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Comparative efficacy of 17-α methyl testosterone on swordtail (*Xiphophorus helleri*) fry via oral feed administration

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Abstract
This current experiment of 90 days was conducted to observe the comparative efficacy of 17 α methyl testosterone on Swordtail (*Xiphophorus helleri*) fry via different dose rate with the help of oral feed administration at wet lab of the College of Fisheries, G.B.P.U.A & T, Pantnagar India. During this whole experiment, we used twelve glass fabricated aquariums with a capacity of 80 liter (Dimensions 4.0 X 1.0 X 1.0 Feet) respectively. Initially, we stocked 120 fish fry of average weight of 0.005 gm in twelve experimental aquarium tanks with 10 numbers of fry in each experimental aquarium respectively. For this experiment we collected Swordtail fish fry from College hatchery. On the other hand, we selected commercially available fish feed for oral administration of 17 α methyl testosterone during this whole experiment. Throughout, the experiment we feed the fish fry @ 5% body weight with interval of 8 hours during day time. Besides this, we mixed commercial available feed with different dose rate of 17 α methyl testosterone like: 30 mg/kg, 60mg/kg and 90 mg/kg respectively. On the other side, we installed one control aquarium tank (T_0) for the comparative study with treatment aquarium tanks like; H_1, H_2 and H_3 respectively. After 90 days of this experiment, we observed efficacy of 17 α methyl testosterone with different dose rate on the masculinization of Swordtail fish fry like; under 90 mg/kg feed we observed maximum male percentage as compared to H_1 and T_0 respectively. Besides this H_3 has a minimum number of female percentage as compare to H_2 tank. Moreover, we observed a high percentage of male population in all treated aquarium tanks with 17 α methyl testosterone as compared to T_0 like; 66.66 %, 60.0 %, 56.66 % and 53.33 % respectively. During our experiment we observed it is crystal clear that 17 α methyl testosterone have satisfactory results on masculinization of swordtail as compare to control experimental aquarium tank respectively.

Keywords: Aquarium tank, Xiphophorus helleri, 17 α methyl testosterone, masculinization

1. Introduction
Masculinization in aquaculture aims to benefit from desirable traits expressed differentially by each sex. In ornamental fish, such manipulation can be profitable, especially in species with high price discrepancy between the sexes (Kipouros *et al*., 2011) [1]. The swordtail (*Xiphophorus helleri*) is a small fresh water fish, native to Central and North America. This fish native range stretches from Honduras in the south, to Central Mexico in the north, but they can now be found on almost every continent. In natural water bodies, swordtails are omnivore, and feed on a diversity of invertebrates, insects, plant matter and algae T. Swordtail (*Xiphophorus helleri*) has commercial importance as ornamental fish (Moghaddam *et al*, 2010) [2]. It is a live-bearer fish. The phenotypic males (i.e., with sword extensions of the caudal fin) can be valued as much as twice the value of phenotypic females (i.e., fish lacking swords) (Yanong *et al*., 2006) [3]. Male swordtail fish is smaller and slimmer than female with gonopodium and pronounced sword like projection on caudal fin. The size of swordtail varies but males are 6-7 cm long while females are 7-9 cm long. Optimum water condition for breeding of swordtails is temperature 23-28 °C, and water hardness 50-100 mg $CaCO_3$ /litre (i.e. moderately soft water) (Ayyappan *et al*, 2011) [4].

1.1 Ornamental Fisheries
Keeping ornamental fishes in the aquarium is an age-old practice for fun and fancy (Ayyappan *et al*, 2011) [4]. Since centuries ago humans are keeping colourful tropical fishes in aquarium and garden ponds as a hobby. Fish keeping in aquarium for ornamental purpose is the second most trending hobby throughout the world after photography.

In China, it is considered as the most popular hobby. Ornamental fishes are living jewels because of their bright body colour, morphology and feeding behaviour. Keeping of fish in aquarium started in 1853 at Regent's Park, England. In India keeping of fishes in the aquarium started during British time and is continuing till the date (Ayyappan *et al*, 2011) [4]. Besides this, Ornamental fish culture and breeding is growing as business in India and it is gifted with a suitable climate, plentiful water resources, rich fauna and flora and abundant manpower base throughout the whole country. But, India's share in the ornamental fish sector is negligible and in present scenario fish export from India is dominated by the wild caught species. The decorative fish sector offers a good opportunity for rural and urban households to enhance income and links them to global trade. Nearly all of the ornamental fishes which are bred in tanks come from the small-scale or backyard type of breeding units. Besides this, in whole states of world business of ornamental fish is estimated to be US$ 6.0 billion. The third world countries continue to be the major producer of ornamental fish like; 60 per cent of ornamental fish is originated from developing countries in 2015. It is reported that global imports and exports of ornamental fish in 2015 were valued at US$ 271 million and US$ 304 million, respectively. In 2015, USA was the largest importer of ornamental fish accounting for US$ 49.67 million followed by United Kingdom (US$ 24.31 million), Germany (US$ 18.62 million) and Japan (US$ 15.71 million), Singapore (US$ 14.33 million). During 2015, India's import for ornamental fish was US$ 0.34 million, with a ranking of 51, in the world. In 2015, top exporters were Singapore (US$ 45.44 million), Spain (US$ 36.07 million), Japan (US$ 31.08 million), Czech Republic (US$ 20.43 million) and Indonesia (US$ 19.67 million). In the same year, India's export was only US$ 1.02 million, with a ranking of 29th in the world. Asia is still the major ornamental fish supplier for USA and Europe with Singapore remaining the hub of Asian ornamental exports. The export of Singapore has decreased recently and still significant player in the trade (Pramod K. Pandey and Sagar C. Mandal. 2017) [5].

1.2 Sex Reversal in Fishes

Masculinization procedure is the unusual development of male sexual characteristics in a female by using hormone treatments or adrenal malfunction. Administration of exogenous steroids can be an effective method in controlling sexual development (Al-ablani and Phelps, 2002) [6]. An important step in establishing an effective regime of hormonal usage to masculinize fish is the identification of the 'labile period' i.e. the period when gonad is highly sensitive to the exogenous factors. Masculinization and feminization in 47 fish species have been successfully induced using 31 steroid hormones (16 androgens, 15 estrogens) (Pandian and Sheela, 1995) [7]. Since the use of steroid hormones for the sex control of food fishes produces environmental and food safety concerns because of the potential for the hormones to enter water bodies and the human food chain, the use of steroids in ornamental fisheries is safer as the fish is not intended for human consumption and the hormone administration is carried out in controlled conditions (Basavaraja *et al*., 2014) [8]. The androgens are classified into four categories. These are aromatizable (natural and synthetic steroids), non-aromatizable (steroids), non-Steroids and medicinal herbs. The success of sex reversal in fish is determined by various factors *viz*. age, size of animals, hormone dose and the duration of hormone exposure and varies from species to species (Nakamura M. and Takahashi H, 1984) [9]. Generally, short time and high doses are recommended for sex reversal. Among androgen, 17α- Methyl testosterone (MT), an anabolic steroid, is being commonly used for masculinization of fish in a number of species (Moghaddam *et al*, 2010) [10]. Expansive use of 17α- Methyl testosterone now a day because of it is easily absorbed and it does not accumulate in fish body and excreted easily (Colborn *et al*., 1993 & Sumpter *et al*., 2005) [11, 12].

2. Material and Methods

2.1 Site of Experiment and Concept

The experimental work was carried out in the Wet Lab of the College of Fisheries, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar. The coordinates of College of Fisheries, Pantnagar are 290ºN latitude, 79.30ºE longitudes and present in the Tarai belt of Himalayan foothills at an altitude of 243.3m above mean sea level (MSL). The humid sub-tropical environment of Pantnagar is characterized by dry and hot summer and very cold winter with the mist usually occurring at the end of December and till February end. The experiment was carried out from February 1, 2018 to May 3, 2018.

During this experiment we would like to observe that efficacy of 17α- Methyl testosterone (MT) on Swordtail fish population with application of hormone on Sword tail fish fry.Total 120 fries were taken for the experiment. The fry were divided into 12 groups, having 10 fry in each. 10 fry of swordtail (*Xiphophorus helleri*) were stocked in each aquarium. Triplicates of each treatment were made with stocking of 10 fry per aquarium in the experiment.

2.2 Source of Fish Fry and Experimental Tanks

Around 200 fry (*Xiphophorus helleri*) were obtained from the available brood stock of Swordtail fish in College of Fisheries and some were procured from the market. On the other side, we selected glass fabricated aquariums for this experiment because it comes under ornamental fishes.

2.3 Experimental Design

During this 90 days experiment we installed twelve well fabricated aquarium of the size 4.0ft x 1.0ft x 1.0ft with an average water depth of 0.65ft (Capacity of 80 litres). Initially, we cleaned and disinfected the experimental aquarium with lime water and KMnO4, then filled up with tap water from bore well and continuous aeration was provided to the water through aerators respectively. Cleaning of the aquarium and removal of leftover feed and excreta were done by siphoning with water exchange of 25% on every alternate day throughout the study period. Fresh tap water from bore well was used to refill the aquarium and continuous aeration was provided. During winters water heaters were used to maintain water temperature. As per experimental design the tanks were identified with different nomenclature and code numbers like; "T^0A, T^0B, T^0C" whereas treatment tanks were named "H^1A, H^1B, H^1C" for 30 mg of hormone per kg of feed, "H^2A, H^2B, H^2C" for 60 mg of hormone per kg of feed and "H^3A, H^3B, H^3C"for 90 mg of hormone per kg of feed.

2.4 Preparation of 17- α methyl testosterone (MT) stock solution and hormone mixed feed

Stock solution of 17- α methyl testosterone was prepared by diluting the hormone in 96% ethanol at a concentration of 1

mg/ml and kept in refrigerator at 4 °C. For the preparation of hormone mixed feed of fry, dry pelleted aquarium feed (Pacific green brand) was taken and ground into powder form. 100 gm feed was taken on three disposable plates. Then the stock solution of MT was added to the feed kept in the plates in different volumes to get the hormone concentration of 30, 60 and 90 mg/kg of feed by following the alcohol evaporation method (Guerrero *et al.,* 1975) [13]. One plate of feed was prepared without hormone and used as control. Hormone mixed feed with different doses were kept separately in the air tight zip poly pack and also kept in refrigerator. Feed were used after exposing them to room temperature. Feeding was done with specific plastic spoons for different concentrations of hormone mixed feed to avoid confusions.

2.5 Mating and breeding of swordtail fish

The breeding experiment was conducted by natural and standardized method. The gravid female has a swollen belly with fries inside. Impregnated gravid female have a dark gravid spot near her anal fin and a large bulging belly. The gravid spot is caused by the fry's eyes pressing against the female's scales. Eight numbers of breeding tanks were selected. In each tank nine numbers of females and three numbers of males were kept (3:1 ratio). Water quality parameters were maintained properly (at optimum level) for better breeding performance. Water depth of tanks was maintained at 8 to 10 inches. In swordtail fertilization is internal and they show specific mating behavior. Male transfer sperms into a female body by a modified anal fin called gonopodium. After 24 to 26 days females exhibit gravid spot at the lower abdominal position near anus which was the indication of the gestation.

2.6 Feeding pattern

The hormone mixed feed of different doses was fed to the fishes twice a day at the rate of 5% of body weight of the fry. Sampling was done after 15 days to adjust the feed quantity based on the weight of the fishes recorded by samples.

2.7 Estimation of masculinization rate

The masculinization rate of the swordtail fry was determined on the basis of their secondary sexual characteristics (Male swordtail fish is smaller and slimmer than female with gonopodium and pronounced sword like projection on caudal fin) at the end of the experiment.

3. Results

3.1 Rate of masculinization of sword tail fry after treatment with different doses of hormone

The objective of this study was to determine the effect of hormonal treatment on masculinization of fries achieved from the treatments with different concentration (30 mg/kg, 60 mg/kg and 90 mg/kg of feed) of 17-α MT. It was observed that the rate of masculinization increased in fries with the increase in hormonal concentration and found significant (P> 0.05). Besides this, a number of male and female found in different groups is presented in Table no. 1 and ratio of male: female in treated swordtail fry with different concentration of hormone is presented in Table no. 2. From the study it was found that the rate of masculinization is in an increasing order with respect to increasing hormonal concentration. The treatment groups, *viz.* H_1 (56.66%), H_2 (60%) and H_3 (66.66%) showed higher percentage of male than the control T_0 (53.33%) group respectively (Table no. 2 & Graph no. 1). The maximum male percentage of 66.66% was observed in H_3 group (Graph no. 1). So in this study it was found that after the dose of 30 mg/kg there was a good effect of further treatment of hormone. This experiment could not be able to produce 100% of male population but the mode of this treatment found to be a great success towards the masculinization of fries of *Xiphophorus helleri.*

Table 1: Number of male and female fry reared in different tanks with hormonal feed

Tanks	T_0		H_1		H_2		H_3	
Sample size	Male no.	Female no.	Male no.	Female no.	Male no.	Female no.	Male no.	Female no.
10	7	3	6	4	5	5	6	4
10	4	6	5	5	6	4	7	3
10	5	5	6	4	7	3	7	3
30	16	14	17	13	18	12	20	10

T_0: Control

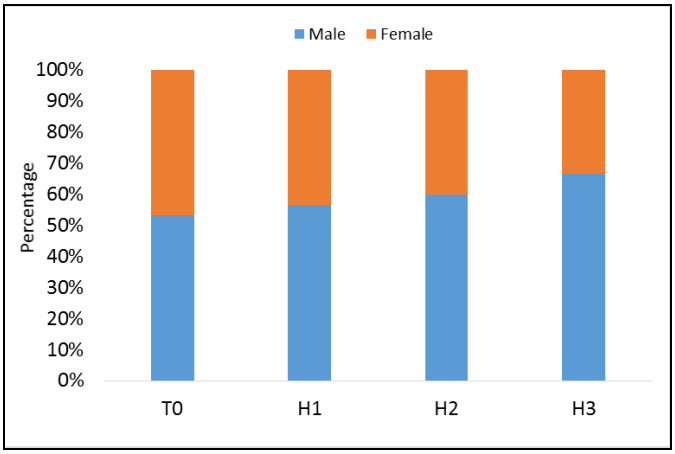
H_1, H_2 and H_3: Feed with different hormone concentration

Table 2: Percentage of male and female fry reared in different tanks with hormonal feed

Treatment	Male percentage	Female percentage
T_0	53.33	46.66
H_1	56.66	43.33
H_2	60.0	40.0
H_3	66.66	33.33

T_0: Control

H_1, H_2 and H_3: Feed with different hormone concentration



T₀: Control
H₁, H₂ and H₃: Feed with different hormone concentration

Graph 1: Representing Male and female percentage of fry in different tanks with treatment

4. Discussion

The present study was done with the objective to assess comparitive efficacy of 17 α methyl testosterone on Swordtail (*Xiphophorus helleri*) fry via different dose rate with the help of oral feed administration. This kind of experimental research already done by various authors related to masculinization on different ornamental fishes like; oral administrations of MT to swordtail can result in 98% masculinization by 75 mg MT kg^{-1} of feed for 30 days. Also, logistic regression obtained from the treatment by 17α-methyltestosterone in different group shown masculinization in swordtail was dose dependent and with increasing concentrations of MT caused an increasing in the number of males (Khiabani A *et al*, 2014) [14]. On the other hand during our experiment we observed a significant role of hormone on fry of swordtail was achieved with the rate of masculinization, 66.66% of the male population was found in 90 mg/kg (H_3) treatment tank where control showed 53.33% of male population respectively (Table no. 2 & graph no. 1). The rate of masculinization increased with increased hormonal application in a significant way (P>0.05). On the other side, the sex ratio in the control group was not statistically different from the expected 50:50 sex ratio (P>0.05). However, immersion treatment of 12.5 and 25 mg/20 L 17α-MT for 30 days resulted in the production of 100% male populations of P. reticulata via Turan *et al*., 2006 [15].

5. Conclusion

As a conclusion, we can say that better masculinization rate of fry of *Xiphophorus helleri* can be found by treating with 17α-MT. Here I found 66.66% of maximum male population at a hormonal concentration of 90 mg/kg (treatment group H_3) of feed at the end of the treatment. This study can help the ornamental fish farmers to produce more number of male fishes to meet the consumer demand. Future scope of this study is as follows to get 100 % male population, manipulate the hormonal doses and duration. This treatment method may be applied for other live bearing ornamental fishes like guppy, mollies, platies and mosquito fish to get more male population.

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