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DOSE OPTIMIZATION OF 17 A-METHYLTESTOSTERONE TO MAXIMIZE SEX REVERSAL OF NILE TILAPIA (*Oreochromis niloticus*)

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ABSTRACT

The study investigated the optimum dose of 17 α-methyltestosterone (17α–MT) for maximum sex reversal and growth performance of Nile tilapia *(Oreochromis niloticus).* Using a completely randomized design, three treatments (Control, T-1, T-2, and T-3) in triplicates were set up. The control group diet was devoid of 17α–MT. The remaining three groups, T-1, T-2, and T-3, were fed feed that contained 30, 60, and 100 mg kg^{-1} of 17α–MT, respectively. Fry of three days old were stocked at a rate of 100 per tank. They were fed with experimental diet for 28 days. After the hormone treatment and 90 days of rearing the highest survival rate was found in the control group. Gonadal squashing of *O. niloticus* showed different sex ratios for different treatments. The control group showed a normal sex ratio of 48.33% males and 51.67% females. The experimental group T-3 showed the highest percentage of males (98.33%) followed by T-2 (93.33%) and T-1 (76.66%). After 90 days of rearing, although the study found the highest mean length (82.12±1.5 mm), weight (26.74±0.08 g) and SGR (9.17±0.05%) in the treatment T2, these growth performance parameters did not differ significantly with the result of treatment T3. The Nile tilapia's sex ratio has been found to be more affected by a higher dose, or 100 mg 17α–MT kg^{-1} of feed. With the increase of hormone inclusion level, sex reversal percentage increases. Higher doses of 17α–MT have also a positive impact on Nile tilapia growth performance. Growth increases with the increasing 17α–MT level up to 60 mg in the diet of *O. niloticus*. Based on the study, the recommended dose for producing maximum mono-sex male tilapia in commercial hatcheries may be 100 mg 17α–MT kg^{-1} feed.

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INTRODUCTION

Tilapia is the second leading aquatic crop globally next to carps in production and is likely to be the most important cultured fish in the 21st century (Ridha, 2006). Rapid growth, high tolerance to low water quality, efficient food conversion, resistance to disease, ease of spawning and good consumer acceptance make tilapia a suitable fish for culture (El-Saidy and Gaber, 2005). Although several species of tilapia are cultured commercially, research on nutrition and culture systems, along with market development and processing including value addition in Nile tilapia made the species as the predominant cultured species worldwide. The Genetically Improved Farmed Tilapia (GIFT) strain developed by the World Fish Center is one of the more successfully introduced farmed Nile tilapia *O. niloticus* that grew up to 60% faster than their relatives (Eknath and Acosta, 1998). However, one of the main problems of Nile tilapia culture is their early maturation (4-5 months old) as they maturate at 20- 30 g and ability to breed every month. This result in successive spawning during the growing season and hence in unwanted reproduction that usually leads to crowded condition in the ponds and consequently reduces growth (Varadaraj and Pandian, 1987). Another problem associated with a mixed sex of tilapia is the sizes of the fish at harvest, varying from small to large due to the faster growth of males. This makes it more difficult to establish uniformity of product.

In order to avoid unwanted spawning in a production unit, all-male populations are preferred (Sultana, et al., 2020). Control of reproduction in tilapia is possible through monosex culture, which may be achieved by various method including manual sexing, hybridization and hormonal sex reversal to produce all male tilapia population (El-Zaeem and Salaam, 2013; Martinez et al., 2014). The first method is culling through a population, discarding the females and keeping the males. The major drawbacks of the first method are human error in sexing and of course the wasting of females (Guerrero, 1982). The main disadvantage of hybridization method is the difficulty in maintaining pure parental stock that consistently produce 100 % male offspring (Pruginin, et al., 1975), as well as the poor spawning success (Lee, 1979). The more common method of generating mostly male populations is through the use of different androgen hormones fed to sexually undifferentiated fry (Phelps, et al., 1995). The most efficient and least expensive method is sex reversal with the use of synthetic steroid 17 α-methyltestosterone (17α-MT).

Tilapia fry are sexually underdeveloped at the time of hatching. Changes in sex hormone levels in their body can therefore have an impact on the genetic sex during the early stages of gonadal development (Andersen et al., 2003). During their first feeding, fry are given male hormones or androgens (17α-MT), which cause the development of male testicles. The involvement of steroid hormones in controlling the gonad differentiation process seems promising (Piferrer, 2000). Hormones are generally included in the diets for several weeks when the fish start eating. However, this technology is recognized as a turning point in aquaculture worldwide. Some researchers recommend 17α-MT into starter fish feed at the dose of 60 mg kg^{-1} feed to produce all male population in tilapia (Popma and Green, 1990). Although the administration of 60 mg 17α-MT kg^{-1} feed dose regularly produces a population with less than 10% females (i.e. >90% males), this has not been shown to be the optimum dose. In this respect, other researchers reported sex reversal in tilapia at the doses lower than 60 mg MT kg^{-1} feed (Varadaraj and Pandian, 1989). The findings from some of these researches are inconsistent, making it difficult to distinguish between treatment and environmental effects. However, based on the previous data, a completely (100%) all-male population has not been observed by using 17α-MT. The lower sex reversal hampers the monosex culture of tilapia. Therefore, it is necessary to determine the ideal MT dosage for consistently 100% sex reversal to male tilapia population. Thus, the present study was conducted with a view to assessing the optimum dose of 17α-MT hormone to produce 100% male tilapia and effects of higher doses on the growth performance.

MATERIAL AND METHODS

Collection of tilapia fry

The experiment was carried out in the hatchery of the department of Genetics and Fish Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur. Just hatched fry of mixed-sex Nile tilapia (*Oreochromis niloticus)* were collected from Biswas fish hatchery, Trishal, Mymensingh. Fry were kept in an indoor plastic tank for one day for acclimatization, after which they were distributed randomly into 120-L glass aquaria (60 x 40 x 50 cm, L x W x H) at a density of 100 fry/aquarium. The average length and weight of fry were 7.5 mm and 5.0 mg respectively. Each aquarium received well aerated tap water. Aquaria were supplied with compressed air via air-stones from air pumps. The temperature was adjusted at 28 ±2 ºC, with a photoperiod of 14 h light and 10 h darkness.

Preparation of hormone treated feed

Four diets with different doses of 17α-methyltestosterone (MT) hormone i.e., 0, 30, 60 and 100 mg MT kg^{-1} diet were prepared through ethanol evaporation method (Mair and Santiago, 1994). The MT hormone used in the present study was obtained from the (Sigma Aldrich Ltd., Germany). A stock solution was made by dissolving 1 g of hormone in 1 L of 95% ethanol. Treatments were made by taking the accurate amount of the hormone from stock solution and brought up to 100 ml by addition 95% ethanol. This solution was evenly sprayed over 1 kg of the diet and mixed thoroughly. The mixture was mixed again and this was repeated to ensure an equal distribution of the MT hormone throughout the feed. In case of control, required amount of feed was prepared by mixing ethanol only. Treated diets were fan dried in shade at 25 ºC for 24 hours. The prepared feeds were preserved in a refrigerator at 4°C.

Dietary treatment

Twelve glass aquaria were randomly allocated into four treatments (T-0, T-1, T-2 and T-3) each with three replications using completely randomized design (CRD). The fries were divided into four groups (T-0, T-1, T-2 and T-3). The control T-0 group diet was devoid of 17α–MT hormone. The remaining groups T-1, T-2, and T-3 were fed with feed containing 30, 60 and 100 mg kg^{-1} of 17α–MT. Triplicate groups of 100 fry/aquarium were assigned to each treatment. Three days old mixed sex fry of Nile tilapia were fed diets four times a day at the rate of 15 % of the total fish biomass. The experiment lasted 28 days. At the end of the experimental period, sex reversal and survival rate were calculated. Semi-dynamic method for removal of excreta was used every 3 days by siphoning a portion of water from the aquarium and replacing it by an equal volume of water.

Sexing of fish

After dietary treatment, sexing of randomly selected juveniles was done by the standard aceto-carmine gonad squashing technique (Guerrero and Shelton, 1974). At sexual maturity, fish was sexed by observing the genital papillae.

Growth performance

After hormone treatment the remaining fishes were transferred from glass aquaria to hapa (5 x 5 x 5 ft, L x W x H) fixed in a pond. Then the fishes were fed with floating pellet feed for 3 months. At the end of the rearing period (90 days), fish were starved for a day, and the final length, weight and specific growth rate (SGR) were measured individually to assess growth performance. The following formula was used to determine the SGR.

Specific growth rate (% day),

$$SGR\ (\%/day) = (\log_e W_2 - \log_e W_1)/T_2 - T_1 \times 100$$

Where,

W1 = the initial live body weight (g) at time T_1 (day)

W2 = the final live body weight (g) at time T_2 (day)

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.

RESULTS AND DISCUSSION

Survival

After 28 days of hormone treatment the survival rate of fishes was calculated for different doses of hormone and the results were presented in Figure 1. Maximum survival rate (92.00%) was obtained in the control group (T-0) during the hormone treatment period. In the treatment T-1, T-2 and T-3, the survival rates were 90.34%, 91.75%, and 88.83% respectively. Then, the different groups of fishes were transferred from all glass aquaria into hapa. At the end of three months of study period, the survival of *O. niloticus* was evaluated for the treated groups and control (Figure 1). Maximum survival rate (86.50%) was observed in the treatment T-0 (control group) whereas minimum survival rate of 81.30 % was observed in T-3 where the concentration of the MT hormone was high. Treatment T-1 and T-2 showed 83.55% and 84.50 % survival rate respectively. However, the higher survival rate was found in the control group than the all the hormone treated groups.

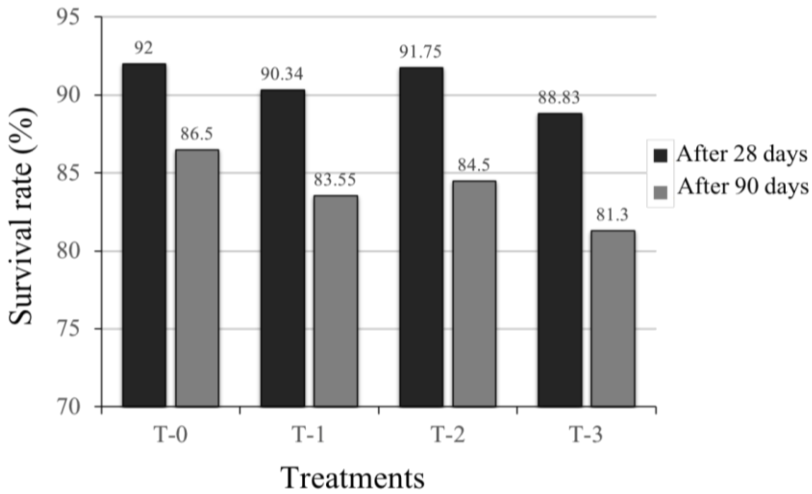

Figure 1. Mean survival rate of *O. niloticus* subjected to oral administration with different doses of 17 α-MT hormone for 28 days and 90 days.

In the present study, after 28 days of hormone treatment and 90 days of rearing the highest survival rate was found in the control group. Celik et al. (2011) reported similarly the highest survival rate (81.6%) in the control group although that rate was a little bit smaller than our result (92.00%). Soto (1992) and Vera-Cruz and Mair (1994) observed the contradictory results that MT administration has no significant effects on survival in the related tilapia species *O. niloticus*. Guerrero (1975) also observed that hormone treatment has no effect on survival in *O. aureus*. Beaven and Muposhi (2012) found a significantly higher survival rate (89.08%) in the individuals that were treated with MT hormone diet compared to the non-MT hormone-treated diet (77%) over a three-month of study period.

Sex reversal

The effectiveness of sex reversal treatments is greatly influenced by the amount of hormone that each individual actually consumes during its labile period of sexual differentiation. Therefore, in the current investigation, several dosages of feed containing 17a-MT were used to observe the effects of these various doses over the first 28 days of feeding. In the current study, 17α-MT was effective in producing phenotypic male of tilapia. The sexing of tilapia through aceto-carmine gonad squashing method revealed that intersex population was not found in all the treatments. The control group showed a normal sex ratio of 48.33% males and 51.67% females. The experimental group T-3 showed the highest percentage of males (98.33%) followed by T-2 (93.33%) and T-1 (76.66%). Lowest percentage of females (1.67%) were observed in T-3, followed by T-2 (6.67 %) and T-1(23.33%). Sex reversal data showed that in all the 17a-MT treated groups, the percentage of male tilapia produced was statistically highly significant (P< 0.01) with respect to the control group (Table 1). The present results support the findings of Ferdous et al. (2011) who reported significantly higher male population of Nile tilapia in hormone-treated groups than the control group.

Table 1. Sex ratio of *O. nilotcus* at different treatments. Fish were sexed by aceto-carmine gonad squashing method.

Treatment	Number of analyzed fish	Male (%)	Female (%)	Sex ratio M:F
T-0 (0 mg MT/kg diet)	60	48.33[a]	51.67 [a]	1:1.07
T-1 (30 mg MT/kg diet)	60	76.66 [b]	23.33 [b]	1:0.30
T-2 (60 mg MT/kg diet)	60	93.33 [c]	6.67 [c]	1:0.07
T-3 (100 mg MT/kg diet)	60	98.33 [d]	1.67 [d]	1:0.02

The highest mean percentage of sex reversal was observed in treatment T-$_3$ (100 mg MT kg^{-1} diet) which is contradictory to the results of Marjani et al. (2009) who reported lower male population (79.38%) for the same dose 100 mg MT kg^{-1} diet in *O. mossambicus*. whereas the highest male percentage (98.09%) was observed in 75 mg MT kg^{-1} diet. Similarly, Jensi et al (2016) reported the lower sex reversal rate (90.0%) at 100 mg MT kg^{-1} diet compared to 60 MT kg^{-1} diet (93.3%). Vera–Cruz and Mair (1994), Smith and Phelps (2001) and Okoko (1996) found higher sex reversal using 60 mg MT kg^{-1} diet of hormone. Interestingly we found somewhat different results compared to the maximum previous findings. Again, the percentage of sex reversal increases with the increase of hormone inclusion that is supported by the findings of Apenuor et al. (2021) who found maximum male population at the dose of 120 mg MT kg^{-1} diet compared to lower doses (30, 60 mg MT). However, hormone treatment could be considered for the masculinization of tilapia fry. Although, in the present study T-2 (60 mg MT/kg diet) was found as an effective dose for the masculinization of Nile tilapia in the hatchery, T-3 (100 mg MT/kg diet) was found as more effective in case of sex-reversal of the tilapia fry.

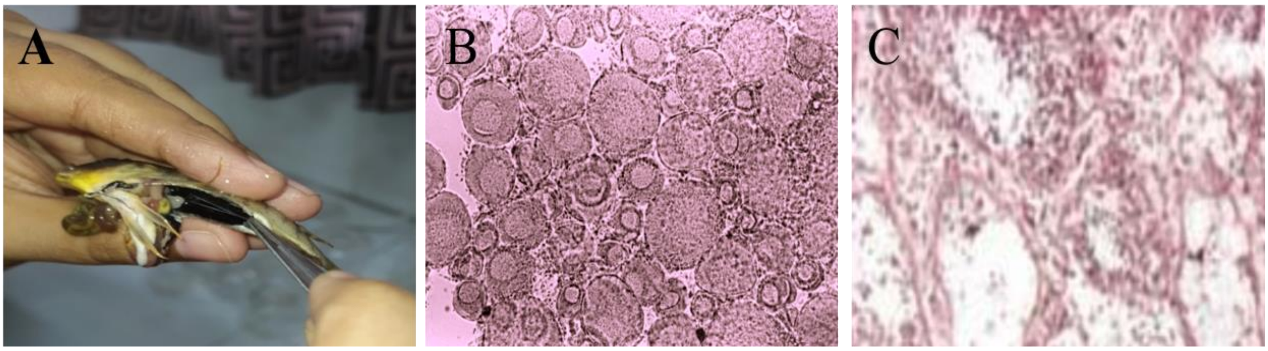

Figure 2. The observed gonad of male and female tilapia after 28 days of MT treatment. (A) Gonad removal (B) Eggs, and (C) Spermatozoa under light microscope.

Growth Performance

Among hormonal masculinization treatments, significant differences in growth parameters ($p<0.05$) were observed especially for the final length, final weight and specific growth rate (SGR) after 90 days of rearing. The different growth parameters of *O. niloticus* fed diets with different doses of 17 α-MT hormone are shown in Figure 2,3 and 4. The mean final length of fish in different treatments T-0, T-1, T-2 and T-3 were 59.43± 1.2, 64.32± 1.3, 82.12± 1.5 and 81.15 ± 2.1 mm respectively (Figure 3) whereas the mean final weight of each fish in those treatments were 18.26± 0.09, 23.31± 0.6, 26.74± 0.08 and 25.42 ± 0.07 g respectively (Figure 4). The increasing MT hormone inclusion level affected the SGR of tilapia. As the hormone increases, an increasing growth rate pattern was observed. The overall mean SGR was found to be higher in MT hormone-based treatments T-1 (9.03±0.08%), T-2 (9.17±0.05%) and T-3(9.12 ±0.07) compared to the control treatment T-0 (8.79±0.03%). Although, the study found the highest mean length (82.12± 1.5 mm), weight (26.74± 0.08 g) and SGR (9.17±0.05%) in the treatment T_2, these growth performance values did not differ significantly with the treatment T3.

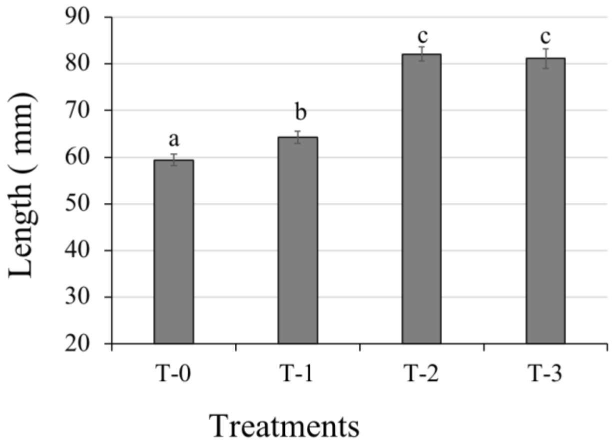

Figure 3. Effects of hormonal masculinization on the length of *O. niloticus.*

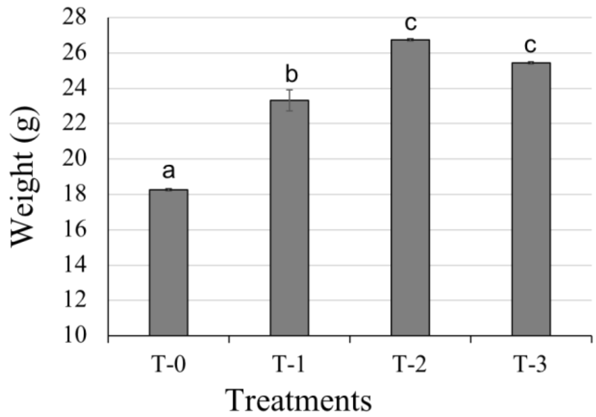

Figure 4. Effects of hormonal masculinization on the weight of *O. niloticus.*

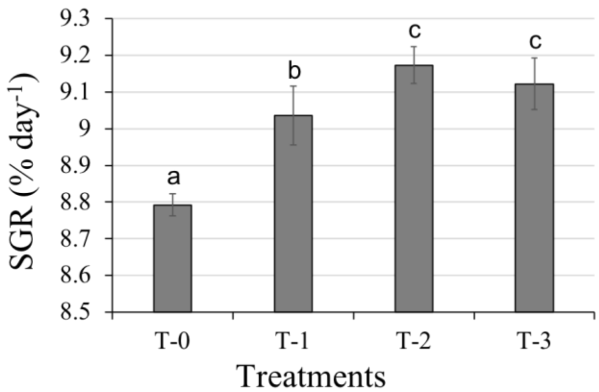

Figure 5. Effects of hormonal masculinization on the SGR of *O. niloticus.*

However, growth parameters were significantly higher in hormone treated fishes compared to the control. These results showed that the different growth parameters were significantly affected by the different doses of MT hormone ($P<0.05$) indicating the inclusion of MT hormone in fish diet is beneficial for fish growth. The increase in fish growth may be occurred due to the MT hormone that may induce the feed digestion and absorption rate causing increase in body weight (Yamazaki, 1976), or MT administration may increase the proteolytic activity of the gut as the case in mirror carp leading to increase the growth rate (Lone and Matty, 1981). The results of the present study were also more or less similar to the findings of Khouraiba (1997) who found that the hormones significantly ($P<0.05$) increased the final weight of fish Nile tilapia as compare to untreated fish.

CONCLUSIONS

According to the study's findings, Nile tilapia's sex reversal and growth performances are positively and significantly influenced by 17-methyltestosterone. The research has shown that feeding Nile tilapia 60 mg MT kg^{-1} feed may help produce monosex populations of the fish (*Oreochromis niloticus*). Additionally, a greater dose, or 100 mg/kg, has been found to have more of an impact on the Nile tilapia's sex ratio. Sex reversal percentage rises along with hormone inclusion level. The growth performance of Nile tilapia is likewise enhanced by higher doses of 17-MT up to a certain inclusion level. This study demonstrated that androgen 17-methyltestosterone can be utilized in feed to produce all male population in *O. niloticus*, however higher dosage will produce higher percentage male population. Hence, 100 mg 17 α-MT $kg^{-1}$of feed is advised for the confirmation of all tilapia feminization. However, the results of this investigation will help enhance Nile tilapia production increasing the sex reversal percentage of *O. niloticus* populations in the hatchery.

COMPETING INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper

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