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Original article

Effect of 17α-methyl testosterone incorporated diets on growth of spotted snakehead, *Channa punctatus* and white carp, *Cirrhinus mrigala*

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ABSTRACT

The effect of different concentrations of 17α-methyl testosterone incorporated diet on growth performance in the fry of *Channa punctatus* and *Cirrhinus mrigala* was evaluated. Four different doses of hormone such as 60, 80, 100 and 120 mg/kg in *C. punctatus* and 40, 60, 80 and 100 mg/kg in *C. mrigala* were administered through diet for a period of 90 days.

Fifth group on a hormone free diet served as a control. The growth performance in terms of length and weight gain of the fry receiving 100 mg/kg in *C. punctatus* and 60 mg/kg in *C. mrigala* were significantly higher than those receiving 80, 120 and 0 (untreated control) mg hormone per kg feed. The highest specific growth rate (0.864 ± 1.18%WG d^{-1}) at 100 mg/Kg diet and (2.47 ± 1.26%WG d^{-1}) at 60 mg/kg diet were observed in *C. punctatus* and *C. mrigala* respectively, showing positive influence of hormone incorporated diet on the growth performance. However, the survival rate of both the species remained unaffected by different dosages of 17α-methyl testosterone.

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). Recent studies have verified that the naturally-occurring, synthetic androgens and estrogens have shown growth-promoting effect in many cultured fishes (Kuwaye et al.,1993; Santandreu and Diaz, 1994; 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). Testosterone is probably the most widely studied natural androgen for growth enhancement in fish. It was found to accelerate growth in common carp (Mattiy and Lone, 1979), Coho salmon (McBride and Fagerland, 1976; Fagerlund et al., 1978; Yu et al., 1979), *Channa striatus* (Nirmala and Pandian, 1983) and *Penaeus indicus* (Vatheeswaran and Ali, 1986). Of the androgens investigated, 17α-methyl testosterone was found to be the most potent. Foremost among these as previously mentioned is 17α methyl testosterone, when tested in *Tilapia* (Guerrero, 1975), goldfish (Yamazaki, 1976), and all salmonids responded with weight increases.

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.

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17α-methyl testosterone, an anabolic steroid, is very much recommended in livestock industry (Sindhu and Pandian, 1984). However, investigations on the impact of 17α-methyl testosterone on the food intake and conversion efficiency of fish are still scanty (Arul, 1986). The present experiment was therefore conducted to evaluate the growth inducing potential of 17α-methyl testosterone on the widely cultured freshwater fishes namely spotted snakehead, *C. punctatus* and white carp, *C. mrigala*.

2. Materials and methods

2.1. Collection of experimental fish

Healthy fry of *C. punctatus* (n = 500) and *C. mrigala* (n = 500) were collected from local fish farm and were rinsed immediately with 0.1% KMnO4 solution to avoid infection upon arrival. The fishes were stocked in cemented rearing tanks with proper aeration and acclimatized to laboratory conditions for one week before commencing the experiment. The test fishes were fed *ad libitum* daily on zooplankton and beef liver. They were provided with well-aerated water (Dissolved oxygen 5.8–6.3 mg/l, pH 6.9–7.4 and temperature 29 ± 2 °C). Feeding was stopped 24 h prior to the start of the experiment in view of making the gut empty and the fish feed formulation in the present study was based on the method adopted by Santhanam et al. (1990). All the experiments were conducted in the laboratory using bore water. The water quality parameters during the experimental period are tabulated in Table 1.

The fry of *C. punctatus* (average length and weight, 1.94 ± 0.34 cm and 0.299 ± 0.02 g) and *C. mrigala* (average length and weight 5.28 ± 0.54 cm and 1.49 ± 0.05 g) were randomly divided into five groups of 90 each in triplicate (5 × 30 × 3 = 450 fry) i.e., 30 fry fishes were introduced into each rearing tank of 80 l capacity containing 50 l water. The experimental fish were not disturbed except on certain occasions such as change of water and other growth observations.

Table 1
Water quality parameters from cement tnks during the experimental period.

Particulars	Cement Tanks		
	January	February	March
Water depth (cm)	60–65[a]	62–66[b]	60–65[a]
Water Temp (°C)	28–31[a]	29 - 32[b]	30–31[c]
pH (Water)	6.9–7.4[a]	7.1–7.3[b]	7.1–7.4[b]
DO_2 (mg/l)	5.8–6.2[a]	5.9–6.3[b]	5.8–6.1[a]
Total alkalinity	89–115[b]	80–120[a]	90–110[c]
Total salinity	0.5–0.7[a]	0.5–0.6[b]	0.5–0.6[b]
CO_2 (mg/l)	0.4–0.7[b]	0.3–0.5[a]	0.3–0.5[a]

[a] Means with similar alphabet did not significantly differ at P < 0.05 level by one-way ANOVA and Tukey's multiple range Test.
[b] Means with similar alphabet did not significantly differ at P < 0.05 level by one-way ANOVA and Tukey's multiple range Test.
[c] Means with similar alphabet did not significantly differ at P < 0.05 level by one-way ANOVA and Tukey's multiple range Test.

2.2. Growth performance

The growth parameters such as increase in length, weight gain (WG), specific growth rate (SGR), food conversion ratio (FCR) and survival rate were calculated employing the following equations:

$$\text{Increase in length (cm)} = \text{Final length} - \text{Initial length}$$

$$\text{Weight gain, WG (g)} = \text{Final weight} - \text{Initial weight}$$

$$\text{Specific growth rate, SGR (\%WG/d)} = 100(\text{Ln W2} - \text{LnW1})/t$$

$$\text{Food conversion ratio (FCR)} = \text{dry feed fed (g)}/\text{Wet weight gain (g)}$$

$$\text{Survival (\%)} = 100 \times (\text{Final number of fish}/\text{Initial number of fish})$$

where Ln = Natural log, W1 = Initial weight, W2 = Final weight, t = Culture period in days.

2.3. Hormonal preparation

The androgenic steroid hormone used for the present study is commercially available 17α-methyl testosterone (MT), from the Sigma Corporation (St. Louis, U.S.A.). Stock solutions of the steroid were prepared by dissolving the appropriate amount of hormone in dimethyl suphoxide (DMSO) [Ranbaxy, India]. The stock solutions of hormone were kept in cool dark place and extreme care was taken to avoid the hormone being exposed to light since some hormones were photosensitive (Varadaraj et al., 1994).

2.4. Feed preparation

The feed for the experiments was prepared using the ingredients *viz:* fish meal (52.5 g) groundnut oil cake (38.5 g), rice bran (18 g), tapioca flour (15.5 g), Soyabean (22.5 g) and Vitamin (2 g) and mineral (1 g) tablets (Table 2) to prepare a total of 150 g feed (protein = 36.11%). The mixture was cooked with steam under pressure at 90 lb. The cooled dough was divided into equal portions and each portion was incorporated with the respective concentration of 17α-methyl testosterone (60, 80,100 and 120 mg/kg for *C. punctatus* and 40, 60, 80 and 100 mg/kg for *C. mrigala*). Each

Table 2
Proximate composition of selected ingredients.

Ingredients	Composition (g)	Protein (%)	Lipid (%)	Carbohydrate (%)
Fish meal (waste)	52.50	51.5	50.7	1.4
Groundnut oil cake	38.50	48.9	10.7	6.7
Tapioca flour	15.5	14.9	0.1	43.7
Rice bran	18.0	15.7	20.4	13.4
Soya Bean	22.50	49.56	8.85	1.37
Vitamin (Supraydin[a])	2	–	–	–
Minerals	1	–	–	–
Total	150 g			

[a] Supraydin: Vitamin A.I.P (as Acetate) -10000 I.U, Cholecaliferol I.P (Vitamin D_3)-1000 I.U, Thamine monzitrats (I.P)-10.0 mg, Ribo flavin I.P-10 mg, Pyridoxine Hydrochloride I.P-3.0 mg, Cyanocobalamine I.P-15.0 mg, Nicotinamide I.P-100.0 mg, Calcium Pantothenate I.P-16.3 mg, Ascorbic Acid I.P -150.0 mg, Tocophenol acetate I.P -25.0 mg, Biotin U.S.P-0.25 mg. Minerals: Calcium Phosphate I.P-129.00 mg, Magnesium Phosphate I.P-60.00 mg, Dried ferrous Sulphate I.P-32.04 mg, Manganese Sulphate I.P-2.03 mg, Total Phosperous in the preparation-25.80 mg. Trace elements: Copper Sulphate I.P-3.39 mg, Zinc Sulphate I.P-2.20 mg, Sodium molybdate I.P-0.25 mg, Sodium borate I.P-88 mg.

sample was mixed thoroughly in order to distribute the hormone uniformly. The control diet was prepared in the same manner except that the hormone was omitted. The hormone was incorporated in the feed after cooking and before extrusion. Each sample of feed was made into pellets by pressing through pelletizer and supplied to the test fish as semi moist pellets. The fish were fed twice a day continued till the end of the experiment. The first feeding was given at 08.00 h and the second at 17.00 h. The food was dispersed in small amounts by hand at a rate of 10% of their body weight per day.

2.5. Statistical analysis

The data obtained from each group was expressed as the mean ± standard error followed by one-way analysis of variance (ANOVA) and Tukey's pairwise comparison test using SPSS (version 16 for windows). The differences were considered statistically significant when $P < 0.05$.

3. Results

The growth performance of *C. punctatus* and *C. mrigala* were determined using different concentrations of 17 α-methyl testosterone. The fish were active and healthy in each diet group when compared to control diet and showed increase in growth rate during the experimental period of 90 days. As shown in Table 1, water quality parameters like temperature, pH, dissolved oxygen showed variations during different phases of the experimental duration.

The impact of different diets on growth performance of *C. punctatus* fry is shown in Table 3. Among the experimental diets, compared to day 0, progressive increase in body weight was observed in all the experimental groups at all the time points tested. As shown in Figs. 1 and 2, growth parameters of *C. punctatus* fry fed control and hormone-treated diets were found to be statistically different ($P < 0.05$). The maximum specific growth rate was observed in 100 mg/Kg diet (0.864 ± 1.18%WG d^{-1}) as compared to 60 mg/Kg (0.523 ± 1.36%WG d^{-1}), 80 mg/Kg (0.653 ± 1.38%WG d^{-1}) and 120 mg/Kg (0.763 ± 0.361%WG d^{-1}). Moreover, 100 mg/kg diet showed the best (lowest) food conversion ratio (0.98 ± 0.03%), whereas the highest FCR (1.21 ± 0.02%) was recorded in control diet and the difference was statistically significant ($p < 0.05$). However, maximum survival rate (93.10%) was obtained in control group, when compared to hormone treated groups.

In *C. mrigala*, 60 mg/Kg 17 α-methyl testosterone diet showed a significantly higher growth performance when compared to the control and other concentrations. Increase in length and weight of *C. mrigala* fry fed control and hormone-treated diets were found to be statistically different ($P < 0.05$) as shown in Figs. 3 and 4. As shown in Table 4, the maximum SGR (2.47 ± 1.26%WG d^{-1}) was observed in 60 mg/Kg compared to 40 mg/Kg (2.31 ± 1.51%WG d^{-1}), 80 mg/Kg (2.04 ± 1.81%WG d^{-1}) and 100 mg/Kg (1.90 ± 1.57%WG d^{-1}). However, growth in control was higher than the groups fed 80 mg/Kg and 100 mg/Kg diets. There was significant difference ($p < 0.05$) in FCR for all treatments, lowest (1.05 ± 0.05%) in 60 mg/Kg diet. Maximum survival (94.75%) was obtained in control groups during the hormone treatment period.

4. Discussion

The present investigation is carried out to find out the optimum concentrations of hormone incorporated feed needed for better growth and consumption rate in *C. punctatus* as well as in *C. mrigala*. The results of the present investigation indicate that dietary administration of testosterone 100 mg/kg diet acted as a better anabolic steroid in *C. punctatus*, as compared to 60, 80, 120 mg/kg. However, the 60 mg/kg concentration showed better anabolic results in

Table 3
Impact of 17α-methyl testosterone on length (cm) and weight (g) in *C. Punctatus* SGR: specific growth rate, FCR: feed conversion ratio.

Concentrations of 17 α-methyl testosterone mg/kg diet	Control	60 mg/kg	80 mg/kg	100 mg/kg	120 mg/kg
Average Initial Length (cm)	1.98 ± 0.376	2.17 ± 0.352	1.99 ± 0.333	1.81 ± 0.314	1.78 ± 0.334
Average Final Length (cm)	3.99 ± 0.398	4.12 ± 0.368	4.06 ± 0.372	4.16 ± 0.576	4.01 ± 0.367
Net gain Length	2.01 ± 0.22	1.95 ± 0.016	2.07 ± 0.039	2.35 ± 0.262	2.23 ± 0.033
Average Initial Weight (g)	0.315 ± 0.068	0.367 ± 0.054	0.302 ± 0.053	0.271 ± 0.081	0.243 ± 0.065
Average final Weight (g)	0.504 ± 0.117	0.588 ± 0.184	0.544 ± 0.184	0.590 ± 0.236	0.483 ± 0.092
Net weight gain (g)	0.189 ± 0.049	0.221 ± 0.130	0.242 ± 0.131	0.319 ± 1.55	0.240 ± 0.027
SGR (% Wt gain/d)	0.522 ± 1.602	0.523 ± 1.36	0.653 ± 1.38	0.864 ± 1.18	0.763 ± 0.361
FCR	1.21 ± 0.02	1.18 ± 0.05	1.15 ± 0.04	0.98 ± 0.03	1.02 ± 0.07
Survival rate (%)	92.87	91.54	90.86	90.10	89.20

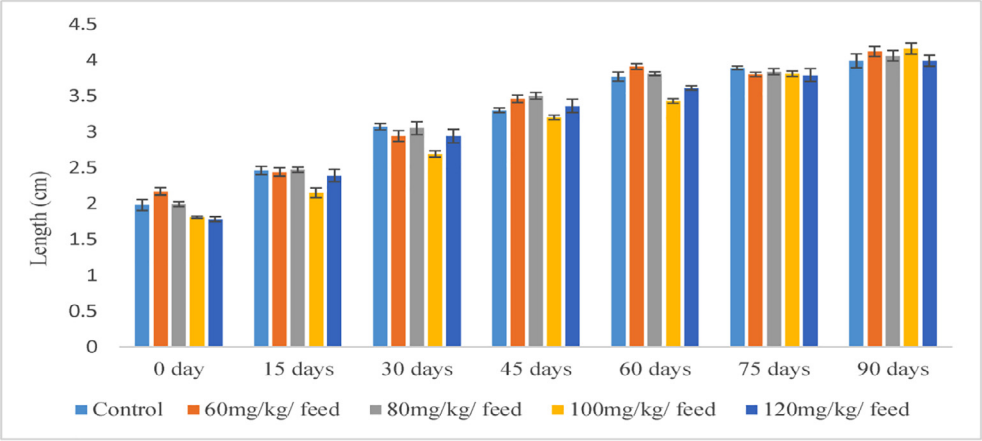

Fig. 1. Growth (Length) performance of *Channa punctatus* using 17α-methyl testosterone.

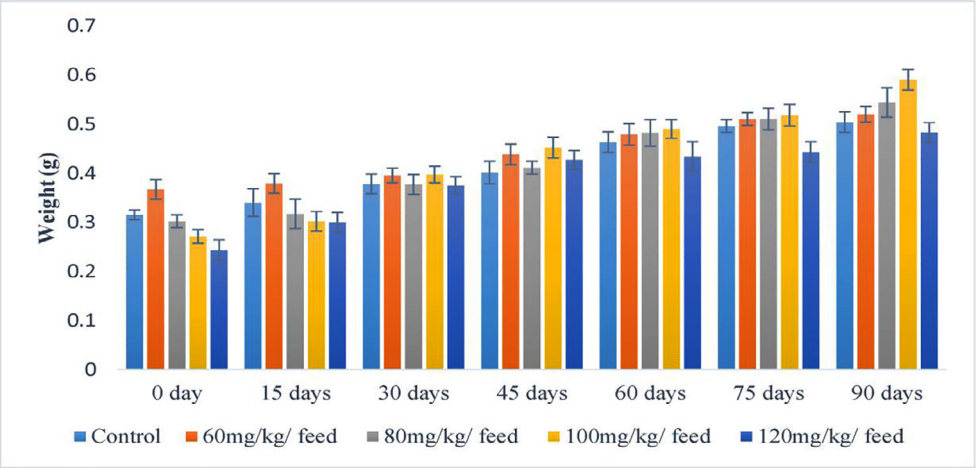

Fig. 2. Growth (weight gain) performance of *Channa punctatus* using 17α-methyl testosterone.

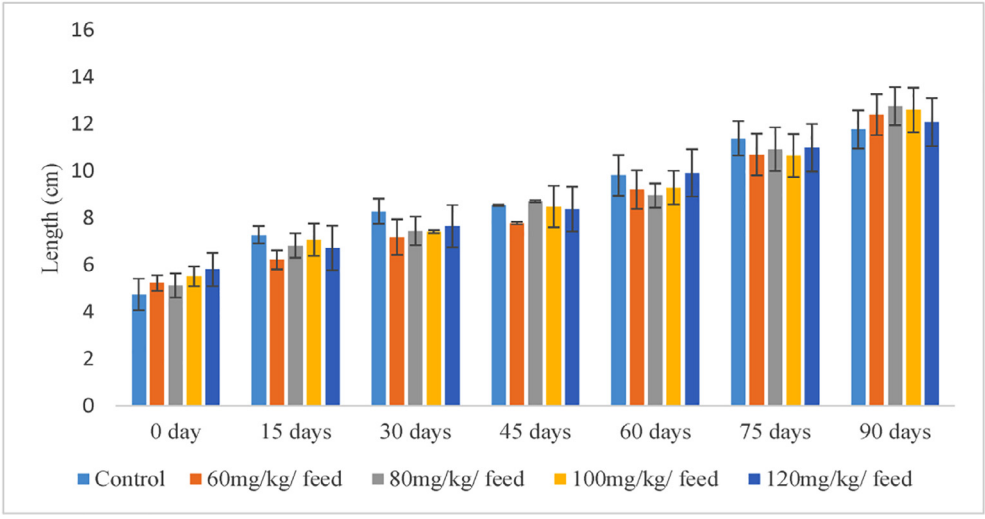

Fig. 3. Growth performance (length) of *C. mrigala* using 17 α-methyl testosterone.

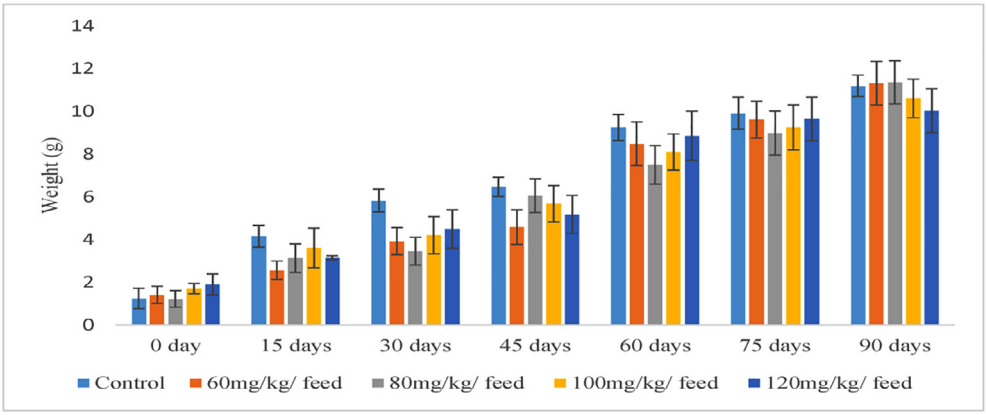

Fig. 4. Growth performance (weight gain) of *C. mrigala* using 17 α-methyl testosterone.

C. mrigala when compared to other treated doses. Faster growth observed in hormone treated *Oreochromis niloticus* by Tayamen and Shelton (1978) and Jensi et al. (2016), was in agreement with the results of the present study. The anabolic effect of testosterone in common carp was reported by Mattiy and Lone (1979) who although at lower concentrations (1.0, 2.5, 5.0 and 10.0 mg/kg diet) observed increased growth in 60 days' culture period. This steroid enhanced growth in *Coho salmon* (McBride and Fagerland, 1976;

Table 4
Impact of 17α-methyl testosterone on length (cm) and weight (g) in *C. mrigala* SGR: specific growth rate, FCR: feed conversion ratio.

Concentrations of 17 α-methyl testosterone mg/kg diet	Control	40 mg/kg	60 mg/kg	80 mg/kg	100 mg/kg
Average Initial Length (cm)	4.74 ± 0.672	5.22 ± 0.334	5.12 ± 0.521	5.51 ± 0.425	5.80 ± 0.714
Average Final Length (cm)	11.76 ± 1.11	12.39 ± 0.87	12.75 ± 0.813	12.59 ± 1.369	12.07 ± 1.446
Net gain Length (cm)	7.02 ± 0.439	7.17 ± 0.536	7.63 ± 0.292	7.08 ± 0.844	6.27 ± 0.752
Average Initial Weight (g)	1.247 ± 0.482	1.407 ± 0.409	1.22 ± 0.39	1.70 ± 0.244	1.899 ± 0.492
Average final Weight (g)	11.19 ± 1.07	11.322 ± 1.60	11.353 ± 1.216	10.67 ± 1.246	10.523 ± 2.03
Net weight gain (g)	9.94 ± 0.588	9.91 ± 1.191	10.13 ± 0.826	8.97 ± 1.002	8.62 ± 1.541
SGR (% Wt gain/d)	2.13 ± 0.66	2.31 ± 1.51	2.47 ± 1.26	2.04 ± 1.81	1.90 ± 1.57
FCR	1.25 ± 0.03	1.22 ± 0.012	1.05 ± 0.05	1.12 ± 0.02	1.21 ± 0.03
Survival rate %	94.75	92.74	92.16	91.17	89.43

Yu et al., 1979), the optimum dosage being 10.0 mg/kg (McBride and Fagerland, 1976). Nirmala and Pandian (1983) found accelerated growth and better food conversion efficiency in *C. striatus* after administration of 5.0, 10.0 and 20.0 mg/kg of testosterone with the best growth occurring at the highest dosage. In *Penaeus indicus*, testosterone at 2.5 mg/kg diet induced growth (Vatheeswaran and Ali, 1986).

Growth depression observed in the present study at two concentrations (80 mg/kg and 100 mg/kg) when compared to control is probably due to the catabolic action as reported by Lone and Matty (1980) in common carp. Unfavorable effects at high doses of this steroid were already reported in *C. striatus* (30 mg/kg) by Nirmala and Pandian (1983). However, Mattiy and Lone (1979) observed increased growth up to 10.0 mg/kg in the European strain of common carp. In the Asian strain of common carp, the optimum dosage appeared to be 2.5 mg/kg diet. The difference in the optimum dosage of testosterone for growth promotion appears to be due to the differential response of the two strains to the hormone treatments.

In the present study the specific growth rate was higher in the four different dosages when compared to the control. In *C. Punctatus*, length 0.924 ± 0.674 cm and weight 0.864 ± 1.18 g was higher at 100 mg/kg., whereas, in *C. mrigala* at 60 mg/kg showed higher results than the control and other dosages. This observation of high specific growth rate and weight showed close resemblance with the results obtained by Basavaraja et al. (1988).

The present study showed considerable increment in consumption rate. In *C. punctatus* fed with 100 mg/kg and *C. mrigala* 60 mg/kg of the FCR was significant than the control. It is known that methyl testosterone induced appetite and enhanced food consumption in *Carassius auratus* (Yamazaki, 1976). Such trends of reduction in consumption rate were reported in trouts and minnows (Fagerlund and McBride 1975; Folmar et al., 2000; Dan and Little 2000). It is also confirmed that at higher dosage a few steroids exhibited some harmful effects (Donaldson et al. 1979). But at low concentration of 40 mg/kg in both the test fishes the consumption rate was less than the control. Diethylstilbestrol and diethylstilbestrol dipropionate in lower dosages (5 and 10 mg/Kg fish) depressed food consumption in *C. striatus*.

Celik et al. (2011) reported 80% survival in *Oreochromis niloticus* which is lower than the result of this study. Control group showed highest survival percentage than treated groups. Survival percentage of control group was 92.87% and 94.75% in *C. punctatus* and *C. mrigala* respectively, which is higher to the results of Celik et al. (2011) and Jensi et al. (2016) who obtained 81.6% and 82% respectively in *Oreochromis niloticus*.

In conclusion, the data presented in the present study, indicate that the hormone 17α-methyl testosterone incorporation in the diet can yield highly significant increases in growth rate and food conversion efficiency of both *C. punctatus* and *C. mrigala*. High doses of this hormone when given can lead to the depression in weight gain as seen in *C. mrigala*. 17 α-methyl testosterone, at a dosage of 100 mg/kg in *C. punctatus* and 60 mg/kg *C. mrigala*, were found to be optimal for these fresh water fishes.

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