalede et al., 2019; Maulana et al., 2023). To boost tilapia farming output, the most effective way to do so is to produce male tilapia larvae for mono-sex production (Malik et al., 2019; Susilawati et al., 2022). This is because, during the tilapia growing process, male tilapia grow more quickly than female tilapia. Fish maleization hormone, or natural MT hormone, is required for this process, which is the stimulation of fish larvae toward males. Compared to female tilapia, male mono-sex (single-sex) farming has several benefits, such as a shorter harvest age, a greater size, and more tender meat (Suyanto in Fitzsimmons, 2004; Omasaki et al., 2016).

One procedure to get male mono-sex tilapia is through sex change procedures by administering male hormones (testosterone). The commonly utilized hormone is the manufactured hormone 17α-MT with a success rate of male sex alter that can reach 96-100% (Zairin, 2003; Mlalila et al., 2015). In addition, masculinization can also be done by controlling the environmental temperature in the form of increased temperature (Bowman et al, 2012). The higher the temperature, the higher the male-sex ratio (Tessema et al., 2006). Abou-El Fotoh et al. (2014) found that a temperature of 28^{o}C produced male tilapia by 52.33% while a temperature of 36^{o}C produced male tilapia by 81%, thus it can be concluded that temperature and sex change are directly proportional.

This study aimed to determine the effect of the duration of the natural Methyltestosterone (MT) hormone treatment on the formation of the male sex tilapia. The expected hypothesis is that immersion of larvae in natural MT hormone with different immersion durations affects the increased percentage of male sex formation in tilapia.

2. MATERIAL AND METHODS

This research was conducted for 2 months from October to December 2021 at the Northern Region Marine and Fisheries Service Branch, Wanayasa Freshwater Fish Seed Development Center, Purwakarta Regency, West Java Province.

Experimental design

The study used a completely randomized design with 5 treatments and 3 replications. Treatment group A as control (-) without hormone, B as control (+) immersed in 17α-MT hormone at a dose of 100 mg/L for 5 h, C, D, and E immersed in natural MT hormone at a dose of 350 mg/L for 5, 10, and 15 h respectively.

Experimental Set-Up

The test fish used in this study were tilapia larvae aged 6 days after hatching (weight between 0.02 - 0.03 g/larvae), the total number of larvae was 750 with a density of 50 larvae/container, and the larvae were obtained from natural spawning. The natural MT hormone used was derived from fresh cow testicles processed into cow testicle flour that

had been previously prepared. The immersion method was made by dissolving the hormone in 70% alcohol in the amount of 3 ml and then put into an immersion jar containing 1 L of water then aerated and homogenized for ± 5 min to remove the alcohol content. Tilapia larvae were immersed according to each treatment and then transferred to a rearing container in the form of a pond that was blocked using nets and fed with pellet (Hi-Pro-Vite brand, type PSP) with a frequency of 3 to 4 times/day ad libitum. After 40 days of rearing (Laheng and Widyastuti, 2019), gonad observations were made by dissecting the abdomen of the fish then the gonads were taken and given 2-3 drops of Eosin solution on a glass object and then observed using a binocular microscope.

Parameters Observed

Percentage of Male Tilapia Fish

The percentage of male fish is the percentage of the number of male fish from the total population of the test fish. Damayanti et al. (2013), stated that to calculate the percentage of male sex using the following formula.

Survival rate

Survival is the percentage of the number of fish that live at the end of the experiment from the total fish population at the beginning of the experiment. The percentage of fish survival rate can be calculated with the formula proposed by (Huisman, 1987):

Data Analysis

The data obtained were analyzed by the one-way Analysis of Variance (ANOVA) method. If the results show a real effect, it is continued with the smallest real difference test (BNT) to determine whether there are significant differences between treatment groups.

3. RESULTS AND DISCUSSION

Based on the results of a study on the masculinization of tilapia larvae by different durations of immersion using natural MT in the form of cow testicle flour. Data on male percentage and survival rate were obtained, as well as water quality data as supporting data.

Percentage of Male Fish

Based on the observation of the percentage of male tilapia with natural MT through immersion, the percentage of males is higher than the negative control treatment (-) without hormones. Data on the percentage of male tilapia in each treatment at the end of the experiment can be seen in Table 1.

Table 1. Percentage of sex of male tilapia at the end of the experiment

Replications	Treatments				
	A C (-)	B C (+)	C (5 h)	D (10 h)	E (15 h)
I	47,7	73	72,7	74,5	77,1
II	46,8	78	72,3	76,7	77,8
III	48,9	70,5	73,3	75,6	85,7
Mean	$47,8^{a}$	$73,8^{bc}$	$72,8^{bc}$	$75,6^{cd}$	$80,2^{d}$

Different letters a, b, c, and d indicate significant differences between treatments.

The table above shows a significant difference ($P < 0.05$) in the percentage value of male tilapia treated with natural MT hormone at different immersion durations. The 15-h treatment produced significantly different results than the C (-) treatment, C (+) treatment, and the 5-h treatment. However, the results were not significantly different from the 10-hour treatment. It can be seen that the sex change of tilapia fish into males happens after immersion in natural MT and is regulated by the immersion duration of tilapia larvae, with a tendency to increase the percentage of male fish produced as the immersion time increases.

Survival of tilapia

Survival of tilapia fish larvae is crucial for determining the effectiveness of treatment methods. The tilapia survival data collected at the end of this study is calculated by subtracting the number of fish at the start of the study from the number of fish that survive at the end of the study. Table 2 shows tilapia survival results for each treatment.

Table 2. Percentage of experimental fish survival at the end of the experiment

Replications	**Treatments**				
	A C (-)	**B C (+)**	**C (5 h)**	**D (10 h)**	**E (15 h)**
I	88	74	88	94	96
II	94	82	94	86	90
III	90	88	90	90	84
Mean	$90,7^{bc}$	$81,3^{a}$	$90,7^{bc}$	90^{ab}	90^{ab}

Different letters a, b, and c indicate significant differences between treatments..

From the table above, the survival rate of tilapia in each treatment using cow testicle flour at different immersion durations did not bring a significant effect ($P > 0.05$) throughout the treatment period until the end of the experiment. However, when the SR of fish treated with the natural hormone MT showed no statistically significant effect on control over hormone-free C(-) treatment, it was significantly different compared to C(+) treatment by immersing the hormone 17α-MT.

Water quality

Water quality is one of the factors that support the survival of fish, but it also affects the formation of male sex when tilapia is still in the period of sexual differentiation. Water quality parameters in this study measured during the experiment included DO, temperature, and pH. According to the results of water quality measurements during the experiment, they are still within the normal range that tilapia can tolerate, thus possibly supporting the survival rate of tilapia. Measurement data are shown in Table 3.

Table 3. Water quality of fish-rearing media during the study

Parameter Measured	Unit	Measurement Results	
		In the Morning	In the Afternoon
Oksigenterlarut	Mg/L	4,0 – 6,8	4,0 – 6,8
Suhu air	^{o}C	24 - 27	24 – 28
pH	Unit	6,3 – 7,9	6,4 – 7,9

Discussion

In this study, the percentage of male tilapia that received natural MT hormone ranged from 72.8 to 80.2%. The administration of natural MT has been shown to influence the hormonal system in the body of tilapia (Sarker et al., 2002), with each treatment increasing the percentage of different male tilapia.

According to Zairin (2003) and Mlalila et al. (2015), MT hormone administration in the sex direction by the immersion method (dipping) is more efficient because the dose given is relatively small and the contact time is shorter. However, the success rate of changing the male sex is below 96%. In this study, sex reassignment was carried out by immersion which aims to determine the best immersion durations in tilapia larvae with a dose of natural MT hormone 350 mg/L in achieving male sex reassignment optimization

The testosterone hormone in cow testicle flour is thought to increase the concentration of androgen hormones in tilapia larvae hence they become male (Huda et al., 2018; Hutagalung, 2020). Furthermore, Iskandar (2010) and Wahidah et al. (2021) explained that the provision of cow testicle flour extract containing the hormone testosterone through immersion can affect the hormonal system in the body of fish thus affecting the formation of male tilapia.

In this study, the total percentage of males in treatment C with a soaking time of 5 hours was 72%, which was smaller than treatment B (positive control) of 73.8%. It is presumed that the cause of the low percentage of male fish produced in treatment C is the presence of other factors such as the material used is still in the form of coarse flour which is not soluble in water therefore it isn't easy to be absorbed by tilapia larvae through the skin, gills, and digestive system. However, the percentage of male fish produced is still higher than in previous studies, where Ridwan (2022) reported 72.14% male fish, previously Phelps and Popma (2000) reported 65% male fish.

The success of the male sex direction through the immersion method is influenced by several factors including size, age, length of treatment, environment, fish species, genetics, hormone type, hormone dose, and treatment time (Muslim, 2011). The application of sex direction techniques is generally performed on fish that are still in the process of differentiation (Fernandino and Hattori, 2019), in this labile period, fish sex can be directed through hormone induction (Baroiller and D'Cotta, 2016). The period of gonad differentiation in tilapia occurs up to 30 min after hatching. Still, the most sensitive period for sex direction in tilapia is 7-14 days after hatching (Nivelle et al., 2019) and according to Yuniarti et al. (2007), sex differentiation in tilapia occurs when the larvae are 6-7 days after hatching until about 27-28 days after hatching.

In that study, the results of SR analysis of experimental fish were still relatively high, ranging from 81.3% to 90.7% compared to Meyer et al. (2008), 40.2% using fresh cow testicles, Yustiati et al. (2018), 86% using oven-dried cow testicles, Shalaby et al. (2007), 60-70% used the synthetic hormone 17α-MT orally, and Yustina et al. (2012), 14.66% in hippo using the synthetic hormone 17α-MT by immersion method.

The use of synthetic hormones leads to higher mortality (Ridha and Lone, 2008). However, the use of chemicals in sex change can affect the survival rate of fish because it is believed that the treated fish are subjected to physiological stress. The experimental fish are still in the larval stage and thus their physiological state is still weak, which also means that these

synthetic materials that enter the body can have a two-step effect on the antibody system, which can initially stimulate the production of antibodies, but the next effect is to suppress immune responses. Long-term administration of synthetic substances, even in low doses, can impair the ability of immune cells to reproduce (spread) (Mariani et al., 2019). Cow testicle flour, on the other hand, is a natural ingredient that does not cause physiological stress to the tilapia larvae, so the survival rate is relatively high.

In addition, cow testicle flour also contains a variety of essential and non-essential amino acids and contains saturated and unsaturated fatty acids (Odin et al., 2011). The amino acids include arginine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, and valine, while non-essentials include aspartate, alanine, glutamate, glycine, Florine, serine, cysteine, and tyrosine. These amino acids are very useful in the formation of androgenic hormones, testosterone, which plays a role in increasing libido and the formation of spermatozoa.

Based on Table 3, the water quality during the study varied from the normal tolerance range of tilapia to very low water temperature. This means that one of the factors responsible for the low percentage of male tilapia fish obtained is the water quality of the rearing environment, especially the temperature. Rohmaniah et al. (2019), the higher the water temperature, the faster the fish's metabolism accelerates, resulting in faster oxygen uptake and respiration and less dissolved oxygen in the water. Without oxygen, fish easily become stressed and even die. Temperature changes can affect the activity of the testosterone hormone; For example, low temperatures reduce the effect of the testosterone hormone, resulting in more female fish than male fish.

According to Chen et al. (2022), water temperature affects larval development stages as well as the duration of gonadal sensitivity to hormone stimulation. Water temperature has a direct impact on the body's metabolic processes, which in turn alter the function of hormones. Meanwhile, Afpriyaningrum (2016) found that tilapia larvae absorbed testosterone levels at 36^{o}C during a 4-h immersion, resulting in 92.50% of males. The optimal water pH for fish survival ranges from 6.5 to 9, and DO levels vary from 5.5 to 6.7 mg/l. Maimunah dan Kilawati (2020) recommend an optimal environment for tilapia rearing with DO 5.3-8.8 mg/l, pH 7.3-8.3, and water temperature 26-30^{o}C. Nivelle et al. (2019) recommend a water temperature of 27-30^{o}C. However, in this study, the water temperature fluctuated between 24 and 28^{o} C.

4. CONCLUSION

It can be concluded that the administration of natural MT by utilizing cow testicle flour through the immersion method at different durations has a significant effect on the formation of male sex in tilapia larvae aged 6 days. Based on the data analysis that has been obtained, the percentage of male tilapia sex during 40 days of the experimental study shows the most effective duration is in treatment E (15 h) with a dose of 350 mg/L, which is 80.2%.

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