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Research Article

Impact of 17α-Methyl testosterone on growth performance of *Tilapia mossambica*

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ABSTRACT

The present study was conducted to assess the impact of an androgenic hormone, 17 α - Methyl testosterone (MT) on growth promoting efficiency in *Tilapia mossambica*. The hormone was incorporated in the feed and fed to the fish upto fours weeks, in the form of three pelleted diets containing 4, 8 and 16 mg MT/ kg diet along with a fourth control group without hormone. From the results obtained it is clear that the hormone MT has promoted the growth of fish over the control. Growth performance was monitored by recording the body weight gain (g), liver weight, Gonad weight and food intake. SGR increases with the increase of hormone dose. The study revealed that the 17 α - Methyl testosterone induced growth enhancement of *Tilapia mossambica*.

1. Introduction

One of the major sources of animal protein for human consumption is fisheries source. Therefore, considerable attention has been given to the production and growth of freshwater fish in aquaculture (Juin et al., 2017). In this context, as the growth improvement of cultured fishes using high protein diets has got constrained application in commercial aquaculture due to the high cost (Pandey et al., 2012) and its dubious role in contributing to the nitrogen load in pond ecosystem (Chakraborty and Chakraborty, 1998; Stibranyiova and Paraova, 2000), incorporation of various types of steroids in the diet of cultivable fishes assumes significance (Higgs et al., 1982; Yamazaki, 1983).

17α -Methyl testosterone (MT) is a synthetically produced anabolic and androgenic steroid hormone; i.e. it promotes both muscle growth and the development of male sexual characters. Hanson *et al*. reported that 10 - 60 MT treatment showed the best growth than control.

The androgen, 17α-Methyltestosterone (MT), an anabolic steroid, is being widely used for masculinizationin of fish in a number of species (Amiri-Moghaddam *et al.,* 2010; Kefi *et al.,* 2013; masculinization by oral administration of feed incorporated with the dosage rates are varied for example in swordtail (*Xiphophorus hellerii*) from 10-100 mg MT kg-1 of diet (Khiabani *et al.,* 2014). Sex reversed swordtail showed a better growth rates than normal (Karayucel *et al.,* 2006). Synthetic androgens are used in fish culture as sex controlling agents and as growth promotors if energy is shut away from developing ovaries towards growth of somatic tissues (El-Asaly, 2004 and Rizkallah *et al*., 2004). Many studies have verified that the naturally-occurring, synthetic androgens and estrogens have shown growth-promoting effect in many cultured fishes (Singh and Pandey, 1995; James and Sampath, 2006). The literature on the hormonal enhancement of growth in fish has been reviewed by many authors (Donaldson et al. 1979; Pelissero and Sumpter, 1992). Based on many reports, different supplementations can be added to feed in order to stimulate the growth parameters of fishes (Ajiboye, 2015; Kumar et al., 2016). A number of anabolic steroids both androgenic and estrogenic increase growth and food conversion efficiency when administered in food (McBride and Fagerlund, 1973; Jensi et al., 2016).

In recent years, the anabolic steroids, which are known to enhance growth parameters and reduce the feed-cost in animal husbandry, have attracted the attention of fish farmers.

2. Material and Methods

2.1 *Procurement and maintenance of fish*

In the present study the fish, *Tilapia mossambica* weighing 11-13g were procured from State fisheries culture tanks. They were transported to the laboratory in oxygenated containers and treated with $KMnO_4$ to avoid dermal infection and acclimatized to laboratory conditions for 10 days. The fish were fed with commercial feed once a day at a rate of 2% of body weight before the experimental period. The temperature was maintained at 27 ± 1°C and water in the containers was replaced by fresh water at every 24 h. During experimental period, the fish were fed with control and experimental - steroid containing diets.

2.2 Preparation of control and steroid containing diets:

A control diet was prepared by mixing 30% of fish meal, 30% of soya bean meal, 18% of wheat bran, 13% of yellow corn, 6% of corn oil, 2% of vitamins and minerals premix and 1% of carboxy methyl cellulose.In addition to the control diet, other three experimental diets were prepared with the addition of 4, 8 and 16 mg of 17α -MT/kg of diet. The diets were prepared by spraying the hormone dissolved in 50 ml of 95% ethyl alcohol and mixed well. Glycerine was added at 0.5%/kg by volume to render the harmful effect of the alcohol. The mixture of diet has been completely dried at room temperature and then sealed in air tight black container and stored in refrigerator until use to avoid bacterial or fungal contamination.The diets containing 17α -MT were characterized as follows:

Diet (1): Control diet (without 17α - MT)
Diet (2): 4 mg of 17α - MT/kg of control diet.
Diet (3): 8 mg of 17α - MT/kg of control diet.
Diet (4): 16 mg of 17α - MT/kg of control diet.

Table-1. Ingradients of control diet.

Ingradients	Dry Matter (g 100 g-1)
Fish meal	30
Solvent-extracted soya been meal	30
Wheat bran	18
Yellow corn	13
Corn oil	6
Vitamins	1
Minerals premix	1
Carboxymethylcullulose (CMC)	1
Total	100

Impact of Anabolic androgenic steroid, 17α- Methyl Testosterone on Weight gain, FCE, SGR, HSI, GSI of fish, *Tilapia mossambica* were studied in three experimental fish groups along with control group.

2.3 Growth Parameters:

Growth performance and feed conversion efficiency were calculated as follows.

I. Weight gain/ loss was estimated by Ricker method (1975)
$= W_2 - W_1$.
Where W_1 and W_2 are the Initial and final weight of fish in grams.

II. Specific Growth Rate (SGR) was estimated by Ricker method (1975)
$SGR = 100(\log W_2 - \log W1) / T$
T is the no. of days of the feeding period.

III. Hepatosomatic Index (HSI) was estimated by Parameswaran *et. al.* method (1974)
HSI = Weight of Liver / Weight of fish x 100.

IV. Gonadosomatic Index (GSI) was estimated by Parameswaran *et. al.* method (1974)
GSI= Weight of Gonad / Weight of fish.

V. Food Conversion Efficiency (FCE) was estimated by Ince *et.al.*method (1982)
FCE **=** Weight gain/Feed Intake x 100

The amount of DPPH radical was calculated following this equation: % inhibition of DPPH = [A0- As]/A0 × 100

2.4 Statistical Analysis

In order to calculate the statistical significance between the growths of the control and those of the groups treated with different doses of 17α -MT, t - test was used. The results were statistically analyzed using Duncan's multiple range tests to determine difference in means Software Program of Statistical Analysis (SPSS, 2008).

3. Results and Discussion

3.1 Chemistry

In the present investigation, the effect of Anabolic androgenic steroid, 17α- Methyl Testosterone (Oral mode - MT in Feed) on Weight gain, FCE, SGR, HSI, GSI of fish, *Tilapia mossambica* ,were studied.

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The weight gain in fish was more at higher concentration of 17 α - methyl testosterone hormone (16mg/kg) and higher duration (28 days). Different concentrations of methyl testosterone hormone have been found to be enhanced the growth of fish, *Tilapia mossambica*. The increase of fish growth difference in grams was observed as 7.25 g control, 9.08 g in 4mg/kg, and 10.49gr in 8mg/1kg and 12.17 g 16mg /kg. All the values were significant at $p< 0.001$.

The increase in fish growth may be due to the MT induced the feed digestion and absorption rate causing increase in body weight (El-Greisy and El-Gamal, 2012), or may be MT administration increased the proteolytic activity of the gut as the case in mirror carp leading to increase in growth rate (Lone and Matty, 1981). Hanson *et al.* (1993) reported that 10-60 ppm of MT-treatment showed the best growth than control. Varadaraj *et al* (1994) have observed the faster growth in *Oreochromis mossambicus* when fed 17α-MT.

In the present study, Specific Growth Rate (SGR) of control fish was 1.58±0.082, in 4 mg/kg was 1.75±0.071 ($p < 0.001$), in 8 mg/kg was 1.96±0.089 ($p < 0.001$) and in 16 mg/kg was 2.52±0.098 ($p < 0.001$). SGR in fish was significantly increased and more increase was at higher concentration of methyl testosterone hormone (16mg/kg) and higher duration (28 days). Mc Andrew & Majumdar (1989) reported that feeding *Oreochromis. aureus* fry with MT-40 for a period of 40 days effected a significant increase in SGR. In carp, androgens increased the SGR during the hormone-feeding phase (Lone & Matty 1980, 1982a, b).

Food Conversion Efficiency (FCE) in control was 39.22±1.090, in 4 mg/kg was 43.15±1.171 ($p < 0.001$), in 8 mg/kg 48.01±1.609 ($p < 0.001$) and in16 mg/kg 53.35±1.459 ($p < 0.001$). FCE of fish was significantly increased and more at higher dosage of 17 α - methyl testosterone hormone (16 mg/kg) and higher duration (28 days). In common carp, *Cyprinus carpio* L., Matty & Lone (1979) and Lone & Matty (1980, 1981) demonstrated that natural androgens and MT treatment enhanced the FCE values.

Hepato Somatic Index (HSI) in fish was significantly increased. HSI in control was 3.198±0.265, in 4 mg/kg was 4.441±0.323 ($p < 0.001$), in 8 mg/kg was 5.057±0.506 ($p < 0.001$) and in 16 mg/kg was 5.977±0.215 ($p < 0.001$). HSI in Fish was

Table-2. Effect of 17 α- Methyl testosterone on growth performance, of *Tilapia mossambica.* For 7 days.

Sl.No	Growth Parameters	Effect of 17 α - MT Containing Diet fed for 7 days MT/10 gm body weight of fish			
		Diet-1 Control	Diet – 2-4 mg/kg	Diet – 3-8 mg/kg	Diet – 4-16 mg/kg
1	Initial body weight (gm)	14.35 ± 0.725	14.29 ± 0.882	14.42 ± 0.641	14.37 ± 0.756
2	Final body weight (gm)	16.13 ± 0.497	17.12 * ± 0.415	18.75 ** ± 0.579	20.74 *** ± 0.508
3	weight gain (gm)	1.78	2.83	4.33	6.37
4	Specific growth rate (SGR)	1.29 ± 0.043	1.47 * ± 0.067	1.64 ** ± 0.052	1.83 *** ± 0.05
5	Hepato Somatic Index (HSI)	2.485 ± 0.090	2.890 * ± 0.146	3.237 ** ± 0.186	3.788 *** ± 0.347
6	Gonado Somatic Index (GSI)	1.053 ± 0.029	1.116 * ± 0.027	1.192 ** ± 0.017	1.271 ** ± 0.026
7	Food conversion Efficiency (FCE)	36.94 ± 0.931	39.83 * ± 1.63	43.46 ** ± 1.568	48.51 *** ± 1.253

Table- 3. Effect of 17 α - Methyl testosterone on growth performance, of *Tilapia mossambica.* For 14 days.

S.No.	Growth Parameters	Effect of 17 α - MT Containing Diet fed for 14 days MT/10 gm body weight of fish			
		Diet - 1 Control	Diet – 2-4 mg/kg	Diet – 3-8 mg/kg	Diet – 4-16 mg/kg
1	Initial body weight (gm)	14.26 ± 0.425	14.31 ± 0.72	14.24 ± 0.446	14.47 ± 0.566
2	Final body weight (gm)	17.87 ± 0.392	19.09 * ± 0.398	20.03 ** ± 0.413	21.85 *** ± 0.401
3	weight gain (gm)	3.61	4.78	5.79	7.38
4	Specific growth rate (SGR)	1.45 ± 0.074	1.57 * ± 0.068	1.72 ** ± 0.071	1.92 *** ± 0.076
5	Hepato Somatic Index (HSI)	2.761 ± 0.299	3.374 * ± 0.213	3.823 ** ± 0.456	4.464 ** ± 0.389
6	Gonado Somatic Index (GSI)	1.075 ± 0.038	1.144 * ± 0.042	1.263 ** ± 0.041	1.368 *** ± 0.046
7	Food conversion Efficiency (FCE)	37.68 ± 1.057	41.12 * ± 1.507	44.83 ** ± 1.399	49.62 *** ± 1.457

Each value in the Mean ± S E of six individual observations.
NS: Not Significant ; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

more at higher dosage of 17α - methyl testosterone hormone (16 mg/kg) and higher duration (28 days).

Hepatosomatic index (HSI) is another biological parameter that helps in studying growth of fish (Weatherley and Gill, 1987). Hepatosomatic index of the Nile tilapia; *Oreochromis niloticus* collected from the different studied sites showed progressive natural increase appeared in the untreated control and treated fish that amounted to a significant increase in the treated fish in the last three months of the study. This is in agreement with Ahmad *et al.* (2002) who found that, HSI was significantly changed at low MT doses (0.5, 1.0 and 2.5 mg MT/kg feed) and slightly increase at high MT doses (5, 10, 20 and 40 mg MT/kg feed).

In the present investigation, Gonado Somatic Index (GSI) in fish was significantly increased. GSI in control was 1.072±0.047, in 4 mg/kg was 1.261±0.042 ($p < 0.001$), in 8 mg/kg was 1.484±0.053 ($p < 0.001$) and in 16 mg/kg was 1.693±0.064 ($p < 0.001$). GSI in Fish was more at higher concentration of 17α - methyl testosterone hormone (16mg/kg) and higher duration (28 days). Sevcikova *et. al.* (2016) have observed that the same result in connection with HSI and VSI, Lower body weight together with unchanged liver weight and Visceral organ led to a reduced HSI and VSI value at the highest copper concentration (70 µg/l) in common carp (*Cyprinus carpio L.*) after sub-chronic exposure to copper.

According to the obtained results, 17α - methyl testosterone hormone in different dosages affected fish growth. Higher dosage (16 mg/kg) showed the most important effects from point of view of all growth parameters. The study clearly indicated that the inclusion of the steroid hormone, 17α-methyltestosterone in the diets significantly enhanced the

Table-4. Effect of 17 α - Methyl testosterone on growth performance, of *Tilapia mossambica.* For 21 days.

S.No	Growth Parameters	Effect of 17 α - MT Containing Diet fed for 21 days MT/10 gm body weight of fish			
		Diet - 1 Control	Diet – 2-4 mg/kg	Diet – 3-8 mg/kg	Diet – 4-16 mg/kg
1	Initial body weight (gm)	14.41 ± 0.74	14.52 ± 0.556	14.36 ± 0.617	14.48 ± 0.591
2	Final body weight (gm)	19.66 ± 0.645	21.37 ** ± 0.392	22.13 *** ± 0.635	23.26 *** ± 0.392
3	weight gain (gm)	5.25	6.85	7.77	8.78
4	Specific growth rate (SGR)	1.53 ± 0.063	1.72 ** ± 0.045	2.09 *** ± 0.063	2.38 *** ± 0.073
5	Food conversion Efficiency (FCE)	38.33 ± 1.017	42.27 * ± 1.149	46.97 *** ± 1.444	51.98 *** ± 1.411
6	Hepato Somatic Index (HSI)	3.015 ± 0.194	3.938 * ± 0.262	4.503 ** ± 0.423	5.223 *** ± 0.290
7	Gonado Somatic Index (GSI)	1.064 ± 0.043	1.197 * ± 0.048	1.33 ** ± 0.056	1.486 *** ± 0.061

Each value in the Mean ± S E of six individual observations.
NS: Not Significant ; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

Table-5. Effect of 17 α - Methyl testosterone on growth performance, of *Tilapia mossambica.* For 28 days.

S. No	Growth Parameters	Effect of 17 α - MT Containing Diet fed for 28 days MT/10 gm body weight of fish			
		Diet - 1 Control	Diet – 2-4 mg/kg	Diet – 3-8 mg/kg	Diet – 4-16 mg/kg
1	Initial body weight (gm)	14.28 ± 0.549	14.53 ± 0.423	14.46 ± 0.612	14.34 ± 0.571
2	Final body weight (gm)	21.53 ± 0.491	23.61 *** ± 0.522	24.65 *** ± 0.508	27.11 *** ± 0.481
3	weight gain (gm)	7.25	9.08	10.49	12.17
4	Specific growth rate (SGR)	1.58 ± 0.082	1.75 ** ± 0.071	1.96 *** ± 0.089	2.52 *** ± 0.098
5	Food conversion Efficiency (FCE)	39.22 ± 1.09	43.15 ** ± 1.171	48.01 ** ± 1.609	53.35 *** ± 1.459
6	Hepato Somatic Index (HSI)	3.198 ± 0.265	4.441 * ± 0.323	5.057 ** ± 0.506	5.977 *** ± 0.215
7	Gonado Somatic Index (GSI)	1.072 ± 0.047	1.261 * ± 0.042	1.484 ** ± 0.053	1.693 *** ± 0.064

Each value in the Mean ± S E of six individual observations.
NS: Not Significant ;* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

growth performance of *Tilapia mossambica.* However, significant enhancements among the treatment groups were observed in weight gain, SGR, FCE, HSI and GSI. Higher dosage of 16 mg/ kg was estimated as more effective in growth performance. These findings suggested that 17α - MT can be used efficiently as growth-promoting agent for young tilapia for at least 4 weeks.

Competing Interests

The authors have declared that no competing interests exist.

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