

POECILIID RESEARCH

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Sex reversal, mortality rate and growth of guppy (*Poecilia reticulata*) affected by 17-alpha methyltestosterone

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Abstract. The effects of different dose rates of synthetic androgen 17-alpha methyltestosterone (MT) i.e. 30, 60, 100, 150, 200 and 300 mg of MT per kg of feed on masculinizing, mortality rate and growth performance were determined in the guppy, *Poecilia reticulata*, since male guppy has more commercial value than female. MT was administrated orally by using pellet dry starter and Ethanol Alcohol, diet to newborn fry guppy for 40 days in glass aquaria. The fry were also kept for seven weeks after feeding to monitor its growth performance; at the end of the experiment the sex ratio was determined by secondary sex characteristics and macroscopic gonad identification. Growth performance was monitored by recording the morphometric characteristics. Wet body weight and total length of fish on 90 day-old guppies were measured. The results showed that ≥ 60 mg MT receiving treatments showed a significantly higher male proportion than other treatments and the control experiments. Dose rate of 200 mg MT/kg of feed resulted in maximum male population with 29.16% loss of fish. While 300 mg MT/kg of feed showed 46.66% mortality rate, it is concluded that the MT has negative effect on survival rate. Also the dose rate of 30 mg MT gave the maximum growth in average body weight i.e. 148 mg and average total length i.e. 37 mm, which are 1.14 and 1.21 times greater than the control groups, respectively.

Key words: aquarium fish, Poeciliidae, dose rate, masculinization, alcohol dry method.

Introduction. Many species of ornamental fish, including both egg-layers and livebearers, exhibit a marked sexual dimorphism due to the more pigmented bodies and larger fins usually observed in males (Piferrer & Lim 1997), are preferred over the female fish by the hobbyists. These species include the livebearers such as guppy, *Poecilia reticulata*, balloon molly, *P. latipinna*, sailfin molly, *P. velifera*, and sunset platy, *Xiphophorus variatus*, as well as the egg-layers such as dwarf gourami, *Colisa lalia*, fighting fish, *Betta splendens*, rosy barb, *Barbus conchoni*, Convict cichlid *Cichlasoma nigrofasciatum*, and red Australian rainbow, *Glossolepis incisus*. This results in male fish commanding up to four times the price of females. Because of this price discrepancy, the culture of monosex (all-male) stocks of ornamental fish could be of a significant economic advantage (Piferrer & Lim 1997; Mousavi-Sabet 2011). The methods to obtain all-male stocks include direct androgen treatment and the production of YY males. The direct approach is easy to implement and straightforward. However, in some livebearers such as the guppy, treatment of newly born fry is complicated because they already have sexually differentiating gonads and the masculinization may be temporary (Piferrer & Lim 1997).

On the other hand, early sexual maturity in some ornamental fish culture is a well recognized problem which resulted, inbreeding in overstocked ponds, reduced production and farmed stocks of a generally low quality (Mousavi-Sabet 2011). To overcome these problems and to develop improved breeding stock of these fishes, researchers decided to produce mono-sex and preferably all male population of cichlids, because the males grow faster than females (Barras & Melard 1997; Myers et al 1995;

Tariq Ezaz et al 2004). Also the males are bigger, more beautiful and more salable than females (Piferrer & Lim 1997). Therefore, the maintenance and breeding of male populations have generated a great amount of interest in terms of commercial applications.

There are different ways such as hormonal sex reversal, manual sexing, hybridization and super male production to attain mono sex population. Sex reversal method has been used as a valuable tool in the elucidation of sex determining mechanisms in addition to its value in production of mono sex population for aquaculture. Sex reversal by oral administration of feed incorporated with methyltestosterone (MT) is the most effective and practical method for the production of all male populations. Dosage of 17-alpha methyltestosterone (MT) used to produce all male ornamental fish, vary wildly. These fishes can be masculinized by direct synthetic hormonal treatment that is efficient and straightforward (Pandian & Sheela 1995; George & Pandian 1996; Gale et al 1999; Mousavi-Sabet 2011).

One of the most important species in ornamental fish culture is the guppy. Livebearers are an important group of relatively large and often colorful aquarium fishes. The family Poeciliidae comprises about 37 genera and about 304 species (Moyle 2002; Nelson 2006). In this study the guppy was used as a model fish because of its wide range of tolerance to water hardness, pH and temperature. It is also easily obtainable and can be propagated inexpensively (Piferrer & Lim 1997; Garcia-Ulloa & Garcia-Olea 2004; Cek et al 2007).

The aim of this study was, therefore, to find out optimum dose rate of MT treatment for sex reversal along with its effects on growth performance and mortality rate of guppy.

Materials and Methods. The sex reversal of newborn fry guppy was achieved by oral administration of 17-alpha methyltestosterone through feed. A stock of *P. reticulata*, comprising males and females was procured from a local ornamental fish dealer. After acclimation to the laboratory conditions, animals were disinfected (Robertson et al 1993) and stocked in three separate aquariums containing recirculating water ($26\pm 1^\circ\text{C}$) and exposed to a 12 (light): 12 (dark) photoperiod, where oxygen concentration was kept above 5 mg/L by air pump. The experimental stocking density was adjusted to eight fish per aquaria at a sexual ratio of two male and six females without emphasis in the male body coloration pattern (Garcia-Ulloa & Garcia-Olea 2004). Each experimental aquarium was filled up to a culture volume of 50 L with municipal freshwater. Daily, faeces and other particles were extracted out from the bottom of each aquarium by siphoning and 50% of water volume was changed every two days. All of the guppy fries used in these treatment studies were obtained from this broodstock. In order to prevent fries from being eaten by their parents, the ripe females were kept in plastic nets. The fries were immediately removed from the aquariums, counted and placed in 24 small glass aquariums, each containing 40 L of freshwater that was continuously aerated with a 5-cm air stone and filtered by a normal sponge filter. Each experimental trial consisted of a control group and a group fed with ethanol treated diet (fish were fed with a diet treated with ethanol). For sex reversal treatment six different dosage groups, 30, 60, 100, 150, 200 and 300 mg of MT per kg of food (each in three replicates), were set out, MT was added to diet with Alcohol (Ethanol 96°) dry method (Elmdoust 2003; Mousavi-Sabet 2011), and each trial was stocked with 40 fish. The fries were fed daily with a commercial diet (dry starter pellet food with 40% crude protein, Biomar brand) six times per day, for 40 days. The important water quality parameters were fixed and recorded, eg: temperature ($26\pm 1^\circ\text{C}$) dissolved oxygen (5.9 ± 0.3 mg/L), hardness (170 ± 10 mg/L) and pH (7.5 ± 0.4). Fries were fed 20% of body weight (BW) per day, and the important growth parameters such as BW and total length were recorded at 90 day-old guppies (50 days after the end of hormonal treatment). Differences between groups in terms of sex ratio of the offspring were determined on the basis of secondary sex characteristics and macroscopic gonad identification (after dissection) and analyses by the chi-square test (Mousavi-Sabet et al 2011). The secondary sex characteristics used included: males are smaller but are more colorful than females; males have a developed gonopodium, a

specialized reproductive fin found only in male fish; longer and more colorful caudal fin in males (Piferrer & Lim 1997; Elmdoust 2003). Differences in mortality and differences in body weight and length between groups were tested with the one-way analysis of variance by ranks (SPSS ver. 10.0 for Windows) followed by the Duncan non-parametric multiple comparison procedure.

Results

Effect of MT on the sex ratio. Result of the present study showed that each hormone treated group (except 30mg MT/kg feed) gave a mean male/female ratio that deviated significantly from the normal 1:1 ratio (chi square analysis) (Table 1), while the control groups and the 30mg MT/kg feed treatment showed normal 1:1 ratio. Two groups (200 and 300 mg MT/kg feed) of the experimental trial gave all male populations. Also, we achieved 82.47% masculinization in *P. reticulata* by feeding with 150 mg MT/kg feed for 40 days. The sex ratio observed in 30mg MT/kg feed treatment groups was 60:56 (male:female), while in the 60mg MT/kg feed treatment groups was 64:49 (male:female), and in the 100mg MT/kg feed treatment groups was 64:42. These results were interpreted as an indication that increasing concentrations of MT to 200mg caused an increase in the number of males produced (Figure 1).

Table 1
Effects of treatment with MT on sex ratio in *Poecilia reticulata*

Dosage MT (mg/kg feed)	Treatment duration (day)	Sex distributions (male:female; M:F)	Sex ratio (%) (M:F)	Chi Square	P
0 (Control)	40	60:57 (<i>n</i> =117)	51.28:48.72	00.11	0.379
0 (Ethanol group)	40	55:61 (<i>n</i> =116)	47.41:52.59	00.83	0.238
30	40	60:56 (<i>n</i> =116)	51.72:48.28	00.17	0.302
60	40	64:49 (<i>n</i> =113)	56.63:43.37	10.06	0.049
100	40	64:42 (<i>n</i> =106)	60.37:39.63	14.98	0.031
150	40	80:17 (<i>n</i> =97)	82.47:17.53	40.19	0.004
200	40	85:00 (<i>n</i> =85)	100.00:0.00	85.00	0.000
300	40	64:00 (<i>n</i> =64)	100.00:0.00	64.00	0.000

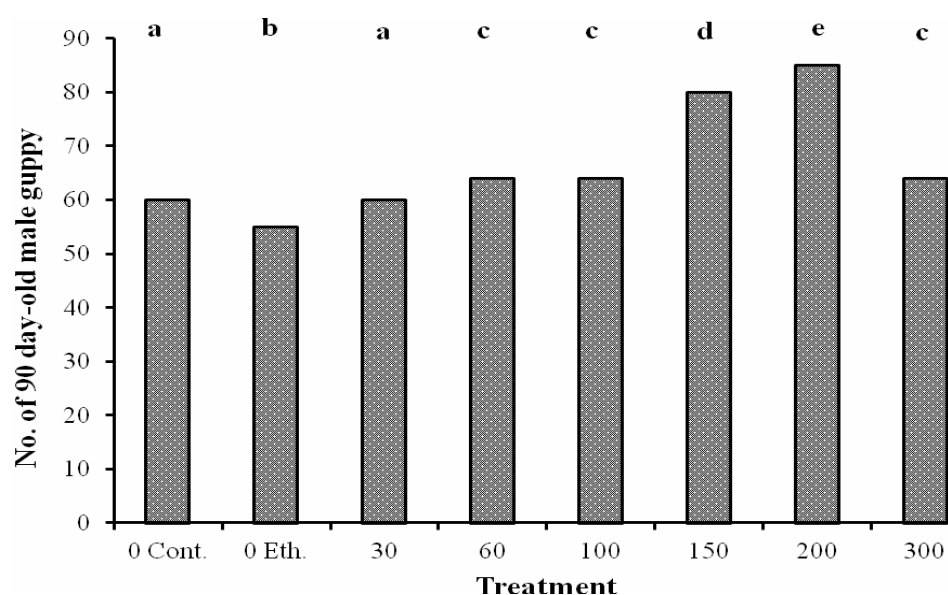


Figure 1. Number of survived 90-day-old male guppies (marketable fish) in different treatments. Different letters indicate significant differences ($p \leq 0.05$).

Mortality and growth of MT-treated fish. Table 2 shows the survival and growth rates of both control and MT-treated *P. reticulata* in terms of total body length and body weight. Significantly higher growth rate was observed in lowest concentration treated fish (30mg MT/kg feed) than other treatments and control groups. When considering mortality, no significant different was observed among control group, ethanol group and dose 30 mg, but mortality in dose 60, 100, 150, 200 and 300 mg MT/kg feed was significantly different among these groups and significantly higher than the other groups. Control, ethanol group, and dose rates of 30 mg MT/kg feed exhibited 3.33, 2.50 and 3.33 percent in mortality rate, respectively. Also, in dose rates, 60 and which were more than 60 mg hormone/kg feed, mortality rate were significantly higher than others. As shown in Figure 2, normally the mortality rate decreased after hormonal treatment (after 40th day). These results were interpreted as an indication that increasing concentrations of MT caused an increase in the mortality rate.

At the end of the experiment (90th day), total body length and weight were significantly increased in 30 mg MT treated group compared to other treatments and the controls (Table 2). Also this indicates that MT has a negative effect on the survival rate of *P. reticulata* (especially when it used 100 and more than 100 mg/kg feed), but it has the ability to increase total body weight and length at the low tested concentrations.

Table 2

Effects of treatment with MT on the survival rate and growth performance (mean \pm SE) in *P. reticulata*

Dosage MT (mg/kg feed)	Survival rate (%)	Mortality rate (%)	Body weight (mg)	Total length (mm)
0 (Control)	96.67 \pm 1.95a	3.33 \pm 1.95a	130 \pm 21.19a	31.03 \pm 2.15a
0 (Ethanol group)	97.50 \pm 0.87a	2.50 \pm 0.87a	129 \pm 19.07a	29.98 \pm 1.87a
30	96.67 \pm 1.74a	3.33 \pm 1.74a	148 \pm 44.72b	37.06 \pm 3.54b
60	94.17 \pm 1.71b	5.83 \pm 1.71b	131 \pm 25.45a	33.68 \pm 2.24c
100	88.34 \pm 2.14c	11.66 \pm 2.14c	128 \pm 20.69a	30.18 \pm 2.09a
150	80.84 \pm 4.62d	19.16 \pm 4.62d	117 \pm 26.12c	27.43 \pm 2.19d
200	70.84 \pm 4.09e	29.16 \pm 4.09e	120 \pm 18.97c	28.02 \pm 2.72d
300	53.34 \pm 11.37f	46.66 \pm 11.37f	119 \pm 23.36c	27.93 \pm 3.01d

Values followed by different letters within the same column are significantly different ($P < 0.05$).

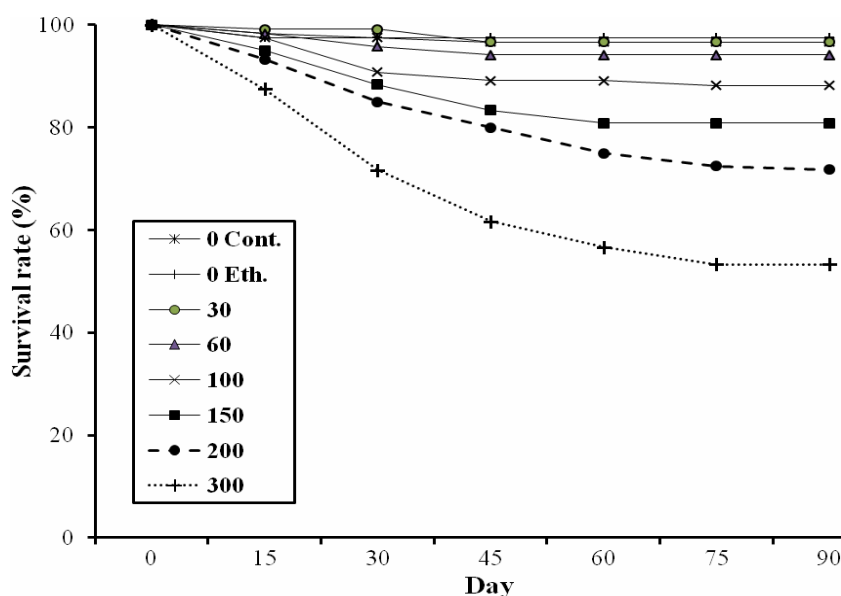


Figure 2. Changes in survival of reared guppy for each treatment at 15, 30, 45, 60, 75 and 90 days after birth.

Discussion. The goal of the present study was to find an optimum dose of MT for sex reversal and improving the growth performance in *P. reticulata*. One of the most important species in ornamental fish culture is the guppy. In this species, sexual differentiation takes place before parturition (Dildine 1936), which compromises the number of approaches to masculinization that can be taken. Initial studies (Eversole 1941; Dzwillo 1962; Clemens et al 1966; Turan et al 2006; Mag & Bud 2006) succeeded in inducing masculinization of guppies after treatment with natural or synthetic androgens. It was observed that the MT was effective at various dose levels in increasing the proportion of males in the population and improving growth performance in *P. reticulata*. However, the present results are consistent with reported results from studies with such fish species as *Sciaenochromis ahli*, *Cyprinus carpio*, *P. reticulata* and *Cichlasoma nigrofasciatum*, all of which were treated with synthetic hormone (Farahmand 1993; George & Pandian 1996; Elmdoust 2003; Mag & Bud 2006; Mousavi-Sabet 2011). George & Pandian (1996) obtained 82% male population in *P. reticulata* at dose rate of 200 mg MT/kg of feed in 20 days and Elmdoust (2003) showed a successful masculinization in *Sciaenochromis ahli* (Cichlidae) with 60 mg MT/kg of feed in 30 days. Mag & Bud (2006) showed that 200 mg 17-alpha-methyltestosterone/kg food administered 5-24 days prior to parturition and, as well, 150 mg 17-alpha-methyltestosterone/kg food can be use in production of all-male guppy stocks. Mousavi-Sabet (2011) obtained 95.49% male population in *Cichlasoma nigrofasciatum* by using 100 mg MT/kg of feed in 40 days. Also, in other fishes, greater than 90% of male population was obtained at a variety of dose rates, for instance, Jae-Yoon et al (1988) obtained 97% of *O. niloticus* males when applying dose rate of 10 mg MT/kg of diet. Other authors have used the higher dose rate to achieve sex reversal. In *Oreochromis* sp, Romerio et al (2000) obtained 98% male population at dose rate of 60 mg MT/kg of feed. The results of other study (Marjani et al 2009) showed a significantly lower male proportion (84.3%) in *O. mossambicus* for highest dose rate of MT, 100 mg MT/kg of feed. These results are in line with the findings of (Okoko 1996) which obtained 71.9% males at the dose rate of 120 mg MT/kg of feed. Other results showed the over dose such as more than 100 mg MT/kg give the sterilized population (Marjani et al 2009). A lower hormone concentration (60 mg of MT) is the same as that used for Eurasian perch, *Perca fluviatilis* (Rougeot et al 2001) and for black crappie, *Pomoxis nigromaculatus* (Alablani & Phelps 1997). However, this dose is lower than that used for the induction of sex reversal in common carp, *Cyprinus carpio* (100 mg of MT /kg feed; Gomelsky et al 1994), but higher than that used in European seabass, *Dicentrarchus labrax* (0.5-5 mg 17 α -methyl-dehydrotestosterone /kg feed; Chatain et al 1999) or the euryhaline tilapia, *Oreochromis mossambicus* (10 mg of MT /kg feed; Ron et al 1995).

Higher growth rate of low-hormone treated fish was also observed. The result conforms to what was reported in pikeperch, *Stizostedion lucioperca* (Demska-Zakes & Zakes 1997). According to Marjani et al (2009) and Mousavi-Sabet (2011), in present study, different dose rates of MT significantly effected on the growth of *P. reticulata*, and 30 mg MT treatment showed more average of the body weight and the body length than others. Dose 75 mg MT for 21 days showed 17.4 g gain in weight followed by dose of 100 mg MT/kg feed and 10.9 g, by dose 50 mg MT/kg feed in *O. mossambicus* (Marjani et al 2009). These results are in line with the findings regarding anabolic effect of MT in fish and all male culture of ornamental fish by different authors observed faster growth of hormone treated fish (Mousavi-Sabet 2007). Hanson et al (1984) reported that 10-60 ppm MT-treatment showed the best growth compared to control group; these are also in line with Dan & Little (2000) who compared the culture performance of different strains of *O. niloticus* and found that considering all strains, MT treatment resulted in a final size of fish 10.7% larger than mixed sex fish. On the other hand in some species the females have better growth performance, so some researchers studied on estrogen hormones. For example, in black crappie and largemouth bass, females grow faster and larger than males (Arslan 2002).

As conclusion in present study and about the *P. reticulata* masculinization, best selected dose rate which resulted in maximum male population with the least loss of fish was the 150-200 mg MT/kg of feed. In addition, the dose 30 mg MT/kg of feed showed

the best growth performance in the fish. The effective dose of 17 α -methyltestosterone in the livebearer *Poecilia reticulata* was higher compared to egg-layer fish species (e.g. Cichlids). The information obtained from this study provides a useful method for sex reversal with lower mortality and commercial scale production of guppy by using 17 α -methyltestosterone.

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