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Effect of 17 α-methyl testosterone on sex reversal and growth of Nile tilapia (*Oreochromis niloticus* L., 1758)

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ABSTRACT

The present study aims at developing a production of monosex population of all male Nile tilapia (*Oreochromis niloticus*) using 17α-methyltestosterone (17α-MT). Three treatments with a control (Control, T-1, T-2, and T-3) in triplicates were set up using completely randomized design (CRD). The control group diet was devoid of 17α–MT. The remaining groups T-1, T-2, T-3 and T-4 were fed with feed containing 50, 60 and 100 mg kg^{-1} of 17α –MT. Three days old fry were stocked at the rate of 300 nos per tank. The fry were fed with experimental diet for 21 days. After that it was shifted to FRP tanks. The maximum mean length and body weight was observed in T-2 is 80 ± 3.87 mm and 59.5 ± 7.4 mm respectively. Gonadal histology from 3 months reared *O. niloticus* for sex reversal was observed that 56.7 %, 83.3 %, 93.3 % and 90 % males respectively. The highest male population of 93.3% males was produced from treated groups of (T-2) 60 mg kg^{-1} 17 α-MT.

Key words : *Oreochromis niloticus, 17α-methyltestosterone, Sex reversal, Histology*

Introduction

The production of a single sex population, especially of male, has several advantages in tilapia aquaculture including enhanced growth and prevention of unwanted reproduction of the various techniques that have been developed to provide male tilapia for culture i.e. manual sexing (Guerrero, 1982), hybridization (Hickling, 1960), genetic manipulation (Pandian and Varadaraj, 1988) and hormonal sex reversal, the later is the most common and economically feasible technique (Clemens and Inslee, 1968; Tayamen and Shelton 1978; Goudie *et al.*, 1983; Jae Yoon *et al*., 1988). The development of hormonal sex-reversal techniques in the 1970s represented a major breakthrough that allowed male monosex populations to be raised to uniform, marketable sizes. 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).

The benefits of the GIFT strain include significantly faster growth rates than other farmed strains, improved survival in polluted waters and that they can be raised in extensive systems without the need for commercial feeds. This species matures at a larger size and is less fecund and thus less prone to overpopulation. Celik *et al.* (2011) tried to produce all male Nile tilapia by feeding the larvae containing

17α-MT at five different doses (0, 20, 30, 40, 50 and 60 mg/kg feed) for 28 days. Since the androgens have both sex reversal and anabolic effects, the sex reversed tilapia shows a better growth performance as compared to normal tilapia. Dan and Little (2000) observed the culture performance of monosex and mixed sex culture of three strains of Nile tilapia and reported that among the three tilapia strains, the GIFT fish attained larger individual final weight. They further narrated that overall monosex fish of three strains grew significantly faster than mixed sex fish. Therefore the study envisages the effect of 17α-Methyl Testosterone on the sex reversal of Nile Tilapia under controlled conditions.

Materials and Methods

Broodstock development

The brood fishes were collected from Barur area, Krishnagiri district in Tamilnadu and transported to Fisheries College and Research Institute, Thoothukudi. The fishes were acclimatized for 15 days in cement tanks (2 m^{-2} dia). The brood fishes were developed in the earthen ponds (25x40 m). Polyethylene net was used for the preparation of rectangular breeding hapa (12 m x 5 m x 1.5 m). Two rectangular polyethylene hapas were installed in the pond and the brooders in the size range of 250-300 g were kept at a stocking density of 4-6 nos m^{-2}. Sex ratios were maintained at 1:3 (male: female). The brood fishes were fed twice in a day with floating pellet feed (Uni-President Tilapia feed) containing 27 % protein @ 3-4 % of body weight.

Fry collection and incubation

After 3 months of maturation the brooders were examined once in 15 days. The fry and eggs were collected from the brooders during the early morning to avoid stress and mortalities. Both fry and eggs were rinsed and counted. Then it was transferred to the hatching tanks and incubated with adequate aeration. The eggs were hatched out after 48-72 hrs depending on the stage of development at 29 ± 1 °C.

Preparation of experimental diet

The experimental diets were prepared with 17á-methyltestosterone (Hi Media) in different concentrations. The diets were Control (without aloe emodin), T-1 (50 mg kg^{-1}), T-2 (60 mg kg^{-1}) and T-3 (100 mg kg^{-1}). A stock solution was made by dissolving 0.3 g of hormone in 60 mL of 95% ethanol. From the stock solution, the working solution 50, 60 and 100 mg MT kg^{-1} was prepared. Each working solution were evenly sprayed separately over 500 g of Irawan prawn starter feed and then air dried for 1 h. The dried pellets were stored in an air sealed container and stored in cool dry place for further use.

Experimental design

The experiment was conducted at Tilapia Hatchery, Fisheries College Research Institute, Thoothukudi, Tamilnadu, India. Three treatments with a control in triplicates were set up using completely randomized design (CRD). The glass aquaria in the size 60 x 30 cm were used for this study. The fry were divided into three groups (Control, T-1, T-2, and T-3) and each group was maintained in triplicate set containing 300 nos. of fry by following a completely randomized design (CRD). The control group diet was devoid of 17α–MT. The remaining groups T-1, T-2, T-3 and T-4 were fed with feed containing 50, 60 and 100 mg kg^{-1} of 17α–MT.

Larval rearing

The fry were reared fed with hormone mixed feed for 21 days at the rate of 3 % body weight for five times and Artemia nauplii for one time per day day. After 21 days of rearing the survival of the fishes was estimated. After hormone treatment the fishes were transferred from glass tank to FRP tank (1.5 m dia) containing 200 fishes for each treatment and control. Now the fishes were fed with floating pellet feed for 3 months. From each fibre tank, to assess the post treatment effect on survival and from each experimental replicate, 10 randomly sampled fish were taken for recording the mean growth (Length and Weight) of fish.

Sex reversal

At the end of the experiment to identify the sex of the fishes each fish was dissected and the gonadal tissue exposed. Morphology of the gonads were examined and recorded. The sex of the fishes was also examined by using histology to differentiate their gonads. Paired gonads were removed with the help of fine forceps and kept in a bottle containing 10 % formalin solution. The tissues were observed under microscope 20 X. Tissues were recorded as testes (male), ovaries (female) or ovo-testes or intersex (gonads containing both ovarian and testicular tissues). The histological studies were also done in Vivek

Laboratory, Nagercoil, Tamilnadu, India after 90 days of rearing for this thirty fishes were sacrificed for different dosages. The slides were examined and photographed using a compound microscope fitted with camera (Nikon Digital Camera D5100). By looking the histological gonadal samples, the male and female rates in the population were found (Nikon Trinocular Microscope Model Eclipse Ni-V, 40X). (Fig. 1 & 2).

Statistical analysis

The data were statistically analysed 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

Collection of fry

The number of embryos/fry collected from the brooder mouth was counted and the observations were presented in the Table 1. The maximum numbers of embryos (1573 nos.) were collected from the fish weighing 350gm. The minimum number of embryos (117 nos.) was collected from the fish weighing 173gm.Younger eggs are light yellow and older eggs are dark orange or brown.

Table 1. Number of eggs/fry collected from the female broodstock of *O. niloticus* reared in hapas.

Size of brood fishes		No of embryos collected
Length (cm)	Weight (gm)	
11.5	212	160
9.4	173	117
16.8	336	237
17.2	350	1573
17.2	355	1291
18.1	400	1179
18.6	425	1158
16.6	350	1573
17.1	355	1291

Survival during the hormone treatment

After 21 days of hormonal treatment the survival percentage of fishes during the hormone treatment period was calculated for different dosages and the results were presented in Table 2. Maximum survival (90.83%) was obtained in the group that received 60 mg kg^{-1} of hormone, when compared to hormone treated and control groups. Maximum survival (95.00%) was obtained in control groups during the hormone treatment period.

The different groups of fishes were transferred from all glass tanks into FRP tanks for growth and

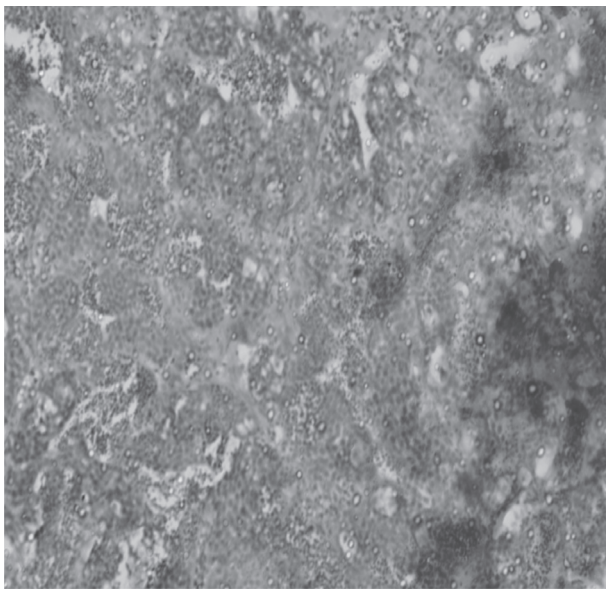

Fig. 1. The spermatozoa observed in male individuals on histology obtained at the end of the experiment and used in specifying sex

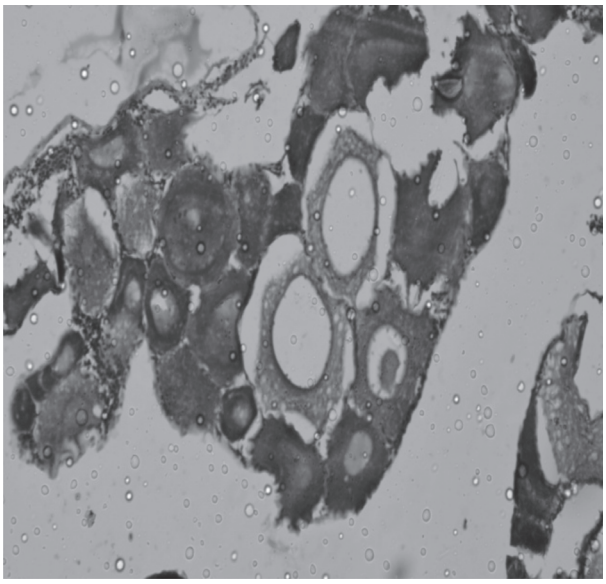

Fig. 2. The egg formations observed on the gonad of female individuals on histology obtained at the end of the experiment and used in specifying sex

Table 2. Mean survival in *O. niloticus* subjected to oral administration with different doses of 17 α-MT for 21 days.

Treatment	Survival
T-1	87.33
T-2	90.83
T-3	87.33
Control	95.00

Growth parameters

sex reversal studies. The maximum mean length (80 ± 3.87mm) was observed in T-2 compared to control (59.5 ± 7.4 mm). Similarly, the maximum mean body weight (11.56 ± 4.027g) was observed in the T-2 compared to control (7.73 ± 0.46 g) (Table 3).

Table 3. Growth parameter Nile Tilapia after 90 days of rearing in FRP tanks.

Treatment	Length	Weight	Survival
T-1	58.2± 1.83	7.47± 0.44	78.5
T-2	80± 3.87	11.56±4.027	80.06
T-3	70.4± 6.35	8.18± 0.50	75.1
Control	59.5±7.4	7.73±0.46	82.0

Survival

The survival of *O. niloticus* was evaluated for the treated and control groups at the end of three months of study (Table 3 and Fig. 5). Maximum survival rate (80.06 %) was observed in T-2 and Minimum survival rate of 75.1 % was observed in T-3 where the concentration of the hormone was high. Controls showed 82 % survival. The higher survival was found in the control than the all the hormone treated groups.

Sex reversal

From the histology of gonadal tissues revealed that inter-sex population was not found in all the treatment and also control groups (Fig. 1 & 2). The control groups showed a normal sex ratio of 56.7 % males and 43.3 % females (Table 4). The experimental groups T-2 showed the highest percentage of males (93.3%) followed by T-3 (90 %) and T-1 (83.3%) respectively. Lowest percentage of females (6.7%) were observed in T-2, followed by T-3 (10 %) and (16.7%) T-1 respectively. 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 groups.

Discussion

The number of eggs and fry collected per female varied, But this result was contradictory with the report of Babiker and Ibrahim (1979) that fecundity in Nile tilapia varied more with body length and weight than with age. Velasco (2003) reported that in the case of Nile tilapia, there is an inverse relationship between the number of eggs and the body weight of female i.e., the lower the weight of mature female, the more will be the number of eggs, similarly, the fishes having the higher weight of mature female, the less will be the number of eggs.

Tayamen and Shelton (1978) observed faster growth of hormone treated *O. niloticus* which agrees to the resulst of the present study. The results of the present study are contradictory with the findings of various authors regarding the anabolic effect of MT in fish and all male culture of tilapia. Howerton *et al*. (1992) and Varadaraj *et al*. (1994) observed faster growth in *O. mossambicus* when fed with MT. These results are line with Dan and Little (2000), who compared the culture performance of different strains of *O. niloticus* and found that considering all strains, MT treatment resulted in a final size of fish 10.7 % larger than mixed sex fish. Contradictory to the results of most of the authors, Jay-Yoon *et al*. (1988) observed a depression in growth of Nile tilapia when hormone concentration was increased from 60 mg/kg in the first 30 days and reduced concentra-

Table 4. Sex proportion of *O. niloticus* after 21 days of 17α L$%L Methyltestosterone treatment

Treatment	No. of fish sexed	Males	Females	Sex ratio M : F	Chi-square value
T-1	30	25(83.3)	5(16.7)	1:0.2	13.4
T-2	30	28(93.3)	2(6.7)	1:0.07	22.4
T-3	30	27(90)	3(10)	1:0.111	19.2
Control	30	17(56.7)	13(43.3)	1:0.764	0.52

tion of 10 mg/kg in the second 27 days of the experiment and 80 mg/kg in the first 30 days and 60 mg/kg in the next 27 days.

Celik *et al.* (2011) reported 80% survival which is similar to the result of this study. Control group showed highest survival percentage than treated groups. Survival percentage of control group was 82% which is similar to the results of Celik *et al.* (2011) who obtained 81.6% in his I experiment. Soto (1992) and Vera-Cruz and Mair (1994) reported contradictory results by observing that MT administration has no significant effect on survival in the related tilapia species *O. niloticus*. Guerrero (1975) also observed that hormone treatment has no effect on survival in *O. aureues*.

The Maximum male population (93.3%) of Nile tilapia was obtained at a dose rate of 60 mg MT kg^{-1} of feed given for 21 days. This result is slightly in agreement with Celik *et al.* (2011) reported 93.7 % males using 60 mg MT kg^{-1} for 28 days. Shamsuddin *et al.* (2012) observed 95% males using 60 mg 17α-MT for 21 days through oral administration in GIFT strain which are similar to the results of the present study. The minimum male proportion (83.3%) was recorded for a dose rate of 50 mg MT kg^{-1}. These findings are also similar to the results of Mateen (2007) who used four different doses viz., 50, 60, 70 and 80 mg MT kg^{-1} for 25 days and got lowest male proportion for 50 mg MT kg^{-1}. The dose rate of 100 mg MT kg^{-1} of feed resulted in 90% males in the present study which is contradictory to the results of Marjani *et al.* (2009) who got lower male proportion (79.38%) for the same dose in *O. mossambicus* for the same duration of treatment. The result of this study has showed that no intersex was found in the treated groups and control groups which is similar the earlier findings of Celik *et al.* (2011). But contradictory results were reported by Mateen (2007) where intersex (6.2% for 50 mg MT kg^{-1}) and (2.7% for 60 mg MT kg^{-1}) was obtained in these dose rate when the hormone was given for a period of 25 days.

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Conflict of interests

The author(s) have not declared any Conflict of interests

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