The Effect of Gynogen (17β-Estradiol) on the Phenotypic, Bioindices and Gonadal Changes of Male Dwarf Gourami, (*Trichogasterlalius)*

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ABSTRACT

The effect of different concentrations of synthetic Gynogen (17β – Estradiol)on the adult fish, *Trichogasterlalius*was studied in the present investigation. It was found that fishes fed with homogenous mixture of the hormone in ethyl alcohol exhibited phenotypical, morphometric, gonadal changes and differences in GSI value. Significant differences for length, weight, body color and GSI values were observed between hormones treated and control groups. Hormone treated groups (i.e. *5* mg/Kg, 10 mg/Kg and 15 mg/Kg) were showing less color than the control groups. However, among the treated group; 15 mg/Kg was showing less coloration than 10 mg/Kg and *5* mg/Kg of hormone treated groups at the end of the experiment. The maximum length of 4.22 cm obtained in *5* mg/kg treated fish in 90th day whereas a minimum of 3.43 cm obtained in initial day in controlled fishes. The maximum weight of 1.3839 g was obtained in control fish in 45th day whereas a minimum of 0.915g was obtained in initial in 15 mg/kg treated fish. Control was showing no significant difference with 10 mg/kg and 15 mg/kg treated groups. The maximum GnSI of 0.1851 was obtained in control group in 45 day whereas a minimum of 0.112 was obtained in the Initial day in 15mg/kg treated fish.

Keywords : *Trichogasterlalius , 17β – Estradiol, Ornamental fish, Gynogen.*

No. of Tables:3 **No. of Figures:1** **No. of References: 21**

INTRODUCTION

Keeping colorful ornamental fishes in aquarium is one of the oldest and most popular hobbies in the world. In India, the hobby of ornamental fish keeping is nearly 70 years old. Keeping of aquarium has emerged as the second most popular hobby in recent years, next to photography. Perhaps, China is the pioneer in adopting aquarium fishas a hobby. The ever-increasing demand for aquarium fishes gradually paved the avenue towards global trade of ornamental fishes. The top exporting countries include Singapore followed by Honkong, Malaysia, Thailand, Philippines, Srilanka, Taiwan, Indonesia and India.

India's overall ornamental fish trade was about 1.06 million US$ during year 2009. India's share to global ornamental fish trade is less than 1 percent but still she is projected as a "sleeping giant" because of yet untapped potential resources (Benjamin, 2012). However India's marginal position is likely to change as trade is gradually increasing (Ghosh *et al.,* 2003). At this moment it is appropriate to delineate India's position and competitiveness in emerging world market of ornamental fishes.

The demand of ornamental fishes is increasing remarkably due to their important role in the world trade for fish and fishery production (Datta et al, 2013).Ornamental fishes are very sweet in their color, peculiar and playful behavior for which they are called 'living jewels'. Atpresent ornamental fish industry is growing rapidly. India has a good resource of natural ornamental fishes. West Bengal is also a wide repository of indigenous ornamental freshwater as well as marine water fish resources having both commercial as well as biological important (Bidisha and Angsuman 2014).In West Bengal, Kolkata and its adjoining areas are the one of the major ornamental fish producing zone of India. About 90% of Indian's exports go from Kolkata followed by 8% of Mumbai and 2% from Chennai (Sarkar and Lakra 2010).

Ornamental fishes are attractive colourful fishes of peaceful nature that are kept as pets in aquarium for recreation purpose. Ornamental fish keeping is the second most popular hobby next to photography. And the ornamental fish industry is one of the most booming one among the World; India having a good share of it. India is blessed with a great resource of different natural ornamental fishes. West Bengal is also sanctified with a wide range of indigenous ornamental fishes of biological as well as commercial importance (Basu et al, 2014).

The Dwarf Gourami *(Trichogasterlalius)* is a peaceful freshwater fish, also known as the "Dwarf Gourami". Gourami is the name used for a big variety of perciform fish characterized by flat body and two elongated rays of pelvic fins used as sense of touch. Since they reach only 2 inches, they can be housed in small tanks and are a good fish' for beginners because of their low aggressiveness, easy care and nice look. Males can be easily distinguished from females for their colors. The male is a bit bigger than the female and has turquoise and orange-red iridescent vertical bands on the entire body and on fins; you can find also color mutations with total orange-red body and turquoise dorsal fin, or total turquoise body with just some red at the edges of the fins. The dwarf gourami female is totally silver with pale turquoise vertical stripes.

West Bengal is one of the states of India having a rich wealth of freshwater resources and fish germ plasm diversity. It is also the pioneer state in ornamental fish production and export. Due to congenial climatic conditions, Kolkata and its surrounding districts have emerged as a promising breeding centre for ornamental fish and a considerable number of small fish farmers and amateurs are engaged in this trade. It is found that 288 exotic varieties of ornamental fishes popular in West Bengal (Bhaskar*et al.,* 1989) and *52* native ornamental fishes are available here. The indigenous fish fauna of

this state includes a wide variety of small fish, which are although unsuitable for conventional farming, but could be gainfully utilized as ornamental fish for their attractive coloration.

MATERIALS AND METHODS

Collection and acclimatization of Fish Species: Male adults of *Trichogasterlalius*ranging from the length 3.2 to 3.8 cm and weight of 0.85 to 1.04 gm were collected from Gullif Street, near to Shyambazar, Kolkata, West Bengal. Fishes were Collected from the market and then transported to the department by Oxygen filled plastic packets. In the laboratory, fishes were given a short bath treatment with 2% potassium permanganate (KMnO4) solution for 3 to 5 minutes as prophylactic measures. Subsequently, they were transferred carefully to the aquarium (60cm, 30cm, and 30cm) containing iron free tap water. For acclimatization to laboratory condition, they were stocked at a density of 30 fish per aquarium in 35 L of water. Fishes were acclimatized in the laboratory condition for 15days before starting of the experiment.

In the laboratory condition they were feed with a commercially available aquarium feed (i.e. Tokyo, Japan). The feed was given to the stocked fishes at the rate of 3% of their body weight daily with two equal rations i.e. during morning and evening hours. Left out feed and accumulated fecal materials ware siphoned out daily morning in order to maintain healthy condition of fishes. The acclimatized fishes were quite active, healthy and normal in body color as well as with their behavior. After 15 days of acclimatization the fishes were taken for experiment.

Preparation of hormone incorporated feed: An Gynogenic steroid hormone 17β-Estradiol (E2) was used in the present study. It was obtained from the Sigma chemicals Ltd. Three different kinds of feed were prepared by adding three doses of E2 as *5* mg of E2 per Kg of feed, 10 mg of E2 per Kg of feed and 15 mg of E2 per Kg of feed. First three different doses of E2 was weighted by electric balance, and kept in the aluminum foils. Then each dose was dissolved separately in 100 ml of 95 % ethanol each. These alcohols were spreaded over the feed (Tokyo, Japan). Then all the feed were left for air drying.

The feed used for controlled fishes were prepared by spreading 100 ml per 1 Kg of feed without hormone and left for air drying. After air drying of overnight the feed were kept separately in the air tight polythene bags for future use. During the preparation hormone treated feed and its future use, a strict precaution was taken like use of Mask, globes, no use of fan etc. Experiments were conducted with 3 replicates having 30 fish in each of aquarium, in which adult *Trichogasterlalius*were fed with diet supplemented with 3 doses of 17β-Estradiol; *5,* 10, 15 mg/Kg diet for a period of 90 days.

Statistical calculation: The means of growth performance parameters, Analysis of variance (ANOVA) using Duncan's multiple range tests (DMRT) and Pearson's correlation were carried out. Results are being summarized in following tables.Water quality parameters were maintained throughout the experiment in the laboratory condition.

RESULTS

Color: For the study of phenotypical characteristics i.e color, of *Trichogasterlalius*was studied with response to the action of female steroid hormonel7β-Estradiol.

Dwarf gourami *(Trichogasterlalius),* is an attractive color fish. Males have an almost translucent blue color, with vertical red to dark orange stripes. Hormone treated groups (i.e. *5* mg/Kg, 10 mg/Kg and 15 mg/Kg) were showing less color than the control groups. However, among the treated group; 15 mg/Kg was showing less coloration than 10

mg/Kg and 5 mg/Kg of hormone treated groups at the end of the experiment.

Length increment of *Trichogasterlalius*: The fortnightly variations in the length have been shown in the figure -1. It indicated the maximum length of 4.22 cm obtained in 5 mg/kg treated fish in 90th day whereas a minimum of 3.43 cm obtained in initial day in controlled fishes.

Weight increment of *Trichogasterlalius*: The fortnightly variations in the weight have been shown in the figure -2. It indicated that the maximum weight of 1.3839 g was obtained incontrol fish in 45th day whereas a minimum of 0.915g was obtained in initial in 15 mg/kg treated fish. Control was showing no significant difference with 10 mg/kg and 15 mg/kg treated groups. The relation between individual treatment group with other treatment groups the 5 mg/kg treatment group was showing highly significant difference (P<0.01) with other treatment groups. Control was not showing any significant difference with 10 mg/kg and 15 mg/kg treatment groups.

Gonado-somatic index of *Trichogasterlalius*: The fortnightly variations in the Gonado-Somatic index have been shown in the figure -3. It indicated the maximum GSI of 0.1851 was obtained in control group in 45 day whereas a minimum of 0.112 was obtained in the Initial day in 15mg/kg treated fish. Initial day was showing highly significant difference (P<0.0l) with other days and however, other days were not showing any significant difference (P>0.05) between them.

DISCUSSION

Externally sex differentiation is possible by observing body color of *Trichogasterlalius.* The Dwarf gourami ***(****Trichogasterlalius****),*** is an attractive color fish. Males have an almost translucent blue color, with vertical red to dark orange stripes. During the present study, color was observed from May to July before and alters the hormone administration. In the present study, no color change was observed in case of control. Alter 30th day nocolor change was observed between treated groups but after 90th day: some color change was observed in treated groups. In between treated groups 15mg/kg was showing less color change than 5mg/kg and 10mg/kg treated groups. The pituitary gland exercises control over the distribution of pigments in the chromatophores due to its hormonal action.

Due to the administration of 17β-Estradiol to the *Trichogasterlalius,* the normal action of the pituitary hormone intermedin is hampered at the pars intermedia. Therefore, the levels of MCH (Melanophore contracting hormone) is dominated over the MSH (Melanophore Stimulating Hormone) resulting in the aggregation of pigments in the chromatophore causing lighter color. Apart from that some other hormones like adrenalin, thyroxine, and gonadal hormone is also responsible for melanin concentration. Khanna, 1988, reported that the internal gonadal hormone of the testis of *Trichogasterlaliuss* influenced by the exogenous hormone I7β-Estradiol. It might be due to action of this exogenous hormone aggregation of the pigments is occurred maximum in higher doses (15mg/kg), medium (10 gm/kg) and minimum in (5mg/kg). Therefore, the decrease in color was found maximum in 15 mg/kg and minimum 5 mg/kg. It might be due to the aggregation pigments in the chromatophores. The similar observations were also seen in *Colisalabiosa*females with the administration of antihormonetestosteron propionate (Forselius, 1957).

In the present study length was in increasing trend. The maximum length of (4.22cm) was obtained in 5 mg/Kg hormone treated group than other treatment and control group and the minimum length of (3.43 cm) was obtained in control treated group in initial time. In overall length, the 15 mg/kg was showing less length increment than other

treatment groups. In the present study weight was in increasing trend. The maximum weight (1 .383g) was obtained in control treated group than other treatment and minimum weight (0.9 15 g) was obtained in 15 mg/kg treated fish at end of experiment. During the present study, weight increment was more in control when compared to other treated groups. It might be due to high concentrations of hormones leads decrease in growth.

In the present study, due to the action of the hormone the growth performance (i.e. the rate of length and weight increment) is comparatively slow with respect to control one. But among the treatment groups, maximum growth was observed in 5 mg/kg and minimum in 15 mg/kg treated groups. This is because, in the high dose (15 mg/kg) the metabolic activity of the fishes are reduced reflecting lower growth rate. On the other hand, it is also believed that at the higher dose of the induced exogenous hormone the secretion of growth hormone (i.e. GH) is reduced. This growth hormone is influencing on metabolism of the fish resulting less growth rate. The above findings were in agreement with the earlier works of Yamazaki, (1976) and *Cleide,etal,* (2000). In the present study GnSI become maximum initially after that it was declining trend. It indicated the maximum GnSI of (.1851) was obtained in control treated group in 45th day whereas a minimum of (.1112) was obtained in the Initial day of 15 mg/kg treated group during the end of the experiment. In the present study, due to the action of the exogenous hormone 17β- Estradiol the rate of gonadal development was minimum in 15 mg/kg and maximum in 5 mg/kg. This is because a gynogen 17β- Estradiol was given to a male of *Trichogasterlalius*which influenced the secretion of the testicular hormone of the male fish.

Palav, A.D., and Belsare S.G., (1995) reported that the hormones are anabolic at lower doses and catabolic at higher doses, causing growth depression. These hormones are known to promote growth by increasing appetite, food Conversion efficiency and protein synthesis. In order for the hormones to be effective for growth promotion if they are fed at lower doses over a longer period of time. Yamazaki (1976) reported that the inhibition of growth in goldfish *(Carassiusauratus)* due to the continuous high treatment (30 mg/kg) with Estradiol-17β in the diet. However, growth was not affected by lower (1 and 10 mg/kg) Estradiol- 17β concentrations. The oral administration of ethynyl Estradiol caused increase in weight-gain, food consumption and food-conversion were reported by Cowey and Sargent (1972) and Coweyet *al.*, (1973) in Plaice, *Pleuroneclesplatessa. Cruz et al.*, (1993) stated that the significant differences in weight gain between most treatments may be due to the short culture period used. According to Cleideet *al.,* (2000) hormone treated group is much heavier than that of the fry feed on hormone free diet. Analysis of log weight data revealed that a dose dependent increasing insizein fish treated with high cstradiol-17 concentrations. No differences were observed during Gynogen treatments in weights of Atlantic salmon fry (Johnstoneet *al.*, 1978). James and Sampath (2006) stated that the gonad weight and Gonado-Somatic Index of Red Swordtail *(Xiphophoroushelleri)* and Siamese fighting fish *(Bettasplendens)* decreased with the increase of hormone dose beyond the Optimum dosage and it negatively reflected on the reproductive performance. Observations of the lower GSI values for hormone treated fishes have also been reported earlier (Basavaraja and Rao, 1988; Das *et al.*, 1990). In the present study, maximum GSI was recorded during 45th day, due to breeding season in the month of June. The present study was coinciding with the earlier statement of Annapaswamyet *al.,* (2008), that the GSI is positively correlated with the breeding season. Very high doses of

Gynogen (15 mg/kg) may result in decrease of feminizing potency and retardation of gonadal development. The numerically lower mean GSI in 17β- Estradiol treated fish seemed to indicate that the hormone suppressed the development of the gonads appeared much thinner than control fish of the same age. Observations of the lower GSI values for hormone treated fishes have been reported by earlier works of (Basavaraja and Rao. 1988).

Conclusion

An excellent aquarium fish needs inherent qualities and traits which improve their demand and market value not only in India but in foreign countries too. This study is an initiation in adult male dwarf Gourami *(Trichogasterlalius),* which will form the basic platform for further research and provide a strategy to study sex ratio of offspring while breeding this hormone treated males. Strategy for hormonal manipulation, standardization of doses, method of injection, age and size of fish, survival of fish after hormonal manipulation which reduces the chance of success and reliability are the major criteria to be studied on a long way.

Table 1: *Mean length (cm ±SD) of Colisalalia by dietary administration of 17ß-estradiol at different treatment level.*

Days	control	5mg/kg	10mg/kg	15mg/kg
Initial	3.4300±0.19322	3.4900±0.21318	3.5400±0.14337	3.4900±0.11738
15th	3.5400±0.13703	3.5900±0.09661	3.6100±0.16465	3.5600±0.13375
30th	3.6900± 0.15055	3.7000±0.15811	3.6800±0.11972	3.6100±0.10328
45th	3.7300±0.15776	3.8204±0.12867	3.7500±0.17512	3.6900±0.10541
60th	3.9000±0.14944	3.9400±0.16193	3.8300±0.13375	3.7600±0.14944
75th	3.9600± 0 .22998	4.0700±0.13984	3.9200±0.12649	3.8500±0.16465
90th	4.1100± 0.17795	4.2200±0.12517*	4.0200±0.16865	3.92700±0.1825

*Denotes highest length enhancement at the end of 90 days

Table 2: *Mean weight (gm ±SD) of Colisalalia by dietary administration of 17ß-estradiol at different treatment level.*

Days	control	5mg/kg	10mg/kg	15mg/kg
Initial	1.0257±0.16190	1.0556±0.16091	0.9217±0.17020	0.9151±0.20834
15th	1.0975±0.09877	1.0942±0.08482	0.9868±0.19414	0.9844±0.14586
30th	1.1544±0.13524	1.1615±0.10203	1.0754±0.17164	1.0310±0.16099
45th	1.3839±0.18125	1.3761±0.13935	1.3025±0.14435	1.2863±0.09987
60th	1.2961±0.19732	1.2757±0.11541	1.2154±0.15544	1.1247±0.09915
75th	1.2747±0.12639	1.2535±0.07978	1.1866±0.15317	1.1069±0.12426
90th	1..2813±0.16993*	1.2706±0.08032	1.1924±0.14820	1.1136±0.17330

*****Denotes highest weight enhancement at the end of 90 days

Table 3: *Mean Gonado-Somatic Index (GSI±SD) of Colisalalia by dietary administration of 17ß-estradiol at different treatment level.*

Days	Control	5 mg/kg	10 mg/kg	15 mg/kg
Initial	0.1188±0.14668	0.1224±0.11026	0.1166±0.15580	0.1122±0.16052
15th	0.1274±0.18123	0.1261±0.20459	0.1217±0.21016	0.1186±0.28533
30th	0.132±0.22537	0.1353±0.25357	0.1336±0.18958	0.1298±0.23809
45th	0.1851±0.33342*	0.1762±0.29632	0.1726±0.09756	0.1696±0.26622
60th	0.1659±0.36115	0.1503±0.27691	0.1492±0.14228	0.1472±0.21621
75th	0.1293±0.32346	0.1285±0.25069	0.127±0.21589	0.1256±0.19721
90th	0.1239±0.21142	0.1216±0.24857	0.1203±0.16284	0.1201±0.08884

*****Denotes highest **GSI** at the end of 45th days

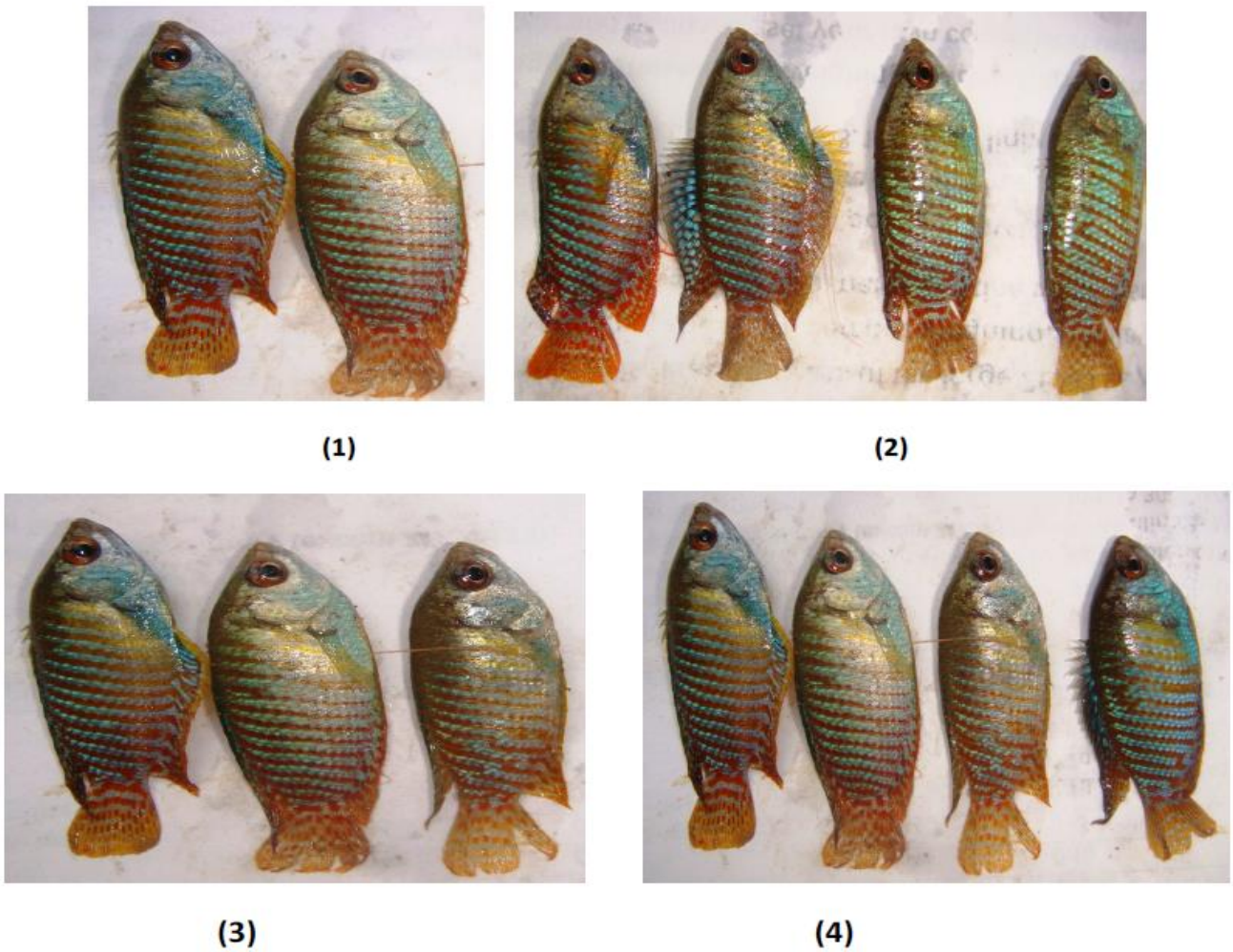

Fig 1: *Plate (1-4): Showing color differences among different groups of male of Trichogasterlalius*

Plate 1: Trichogasterlalius from control group
Plate 2: Trichogasterlalius from 5mg/Kg hormone treated group,
Plate 3: Trichogasterlalius from10mg/Kg hormone treated group,
Plate 4: Trichogasterlalius from15mg/Kg hormone treated group.

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