Bangladesh Journal of Veterinary and Animal Sciences
Journal home page: www.bjvas.com
pISSN 2227-6416
eISSN 2709-2542

Research article

Effect of hormonal masculinization on growth performance of tilapia (*Oreochromis niloticus*)

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A R T I C L E I N F O

Article history:

Received: 07/05/2021

Accepted: 28/06/2021

Keywords:

Nile Tilapia (*Oreochromis niloticus*), Growth Performances, Sex Reverse, 17α-methyltestosterone.

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A B S T R A C T

The present study was conducted in the Meridian hatchery, Sonagazi, Feni, to evaluate the effects of 17α-methyltestosterone hormone on the growth performance of Nile Tilapia *Oreochromis niloticus*). Brood fishes were collected from NAMSAI farm, Thailand and BFRI, Bangladesh and then stocked in the breeding hapa. A total of 1600 brood fishes were used in 10 hapas where stocking density was maintained at 5 fishes/ sq. m and sex ratio was maintained at 3:1. Eggs were collected from the fish mouth, disinfected and finally placed on the hatching trays and jars. The hatched larvae were placed in the 12 SRT tank and different doses of 17α-methyltestosterone hormone applied. The experimental treatments were T_0 (Control, without MT), T_1 (50 mg MT/kg), T_2 (60 mg MT/kg), T_3 (80 mg MT/Kg) and each treatment had three replications. The study showed significantly higher growth performance in the T_2 treatment. The results showed that the mean weight of fish was significantly higher under T_2 (533.5±1.249 g), mean length was under T_2 (29.97±0.153 cm) and SGR was under T_2 (4.259±0.0013) in comparison with the other three treatments. The highest survival rate 87.6% was obtained in T_2. The highest value of FCR (1.8) was observed in T_1 and the lowest (1.2) was in both T_2, T_3, respectively. The highest sex reverse percentage was found in T_2 (98%) followed by T_3 (97.883%) and T_1 (77.833%). There was a strong interaction between 17α-methyltestosterone hormone and growth performance and sex reversal of Nile Tilapia ($p < 0.05$, n=4). This study suggests that the use of 17α-methyltestosterone hormone can improve the growth performance of Nile Tilapia.

To cite this paper: *Hasanuzzaman, M. D. Hossain, S. Rana, D. A. Khanom and S. A. A. Nahid, 2021. Effect of hormonal masculinization on growth performance of tilapia (Oreochromis niloticus). Bangladesh Journal of Veterinary and Animal Sciences, 9(1):52-58.*

1. INTRODUCTION

Nile tilapia (*Oreochromis niloticus*) is a native fish species of Egypt that has become popular worldwide mainly as a valuable fish, easy to breed, and grows in various aquaculture systems (El-Sayed, 2006). In Bangladesh, UNICEF introduced Nile Tilapia (*Oreochromis niloticus*) in 1974, but this was not flourishing due to a lack of fruitful research. Then it was further introduced in Bangladesh at 1987 by BFRI from Thailand (Gupta et al., 1992). Since then, development agencies and scientists have developed technologies for better cultural techniques at the farm level. Tilapia is likely to be the most important of all aquaculture fish in the 21st century.

Tilapia has specific favorable characteristics, like tolerance to adverse environmental conditions, can survive at low dissolved O_2, euryhaline, relatively fast growth, and efficient food conversion. These characteristics make tilapia one of the best choices for farmers (Yi et al., 1996; Penna-Mendoza et al., 2005). Despite having many good characteristics, one of the main impediments in tilapia production at a commercial scale is its precocious reproduction. It attains sexual maturity early and reproduces after every 4-6 weeks in the pond. The mono-sex culture technique can be used to control this unwanted reproduction of tilapia by culturing all-male tilapia in the pond. Tilapia has sexual growth dimorphism in which males grow faster and have more standard size than females (Muir and Little, 1991).

There are four strategies for mono-sex male culture i.e., the manual process by visual examination; hybridization; gene manipulation; and masculinization via steroid hormone. At the time of hatching, tilapia fry is sexually undeveloped. Hence, during the early period of gonad differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen et al., 2003). One of the most common techniques for producing mono-sex populations is steroid-induced sex inversion. This involves the administration of synthetic androgens or estrogens for differentiating the fry. The production of mono-sex tilapia with 17α-methyltestosterone (MT) is well established and could be incorporated in starter feed with different doses of MT hormone (Popma and Green, 1990). The present study is aimed to evaluate the effects of different doses of 17α-methyltestosterone hormone in masculinization and growth performance of Nile Tilapia.

2. MATERIALS AND METHODS

Study area

The experiment was conducted on Nile Tilapia (*Oreochromis niloticus*) in the Meridian hatchery which was located at Sonagazi upazila in Feni district for 12 months. The study area's coordinates were obtained using 'Google Maps' software, and a map was generated by 'Arc-GIS' software (Figure 1).

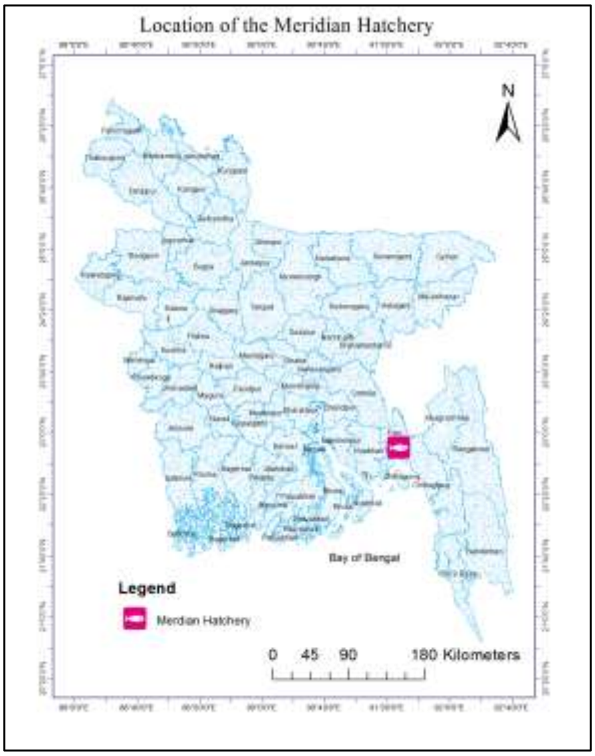

Figure 1. Study area (Meridian Hatchery)

Preparation of the brood hapa and collection, stocking, and feeding of the brood fishes

Brood fishes were collected from Bangladesh Fisheries Research Institute (BFRI), Mymensigh and NAMSAI farm, Thailand. A total of 10 hapas of 32 sq. m. (8 m x 4 m) was used for the breeding purpose and water height was maintained 1 meter for each hapa. A total of 1600 brood fishes were used in the 10 hapas and stocking density was maintained at 5 fishes/ sq. m and sex ratio was maintained 3:1 (120 females:40 male). Feeding was done in relation to the 1% per day of the bodyweight of the brood fishes and feeding frequency was twice/day. The protein content of the feed was maintained at 30% in the brood fish feed.

Egg collection, disinfection and incubation

After 3 weeks of stocking, eggs were collected from the mouths of the brood fishes and classified into various categories. The eggs were collected and washed in clean water to remove any foreign materials. The eggs were formalin-fixed (2 ml formalin/liter solution) for 2-3 minutes before being washed with clean water. Finally, the eggs were disinfected by keeping them in a 0.9% NaCl solution. The eggs were incubated using both tray and jar methods. After the disinfection, eggs were moved to the tray and jar, and sufficient water flow was maintained for hatching. During the incubation period (5-7 days) continuous aeration was ensured for proper hatching.

Transfer of swim up fry to SRT (Sex Reversed Tilapia) hapa

Swim-up fry were moved to SRT hapa for MT feed treatment after hatching. A total of 12 hapas were used, each with an area of 18 sq. m. (6 m x 3 m). Nine of the 12 hapas were used for hormonal treatment, while three were used to apply a control treatment (normal feeding). All of the hapas were placed in the 40 decimal ponds. Swim-up fry stocking density was 3 thousand per hapa. The average weight of the swim-up fry was 0.0117 g.

Preparation of MT hormone treated feed

A stock solution was prepared by using 5 gm 17α-methyltestosterone with 10 litre 95% ethyl alcohol. The solution was preserved at 4^oC temperature in the refrigerator. Then, to prepared MT mixed feed for experimentation, 50 mg, 60 mg and 80 mg of MT and 120 ml solution and 120 ml additional alcohol (for rinsing and better distribution) were mixed with 1 kg feed respectively. The mixture of the feed had been completely dried at room temperature or sunlight of the early morning and then sealed in air-tight black container and stored in refrigerators until use to retard bacterial or fungal contamination.

Sex-reversed protocol and treatments of the experiment

To measure sex reversal percentages and the impact of MT on growth efficiency, three separate doses of MT hormone were used as treatments (T_1 = 50 mg MT/kg hormone, T_2 = 60 mg MT/kg hormone, T_3 = 80 mg MT/kg hormone). T_0 was the control treatment (without MT hormone). Nine (9) of the 12 SRT hapas were used for hormone treatment (Three for each treatment). Twenty-eight (28) days of hormonal treatment (MT feed feeding protocol) was maintained for the sex reversal in the sex reversal tank.

Fry grading and transfer to nursery and grow out pond

The fry were graded using a plastic net (mesh size: 4 mm). The average weight of the fry was 0.25 g/fry. The graded 2800 fry were then moved to each nursery hapa. Each hapa was 40.5 sq. m (9 m x 4.5 m) and water height was maintained at 1.5-2 m for each hapa. A total 12 hapas were used for the experiment; 9 for hormone-treated fry and 3 for control feed treated fry. Feed was given according to the 20% body wt. and 32% protein ratio was maintained during feed formulation. After a month of rearing, the fry were moved to the grow-out pond. Water quality was periodically monitored during the whole experimentation period.

Sampling and evaluation of the growth performance and survival rate

Sampling was done every 15 days interval in the nursery and grow-out pond. Weight and length were measured and data were recorded. To evaluate the growth performance of tilapia under different hormone treatments following growth parameters were calculated based on the collected data.

Mean weight (g)

$$= \frac{\text{Sum of final weight value of each treatment}}{\text{No of replications of each treatment}}$$

Mean length (cm)

$$= \frac{\text{Sum of final length value of each treatment}}{\text{No of replications of each treatment}}$$

Weight gain (g) = Final body weight – Initial body weight

Length gain (cm) = Final length of the fish body – Initial length of the fish body

$$\text{Survival rate (\%)} = \frac{\text{Final no. of live fishes}}{\text{Initial no. of fishes}} \text{x } 100$$

Specific Growth Rate (SGR)

$$= \frac{\ln(\text{final weight}) - \ln(\text{initial weight})}{\text{duration in days}} * 100$$

$$\text{Condition Factor (CF)} = \frac{\text{Weight of fish}}{(\text{Length of fish})\hat{}3} * 100$$

Specifying of sex rates

To specify sex rates, aceto-cramine solution was used. 1 gm carmine powder was mixed with 100 ml glacial acetic acid (45%) to prepare 1% aceto-cramine solution. After dissection of the fish, gonads were taken carefully in the glass slides and 1 drop of aceto-cramine solution was added. Then the slide was placed under the microscope and gonad was observed. Web-like structured gonad was identified as female fish, and finely grainy gonad as male fish. Determination of the sex was carried out in each sampling.

Statistical Analysis

The one-way analysis of variance (One way ANOVA) was performed using SPSS (Statistic Package for social science) version IBM SPSS Statistics 23 software to determine the significant differences among means. For all tests, a criterion of $P<0.05$ was used to determine statistical significance.

3. RESULTS

Growth performance and sex reversal of Nile Tilapia

Growth parameters were significantly higher in the T_2 treatment that was shown in Table 1. Among hormonal masculinization treatments, significant differences ($p<0.05$) were observed for final weight, weight gain, final length, SGR, and CF. The mean weight of each treatment such as T_0, T_1, T_2 and T_3 were 481.57±1.19, 505.0±1.58, 533.5±1.24 and 530.36±1.09 g (Figure 2) and mean weight gain were 481.31±1.19, 504.75±1.58, 533.25±1.24 and 530.11±1.09 g (Figure 3), respectively. The mean length of each treatment such as T_0, T_1, T_2 and T_3 were 29.03±0.05, 29.3±0.15, 29.97±0.15 and 29.67±0.05 cm (Figure 4) respectively. The study found the highest mean SGR (4.25±0.00) and CF (2.03±0.00) in the T_2 treatment (Figure 5 and 6).

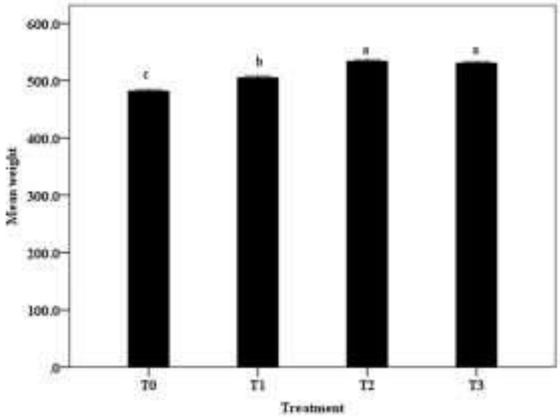

Figure 2. Effects of hormonal masculinization on fish weight

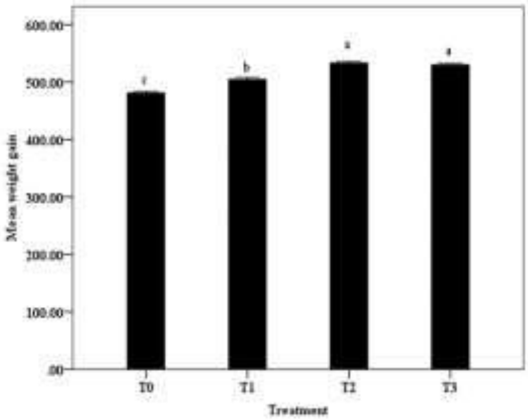

Figure 3. Effects of hormonal masculinization on fish weight gain

Table 1. Growth performance of tilapia

	T_0	T_1	T_2	T_3	Level of Significance
Mean weight (g)	481.57±1.19^c (478.61-484.53)	505.0±1.58^b (501.06-508.94)	533.5±1.24^a (530.39-536.61)	530.37±1.09^a (527.64-533.09)	0.000
Mean weight gain (g)	481.31±1.19^c (478.35-484.28)	504.75±1.58^b (500.81-508.69)	533.25±1.24^a (530.15-536.35)	530.11±1.09^a (527.39-532.84)	0.000
Mean Length (cm)	29.03±0.05^d (28.89-29.18)	29.3±0.15^c (29.05-29.55)	29.97±0.15^a (29.59-30.346)	29.67±0.05^b (29.52-29.81)	0.000
SGR	4.20±0.00^c (4.19 -4.20)	4.22±0.00^b (4.22-4.23)	4.25±0.00^a (4.26-4.26)	4.25±0.00^a (4.25-4.25)	0.000
CF	1.96±0.13^b (1.93-2.00)	2.00±0.01^{ab} (1.97-2.04)	1.98±0.027^b (1.92-2.05)	2.03±0.00^a (2.00-2.05)	0.012

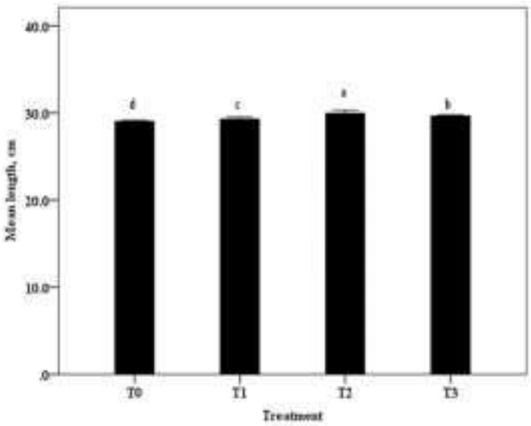

Figure 4. Effects of hormonal masculinization on fish length

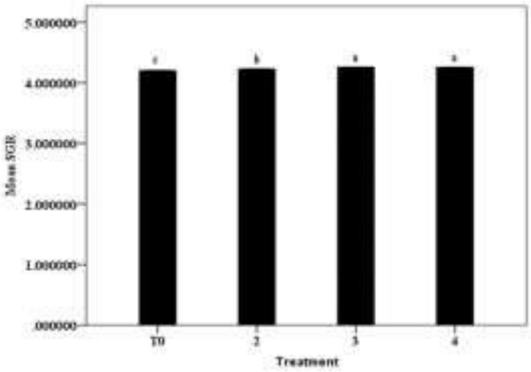

Figure 5. Specific Growth rate of fish at different treatment

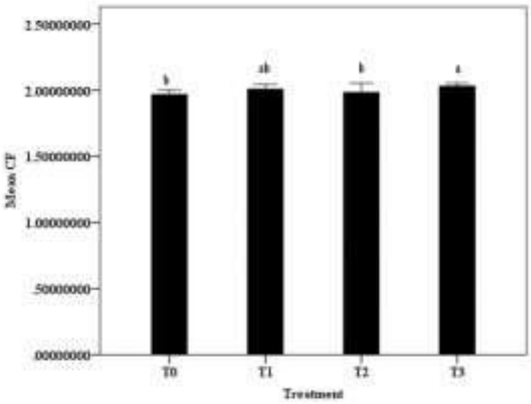

Figure 6. Condition factor of fish at different treatment

Effects of hormonal masculinization on sex reversal

The sex reversing percentages of T_1, T_2 and T_3 were 77.83±0.76, 98.0±0.5 and 97.83±0.76, respectively (Figure 7). T_2 showed the highest sex reverse percentage among all the treatments.

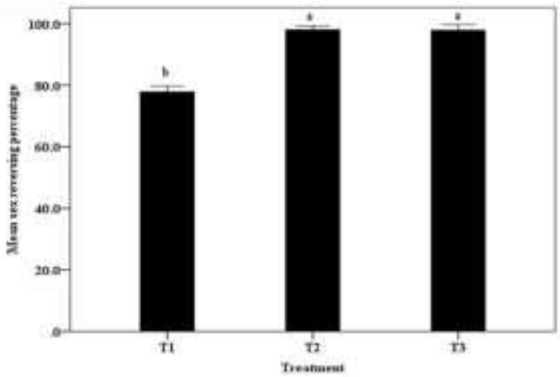

Figure 7. Percentage of fish sex reversing at different treatment

Effects of hormonal masculinization on FCR

Experimental fish showed the best FCR (1.2) in the T_2 and T_3 hormone-treated feed. All hormonally treated fish showed better FCR performance than control fish (1.7) (Figure 8).

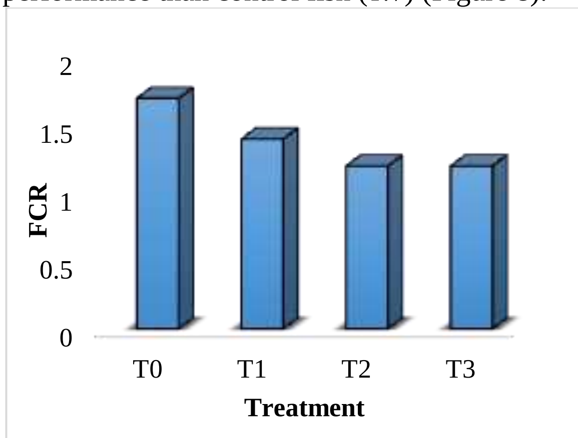

Figure 8. FCR value

Effects of hormonal masculinization on survival rate

Effects of hormonal masculinization on the survival rate of tilapia are recorded in Table 2. The highest survival rate was 87.6% in T_2 and the lowest was 65.7% in T_0 in the experiment.

Table 2. Effects of hormonal masculinization on survival rate

Treatment	Survival Rate (%)
T_0	65.7
T_1	78.1
T_2	87.6
T_3	84.9

4. DISCUSSION

Effects of hormonal masculinization in the growth performance

Growth parameters were observed significantly higher in the Treatment-2 (60 mg MT hormone/kg) of the present study (Table 01). Hanson et al. (1983) found that the use of 60 ppm MT-treatment provides higher growth of Nile Tilapia than control treatments which agreed with the present study. Hormone-treated group showed higher growth than the control group which agreed with Varadaraj et al. (1994), Tayamen and Shelton (1978), Jensi et al. (2016) and Sarbajna et al. (2010); who observed higher growth when fry fed 17-alphamethyl testosterone hormone.

Effects of hormonal masculinization on sex reversal

The highest mean percentage of sex reversal was observed in the T_2 (60 mg MT hormone/kg) in the present study (Figure 7). Ferdous et al. (2011) found that hormone-treated group gave a significantly higher male population of Nile Tilapia than the control group. Vera–Cruz and Mair (1994), Smith and Phelps (2001) and Okoko (1996) also found higher sex reversal percentage using 60 mg MT/kg dose of hormone. Hence, hormone treatment could be considered for the masculinization of Tilapia fry. The present study found T_2 (60 mg MT/kg) as an effective dose for the masculinization of Nile Tilapia in the hatchery. T_3 (80 mg MT/kg) was also found effective in case of sex-reversal of the fry but it might increase the cost of the hatchery operations.

Effects of hormonal masculinization on FCR

Experimental fish showed the best FCR (1.2) in T_2 and T_3 hormonal treated feed (Figure 8). All hormone-treated fish showed better FCR performance than control fish (1.7). Beaven and Muposhi (2012) observed a value of food conversion ratio (FCR) of 1.6 for MT hormone-treated diet fish individuals and 1.98 for those individuals not treated with MT hormone. That provides clear evidence of the effects of 17-alpha methyltestosterone on FCR in the case of Nile Tilapia which also makes the findings of the present study evident. Ahmad et al. (2002) found a better FCR value in the case of hormone-treated fish at the end of the experiment which too agrees with the findings of the present study.

Effects of hormonal masculinization on survival rate

The highest survival rate was 87.6% in T_2 and the lowest was 65.7% in T_2 (Table 2). The highest mortality was observed in the SRT tank during the experimentation. Beaven and Muposhi (2012) found a significantly high 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 study period. Jensi et al. (2016) found the highest survival rate (80.06%) in the 60 mg/kg hormone-treated fish tank which is substantiated by the present study.

5. CONCLUSION

The study concludes that 17αmethyltestosterone has a positive and significant impact on the growth performances, survival rate, sex reversal, and Food Conversion Ratio (FCR) of Nile tilapia (*Oreochromis niloticus*). The study has evidenced that 60 mg MT/kg 17α-methyl testosterone hormone dose through diet might be useful in the production of mono-sex populations of Nile tilapia (*Oreochromis niloticus*). It has also been observed that a higher dose than 60 mg/kg has more or less similar effects on the growth performance and sex ratio of Nile tilapia but that might increase the production cost. The findings of this study will help to increase Nile tilapia production from the culture pond and will minimize the cost for the production of mono-sex Nile tilapia populations in the hatchery.

ACKNOWLEDGEMENTS

The author would like to express his gratitude to the 'Meridian Hatchery Limited' for their priceless support during the research period.

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