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ANDROGEN SEX REVERSAL, SUBSEQUENT GROWTH AND MEAT QUALITY OF NILE TILAPIA (*Oreochromis niloticus*)

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Synthetic androgen, 17 α-methyl testosterone (MT), is widely used to reverse the sex in order to get all male progeny in Nile tilapia (*Oreochromis niloticus*). Present project was therefore planned to monitor the effect of different dose rates of MT i.e. 50, 60, 70 and 80 mg/ kg of feed on the sex reversal, growth increments and meat quality (proximate body composition and organo-leptic qualities) of Nile tilapia. MT was administrated orally by using 40% crude protein feed to tilapia hatchling in glass aquaria for 25 days. Then the hatchling was shifted to earthen ponds in order to monitor its growth for 20 fortnights. Organic manure and 40% CP was used in ponds at 1:1. After the experiment the sex ratio was assessed by gonad squash method. Growth performance was evaluated by recording morpho-metric characteristics i.e. body weight and total body length. Meat quality of the experimented fish was determined by proximate body composition following the standard method of AOAC and organo-leptic evaluation. Important water quality parameters were also recorded throughout the experiment. All the treatments MT treatments showed a highly significantly higher male progeny than control which showed normal sex ratio 1:1 (male: female). The dose of 70 mg MT/ kg of feed yielded maximum male proportion (98%) with no female and fewer intersexes (2%). Among the other MT treatments, dose rate of 50 mg resulted in minimum male proportion (80 %), highest female (13.8%) and highest intersexes (6.2%). Dose rate of 60 and 80 mg resulted in 89 and 90.7% male population. Dose rate of 70 mg also gave significantly maximum fish production i.e. 3342 kg/ ha./year, 1.6 times greater than control. Control treatment showed maximum body protein contents i.e. 15.69%, significantly different from all other treatments. 70 mg treatment showed a highly significant difference with respect to total body fats contents having maximum value of 3.52% fat. The lowest bone: meat ratio was exhibited by dose rate of 70 mg i.e. 1:27. All treatments showed a non significant difference in respect of different organo-leptic qualities of meat i.e. colour, texture, softness and overall acceptability except flavour, in which they differed significantly. Water quality parameters recorded during the entire period remained under favourable limits for fish culture. Regression studies revealed a highly significant dependence of fish growth on water temperature and planktonic biomass.

Keywords: Sex reversal, Nile tilapia, growth performance, body composition.

INTRODUCTION

In view of food security and economics, aquaculture and fisheries are the promising fields that supplied the world with high quality food stuff more than 154 million tons of fish in 2011 (FAO, 2012). As a result, the estimated apparent per capita supply is raised to 17 kg, which is an all-time highest so for. According to FAO assessment, an additional 37 million tons of fish per year will be required by 2030 to maintain present levels of fish consumption for an increased world population. The addition of 2 billion more people to the world population by the 2030 will mean that aquaculture will require to produce nearly double that, 85 million tons of fish per year, just to maintain current per capita consumption levels (FAO, 2007).

Among the largest fish species groups contributing towards world aquaculture production, Tilapia is second major species which achieved this remarkable position within few years (FAO, 2012). Tilapia is an optimal agent for fish culture in saline, arid and semi-arid conditions like Pakistan. There is very fast and significant growth trends in tilapia production as exhibited in the last 10 years in different countries of the world by adopting advanced techniques for tilapia culture.

Despite having many good characteristics, the major drawback to tilapia culture is the tendency to breed early and overpopulate a pond. This over population results in competition for food and stunted growth, making it difficult to obtain fish above 150 g. The optimal solution to the said problem is to consider culture of masculine tilapia. Male tilapia exhibits better growth than female. All male/ no female progeny of tilapia could be produced by adopting different methodologies i.e. hormonal sex reversal, hybridization, super male production and applying heat shock during early fry stage.

Inversion of sex through use of hormones has been adopted to develop all male tilapia culture, widely. The synthetic androgen 17α- methyl testosterone (MT), has been an effective tool in producing all male progeny in Nile tilapia, *Oreochromis niloticus* (Mair and Little 1991; Vera Cruz and

Mair, 1994). The hormone is eliminated readily from treated animal and does not have any withdrawal effects (Curtis *et al*., 1991). So, the present research was planned to evaluate the effective dose rates of MT to masculine Nile tilapia and monitoring its growth and meat quality.

MATERIALS AND METHODS

The experiment was conducted in two steps i.e., a) sex reversal, b) growth phase, at Fisheries Research Farms, University of Agriculture, Faisalabad, Pakistan for a period of 22 fortnights. In the first step 3-day old fry of Nile tilapia (*Oreochromis niloticus*) was stocked in 5 glass aquaria, (1 control + 4 treatments) along with three replications in order to reverse its sex. The fry was administrated orally by the androgen, 17 α-methyl testosterone (MT), mixed with 40% CP feed @ 50, 60, 70 and 80 mg MT/kg of feed in four treatments except control feed, which was MT free, for 25 days. The aquaria were supplied with pumped air throughout the experiment to maintain the concentration of dissolved oxygen up to 5-6 ppm. Feed was given to fry three times a day at the rate of 10% of body weight. After 40 minutes of feed application the remaining feed and excreta was removed from the aquaria by siphoning. After 25 days, these treated fry were transferred to earthen ponds to carry out growth phase for 20 fortnights. Organic manure and 40% CP was used in ponds in 1:1 ratio. After the growth period the sex proportion was assayed by using gonad squash method. Growth performance was evaluated by recording morpho-metric characteristics i.e. body weight (g) and total body length (cm). Meat quality of the experimented fish was determined by proximate body composition following the standard method of AOAC (2003) and organo-leptic evaluation. Important water quality parameters were also recorded throughout the experiment (AOAC, 2003). The data of sex reversal was analyzed statistically by using Logistic Model Regression. The growth, meat quality and water quality data was analyzed through ANOVA followed by DMR test (Steel *et al.,* 2007).

RESULTS AND DISCUSSION

The present research endeavor focused on dose optimization of 17 ∝ methyl testosterone for 25 days in order to achieve masculinized progeny of Nile tilapia (*Oreochromis niloticus*). Results of this study revealed that the treatment groups exhibited a mean male/female ratio which departed significantly from the expected 1:1 ratio, with the male portion significantly higher than females. The control group showed a normal 1:1 (male: female) ratio. In this study, the maximum male population (98%) of Nile tilapia was obtained at a dose rate of 70 mg MT/kg of feed for 25 days, while the minimum male proportion (80%) was recorded for a dose rate of 50 mg MT/kg of feed for 25 days. The dose rates of 60 and 80 mg MT/kg of feed resulted in 89 and 90.7% males, respectively (Table 1). Greater than 90% male populations (not including intersex fish) have been obtained at a variety of dose rates. Jay-Yoon *et al.* (1988) reported that the dose of 10 mg MT/ kg of diet yielded 97% *O. niloticus* males, in certain studies, relatively higher dose have been suggested to sex reverse *O. niloticus*. Tayamen and Shelton (1978) obtained 99 to 100% males after treating fish for 25 days at 30 mg and 60 mg MT. Guerrero and Guerrero (1988) obtained 99% males with 30 mg of 17-alpha MT fed for 21 days. 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 a 100% male sex population of Nile tilapia at 40 mg MT/kg of feed for each of 15, 20 and 25 days of treatment. Romerio *et al*. (2000) acheived 98% male progeny by using 60 mg MT/kg of feed. Smith and Philps (2001) reported a 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 a dose rate of 50 mg MT/kg of feed.

Table 1. Percentage of *Oreochromis niloticus* classified as male, female and intersex under control and MT treatments of 25 days

Treatments	% Male	% Female	% Intersex
Control (T0)	51.0d	49.0a	0.0
T1 (50 mg MT/Kg feed)	80.0c	13.8b	6.2a
T2 (60 mg MT/Kg feed)	89.0b	8.3c	2.7c
T3 (70 mg MT/Kg feed)	98.0a	0.0	2.0c
T4 (80 mg MT/Kg feed)	90.7b	4.0d	5.3b

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

In this study, the hormone mixed feed was given at 10% of body weight daily which resulted in up to a 98% male population (Table 1, Fig. 1). Many authors reported a different daily dose rate to obtain more than 95% sex reversal in Nile tilapia. Owuse-Frimpong and Nijjar (1981) achieved 100% male *O. niloticus* at a daily dose rate of 4, 5, and 6% of body weight with 60 mg/kg of 17-alpha MT for 14 days. Okoko (1996) fed hormone mixed feed at 15 % of body weight daily to obtain 99.3% males for 30 mg MT/kg of feed and 97% male for 60 mg MT/kg of feed.

The treatment group that received 70 mg MT/kg of feed for 25 days (T3) resulted in the highest total fish production of 3342 kg/hectare/year (Table 2). The production in the group was 1.6 times higher than the control and these results are harmonious with the findings about the anabolic effect of MT on fish masculanization by various authors. Tayamen and Shelton (1978) reported increased growth rate in hormone treated *O. niloticus*. Hanson *et al. (*1983) described that 10-60 ppm MT-treatment yielded higher growth as

Table 2. Fish production of *Oreochromis niloticus* as observed under control and different sex reversal treatments

Parameters	Control	T 1	T 2	T 3	T 4
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

Table 3. Comparison of means of proximate body composition of *Oreochromis niloticus* under various treatments

Treatments	Moisture %	Protein %	Total Fats %	Ash %	Carbohydrates
Control (T0)	79.73a	15.69ab	2.12d	1.05f	1.41d
T1 (50 mg MT/Kg feed)	79.58a	14.84c	2.20d	1.40e	1.98b
T2 (60 mg MT/Kg feed)	79.43a	15.00bc	2.50c	2.00ab	1.07e
T3 (70 mg MT/Kg feed)	77.11a	15.91a	3.15b	1.95bc	1.07e
T4 (80 mg MT/Kg feed)	78.88a	14.96bc	3.10ab	1.76cd	1.10e

Means sharing similar letter in a column are statistically non-significant P > 0.05.

compared to the control. Howerton *et al*. (1992) and Varadaraj *et al*. (1994) observed faster growth in *O. mossambicus* when fed MT. These findings of the present work corroborate with Dan and Little (2000), who analyzed the comparative growth performance of different strains of *O. niloticus* and concluded that MT treatment led to final size of fish 10.7% higher than mixed sex tilapia.

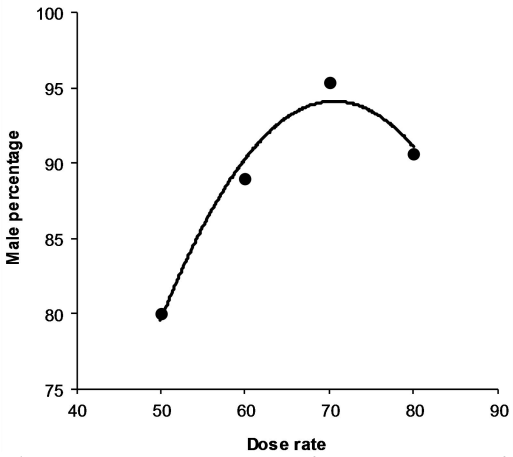

Figure 1. Response curve showing the probability of being male in an *Oreochromis niloticus* population when methyl testosterone was given for 25 days @ 50, 60, 70 and 80 mg/kg of feed.

Logistic regression for the treatments in which Oreochromis niloticus fed 17 alpha methyl testosterone @ 50, 60, 70 and 80 mg /kg of feed for 25 days, respectively.

Y = 0.065 + 0.93 x dose (Y = occurrence of male)

The results of proximate body composition revealed that there was a marked influence of hormone induced masculization on the meat profile of fish. Statistically, the total fats, ash and carbohydrate content showed a highly significant difference among different treatments. The crude protein contents showed a significant difference and moisture content remained non-significant under all treatments. There was a trend towards accumulation of fat that was correlated with MT concentration. Fat content was highest (3.15%) in the treatment group which received 70 mg MT for 25 days and exhibited maximum sex reversal and highest total fish production. The control treatment group had 2.12% fat content (Table 3). These findings are in line with the results reported by Mamun *et. al*. 2004, who found the maximum fat content in sex reversed genetically improved farmed tilapia (GIFT) 3.85% as compared to other strains of Nile tilapia. They also concluded that the superiority of the GIFT strain over other strains might be due to high energy retention which is the ultimate result of higher body fat. In the control (received no MT), the fat content was 2.12% which is similar to the findings of El-Saidy and Gaber 2005, who recorded 2.33% fat in the flesh of normal Nile tilapia when fed a 30 % protein feed. Fat content of meat of Nile tilapia decreased with the increase in moisture content in this study. These findings are also confirmatory to those of El-Sayed (2002) who reported similar results in *O. niloticus*.

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