# MASCULINIZATION OF NILE TILAPIA (OROCHROMIS NILOTICS) FRY BY IMMERSION IN 17α-METHYLTETOSTERONE

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***Abstract***

***This study was conducted to develop a short-term immersion procedure for masculinization of Nile Tilapia (Oreochromis niloticus) by using 17α-methyltestostrone at 100, 200 or 400 µg/l for 3, 6 or 12 h. Fry were immersed tow successive times with 3 days interval period. The highest percentage of male Oreochromis niloticus (96±4%) and the lowest gonado-somatic index of female Oreochromis niloticus (1.89 ±0.02) were obtained by immersion of Fry in 17α-methyltestosterone at the level of 400 µg/l for 6 h. However, survival rate of Oreochromis niloticus Fry during hormone treatment period did not differ significantly from survival rate in the control group.***

*Tilapia culture is widespread allover the world, The problem of over population in fish ponds caused by uncontrolled reproduction is a major constraint to the further development of the Tilapia culture industry. This problem could be overcome by culturing all-male populations of Tilapia (Gale et al., 1995). oneof the most common techniques for producing all-male populations of Tilapia is androgen-induced-sex-reversal by using androgen-treated feed (Veracuz and Mair,1994). Van Dentturk et al. (1989)who found that GSIof female claries gariepinus decreased when high dose of MT (300 µg/l)administered, Chatain et al. (1999) who found that MDHT did not significantly affectthe gonadal development of Dicentrachus labrax. However, The immersion of Fry is not fully developed for practical usage. (Gale et al. 1999). Feeding androgen carries some potential disadvantages as in efficiency in masculinization immersion of Tilapia fry in androgen solutions may be an alternative tooral administration of androgen, this technique is well developed in salmonid culture; however it remains largely experimental in Tilapia culture. The objective of this research was to develop short-term immersion procedure for the masculinization ofNile Tilapia by using 17α-methyltestosterone and evaluating the most proper dose concentration and hormone treatment period.*

**key words:** ***tilapia; androgen; methyltestosterone; oreochromis niloticus; sex reversal; males; sex ratio.***

## MATERIALS AND METHODS

The experiments were conducted at Shemese Fish Hatchery, Edko, Behera Governorate, Egypt and Laboratories of Faculty of Veterinary Medicine, Alexandria University, Egypt.

Newly hatched *Oreochromis niloticus (O. niloticus)* fry were obtained by artificial spawning using hapa according to the method described by Bautista et al. *(1988)* and randomly assigned to 10 groups. Each group was being replicated 3 times. Fry were stocked in 4 liter glass jars with 3 liters of fresh water at stocking density of 33 fry/liter *(Gale et al., 1999)*. The water in the jars was maintained at 25 ±2 °C under constant aeration. Fry were immersed in *17α-methyltestosterone* hormone at 100, 200 or 400 µg/l for 3, 6 or 12 h immersion period. Control group included the immersion of fry in the water only. After immersion, the fry were collected and stocked in jars that contained fresh water. The immersion was repeated after 3 days for all groups.

During hormone treatment period, fry were fed a powdered feed consisting of 30 % (by weight) fish meal and 70% rice bran *(Veracruz and Mair, 1994)*. Fry were fed to satiation 4 times daily.

After the immersion period, 150 randomly selected fry per group were transferred from the jars to 2 m² nursing hapas suspended in their respective 150 m² earthen ponds for 3 weeks prior to their release into the ponds. At the end of grow-out period which lasts for 112 days, 50 fish from each group were individually weighted and the gonads were weighted for calculation of gonado-somatic index *(GSI)* according to Crivelli *(1981)*. Moreover, the fish were individually sexed by examination of squash preparation of gonads after a ceto-carmine squash method *(Guerrero and Shelton, 1974)*. Sex ratio was expressed as a percentage.

Survival rate was estimated for Tilapia fry during hormone treatment period. Statistical analysis of the obtained data was performed using Statistical Analysis System, SAS *(1987)*.

## RESULTS AND DISCUSSION

It is clear from the obtained data that the immersion of *O. niloticus* fry in *17α-methyltestosterone (MT)* at 400 µg/liter for 6 hours resulted in the highest percentage of male populations (96 ±5 %), followed by 88 ±4 % and 86 ±5 % males in groups 9 and 7, which immersed in 400 µg *MT*/l for 12 h and 3 h, respectively *(Table 1)*, while using the hormone at the level of 200 µg *MT*/l, produced lower male percentage (80 ±6, 78 ±4 and 82 ±4 % males in groups 4, 5 and 6, respectively) than the results obtained in fish groups which were treated at 400 µg *MT*/l. The lowest male percentage was obtained in fish groups which were treated at the hormonal level of 100 µg *MT*/l. These results agree with those obtained by Gale et al. *(1999)*.

Immersion of *O. niloticus* fry for 3h at the hormonal level of 400 µg *MT*/l produced 86 ±5 % males; this result is lower then that obtained by Gale et al. *(1995)* who has produced 93 – 100% male *O. niloticus* fry by immersing the fry in 500 µg/l of *17α-methyldihydrotestosterone (MDHT)* for 3h. These results can be explained on the basis that the treatment used in the present research may not represent an optimal dose of steroid or it may be due to conversion of *MT* to a less active form or simply a faster rate of clearance from the body than *MDHT*. Another possible explanation for the differing effects of the two steroids is that MDHT is more potent masculinizing agent than *MT*. The present research may overcome these problems by increasing immersion period of *MT* from 3h to 6h.

Inter-sexes appeared as a low percentage, 2 % and 4 % in groups 2 and 5 which were treated at 100 and 200 µg *MT*/l for 6 h. However, no inter-sexes individuals were found at the higher (400 µg *MT*/l) hormonal levels. These results agree with those obtained by Berger and Rothbard *(1988)* who found that the higher percentage of inter-sexes Red Tilapia (received diets containing *17α-methyltestosterone*) appeared at the lower hormonal doses.

It is clear from the obtained data *(Table 2)* that both male and female hormone-treated *O. niloticus* had significantly lower *GSI* compared to untreated fish (control groups). These results are confirmed by those of Veracruz and Mair *(1994)*.

The lowest *GSI* of female *O. niloticus* was obtained in group 8 (1.89 ±0.02), which was treated at the highest hormonal level (400 µg *MT*/l) for 6h. This result is confirmed by the work of Van Dentturk et al. *(1989)* who found that *GSI* of female *claries gariepinus* decreased when high dose of *MT* (300 µg *MT*/l) was administered. It is known that high doses of androgen may result in a decrease of masculinizing potency and retardation of gonad development.

Gonado-somatic index of male *O. niloticus* which immersed for 3h at the levels of 100, 200 and 400 µg *MT*/l (0.82 ±0.04, 0.77 ±0.04 and 0.79 ±0.03, respectively) was not affected significantly by the level of hormone. These results agree with those obtained by Chatain et al. *(1999)* who found that *MDHT* did not significantly affect the gonadal development of *Dicentrachus labrax*.

Survival rates *(Fig 1)* of *O. niloticus* fry in different groups during hormone treatment period were nearly similar (ranged from 88.9 ±2.18 to 93.6 ±0.48 %) and did not differ significantly from survival rate in the control group (91.9).

## REFERENCE

Bautitsta, A. Ma., Carlos, M. And SabAntonio, A. I. ***(1988). Hatchery production of Oreochromis niloticus L. At different sex rations and stocking densities. Aquaculture, 73: 83-95.***

Chatain, B.,Saillant, E. And Peruzzi, S.***(1999): Prodution of monosex male populations of European seabass, Diccentrarcus labrax L. By use of the synthetic androgen 17α-methydehydroltestosterone.Aquaculture,178: 225-234.***

Crivelli, A. J***, (1981): The biology of the common carp, cyprinus carpio L. In the camague, southrn France. J. Fish Biol, 18, 271-290.***

Gale, W. L. Fritzpatrick, M. S., Luceero, M. Concentras-sanchez, W. M. And Schreck, C.B.***(1999) Masculinization of Nile Tilapia (Oreochromis Niloticus) by immersion in androgens.Aquaculture, 178: 349-357.***

Gale, W. l. Fitzpatrick, M/ S. And schreck, C. V. ***(1995): Immersion of Nile Tilapia (Oreochromis niloticus) in 17α-methyltesostrone and mestanolone for the production of all-mall populations. In Goetze. F. W. And Thomas. P. Eds., proceeding of the Fish International Symposium on Reproductive physiology of Fish. Fish symposium 95, Austin. Tx. 1995, 117.***

Guerrero, R. D. And Shelton, W. L. (1974)***: An aceto-carmine squash methode for sexing juvenile fishes.Prog. Fish Cult., 36:56.***

***SAS (1987): Statistical Analysis systém. User's guide: Statistics. SAS institute, Cary, North Carolina.***

Van Den Hurk, R., Richter, C. J. J. And Janssen-Dommerholt. J. ***(1989): Effects of 17α-methyltestostrone and 11β-hydroxyandrotenedione on gonad differentiation in the African Catfish, Clarias gariepinus. Aquacultre, 83: 179-191.***

Veracruz, E. M. And Mair, G. C. ***(1994): Conditions for effective androgen sex reversal in Oreochromis niloticus (l). Aquaculture, 122:237-248.***

Table 1: Percentage of males, females and intersexes *Oreochromis niloticus* in different group:

| Groups | Males | Females | Intersexes |
|---|---|---|---|
| 1. 100 *MT*** 3h | 76 ±*5[d] | 24 ±5[b] | — |
| 2. 100 *MT* 6h | 80 ±6[c] | 18 ±5[c] | 2 ±4[b] |
| 3. 100 *MT* 12h | 78 ±7[cd] | 22 ±5[b] | — |
| 4. 200 *MT* 3h | 80 ±6[c] | 20 ±5[bc] | — |
| 5. 200 *MT* 6h | 78 ±4[cd] | 18 ±5[c] | 4 ±8[a] |
| 6. 200 *MT* 12h | 82 ±4[bc] | 18 ±5[c] | — |
| 7. 400 *MT* 3h | 86 ±5[b] | 14 ±5[d] | — |
| 8. 400 *MT* 6h | 96 ±5[a] | 4 ±5[e] | — |
| 9. 400 *MT* 12h | 88 ±4[b] | 12 ±5[d] | — |
| 10. Control | 52 ±4[e] | 48 ±5[a] | — |

*Means ± standard error.
Figures with different superscripts in each vertical row are significantly different ($p<0.05$).
**MT*: *17α-methyltestosterone*. h: Immersion period in hours.

Table 2: Gonado-somatic index (*GSI*) of males and females *Oreochromis niloticus* in different groups:

| Groups | *GSI* | | |
|---|---|---|---|
| | Males | Females | Intersexes |
| 1. 100 *MT* 3h | 0.82 ±0.04[b*] | 2.20 ±0.18[d] | — |
| 2. 100 *MT* 6h | 0.83 ±0.03[b] | 1.95 ±0.11[e] | 1.24 ±0.00[a] |
| 3. 100 *MT* 12h | 0.68 ±0.03[d] | 2.60 ±0.08[b] | — |
| 4. 200 *MT* 3h | 0.77 ±0.04[bc] | 2.37 ±0.09[c] | — |
| 5. 200 *MT* 6h | 0.76 ±0.05[c] | 2.51 ±0.08[b] | 1.02 ±0.00[b] |
| 6. 200 *MT* 12h | 0.82 ±0.06[b] | 2.30 ±0.09[cd] | — |
| 7. 400 *MT* 3h | 0.79 ±0.03[bc] | 2.37 ±0.02[c] | — |
| 8. 400 *MT* 6h | 0.83 ±0.04[b] | 1.89 ±0.02[e] | — |
| 9. 400 *MT* 12h | 0.71 ±0.03[cd] | 2.62 ±0.09[b] | — |
| 10. Control | 0.93 ±0.03[a] | 2.75 ±0.09[a] | — |

*Means ± standard error.
Figures with different superscripts in each vertical row are significantly different ($p<0.05$).
*MT*: *17α-methyltestosterone*. h: Immersion period in hours.

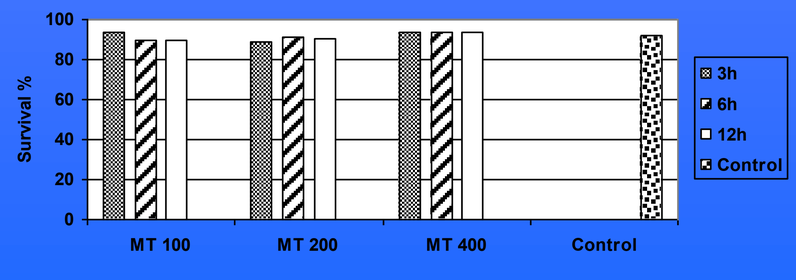


Fig. 1: Survival rate of *Oreochromis niloticus* fry in different groups.