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ANIMAL SCIENCE

Effect of Methyl Testosterone (17α-MT) on the phenotype, bioindices and gonads of adult male dwarf Gourami (*Colisa lalia*)

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Abstract

The effect of different concentrations of synthetic androgen 17α- methyl-testosterone (MT) on the adult fish, *Colisa lalia* was studied in the present investigation. It was found that fishes fed with homogenous mixture of the hormone in ethyl alcohol, exhibited phenotypically, 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. Fishes fed with hormonal doses of 10 and 15 mg/Kg feed showed significantly elevated body color compared to 5 mg/Kg feed. Length, weight and GSI values were found significantly higher in 10 mg than 5mg/Kg and control group. The highest mean length and mean weight of fish was recorded as 4.76±0.13 mm and 1.50±0.15g respectively with the hormone treatment of 10mg/Kg of feed. The present study revealed that the synthetic hormone had no significant effect on the gonad development of *C. lalia*.

Key words: *Colisa lalia,* 17α- methyl-testosterone, Ornamental fish, Synthetic androgen

Introduction

The dwarf gourami (*Colisa lalia*) also called *Trichogaster lalius* belonging to Order- Perciformes and Family- Ospheronemidae / Belontidae is inhabitant of slow moving streams, rivulets and lakes with plenty of vegetation. They generally grow to a size of 3.6-5.0 cm or to a maximum of 8.8 cm. Males can be easily distinguished from females for their colour. The dwarf gourami male is a bit bigger than the female and has turquoise and orange-red iridescent vertical bands on the entire body and on fins, mutants with total orange-red body and turquoise dorsal fin, or total turquoise body with just some red at the edges of the fins are available. The dwarf gourami female is totally silver with pale turquoise vertical stripes (www.aqua-fish.net).

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. 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 share in ornamental fish trade is estimated to be less than 1 % of the global trade. The largest importer of ornamental fish is the USA importing fish worth over US$ 500 million every year followed by Europe and Japan. The emerging markets are China and South Africa.

India hosts 11% of global ichthyic diversity of about 31,300 fish species, so far it possess 930 described ornamental fish species and of these approximately 250 are now available to aquarium hobbyists. Despite the huge potential offered by the rich diversity and environment, export of ornamental fish from India continues to remain negligible. Approximately 267 fish species belonging to 38 families has been identified as ornamental fishes from North East India comprising about 85% of the total aquarium fish trade of India.

Ornamental fishes are acceptable to consumers if they have striking and vibrant colours. Colouration, which is one of the most important factors deciding the market value of the ornamental

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fish, is controlled by the endocrine and nervous systems (Asimi, 2009). However, dietary sources supplemented with external hormones also play a role in enhancing the fish colour. People involved in the trade of ornamental fish are constantly exploring methods of enhancing skin coloration. 17α- methyl-testosterone was the first successful hormone used to produce functional males from female genotype in Medaka (*Oryzias latipes*) (Yamamoto, 1958). Several methods for hormone administration include dietary implementation, oral administration, injection and silastic implantation. Dietary administration of hormone is most practical and effective methodology (Yamamoto, 1953). The diet supplementation generally involves homogenous mixing of the steroid in the diet and the alcohol evaporation method is the most widely used for steroid application (Guerrero, 1975). The hormone has been applied for masculinization, inducing colour and anabolic consideration in many ornamental fishes.

Current investigation was carried out to study the effect of exogenous steroid, methyl testosterone (MT) on phenotypical and gonadal changes in adult male Dwarf Gourami, *Colisa lalia* and its response in the induction of body colour, enhancement in the length and weight without suppressing the gonad of adult fish.

Materials and Methods

Collection and acclimatization of fish species

The study was conducted at the Department of Fishery Biology and Resources Management of West Bengal University of Animal and Fishery Sciences at Chakgaria during 2011. Male adults of *Colisa lalia* ranging from the length 3.2 to 4.2 cm and weight of 0.85 to 1.44 mg were collected from Gullif Street, Kolkata, West Bengal. In the laboratory, the fishes were given a short bath treatment with 2% potassium permanganate ($KMnO_4$) solution for 3 to 5 minutes as prophylactic measures. Subsequently, they were transferred carefully to the aquarium (60 x 30 x 30) cm^3 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 for 15 days before starting the experiment. Experiment was conducted with 3 replicates having 30 fish in each aquarium, in which adult *Colisa lalia* were fed with diet supplemented with 3 doses of 17 α -MT i.e. 5, 10, 15 mg/Kg diet for 90 days.

A control without hormone dosage fed with a commercially available aquarium feed was maintained. The feed was given to the stocked fishes at the rate of 3% of their body weight daily with equal rations i.e. during morning to evening hours. Left out feed and accumulated fecal matter was siphoned out daily morning in order to maintain healthy condition of fishes. The important water quality parameters were fixed and recorded i.e. water temperature (30.21± 3.1°C), pH (7.39 ±0.12) and D.O. (4.65± 0.45ppm). The biotic factors like length, weight and colour of fish as well as the GSI (Gonado Somatic Index) were measured and documented in every 15 days interval. For each sampling 10 fishes were randomly selected from 3 replicates of each treatment doses. For the colour estimation the views of ten different persons were collected secretly and separately. Gonado Somatic Index was calculated with the help of following formulae

GSI (Gonado Somatic Index) =Weight of gonad / Weight of fish x 100

Statistical Calculation

The average body weight and length of the hormone treated fish were compared to that of the control by multivariate ANOVA using time and treatment followed by Duncan's multiple range test (DMRT) between the treatments. Similarly the biological end points like GSI and treatment doses were analyzed through Pearson's correlation to determine the influence of hormones on GSI. All this statistical analysis were done using SPSS software package 17.

Preparation of hormone incorporated feed

Three different feeds containing different concentrations (5, 10 and 15 mg per Kg of feed) of MT hormone (obtained from Sigma Chemicals Ltd., USA) were used for the analysis. Each dose was dissolved separately in 100 ml of 95% ethanol and the hormone mixture was spread over the feed and air dried. Control fishes were fed with feed speeded with 10 ml of 95% ethanol / kg without any hormone. The prepared feed was kept in sealed packets and stored in the freeze (4°C). Feed were taken out from the freeze before 15 minutes of use. During use maximum pre-cautionary measures are taken.

Results

Current investigation resulted in the enhancement of body color in hormone treated groups (5, 10 and 15 mg/Kg feed) in contrast to control where no color was detected after 90 days experiment. Among the hormone treated group 10 and 15 mg/Kg feed treated fishes showed more coloration compared to 5mg/Kg treated fishes (Plate 1 to 4). The average length and weight of fishes ranged between 3.49±0.18 to 3.54±0.12 mm

and weight 0.90±0.24 to 1.08±0.19 gm respectively. The average body weight and length of the hormone treated fish were interpreted to find the probability of occurrence and represented in Table 1, 2.

Plate 1. Control group of *Colisa lalia* fishes.

Plate 2. 5 mg/Kg treated group of *Colisa lalia* fishes.

Measurement of male gonads (testis)

In the initial stages of the experiment no significant differences were found between control and hormone treated groups, but at the end of experiment control fish showed difference in average length 3.99±0.12 mm and weight 1.28±0.03g compared to hormone treated group (Figure 1, 2). The highest mean length and mean weight at the end of experiment was 4.76±0.13 mm and 1.50±0.15g in 10 mg/Kg feed respectively. GSI values also increased in MT treated fish than control fish that means the hormone was not suppressing the gonadal development (Figure 3).

Plate 3. 10 mg/Kg treated group of *Colisa lalia* fishes.

Plate 4. 15 mg/Kg treated group *Colisa lalia* fishes.

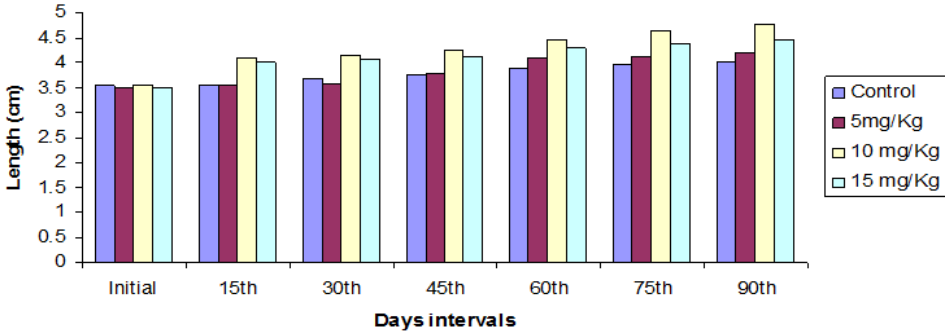

Figure 1. Fortnightly variations in length of *Colisa lalia* fishes upon treatment with exogenous steroid, methyl testosterone (MT).

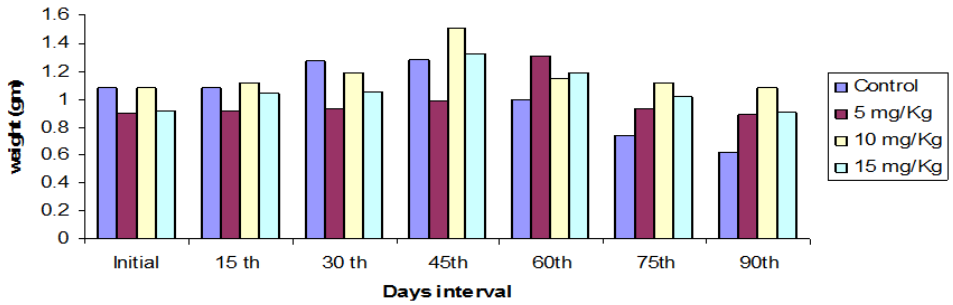

Figure 1. Fortnightly variations in weight of *Colisa lalia* fishes upon treatment with exogenous steroid, methyl testosterone (MT).

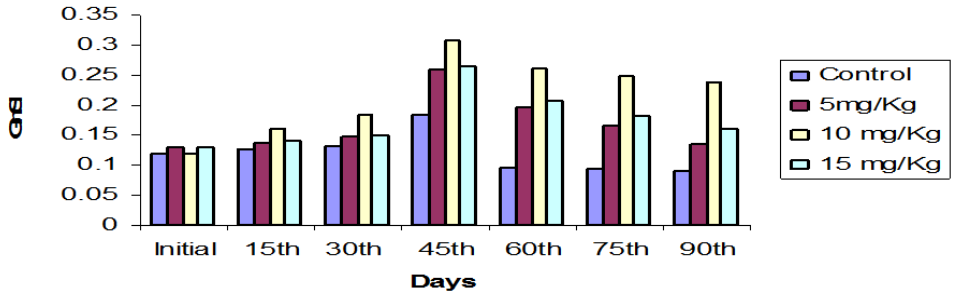

Figure 1. Fortnightly variations in Gonado-Somatic Index (GnSI) of *Colisa lalia* fishes upon treatment with exogenous steroid, methyl testosterone (MT).

Table 1. Comparison of length, weight and GSI value with time through Post-Hoc Test.

Time	Number	Length	Weight	GSI
Initial	40	3.5150^{a}	0.8979^{a}	0.1243^{a}
15^{th} day	40	3.7975^{b}	0.9966^{b}	0.1817^{ab}
30^{th} day	40	3.9175^{c}	1.0868^{c}	0.2587^{bc}
45^{th} day	40	3.9400^{c}	1.1042^{c}	0.3267^{c}
60^{th} day	40	3.9500^{c}	1.1833^{c}	0.4429^{c}
75^{th} day	40	4.0450^{d}	1.2750^{d}	0.4592^{d}
90^{th} day	40	4.0650^{d}	0.9511^{ab}	0.3778^{bc}

Values with a common superscript in the same column with the same letter index did not differ significantly (P>0.05).

Table 2. Post-Hoc Test comparison with treatment groups in case of Length, Weight, and Gonado-Somatic Index.

Treatment	Number	Length	Weight	GSI
Control	70	3.7314^{a}	1.0063^{a}	0.2533^{a}
5mg/Kg	70	3.8571^{b}	1.0098^{a}	0.2765^{ab}
10mg/Kg	70	4.0114^{cd}	1.1617^{b}	0.3720^{c}
15mg/Kg	70	4.0543^{bc}	1.0488^{a}	0.3617^{bc}

Values with a common superscript in the same column with the same letter index did not differ significantly (P>0.05).

Discussion

The colour changes in treated fishes were found more than that of the controlled fishes (Plate 1 to 4). The change in fish color may be due to the actions of hormone on neuropeptide system of fish. The hormone (MT) is triggering the pituitary to produce more melanophore dispersing hormone (MDH) thus increasing the level of concentration in the blood stream, which is resulting in the color variations according to hormone concentration.

In the present study length, weight and Gonado-Somatic Index showed an increasing trend (Fig-1, 2 & 3). The maximum length was attained in 10 mg/Kg hormone treated fish than other treatments and control groups which was in well accordance of the finding of Simone (1990) in channel cat fish, *Ictalurus punctatus*. At the end of experiment smallest length and weight was observed in control than other hormone treated group (Table-1&2). Lone and Matty (1980)

reported that 17 α -MT induced better growth by acting in three different ways - improved food conversion, activation of other exogenous anabolic hormones and direct effect on gene expression in muscle cells. MT has shown its impact on the growth enhancement of various fish species such as Pacific salmon, *Onchorhynchus tschawytscha* (Bride and Fagerlund, 1973), Common carp, *Cyprinus carpio* (Lone and Matty, 1980) and Nile tilapia, *Oreohromis niloticus* (Tayamen and Shelton, 1978). Androgenic activity of the gut lead to the growth enhancement in Mirror carp (Lone and Matty, 1981).

Fry of *Oreochromis niloticus* treated with MT @5-25 mg/Kg diet was found to be significantly heavier than the control (Jo et al., 1995). Best growth was observed with MT compared to control at 10-60 ppm (Hanson et al., 1983). Hormone treated group was much heavier than that of the fry feed on hormone free diet (Cleide et al., 2000). Contradictory to the present findings stated that growth depression was observed in the Gold fish, *Carassius auratus* treated with MT at higher concentration than 10 mg/Kg and growth enhancement at low concentration i.e. 1mg/Kg (Yamazaki, 1976).

GSI value in this research was higher among hormone treated adult fish compared to control group at the end of experiment. In the present study the development of gonadal materials (Primary spermatocytes, Secondary spermatocytes and Spermtozoa *i.e.* sperms) inside the follicles of the male indicated the maturation stages of gonads (Plate 5&6). It revealed that hormone had no effect on gonadal development in adult fish. The length, weight and the GSI values were increasing with respect to the experimental period (Fig-1,2&3). Contradictory to the present findings 100% sterility of gonad was reported in grass carp by using mibolerone hormone (Kavumpurath and Sampath,1990) and in common carp of same age group (Rao and Rao,1983; Basavaraja and Rao,1988; Das et al.,1990). Gonad weight and GSI value of Red swordtail, *Xiphophorous helleri* and Siamese fighting fish, *Betta splendens* decreased with the increase of hormone dose beyond the optimum dosage and it negatively reflected on the reproductive performance (James and Sampath, 2006).

Gonads sterility of Salmon was achieved at a dose of 30 mg/Kg diet (Simpson et al, 1978). Rainbow trout fry with 10 ppm MT for 8 week and 30 ppm MT for 4 week obtained sterility (Hurk and Slof, 1981). The administration of steroid hormones has been reported to cause sterility in rainbow trout (Yamazaki, 1983), grass carp (Boney et al., 1984) and carp (Basavaraja, 1984). Sterility due to suppression of gonad development in common carp was reported by Pandian and Sheela (1998). Present study indicated neither hormonal doses (5, 10 and 15 mg of MT per Kg of feed) nor duration of treatment (90 days) could result in degeneration of the testis. This may be either due the inadequate hormonal dose or lesser experimental time period in our study as in other earlier studies time period was longer and fishes been encountered at higher doses of the Methyl Testosterone (17α-MT).

Conclusion

Despite the marketability and commercialization of Gourami (*Colisa lalia*) on large scale, its captive breeding with the help of inducing agents specially colour inducer i.e. MT hormone is still a virgin field.

This study is an initiation in adult male dwarf Gourami (*Colisa lalia),* 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. In the present study it was revealed that hormone doses 5 mg/kg-15 mg/kg for a maximum period of 90 was not enough to depress the gonadal development. Moreover, a marked increment was observed in weight, length and Gonado-somatic Index value at 10 and 15 mg/kg MT harmone in feed. Therefore, along term study is necessary to optimize the hormonal dose and experimental time in Gourami (*Colisa lalia*). More important, 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.

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