# Aqua-Internship Program
# Asia Link Project
# Faculty of Fisheries

## Bangladesh Agricultural University, Mymensingh

## OPTIMIZATION OF DOSE OF 17α-METHYLTESTOSTERONE DURING MASCULINIZATION OF TILAPIA (*Oreochromis niloticus*)


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## Background

Hormonal sex reversal is a technique of changing of sexes from one sex to another in fish by administering synthetic steroid hormones before and/or during the period of sexual differentiation. In this technique the first feeding fry are treated with steroid hormones or androgens (i.e. 17α-methyltestosterone), which develops testes and male sexual characteristics at maturity. The choice of conversion of sexes (either all males or females) depend on growth characteristics of individual sexes of fish species. For example, in tilapia males grow approximately 30% as fast as the females, masculinization using androgen hormones (Shelton *et al.*, 1978; Guerrero, 1979; Guerrero and Guerrero, 1988) have become a popular practice. The use of male sex steroids to induce sex inversions of genotype females into phenotypic males has proven to be one of the successful methods to produce monosex population (Hunter and Donaldson, 1983). The androgen 17α-methyltestosterone are widely used hormones for sex inversion in tilapia. Philippines and Thailand are the two pioneer countries in Asia where mass seed production of all male Nile tilapia (*Oreochromis niloticus*) using androgen hormones are popularly practiced. With regards to hormonal sex reversal of fry, the skill required for administration, the variable success of hormone treatment and aversion to the use of hormone in the production of fish for human consumption are among the factors contributing to the general difficulties in using this method of production of monosex broods in aquaculture (Rahman and Sarder, 2000). Some concerns have been raised questions that consumption of hormone treated tilapia might be harmful for human health. But there is no evidence for any health hazard (Green and Teicher-Coddington, 2000). Recent studies have demonstrated that exogenous steroids are rapidly cleared from tissue after the end of treatment; no residual can be detected within one month of the termination of 17α-methyltestosteron hormone during masculinization of tilapia. I was worked at “Reliance Aqua Farms” in Trishal upazila, Mymensingh for a period of 3 months to acquire practical knowledge and skill on optimization of dose of 17α-methyltestosteron hormone during masculinization of tilapia (*Oreochromis niloticus*).

## Objectives

I. Masculinization of spawn by feeding androgen hormone (MT);
II. To optimize the dose for production of monosex population of tilapia;
III. To analysis the percentage of male tilapia by feeding androgen hormone.

## Methodology

### Study period and area:

The experiment were conducted in the "Reliance Aqua Farms", Bailure, Trishal, Mymensingh for a period of 30 days. Three day old spawns of tilapia were stocked in 05 (five) transitory hapa (6.7 ft × 5.7 ft × 2.5 ft). After 4 days they were transferred in primary treatment hapa (5.7 ft × 10 ft) and after 9 days they transferred in secondary treatment hapa (10 ft × 20 ft).

Three day old spawn of tilapia fry in a transitory tank

Sex reversed fry rearing hapas fixed in a pond

### Experimental designs:

The experiment was designed with 5 treatments ($T_1$, $T_2$, $T_3$, $T_4$ and $T_5$). Each treatment contained 2000 spawns. The spawns were reared for 30 days. Five different doses of MT hormone were used in this experiment. Table 1 showing the dose of hormone used in different treatments.

**Table -1. MT hormone incorporation level in five different diets designated for five treatments.**

| Diet | mg hormone/kg feed |
|---|---|
| $T_1$ | 0 (control) |
| $T_2$ | 40 |
| $T_3$ | 50 |
| $T_4$ | 60 |
| $T_5$ | 70 |

### Diet formulations:

Four diets with different doses of MT hormone i.e., 40, 50, 60 and 70 mg/kg were prepared through ethanol (98%) evaporation method (Mair and Santiago, 1994). To prepare 100g

feed for each treatment; required amount of MT hormone (i.e. 4, 5, 6, 7 mg MT hormone for $T_1$, $T_2$, $T_3$, $T_4$ and $T_5$ respectively) was diluted with 20ml of alcohol for homogenous mixing with the feed in each treatment. The feed which was mixed with the hormone was Mega zero nursery feed. Incase of control required amount of feed not prepared by hormone and ethanol.

Preparation of hormone treated feed

Machine used for mixing hormone with feed

**Process of diet formulation and preservation:**

The required amount of hormones and nursery feed was measured by electrical balance and ethanol was measured by measuring cylinder. At first MT hormone was diluted with ethanol then the hormone mixed ethanol poured into the plastic container, which was already filled with feed. The feed was then stired vigorously for homogenous mixing the hormone. Finally a pest like diet was formed. After completion of the diet, prepared five diets were air dried separately with keeping the lid of the containers open until the diets become fully dry. The diets were then converted into powdery form and preserved in the refrigeration at $4^oC$ for future use.

**Starting of the experiment**

The experiment was comprised of five treatment of different doses (except control which was hormone free) of MT hormone each, with no replication. 2000 spawns were stocked in each hapa.

**Rearing of spawns:**

The spawns were fed with hormone mixed feed 5 times (started at 8.00 am and continued upto 6.00 pm with two hour interval) a day upto satiation.

Fish feed with hormone mixed feed

**Sampling of the fish:**

The fish were sampled at weekly interval to determine the increase in their size. Sampling was done in the early morning when the fish stomach was about to be empty to avoid the biasness of weight due to the presence of excessive feed. The weight was taken in an analytical balance.

**Fish sexing:**

The fish was sexed by gonad squashing and aceto-carmine staining method. The fish was killed and the viscera was removed to reveal the two thread like gonads lying along the surface of the body cavity on either side of the kidney. The gonads were removed and placed on a clean glass slide. A few drops of aceto-carmine stain were added and the gonad squashed with a coverslip. The male sex of the fish was identified by examining the slides under a microscope.

Dissection of fry to reveal the gonad

Addition of aceto-carmine stain with gonad

Sex-identification with a microscope

## Results and Discussion

During starting the experiments, the initial weight of 100 fry were taken. The average increment of weight of fry for the period of 30 days are shown in Table-2 and which are graphically presented in Fig. 1. During sampling at 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ days of experiment significant variations in weight of fry was found, although five different doses of hormone were administered.

Fish from five treatments i.e. fish feed with 0, 40, 50, 60 and 70 mg MT hormone per kg of feed were sexed and the results of sexratios in different treatments are given in Table-3, which are graphically presented in Fig. 2.

**Table-2. Growth in weight (g) of tilapia fry (*O. niloticus*) during the hormonal feeding period.**

| Treatments/days | Av. wt. days 0 | Av. wt. days 7 | Av. wt. days 14 | Av. wt. days 21 | Av. wt. days 28 |
|---|---|---|---|---|---|
| $T_1$ (0 mg/kg) control | 0.01 | 0.02 | 0.05 | 0.12 | 0.20 |
| $T_2$ (40 mg/kg) | | 0.0175 | 0.05 | 0.11 | 0.16 |
| $T_3$ (50 mg/kg) | | 0.019 | 0.055 | 0.115 | 0.17 |
| $T_4$ (60 mg/kg) | | 0.022 | 0.065 | 0.12 | 0.18 |
| $T_5$ (70 mg/kg) | | 0.018 | 0.058 | 0.118 | 0.175 |

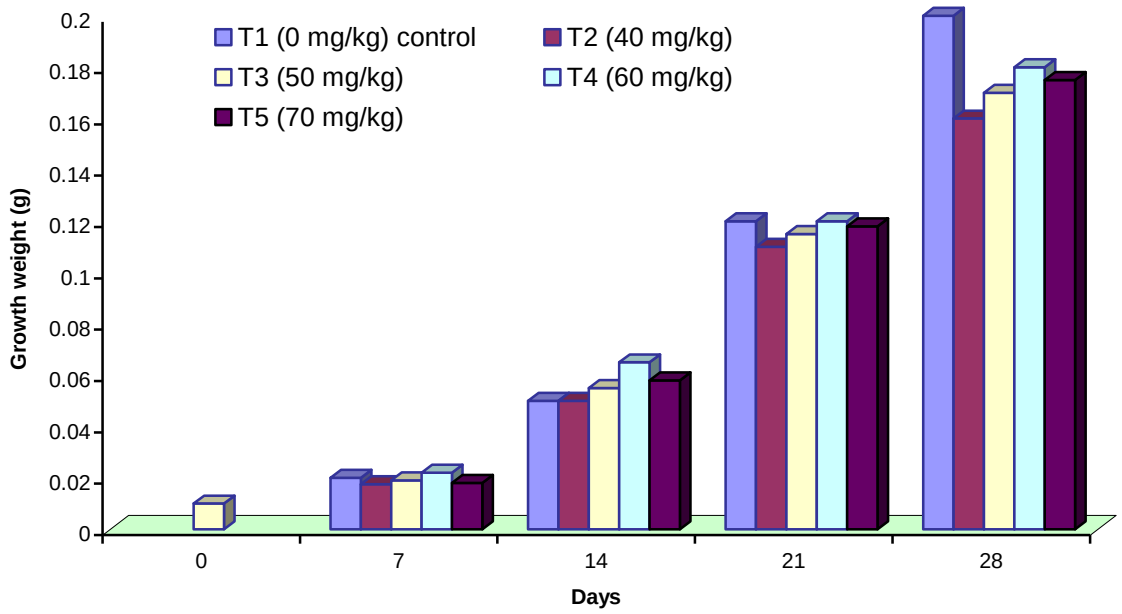


**Fig. 1. Days average growth in weight (g) of tilapia fry *O. niloticus* during the hormonal (MT) feeding of experimental period.**

From above table, we show that the weight of tilapia fry is lower, due to winter month. During winter period, the weight of fry decreases gradually.

**Table-3. Sex ratio of fish at different treatment**

| Treatments | No. of fish dissected | No. of female | No. of male | % of male |
|---|---|---|---|---|
| $T_1$ (0 mg/kg) | 35 | 18 | 17 | 48.57% |
| $T_2$ (40 mg/kg) | 35 | 5 | 30 | 85.71% |
| $T_3$ (50 mg/kg) | 35 | 3 | 32 | 91.42% |
| $T_4$ (60 mg/kg) | 35 | 2 | 33 | 94.29% |
| $T_5$ (70 mg/kg) | 35 | 1 | 34 | 97.14% |

From above table $T_3$ showed 91.42% and $T_4$ showed 94.29% male sex, while $T_2$ showed 85.71% and $T_5$ showed 97.14% male sex. The control group $T_1$ (0 mg/kg) had 48.57% male sex.

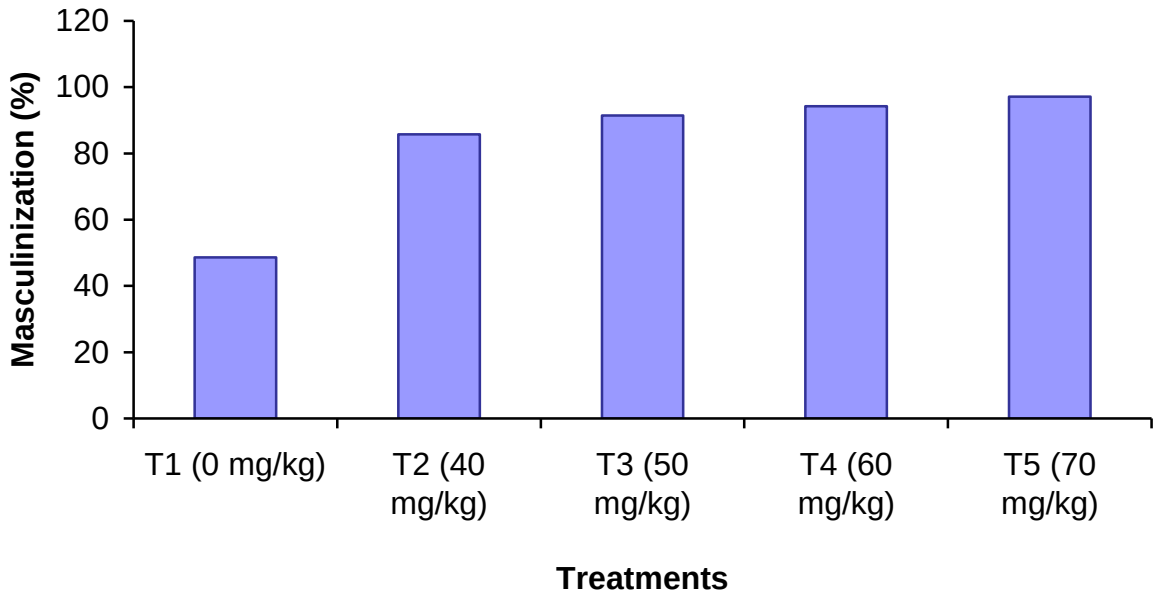


**Fig. 2. Percentage (%) of masculinization at different treatments of MT hormone**

From above Figure, we show that the percentage of masculinization at all the treatment were higher than those of control group (48.57%).

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. In case of masculinization, fish size at the end of

treatment period could be another factor. The masculinization of *Oreochromis niloticus* fry could be unsuccessful if fry failed to attain a standard length of 12 mm by the end of hormone treatment (Dunhum, 1990).

The results of the present research, suggest that the optimum dose of MT hormone were 60 mg/kg and 70 mg/kg with a feeding period of 30 days.

**Benefit derived from my research**

**Benefits for myself:**

- By conducting this research/work we able to know the optimum dose of 17α-methyltestosterone and which dose produce higher percentage of male.
- Four treatments were used to make masculinization. So, progenies from the dose of 60 mg/kg and 70 mg/kg are selected for further rearing and testing whether they are fully YY sex-reversed male, so, this research will helpus further to produce YY supermales in *O. niloticus.*
- We also acquire knowledge and skill of various important factor, that overdosing makes the fish sterile. So, we should not use overdose of hormone.

**Benefits for entrepreneur:**

- The growth of male tilapia is higher than female and female create various problems, because they are prolific breeder. So this experiment will encourage them to produce monosex tilapia (all male) by using optimum dose of hormone (MT).
- By using 60 mg/kg and 70 mg/kg, 94-97% male tilapia production is possible; so they obtain high profit.
- And they do not apply overdose of hormone and their money is saving.

**Constraints:**

During the period of experiments there are many problems, most major problems are-

- Unavailability of fry during research period.
- Increase temperature gradually so egg production of tilapia ultimately reduced, because tilapia do not release eggs until they find suitable environmental parameter.

**Recommendation:**

Before entering this culture technique and optimization of hormonal dose, following point must be followed and should be considered in mind-

- Recommended stocking density for optimum masculinization of tilapia 12 fry/l must be followed during practices;
- During hormone treatment, treatment duration, age and size of fry, availability of natural feed, feeding frequency must be checked carefully, to obtain high rate of masculinization in tilapia.
- Apply of high dose of hormone or overdose should be prevented.
- To get high percentage of male tilapia, the optimum dose of 17α-methyltestosterone hormone and 60 mg/kg, and 70 mg/kg with a feeding period of 30 days must be followed;
- During preparation of hormone made feed, the worker should use gloves and mask, for their safety.

| **Signature of Supervisor** | **Signature of Field Supervisor** | **Signature of Intern** |
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