EFFECT OF DIFFERENT CONCENTRATIONS OF 17-A-METHYLTESTOSTERONE ON SEX REVERSAL OF GUPPY (*Poecilia reticulata*)

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Abstract

Guppy (Poecilia reticulata) is a highly demanded, commercially valuable ornamental fish in the world. The male fish has higher economic value due to its bright color than the female fish. However, in general there are high proportions of occurrence of female fish in breeding progeny population. Hence, the current study was carried out to identify the effect of different doses of synthetic androgen 17- α Methyltestosterone on morphometric characteristics of guppy. The *17- α Methyltestosterone 40 mg/kg, 60 mg/kg and 80 mg/kg concentrations were used as treatments with the control having zero concentration. The treatment, 17- α Methyltestosterone hormone was fed to fish as powdered fish feed for 21 days. Hormone was mixed with feed by using ethanol spray method at different concentrations, and. four different diets including the control were fed to day-old guppy for 71 days in glass aquaria. Each treatment and the control had three replicates each carrying 100 fries. Treatments were given during the labile period of 21 days followed by control feed for the rest of the period in their life cycle. At the end of the experimental period, morphometric features such as color intensity, body weight gain, survival percentage, and masculinization were assessed. Presence of gonopodium was used to determine the sexuality. The results showed that the 80 mg/kg 17- α Methyltestosterone fed group had the highest male percentage compared to those in the other treatment and the control group. However, 40 mg/kg treatment resulted a higher weight gain and higher survival rate than 60 mg/kg, 80 mg/kg treatments. All treatments increased color intensity, but no difference (p>0.05) was observed among treatments. Findings of this study concluded that feeding of 40 mg/kg 17-α Methyltestosterone containing feed showed the best morphological performances in commercial guppy with higher male percentage under aquarium condition.*

Keywords: *Guppy, Poecilia reticulata, 17- α Methyltestosterone, Sex reversal*

INTRODUCTION

Ornamental fish can be identified as an attractive, eye-catching finned creature. They can help minimizing mental stresses and illnesses. Being a partner of ornamental fish industry for more than nine decades, Sri Lanka exports fresh water, marine water and brackish water fish species and marine invertebrates. Freshwater aquariums mainly trade colorful species. According to Rathnamurthi et al (2019), export fish varieties of Sri Lanka include mainly Guppy (87%) followed by Platy (3.3%), Molly (2.2%), Tetra (2%), Zebrafish (1.7%) and Barb (0.9%). However, there is a variation of export demand according to the quarter of the year where the demand is high in 1st quarter for species such as Guppy, Platy, Molly and Tetra. The main target of ornamental fish production is the export market. Sri Lanka earned about US$ 8 million per year from the exportation of ornamental fish and plants to over 52 countries in 1998 (EDB, 1999). Over the

past 25 years, it has grown only to US$ 17.37 million in 2022.

Sri Lanka has developed a good reputation in the international market for exporting high quality wild caught and tank bred fish as well as wild caught marine fish species (Wijesekara and Yakupityage, 2001). The main export markets for Sri Lankan freshwater fish are found in Japan, USA, Germany, France, UK, the Netherlands, Italy, Spain and United Arab Emirates. On the other hand, there is a considerable local market where both imported and local fish species are marketed. Exotic ornamental fish are received from different countries such as Singapore, Thailand, Indonesia, Malaysia, Japan and the Maldives, a small proportion of which ending up as brood stock for the fish breeders. Thus, entering to the cycle with genetic improvement and value addition.

Guppy being the dominating species (67%) the exports for many decades (Wijesekara and Yakupityage, 2001), the improvement of the fish will have a direct impact on the volumes of export earnings. There are some differences between male and female guppy fish, among which the main difference is the colorful and large dorsal and caudal fins. In addition, male fish is small, colorful body compared to females (Gideon *et al*., 2007). Thus, the market demand of male guppy is obviously higher than that for female guppy, low occurrence of which in the population is beneficial in aquarium industry.

The sex reversal of female guppies could be a strategic intervention for maximizing the profit, because female guppy could then be sold to a price close to male guppy. In this study we attempted to identify the optimum dose of 17 –α Methyltestosterone required to sex reversal of guppy with high survival and growth rate.

MATERIALS AND METHODS

Day old Red blond guppy fry were brought from the NAQDA- Rambadagalla and introduced to the experimental tanks, after acclimatization for 20 to 30 minutes upon arrival, maintained at the Laboratory at the Department of Animal Science, University of Peradeniya. Fry were allocated (100 fry per tank) into each experimental glass-tank (1ft×1.5ft×3ft) randomly. There were 12 tanks used in the experimental setup where each treatment and the control carried three replicates.

Preparation of fish feed and feeding

Feeds were prepared the day before the fry were brought to the lab. Commercially available fish feed and fish meal were purchased from the market. Then, 17- α Methyltestosterone and Vitamin were mixed with commercial feed as follow: 80% commercial fish feed, 20% fish meal, 10g vitamin and three concentrations (40 mg/kg (Treatment 1), 60 mg/kg (Treatment 2) and 80 mg/kg (Treatment 3)) of 17–α methyl testosterone. 17 –α Methyl testosterone were incorporated in to powdered feed in formulating treatments and the control carries no hormone. Ethanol was added to feed by ethanol spraying method. Hormone incorporated feed was stored under refrigerated condition under airtight condition until used.

Hormone treated feed were fed to experimental fish through the first 21 days of their life cycle (labile period). The feeding schedule of the experimental diet was as follow; 15% of the body weight for the first month, 10% of the body weight during the second month and after that 3% of the body weight till 71 days. At the end of experiment period (71 days) the growth rate, sex reversal, survival rate and the color development of guppies were recorded. The laboratory conditions maintained during the experiment were 25 ^{0}C of temperature, 7.5 pH, 5-6 ppm dissolved oxygen.

Feeding was carried out by dividing the total amount of feed to be given in a particular day into 5 parts (35%,30%,20%,10%,5%) and feeding fish five times a day. In the second month it was only 4 times a day with 4 parts (35%,25%,25%,15%). Syphoning was practiced after each feeding to remove excess feed remains at the bottom of the tanks.

RESULTS AND DISCUSSION

Chromo color meter measurement of the color intensity of fish body (mainly on tail fin) are given in Table 1. As shown, there was a significant difference in color intensity ($P<0.05$) between the control group and the treatment groups. There was no significant difference ($P>0.05$) among treatments groups though there was change of red coloration in guppy fish population with the increase concentration of hormones. The maximum color measurement was observed in 80mg/kg treatment.

When body color changes were considered, it was clear that fish in the control group remained with the light stint of red color. According to the chromo colour meter reading, it was clearly evident that the fish body color turned more red color in the treatment groups. This observation could be explained by taking the changes in pigment production, i.e. melanin, drozopetrin and carotenoid pigments, in the skin of fish with 17-α Methyltestosterone treatment. Drozopetrin and carotenoid are critically important to creation of chroma of red color. Amount of light that reflects or passes through the body surface causes the distinction between light and dark intensities of respective colors. The physiological color change occurs in fish body by collecting and distributing pigments in chromatophore. Further, by changing the rate of pigments or the number of chromatophore also make changes in the body color. When 17-α Methyl testosterone is applied, it tends to increase the tint towards red by creating pigments. In some initial studies about hue and color measurements of spotted scat fish (*Scatophagus argus*) showed that when the fish skin is exposed to 17-α Methyltestosterone, the color intensity increased (Shahidian *et al*., 2004). In the present study, the color improving rate was higher in 17-α Methyltestosterone treated guppy than that in control. In the control group, the color improvement was observed after 21 days while the treatment groups showed the color improvement from the 12^{th} day.

Effect on sex ratio

The effect of different levels of 17-α Methyltestosterone on the sex ratio is shown in Table 2.

Table 1: Effect of 17-α Methyltestosterone on color Intensity of treatments

Treatment	Dose of 17-α Methyltestosterone	Color intensity
Control	0 mg/kg	17.27^{a}
Treatment 1	40 mg/kg	28.32^{b}
Treatment 2	60 mg/kg	29.39^{b}
Treatment 3	80 mg/kg	29.71^{b}

The different letter superscripts indicate the significant difference of color intensity at $P<0.05$.

Table 2: Male percentage and female percentage of treatments and control

	Male	Female
Control	11±0.67	87±0.67
Treatment 1	57±0.33	40±0.33
Treatment 2	51±0.33	26.±0.33
Treatment 3	52±0.33	14±0.33

The experiment was conducted under controlled conditions where temperature, pH and dissolved oxygen levels varied within narrow ranges (24.5 - 26.5^{o}C temperature, 6.5 - 8.5 pH and 5 - 6 ppm dissolved oxygen). The labile period of *Poecilia reticulata* is 21 days. When androgen hormone is applied during the labile period, sex of a fish could be changed phenotypically, but not genotypically. In the present study, three concentrations of 17-α Methyltestosterone were applied for day-old fish. According to Table 2, the control showed a higher number of females. However, the female to male ratio of normal guppy population is 55 to 45. Thus, the control was a female skewed population, probably due to the random genotypic effect of the cohort. However, in the treatment groups, there were significantly higher($P<0.05$) numbers of males than females irrespective to the level of 17-α Methyltestosterone. However, this sex reversal method is not practiced in the commercial level at present for guppy. Phenotypic males were identified by morphological characteristics, i.e. gonopodium and body coloration. In this experiment, the gonopodium was used to differentiate the males and the females.

According to the male to female ratio shown in Table 2, increase of the concentration of 17-α Methyltestosterone hormone has resulted a higher number of males in the population. This observation is in agreement with previous studies done using 17-α Methyltestosterone hormone where it was reported the change of sex ratio of normal guppy population changing the sex ratio of male to female from 60:56 to 64:42 when the concentration of 17-α Methyltestosterone changed 30 mg/kg to 100 mg/kg concentration (Moussavi-Sabet *et al*., 2012). Therefore, the sex reversal could be used as a strategy to reduce the discard of numbers of female guppies in ornamental fish industry, and thereby increasing the total output of the hatchery with high economics benefits in commercial scale operations.

Effect on survival

Survival rate of control and the 40mg/kg 17 –α Methyltestosterone treatment group were significantly higher than other two treatments hormone treated groups (80 mg/kg and 60 mg/kg treatments with 77.6% and 99.3% survival rates, respectively). Previous studies also showed similar results, where it was reported that increasing the hormonal concentration gradually reduced the survival rate (Samantha *et al*., 1859). According to the present study 40 mg/kg 17-α Methyltestosterone concentration had the highest survival percentage among all three treatments.

Effect on Weight gain

The objective of this study was to identify the optimum dose of 17 –α Methyltestosterone that requires for sex reversal of guppy with high survival rate where any effect of body weight gain is important. According to the results, the control group had the highest weight gain than treatments followed by 40 mg/kg

group and 60 mg/kg treatment group, respectively. The lowest weight gain was recorded in the 80 mg/kg group.

Control of environmental factors during the experiment

Water pH

Since fish perform all their bodily functions in water, they totally depend upon water to breathe, feed and grow, excrete wastes, maintain a salt balance and reproduce, understanding the physical and chemical qualities of water is critical to successful aquaculture. Thus, water determines the success or failure of an aquaculture operation to great extent, very high and low pH values are very harmful to young fry, because they are extremely sensitive to pH levels below 5. In this study pH in water was in between the values of 6 to 8. Because guppy prefer neutral pH level to get enough oxygen. Any deviation from the optimum pH levels will cause for fish appear ill or have low energy. However, the remaining feed can cause changes of pH in the water by generating ammonia in the water. Ammonia increases the pH level in the water. Multi meter was used to measure the pH in water. In this experiment bottom, syphoning was done twice a day to prevent the changing pH of water.

Dissolved Oxygen level

The average dissolved oxygen level in the water was 5 ppm to 6 ppm. It was the most suitable dissolved oxygen level for guppy. The present study used air pumps to maintain the dissolved oxygen level inside the tanks. Temperature was not varied during the experimental period. Fish were carefully observed during the experimental period, as fish tend to retain at the water surface to receive oxygen from the air.Fish remained active and distributed uniformly inside the tank during experimental period.

Temperature

Being cold blooded, fish cannot regulate their internal body temperature. When the water temperature changes, so does their body temperature. During the experimental period there was no water temperature changes inside the tank. There was a uniformity of temperature among treatment tanks.

CONCLUSIONS

According to the findings of this study the best concentration of 17-α Methyltestosterone that could be incorporated into fish feed to produce the highest proportion of male guppy population along with the highest survival rate and the body weight gain is 40 mg/kg. Further, the cost (50 cents/fish) incurred for hormonal sex reversal was very negligible compared to the income that could be obtained for the sex reversed guppy compared to wastage occur with natural female guppy in commercial scale aquarium operations.

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