

The Effect of 17-Alpha Methyl Testosterone on Masculinization, Mortality Rate and Growth in Convict Cichlid (*Cichlasoma nigrofasciatum*)

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Abstract: In the present study, effect of different doses of synthetic androgen 17-alpha methyl testosterone (MT) 30, 60, 100, 150, 200 and 300 mg of MT per kg of feed, on sex reversal, mortality rate and growth performance of Convict Cichlid was evaluated. MT was administrated orally by using pellet dry starter and Ethanol Alcohol, diet to Cichlid fry for 40 days in glass aquaria. The fry were also kept for 6 weeks after feeding to monitor its growth performance, at the end of the experiment the sex ratio was determined by examining the gonads after dissecting the fish. Growth performance was monitored by recording the morphometric characteristics. Wet body weight and total length of fish on six weeks after feeding were measured. The results showed that all MT receiving treatments showed a significantly higher male proportion than the control experiment. Dose rate of 100 mg MT /kg of feed resulted in maximum male population (95.49%) with the least loss of fish. Also the dose rate of 100 mg MT gave the maximum growth in body weight (2.41 g) and total length (46.19 mm), which are 1.8 and 1.4 times greater than the control group, respectively.

Key words: Ornamental Fish • Cichlidae • Dose Rate • Sex Reversal • Alcohol Dry Method

INTRODUCTION

Many species of ornamental fishes exhibit a marked sexual dimorphism and in most cases the male fish, due to their more heavily pigmented bodies and usually more developed fins, are preferred over the female fish by the hobbyists [1]. On the other hand, early sexual maturity in Cichlids culture is a well recognized problem which resulted, inbreeding in overstocked ponds, reduced production and farmed stocks of a generally low quality. To overcome these problems and to develop improved breeding stock of cichlids, researchers decided to produce mono-sex and preferably all male population of cichlids, because the males grows faster than females [2, 3, 4]. Also the males are bigger, more beautiful and more salable than females. Therefore, the maintenance and breeding of male populations have generated a great amount of interest in terms of commercial applications.

There are different ways such as hormonal sex reversal, manual sexing, hybridization and super male production to attain, mono sex population. Sex reversal method has been used as a valuable tool in the elucidation of sex determining mechanisms in addition to its value in production of mono sex population for aquaculture. Sex reversal by oral administration of feed

incorporated with methyl testosterone (MT) is the most effective and practical method for the production of all male populations. Dosage of 17-alpha methyl testosterone (MT) used to produce all male cichlids, vary wildly. Cichlid fishes can be masculinized by direct synthetic hormonal treatment that is efficient and straightforward [5, 6, 7].

Cichlids are an important group of relatively large and often colorful aquarium fishes. The family Cichlidae comprises about 105 genera and 1300 species and it is the second largest family in the Perciformes order [8]. Convict Cichlid *Cichlasoma nigrofasciatum* is a medium-sized cichlid from Central America [9]. In this study it was used as a model fish because of its wide range of tolerance to water hardness, pH and temperature. It is also easily obtainable and can be propagated inexpensively [10-13]. Under optimum conditions the fish produce large numbers of viable eggs (100-800 per batch). The development period from fertilization to hatching is very brief, approximating 3 days at 27°C [14]. This species also shows notable reproductive features, such as a complex social and breeding behavior, which includes parental care and, most importantly, a high spawning rate (about every 20 days during a 9-month period) [11, 13, 15].

The aim of this study was, therefore, to find out optimum dose rate of MT treatment for sex reversal along with its effects on growth performance and mortality rate of Convict Cichlid (*Cichlasoma nigrofasciatum*).

MATERIALS AND METHODS

The sex reversal of Convict cichlid fry was achieved by oral administration of 17-alpha methyl testosterone through feed. A stock of *C. nigrofasciatum*, comprising males (mean weight: 3.53 ± 0.11 g; mean length: 67.04 ± 3.15 mm) and females (mean weight: 2.79 ± 0.14 g; mean length: 56.74 ± 4.73 mm) was procured from a local ornamental fish dealer. Variability in sex ratios among individual spawns in cichlids has been observed by Shelton *et al.* [16]. Therefore, three pairs from this stock were placed in three separate aquariums containing recirculating water ($28 \pm 1^\circ\text{C}$) and exposed to a 16 (light): 8 (dark) photoperiod. All of the larvae used in these treatment studies were obtained from these pairs. These females had spawned -in an earthen jug- the eggs hatched approximately 3 days past fertilizing (dpf) and within 3 days the larvae had free swimming. The fries were immediately removed from the aquariums, counted and placed in 24 small glass aquariums, each containing 40 liter of water that was continuously aerated with a 5-cm air stone and filtered by a normal sponge filter. Each experimental trial consisted of a control group and a group fed with ethanol treated diet (fish were fed with a diet treated with ethanol). For sex reversal treatment six different dosage groups, 30, 60, 100, 150, 200 and 300 mg of MT per kg of food (each in 3 replicates), were set out, MT was added to diet with Alcohol (Ethanol 96°) dry method and each trial was stocked with 40 fish. The fries were fed daily with a commercial diet (dry starter pellet food with 40% crude protein, Biomar brand) 6 times per day, for 40 days. The important water quality parameters were fixed and recorded, temperature (28 ± 1), dissolved oxygen (6.9 ± 0.3 mg/l), hardness (170 ± 10 mg/l) and pH (7.5 ± 0.4). Fries were maintained by increase in 20% body weight (BW) per day and the important growth parameters such as body weight and total length were recorded at 90 dpf (45 days after the end of hormonal treatment). Differences between groups in terms of sex ratio of the offspring were determined on the basis of secondary sex characteristics and macroscopic gonad identification (after dissection) and analyses by the chi-square test [17, 18]. The

secondary sex characteristics used included: males grow larger but are less colorful than females and they develop longer dorsal and anal fins as well as a fatty lump on their foreheads; females develop a pinkish coloration at the base of the dorsal and caudal fins and may also develop a fatty lump on their foreheads but one that is smaller than that found in males. Differences in mortality and differences in body weight and length between groups were tested with the one-way analysis of variance by ranks (SPSS ver. 10.0 for Windows) followed by the Duncan non-parametric multiple comparison procedure.

RESULTS

Effect of MT on the sex ratio; The obtained results showed that each hormone treated group gave a mean male/female ration that deviate significantly from the normal 1:1 ratio (chi square analysis), (Table 1), with significantly higher growth rate in males than females, while the control group showed normal 1:1 ration. Three groups (150, 200 and 300 mg MT /kg feed) of the experimental trial gave all male populations. Also, we achieved 95.49% masculinization in *C. nigrofasciatum* by feeding with 100 mg MT /kg feed for 40 days. The sex ratio observed in 30mg MT /kg feed treatment groups was 84:34 (male: female), while in the 60mg MT /kg feed treatment groups was 91:26 (male: female). These results were interpreted as an indication that increasing concentrations of MT caused an increase in the number of males produced.

Mortality and growth of MT-treated fish; Table 2 shows the survival and growth rates of both control and MT-treated *C. nigrofasciatum* in terms of total body length and body weight. When considering mortality, no significant different was observed between control group, ethanol group, dose 30 and dose 60, but mortality in dose 100, 150, 200 and 300 mg MT /kg feed was significantly different between these groups and significantly higher than the other groups. Control and ethanol groups exhibited 0.83% and 0.0% mortality, respectively. Application of dose rates of 30 and 60 mg MT /kg feed showed 1.66% and 2.50% mortality, respectively. Also, in dose rates, 100 and which were more than 100 mg hormone/kg feed, mortality rate were significantly higher than other treatments. These results were interpreted as an indication that increasing concentrations of MT caused an increase in the mortality rate.

Table 1: Effects of treatment with MT on sex ratio in *Cichlasoma nigrofasciatum*

Dosage MT(mg/kg feed)	Treatment duration (day)	Sex distributions (male:female; M:F)	Sex ratio (%) (M:F)	Chi Square	P
0 (Control)	40	58:61 (n=119)	48.74:51.26	0.08	0.324
0 (Ethanol group)	40	55:65 (n=120)	45.83:54.17	0.83	0.225
30	40	84:34 (n=118)	71.19:28.81	21.19	0.008
60	40	91:26 (n=117)	77.78:22.22	36.11	0.004
100	40	106:5 (n=111)	95.49:04.51	91.90	0.001
150	40	101:0 (n=101)	100.00:0.00	101.00	0.000
200	40	90:01 (n=91)	98.90:01.10	87.04	0.000
300	40	75:00 (n=75)	100.00:0.00	75.00	0.000

Table 2: Effects of treatment with MT on the survival rate and growth performance (mean \pm SE) in *C. nigrofasciatum*

Dosage MT (mg/kg feed)	Survival rate (%)	Mortality rate (%)	Body weight (g)	Total length (mm)
0 (Control)	99.17 \pm 0.83a	0.83 \pm 0.83a	1.35 \pm 0.09a	32.31 \pm 1.12a
0 (Ethanol group)	100.0 \pm 0.00a	0.00 \pm 0.00a	1.29 \pm 0.12a	31.09 \pm 1.10a
30	98.34 \pm 0.83a	1.66 \pm 0.83a	1.42 \pm 0.15a	33.14 \pm 1.83a
60	97.50 \pm 1.44a	2.50 \pm 1.44a	2.02 \pm 0.23b	38.05 \pm 2.24b
100	92.50 \pm 1.44b	7.50 \pm 1.44b	2.41 \pm 0.39c	46.19 \pm 3.65c
150	84.17 \pm 2.20c	15.83 \pm 2.20c	2.08 \pm 0.24b	43.06 \pm 2.18c
200	75.84 \pm 2.20d	24.16 \pm 2.20d	1.97 \pm 0.20b	36.85 \pm 2.72b
300	62.50 \pm 2.50e	37.50 \pm 2.50e	2.05 \pm 0.33b	37.33 \pm 4.14b

Values followed by the same letters within the same column are not significantly different ($P < 0.05$)

At the end of the experiment (90 dpf), total body length and weight were significantly increased in MT treated groups compared to the controls (Table 2). This indicates that MT has a negative effect on the survival rate of *C. nigrofasciatum* (when it used 100 and more than 100 mg/kg feed), but it has the ability to increase total body weight and length at the tested concentrations.

DISCUSSION

The goal of the present study was to find an optimum dose of MT for sex reversal and improving the growth performance in *C. nigrofasciatum*. It was observed that the MT was effective at various dose levels in increasing the proportion of males in the population and improving growth performance in *C. nigrofasciatum*. However, the present results are consistent with reported results from studies with such fish species as *Sciaenochromis ahli*, *Cyprinus carpio* and *Cichlasoma nigrofasciatum*, all of which were treated with synthetic hormone [19, 20, 6].

George and Pandian [6] obtained 82% male population in *C. nigrofasciatum* at dose rate of 200 mg MT /kg of feed in 20 days and Elmdoust [19] showed a successful masculinization in *Sciaenochromis ahli* with 60 mg MT /kg of feed in 30 days. Also, in other

Cichlid fishes, greater than 90% of male population was obtained at a variety of dose rates, Jae-Yoon *et al.* [21] obtained 97% of *O. niloticus* males when applying dose rate of 10 mg MT/kg of diet. Other Authors have used the higher dose rate to achieve sex reversal. *Oreochromis* sp. [22] obtained 98% male population at dose rate of 60 mg MT /kg of feed. The results of other study [23] showed a significantly lower male proportion (84.3%) in *O. mossambicus* for highest dose rate of MT, 100 mg MT /kg of feed. These results are in line with the findings of [24] which obtained 71.9% males at the dose rate of 120 mg MT /kg of feed. Other results showed the over dose such as more than 100 mg MT /Kg give the sterilized population [23].

According to Marjani *et al.* [23], in present study different doses of MT, significantly effected on the growth of *C. nigrofasciatum* and all the treatments which received MT, showed more average of the body weight and the body length than the controls. Dose 75 mg MT for 21 days showed 17.4 g gain in weight followed by dose of 100 mg MT/kg feed and 10.9 g, by dose 50 mg MT/kg feed in *O. mossambicus* [23]. These results are in line with the findings regarding anabolic effect of MT in fish and all male culture of Cichlids by different authors observed faster growth of hormone treated fish [25]. Hanson *et al.* [26] reported that 10-60 ppm MT-treatment showed the best Growth than control,

these are also in line with Dan and Little [27] 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 10.7% larger than mixed sex fish. As a finally result in present study and about the *C. nigrofasciatum* masculinization, best selected dose rate which resulted in maximum male population with the least loss of fish was the 100 mg MT /kg of feed. In addition, the dose rate showed the best growth performance in the fish.

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