



Effect of Testosterone Propionate in Enhancing Growth in *Barilius bakeri*, (1865) Day

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Abstract

Effect of anabolic steroid testosterone propionate (TP) in enhancing growth of *Barilius bakeri* under lab conditions was studied. *B. bakeri* of average size 2.19 ± 0.92 g were collected from river Kallar, Thiruvananthapuram, Kerala for the experiment. Experimental groups were fed with formulated feed supplemented with 1, 2, 3 and 4 ppm of TP respectively. A control group fed without TP was also administered. Each treatment was done in triplicate and length and weight of the fishes were recorded fortnightly. Formulated feed with 3 ppm of TP level showed maximum values in percentage gain in length, weight and specific growth rate (SGR). Feed utilization studies showed that the feed conversion ratio (FCR) was highest in the control group. The food conversion efficiency (FCE), food assimilation efficiency (FAE), protein efficiency ratio (PER) and protein digestibility were highest in fishes fed with 3 ppm TP. There was no significant variation in the proximate body composition among the different treatment groups. The study showed that the administration of TP at 3 ppm level increases growth rate thereby reducing the period of growth and cost of production in *B. bakeri* which may be highly advantageous from a commercial aquarist's point of view.

Keywords: *Barilius bakeri*, testosterone propionate, feed, growth

Introduction

Studies on indigenous ornamental fishes were confined mainly to the breeding aspects and surveys on their availability in different water bodies. There is a limit to the maximum growth in fishes which can be obtained in normal conditions. If fish has to be grown beyond physiological limits, it has to be either genetically manipulated or should be administered hormones or growth promoters (Keshavanath, 2005). The androgen, 17 α -methyl testosterone (17 α -MT) has been extensively experimented to promote growth in several species of fishes. James & Sampath (2006) recommended the optimum dosage of 10 mg kg⁻¹ of MT for attaining higher SGR in *Xiphophorus helleri* and *Betta splendens*. Kortner et al. (2009) in their work on the effect of keto testosterone has found that this hormone plays a significant role in growth and development of oocytes in Atlantic cod. *B. bakeri* is an endemic indigenous ornamental fish of Kerala. The fish is being caught from wild in large quantities and is being exported as aquarium fish. Though there have been studies regarding their abundance as well as breeding behaviour of indigenous ornamental fishes in the water bodies of Kerala (India), there are only limited studies related to their growth performance using growth promoters. The objective of the present study is to arrive at optimum dosage of testosterone propionate required to bring about maximum growth in *B. bakeri*. This knowledge would be useful for aquarists as the attainment of maximum size of the fish at the shortest time is important for better market value for the ornamental fish.

Materials and Methods

Juveniles of *B. bakeri* of average size 2.19 ± 0.92 g were collected from river Kallar, a hill stream near

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Ponmudi Hills in Trivandrum, Kerala, India and were acclimatized for a week. Subsequent to acclimatization, they were introduced into experimental tanks of size 90 x 45 x 45 cm supplied with aged tap water and provided with a biological filter. Both long term experiments and short term experiments were conducted and a triplicate was maintained for each treatment. For long term experiment for 75 days, fishes were stocked at the rate of 12 per tank. Fishes were fed @ 5% body weight twice daily at 10.00 and 16.00 h. Water temperature was recorded with a mercury thermometer and pH using a digital pH meter. Dissolved oxygen, total alkalinity, ammonia, nitrite and nitrate were estimated following standard methods of APHA (1992) and were monitored throughout the experimental period. About half the quantity of water in the tank was replaced weekly and water quality parameters were kept within tolerable limits. Fishes were taken out from each experimental tank fortnightly and their length and weight were recorded. The gross body or carcass composition of the control and experimental fish were analysed after 75 days. Five fish from each tank were collected, killed and weighed. They were minced properly, dried and stored in airtight vials. In the case of short term experiment, the quantity of feed was fixed @ 10% dry weight and each time the feed was left in the tank for 6 h. Unconsumed feed was siphoned out and dried in the oven at 60°C till it reached a constant weight and weighed. Faecal matter was siphoned out before the next feeding. The faecal matter thus collected was sieved through blotting silk and residue weighed after drying in the oven at 60°C till it reached a constant weight. The dried faecal matter was then stored in the desiccator until further analysis. The quantity of feed was reassessed at an interval of 15 days based on the weight of the fish. After 30 days, the final weight of all the fish was recorded. The calculations were done based on the formula of Kesavanath et al. (1991).

Dried anchovy (fishmeal), groundnut oil cake (GOC), tapioca powder and rice bran (RB) were used for the preparation of the feed. All the ingredients were dried, powdered and sieved thoroughly into fine powder. The proportion of the ingredients for making the feed and their composition are given in Table 1.

The ingredients were mixed, hand kneaded with sufficient quantity of water and made into a soft dough. The dough was steam cooked for 20 min to improve the digestibility of feed. It was then cooled before adding Testosterone propionate, vitamin and minerals. Testosterone propionate under the trade name Testosterone (Infar India Limited) at 1, 2, 3 and 4 ppm were incorporated in to the diet. The final product was pelletised with a pelletiser and dried in an oven below 40°C. The dried pellets were broken into small pieces and stored separately in air tight containers. The control feed without TP was also prepared in a similar way.

Total protein, lipid, moisture, ash and fibre of the samples were determined based on the methodology followed for the proximate composition of feed ingredients. The glycogen content was estimated by anthrone method by Oser (1965). Total nitrogen was estimated by Micro-Kjeldahl method and the protein was computed by multiplying the nitrogen value with the factor 6.25 based on Belcher & Godbert (1954). For carbohydrate estimation, phenol-sulphuric acid method by Dubois et al. (1956) was followed. Total lipid estimation was done by the method of Bligh & Dyer (1959). Gross energy (KJ g⁻¹) was calculated using the method of Jia et al. (1991). Moisture content was determined by keeping the samples in hot air oven at 95 to 100°C for 30 min and then dried at 60°C till a constant weight was obtained. The crude fibre content in the samples was determined by acid base digestion and ash content by burning the sample at 550 ± 20°C for 6 h in a muffle furnace based on AOAC (2000).

Table 1. Proximate composition of feed ingredients (% dry weight basis) and formulated feed

Feed ingredients	Moisture (%)	Protein (%)	Carbohydrate (%)	Lipid (%)	Ash (%)	Fibre (%)
Tapioca Flour	12.050 ± 0.602	5.825 ± 0.291	80.000 ± 3.750	2.050 ± 0.103	0.530 ± 0.027	1.520 ± 0.076
Rice Bran	7.040 ± 0.352	10.550 ± 0.527	37.470 ± 1.573	3.350 ± 0.167	20.620 ± 1.031	21.920 ± 1.096
Groundnut Oil Cake	4.970 ± 0.248	47.910 ± 1.446	30.400 ± 1.520	6.925 ± 0.962	9.580 ± 0.479	3.620 ± 0.181
Fish Meal	5.730 ± 0.641	54.030 ± 3.626	5.720 ± 0.586	6.720 ± 0.575	25.650 ± 0.666	6.600 ± 0.480
Feed	11.180 ± 0.609	39.893 ± 1.115	27.780 ± 1.389	5.600 ± 0.330	12.500 ± 0.625	4.260 ± 0.213

Data from each glass tank in replicates were analyzed as a unit for comparison among different dietary treatments. Results of the study were subjected to one way analysis of variance (ANOVA) using statistical package SPSS version 10.1. The means were compared using Duncans multiple range test at 1% significance level.

Results and Discussion

All the feeding experiments with hormone incorporated feed were carried out with feeds formulated at 40% protein level. Water quality was regularly checked and were found to be within the optimum range (Table 2). Only variation was in the level of hormone that was incorporated to the feed. Mean weight of *B. bakeri* fed on control and experimental diets at the end of experiment are shown in Table 3. The percentage weight gain for the different treatments were found to be varying with the highest percentage gain in 3 ppm (143.84%) and the lowest percentage gain in control (23.29%). There was significant ($p < 0.01$) difference in the percentage increase in weight between the different groups. The SGR for the different treatments was found to be increasing upto 3 ppm and then decreasing. The highest value was recorded in 3 ppm (0.52%) and the lowest value for the control (0.12%). There was significant ($p < 0.01$) difference in the SGR between the different groups. The proximate composition of the fish muscle showed that there was no significant variation in any of the parameters examined between the different groups. The highest protein content in fish muscle was recorded for the 3 ppm TP fed group and the lowest for the control. Feed

Table 2. Water quality parameters

Parameters	Value
Temperature (°C)	28.00 ± 1.40°C
pH	7.00 ± 0.35
Dissolved oxygen (mg l ⁻¹)	4.97 ± 0.25
Total alkalinity	28.70 ± 1.43
Ammonia (mg l ⁻¹)	0.25 ± 0.01
Nitrite (mg l ⁻¹)	2.19 ± 0.11
Nitrate (mg l ⁻¹)	1.23 ± 0.06

utilization studies of *B. bakeri* fed on control and experimental diets for 30 days are given in Table 4.

The production (0.68 g), FCE (5.49), assimilation (11.20 g), FAE (91.15%), SGR (0.69%), PER (0.14) and protein digestibility (86.32%) was highest for 3 ppm and lowest for the control. There was statistically significant ($p < 0.01$) difference in the production, consumption, FCR, FCE, assimilation, SGR and PER values between the different treatments.

There was an increase in length, weight and SGR with increasing levels of hormone incorporated feed which reached a peak at 3 ppm and then started declining. Growth reduction in *Xiphophorus helleri* and *Betta splendens* at higher doses of 17 α -MT due to the catabolic action of hormones was observed by James & Sampath, 2006. Huggard et al. (2011) reported that gold fish administered testosterone at physiological doses increased the growth hormone mRNA production above basal level and supra

Table 3. Growth parameters of *Barilius bakeri* fed with different levels of TP for 75 days

Parameters	F ratio	Treatments Control	1 ppm	2 ppm	3 ppm	4 ppm
Initial length (cm)		6.600 ± 0.330	6.600 ± 0.330	6.625 ± 0.331	6.250 ± 0.313	6.550 ± 0.328
Final length (cm)		7.133 ± 0.357	7.210 ± 0.361	7.350 ± 0.368	7.100 ± 0.355	7.300 ± 0.365
Percentage gain in length (%)	46.131**	8.076 ± 0.404 ^a	9.242 ± 0.462 ^b	10.943 ± 0.547 ^c	13.600 ± 0.680 ^c	11.450 ± 0.572 ^d
Initial weight (g)		2.533 ± 0.127	2.467 ± 0.123	2.475 ± 0.124	1.825 ± 0.091	2.525 ± 0.126
Final weight (g)		3.123 ± 0.156	3.917 ± 0.196	4.025 ± 0.201	4.450 ± 0.222	4.300 ± 0.215
Percentage gain in weight (%)	350.84**	23.293 ± 1.165 ^a	58.776 ± 2.939 ^b	62.626 ± 3.131 ^{bc}	143.836 ± 7.192 ^c	70.297 ± 3.515 ^d
SGR (%)	229.08**	0.121 ± 0.006	0.268 ± 0.013 ^b	0.282 ± 0.014 ^{bc}	0.516 ± 0.026 ^d	0.303 ± 0.015 ^c

a, b, c, d– Means with the same superscript do not differ significantly

** ($p < 0.01$)

Table 4. Feed utilization studies of *Barilius bakeri* fed with different levels of TP for 30 days

Parameters	F ratio	Control	1 ppm	2 ppm	3 ppm	4 ppm
Initial weight (g)		1.230 ± 0.061	1.200 ± 0.060	1.220 ± 0.061	1.120 ± 0.056	1.190 ± 0.059
Final weight (g)		1.670 ± 0.080	1.720 ± 0.090	1.800 ± 0.090	1.80 ± 0.086	1.750 ± 0.090
Production (g)	29.231**	0.440 ± 0.020 ^a	0.520 ± 0.030 ^b	0.580 ± 0.030 ^c	0.680 ± 0.030 ^d	0.560 ± 0.030 ^{bc}
Consumption (g)	42.862**	10.030 ± 0.500 ^a	11.070 ± 0.550 ^b	11.560 ± 0.580 ^c	12.390 ± 0.620 ^d	11.350 ± 0.570 ^{bc}
Faecal output (g)	0.651 ^{NS}	1.120 ± 0.060	1.150 ± 0.060	1.175 ± 0.060	1.185 ± 0.060	1.180 ± 0.060
FCR	8.139**	22.795 ± 1.140 ^c	21.288 ± 1.060 ^{bc}	19.931 ± 1.000 ^{ab}	18.220 ± 0.910 ^a	20.268 ± 1.010 ^b
FCE (%)	8.246**	4.387 ± 0.220 ^a	4.697 ± 0.230 ^{ab}	5.017 ± 0.250 ^b	5.488 ± 0.270 ^c	4.934 ± 0.250 ^b
Assimilation (g)	802.731**	8.910 ± 0.440 ^a	9.920 ± 0.500 ^b	10.385 ± 0.520 ^d	11.205 ± 0.560 ^e	10.170 ± 0.510 ^c
FAE (%)	0.049 ^{NS}	89.846 ± 4.490	90.472 ± 4.520	90.645 ± 4.530	91.150 ± 4.560	90.445 ± 4.520
SGR (%)	29.842**	0.443 ± 0.020 ^a	0.521 ± 0.030 ^b	0.563 ± 0.030 ^b	0.687 ± 0.03 ^c	0.558 ± 0.030 ^b
PER	8.031**	0.110 ± 0.010 ^a	0.117 ± 0.010 ^{ab}	0.125 ± 0.010 ^b	0.137 ± 0.010 ^c	0.123 ± 0.010 ^b
Protein methodology digestibility (%)	NS 1.059	80.070 ± 0.801	81.310 ± 1.626	82.470 ± 1.649	86.325 ± 1.726	84.250 ± 1.685

a, b, c, d– Means with the same superscript do not differ significantly

** p<0.01

NS-Non-significant

physiological doses of the same resulted in inhibition of mRNA production resulting in decreased growth. Present experiment showed a similar trend. The food utilization studies also showed that the different efficiency ratios were found to be increasing with the increase in the level of hormones. Such findings have been cited by several other authors. Felix et al. (1990), in their experiments with black molly found significant influence of 17 α -MT on the feeding rate and food conversion efficiency. Turan & Akyurt (2005) found that *Clarias gariepinus*, fed with diets incorporated with sex steroids had significantly better food conversion and protein efficiency ratios and net protein utilization. The appetizing and anabolic effect of hormone incorporated feed as recognized by several authors is confirmed in the present experiment. The reason for the improved growth efficiency with increase in the level of hormones has been explained by several authors. Buddington & Krogdahl (2004) stated that hormones regulate the gastrointestinal tract functions to match changes in environmental conditions and physiological states. Norbeck & Sheridan (2011) found that testosterone increased the growth hormone receptors levels and binding capacity of insulin like growth hormone in *O. mykiss*.

The present experiment showed that the oral administration of TP is effective in enhancing

growth of *B. bakeri*. The higher growth of fish can be correlated with better assimilation and high conversion efficiency associated with hormone incorporated diet. The study shows the most effective dosage of hormone for the species, that brings about maximum growth is 3 ppm and beyond that an increase of dosage produced no growth or negative growth of fishes. The study concludes that administration of hormones through feed increases growth rate thus reducing the grow out period which will be highly advantageous from a commercial aquarist's point of view.

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