

## Effects of testosterone propionate on growth, survival and sex-ratio of African catfish (*Clarias gariepinus* Burchell)

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### Abstract

For studying the effects of different levels of testosterone propionate on growth, survival and sex-ratio, five different doses such as 125, 100, 75, 50, 25 mg hormone per kg feed were administered to 5-day old *Clarias gariepinus* fry through diet for a period of 40 days. The growth performance in terms of weight and length gain of the fry receiving 100 and 75 mg hormone per kg feed were significantly higher than those receiving 50, 25 and 0 (untreated control) mg hormone per kg feed. The groups of fry treated with higher doses of hormone showed lower survival compared to those with lower doses of hormone. The frequency of male fish in all the hormone treated groups except the 125mg/kg group were significantly higher than that of the expected frequency of male fish in a normal population. The highest frequency of male fish, 92.08%, was obtained with the diet containing 50 mg hormone/kg diet however, the highest levels of hormone (125mg/kg diet) resulted in relatively lower frequency of male fish.

**Key words** : Testosterone-propionate, African catfish, Sex-ratio

### Introduction

The diversified and labile sex-differentiation systems in fishes have made it possible to induce sex-reversal by hormonal treatment in many gonochoristic and some hermaphrodite fishes (Pandian and Sheela 1995). Hormonal sex-reversal technique may be used to understand the mechanism of sex differentiation and to produce mono-sex population for aquaculture. Fish production can be improved by adopting mono-sex culture technique either by involving male or female fish, depending on the superiority of growth performance. The adequate supply of fry of a single sex is, however, the main prerequisite of this practice. In the fry or fingerling stages, it is usually not possible to separate physically the fish according to their sex. So it is presumed that the whole population of fry be produced as a single sex. Dietary administration of synthetic steroid hormones has been proved to be the most effective and easy means of producing mono-sex population through hormonal

sex-reversal (Hunter and Donaldson 1983). Steroids can also be administered by immersion (Pifferer *et al.* 1994) and by injection (Shelton 1986). Monosex population can also be produced by producing homogametic parents of both sex (Scott *et al.* 1989, Kavumpurath and Pandian 1992). Depending on the sex determining mechanism neo-males (genotypic females) or neo-females (genotypic male) are produced by administering sex hormone (Shelton 1986, Scott *et al.* 1989, Lahav 1993). After repeated crossing and progeny testing the homogametic broodfish are produced.

The African catfish, *Clarias gariepinus*, has been introduced in Bangladesh in 1989. This fish possesses many characteristics such as faster growth rate, disease resistance and hardiness which are suitable for aquaculture. Production of this fish can be improved by adopting the culture of mono-sex population. Van den Hurk *et al.* (1989) studied the effect of 17 $\alpha$ -methyl testosterone and 11 $\beta$ -hydroxyandrostenedione on gonadal differentiation of *C. gariepinus* and found that from day 28 after hatching these hormones were found to be effective to change the sex-ratio in favour of male. In the present study, an androgenic hormone testosterone propionate has been administered in the first feeding fry of *C. gariepinus* with variable doses in order to optimise the dose for the production of maximum male population. We also report in this paper the comparative growth performance and survival of the fry during the hormone feeding phase receiving different levels of hormone dose.

## Materials and methods

### *Sources of fry and experimental design*

The experiment was conducted with first feeding larvae of *C. gariepinus* which were produced through induced breeding by using human chorionic gonadotropin (HCG) and carp pituitary extracts as inducing agents. The experiment was conducted with 5-day old larvae having an average total length of 7.4 mm and average body weight of 6.8 mg.

Eighteen plastic bowls of 20 cm radius and 21 cm depth were used for the hormonal treatment. The plastic bowls were arranged into 3 rows, 6 bowls each and each bowl contained 300 *C. gariepinus* larvae. The plastic bowls were randomly arranged for six treatments (T<sub>1</sub>-T<sub>6</sub>) each with 3 replications. The fry of treatments T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, T<sub>4</sub>, T<sub>5</sub> and T<sub>6</sub> were fed with diet containing 125, 100, 75, 50, 25 and 0 mg of hormone per kg feed respectively.

### *Preparation of hormone treated feed*

The androgenic hormone testosterone propionate used in this study was collected from local market with the trade name of 'Testoviron' (Germany) in oily preparation. For preparing the hormone-mixed feed 250 mg of hormone was dissolved in 250 ml ethanol which was considered as stock solution. For the diets containing 125, 100, 75, 50 and 25 mg of hormone per kg of feed 75 ml,

60ml, 45ml, 30ml, and 15ml of the stock solution was mixed with 600g of 'SABINCO' shrimp nursery feed respectively. Additional ethanol was added to each of the diet to equalize the volume up to 75ml. The untreated control (T<sub>0</sub>) was also mixed with 75ml of ethanol without hormone. The feeds were then air-dried at room temperature to remove ethanol by evaporation and then oven dried at 30° C for 24 hours. All the feeds were stored in a vacuum polythene bag by sealing the opening and then kept in refrigerator (4°C).

### **Feeding trial and rearing**

The larvae were fed regularly three times daily at 08.00, 14.00 and 22.00 hours at the rates of 35%, 30%, 25% and 20% of body weight per day for the 1st, 2nd, 3rd and 4th 10 days respectively of the 40 days hormone treatment phase. The uneaten feed, debris and faeces were siphoned once daily. The bowls were provided with a continuous supply of water through perforated PVC pipes.

After completion of the hormonal trial the fish were transferred to fibre glass tanks and fed with hormone free artificial feed for two month. The fish were then transferred in a race-way and reared for another four months when sexing of the fish could be done easily with normal 'SABINCO' catfish feed containing 30% protein, 6% lipid, 6% crude fibre and 17% ash.

### **Collection and analysis of data**

A representative of 15 fry from each bowl were randomly sampled for the record of weight (mg) and length (mm) at 10 days interval. The dead fry were removed from the bowl and recorded daily. For studying the sex-ratio in different treatments the fish were sexed morphologically by the genital papillae at the age of about eight months. Sexually mature *C. gariepinus* can be easily sexed on the basis of genital papilla which is elongated and pointed for the males, and short and round for the females. The sex of the relatively smaller fish were confirmed by examining the gonadal condition following dissection.

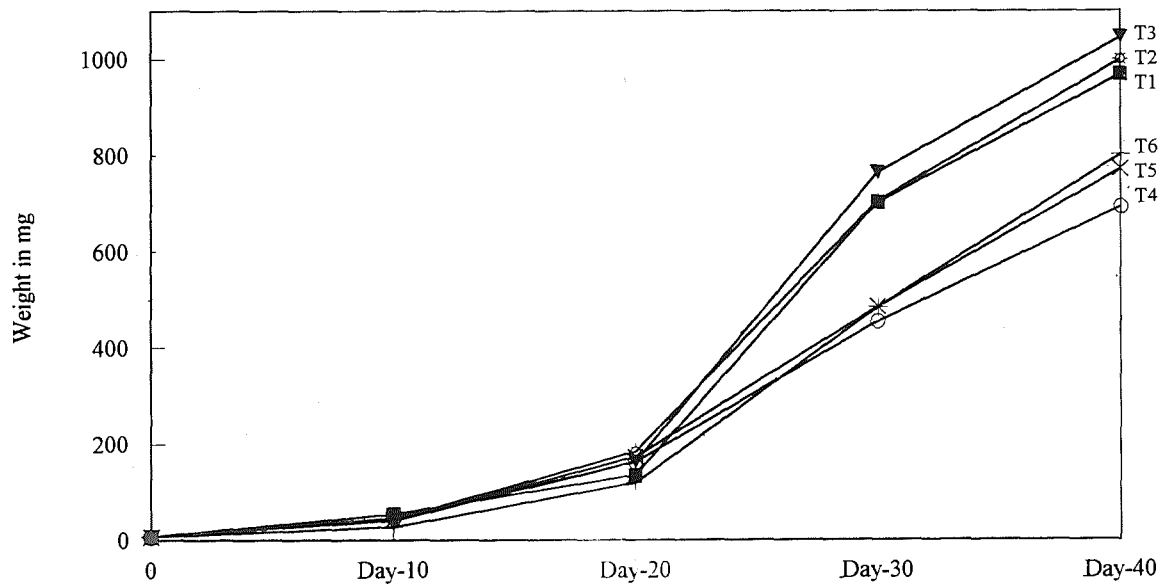
The growth parameters of the larvae were analysed by Analysis of Variance (ANOVA) following completely randomised design and Duncan's New Multiple Range Test (DMRT). The analysis was done by using a computer package 'STATGRAPHICS' version-7. The frequencies of male population in the different treatments were compared with that of the expected frequency of male fish (50%) in a random normal population by Chi-square Test (Zar 1996).

## **Results**

### **Growth and survival**

The increase in body weights of fry of all the treatment groups appeared to be similar up to day-20, variations between the body weights of fry under higher and lower doses became clear later on (Figure 1). Similar trend was also observed in the case of length increments (data not shown). The mean weight

gain of the fry receiving 100 mg ( $T_2$ ) and 75 mg ( $T_3$ ) hormone per kg feed at the end of the 40 days hormone trial phase were found to be significantly higher ( $p < 0.05$ ) than those receiving 50 ( $T_4$ ), 25 ( $T_5$ ) and 0 ( $T_6$ ) mg hormone per kg feed (Table 1). The mean weight gain of the fry receiving 125mg of hormone ( $T_1$ ) was significantly higher than that of  $T_4$  but similar to  $T_5$  and  $T_6$ . No Significant differences were observed among the weight gain of the fry of  $T_1$ ,  $T_2$ , and  $T_3$ , and among  $T_4$ ,  $T_5$  and  $T_6$  ( $P > 0.05$ ) (Table 1). The mean length-gain of the fry of  $T_2$  and  $T_3$  at the end of the 40 days hormone feeding phase were significantly higher than that of  $T_4$ ,  $T_5$  and  $T_6$  ( $P < 0.05$ ). No significant differences were observed among the length-gain of the larvae of  $T_1$ ,  $T_2$  and  $T_3$  and among  $T_4$ ,  $T_5$  and  $T_6$  ( $P > 0.05$ ) The condition factor of the fry of  $T_1$  were significantly higher than those of rest of the treatments ( $P < 0.05$ ). No significant differences were observed among the condition factors of the fry of  $T_1$ ,  $T_2$ ,  $T_3$  and  $T_5$ . The condition factor of the fry of  $T_6$  was found to be the lowest (Table 1).



**Fig. 1.** Mean weight of *C. gariepinus* fry under different treatments during the 40 days hormone feeding phase.  $T_1$ ,  $T_2$ ,  $T_3$ ,  $T_4$ ,  $T_5$  and  $T_6$  represents six different diets containing 125, 100, 75, 50, 25 and 0 mg of testosterone propionate per kg feed respectively.

**Table 1.** Various growth parameters and survival rates of *C. gariepinus* fry fed on diets containing different levels of testosterone propionate

Parameters	Treatments					
	T <sub>1</sub> (125mg/kg)	T <sub>2</sub> (100mg/kg)	T <sub>3</sub> (75mg/kg)	T <sub>4</sub> (50mg/kg)	T <sub>5</sub> (25mg/kg)	T <sub>6</sub> (0mg/kg)
Initial weight (mg)	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7	6.8±1.7
Final weight (mg)	968.4	998.8	1045.3	692.2	770.4	800.7
Weight-gain (mg)	961.6 <sup>ab</sup>	992.0 <sup>a</sup>	1038.5 <sup>a</sup>	685.4 <sup>c</sup>	763.6 <sup>bc</sup>	793.9 <sup>bc</sup>
Initial length (mm)	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8	7.9±0.8
Final length (mm)	50.2	52.0	51.9	46.1	47.6	46.0
Length-gain (mm)	42.3 <sup>ab</sup>	44.1 <sup>a</sup>	44.0 <sup>a</sup>	38.2 <sup>b</sup>	39.7 <sup>b</sup>	38.6 <sup>b</sup>
Condition factor*	0.73±0.1 <sup>a</sup>	0.68±0.1 <sup>b</sup>	0.68±0.1 <sup>b</sup>	0.67±0.1 <sup>b</sup>	0.68±0.08 <sup>b</sup>	0.61±0.16 <sup>c</sup>
Survival (%)	10.33 <sup>c</sup>	10.89 <sup>c</sup>	10.44 <sup>c</sup>	25.44 <sup>a</sup>	23.33 <sup>a</sup>	17.67 <sup>b</sup>

Figures in the same row followed by different superscripts are significantly different at  $P < 0.05$

W

\*Condition factor =  $\frac{W}{L^3} \times 10^5$  where L= Length in mm and W= Weight in g

The survival rates of the fry of T<sub>4</sub> and T<sub>5</sub> were significantly higher ( $P < 0.05$ ) than those of T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub>, and T<sub>6</sub>. The survival rate of the fry of T<sub>6</sub> was significantly higher ( $P < 0.05$ ) than those of T<sub>1</sub>, T<sub>2</sub> and T<sub>3</sub>. No significant differences were observed among the survival rates of T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> (Table 1).

### Sex-ratio

The sex-ratio data of the fish of different treatment groups receiving different doses of testosterone propionate are presented in Table 2. The frequencies of male fish obtained in the treatments having diets containing 25, 50, 75 and 100 mg of testosterone propionate per kg feed (T<sub>2</sub>-T<sub>5</sub>) were found to be significantly higher than that of the expected frequency of male fish (50%) in a random normal population ( $P < 0.05$ ). The frequency of males in the group of fish growing from fry fed on 50mg/kg of feed was found to be the highest (92.08%). On the other hand, the frequency of male fish in T<sub>1</sub> (125 mg/kg) was found to be the lowest and was not significantly different from that of the expected frequency of 50%. The frequency of female and male fish in the untreated control group (T<sub>6</sub>) was also not significantly different from that of the expected ratio of 1:1 in a normal population.

**Table 2.** Frequency of males and females in the groups of fish grown from the fry fed on different levels of testosterone propionate

Sex of fish	Numbers/frequency of fish in different treatments					
	T <sub>1</sub> (125mg/kg)	T <sub>2</sub> (100mg/kg)	T <sub>3</sub> (75mg/kg)	T <sub>4</sub> (50mg/kg)	T <sub>5</sub> (25mg/kg)	T <sub>6</sub> (0mg/kg)
Male	19	34	47	93	40	43
Female	8	9	7	8	10	38
Total	27	43	54	101	50	81
Frequency of female(%)	29.63	20.93	12.96	7.92	20.00	46.91
Frequency of male(%)	70.37	79.07	87.04	92.08	80.00	53.09

## Discussion

### *Growth and survival*

Anabolic steroids, both androgens and estrogens enhance growth and feed conversion efficiency when administered at optimal level in fish (Matty 1985). The anabolic effects of the most frequently used synthetic steroid, 17 $\beta$ -methyltestosterone, have been found to be dose dependent which was reported by a number of authors (Donaldson *et al.* 1979). Yamazaki (1976) found best growth rate of goldfish using 17 $\beta$ -methyltestosterone at a concentration of 1ppm, the growth rate was decreased at a concentration of 10 ppm and weight loss was noticed at a concentration of 30ppm. In the present study, though the effects of various levels of hormone on growth were not clear up to day-20, a dose-dependent response in growth became clear on day-30 onward (Figure 1). The fry receiving testosterone propionate at the doses of 100 and 75mg per kg diet showed significantly higher weight and length gain than those receiving 50, 25 and 0 mg per kg diet (Table 1). No difference in growth performance was observed among the groups of fry receiving 125, 100 and 75 mg of hormone per kg diet. No difference in growth performance was also observed between the groups of fry receiving the highest and the lowest levels of hormone. These findings support that the higher doses of the hormone do not induce growth enhancement rather tend to be catabolic or exert deleterious effects which interfere with the normal processes and retard any gain in anabolic response expected by the increase in dose. In contrast to the findings of the present study, Eversole (1939) found retarded growth when testosterone propionate was administered to 2 months old guppy (*Lebistes reticulatus*) twice weekly for a period of 17 weeks. However, Svårdson (1943) observed (cited by Donaldson *et al.* 1979) a sex and age dependent response of guppy to testosterone propionate. This author found significant increase in length in the sexually mature female fish when the hormone was administered through diet.

From the present study it appears that up to a certain level testosterone propionate has positive effects on growth of *C. gariepinus* fry though these effects could be visible from day-25 after hatching.

The survival rates of the fry during the 40 days experimental period were found to be related to the doses of hormone (Table 1). The groups of larvae receiving higher levels of hormone (125-75 mg/kg feed) showed significantly lower survival rates compared to those receiving lower doses or no hormone. Pandian and Varadaraj (1990) found that the mortality of *Oreochromis mossambicus* increased as the doses of methyl testosterone increased. Torrans *et al.* (1988) also reported that survival of *O. aureus* negatively correlated with the doses of mibolerone when applied by immersion for sex-reversal. As mentioned earlier, the levels of androgens higher than that of the optimal may have deleterious effects on fish which might be the cause of higher mortality. The effects of hormone however, can not be considered as the only factor for such low survival rates because the survival rate of the fry of untreated control was also found to be low (17.67%). The *C. gariepinus* fry in this study were reared on completely artificial feeding that might be the cause of such poor survival rates. It is now established that *Clarias* fry show poor growth and survival with artificial feed (Uys and Hecht 1985, Alam 1988). Administration of hormones through live food such as *Tubifex* sp. worms could be tried to overcome this problem in *Clarias* fry.

### **Sex-ratio**

In the present, study the synthetic androgen testosterone propionate was found to play a significant role in altering the sex of *C. gariepinus* into males. Among the five doses of hormone tested, the dose of 50mg/kg feed was found to be most potent and resulted in 92.08% male fish. Ridha and Lone (1990) reported 90.3% male *O. spilurus* after oral administration of 70 mg 17 $\beta$ -methyltestosterone per kg feed for a period of 38 days. Administration of 50ppm 17 $\beta$ -methyltestosterone for 5 weeks (from 6 to 11 week), resulted in 92.7% males in *Cyprinus carpio* (Komen *et al.* 1989). These findings are more or less similar to the findings of the present study. Goudie *et al.* (1989) reported that due to the antagonistic action of 17 $\beta$ -methyltestosterone the frequency of female fish was higher at higher dosages. The occurrence of relatively more female (less male) at higher levels of testosterone propionate may have been resulted from the same reason. In contrast to the present study, Van den Hurk *et al.* (1989) reported that 17 $\beta$ -methyltestosterone had a feminizing effect at a dose of 100 $\mu$ g/l (immersion treatment) from day 14 after hatching in *C. gariepinus*. However, they mentioned this effect as pharmacological rather than physiological. The same dose of a particular hormone is not equally effective in all species of fish. It has also been reported that the higher doses of hormone might not necessarily produced higher induction in sex-reversal (Guerrero 1975, Woiwode 1978, Ridha and Lone 1990). As the androgenic hormone action is

species specific, for maximum induction of sex-reversal the dose of a particular hormone to be optimised for each species individually. From the present study, it appears that the optimum dose of testosterone propionate in *C. gariepinus* lies within the range of 50-75mg/kg feed.

Like the dose of hormone the success of sex-reversal experiment also depends on optimal duration as well as date of starting of hormone treatment for a particular species. Since the higher doses of hormone produced relatively higher mortality and lower frequency of male fish it is recommended to optimise the duration of testosterone propionate treatment for better masculinization in *C. gariepinus*. As mentioned earlier, the overall survival rates of fry during the hormone feeding phase was found to be low which might also affect the success of the sex-reversal experiment. In the present study, the hormone treatment was initiated from day-5 after hatching. Starting of hormone treatment at later stages would have resulted in better survival and thus better result in sex-reversal.

The objective of a sex-reversal experiment is to produce 100% mono-sex population of a particular sex. The highest frequency of male fish obtained in the present study is 92.08% which indicates that it is possible to reverse the sex mostly or entirely by dietary administration of testosterone propionate in *C. gariepinus*. Further research in this species is however, extremely essential.

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