EFFECT OF ANDROGEN ON SEX REVERSAL AND GROWTH OF NILE TILAPIA (*Oreochromis niloticus*)

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In the present study, effect of different dose rates of synthetic androgen 17 alpha methyl testosterone (MT) i.e. 50, 60, 70 and 80 mg/ kg of feed, on sex reversal and growth performance of Nile tilapia was evaluated. MT was administrated orally by using 40% crude protein diet to tilapia fry for 25 days in glass aquaria. After this period fry was shifted to earthen ponds to monitor its growth performance for 280 days (20 fortnights). At the end of experiment the sex ratio was determined by examining gonads after dissecting the fish. Growth performance was monitored by recording the morpho-metric characteristics i.e. wet body weight and total length of fish on fortnightly basis. The results of the present study showed that all MT receiving treatments showed a significantly higher male proportion than control. Dose rate of 70 mg MT/ kg of feed resulted in maximum male population (95.4%) with 1% female and fewer intersexes (2%). The dose rate of 70 mg MT gave the maximum gain in body weight i.e. 283.2 g, resulting in maximum fish production i.e. 3342 kg/ha./year, 1.6 times greater than control.
Keywords: Sex reversal, Nile Tilapia, growth

INTRODUCTION

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 Nile tilapia, fish scientists, economists and commercial fish producers joined together to produce mono-sex, preferably all male population of this species by adopting different techniques i.e. hormonal sex reversal, manual sexing, hybridization and super male etc., because the males of Nile tilapia grows faster than females (Baras and Melard, 1997). 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 monosex 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 17α- methyl testosterone (MT), used to produce all male tilapia, vary widely. The dosage rates vary from 10-70 mg hormone/Kg of diet for Nile tilapia, *Oreochromis niloticus*, (Abidi *et al.* 1992; 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 reduces the growth rate due to their slower growth rate or reproduction (Macintosh *et al.* 1985). Sex reversal may also affect the meat quality of tilapia. There are several studies 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 Nile tilapia.

The aim of this study was, therefore, to find out optimum dose rate of MT treatment for sex reversal along with its effect on growth performance of Nile tilapia (*Oreochromis niloticus*).

MATERIALS AND METHODS

The experiment was conducted at Fisheries Research Farms, Department of Zoology and Fisheries, University of Agriculture, Faisalabad, Pakistan in two phases. In the first phase the sex reversal of tilapia fry was done by oral administration of 17 alpha methyl testosterone through feed and in second phase the growth performance and of this fry was tested.

During phase I, the fertilized eggs of Nile tilapia were obtained from the mouth of female tilapia and shifted to circular tank in order to get the tilapia fry. After three days of hatching, the fry was shifted to five glass aquaria (1 control + 4 treatments) having 65 L of water each, for sex reversal treatment. Three days old fry was administrated orally the androgen; 17 alpha methyl testosterone (MT) mixed in 40% crude protein diet at four different dose rates viz. (T1) 50; (T2) 60; (T,3) 70 and (T4), 80 mg MT/ kg of feed for 25 days. The important water quality parameters viz. water temperature; dissolved oxygen and pH were also recorded. The two parallel replicates were also run. After this the fry were transferred to earthen ponds for their growth studies for a period of 280 days (20 fortnights). The important growth parameters viz. body

weight and total length were recorded after every fortnight by using nylon drag net. At the completion of 20 fortnights all fishes of each treatment were captured by draining out the pond water. Their body weights and total length was measured.

The fishes were examined by microscope examination to differentiate their gonads. Each fish was dissected and the gonadal tissue exposed. Paired gonads were removed with the help of fine forceps and placed on a microscope slide, stained with fast green, covered with another slide and squashed. The tissues were observed under microscope 10 x power. Tissues were recorded as testes (male), ovaries (female) or ovo-testes or intersex (gonads containing both ovarian and testicular tissues).

The experimental design of both experiments i.e. aquarium experiment and pond experiment, was RCBD (randomized complete block design). Logistic model regression was used to analyze the data of sex reversal. These model are 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., while for the statistical analysis of the means of growth performance parameters Analysis of Variance (ANOVA) and Duncan's Multiple Range (DMR) test, was used.. SPSS (1999) package of the software was used for statistical analyses of the data.

RESULTS AND DISCUSSION

An objective of this study was to find out the minimum dose of 17-alpha methyl testosterone to obtain all male population of Nile tilapia (*Oreochromis niloticus*). Results of this study showed that each hormone treated group gave a mean male/female ratio that deviate significantly from the normal 1:1 ratio (Table 1), with the males significantly higher than females, while the control group showed normal 1:1 ratio. No dose rate of MT gave 100% male population of *O. niloticus.* In this study maximum male population (95.4%) of Nile tilapia was obtained at the dose rate of 70 mg MT/kg of feed for 25 days, while the minimum male proportion (80%) was recorded for dose rate of 50 mg MT/kg of feed for 25 days (Table 1). The dose rates of 60 and 80 mg MT/kg of feed were resulted in 89 and 90.7 % males, respectively(Table1). Statistical analysis of the sex reversal data showed a highly significant difference ($P< 0.01$) among all the three sexes i.e. male, female and inter-sex, under all the treatments (Table 1). The logistic regression obtained from the results of present study remained as: logit $\pi = 0.065 + 0.93$ x dose.

Table 1. Percentage of *Oreochromis niloticus* classified as male, female and intersex under control and different MT sex reversal treatments

Treatments	Male %	Female %	Intersex
Control	51.0 d	49.0 a	0.0
T 1	80.0 c	13.8 b	6.2 a
T 2	89.0 b	8.3 c	2.7 d
T 3	95.4 a	1.00 e	3.6 c
T 4	90.7 b	4.0 d	5.3 b

(Values sharing similar letter in a column are statistically non-significant $P > 0.05$)

Logistic regression for the treatments of 'Experiment B' in which Oreochromis niloticus fed 17 alpha methyl testosterone @ 50, 60, 70 and 80 mg /kg of feed for 25 days i.e. T 1, T 2, T 3 and T 4 respectively.

Y = 0.065 + 0.93 x dose

(Y = occurrence of male)

Greater than 90% male populations have been obtained at a variety of dose rates. Jay-Yoon *et al.* (1988) obtained 97% *O. niloticus* males at dose rate of 10 mg MT/ kg of diet. Other authors have used the higher dose rates to sex reverse *O. niloticus*. Vera–Cruiz and Mair (1994) obtained 95 to 98% males with 40 mg MT/kg of diet and 99% with 60 mg MT/kg of diet fed at 20% body weight for 25 days. Abucay and Mair (1997) produced 100% male sex population of Nile tilapia at 40 mg MT/kg of feed for each 15, 20 and 25 days of treatment. Romerio *et al*. (2000) obtained 98 % male population at dose rate of 60 mg MT/kg of feed. Smith and Philps (2001), reported 99-100 % male proportion of Nile tilapia when given MT at 60 mg/kg of feed. Bhandari *et al*. (2006) achieved 100 % masculinization of *O. niloticus* at the dose rate of 50 mg MT /kg of feed. The results of this study showed a significantly lower male proportion (90.7%) for highest dose rate of androgen i.e. 80 mg MT/kg of feed. These results are in line with the findings of Okoko (1996), who obtained 99.3 % males at 30 mg MT, while 97 and 71.9 % males at the dose rates of 60 and 120 mg MT respectively. He further reported that higher dose rates i.e. 240, 480, 600 and 1200 mg MT/kg of feed resulted in no increase of male percentage.

Different dose rates of MT, significantly effected the growth of *Oreochromis niloticus.* All the treatments which received MT, showed more average body weight and gain in body weight of *Oreochromis niloticus* than the control (Table 2, Figure 1). Treatment T3 (70 mg MT for 25 days) showed 283.2 g gain in weight followed by T2 (60mg MT for 25 days) 238.3 g, Treatment T4 (80 mg MT for 25 days) 217.7 g and T1 (50 mg MT for 25 days) 189.8 g at the end of

Table 2. Fortnightly observations on average and gain in body weight (g) of *Oreochromis niloticus* under different treatments

Fort-Night	Control		T1		T2		T3		T4	
	Av. wt.	Gain in wt.	Av. wt.	Gain in wt.	Av. wt.	Gain in wt.	Av. wt.	Gain in wt.	Av. wt.	Gain in wt.
Stocking	0.5	-----	0.4	-----	0.5	-----	0.6	-----	0.4	-----
1	4.7	4.2	4.5	4.1	5.1	4.6	5.4	4.8	4.9	4.5
2	11.4	6.7	10.7	6.2	11.2	6.1	12.9	7.5	11.1	6.2
3	22.6	11.2	21.2	10.5	25.5	14.3	29.0	16.1	26.4	15.3
4	30.2	7.6	33.4	12.3	43.0	17.5	47.4	18.4	44.4	18.0
5	42.5	12.3	47.5	14.0	63.2	20.2	67.6	20.2	65.6	21.2
6	57.4	14.9	65.1	17.6	84.3	21.1	90.0	22.4	85.9	20.3
7	69.3	11.9	82.2	17.1	106.9	22.6	115.5	25.5	107.3	21.4
8	81.2	11.9	100	17.8	129.4	22.5	141.6	26.1	130.0	22.7
9	92.6	11.4	114.3	14.3	149.5	20.1	166.7	25.1	148.2	18.2
10	102.5	9.9	125.1	10.8	168.1	18.6	191.1	24.4	163.6	15.4
11	113.1	10.6	136.2	11.1	186.0	17.9	210.3	19.2	178.7	15.1
12	121.7	8.6	146.3	10.1	200.4	14.4	229.3	19.0	189.3	10.6
13	129.8	8.1	155.8	9.5	210.1	9.7	243.8	14.5	198.2	8.9
14	138.2	8.4	163.9	8.1	217.3	7.2	255.2	11.4	202.7	4.5
15	145.6	7.4	168.4	4.5	223.8	6.5	265.8	10.6	205.7	3.0
16	154.0	8.4	173.6	5.2	228.8	5.0	272.4	6.6	208.8	3.1
17	158.2	4.2	179.8	6.2	233.0	4.2	276.2	3.8	212.9	4.1
18	159.8	1.6	186.1	6.3	236.1	3.1	280.1	3.9	216.0	3.1
19	162.2	2.4	190.2	4.1	238.8	2.7	283.8	3.7	218.1	2.1
Total		**161.7 e**		**189.8 d**		**238.3 b**		**283.2 a**		**217.7 c**

Analysis of variance on gain in body weight of *Oreochromis niloticus*

S O V	d f	Sum of squares	Mean squares	F value
Fortnights	18	6199.021	344.390	48.1530 **
Treatments	4	915.679	288.919	16.0039 **
Error	72	1029.888	14.304	
Total	104	8144.587		

** = Highly significant (at P < 0.01)

S.E. (treatments) = ± 0.8914 **S. E. (fortnights)** = ± 0.6135

Table 3. Total fish production of *Oreochromis niloticus* observed under control and different sex reversal treatments

	Control	T1	T2	T3	T4
Total No. of fish stocked	200	200	200	200	200
Total No. of fish recovered	193	184	194	183	185
Survival	96.5 %	92.0 %	97.0 %	91.5 %	92.5 %
Initial average weight (g)	0.5	0.4	0.5	0.6	0.4
Final average weight (g)	162.2	190.2	238.8	283.8	218.1
Average gain in weight (g)	161.7	189.8	238.3	283.2	217.7
Specific Growth Rate (SGR)	2.07	2.20	2.20	2.20	2.25
Fish production pond/280 days (Kg)	31.21	34.92	46.23	51.82	40.27
Fish production/pond/year (Kg)	40.68	45.52	60.26	66.85	52.49
Fish production/acre/year (Kg)	823.36	921.32	1219.66	1353.04	1062.04
Fish production/hectare/year (Kg)	2033.70	2275.66	3012.56	3342.00	2624.12

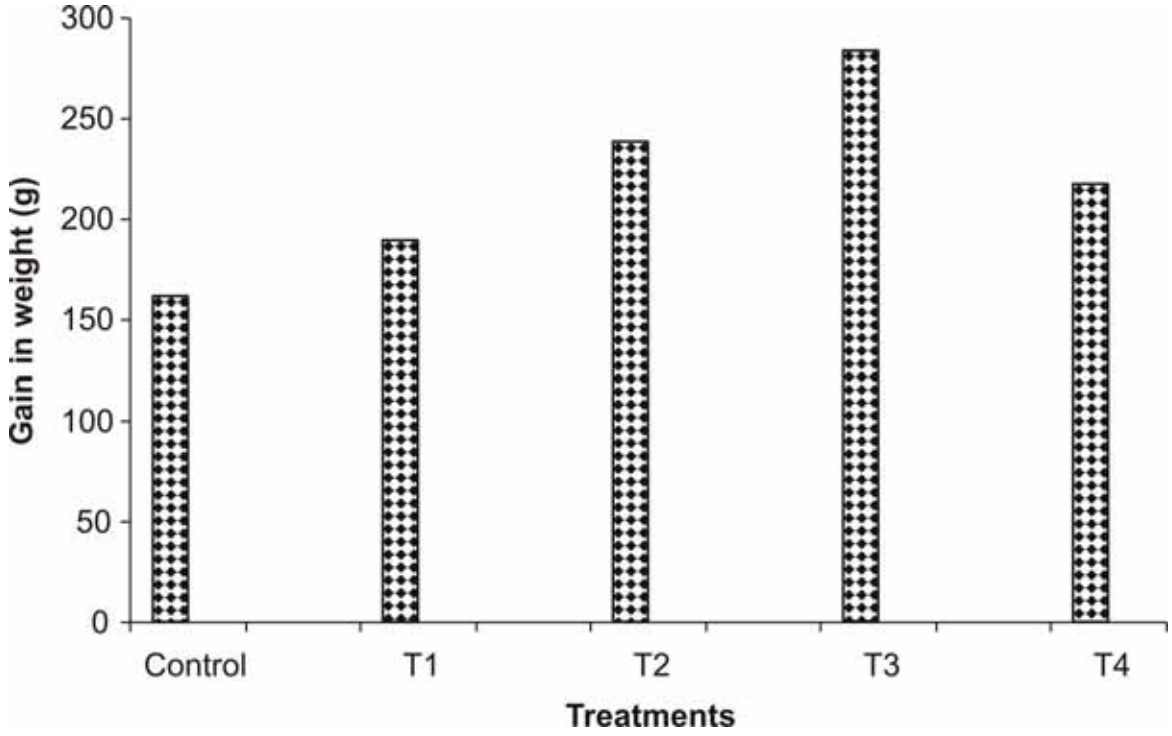

Figure 1. Average gain in body weight of *Oreochromis niloticus* under different sex reversal treatments.

experiment. Statistical analysis on gain in body weight, showed a highly significant difference among different sex reversal treatments and fortnights. Treatment received 70 mg MT/kg of feed for 25 days (having the highest male percentage of 95.4%) showed the highest total fish production of 3342.0.kg/hectare/year (Table 3). This fish production is 1.6 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. Tayamen and Shelton (1978) observed faster growth of hormone treated *O. niloticus*. Hanson *et al. (*1983) reported that 10-60 ppm MT-treatment showed the best growth than control. Varadaraj *et al*. (1994) observed faster growth in *O. mossambicus* when fed 17-alpha methyl testosterone. These results 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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