Sex reversal using 17 α-methyltestosterone immersion and its effect on sex reversal and growth performance of Nile tilapia (*Oreochromis niloticus* L., 1758)

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ABSTRACT

The study aimed to evaluate the optimal dose of 17 α-methyltestosterone (MT) and stocking density using immersion method for maximum survival, masculinization rates and growth performance of Nile tilapia (*Oreochromis niloticus*). The experimental design employed complete randomization including three concentrations of 17 α-methyltestosterone (1.5, 2.0, and 2.5 mg/L) and three stocking densities of 500, 750, and 1000 fish/L and a control group, arranged in three replicates. The control groups consisted of 3 stocking densities of 500, 750, & 1000 fish/L and fish were kept in glass aquaria containing MT-free water. Fish were exposed to MT solution for 2 h, and the fish were then transferred to nursery in hapas in earthen ponds at a density of 1,000 fish/m^2 for 60 days. After the hormone treatment and 60 days of rearing, the highest survival rate was found in the control group. The male ratios in the MT treatments ranged from 78.9 to 91.1% and were statistically higher than that in the control (55.1%) ($P < 0.05$). The most effective doses and stocking density in sex-reversal of the tilapia fry using immersion method were 2.0 mg 17 α-MT/L and 750 fish/L, respectively. The mean weight and length of fish in the MT treatments were greater than those in the control treatment, although the difference was not statistically significant ($P > 0.05$). The results also showed that the average weight of experimental fish was directly proportional to the hormone concentration but inversely proportional to the stocking density. Based on the study findings, the recommended dose for producing maximum mono-sex male tilapia is 2.0 mg 17 α-MT /L, and the most suitable stocking density is 750 fish/L.

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1. Introduction

Tilapia is an economically important fish species that is widely cultured in the world with the second highest total global production next to carps (Ridha, 2006). The reasons are due to its fast growth rate, high tolerance to low water quality and adverse environmental conditions, efficient use of a variety of available feeds, good disease resistance, easy reproduction, and good meat quality and acceptable by different groups of consumers (El-Saidy & Gaber, 2005). To promote tilapia production, studies on nutritional requirements, farming systems, as well as post-harvest handling/storage processes, and market development have been carried out worldwide. However, the advantage of easy reproduction in captivity has become the biggest obstacle to intensive farming of tilapia on a commercial scale. Usually tilapias are very early reproductive maturity species (4 - 5 months old) at a fairly small average weight (20 - 30 g/individual) and spawning continuously every month. This leads to the increasing density of pond culture, which reduces the efficient use of feed and growth rate, thereby reducing the economic efficiency of the culture model (Varadaraj & Pandian, 1987). In addition, male tilapia has a faster growth rate than female tilapia of the same age (Hossain et al., 2022), which make a lot of fish that are smaller than commercial size when harvested, so it is difficult to apply industrial farming scale with a mixed sex of tilapia.

To avoid these undesirable obstacles in intensive culture, farmers have adopted an all-male tilapia culture (Sultana et al., 2020). There are many ways to produce the male monogamous tilapia (Hossain et al., 2022). The first method is manual sexing based on the difference in secondary sexual characteristics, external genitalia morphology, eliminating the females and keeping the males. The main disadvantages of this method are human errors and the waste of females. The second method is hybridization method including: i) using interspecific hybridization with different sex-determining chromosomal mechanisms (XY & ZW) (Lovshin, 1982); ii) using cross breeding between normal female and super male (YY) (Mair et al., 1995). According to Pruginin et al. (1975), the main advantage of the crossbreeding method is that it can produce 100% males; however the biggest drawbacks of this method are the cost and technique to maintain the purebred brood stock. The third method is sex-reversal using male sex hormones to masculinize tilapia (Le, 2008; El-Zaeem, 2013). According to Andersen et al. (2003), tilapia fry are sexually underdeveloped at the hatching time. Thus, changes in sex hormone levels in their bodies may have an impact on genetic sex during early gonadal development. Sex-reversal by feeding fish with food containing 17 α-methyltestosterone is one of the most effective and commonly used methods because of its low investment cost and short processing time (Prabu et al., 2019). Moreover, the obtained male ratio is always high and stable compared to other methods. Recently, the immersion method that exposing Tilapia fry before gonadal differentiation in water containing 17 α-methyltestosterone showed more advantages such as high male and survival rate, simplicity, short implementation time, lower MT dose and workers are not directly exposed to the hormone so it is safe for both consumers and producers. However, the MT concentration and treatment density in the immersion method have not been fully studied on Nile tilapia in Vietnam. Therefore, this study was conducted to determine the optimal dose of 17 α-methyltestosterone hormone and effective treatment density to improve the survival rate, sex conversion rate and growth performances of tilapia treated by immersion method.

2. Material and methods

Broodstock development

The brood fishes (200 - 250 g) were purchased from National Breeding Centre for Southern Freshwater Aquaculture, Research Institute for Aquaculture No. 2, Vietnam. The fish were acclimatized for 15 days and raised to reproductive maturity in 60 days in hapa (12 m x 5 m x 1.5 m) suspended in the earthen ponds (15 m x 30 m) in the Plants and Livestock Hatcheries, Agricultural Service Center of Binh Phuoc province. The ready spawning brood fishes were then paired for spawning in six matting hapas (12 m x 5 m x 1.5 m) with a ratio of 3 females: 1 male/m^2 in the earthen ponds (30 m x 50 m). The brood fishes were fed twice in a day at 3% body weight on a commercial floating pelleted feed (Brand Tilapia feed, Cargill company), with approximately 27% of crude protein.

Fry collection

After 15 days of pairing, fry were collected every seven days. The fry were collected using a soft net in the early morning to avoid stress and mortality. The fish were acclimated in the one m^3 composite tanks for one day, then removing weak/dead fish and sorting out fish by mesh size.

Preparation of hormone treated solutions

The 17 α-methyltestosterone hormone used in the present study was obtained from the Sigma Aldrich Ltd., Germany. A stock solution was made by dissolving 400 mg of hormone in 1 L of 95% ethanol to achieve the stock solution with the nominal concentration of 400 mg. Treatment concentrations were prepared by dissolving the accurate amount of the hormone from stock solution in 20 L glass tanks containing 10 L of water and with vigorously aerating to allow the alcohol to evaporate.

Experimental design

The experiment was carried out in 20 L glass tanks using completely randomized design. The experiment included three 17 α-methyltestosterone (MT) concentrations of 1.5, 2.0, & 2.5 mg/L and three stocking densities of 500, 750, & 1000 fish/L and a control in triplicates (Table 1). The control groups consisted of three stocking densities of 500, 750, & 1000 fish/L and fish were kept in glass aquaria containing MT-free water. One day after acclimatization in the composite tank that is continuously aerated, tilapia fry (0.24 g ± 0.06) were randomly stocked into the glass aquaria according to the treatments of stocking densities, MT concentrations and control experiment. Fish were exposed to MT solution for two hours, and the fry were then transferred to nursery in hapas (4 m x 2 m x 1.6 m) suspended in earthen ponds at a density of 1,000 fish/m^2 for 60 days. The fishes were fed twice a day on a commercial floating pelleted feed (Brand Tilapia feed, Cargill company)with approximately 40% of crude protein, at a feeding rate of 5% of body weight.

Sex reversal

At the end of experiment, 50 fish were randomly collected in each experimental replicate to identify the sex of the fishes. Morphology of the gonads were examined and recorded. Sexing determination was done by standard aceto-carmine gonad squashing technique and hematoxylen & eosin staining (Guerrero & Shelton, 1974).

Table 1. Treatment combination details of the experiment

Treatment	Stoking density (fish/L)	17 α-methyltestosterone concentration (mg/L)
C1	500	0
C2	750	0
C3	1000	0
T1	500	1.5
T2	750	1.5
T3	1000	1.5
T4	500	2.0
T5	750	2.0
T6	1000	2.0
T7	500	2.5
T8	750	2.5
T9	1000	2.5

Growth performance

Every 30 days, 10 fish were randomly collected in each experimental replica te to measure the mean growth (average weight and total length), then released the fish back into experimental hapa. Survival rates were recorded at 2 h after immersion of fish in MT solutions and at the end of the growth experiment (day 60).

Statistical analysis

The data were statistically analyzed by statistical package SPSS version 16.0 in which data were subjected to one-way ANOVA and Duncan's multiple range test (DMRT) was used to determine the significant differences between the means at 5% level of significance.

3. Results and Discussion

Survival

Survival rate of fish after 2 h of treatment with MT and at the end of the experiment (60 days) was presented in Figure 1.

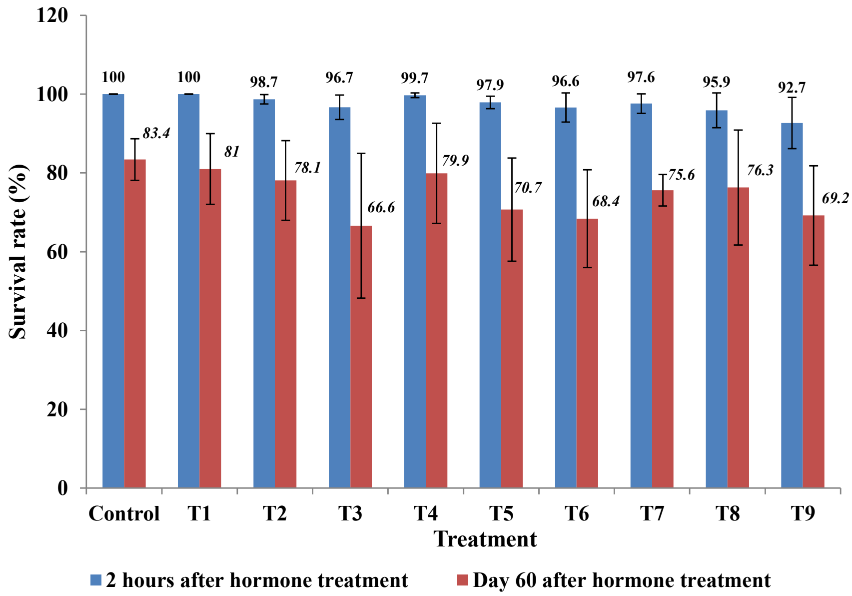

Figure 1. The survival rate of tested fish after 2 h of hormone treatment.

The results showed that the survival rate of tilapia fry after 2 h of hormone treatment ranged from 92.7 to 100%, in which the fish in the control and T1 (1.5 mg MT/L + stocking density of 500 fish/L) had the highest survival rates of 100%, followed by treatments T4 (2.0 mg MT/L + stocking density of 500 fish/L), T2 (1.5 mg MT/L + stocking density of 750 fish/L), T5 (2.0 mg MT/L + stocking density of 750 fish/L) and T7 (2.5 mg MT/L + stocking density of 500 fish/L), respectively. However, there was no statistically significant difference in survival rate among treatments ($P > 0.05$).

At the end of 60 day of nursing period, the maximum survival rate (83.4%) was found in control group, while the lowest survival rate (66.6%) was found in T3 group. Experimental results showed that the survival rates of fish in the control treatment at all experimental stages were higher than the survival rates in all MT treatments, but the difference was not statistically significant ($P > 0.05$). The result also indicated that the survival rate was inversely proportional to the MT concentrations and stocking densities. In general, the MT hormone immersing method is not significantly affect the survival rate of tilapia fry during treatment and rearing periods.

The survival rate of tilapia in this experiment is consistent to that of tilapia treated with oral administration method for 21 days of authors such as Tu (2006) (69.5 - 83.4%), Jensi et al. (2016) (87.33 - 95.00%) and Alam et al. (2023) (81.3 - 92.0%). In addition, this result is similar to the survival rate of other fish when treated with feeding methods such as Aulonocara nyassae (62.2 - 93.3%) (Karslı, 2021) and Clarias gariepinus (81.1 - 92.2%) (Alam et al., 2023).

Sex reversal

The effectiveness of sex reversal treatments is greatly depended on the amount of hormone, the duration of use, the administration method that the fish can absorb hormone during its labile period of sexual differentiation. The rate of masculinization is considered one of the important indicators to help evaluate the quality and effectiveness of the hormone used as well as the efficiency of the all-male tilapia production process. Therefore, in this study, different concentrations of MT were used to observe the effects of these different doses on sex-reversal of tilapia fry after exposed to MT in 2 h.

From the histological analysis of gonadal tissues (Figure 2), there was no intersex individuals found in all MT treatments as well as in control groups (Table 2). The control group showed the normal sex ratio of 1 male: 0.81 female. The male ratios in the MT treatments ranged from 78.9 to 91.1% and were statistically significant higher than that in the control treatment (55.1%) ($P < 0.05$). Experimental results showed that the highest rates of masculinization of 91.1%, 90.0% and 87.8% were found in treatment T8 (MT concentration of 2.5 mg/L and stocking density of 750 fish/L), treatment T7 (MT concentration of 2.5 mg/L and density 500 fish/L) and treatment T5 (MT concentration of 2.0 mg/L and density of 750 fish/L), respectively, and the difference was not statistically significant among these treatments ($P > 0.05$). It indicated that MT at concentrations of 2 and 2.5 mg/L are effective doses for the masculinization of tilapia using immersion method. The finding showed that treatment T8 (MT concentration of 2.0 mg/L and density of 750 fish/L) is the most appropriate concentration and stocking density in sex-reversal of the tilapia fry using immersion method.

The results of this experiment are consistent with the research results of tilapia sex-reversal using the MT immersion method of other authors. Wassermann & Afonso (2003) masculinized the 14 days post hatching (DPH) tilapia fry by MT immersion method with the rate of masculinization ranging from 86.0 to 91.6%; Male proportion (68.5 - 83.3%) was observed when Duong & Pham (2006) treated tilapia fry (15 - 17 DPH) with the MT immersion method. Le (2008) reported the higher sex reversal rate (88.89 - 98.89%) when exposing tilapia fry (13 - 15 DPH) to MT solution. The results of this study are also consistent with the results of sex-

Table 2. Sex ratio of *O. niloticus* in different 17 α-methyltestosterone immersion treatments and stocking density treatments

Treatment	Number of analyzed fish	Male (%)	Female (%)	Sex ratio male:female
Control	100	55.1a	44.9	1:0.81
T1	100	81.1b	18.9	1:0.23
T2	100	81.1b	18.9	1:0.23
T3	100	84.5b	15.5	1:0.18
T4	100	78.9b	21.1	1:0.27
T5	100	87.8bc	12.2	1:0.14
T6	100	80.0b	20.0	1:0.25
T7	100	90.0bc	10.0	1:0.11
T8	100	91.1c	8.9	1:0.10
T9	100	80.0b	20.0	1:0.25

Values (mean ± standard deviation of data for triplicate groups) with different superscripts in the same column are significantly different (one-way ANOVA and Tukey test, $P < 0.05$).

reversal studies using feeding method. Jensi et al. (2016) reported that the male populations (83.3 to 93.3%) were obtained when feed new hatching tilapia fry with feed containing MT at doses of 50, 60 and 100 mg/kg feed in 21 days. The results show that the masculinization rate in this study is slightly lower than that of male proportions of other studies. However, the optimal MT concentration in this study was 2.0 mg/L, which was 33.33% lower than the dose used by Wassermann & Afonso (2003) and 4.17 times lower than that used by Duong & Pham (2006). In addition, the optimal stocking density of this study was 750 fish/L, which was much higher than the stocking densities in Wassermann & Afonso (2003) and Le (2008) studies of 60 fish/L, and Karaket et al. (2023) study of 100 fish/L.

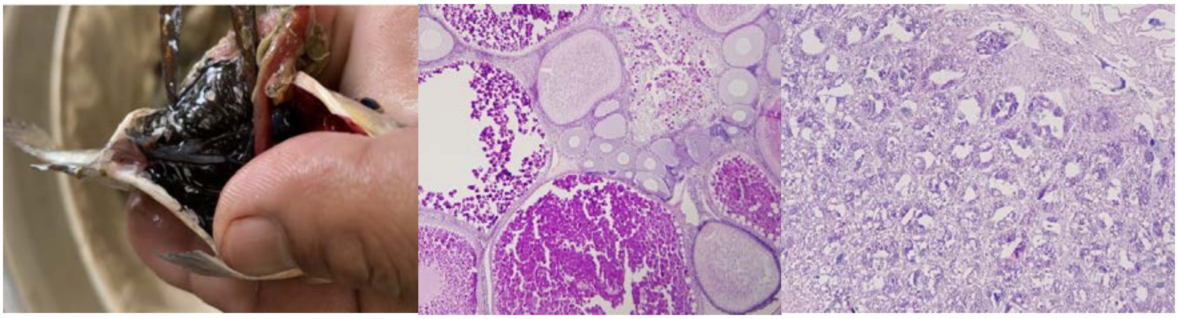

Figure 2. The observed gonad of male and female tilapia after 2 h of 17 α-methyltestosterone treatment. (A) Gonad removal (B) Eggs, and (C) Spermatozoa under light microscope.

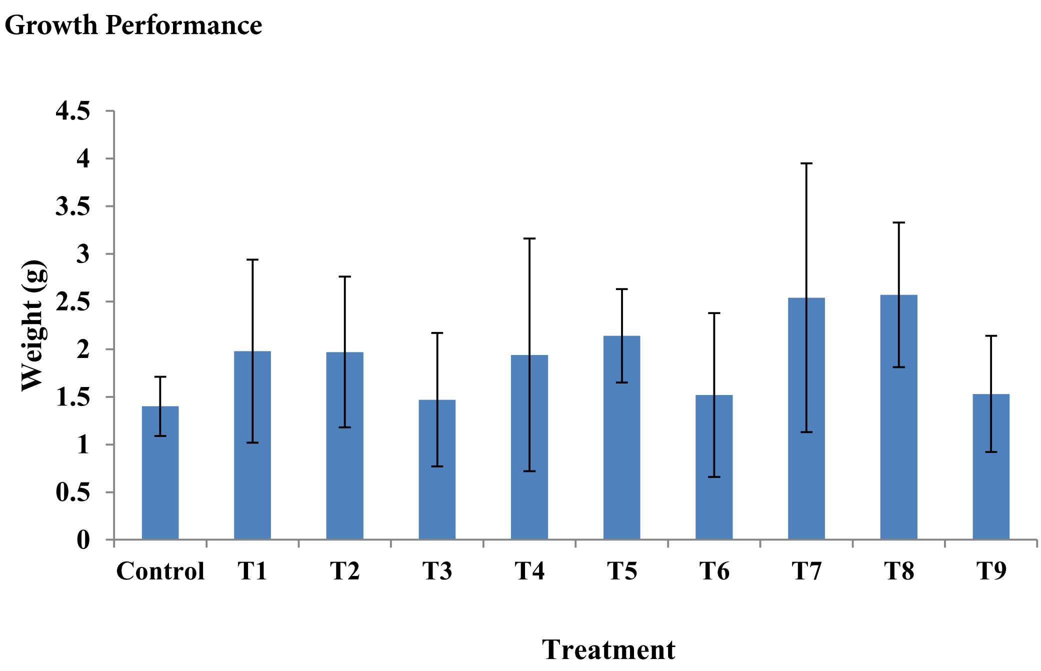

Figure 3. Effects of hormone sex-reversal on the weight of *O. niloticus*.

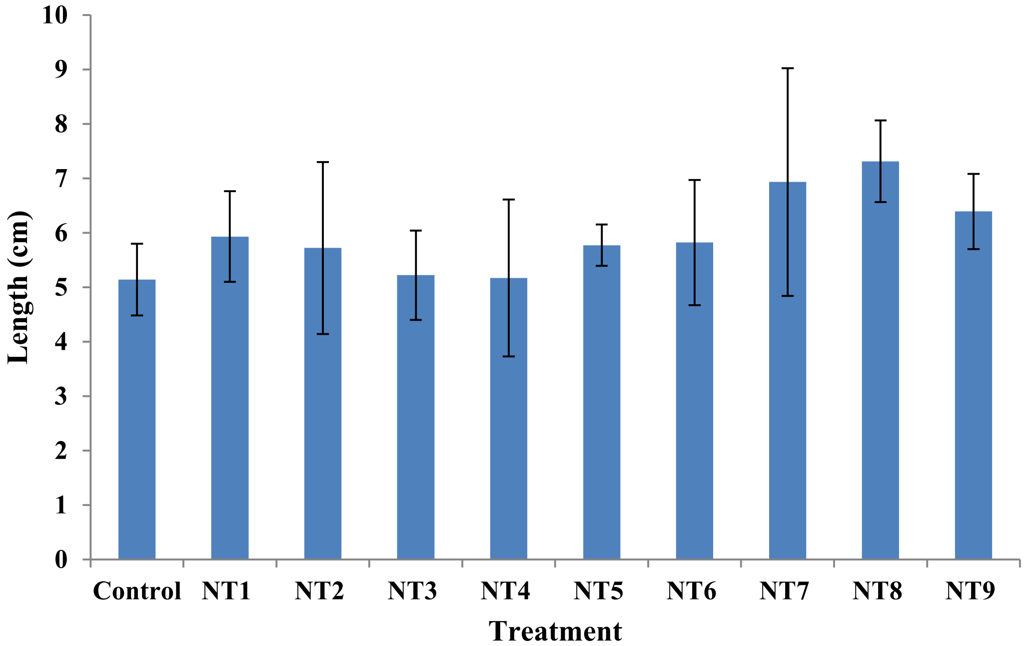

Figure 4. Effects of hormone sex-reversal on the length of *O. niloticus*.

Experimental results indicated that the average weight and total length of tested fish ranged from 1.40 to 2.57 g and from 5.14 to 7.13 cm, respectively (Figures 3 & 4). The mean weight and length of fish in the MT treatments were greater than that of the fish in the control treatment, although the difference was not statistically significant ($P > 0.05$). The results showed that the mean growth of experimental fish was not affected by hormonal masculinization over a period of 60 days after exposure to MT. In addition, the study results also showed that the average weight of experimental fish was directly proportional to the hormone concentration but inversely proportional to the stocking density.

4. Conclusions

According to the research findings, tilapia sex reversal using 17 α-methyltestosterone immersion method gives the same rate of masculinization as the feeding method, but the processing time is much shorter, only 2h compared to 21 days of oral administration method. The most effective 17 α-methyltestosterone concentration in tilapia sex-reversal of the immersion method is 2 mg/L and the most appropriate stocking density is 750 fish/L. In addition, the mean growth of experimental fish was not affected by hormonal masculinization over a period of 60 days after exposure to 17 α-methyltestosterone.

Conflict of interest

The authors have no conflicts of interest to declare.

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