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Influence of 17-Alpha Methyl Testosterone on Masculinization and Growth in Tilapia (*Oreochromis mossambicus*)

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Abstract: In the present study, effect of different dose rates of synthetic androgen 17-alpha Methyl Testosterone (MT) i.e., 50, 75 and 100 mg of hormone in per kg of feed, on sex reversal and growth performance of Mozambique tilapia was evaluated. MT was administrated orally by using pellet dry starter and Ethanol Alcohol, diet to tilapia fry for 21 days in glass aquaria. The fry were also kept for 2 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 start of feeding end of feeding and two weeks after feeding were measured. The results of the present study showed that all MT receiving treatment showed a significantly higher male proportion than the control experiment. Dose rate of 75 mg kg⁻¹ MT of feed resulted in maximum male population (98.09%) with 1.91% sterilized fish. The dose rate of 75 mg kg⁻¹ MT gave the maximum gain in body weight i.e., 11.8 g, which is 1.2 times greater than the control.

Key words: Feeding method, tilapia fry, dose rate, sex reversal, growth performance

INTRODUCTION

An objective of this study was to find out the minimum dose and time of 17-alpha methyl testosterone to obtain all male population of Mozambique tilapia (*Oreochromis mossambicus*).

Aquaculture now accounts for over 20% of the world fish production for human consumption.

The consultative group on International Agricultural Research has recently predicted that within 15 years fish farming and sea ranching could provide more than 40% of fish for the human diet and more than half of the value of the global fish catch. The world is in a state of transition from the hunting and gathering of fish to the production of fish by aquaculture. In these days one of the most popular choices for aquaculture is tilapia fishes.

Early sexual maturity in tilapia 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 tilapia, fish scientists, economists and commercial fish producers joined together and decided to produce mono-sex and preferably all male population of tilapia because the male of tilapia grows faster than females (Barras and Melard, 1997).

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 tilapia. Dosage of 17-alpha Methyl Testosterone (MT) used to produce all male tilapia, vary wildly. The dosage rates vary from 10-100 mg kg⁻¹ MT of diet for tilapia (Abucay and Mair, 1997). Sex reversed tilapia showed a better growth rates than normal because administration of androgen have both an androgenic and anabolic effect. There are several studies comparing the growth of sex reversed, near all male populations, to that of a mixed sex population after hormone treatment showed the improved growth of sex reversed fish than non-treated because the presence of females reduce the growth rate due to their slower growth rate or reproduction (Mocintosh *et al.*, 1985). Sex reversal may also affect the meat quality of tilapia (Bhandari Nakamura *et al.*, 2006). There are several stasis in regard of effect of different feed ingredients on meat quality of tilapia but a scarcity of work is persisted about the effect of sex reversal on meat quality (proximate body composition) of tilapia. 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 of tilapia (*Oreochromis mossambicus*) in hydrochemistry factors of Iranian waters.

MATERIALS AND METHODS

The experiment was conducted by Marine Biology Group, Department of Marine Science and Oceanography, Sciences and Researches Branch, Azad Islamic University, Tehran, Iran.

The sex reversal of tilapia fry was achieved by oral administration of 17 alpha methyl testosterone through feed. MT was obtained from Aborihan drug company. At first 400 fertilized eggs were obtained from the moth of several parent females and shifted to circular tank in order to get the tilapia fry after three days (7 day post fertilization (1 dpf)) of hatching, the fries were placed into 12 glass tank each having 65 L water each experimental trial consisted a control group and a group fed with ethanol treated diet (fish were fed with a diet treated with ethanol). For sex reversal treatment three different dosage groups (50, 75, 100 mg kg⁻¹ MT of food (each in 2 replicates) were set out, MT was added to diet with Alcohol (Ethanol 96°) dry method. And each trial was stocked with 35 fish.

The fry were fed daily with a commercial diet (dry pellet food, copens Brand) for 21 days (28 dpf). The important water quality parameters was fixed and recorded, for e.g., temperature (28±1*), dissolved oxygen (7.2±0.2 ms L⁻¹) and pH (7.7±0.3). Fry 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 7 dpf (start of feeding), 28 dpf (end of feeding) and 42 dpf (2 weeks after the end of feeding).

The experimental design was tested by RCBD (Randomized Complete Block Design). At the end of the experiment, the head and tail of the fry were cut off and the body was fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin sectioned at (5 µm thicknesses) and stained with haematoxylin and eosin for histological examination. The tissues were observed under microscope 40x powers. Tissues were recorded as testes (male), ovaries (female), ovotestes or intersex (gonads containing both ovarian and testicular tissues) and sterilized gonads.

Logistic model regression was used to analyze the data of sex reversal. These models were used to study such type of experiment where there is a binary response, Y = 1 (male in this case), Y = 0 (not male), which may be influenced by explanatory variables. For the statistical analysis of the means of growth performance parameters Analysis of Variance (ANOVA) and Tukey test, were carried out. SPSS software was used for statistical analysis and chi square analysis was done to analyze the sex ratio. The present research was conducted from 4 May 2007 to 20 August 2008.

RESULTS AND DISCUSSION

Result of the present study 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. Non of the experimental trial (for dose rate of MT) gave 100% male population of *O. mossambicus*. The results of the present study showed that all MT receiving treatment showed a significantly higher male proportion than control. When considering mortality, no significant different was observed between control group, dose 50 and dose 75 but mortality in dose 100 mg kg⁻¹ MT was significantly higher than the other groups. Control group exhibited 51.96% male population and 0.95% mortality. Application of dose rate of 50 mg kg⁻¹ of MT showed 74.29% male population and 0% mortality. Application of dose rate of 75 mg kg⁻¹ MT of feed resulted in maximum male population (98.09%) with 1.91% sterilized fish. Mortality in this trial was 0%. Application of dose rate of 100 mg kg⁻¹ hormone showed 79.38% male population, 20.61% sterilized fish with 7.62% mortality (Table 1). Mortality and sterilized fish when applying 100 mg kg⁻¹ MT was significantly higher than other treatment.

Greater than 90% of male population were obtained at a variety of dose rates, for e.g., Jae-Yoon *et al.* (1988) obtained 97% of *O. niloticus* males when applying dose rate of 10 mg kg⁻¹ MT of diet. Other researchers have used the higher dose rate to achieve sex reversal.

Oreochromis sp., Romerio *et al.* (2000) obtained 98% male population at dose rate of 60 mg kg⁻¹ MT of feed. The results of this study showed a significantly lower male proportion (84.3%) for highest dose rate of MT, 100 mg kg⁻¹ of feed. These results are in line with the findings of Okoko (1996), who obtained 71.9% males at the dose rate of 120 mg kg⁻¹ MT of feed. Also other results showed the over dose such as 100 mg kg⁻¹ MT give the sterilized population.

Different dose rates of MT, significantly effected the growth of *Oreochromis mossambicus*, all the treatments which received MT, showed more average (Av) Body Weight (WB) and gain in body weight of *Oreochromis mossambicus*, than the control (Table 2). Dose 7.5 mg MT for 21 days showed 17.4 g gain in weight followed by dose of 100 mg kg⁻¹ MT and 10.9 g, by dose 50, 10.1 g at the end of the experiment.

Table 1: Percentage of *Oreochromis mossambicus* classified as male, female and sterilize under control, ethanol group and different MT sex reversal treatment

Treatments	Male (%)	Female (%)	Sterility (%)	Mortality (%)
Control	51.80A	48.20A	0.00C	0.00A
Ethanol group	52.06A	47.94A	0.00C	0.95A
Dose (50 mg kg ⁻¹)	74.29B	25.71B	0.00C	0.00A
Dose (75 mg kg ⁻¹)	98.09C	0.00C	1.91B	0.00A
Dose (100 mg kg ⁻¹)	79.38B	0.00C	20.61A	7.62B

*The Latin character show the result of Tukey analyze, same character don't have significantly difference but unlike character have a significantly difference

Table 2: Observation on average and gain in weight body of *Oreochromis mossambicus* under different treatments

	Control		Ethanol group		Dose (mg kg ⁻¹)					
					50		75		100	
	Av. BW	Gain in BW	Av. BW	Gain in BW	Av. BW	Gain in BW	Av. BW	Gain in BW	Av. BW	Gain in BW
Start of feeding 7 pdf	0.5	-	0.4	-	0.5	-	0.5	-	0.6	-
End of feeding 28 pdf	6.4	6.1	6.6	6.2	7.3	6.8	7.9	7.4	7.5	6.9
2 weeks after end of feeding 42 pdf	16.1	9.7	16.4	9.8	17.4	10.1	19.7	11.8	18.4	10.9

Av: Average, BW: Body Weight

Statistical analysis on gain in body weight, showed a significant difference among different reversal treatments. Treatment received 75 mg kg⁻¹ MT of feed for 21 days (having the highest male percentage of 98.01%). Showed the highest gain in body weight, this fish production is 1.3 times greater than control (received no hormone), these results are in line with the findings regarding anabolic effect of MT in fish and all male culture of tilapia by different authors observed faster growth of hormone treated *O. niloticus*.

Hanson *et al.* (1984) reported that 10-60 ppm MT-treatment showed the best growth than control, these 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 10.7% larger than mixed sex fish.

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