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# EFFECT OF DIFFERENTIAL DURATION OF ANDROGEN TREATMENT ON GROWTH OF *NILE TILAPIA* UNDER INDIAN CONDITION

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## Abstract
Little is known about the effect of differential duration of androgen treatment on growth performance of *Oreochromis niloticus* in India. In this study, 3 days old juveniles of tilapia were fed with 17α-methyltestosterone treated diet for three different duration regimes: 0 days (control), 30 days (30 days treatment) and 180 days (throughout treatment). After six months of culture, different growth parameters like body weight, length, depth, daily weight gain (DWG), specific growth rate (SGR) and proximate body composition were analyzed. The serum testosterone concentrations of the fish were measured from the 3rd month of culture. It was found that both the 30 days treated and throughout treated categories yielded significantly higher weight, length, depth, DWG, SGR and protein content compared to the control fish. But, there was no significant difference in growth parameters among the two hormone treated groups. Achievement of an optimum saturation level of hormone activity after the application of a particular titre of testosterone may attribute to such trend in growth pattern. The serum testosterone concentration of the 30 days treated fish at the end of culture period is similar to that of the control males. This can validate the human consumption of the 30 days treated tilapia rather than throughout treated ones that have ~2 times more testosterone concentration at the same time. Thus, dietary androgen treatment for the first month of culture followed by rearing with control diet can be regarded as the ideal method for sustainable augmented fish production in India.

**Key Words**: *Nile tilapia*; Androgen treatment; Differential duration; Growth pattern.

## Introduction

Nile tilapia (*Oreochromis niloticus*) has considerable potential for aquaculture in many tropical and subtropical regions in the world (Fitzsimmons 2000). It is currently ranked second only to carps in global production and is likely to be the most important cultured fish in the 21st century (Ridha 2006). In 2003, the global production of tilapia was around 1.5 million mt (Fitzsimmons 2004). Rapid growth rates, high tolerance to low water quality, efficient food conversion, resistance to disease, good consumer acceptance and ease of spawning make tilapia a suitable fish for culture (El-Saidy and Gaber 2005). The climatic and ecological conditions in the eastern region of India are suitable for culture of tilapia and it is widely introduced in the shallow and seasonal ponds of the region. However, ecologists are concerned with the potential adverse effect of this exotic species on the indigenous fish population (Bartley and Martin 2004, De Silva et al. 2004). Moreover, its performance in open water reservoirs of India has been discouraging (Sugunan 2000). Failure of tilapia culture has often been attributed to uncontrolled spawning, producing large numbers of fry and stunted populations. In spite of this, it is felt that tilapia can contribute to the animal protein intake of low-income rural and urban population. Hence, new techniques for maintenance of high growth rate of tilapia are the need of the day.

Tilapia exhibits sexual dimorphic growth where males grow significantly faster, larger and more uniform in size than females (Guerrero and Guerrero 1975). Dietary treatment of fish with exogenous androgens can cause sex reversal to produce all-male tilapia population (Smith and Phelps 2001). Several studies have suggested that hormone treated, sex reversed fish grow faster than nontreated fish (Guerrero 1975, Hanson et al. 1983, Muhaya 1985). But, limited data are available on the growout performance of androgen treated *Oreochromis niloticus* in the Indian context (Pandian and Varadaraj 1988). Besides, effect of hormone treatment

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for different time period on fish growth and validation of human consumption of such androgen treated fish needs to be documented. The objective of the present study was therefore to compare the efficacy of androgen treatment for differential duration to induce growth in tilapia during its intensive monoculture under the climatic and ecological conditions prevailing in the Gangetic plains of India.

## Materials and Methods

Three days old mixed sex juveniles of Nile tilapia (mean weight 0.025 ± 0.009 g; mean length 1.25 ± 0.012 cm) were collected from the Fish Hatchery at Naihati, West Bengal and randomly stocked in three 9 $m^3$ rectangular concrete tanks at a density of 50 fish $m^{-3}$. The fish were cultured in the tanks for six months. Throughout the entire culture period different water quality parameters like temperature, $DO_2$, free $CO_2$, pH, total alkalinity and turbidity were regularly monitored using the standard procedures of American Public Health Association (APHA 1998) and maintained within ideal value limits for all the culture systems (data not shown). During this period, the fish were fed twice daily at a constant rate of 20% body weight/day for the first month, 10% body weight/day for the next two months and 5% body weight/day for the rest of the culture period. Fish in one concrete tank were fed with hormone untreated control diet throughout the entire culture period. Fish in the second tank were given 17αMT treated diet with a dose of 10 mg/kg for the first 30 days and control diet for the rest of the culture duration. Fish in the third tank were provided with 17αMT treated food with a dose of 10mg/kg for the entire culture period. The hormone treated diets were prepared by the alcohol evaporation technique (Shelton et al. 1978). The entire experiment was conducted in three replicating units for statistical validation.

Fish from each tank were measured individually for weight, length and depth every 4 weeks and at the end of the trial. Besides, growth parameters like specific growth rate (SGR), daily weight gain (DWG), food conversion ratio (FCR), protein efficiency ratio (PER) and apparent net protein utilization (ANPU) were measured according to standard formulation (Pechsiri and Yakupitiyage 2005) at the end of the culture period. Equal amount of wet muscle tissue from 10 fish from each experimental set were taken to determine proximate body composition using standard methods (AOAC 1984). Moisture content was measured by drying a sample at $105^0$C in an oven for 24 hours and ash content was estimated by burning the sample at $550^0$C overnight in a muffle furnace. Crude protein and crude lipid were determined using the Kjeltec system 1026 distilling unit and Soxtec system HT 1043 (Tecator, Hognas, Sweden) respectively. To determine these body composites by wet mass, the proportion of dry tissue composed of protein, fat and ash was multiplied by percent solids from the original sample. The percent solid was calculated as the ratio of dry mass to wet mass.

Serum testosterone concentration of fish of same age from the three different treatment categories (control, 30-days treated and throughout-treated) was determined using a commercial ELISA kit from Omega Diagnostics (UK) according to the protocol provided. Blood was collected from the caudal vein of the fish and placed in 1.5 ml Eppendoff tubes without the addition of any anticoagulant. After certain time period the tubes were subjected to centrifugation and the clear upper layer of plasma was isolated with micropipette. This was stored in –$20^0$C until further use as the test sample for the ELISA kit. The samples were brought back to room temperature ($20^0$C to $25^0$C) prior to the start of the assay. At first, 10 µl of the test sample was dispensed into the microtitre well. Then 100 µl of testosterone HRP conjugate reagent and 50 µl of rabbit anti-testosterone reagent was dispensed respectively into the well and mixed thoroughly for 30 seconds. It was then incubated at $37^0$C for 90 minutes. Next, the well was rinsed carefully with distilled water and 100 µl of substrate solution was added into the well. It was gently mixed for 5 seconds and again incubated in the dark at room temperature ($20^0$C to $25^0$C) for 20 minutes. Finally, the reaction was stopped by adding 100 µl of stop solution that was gently mixed for 30 seconds and absorbance at 450 nm was read immediately within 10 minutes using a microplate reader.

The data were expressed in terms of mean ± standard error. All data were subjected to one-way ANOVA. When appropriate, Duncan's multiple tests (at 5%) (Duncan, 1955) was applied to evaluate the differences among means. The statistically homogenous means were denoted by similar alphabets.

## Results

The survival percentage of the fish for control and both the treated categories was around 90%. Hormone untreated control tilapia population showed 50% males while both the treatment duration categories yielded almost 100% male tilapia population with no significant difference (P-value > 0.05) between the two treatment duration groups.

The initial weight, length and depth of all the fish were identical. But after the first month of culture, the

treated fish under both the treatment duration categories grew significantly larger than the control ones (P-value < 0.05) (Figure 1). This trend of growth performance was maintained till the end of the culture period (Figure 1).

Figure 1. Comparative growth patterns (A: Weight; B: Length; C: Depth) of Nile tilapia for differential durations of hormone treatment.

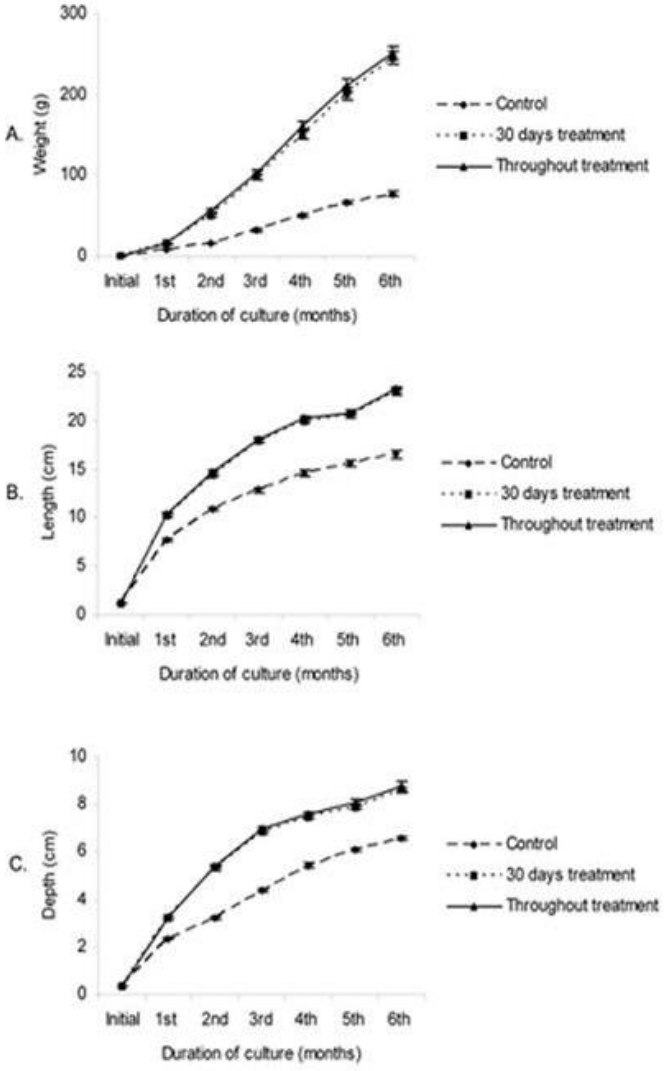


Figure 1

DWG and SGR of fish in both the treated categories were significantly greater compared to the control fish but, ANPU of the treated groups was significantly lower than the control (Table 1). The control fish showed a significantly greater FCR but significantly lower PER than the 30-days treated category while the FCR and PER of throughout treated category belonged to both the higher and lower homogenous subsets (Table 1). Interestingly, treating the fry with hormone for the entire culture period did not yield a significantly better growth than the 30 days treatment duration category as they belonged to the same homogenous subsets in terms of weight, length and depth (Figure 1). Moreover, they showed no significant difference for SGR, DWG, FCR, PER and ANPU values as they belong to the same homogenous subset for all these parameters (Table 1).

In terms of body composition, both the treated groups showed significantly lower water content but significantly higher protein content compared to the control (Table 2). Hormone treatment for both the duration categories had no significant impact on the fat and ash content of the fish (Table 2). Again, there was no significant difference between the two treatment categories as both belonged to same homogenous subset for water, protein, fat and ash content (Table 2).

Table 1. Comparative account of growth parameters for differential durations of hormone treatment. Similar alphabets denote homogenous means.

| Treatment category | Growth parameters | | | | |
|---|---|---|---|---|---|
| | SGR (%) | DWG (g/day) | FCR | PER | ANPU (%) |
| Control | $4.61^{b} \pm 0.05$ | $0.43^{b} \pm 0.02$ | $3.83^{a} \pm 0.08$ | $0.87^{b} \pm 0.02$ | $12.0^{a} \pm 0.4$ |
| 30 days treatment | $5.25^{a} \pm 0.05$ | $1.37^{a} \pm 0.08$ | $3.55^{b} \pm 0.05$ | $0.95^{a} \pm 0.01$ | $6.0^{b} \pm 0.4$ |
| Throughout treatment | $5.27^{a} \pm 0.05$ | $1.4^{a} \pm 0.08$ | $3.68^{ab} \pm 0.04$ | $0.91^{ab} \pm 0.01$ | $5.0^{b} \pm 0.3$ |

Table 2. Comparative account of proximate body composition for different hormone treatment duration categories. Similar alphabets denote homogenous means.

| Treatment category | Body composition (% wet weight) | | | |
|---|---|---|---|---|
| | Water | Protein | Fat | Ash |
| Control | $78.34^{a} \pm 0.6$ | $12.3^{b} \pm 0.4$ | $5.1^{a} \pm 0.3$ | $3.89^{a} \pm 0.2$ |
| 30 days treatment | $75.49^{b} \pm 0.9$ | $15.4^{a} \pm 0.2$ | $4.3^{a} \pm 0.5$ | $3.84^{a} \pm 0.3$ |
| Throughout treatment | $75.73^{b} \pm 0.7$ | $15.6^{a} \pm 0.2$ | $3.98^{a} \pm 0.5$ | $3.89^{a} \pm 0.3$ |

In the 30 days treated group, there was ~1.5 fold increase ($1.2 \pm 0.002$ ng/ml compared to $0.82 \pm 0.03$ ng/ml at 4th month) in serum testosterone level compared to that of control males of same age upto four month stage (Table 3). Beyond that no significant difference in the serum hormone concentration could be detected between the control and the 30 days treated group. For the throughout treatment group, there was ~2 fold increase ($1.6 \pm 0.003$ ng/ml at 4th month) in serum testosterone level compared to the control at the 4th month stage and that value was almost maintained till the end of the culture (Table 3).

Table 3. Concentration of free serum testosterone (ng/ml) in the control males and two groups of hormone treated males from third month to the end of the culture period.

| Treatment category | Culture period | | | |
|---|---|---|---|---|
| | 3rd month | 4th month | 5th month | 6th month |
| Control male | $0.751 \pm 0.02$ | $0.82 \pm 0.03$ | $0.9 \pm 0.01$ | $0.9 \pm 0.01$ |
| 30 days treated male | $1.2 \pm 0.005$ | $1.2 \pm 0.002$ | $0.9 \pm 0.006$ | $0.9 \pm 0.005$ |
| Throughout treated male | $1.5 \pm 0.006$ | $1.6 \pm 0.003$ | $1.6 \pm 0.002$ | $1.6 \pm 0.007$ |

## Discussion

Tilapia belongs to one of the most commercially important groups of freshwater fish in world aquaculture (Coimbra and Reis-Henriques 2005). Farmed tilapia production throughout the world increased dramatically in recent years, increasing from 383,654 mt in 1990 to 2,326,413 mt in 2006 (FAO 2007). India contributes about 9.90% of the total freshwater fish production in the world but its contribution is gradually declining. Tilapia has good potential for the enhancement of production in the fishery sector of India but considerable research is required to adopt different techniques of monosex tilapia culture that are practiced in other countries.

A significantly higher percentage of males in both the androgen treated fish groups compared to the control denote the validity of hormone treatment to produce all-male monosex tilapia population. But, no significant difference in percentage of males between the throughout treated and 30 days treated categories advocates the use of the synthetic androgen for the lower treatment regime as evidenced by a previous study (Chakraborty *et al* 2007). Besides, treatments of fish with excessive androgen can also lead to reduced masculinization, and in some cases induce paradoxical feminization (Devlin and Nagahama 2002). The high survival percentage of the monosex fish indicates that androgen treatment has no adverse effect on the general health of the juvenile fish. Male tilapia grow faster than the females as the females use considerable energy for egg production and eat comparatively less when they are incubating eggs (Tran-Duy *et al*. 2008). Several studies are in agreement that testosterone produces muscle hypertrophy by increasing muscle protein synthesis

(Bhasin *et al* 2001). The higher serum testosterone concentration upto a particular time period, increased growth performance and greater protein content of the treated fish can surely be analyzed considering this knowledge. Failure of throughout treated categories to make a strong impact on the growth performance over the 30 days treated groups may be attributed to the achievement of an optimum saturation level of hormone activity after a particular titre of testosterone is applied. The serum testosterone concentration of the 30 days treated fish at the end of 6 month culture period is similar to that of the control males. This can validate the human consumption of the 30 days treated tilapia rather than throughout treated ones that have ~2 times more hormone concentration at the same time (Table 3). The synthetic male steroid hormone 17α-MT, which is used in this study, has a short half-life of 4 days. Thus, in case of 30 days treatment duration, the administered steroid level should easily be decreased to that in the control males at the time of human consumption after six months of culture.

In many fish including tilapia, it has been reported that the protein requirement of the fish decreased with increasing size (El-Saidy and Gaber 2005). Our study has also indicated the same as observed from the decreased ANPU value with increased size in hormone treated fish than that in the smaller controls (Table 1). There was a general decrease in FCR and increase in PER for treated fish than the controls. Throughout treatment category showed a better food conversion capacity than their corresponding 30 days treated counterpart (Table 1). But, just the opposite scenario was found for capacity to effective protein utilization as 30 days treated fish had better protein efficiency than the throughout treated group (Table 1). Such observation may be related to the fact that FCR decreases while PER increases with increased feeding rate (Pechsiri and Yakupitiyage 2005). Though hormone treatment can increase the SGR and DWG of the fish, differential treatment duration showed no significant effect on these factors as the growth potential of the two treated groups are almost similar. It has been observed that for a given food composition, the body protein percentage on a wet weight basis is mainly affected by the body weight in salmonids (Shearer 1994). Similar observations have been noticed in Nile tilapia also where body protein content increases with wet weight (Pouomogne and Mbongblang 1993; Abdelghany and Mohammed 2002). This explains the higher protein content of the hormone treated fish than controls and more percentage of protein in the throughout treated fish than their 30 days treated counterparts as well.

The main objectives of fishery sector in India are to increase fish production, improve export earnings, provide more animal protein and expand employment opportunities. Culture of tilapia as a cash crop has two basic options: regular culture without sex separation and the culture of monosex male populations. Regular culture without sex separation has often failed in the past because of the "wild spawning" of the tilapia that produces a large number of fry which stunt the entire population (Lèveque 2002). Culture of monosex male tilapia resolves this problem. Moreover, the androgen treated males have higher growth rates than the control males. Thus, additional advantages of larger fish and higher yields are gained through culture of androgen treated sex-reversed tilapia. But, considering the economical aspect of hormone treatment and validation of human consumption of such androgen treated fish, hormone treatment for the first 30 days followed by further rearing with control diet should be the ideal method of choice for a sustainable monosex tilapia culture.

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