

Optimization of hormonal dose during masculinization of tilapia (*Oreochromis niloticus*) fry

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Abstract

The experiment was conducted to optimize the dose of 17 α -methyl testosterone (MT) during masculinization of tilapia (*Oreochromis niloticus*) fry. Five treatments were designed with various doses of hormone to find out the most effective one. The treatments were designated as T₁ (0 mg MT/kg), T₂ (40 mg MT/kg), T₃ (50 mg MT/kg), T₄ (60 mg MT/kg) and T₅ (70 mg MT/kg). MT was administered orally by using nursery feed and Ethanol (as hormone carrier solvents) diet to tilapia fry for 28 days in hapa. After completion of the trial period of 28 days nursing of the experimental fry was continued for further 2 months with commercial feed. At the end of experiment the sex ratio was determined by examining gonad after dissecting the fish. Growth performance was monitored by recording the morphometric characteristics *i.e.* weight gain (g), average daily weight gain (g). The analysis of growth data showed significant variation in weight, % weight gain, SGR (% /day) of fish among the different treatments. SGR increases with the increase of hormone dose. The present study demonstrated that all MT receiving treatments showed a significantly ($p < 0.01$) higher male proportion (94.28%) than control (48.57%). The dose of 60 mg MT/kg of feed resulted in maximum male population (94.28%). The result indicated that the optimum dose of MT hormone was 60 mg /kg with a feeding period of 28 days after hatching.

Keywords: 17 α -methyl testosterone (MT), Masculinization, Growth

Introduction

Tilapia is likely to be the most important of all aquaculture fish in 21st century. Tilapia has certain favourable characteristics, like tolerant to adverse environmental conditions, can survive at low dissolved O₂, euryhaline, relatively fast growth and efficient food conversion. All 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 commercial scale is its precocious reproduction. It attains sexual maturity at early age 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 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.* manual process by visual examination; hybridization; gene manipulation and masculinization via steroid hormone. At the time of hatching tilapia fry are sexually undeveloped. Hence, during the early period of gonadal differentiation, changes in sex hormone level can affect the final sex independently of the genetic sex (Andersen *et al.*, 2003).

Hormonal sex reversal is a technique of changing of sexes from one sex to another in fish by administering synthetic steroid hormones before or during the period of sexual differentiation. In this technique, the first feeding fry are treated with male hormone or androgens (17 α -methyl testosterone), which develops testes and male sexual characteristics at maturity.

Hormone treatment does not alter the genotype of the fish but directs the expression of the phenotype. Hormonal sex reversal quickly became the methodology of choice to produce single sex population. Low dose of hormone, usually 17 α -methyl testosterone, added to the early fry diet in between 40-60 ppm, when correctly administered, could guarantee almost complete sex reversal of female tilapia into phenotypic male. In tilapia, sex steroid hormones play a promising role in directing the gonadal differentiation process (Piferrer, 2001). 17 α -methyl testosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone. This hormone promotes both muscle growth and development of male sexual characters. However, it is important to identify the optimal level of MT as well as duration and timing of treatment for consistent, successful sex reversal (Dunhan, 1990).

Tilapia culture is increasing in Bangladesh and farmers need sufficient good quality monosex tilapia. The aim of this study was, therefore, to find out optimum dose rate of MT treatment for sex reversal along with its effect on growth performance of Nile tilapia (*Oreochromis niloticus*).

Materials and Methods

Sources of fry and experimental design

Nile tilapia (*O. niloticus*) fry used in the present experiment were collected from "Reliance Aqua Farm", Boilor, Trishal, Mymensingh. Three days old spawns (first feeding stage) of tilapia (*O. niloticus*) were stocked in 10 (Ten) hapa (Size 6ft×6ft).

The experiment was conducted in duplicate with 5 treatments designated as T₁ (0 mg MT/kg), T₂ (40 mg MT/kg), T₃ (50 mg MT/kg), T₄ (60 mg MT/kg) and T₅ (70 mg MT/kg). The fry were reared for 28 days with hormone containing feed.

Hormone-feed preparation and feeding trial

Four diets with different doses of MT hormone i.e. 40, 50, 60 and 70 mg/kg were prepared through ethanol evaporation method (Muir and Santiago, 1994). To prepare 5 kg feed for each treatment required amount of MT hormone (i.e. 200, 250, 300, 350 mg MT hormone for T₂, T₃, T₄ and T₅ respectively) was diluted with 750 ml ethanol carrier for homogenous mixing with the nursery feed. In case of control, only nursery feed was used without hormone treatment. The fry were fed with hormone mixed feed 5 times (started at 8.00 am and continued up to 6.00 pm with two hour interval) a day up to satiation. The hormone feeding phase was continued for a period of 28 days. The fish were then fed with hormone free commercially available diet for further two months.

Growth Parameters

Growth performance was calculated according to the following formulae after Rashid (2008).

Weight gain (g) = Mean final weight (g) - mean initial weight (g)

$$\% \text{ Weight gain} = \frac{\text{Mean final fish weight} - \text{mean initial fish weight}}{\text{Mean initial fish weight}} \times 100$$

$$\text{Average daily weight gain (g)} = \frac{\text{Mean final weight} - \text{Mean initial weight}}{T_2 - T_1}$$

$$\text{SGR (\%/day)} = \frac{\log_e W_2 - \log_e W_1}{T_2 - T_1}$$

Collection and analysis of data

Ten fry from each of the hapa were randomly selected for measuring weight (g) at an interval of 7 days. At the end of the rearing period, the male and female fish were sexed by gonad squashing and aceto-carmine staining method (Guerrero and Shelton, 1974).

The weight gain (g), average daily weight gain (g) and specific growth rate (%/day) of fish during experiment with different hormone feeds were tested using one way analysis of variance (ANOVA). The statistical analysis was performed with the aid of the computer software SPSS programme and MS Excel Programme.

Results and Discussion

Sex reversal

An objective of this study was to find out the minimum dose of 17-alpha methyl testosterone to obtain all male population of Nile tilapia (*Oreochromis niloticus*). Results of this study showed that each hormone treated group gave a significantly higher male/female ratio (Table 1), while the control group showed normal male and female ratio. No treatment gave 100% male population of *O. niloticus*. In this study maximum male population (94.28 %) of Nile tilapia was obtained at the dose of 60 mg MT/kg of feed for 28 days, while the minimum male proportion (88.57%) was recorded for dose of 40 mg MT/kg of feed for 28 days (Table 1, Fig. 1). The dose of 50 and 70 mg MT/kg of feed were resulted in 91.43% males (Table 1). Statistical analysis of the sex reversal data showed a highly significant difference ($P < 0.01$) between treatments (Table 1). The percentage of male fish observed all treatments receiving MT showed a greater and significantly higher ($p < 0.01$) male proportion as compared to control treatment.

17 α -methyl testosterone was effective in producing phenotypic male of tilapia. Greater than 90% male populations have been obtained at a variety of dose rates. Jae-Yoon *et al.* (1988) obtained 97% *O. niloticus* males at dose rate of 10 mg MT/ kg of diet. Vera-Cruz and Mair (1994) obtained 95 to 98% males with 40 mg MT/kg of diet and 99% with 60 mg MT/kg of diet fed at 20% body weight for 25 days. Romerio *et al.* (2000) obtained 98 % male population at the dose of 60 mg MT/kg of feed. Smith and Phelps (2001), reported 99-100 % male Nile tilapia when given MT at 60 mg/kg of feed. The results of this study showed a significantly lower male proportion (91.43 %) for highest dose rate of androgen *i.e.* 70 mg MT/kg of feed. These results are in line with the findings of Okoko (1996), who obtained 99.3% males at 30 mg MT/kg, while 97 and 71.9% males at the dose rates of 60 and 120 mg MT respectively. He further reported that higher dose rates of MT/kg of feed resulted in no increase of male percentage.

However, duration of treatment for successful sex-reversal of tilapia fry might be an important consideration and the duration used in the present study closely followed the duration of Okoko (1996). High rate of masculinization in tilapia can be influenced by some important factors like hormone concentration, treatment duration, age and size of fry, availability of natural feed, stocking density and feeding frequency (Muir and Little, 1991).

Table 1. Sex-ratios of fish at different hormone treatment

Treatments	No. of fish dissected	No. of female	No. of male	% of male
T ₁ (0mg/kg) control	35	14 ^a	17 ^b	48.57 ^d
T ₂ (40mg/kg)	35	4 ^b	31 ^a	88.57 ^c
T ₃ (50mg/kg)	35	3 ^{bc}	32 ^a	91.43 ^b
T ₄ (60mg/kg)	35	2 ^c	33 ^a	94.28 ^a
T ₅ (70mg/kg)	35	3 ^{bc}	32 ^a	91.43 ^b

Figures followed by different superscripts are significantly different ($p < 0.01$)

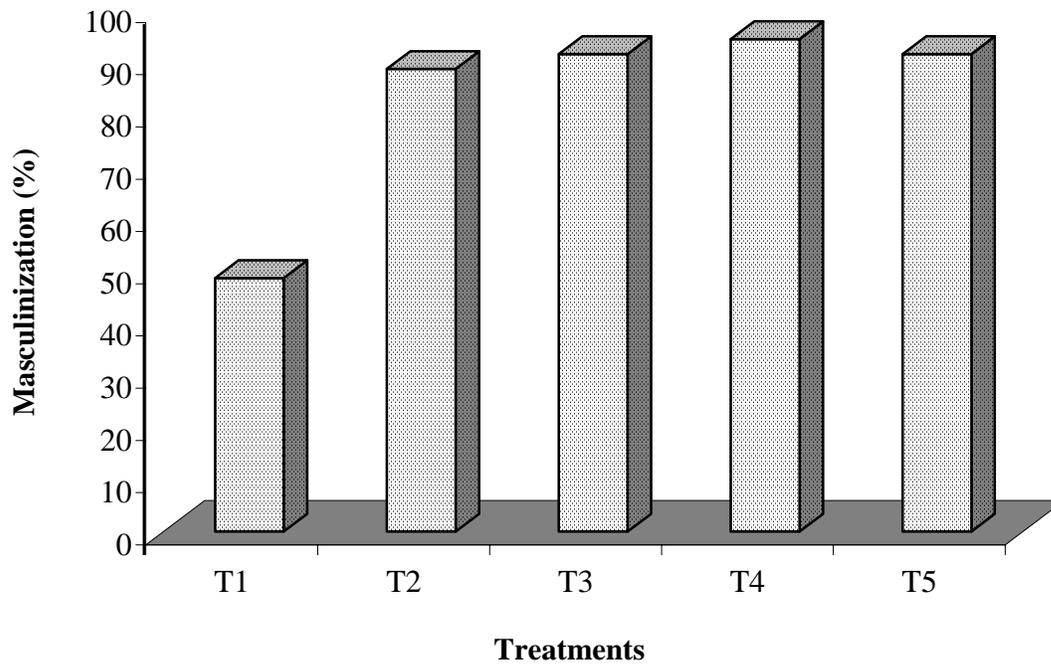


Fig. 1. Percentage (%) of masculinization at different treatments of 17 α -methyl testosterone (MT) hormone

Growth performance parameters

Different dose of MT, significantly influences the growth of *Oreochromis niloticus*. All the treatments which received MT, showed more average body weight and gain in body weight of *Oreochromis niloticus* than the control (Table 2, Fig. 2). Treatment T₅ (70 mg MT/kg) showed 0.27g gain in weight followed by T₄ (60 mg MT/kg) 0.16g, Treatment T₃ (50 mg MT/kg) 0.14g, Treatment T₂ (40 mg MT/kg) 0.12 g and T₁ (control) 0.098g at the end of 28 days. The best percent weight gain (1350%) was observed in case of the higher doses of hormone T₅ (70 mg MT/kg) (Table 2). The specific growth rate (%/day) of Nile tilapia ranged between 6.317 (control) and 9.516 % (70mg MT/kg) during this study (Table 2). Statistical analysis of SGR data revealed that all treatments receiving methyl testosterone showed a significant effect on the specific growth rate of fish.

Table 2. Various growth parameters of *O. niloticus* fry fed on five diets containing different amount of hormone at the end of the 28 days experimental period

Parameters	Treatment 1 control (T ₁)	Treatment 2 (T ₂)	Treatment 3 (T ₃)	Treatment 4 (T ₄)	Treatment 5 (T ₅)	LSD	level of significance
Initial weight (g)	0.020±0.00	0.020±0.00	0.020±0.00	0.020±0.00	0.020±0.00	-	ND
Final weight (g)	0.118±0.011 ^b	0.134±0.013 ^b	0.155±0.016 ^b	0.183±0.024 ^b	0.290±0.057 ^a	0.023	**
Weight gain (g)	0.098±0.011 ^b	0.114±0.013 ^b	0.135±0.016 ^b	0.163±0.024 ^b	0.270±0.057 ^a	0.023	**
% weight gain	487.5±53.03 ^b	570± 63.64 ^b	672.5± 81.32 ^b	815 ± 120.20 ^b	1350± 282.84 ^a	106.82	**
Average daily weight gain (g)	0.00348±0.000 ³	0.00407±0.000 ⁴	0.00480±0.000 ⁵	0.00582±0.000 ⁸	0.00964±0.002 ^a	0.0325	**
SGR (%/day)	6.317±0.323 ^c	6.785±0.340 ^{bc}	7.292±0.377 ^{bc}	7.891±0.471 ^b	9.516±0.701 ^b	0.337	**

** Significant at 1% level of probability

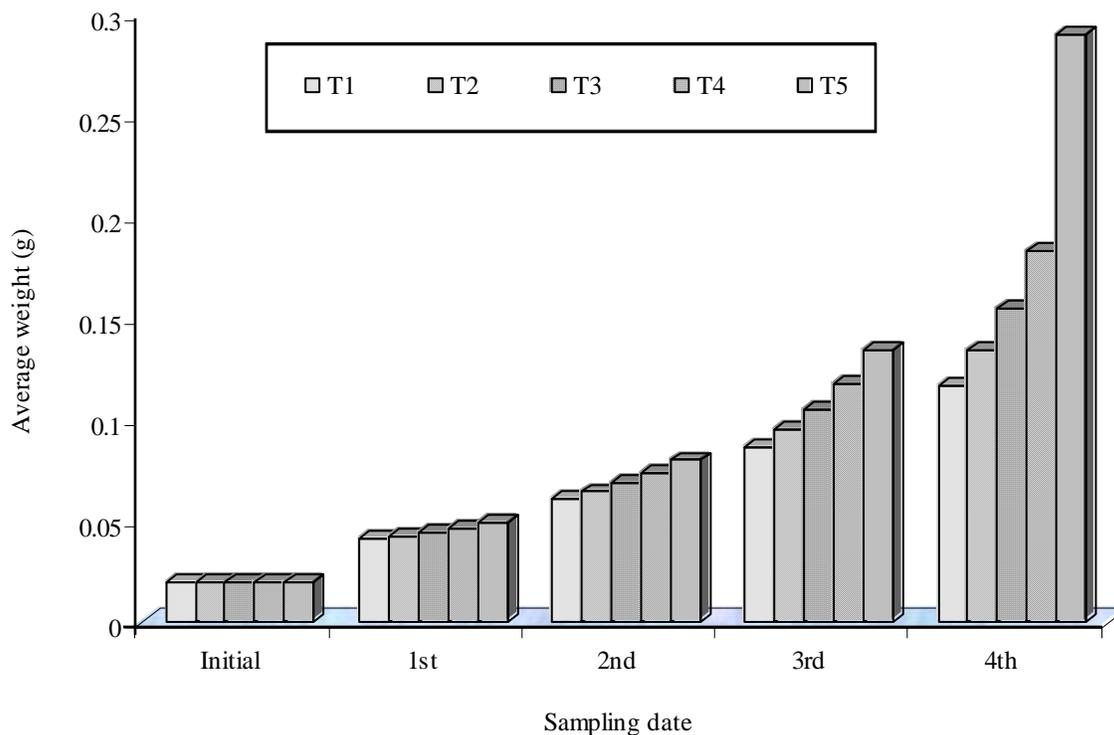


Fig. 2. Weight (g) of *Oreochromis niloticus* fry under five different treatments

Statistical analysis on gain in body weight, showed a highly significant difference among different sex reversal treatments. Hanson *et al.* (1983) reported that 10-60 ppm MT-treatment showed the best growth than control. Varadaraj *et al.* (1994) observed faster growth in *O. mossambicus* when fed 17-alpha methyl testosterone. These results are also in 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 larger than mixed sex fish.

The present study indicates that there is a positive relationship between doses of androgen and the percentage of male fish, but higher dose (70 mg/kg) of hormone resulted in no increase of male fish. The result of the present research revealed that the optimum dose of MT hormone was 60 mg/kg with a feeding period of 28 days. The dose of 60 mg 17 α - methyl testosterone per kg of feed was found to be best for inducing sex reversal in *O. niloticus* resulting 94.28% (higher) male in the population.

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References

- Andersen, L., Holbech, H., Gessbo, A., Norrgen, L. and Petersen, G.I. 2003. Effect of exposure to 17 α -ethinyltradiol during early development on sexual differentiation and induction of vitellogenin in zebra fish (*Danio rerio*). *Comp. Biochem. Physiol. C. Toxicol. Pharmacol.*, **134**: 365–374.
- Dan, N.C. and Little, D.C. 2000. The culture performance of mono-sex and mixed-sex new-season and over wintered fry in three strains of Nile tilapia (*Oreochromis niloticus*) in northern Vietnam. *Aquaculture*, **184**: 221-231.
- Dunhan, R.A. 1990. Production and use of mono sex or sterile fishes in aquaculture. *Rev. Aquat. Sci.*, **2**: 1–17.

- Guerrero, R.D. and Shelton, W.L. 1974. An aceto-carmine squash method for sexing juvenile fishes. *Progress Fish Cult.*, **36(1)**: 56.
- Hanson, T. R., Smitherman, R.O., Shelton, W.L. and Dunham, R.A. 1983. Growth comparison of mono sex tilapia produced by separation of sexes, hybridization and sex reversal. *In: Proceedings of International Symposium on Tilapia in Aquaculture.* (ed. by L. Fishelson and Z. Yaron), Tel Aviv University, Tel Aviv. pp. 570-579.
- Jae-Yoon, J., Smitherman, R.O. and Behrennds, L.L. 1988. Effect of dietary 17alpha-methyl testosterone on sex reversal and growth of *Oreochromis niloticus*. *In: The Second Symposium on Tilapia in Aquaculture.* (ed. by R.S.V. Pullin, T. Bhukaswan, K. Tonguthai and J.L. Maclean), ICLARM conference Proceedings 15, Department of Fisheries, Bangkok, Thailand and ICLARM, Manila, Philippines. pp. 203-207.
- Muir, G.C. and Little, D.C. 1991. Population control in farmed tilapia. *NAGA, ICLARM Quarterly, Manila, Philippines*, **14 (3)**: 8-13.
- Muir, G.C. and Santiago, L.P. 1994. Feminization of Nile tilapia, *Oreochromis niloticus* (L.), by oral application of Diethylstilbestrol (DES). *In: Third Asian Fisheries Forum.* (ed. by L.M. Chou, A.D. Munro, T.J. Lam, T.W. Chen, L.K.K. Cheong, J.K. Ding, K.K. Hooi, H.W. Khoo, V.P.E. Phang, K.F. Shim and C.H. Tan), Asian Fisheries Society, Manila, Philippines. pp. 94-97.
- Okoko, M. 1996. Effect of 17-alpha methyl testosterone concentrations on the sex ratio and gonadal development of Nile tilapia *Oreochromis niloticus*. MS Thesis. Auburn University, Alabama, US. 121pp.
- Penna-Mendoza, B., Gomez-Marquez, J.L., Salgado-Ugerte, I.H. and Ramirez-Nogguera, D. 2005. Reproductive biology of *Oreochromis niloticus* (Perciformes: Cichlidae) at Emiliano Zapata dam, Morelos, Mexico. *Re. Biol. Trop.*, **53**: 515-522.
- Piferrer, C. 2001. Endocrine sex control strategies for feminization of Teleost fish. *Aquaculture*, **197**: 229-281.
- Rashid, M.H. 2008. Effect of stocking density on the growth, survival and production of mono-sex GIFT Tilapia (*Oreochromis niloticus* L.) reared in recirculatory system in cisterns. MS Thesis. Department of Aquaculture, BAU, Mymensingh. 68pp.
- Romerio M-P, C.S., Fenerich-Verani, N., Santo De-Campos, B.E. and Da-Silva, A.S. 2000. Masculinization of Nile tilapia, *Oreochromis niloticus*, using different diets and different doses of 17alpha- methyl testosterone. *Revista Brasileira de Zootecnia*, **29 (3)**: 654-659.
- Smith, E.S. and Phelps, R.P. 2001. Impact of feed storage conditions on growth and efficiency of sex reversal of Nile tilapia. *North American J. Aquacult.*, **63(2)**: 242-245.
- Varadaraj, K., Kumari, S.S. and Pandian, T.J. 1994. Comparison of conditions for hormonal sex reversal of Mossambique tilapias. *Progress. Fish. Cult.*, **56 (2)**: 81-90.
- Vera-Cruz, E.M., and Mair, G.C. 1994. Conditions for effective androgen sex reversal in *Oreochromis niloticus* (L.). *Aquaculture*, **122**: 237-248.
- Yi, Y., Kwei, L.C. and Diana, J.S. 1996. Influence of Nile tilapia (*Oreochromis niloticus*) stocking density in cages on their growth and yield in cages and in ponds containing the cages. *Aquaculture*, **146(3-4)**: 205-215.